DNAの高次構造と
物理的特性に印された遺伝情報の解読

（課題番号　18570165）

平成18年度ー平成19年度科学研究費補助金（基盤（C））
研究成果報告書

平成20年5月

研究代表者　　大山　隆
（早稲田大学教育・総合科学学術院・教授）

はしがき

遺伝情報とは、生物が自己と同じものを複製するために次世代に伝える情報である。そして今や、「遺伝情報は核酸塩基の配列として符号化されている」という概念が定着している。例えば、Oxford Dictionary of Biochemistry and Molecular Biology (2nd ed.)の「genetic information」の項には、“the information carried in a sequence of nucleotides in a molecule of DNA or RNA.”と書かれている。確かに遺伝暗号は塩基配列に“記”され、遺伝子発現にかかわる遺伝情報の多くも、特定の塩基配列が担っている。それでは遺伝情報は塩基配列にしか記されていないのだろうか。答えは「No」である。DNA の高次構造や特性にも情報が“印”されていることが明らかになってきた。エピジェネティクスの時代を迎え、今日、このような高次の遺伝情報が注目を集めている。本研究では、DNA の高次構造と物理的特性に印された遺伝情報の解読を試みた。

研究組織

研究代表者：　大山　隆　（早稲田大学教育・総合科学学術院・教授）
研究分担者：　三谷　匡　（近畿大学先端技術総合研究所・准教授）

研究経費

直接経費

平成 18 年度	2,000 千円
平成 19 年度	1,500 千円

間接経費

平成 18 年度	0 千円
平成 19 年度	450 千円

計　3,950 千円

研究成果

研究成果の概要を内容別に記すとともに、当該研究期間中に公表した主要関連論文の別刷りを添付することで本研究の成果報告とする。

I. ベントDNAが担う遺伝情報の解析

T20は、負の超らせんを擬態した180塩基対の合成ベントDNAで（図1）、我々の研究室で作製された。我々はこれまでに、HeLa細胞のゲノム内でT20がクロマチンを制御して転写を活性化できることを明らかにしていた。本研究では、T20をもつレポーターをマウスES細胞のゲノムに導入し、ES細胞におけるその機能と細胞分化がその機能に及ぼす影響について解析した。レポーターコンストラクトには、ニワトリのβ-アクチンプロモーターの上流に二つのloxP配列で挟んだT20を配置し、GFP遺伝子をレポーター遺伝子としたものを用いた。3組の細胞株（3種類の試験株とそのそれぞれに対応する対照株）を用いた

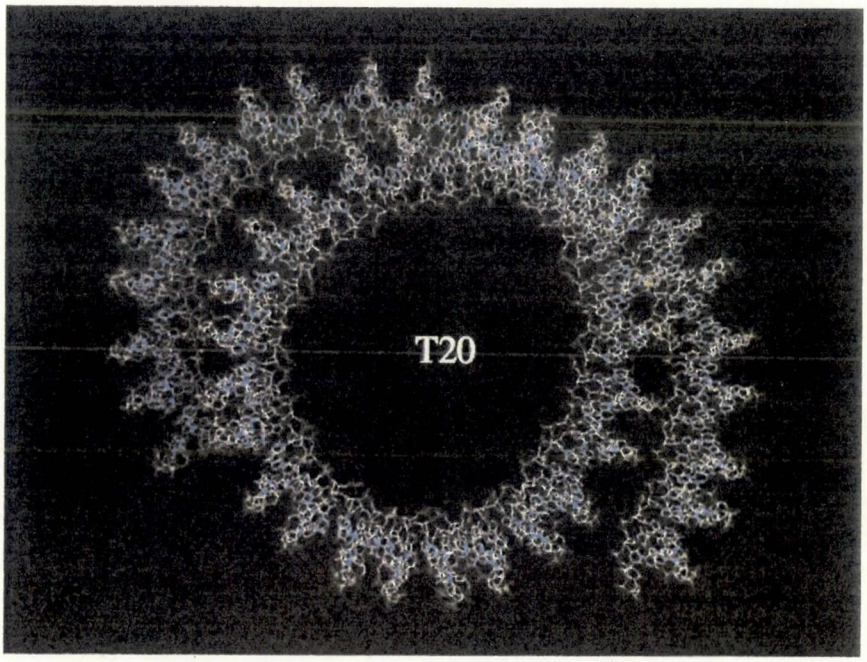


図1．人工ベントDNA T20

解析の結果、T20 はマウス ES 細胞内でも、また同細胞を肝細胞に分化させた後にも、下流のプロモーターを活性化できることが明らかになった。さらに、lacO/lacI-GFP 法を用いて、ES 細胞におけるレポーターの存在部位を解析した。64 コピーの lacO 配列をタンデムに並べた DNA をレポーター（二つの loxP 配列で挟んだ T20、単純ヘルペスウイルスのチミジンキナーゼプロモーター、ルシフェラーゼ遺伝子から構成される）につなぎ ES 細胞ゲノムに 1 コピー導入して解析したところ、レポーターが核の周縁部に局在していることが分かった。このほか本研究では、ES 細胞を肝細胞に分化誘導する際に興味深い現象を見出した。これは、レポーターが導入された細胞は総じて分化に“抵抗性”を示す（最終的には分化する）という現象で、外来遺伝子の存在が細胞分化にも影響を及ぼしうるという点で興味深い。この現象の分子機構については、継続検討課題として現在も調べている。

II. DNA の物理的特性が担う遺伝情報の解析

DNA の柔軟性をゲノムワイドに解析できるコンピュータプログラムを開発し、出芽酵母第 3 染色体に存在する全ての高頻度組換え部位の柔軟性を解析した。その結果、高頻度組換え部位にはゲノムの平均的硬さに比べるとかなり柔らかい DNA が含まれていることが分かった。ゲノム全体を解析した結果からは、エクソン領域がイントロンや遺伝子間領域よりも柔らかい DNA でできていることが明らかになり、DNA の柔軟性がエクソンとイントロンの違いを“表示する”情報として機能している可能性が示唆された。

研究成果発表

1. 論文

(1) Matsugami, A., Tani, K., Ouhashi, K., Uesugi, S., Morita, M., Ohyama, T. and Katahira, M.
Structural property of DNA that migrates faster in gel electrophoresis, as deduced by CD spectroscopy.
***Nucleosides, Nucleotides and Nucleic Acids,* 25**, 417-425 (2006)

(2) Sumida, N., Nishikawa, J., Kishi, H., Amano, M., Furuya, T., Sonobe, H. and Ohyama, T.
A designed curved DNA segment that is a remarkable activator of eukaryotic transcription.
***FEBS J.*, 273**, 5691-5702 (2006)

(3) Inoue, S., Sugiyama, S., Travers, A.A. and Ohyama, T.
Self-assembly of double-stranded DNA molecules at nanomolar concentrations.
***Biochemistry*, 46**, 164-171 (2007)

(4) Kamiya, H., Fukunaga, S., Ohyama, T. and Harashima, H.
The location of the left-handedly curved DNA sequence affects exogenous DNA expression *in vivo*.
***Arch. Biochem. Biophys.*, 461**, 7-12 (2007)

(5) Sumida, N., Sonobe, H. and Ohyama, T.
Chromatin structure formed on a eukaryotic promoter activated by a left-handed superhelical bent DNA of 180 bp.
***J. Adv. Sci.*, 19**, 22-28 (2007)

2. 著書

(1) Ohyama, T., Sumida, N., Tanase, J., Inoue, S. and Okabe T.
Genetic information carried in DNA conformation and properties.
In Kiyama, R. and Shimizu, M. (eds), ***DNA Structure, Chromatin and Gene Expression***. Transworld Research Network (Kerala), 71-84 (2006)

(2) Ohyama T.
Regulation of chromatin structure by curved DNA: how activator binding sites become accessible.
In Nagata, K. and Takeyasu, K. (eds.), ***Nuclear Dynamics-Molecular Biology and Visualization of the Nucleus***. Springer-Verlag (Tokyo), 227-238 (2007)

3. 総説

(1) 大山隆
DNA の高次構造と物理的特性に印された遺伝情報.
蛋白質核酸酵素, 53, 1-11 (2008)

4. 学会発表

(1) 大山隆、隅田周志、棚瀬潤一
クロマチン工学の開拓
第 6 回細胞核ダイナミクス研究会　2006 年 5 月（熊本）

(2) Takashi Ohyama, Noriyuki Sumida, Yoshiro Fukue, Jun-ichi Tanase, Shotaro Inoue
Genetic information written in DNA conformation and properties
20th IUBMB International Congress of Biochemistry and Molecular Biology and 11th FAOBMB Congress　2006 年 6 月（京都）

(3) 大山　隆、棚瀬潤一、隅田周志、井上正太郎
細胞核ダイナミクスとゲノムの折り畳み
特定領域研究「細胞核ダイナミクス」第 3 回班会議　2006 年 7 月（大阪）

(4) Takashi Ohyama, Noriyuki Sumida, Jun-ichi Tanase
How promoters are recognized
7th EMBL Transcription Meeting　2006 年 8 月 (Heidelberg)

(5) 大山隆
DNA の高次構造と特性に印された遺伝情報の解読
国立遺伝学研究所研究会「クロマチンダイナミクスとゲノム機能制御」
2006 年 10 月（三島）

(6) 大山　隆、隅田周志、棚瀬潤一
クロマチン工学の開拓
生理学研究所研究会「DNA 構造を基盤とするゲノム生理学の展開−DNA、蛋白質、膜の相互作用」 2006 年 11 月（岡崎）

(7) Takashi Ohyama, Noriyuki Sumida, Shotaro Inoue, Jun-ichi Tanase, Hajime Kimura, Mariko Miura
How genomic DNA is functionally folded in a nucleus
International Symposium 「Functional Organization of the Nucleus」　2007 年 1 月（Awaji）

(8) 大山　隆、木村　元
DNA の機械的特性と染色体構造
第 24 回染色体ワークショップ　2007 年 1 月（佐賀）

(9) 大山　隆、木村　元
ヒトゲノムの機械的特性地図
特定領域研究「核ダイナミクス」第 4 回班会議　2007 年 7 月（湯沢）

(10) Takashi Ohyama, Jun-ichi Tanase, Hajime Kimura
Genetic information carried in DNA conformation and properties
EMBO Conference Series on Nuclear Structure and Dynamics　2007 年 9 月 (Montpellier)

(11) 大山隆、棚瀬潤一、宇田川紘司、木村元、景山大、古屋美香、藤井俊輔、三谷匡
ゲノム DNA の特性解析と高次遺伝情報の解読
第 7 回細胞核ダイナミクス研究会　2007 年 9 月（札幌）

(12) 大山隆
高次遺伝情報の解読
国立遺伝学研究所研究会「クロマチン研究会−ゲノム・細胞核から個体発生まで−」　2007 年 10 月（三島）

(13) 大山隆、棚瀬潤一、伊藤美月、三谷匡、清水光弘
クロマチン工学のストラテジー
BMB2007（第 30 回日本分子生物学会年会・第 80 回日本生化学会大会　合同大会）ワークショップ「クロマチン工学の開拓」　2007 年 12 月（横浜）

(14) 棚瀬潤一、宇田川紘司、山本拓弥、伊藤美月、藤井俊輔、三谷匡、大山隆
クロマチン工学による外来遺伝子の安定的発現
BMB2007（第 30 回日本分子生物学会年会・第 80 回日本生化学会大会　合同大会）　2007 年 12 月（横浜）

(15) 木村元、古屋美香、景山大、村田昇、大山隆
ヒトゲノムの機械的特性地図
BMB2007（第 30 回日本分子生物学会年会・第 80 回日本生化学会大会　合同大会）　2007 年 12 月（横浜）

(16) 小平玲菜、西村恵美、鎌田康之、岡部隆宏、大山隆
食虫植物 S-like リボヌクレアーゼ遺伝子の構造と発現
BMB2007（第 30 回日本分子生物学会年会・第 80 回日本生化学会大会　合同大会）　2007 年 12 月（横浜）

(17) 清水光弘、諸橋伸行、森智、神庭昂平、大山隆
酵母ゲノムにおけるクロマチン工学
BMB2007（第 30 回日本分子生物学会年会・第 80 回日本生化学会大会　合同大会）ワークショップ「クロマチン工学の開拓」　2007 年 12 月（横浜）

(18) 三谷匡、川村紘子、笹栗弘貴、横田隆徳、棚瀬潤一、大山隆、田口善智
マウスES細胞における非B-型DNAによるshRNA発現活性化に対する効果
BMB2007（第 30 回日本分子生物学会年会・第 80 回日本生化学会大会　合同大会）　2007 年 12 月（横浜）

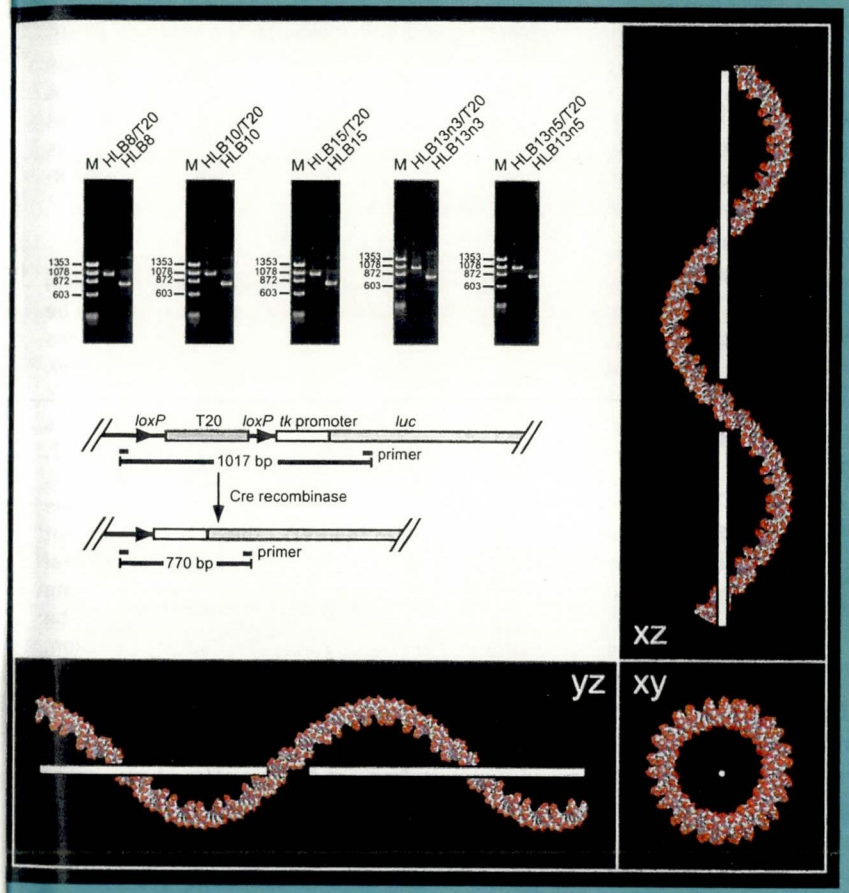
(19) 木村元、景山大、古屋美香、大山隆
ヒトゲノムの機械的特性と染色体構造
第 25 回染色体ワークショップ　2008 年 1 月（湯河原）

ISSN 1742-464X

www.febsjournal.org

the FEBS Journal

Volume 273 Number 24 December 2006



Blackwell Publishing

the FEBS Journal

Print ISSN 1742-464X
Online ISSN 1742-4658

Formerly *European Journal of Biochemistry*

SUBSCRIPTION RATES

FEBS Journal is published twice a month in 2007 (24 issues). Subscription prices for 2007 are as follows:

	Europe	The Americas	Rest of World
Institutional Premium Rate*	£2391.00	$4418.00	£2630.00

*Includes online access to current content and all online backfiles to January 1st 1997, where available.
For submission instructions, subscription and all other information visit: www.febsjournal.org

Customers in the UK should add VAT at 6%; customers in the EU should also add VAT at 6%, or provide a VAT registration number or evidence of entitlement to exemption. Customers in Canada should add 6% GST or provide evidence of entitlement to exception. The Premium institutional price includes online access to the current and all available previous year electronic issues. For other pricing options or more information about online access to Blackwell Publishing journals, including access information and terms and conditions, please visit www.blackwellpublishing.com

Journal e-mail: febsjournal@oxon.blackwellpublishing.com

DELIVERY TERMS AND LEGAL TITLE

Prices include delivery of print journals to the recipient's address. Delivery terms are Delivered Duty Unpaid (DDU); the recipient is responsible for paying any import duty or taxes. Legal title passes to the customer on despatch by our distributors.

PUBLISHER

FEBS Journal is published by Blackwell Publishing Ltd, 9600 Garsington Road, Oxford, OX4 2DQ, UK. Tel: +44 (0) 1865 776868, Fax: +44 (0) 1865 714591.

Journal Customer Services: For ordering information, claims and any enquiry concerning your journal subscription please contact your nearest office:
UK: Email: customerservices@blackwellpublishing.com; Tel: +44 (0) 1865 778315; Fax: +44 (0) 1865 471775
USA: Email: customerservices@blackwellpublishing.com; Tel: +1 781 388 8206 or 1 800 835 6770 (Toll free in the USA); Fax: +44 (0) 1865 471775
Asia: Email: customerservices@blackwellpublishing.com; Tel: +65 6511 8000; Fax: +44 (0) 1865 471775

This journal is available online at:
Blackwell Synergy. Visit www.blackwell-synergy.com to search the articles and register for table of contents e-mail alerts.

Highwire. Visit content.febsjournal.org to search the articles.

Access to this journal is available free online within institutions in the developing world through the AGORA initiative with the FAO and the HINARI initiative with the WHO. For information, visit http://www.aginternetwork.org or www.healthinternetwork.org

Back issues: Single issues from current and recent volumes are available at the current single issue price from Blackwell Publishing Journals. Earlier issues may be obtained from Periodicals Service Co. (www.periodicals.com)

PERIODICAL ID STATEMENT

FEBS JOURNAL, (1742-464x), is published twice a month. US mailing agent: G3 Worldwide (US) Inc., 8701 Bellanca Ave., Los Angeles, CA 90045. Periodical postage paid at Los Angeles, CA and additional mailing offices. POSTMASTER: Send all address changes to FEBS JOURNAL, Blackwell Publishing Inc., Journals Subscription Department, 350 Main St., Malden, MA 02148-5020.

AUTHOR MATERIAL ARCHIVE POLICY

Please note that unless specifically requested, Blackwell Publishing will dispose of all hardcopy or electronic material submitted two issues after publication. If you require the return of any material submitted, please inform the Editorial Office or Production Editor as soon as possible if you have not yet done so.

DISCLAIMER

The Publisher, FEBS and Editors cannot be held responsible for errors or any consequences arising from the use of information contained in this journal; the views and opinions expressed do not necessarily reflect those of the Publisher, FEBS and Editors, neither does the publication of advertisements constitute any endorsement by the Publisher, FEBS and Editors of the products advertised.

PAPER

Blackwell Publishing's policy is to use permanent paper from mills that operate a sustainable forestry policy, and which has been manufactured from pulp that is processed using acid-free and elementary chlorine-free practices. Furthermore, Blackwell Publishing ensures that the text paper and cover board used in all our journals has met acceptable environmental accreditation standards.

the FEBS Journal

A designed curved DNA segment that is a remarkable activator of eukaryotic transcription

Noriyuki Sumida[1], Jun-ichi Nishikawa[1], Haruka Kishi[1], Miho Amano[1], Takayo Furuya[1], Haruyuki Sonobe[1] and Takashi Ohyama[2,3]

1 Department of Biology, Faculty of Science and Engineering, Konan University, Kobe, Japan
2 Department of Biology, School of Education, Waseda University, Tokyo, Japan
3 Graduate School of Science and Engineering, Waseda University, Tokyo, Japan

Keywords
chromatin; chromatin engineering; curved DNA; supercoil; transcription activator

Correspondence
T. Ohyama, Department of Biology, School of Education, Waseda University, 1-6-1 Nishi-Waseda, Shinjuku-ku, Tokyo 169-8050, Japan
Fax: +81 3 3207 9694
Tel: +81 3 5286 1520
E-mail: ohyama@waseda.jp

(Received 11 September 2006, revised 19 October 2006, accepted 25 October 2006)

doi:10.1111/j.1742-4658.2006.05557.x

To identify artificial DNA segments that can stably express transgenes in the genome of host cells, we built a series of curved DNA segments that mimic a left-handed superhelical structure. Curved DNA segments of 288 bp (T32) and 180 bp (T20) were able to activate transcription from the herpes simplex virus thymidine kinase (*tk*) promoter by approximately 150-fold and 70-fold, respectively, compared to a control in a transient transfection assay in COS-7 cells. The T20 segment was also able to activate transcription from the human adenovirus type 2 *E1A* promoter with an 18-fold increase in the same assay system, and also activated transcription from the *tk* promoter on episomes in COS-7 cells. We also established five HeLa cell lines with genomes containing T20 upstream of the transgene promoter and control cell lines with T20 deleted from the transgene locus. Interestingly, T20 was found to activate transcription in all the stable transformants, irrespective of the locus. This suggests that the T20 segment may allow stable expression of transgenes, which is of importance in many fields, and may also be useful for the construction of nonviral vectors for gene therapy.

DNA is packaged into chromatin in eukaryotes, thereby maintaining genes in an inactive state by restricting access to the general transcription machinery. Proteins that turn on or activate gene transcription are called activators, and these proteins recruit the chromatin remodeling complex to facilitate transcription [1–3]. Activators can bind to a target element in a regulatory promoter or enhancer [4], even when the target is adjacent to or actually within a nucleosome [5–9]. The regulatory promoter is typically located immediately upstream of the core promoter, which is positioned immediately adjacent to and upstream of the gene [4].

Regulatory promoters often include intrinsically curved DNA structures and poly(dA·dT) sequences [10,11]. Recent studies have shown that such structures are used to organize local chromatin structure to allow activator-binding sites to be accessible [11]. This suggests that engineering of chromatin structure for gene expression may be possible using these promoter structures or artificial mimics. This new technology, which might be referred to as 'chromatin engineering', would also permit stable expression of transgenes, which is of importance in many areas of the biological sciences. Moreover, such technology could lead to the development of useful nonviral vectors for gene therapy. Therefore, the goal of the current study was to construct artificial bent DNA segments that can stably express transgenes in the genome of living cells, as a first step in chromatin engineering.

We have reported that a 36 bp left-handed curved DNA segment, which we refer to as T4 in the present study (T indicates a dT·dA tract and the numeral indicates the number of tracts), activates the herpes simplex virus thymidine kinase (HSV *tk*) promoter in a

Abbreviations
Ad2, adenovirus type 2; EBV, Epstein–Barr virus; HSV, herpes simplex virus; mcs, multiple cloning site.

transient transfection assay system, at a specific rotational phase and distance between T4 and the promoter [12]. We concluded that T4 formed part of the nucleosome, leaving the TATA box in the linker DNA with its minor groove facing outwards, which led to activation of transcription by approximately 10-fold. Here, we investigated the effect on transcription of curved DNA segments that are longer than T4, using episomes and stable transformants, in addition to a transient transfection assay system. We show that a 180 bp left-handed curved DNA segment (T20) causes marked activation of transcription, irrespective of the assay system employed.

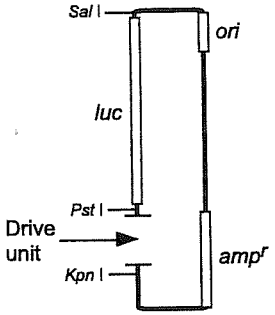
Results

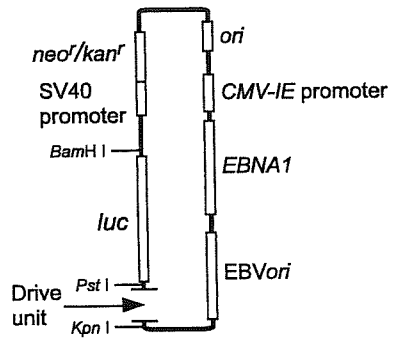
Plasmid constructs

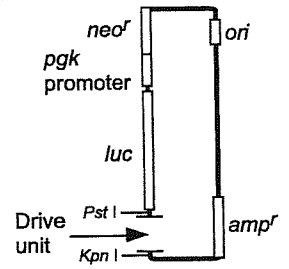
The constructs used in this study are shown in Table 1 and Fig. 1. The periodicity of A-tracts determines the

Table 1. Reporter constructs used in this study. The 'drive unit', which is shown in detail in Fig. 1, indicates the segment containing a promoter and an upstream synthetic DNA with a defined geometry. *luc*, luciferase gene; *CMV-IE*, cytomegalovirus immediate-early gene; *pgk*, mouse phosphoglycerate kinase gene; *neo*, neomycin phosphotransferase gene; EBV*ori*, Epstein-Barr virus replication origin; *EBNA1*, Epstein–Barr virus nuclear antigen 1 gene.

Group	Name	Drive unit	Vector
I	pST0/TLN-7[a]	1	
I	pLHC4/TLN-6[a]	2	
I	pLHC8/TLN-6	3	
I	pLHC12/TLN-6	4	
I	pLHC16/TLN-6	5	
I	pLHC20/TLN-6	6	
I	pLHC24/TLN-6	7	
I	pLHC28/TLN-6	8	
I	pLHC32/TLN-6	9	
I	pLHC36/TLN-6	10	
I	pLHC40/TLN-6	11	
II	pRHC4/TLN[a]		
II	pRHC4/TLN+40	12	
II	pRHC8/TLN+40	13	
II	pRHC12/TLN+40	14	
III	pST0/ELN	15	
III	pLHC20/ELN	16	
IV	pST0/TLN-7/EBV*ori*[a]	1	
IV	pLHC20/TLN-6/EBV*ori*	6	
IV	pLHC32/TLN-6/EBV*ori*	9	
V	pLHC20/*loxP*/TLN-6	17	
V	pLHC20/*loxP*/-2/TLN-6	18	
V	pLHC20/*loxP*/-4/TLN-6	19	
V	pLHC20/*loxP*/-5/TLN-6	20	
V	pLHC20/*loxP*/-8/TLN-6	21	
V	pLHC20/*loxP*/-10/TLN-6	22	







[a]Plasmids reported previously [12,14].

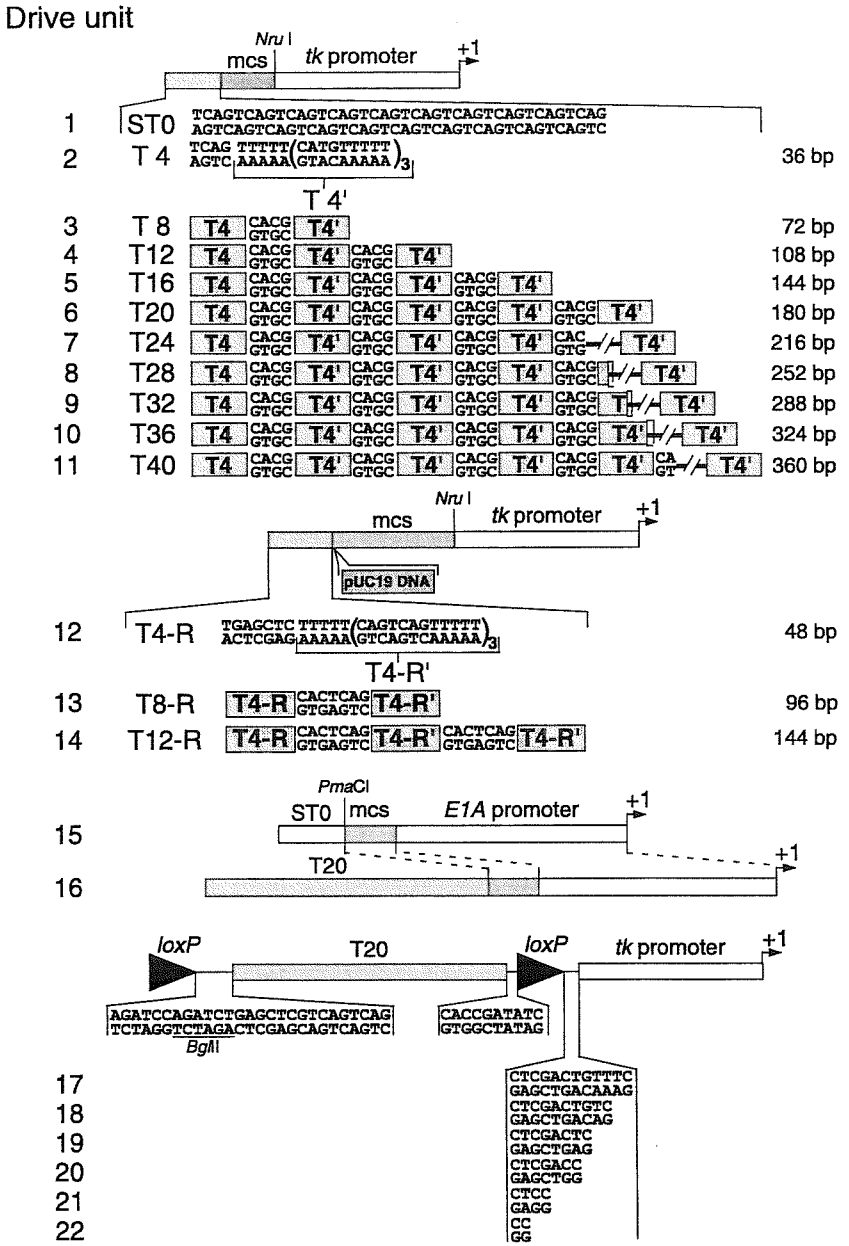


Fig. 1. Structure of the 'drive unit' shown in Table 1. 'mcs' and '*loxP*' indicate the multiple cloning site and the *loxP* sequence [18], respectively. The complete nucleotide sequences of drive units 1 and 2 are given in Nishikawa *et al.* [12]. The *E1A* promoter spans the region from nucleotides 357 to 498 of human Ad2 DNA.

three-dimensional architecture of DNA: that is, periodicities smaller than 10.5 bp give left-handed curves, and those larger than 10.5 bp give right-handed curves [12,13]. Based on this knowledge, left-handed or right-handed curved DNA segments were prepared. The plasmids pST0/TLN-7, pLHC4/TLN-6, pRHC4/TLN and pST0/TLN-7/EBV*ori* have been reported previously [12,14]. In the construct names, 'ST', 'LHC' and 'RHC' indicate straight, left-handed curved and right-handed curved, respectively, and the numerals following these indicate the number of $(T/A)_5$ tracts in the construct. 'TL' in 'TLN' refers to the *tk* promoter and the luciferase gene, and 'EL' in 'ELN' refers to the *E1A* promoter of human adenovirus type 2 (Ad2) and the luciferase gene. In the *tk* promoter-based constructs, the best position of the left-handed curved segment for transcriptional activation was determined to be 125 bp upstream from the center of the TATA box [12]. Thus, we inserted longer curved DNA segments into that position. For convenience, the constructs were divided into five groups. Groups I, II and III were used in transient transfection assays: group I constructs each contained a left-handed curved DNA, except for the control construct pST0/TLN-7; group II constructs each contained a right-handed curved DNA; group III constructs contained the Ad2 *E1A* promoter instead of the *tk*

promoter. The group IV constructs were replicable in host cells, and the group V constructs were prepared for the examinination of transcription levels on the genome of stable transformants. In the group II constructs, '+40' indicates an insertion of a 40 bp DNA fragment within the multiple cloning site (mcs). In the group V constructs, a number between two slashes indicates the number of base pairs that were deleted from the region between the downstream *loxP* sequence and the *tk* promoter.

Marked activation of transcription by left-handed curved DNA segments in a transient expression system

Promoter activity was studied by introducing each construct into COS-7 cells by electroporation and performing a luciferase assay after 21 h in culture. The results are shown in Fig. 2. The promoter activity of pST0/TLN-7, which has a straight DNA segment upstream of the *tk* promoter, was used as a control, and the data are presented as a fold increase over the control. The previously reported activity of pLHC4/TLN-6 [12] is also shown. As shown in Fig. 2A, all left-handed curved DNA segments (named T*n*, where $n = 4$, 8, 12, 16, 20, 24, 28, 32, 36 and 40; Fig. 1) activated transcription from the *tk* promoter, although the extent of activation differed among these segments. For the segments from T4 to T32, the extent of transcriptional activation correlated with the length of the curved segment; however, T36 was less effective than T32, and T40 was less effective than T36. Thus, the most effective segment for transcriptional activation was T32, which activated the *tk* promoter in COS-7 cells by approximately 150-fold, relative to the control construct. The fragment length itself might generate some positive effects on transcription. To examine this possibility, we substituted the T*n* region with a 196 bp straight DNA fragment

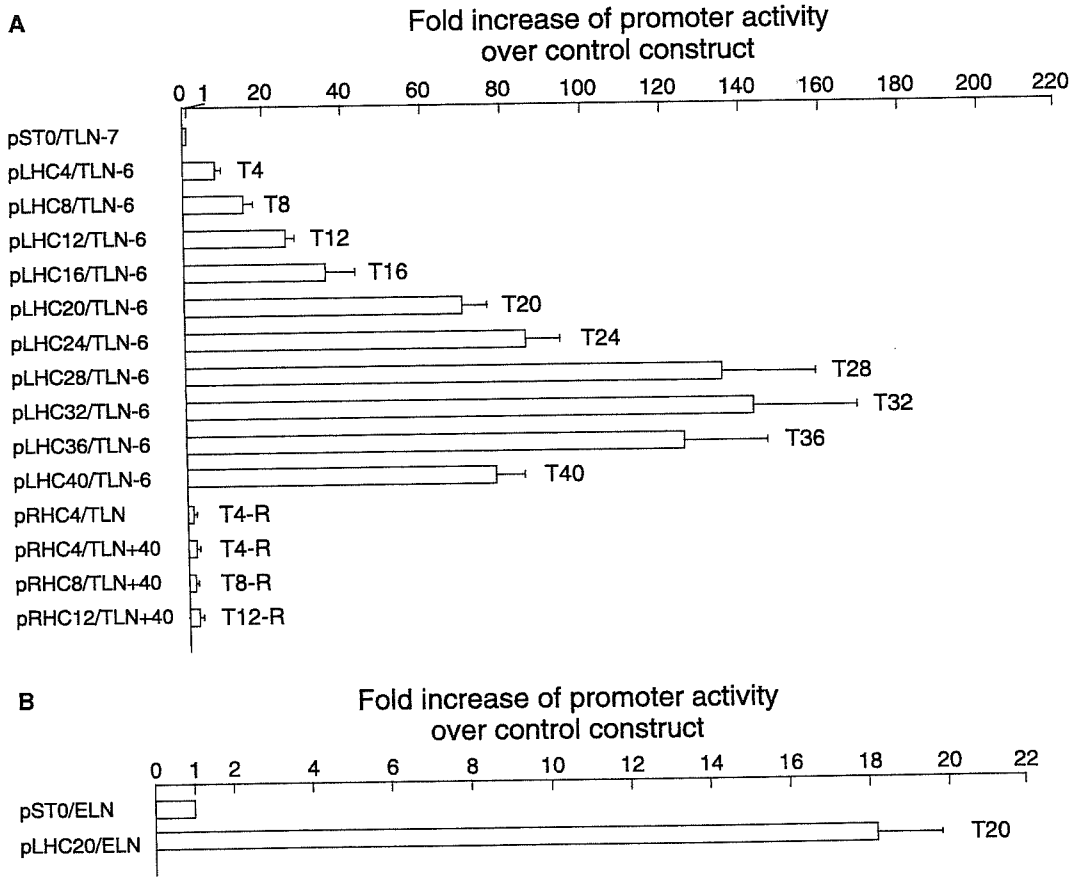


Fig. 2. Effect of curved DNA segments on transcription, as examined in a transient transfection assay. (A) Effect on transcription from the HSV *tk* promoter. The promoter activity was determined in a luciferase assay, with the activity of pST0/TLN-7, which includes a straight DNA segment, used as a standard. The promoter activity of pLHC4/TLN-6 is cited from Nishikawa *et al.* [12]. Values are shown as means ± SD ($n = 6$ or 4). (B) Effect of T20 on transcription from the human Ad2 *E1A* promoter. The activity of pST0/ELN, which includes a straight DNA segment, was used as a standard. Values are shown as means ± SD ($n = 6$).

derived from pUC19 (spanning nucleotides 1619–1814). The transcription level of this construct was almost the same as that of pST0/TLN-7 (not shown). Thus, it was confirmed that the fragment length was irrelevant to the transcriptional activation.

The effects of right-handed curved segments on transcriptional activation were also investigated. In a preliminary test, insertion of a 40 bp DNA fragment into the mcs slightly increased the promoter activity compared to that of pRHC4/TLN (but the effect was only slight). Therefore, the effect of longer right-handed curved segments was examined in constructs with a 40 bp insertion. However, the effects of all these segments on transcriptional activation were very slight, compared with the activity of the pST0/TLN-7 control construct (Fig. 2A).

We also investigated the effect of T20 using the human Ad2 *E1A* promoter. Control promoter activity was obtained using pST0/ELN, which carries a straight DNA segment upstream of the *E1A* promoter. As shown in Fig. 2B, T20 was able to activate the *E1A* promoter by 18-fold over the control, showing that this segment can activate another eukaryotic promoter, in addition to the *tk* promoter.

Effects of left-handed curved DNA segments on transcription on episomes

Chromatin structures formed on DNA templates that can replicate in the nucleus are likely be more uniform than those formed on nonreplicable DNA templates. To examine whether the phenomenon observed in Fig. 2A was reproducible on replicable DNA templates, the episomes pLHC20/TLN-6/EBV*ori* and pLHC32/TLN-6/EBV*ori* were constructed (group IV constructs in Table 1). They were introduced into COS-7 cells and allowed to replicate, and then promoter activities were assayed 21 days after transfection (Fig. 3). Although both T20 and T32 activated the *tk* promoter, the extent of activation was greatly reduced in each case, compared with the results obtained in the transient transfection assay. The T20 segment activated transcription five-fold and T32 did so approximately four-fold, relative to control data. In contrast to the results for transient transfection, T32 gave less activation of transcription than T20 in episomes.

Effect of left-handed curved DNA segments on transcription in genomic chromatin

As T20 gave greater activation of transcription than T32 in episomes (Fig. 3), we studied the effect of the T20 segment on transcription in the context of genomic chromatin. The reporter constructs used for this purpose are shown in Table 1 and Fig. 1. The T20 sequence was placed between two *loxP* sequences, and this region was placed upstream of the *tk* promoter. The *loxP* sequences were included to allow establishment of control cell lines containing genomes in which T20 is deleted, as described below. The rotational orientation of the upstream curved DNA relative to the promoter has previously been shown to influence the promoter activity [12]. Therefore, to optimize the effect of T20, we initially constructed several derivatives with T20 oriented differently relative to the *tk* promoter (group V in Table 1), and investigated the resulting effects on transcription in the transient transfection assay used in Fig. 2. A deletion of two nucleotide pairs from the region between the downstream *loxP* sequence and the *tk* promoter generated pLHC20/*loxP*/-2/TLN-6. As the deletion generates a rotation of 69° [(2/10.5) × 360°] between both sides of the deletion, the rotational phase between T20 and the promoter in the construct differs by 69° from that in pLHC20/*loxP*/TLN-6. Deletions of four, five, eight and 10 nucleotide pairs generated differences of 137°, 171°, 274° and 343° [(4/10.5) × 360°, (5/10.5) × 360°, (8/10.5) × 360° and (10/10.5) × 360°, respectively] in the rotational phase, compared with the pLHC20/*loxP*/TLN-6 construct.

Although the promoter activity of pLHC20/*loxP*/TLN-6 was slightly higher (2.2 ± 1.3-fold) than that of

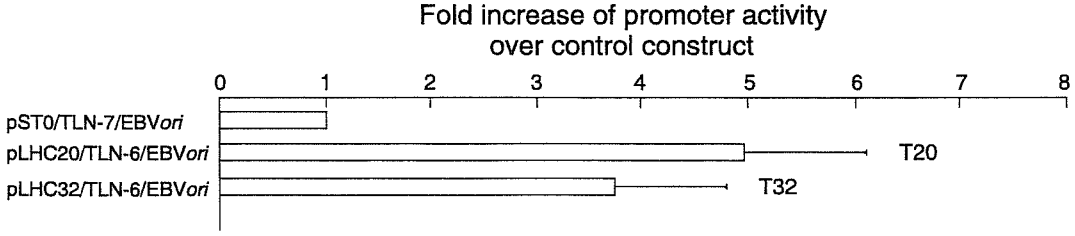


Fig. 3. Influence of template DNA replication on transcriptional activation by left-handed curved DNA segments. The promoter activity was determined in a luciferase assay performed on day 21 after transfection, with the activity of pST0/TLN-7/EBV*ori* used as a standard. Values are shown as means ± SD ($n = 3$).

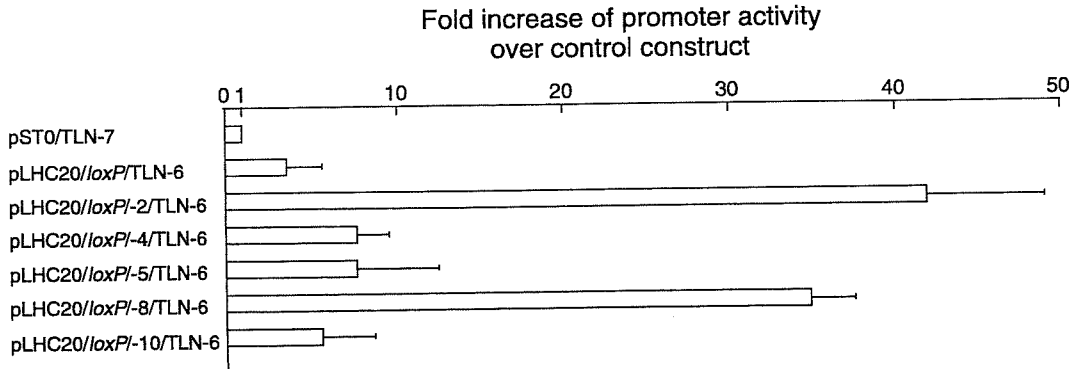


Fig. 4. Optimization of the position of the T20 segment, as examined in a transient transfection assay. The promoter activity was determined in a luciferase assay, with the activity of pST0/TLN-7 used as a standard. Values are shown as means ± SD (n = 5 or 4).

the control plasmid pST0/TLN-7 (Fig. 4), it was much lower than the promoter activity of pLHC20/TLN-6 (Fig. 2A). This was presumably because the downstream *loxP* sequence located between T20 and the *tk* promoter interfered with the position of T20 relative to the promoter. Alteration of the rotational phase between T20 and the *tk* promoter by 69° (pLHC20/*loxP*/-2/TLN-6) and 274° (pLHC20/*loxP*/-8/TLN-6) increased promoter activation significantly (24.9 ± 4.4-fold and 20.6 ± 1.5-fold, respectively); the activity of the former construct was 11-fold (calculated from the mean values; 24.9/2.2) higher and that of the latter was nine-fold (20.6/2.2) higher than the activity of pLHC20/*loxP*/TLN-6.

The constructs pLHC20/*loxP*/TLN-6 and pLHC20/*loxP*/-2/TLN-6 were used to test the effect of T20 on transcription in the context of genomic chromatin. These constructs were selected to determine whether the difference in transcriptional activation found for transient transfection is maintained in the genome. After the constructs were cleaved at the *Kpn*I site, they were introduced into the HeLa genome as described in Experimental procedures. Among the cell lines with a genome that harbored the linearized pLHC20/*loxP*/TLN-6 or linearized pLHC20/*loxP*/-2/TLN-6, we screened for those that harbored a single copy of each plasmid using Southern blot analysis (Fig. 5). Five cell lines were finally established: HLB8/T20, HLB10/T20, HLB15/T20, HLB13n3/T20 and HLB13n5/T20; the first three of these contain a single copy of linearized pLHC20/*loxP*/TLN-6, and the other two contain a single copy of linearized pLHC20/*loxP*/-2/TLN-6. By

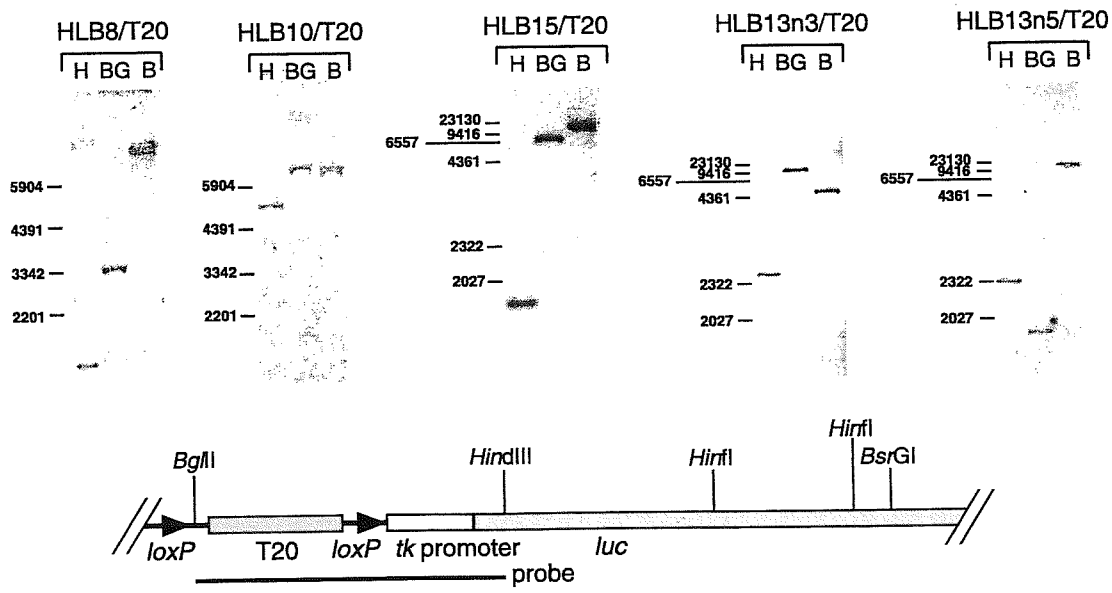


Fig. 5. Southern blot analysis of genomes of the established cell lines. Genomic DNAs from HLB8/T20, HLB10/T20, HLB15/T20, HLB13n3/T20 and HLB13n5/T20 were restricted with *Hinf*I (H), *Bsr*GI (BG) or *Bgl*I (B). After separation by gel electrophoresis and blotting, each digest was hybridized with a probe that is indicated below the autoradiograms.

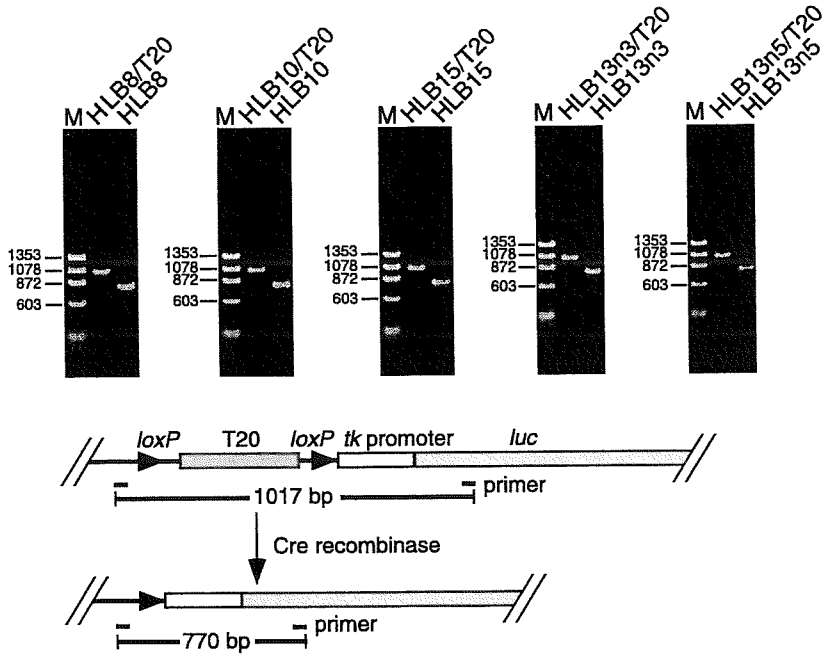


Fig. 6. Demonstration of the absence of the T20 segment in control cell lines. The target region for PCR amplification is illustrated at the bottom of the figure. 'M' indicates the size marker.

expressing the bacteriophage P1 Cre recombinase in these cell lines, we established control cell lines in which T20 was deleted from the reporter locus in the genome (Fig. 6).

The sites of reporter integration were determined by isolating the genomic DNA adjacent to the downstream end of the reporter construct and subsequent sequencing of this DNA (Fig. 7A). In the cell lines HLB8/T20, HLB10/T20, HLB15/T20 and HLB13n5/T20, each reporter was found to be integrated into an intergenic region, whereas in HLB13n3/T20, the reporter was integrated into the coding region of a gene. Interestingly, T20 was found to activate transcription irrespective of the position of the reporter construct (Fig. 7B). The difference in promoter activation observed between pLHC20/*loxP*/TLN-6 and pLHC20/*loxP*/-2/TLN-6 with transient transfection (Fig. 4) was not observed for transcription in the HeLa genome.

Discussion

We constructed several synthetic left-handed curved DNA segments that are able to activate eukaryotic promoters; in particular, the T32 and T20 segments activated transcription from the HSV *tk* promoter with approximately a 150-fold and a 70-fold increase over a straight control, respectively, in a transient transfection assay. T20 also activated transcription from the human Ad2 *E1A* promoter with approximately a 20-fold increase in the same assay system. In addition, T20 activated transcription in an EBV*ori*-containing episome and, most interestingly, in the genome of all stable transformants established in the study.

In our earlier study, we showed that a T4 segment has high affinity for the histone core in a transient transfection assay using pLHC4/TLN-6; nucleosome formation on this segment arranges the TATA box in the linker DNA with its minor groove facing outwards, which facilitates initiation of transcription [12]. The left-handed curved structure of T20, which is illustrated in Fig. 8, is five times longer than that of T4. Therefore, T20 seems to have higher affinity for the histone core, compared with T4. Compressing T20 along the superhelical axis easily allows formation of 1.75 turns of a left-handed supercoil that mimics nucleosomal DNA. Indeed, nucleosome formation on T20 was detected in chromatin formed on both transiently transfected constructs and constructs integrated into the HeLa genome (data not shown). Thus, T20-mediated activation of transcription may have occurred through the same mechanism reported for T4-containing constructs. However, the nucleosome-deposited population may not have been implicated in the transcriptional activation. As T20 seems to be compatible with the stabilization of an apical loop within a negatively supercoiled plectoneme, the promoter DNA sequence might be highly exposed in chromatin, or an altered promoter architecture might be effective in transcription. In addition, COS-7 and HeLa cells may contain proteins that preferentially bind to the left-handed curved DNA and activate transcription. Thus,

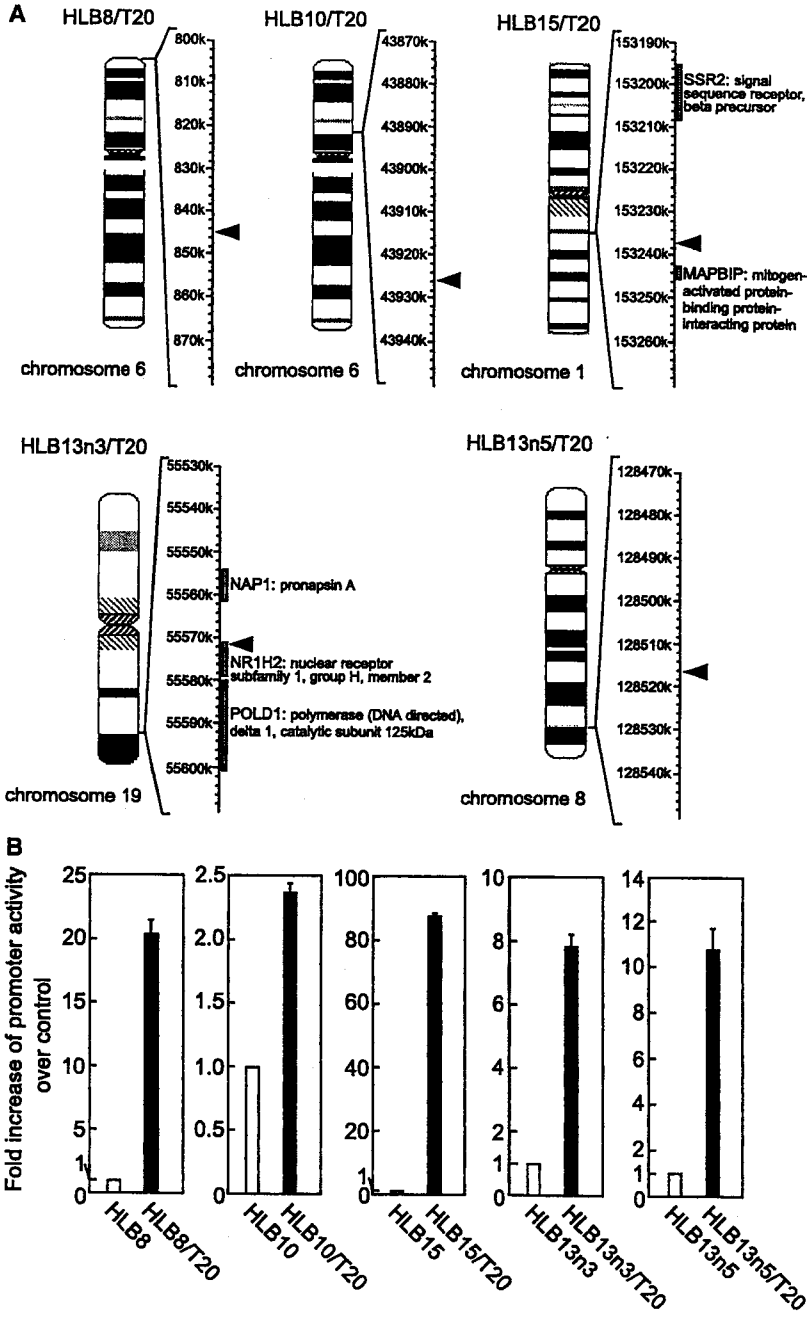


Fig. 7. Loci of reporter constructs in the established cell lines and expression levels of the luciferase gene. (A) Reporter loci. The HeLa genome region located adjacent to and downstream of each reporter construct was sequenced as described in Experimental procedures. The corresponding regions in normal human chromosomes are shown. The loci of integration are indicated with arrowheads. (B) Luciferase gene expression in each cell line. Values are shown as means ± SD ($n = 6$ or 4).

several explanations are possible for the mechanism of the observed transcriptional activation. We are currently examining the activation mechanism further.

We found a clear relationship between the length of T*n* ($n = 4, 8, 12, 16, 20, 24, 28, 32, 36, 40$) and promoter activity, with optimum promoter activation being achieved with T32 (Fig. 2A). However, the effects of T20 and T32 were reversed in transcription on episomes (Fig. 3). This difference was presumably caused by differences in local chromatin structures formed on nonreplicable and replicable constructs. The positioning of nucleosomes seems to be less uniform, differing from construct to construct, on nonreplicable constructs than on replicable constructs. In addition, nucleosome density was presumably lower on the nonreplicable constructs. These differences seem to have been reflected in the 'nakedness' of the promoter, which may in turn have influenced transcription.

The T20 segment caused greater activation of transcription in pLHC20/*loxP*/-2/TLN-6 than in

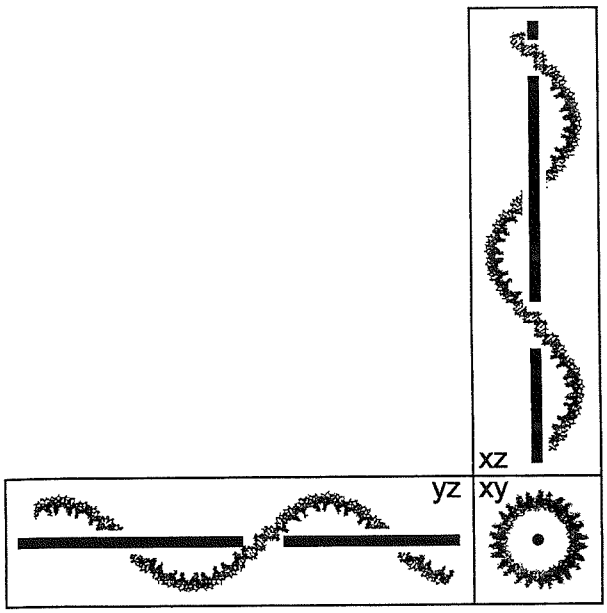


Fig. 8. Three-dimensional architecture of T20. The figure was drawn using a combination of DIAMOD [19] and RASMOL [20]. The modeling algorithm was based on that of Bansal *et al.* [21]. The bold line indicates the superhelical axis.

pLHC20/*loxP*/TLN-6 (Fig. 4). However, this difference was not observed in stable transformants; that is, the effect of T20 seemed to be about the same in transformants when either of the two constructs was used (Fig. 7B). The difference in the rotational phase between T20 and the promoter may have generated a more pronounced effect in a closed circular plasmid than in a linear genome, or alternatively the local chromatin structure formed in the promoter region may have differed between the plasmids and the genome. Whichever is the case, an important finding in this study is that T20 (and presumably the other T*n* constructs) can activate transcription in the context of genomic chromatin, irrespective of the locus of integration. To our knowledge, this is the first designed DNA segment that can stably activate transcription in the genome of host cells; therefore, T20 seems to be a promising tool for high-level and stable expression of transgenes, and may also be useful in the construction of nonviral vectors for gene therapy.

Experimental procedures

Plasmid construction

Group I

A synthetic double-stranded (ds) oligonucleotide (T4″), obtained by annealing oligonucleotides 5′-GTTTTTCATG TTTTTCATGTTTTTCATGTTTTTCAC-3′ and 5′-GTG AAAAACATGAAAAACATGAAAAACATGAAAAAC-3′, was inserted into the *Pma*CI site of pLHC4/TLN [12] by blunt-end ligation. After a conventional cloning and screening procedure, a plasmid with a tandem-repeated T4″ was selected. A unique *Pma*CI site was created in the plasmid, and the number of T4″ segments was increased one by one, using the same procedure. Finally, each resulting construct was digested with *Pma*CI and *Pvu*II, and these ends were closed to generate pLHC8/TLN-6, pLHC12/TLN-6, pLHC16/TLN-6, pLHC20/TLN-6, pLHC24/TLN-6, pLHC28/TLN-6, pLHC32/TLN-6, pLHC36/TLN-6 and pLHC40/TLN-6, respectively.

Group II

Synthetic oligonucleotides 5′-TCAGTTTTTCAGTCAG TTTTTCAGTCAGTTTTTCAGTCAGTTTTTCAC-3′ and 5′-GTGAAAAACTGACTGAAAAACTGACTGAAAAAC TGACTGAAAAACTGA-3′ were annealed to generate a dsDNA fragment, which we named T4-R″. T4-R″ was inserted into the *Pma*CI site of pRHC4/TLN [12] to produce pRHC8/TLN. Subsequently, T4-R″ was inserted into a newly created unique *Pma*CI site in pRHC8/TLN to generate pRHC12/TLN. A DNA fragment from pUC19 (positions 672–711) was inserted into the *Pma*CI site in the mcs in each construct to generate pRHC4/TLN+40, pRHC8/TLN+40, and pRHC12/TLN+40, respectively.

Group III

The construct pST0/ELN was prepared as follows. A DNA fragment containing the *E1A* promoter (nucleotides 357–498 in human Ad2) was obtained by digesting the plasmid pEKS (a gift from Y. Kadokawa, Fujita Health University, Toyoake, Japan) with *Eco*RI and *Bam*HI. The fragment was then blunted and ligated to phosphorylated *Pst*I linkers (5′-GCTGCAGC-3′). Subsequently, the resulting fragment was digested with *Sac*II, and blunted and digested with *Pst*I. Using the resulting product, the *Nru*I–*Pst*I region of pST0/TLN [12] was replaced. To construct pLHC20/ELN, the upstream straight region of pST0/ELN was removed by digestion with *Kpn*I and *Pma*CI, and this region was filled with the T20-containing *Kpn*I–*Pma*CI fragment of pLHC20/TLN.

Group IV

The construction of pST0/TLN-7/EBV*ori* has been reported previously [14]. Construction of the other plasmids was performed as follows. pEB6CAGFP [15] was digested with *Ssp*I, and a phosphorylated *Bam*HI linker (5′-CGGATCCG-3′) was ligated to the linearized plasmid. The resulting product was digested with *Spe*I, treated with T4 DNA polymerase, and subsequently digested with *Bam*HI. The fragment

containing an Epstein–Barr virus (EBV) replication origin (*oriP*) and the EBV nuclear antigen 1 (EBNA1) gene was purified and ligated to the *Xmn*I–*Bam*HI fragments of pLHC20/TLN-6 and pLHC32/TLN-6 to generate pLHC20/TLN-6/EBV*ori* and pLHC32/TLN-6/EBV*ori*, respectively.

Group V

The *loxP* sequence was derived from pLNX (a gift from S. Noguchi, Meiji Institute of Health Science, Odawara, Japan, and Y. Kadokawa). The plasmid was digested with *Sal*I, blunted with S1 nuclease, and digested with *Xho*I. A resulting fragment containing a *loxP* site was isolated and inserted between the *Pma*CI and *Xho*I sites of pLHC20/TLN. Then, the *Kpn*I–*Dra*I region of the resulting construct was isolated and inserted between the *Kpn*I and *Nru*I sites of pLHC20/TLN-6. Finally, to generate pLHC20/*loxP*/TLN-6, the *Bgl*II–*Eco*RI fragment, which also contains a *loxP* sequence, and the *Bgl*II–*Eco*RV fragment, which contains the mouse *pgk* promoter and the neomycin phosphotransferase gene, of pLNX were isolated and inserted into the *Kpn*I site and *Sal*I site, respectively, of the pLHC20/TLN-6 derivative described above. The variant plasmids pLHC20/*loxP*/-2/TLN-6, pLHC20/*loxP*/-4/TLN-6, pLHC20/*loxP*/-5/TLN-6, pLHC20/*loxP*/-8/TLN-6 and pLHC20/*loxP*/-10/TLN-6 were made by deleting the indicated number of nucleotide pairs between the downstream *loxP* sequence and the *tk* promoter of pLHC20/*loxP*/TLN-6. The procedure was as follows. Initially, PCRs were carried out using pLHC20/*loxP*/TLN-6 and the following sets of primers: for pLHC20/*loxP*/-2/TLN-6, 5′-TAACCCGGGAGAATTCGAGC-3′ (P-del) and 5′-ACAGTCGAGATAACTTCGTA-3′; for pLHC20/*loxP*/-4/TLN-6, P-del and 5′-AGTCGAGATAACTTCGTATA-3′; for pLHC20/*loxP*/-5/TLN-6, P-del and 5′-GTCGAGATAACTTCGTATAG-3′; for pLHC20/*loxP*/-8/TLN-6, P-del and 5′-GAGATAACTTCGTATAGCAT-3′; and for pLHC20/*loxP*/-10/TLN-6, P-del and 5′-GATAACTTCGTATAGCATAC-3′. The PCR conditions were as follows: 95 °C for 5 min; 30 cycles with 1 min for denaturation at 95 °C, 1 min for annealing at 57 °C and 1 min for extension at 72 °C; and a final extension at 72 °C for 10 min. All amplified products were digested with *Kpn*I and inserted between the *Kpn*I and *Nru*I sites of pLHC20/TLN-6. Finally, each of the resulting constructs was cleaved with *Sal*I, and the *Bgl*II–*Eco*RV fragment of pLNX, which carries the neomycin phosphotransferase gene, was inserted into the site. All constructs were sequenced for verification.

Generation of HeLa cell lines

HeLa cells were grown in Eagle's MEM containing 5% fetal bovine serum at 37 °C in 5% CO_2. They were transfected with 1 μg of *Kpn*I-digested pLHC20/*loxP*/TLN-6 or pLHC20/*loxP*/-2/TLN-6 using FuGENE6 transfection reagent (Roche Diagnostics, Basel, Switzerland) and cultured for 8–10 days in medium containing 0.4 mg·mL^{-1} G418 (Sigma-Aldrich, St Louis, MO, USA). Surviving cells were then plated at limiting dilution and recultured for 8–10 days in the presence of 0.4 mg·mL^{-1} G418, and finally, several colonies were isolated.

Integration of the transgenes was confirmed by PCR using primers 5′-GACAATCGGCTGCTCTGATG-3′ and 5′-TGCGATGTTTCGCTTGGTGG-3′, which are specific for the neomycin phosphotransferase gene, and colonies harboring a single reporter were selected by Southern blot analysis, as described below. Finally, we established five cell lines, which we named HLB8/T20, HLB10/T20, HLB15/T20, HLB13n3/T20 and HLB13n5/T20. The first three of these contain a single copy of pLHC20/*loxP*/TLN-6, and the other two contain a single copy of pLHC20/*loxP*/-2/TLN-6 in their genomes. To establish control cell lines, the cells were transfected with pBS185 (Invitrogen, Carlsbad, CA, USA), which expresses Cre recombinase. After cell cloning, T20-deletion clones were selected based on PCR analysis using primers 5′-GCGCCGGATCCTTAATTAAG-3′ and 5′-GGAGGTAGATGAGATGTGACGAACG-3′. The established cell lines were named HLB8, HLB10, HLB15, HLB13n3 and HLB13n5, respectively.

Southern blot analysis

Genomic DNA was isolated using a standard method [16]. DNA was digested with *Bgl*II, *Bsr*GI or *Hin*fI, electrophoresed on a 1.2% (w/v) agarose gel, and blotted onto a nylon membrane, which was then hybridized with the 402 bp *Bgl*II–*Hin*dIII fragment of pLHC20/*loxP*/TLN-6 labeled with [α-^{32}P]dCTP (3000 Ci·mmol^{-1}) by random priming.

Determination of transgene loci

Loci of transgenes in the cell lines HLB8/T20, HLB10/T20, HLB15/T20, HLB13n3/T20 and HLB13n5/T20 were determined by ligation-mediated-PCR, which was performed according to the method described by Pfeifer *et al.* [17]. Briefly, genomic DNAs of the above cell lines were digested with either *Hae*III, *Hin*cII, *Hin*fI, *Hin*fI or *Spe*I. Samples of 3 μg were then annealed with 5′-GTACTGTAACTGAGCTAACATAACC-3′. Primer extension reactions were performed using a mixture of $Vent_R$ DNA polymerase and $Vent_R$ (exo$^-$) DNA polymerase (New England Biolabs, Hitchin, UK) [17] at 95 °C for 5 min, 58 °C for 30 min, and 76 °C for 10 min. The products were purified and ligated to the linker DNA obtained by annealing of oligonucleotides 5′-GCGGTGACCCGGGAGATCTGAATTC-3′ (oligo A) and 5′-GAATTCAGATC-3′. The resulting products were purified and amplified by PCR with oligo A and oligonucleotide 5′-ACTGAGCTAACATAACCCGG-3′, using the following PCR conditions: 95 °C for 5 min;

20 cycles with 1 min for denaturation at 95 °C, 2 min for annealing at 60 °C and 3 min for extension at 72 °C; and a final extension at 72 °C for 10 min. One-tenth of the volume of the PCR products was used for a nested PCR with primers 5′-TAACCCGGGAGAATTCGAGC-3′ and oligo A, under the following conditions: 95 °C for 5 min; 20 cycles with 1 min for denaturation at 95 °C, 2 min for annealing at 65 °C and 3 min for extension at 72 °C; and a final extension at 72 °C for 10 min. All amplified products were purified on a 1.5% (w/v) agarose gel and sequenced.

Luciferase assay

Transcription occurring on transiently transfected plasmids or on episomes that replicated in the nucleus was assayed as described previously [12,14]. Quantification of episomes in nuclei was performed using primer extension analysis. The DNA was recovered from 3×10^5 cells, digested with *Eco*RI, and subjected to primer extension analysis using a 5′-^{32}P-labeled primer: 5′-GGAATGCCAAGCTTACTTAG-3′. The reaction products were resolved on 6% polyacrylamide-7M urea gels, and the radioactivity was analyzed using a Fujix BAS2000 Bio-image analyzer (Fuji Photo Film, Tokyo, Japan). These data were used for normalization of template molecules. Transcription occurring in the genome of each stable transformant was assayed using 1.0×10^6 cells. The cells were lysed with lysis buffer from the Promega luciferase assay system (Promega, Madison, WI, USA), and luciferase activity was measured according to the manufacturer's instructions.

Acknowledgements

The authors would like to acknowledge the contributions of Y. Kadokawa and S. Noguchi. This study was supported in part by JSPS and MEXT research grants to TO.

References

1 Workman JL & Kingston RE (1998) Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu Rev Biochem* **67**, 545–579.
2 Aalfs JD & Kingston RE (2000) What does 'chromatin remodeling' mean? *Trends Biochem Sci* **25**, 548–555.
3 Prochasson P, Neely KE, Hassan AH, Li B & Workman JL (2003) Targeting activity is required for SWI/SNF function in vivo and is accomplished through two partially redundant activator-interaction domains. *Mol Cell* **12**, 983–990.
4 Carey M & Smale ST (2000) *Transcriptional Regulation in Eukaryotes: Concepts, Strategies, and Techniques.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
5 Almer A, Rudolph H, Hinnen A & Horz W (1986) Removal of positioned nucleosomes from the yeast PHO5 promoter upon PHO5 induction releases additional upstream activating DNA elements. *EMBO J* **5**, 2689–2696.
6 Archer TK, Cordingley MG, Wolford RG & Hager GL (1991) Transcription factor access is mediated by accurately positioned nucleosomes on the mouse mammary tumor virus promoter. *Mol Cell Biol* **11**, 688–698.
7 Zhu Z & Thiele DJ (1996) A specialized nucleosome modulates transcription factor access to a *C. glabrata* metal responsive promoter. *Cell* **87**, 459–470.
8 Wolffe AP (1998) *Chromatin: Structure and Function*, 3rd edn. Academic Press, London.
9 Onishi Y & Kiyama R (2003) Interaction of NF-E2 in the Human β-globin locus control region before chromatin remodeling. *J Biol Chem* **278**, 8163–8171.
10 Ohyama T (2005) Curved DNA and transcription in eukaryotes. In *DNA Conformation and Transcription* (Ohyama T, ed.), pp. 66–74. Landes Bioscience, Georgetown, TX.
11 Ohyama T (2005) The role of unusual DNA structures in chromatin organization for transcription. In *DNA Conformation and Transcription* (Ohyama T, ed.), pp. 177–188. Landes Bioscience, Georgetown, TX.
12 Nishikawa J, Amano M, Fukue Y, Tanaka S, Kishi H, Hirota Y, Yoda K & Ohyama T (2003) Left-handedly curved DNA regulates accessibility to *cis*-DNA elements in chromatin. *Nucleic Acids Res* **31**, 6651–6662.
13 Hirota Y & Ohyama T (1995) Adjacent upstream superhelical writhe influences an *Escherichia coli* promoter as measured by *in vivo* strength and *in vitro* open complex formation. *J Mol Biol* **254**, 566–578.
14 Nishikawa J, Fukue Y & Ohyama T (2004) Left-handedly curved DNA introduced into episomes can activate transcription in a TATA box-dependent manner. *J Adv Sci* **16**, 99–103.
15 Tanaka J, Miwa Y, Miyoshi K, Ueno A & Inoue H (1999) Construction of Epstein–Barr virus-based expression vector containing mini-oriP. *Biochem Biophys Res Commun* **264**, 938–943.
16 Sambrook J & Russell DW (2001) *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
17 Pfeifer GP, Chen HH, Komura J & Riggs AD (1999) Chromatin structure analysis by ligation-mediated and terminal transferase-mediated polymerase chain reaction. *Methods Enzymol* **304**, 548–571.
18 Hoess RH, Ziese M & Sternberg N (1982) P1 site-specific recombination: nucleotide sequence of the

recombining sites. *Proc Natl Acad Sci USA* **79**, 3398–3402.
19 Dlakic M & Harrington RE (1998) DIAMOD: display and modeling of DNA bending. *Bioinformatics* **14**, 326–331.
20 Sayle RA & Milner-White EJ (1995) RASMOL: biomolecular graphics for all. *Trends Biochem Sci* **20**, 374–376.
21 Bansal M, Bhattacharyya D & Ravi B (1995) NUPARM and NUCGEN: software for analysis and generation of sequence dependent nucleic acid structures. *Comput Applic Biosci* **11**, 281–287.

the FEBS Journal

Volume 273 Number 24 December 2006

Original Articles

Continued inside

Cover Illustration
A designed curved DNA segment that activates eukaryotic transcription (Figure adapted from N. Sumida *et al.*, pp. 5691–5702).

Abstracted and indexed by *Biological & Agricultural Index, Biosis, CAS, ISI (Current Contents), EMBASE/Excerpta Medica, INIS, Medlars, MEDLINE, Physics Briefs, Sociedad Iberoamericana de Información Científica (SIIC)*

Typeset in India
Printed in Singapore

Blackwell Publishing

Blackwell Synergy
This journal is available online at Blackwell Synergy.
Visit www.blackwell-synergy.com to search the articles and register for table of contents e-mail alerts.

1742-464X(200612)273:24;1-5

Nucleosides, Nucleotides, and Nucleic Acids, 25:417–425, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600684068

Taylor & Francis
Taylor & Francis Group

STRUCTURAL PROPERTY OF DNA THAT MIGRATES FASTER IN GEL ELECTROPHORESIS, AS DEDUCED BY CD SPECTROSCOPY

Akimasa Matsugami □ *Supramolecular Biology, International Graduate School of Arts and Sciences, Yokohama City University, Yokohama, Japan*

Keiko Tani, Kiyoshi Ouhashi, and Seiichi Uesugi □ *Department of Environment and Natural Sciences, Graduate School of Environment and Information Sciences, Yokohama National University, Yokohama, Japan*

Mitsunori Morita and Takashi Ohyama □ *Department of Biology, Faculty of Science and Engineering, Konan University, Kobe, Japan*

Masato Katahira □ *Supramolecular Biology, International Graduate School of Arts and Sciences, Yokohama City University, Yokohama, Japan, Genome Science Center, RIKEN, Yokohama, Japan, and PRESTO, JST, Yokohama, Japan*

□ *Bent DNAs are known to migrate slower than ordinary DNA in non-denaturing polyacrylamide gel electrophoresis. In contrast, several satellite DNAs have been shown to migrate fast. The structural property that causes the fast migration, however, is not clarified so far on molecular basis. We have investigated the structural property of a satellite DNA, which contains consecutive purine sequences and migrates faster in gel, by CD spectroscopy. Partial formation of an A-form–like structure has been suggested. Reduction in DNA length due to the formation of the A-form–like structure may be responsible for the fast migration. The pronounced rigidity of DNA may also contribute to the behavior.*

Keywords Bent DNA; CD; Satellite DNA; A-Form

INTRODUCTION

Eukaryotic genomes contain tandemly repeated DNA sequences known as satellites.[1,2] The satellites cover a few percent to >50% of mammalian

Received 26 December 2005; accepted 23 January 2006.
We thank Dr. Hideki Tagashira for technical assistance. M.K. was supported by Grants-in-Aid for Scientific Research (Nos. 17026011, 17048009, 18011008, and 18370046) and the Protein 3000 Project of MEXT and by a PRESTO grant of JST. T.O. was supported by MEXT (No. 17050022). A.M. is supported by a postdoctoral fellow program of JSPS.
This article is dedicated to Professor Eiko Ohtsuka on the occasion of her 70th birthday.
Address correspondence to Masato Katahira, Supramolecular Biology, International Graduate School of Arts and Sciences, Yokohama City University, 1-7-29 Suehiro, Tsurumi-ku, Yokohama 230-0045, Japan. E-mail: katahira@tsurumi.yokohama-cu.ac.jp

genomes.[2] Satellites are usually associated with regions of constitutive heterochromatin. Intrinsically bent structure has been found for many satellite DNAs and has been suggested to function in folding of DNA into chromosomes.[3–8] DNA curvature may aid tight winding of DNA in constitutive heterochromatin.

It is known that bent DNA migrates slower than ordinary DNA in nondenaturing polyacrylamide gel electrophoresis. In contrast, some satellite DNAs of box turtle, Komodo dragon, vulture, and cow migrate fast.[9] Fast migration is reported for DNA fragments from yeast centromeres[10] and those found in a study of illegitimate chromosomal recombination in mammalian cells.[11] Bovine satellite I DNA localizes in the centromeres of all autosomes.[12] Predominance (>80%) of purine–purine (or pyrimidine–pyrimidine) sequence over purine-pyrimidine (or pyrimidine–purine) sequence throughout the entire satellite I was noted.[13] Furthermore, it contains many runs of three or more consecutive purine (or pyrimidine) residues. It is found that one half of bovine satellite I DNA, an F fragment (704 bps), migrates faster, while the other half, an N fragment (710 bps), migrates normally.[13] The phenomenon of the fast migration of the F fragment is pronounced at lower temperature and the phenomenon disappears completely above 50°C.[13] Although it is well established that bent DNA structure causes slow migration, the structural property that causes fast migration is not clarified on molecular basis. The elucidation of the structural property will give us a clue to understand biological roles of DNA fragments that exhibit faster migration.

Here, we have studied structural properties of F and N fragments, together with another non-related fragment with a similar size (692 bps), by CD spectroscopy. It is suggested that the F fragment partially forms an A-form–like structure. The length of an A-form is known to be shorter than that of a B-form. Thus, the reduction in DNA length due to the partial formation of the A-form–like structure may account for the fast migration of the F fragment.

MATERIALS AND METHODS

Preparation of DNA Fragments

The F and N fragments of bovine satellite I DNA were prepared as described previously.[13] Briefly, the construct carrying the F fragment and that carrying the N fragment were digested with *Pst*I. Each digest was extracted with phenol, precipitated with ethanol, rinsed with 70% ethanol, and dried. A control fragment (692 bps), which is not related to the bovine satellite I DNA was obtained through digestion of a plasmid pUC19 with *Dra*I, and prepared in a similar way.

CD Analysis

Each fragment was dissolved in the solution containing 10 mM Tris-HCl (pH 7.4) and 1 mM EDTA. The concentration of each fragment was 0.66 μM. CD spectra and thermal CD melting curves of each fragment were recorded with a Jasco J-720 spectropolarimeter. For melting, the temperature of the solution was raised from 5′ to 95°C at the rate of 1°C/min. The melting temperature was determined by the use of derivative of the melting curve.

RESULTS AND DISCUSSION

Characteristics of Nucleotide Sequences of F, N, and Control Fragments

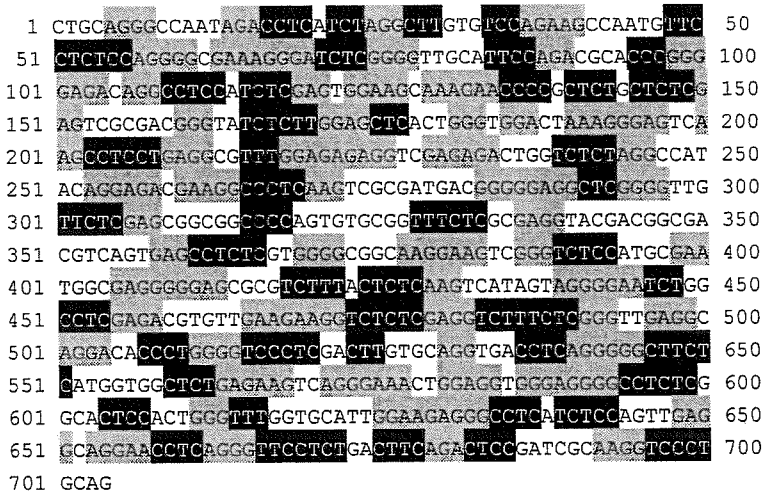
Figure 1 shows nucleotide sequences of F, N, and control fragments. Predominance of purine–purine (or pyrimidine–pyrimidine) sequence over purine–pyrimidine (or pyrimidine–purine) sequence is seen for the F and N fragments.[13] Furthermore, these fragments contain many runs of three or more consecutive purine (or pyrimidine) residues, as revealed by shading in Figure 1. This characteristic is less pronounced for the control fragment.

Temperature-Dependent Spectral Change of the F Fragment Related to the Dissolution of a Certain Structure Responsible for the Fast Migration

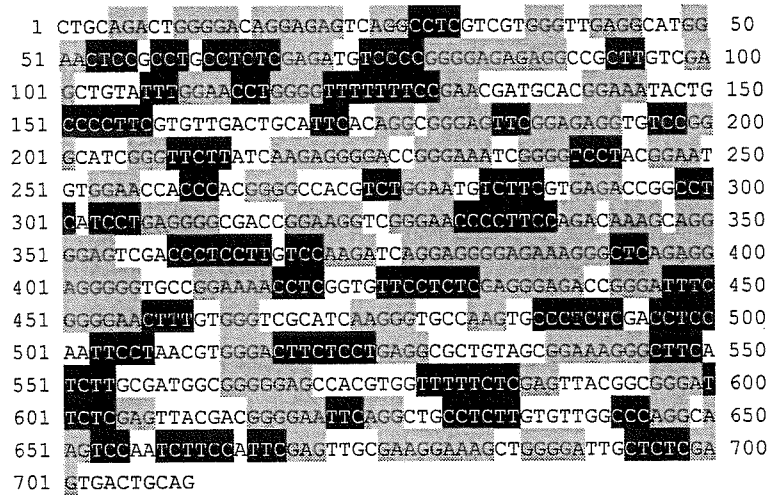
It was reported that the F fragment migrates fast in non-denaturing polyacrylamide gel at low temperature.[13] Relative size, R_s, defined as (apparent size)/(actual size) was ca. 0.9 at 5°C. R_s gradually approached 1.0 when temperature was raised, and reached 1.0 at 50°C. Thus, the phenomenon of fast migration of the F fragment disappears at 50°C. In contrast, both N and control fragments migrate normally at any temperature between 5° and 50°C, an R_s value being ca. 1.0.[13] Therefore, it is expected that the difference in temperature dependency of mobility among three fragments may be reflected on their CD spectra.

Figures 2A–2C show CD spectra of each fragment at 5–80°C. For the F fragment, a gradual change of the CD spectrum was observed up to 50 (or 65)°C. When temperature was further raised to 80°C, the CD spectrum changed drastically, which implied melting of the F fragment duplex into single strands. In fact, the melting temperature of the F fragment was determined to be 73.4°C (Table 1). The spectral change up to 50 (or 65)°C, in turn, is supposed to reflect a structural change of the F fragment in the duplex state. A rate of the spectral change between 5 and 50°C $([\theta]_{5°C}-[\theta]_{50°C})/[\theta]_{50°C} * 100$ was 15% for the F fragment, as monitored at 265 nm. It is supposed that the gradual spectral change of the F fragment in the 5–50°C

F



N



Control

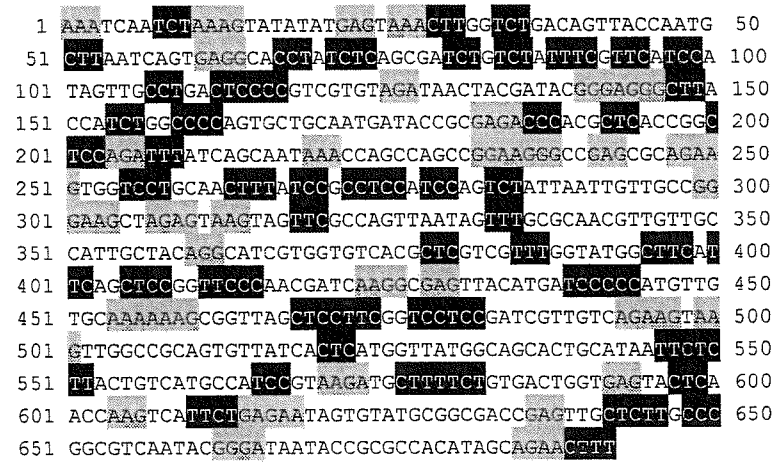


FIGURE 1 Nucleotide sequences of F, N, and control fragments. Runs of three or more consecutive purine or pyrimidine residues are shaded with gray and black, respectively.

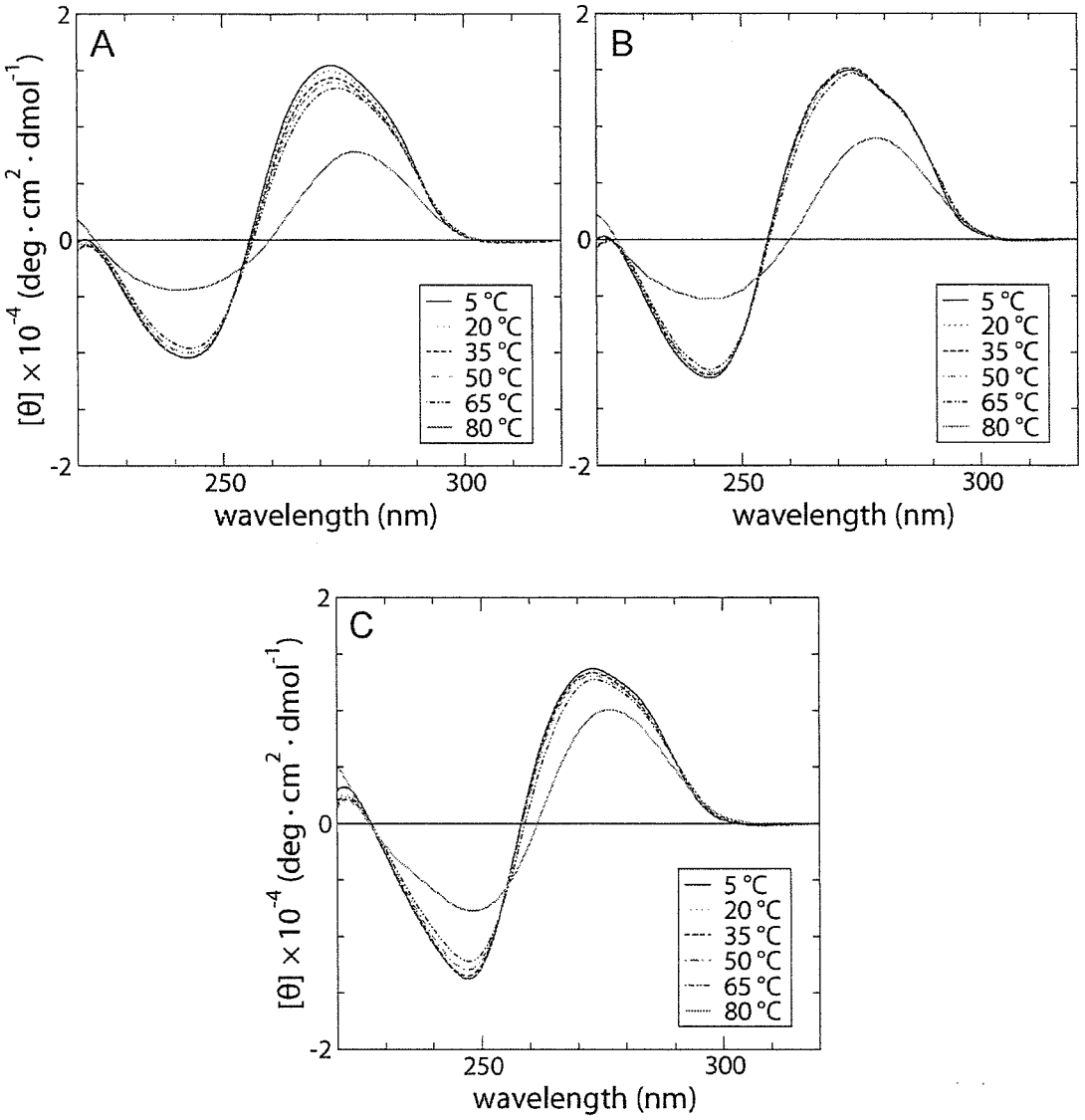


FIGURE 2 CD spectra of the F fragment (A), the N fragment (B), and the control fragment (C).

range correlates with the gradual disappearance of the phenomenon of the fast migration in the same temperature range. Temperature-dependent spectral change may reflect the disappearance of a certain structure that is responsible for the fast migration.

For the N fragment, in contrast, a spectral change is quite limited up to 50°C. The rate of the spectral change between 5 and 50°C is only 2%. At 80°C, a drastic change of the spectrum corresponding to melting of the N fragment duplex was observed, as observed for the F fragment. The melting temperature of the N fragment was determined to be 70.6°C. A very limited change of the spectrum up to 50°C suggests that the N fragment undergoes almost no structural change up to this temperature. A spectral change was comparatively modest also for the control fragment up to 50°C, the rate of the spectral change between 5 and 50°C being 6%. The melting

TABLE 1 Summary of Properties of the F, N, and Control Fragments

	F fragment	N fragment	Control fragment
Origin	One half of bovine satellite I DNA	The other half of bovine satellite I DNA	pUC19 plasmid
Size (bps)	704	710	692
Mobility in gel			
at 5°C	Fast	Normal	Normal
at 50°C	Normal	Normal	Normal
Melting temperature (°C)	73.4	70.6	65.7
Rate of CD spectral change between 5 and 50°C (%)	15	2	6

temperature of the control fragment was determined to be 65.7°C. The modest spectral changes suggesting modest structural changes, if any, of N and control fragments in the 5–50°C range are consistent with the observation that these fragments exhibited an almost constant R_s value of 1.0 in this temperature range.

Partial Formation of an A-Form–Like Structure for the F Fragment at Low Temperature

For the temperature range of 5 to 50°C, F, N, and control fragments all gave CD spectra basically characteristic of B-form, a positive peak around at 275 nm and a negative peak at around 245 nm with the comparable intensity.[14–16] This indicates that not only the N and control fragments, but also the F fragment mainly takes on B-form, even at 5°C. It is supposed that the population of a certain structure that is responsible for fast migration is small even at 5°C, although the phenomenon of the fast migration of the F fragment is pronounced at this temperature. In order to extract the CD spectrum corresponding to the structure responsible for the fast migration, a difference spectrum was obtained by subtracting the CD spectrum at 50°C from that at 5°C (Figure 3). It should be remembered that the phenomenon of the fast migration of the F fragment becomes less pronounced at higher temperature and disappears at 50°C. The difference spectrum is different from the spectrum of a canonical B-form. The difference spectrum gives a positive peak around at 265 nm and a comparatively rather weak negative peak around at 240 nm. These features are characteristic of the spectrum of an A-form.[14–16] Thus, it is suggested that the F fragment may partially take on an A-form–like structure at low temperature and that this structure is responsible for the fast migration in gel. Partial formation of the A-form–like structure for the F fragment which contains many runs of three or more consecutive purine (or pyrimidine) residues is consistent with the claim that runs of consecutive purine sequence have a tendency to set up an A-form–like structure.[17,18]

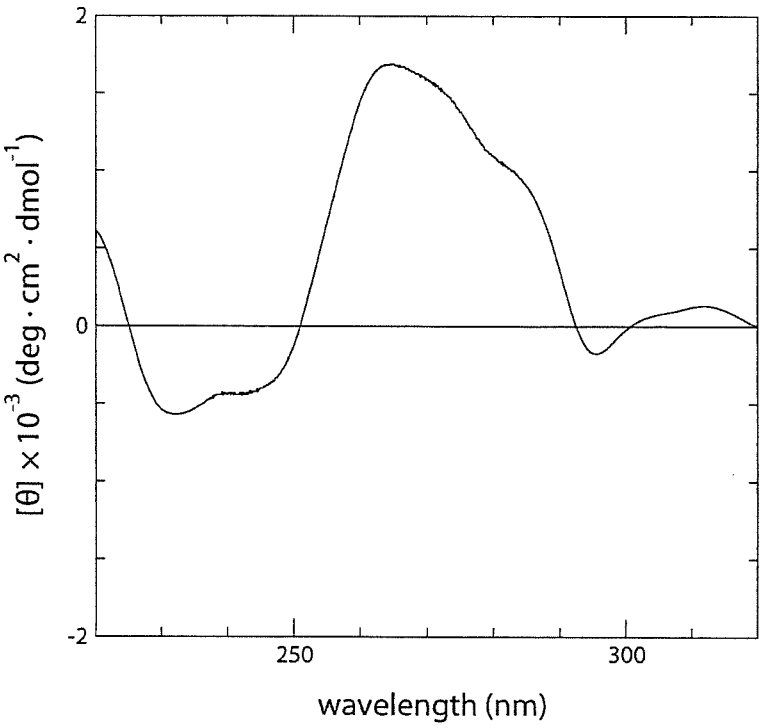


FIGURE 3 A CD difference spectrum for the F fragment between 5 and 50°C.

On the addition of 10 mM of Mg^{2+}, R_s value of the F fragment changed from 0.9 to ca. 1.0.[13] We have tried to examine the effect of Mg^{2+} on the CD spectrum of the F fragment. Under the CD conditions, however, the addition of Mg^{2+} caused some kind of precipitation and qualitative CD analysis was not possible. Higher DNA concentration applied for the CD experiment, compared to that applied for the gel electrophoresis experiment, may be responsible for the precipitation, although the exact reason of the precipitation is not clear.

Possible Explanation on the Fast Migration of the F Fragment from the Structural Viewpoint

The length of A-form DNA is ca. 30% shorter than that of B-form DNA with the same base pairs.[19] Thus, reduced length of the F fragment DNA due to the partial formation of the A-form–like structure may account for the fast migration in gel. It should be noted that a complete switch to the A-form–like structure is not required to explain the observed R_s value of 0.9. Partial formation of the A-form–like structure is enough to achieve the reduction of the length to 90% of ordinary B-form DNA. This is qualitatively consistent with the result that the spectral change of the F fragment between 5 and 50°C is not so drastic.

The F fragment contains many runs of three or more consecutive purine (or pyrimidine) residues, as described above. It was proposed that

purine–purine stacking interactions provide most of the mechanical rigidity of the DNA helix.[20] A stiff and rod-like molecule may exhibit minimal frictional drag as it passes through tight pores of polyacrylamide gel. The rigidity or the narrowness of allowed conformational space of the F fragment might also contribute to the fast migration.

Interpretation of the Mobility of the N Fragment

It was found that a part of the N fragment forms bent structure that causes slow migration.[13] The center of the curvature locates approximately between positions 600 and 610 of the N fragment. It was suggested that periodically spaced $(dT)_{2-5}$ tracts between positions 570 and 640 are responsible for the formation of the bent structure. In spite of the existence of the bent structure at a part of the N fragment, however, the N fragment migrates normally, R_s being 1.0 at any temperature. In order to interpret this phenomenon, it was proposed that like the F fragment, the N fragment containing many runs of three or more consecutive purine (or pyrimidine) residues also possesses the A-form–like structural property which causes fast migration and that the tendency of this fast migration cancels the tendency of the slow migration caused by the bent structure.[13] The CD spectral change for the N fragment between 5 and 50°C was very limited compared to those for the F and control fragments. The result may imply that both of the bent structure and the A-form–like structure remain in this range of temperature, which would result in the continuation of the cancellation. This may account for the observation that R_s of the N fragment is kept at 1.0 in this range of temperature, although further research is needed to clarify the behavior of the N fragment.

REFERENCES

1. John, B.; Miklos, G.L.G. Functional aspects of satellite DNA and heterochromatin. International Review of Cytology **1979**, 58, 1–114.
2. Singer, M.F. Highly repeated sequences in mammalian genomes. International Review of Cytology **1982**, 76, 67–112.
3. Trifonov, E.N.; Sussman, J.L. The pitch of chromatin DNA is reflected in its nucleotide sequence. Proceedings of the National Academy of Sciences USA **1980**, 77, 3816–3820.
4. Drew, H.R.; Travers, A.A. DNA bending and its relation to nucleosome positioning. Journal of Molecular Biology **1985**, 186, 773–790.
5. Wada-Kiyama, Y.; Kiyama, R. An intrachromosomal repeating unit based on DNA bending. Molecular and Cellular Biology **1996**, 16, 5664–5673.
6. Radic, M.Z.; Lundgren, K.; Hamkalo, B.A. Curvature of mouse satellite DNA and condensation of heterochromatin. Cell **1987**, 50, 1101–1108.
7. Kodama, H.; Saitoh, H.; Tone, M.; Kuhara, S.; Sakaki, Y.; Mizuno, S. Nucleotide sequences and unusual electrophoretic behavior of the W chromosome-specific repeating DNA units of the domestic fowl, Gallus gallus domesticus. Chromosoma **1987**, 96, 18–25.
8. Benfante, R.; Landsberger, N.; Tubiello, G.; Badaracco, G. Sequence-directed curvature of repetitive AluI DNA in constitutive heterochromatin of *Artemia franciscana*. Nucleic Acids Research **1989**, 17, 8273–8282.

9. Fitzgerald, D.J.; Dryden, G.L.; Bronson, E.C.; Williams, J.S.; Anderson, J.N. Conserved patterns of bending in satellite and nucleosome positioning DNA. Journal of Biological Chemistry **1994**, 269, 21303–21314.
10. Anderson, J.N. Detection, sequence patterns and function of unusual DNA structures. Nucleic Acids Research **1986**, 14, 8513-8533.
11. Milot, E.; Belmaaza, A.; Wallenburg, J.C.; Gusew, N.; Bradley, W.E.C.; Chartrand, P. Chromosomal illegitimate recombination in mammalian cells is associated with intrinsically bent DNA elements. EMBO Journal **1992**, 11, 5063–5070.
12. Kurnit, D.M.; Shafit, B.R.; Maio, J.J. Multiple satellite deoxyribonucleic acids in the calf and their relation to the sex chromosomes. Journal of Molecular Biology **1973**, 81, 273–284.
13. Ohyama, T.; Tsujibayashi, H.; Tagashira, H.; Inano, K.; Ueda, T.; Hirota, Y.; Hashimoto, K. Suppression of electrophoretic anomaly of bent DNA segments by the structural property that causes rapid migration. Nucleic Acids Research **1998**, 26, 4811–4817.
14. Ivanov, V.I.; Minchenkova, L.E.; Schyolkina, A. K.; Poletayev, A.I. Different conformations of double stranded nucleic acids in solution as revealed by circular dichroism. Biopolymers **1973**, 12, 89–100.
15. Moore, D.S.; Wagner, T.E. Double-helical DNA and RNA circular dichroism. Calculations on base-sugar-phosphatehelix interactions. Biopolymers **1974**, 13, 977–986.
16. Saenger, W. *Principle of Nucleic Acid Structure.* Springer-Verlag, New York, **1984**.
17. Nickol, J.; Rau, D.C. Zinc induces a bend within the transcription factor IIIA-binding region of the 5 S RNA gene. Journal of Molecular Biology **1992**, 228, 1115–1123.
18. Fairall, L.; Martin, S.; Rhodes, D. The DNA binding site of the Xenopus transcription factor IIIA has a non-B-form structure. EMBO Journal **1989**, 8, 1809–1817.
19. Dickerson, R.E.; Drew, H.R.; Conner, B.N.; Kopka, M.L.; Pjura, P.E.; Helix geometry and hydration in A-DNA, B-DNA, and Z-DNA. Cold Spring Harbor Symp. Quant. Biol. **1983**, 47, 13–24.
20. Hagerman, K.R.; Hagerman, P.J. Helix rigidity of DNA: the meroduplex as an experimental paradigm. Molecular Biology **1996**, 260, 207–223.

Self-Assembly of Double-Stranded DNA Molecules at Nanomolar Concentrations

Shotaro Inoue, Shigeru Sugiyama, Andrew A. Travers, and Takashi Ohyama

Department of Biology, Faculty of Science and Engineering, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan, Instrumentation Engineering Laboratory, National Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K., Department of Biology, Faculty of Education and Integrated Arts and Sciences, Waseda University, 1-6-1 Nishi-Waseda, Shinjuku-ku, Tokyo 169-8050, Japan, and Major in Integrative Bioscience and Biomedical Engineering, Graduate School of Science and Engineering, Waseda University, 1-6-1 Nishi-Waseda, Shinjuku-ku, Tokyo 169-8050, Japan

Biochemistry®

Reprinted from
Volume 46, Number 1, Pages 164–171

Self-Assembly of Double-Stranded DNA Molecules at Nanomolar Concentrations†

Shotaro Inoue,‡ Shigeru Sugiyama,§ Andrew A. Travers,|| and Takashi Ohyama*,⊥

Department of Biology, Faculty of Science and Engineering, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan, Instrumentation Engineering Laboratory, National Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K., Department of Biology, Faculty of Education and Integrated Arts and Sciences, Waseda University, 1-6-1 Nishi-Waseda, Shinjuku-ku, Tokyo 169-8050, Japan, and Major in Integrative Bioscience and Biomedical Engineering, Graduate School of Science and Engineering, Waseda University, 1-6-1 Nishi-Waseda, Shinjuku-ku, Tokyo 169-8050, Japan

Received July 31, 2006; Revised Manuscript Received November 8, 2006

ABSTRACT: Some proteins have the property of self-assembly, known to be an important mechanism in constructing supramolecular architectures for cellular functions. However, as yet, the ability of double-stranded (ds) DNA molecules to self-assemble has not been established. Here we report that dsDNA molecules also have a property of self-assembly in aqueous solutions containing physiological concentrations of Mg^{2+}. We show that DNA molecules preferentially interact with molecules with an identical sequence and length even in a solution composed of heterogeneous DNA species. Curved DNA and DNA with an unusual conformation and property also exhibit this phenomenon, indicating that it is not specific to usual B-form DNA. Atomic force microscopy (AFM) directly reveals the assembled DNA molecules formed at concentrations of 10 nM but rarely at 1 nM. The self-assembly is concentration-dependent. We suggest that the attractive force causing DNA self-assembly may function in biological processes such as folding of repetitive DNA, recombination between homologous sequences, and synapsis in meiosis.

Protein molecules make assemblies by intermolecular interactions, and various molecular machines and subcellular structures are formed by these assemblies. When a molecule or a few kinds of molecules assemble, "protein polymers" which have regular structures are formed. Tobacco mosaic virus (TMV)[1] is an early example of such a phenomenon. TMV is decomposed into an RNA and proteins at acidic pH, and the virus is reformed by forming a protein polymer on the RNA with rising pH (*1*). In this case, polymerization occurs toward the state of minimum free energy, which is determined by such conditions including the chemical potentials of molecules and ions, temperature, and pH (*2*). This kind of protein polymerization has been called "self-assembly" (*2*, *3*). Actin, myosin, and tubulin in eukaryotic cells and flagellin found in bacterial flagella are also well-known examples of the molecules that exhibit a self-assembly phenomenon (*4*−*7*).

Two single-stranded DNA or RNA strands with complementary nucleotide sequences form double-stranded helices, which may be considered to be the simplest form of the assembly of nucleic acids. It is also known that nine GGA sequence repeats form a parallel-stranded DNA homoduplex in the presence of 50 mM NaCl at pH 4, 7, and 9 (*8*). Furthermore, DNA and RNA molecules can also assemble to form multistranded structures such as triplex and quadruplex structures in a nucleotide sequence-dependent manner, through base pairing [e.g., Watson−Crick, Hoogsteen, and reverse Hoogsteen (*9*, *10*)]. In very concentrated solutions, even dsDNA molecules assemble regularly, which finally results in generation of DNA crystals (*11*). However, the ability of dsDNA molecules to assemble at nanomolar concentrations has not been demonstrated.

We have previously reported that electrophoresis using physiological concentrations of Mg^{2+} can cause a mobility shift of restriction fragments in nondenaturing polyacrylamide gels as the overhangs interact (*12*). Theoretically, this phenomenon can be used to detect preferential intermolecular DNA interactions, if any, in aqueous solutions. Using a facile method, we studied the intermolecular DNA interactions and found that DNA molecules with an identical sequence and length preferentially interact with each other and self-assemble even in a solution composed of heterogeneous DNA species. In this report, we show for the first time that dsDNA molecules have the property of self-assembly.

MATERIALS AND METHODS

DNA Preparation. Fragments A and AR, B and BR, and C and CR originated from *Hin*dIII−*Hin*cII, *Hin*dIII−*Bam*HI, and *Hin*dIII−*Eco*RV fragments of pBR322, respectively. The *Hin*dIII site of the plasmid was converted to a *Mlu*I site in constructs A, B, and C. In the construction of fragments AR, BR, and CR, the *Hin*cII, *Bam*HI, and *Eco*RV sites, respectively, were converted to *Mlu*I sites. Fragment D originated from the *Eco*RV−*Eco*T14I fragment of pGL2-Basic Vector

† This study was supported in part by JSPS and MEXT research grants to T.O.

* To whom correspondence should be addressed. E-mail: ohyama@waseda.jp. Telephone: +81 3 5286 1520. Fax: +81 3 3207 9694.

‡ Konan University.

§ National Food Research Institute.

|| MRC Laboratory of Molecular Biology.

⊥ Faculty of Education and Integrated Arts and Sciences and Graduate School of Science and Engineering, Waseda University.

[1] Abbreviations: ds, double-stranded; AFM, atomic force microscopy; TMV, tobacco mosaic virus.

10.1021/bi061539y CCC: $37.00
Published on Web 12/15/2006

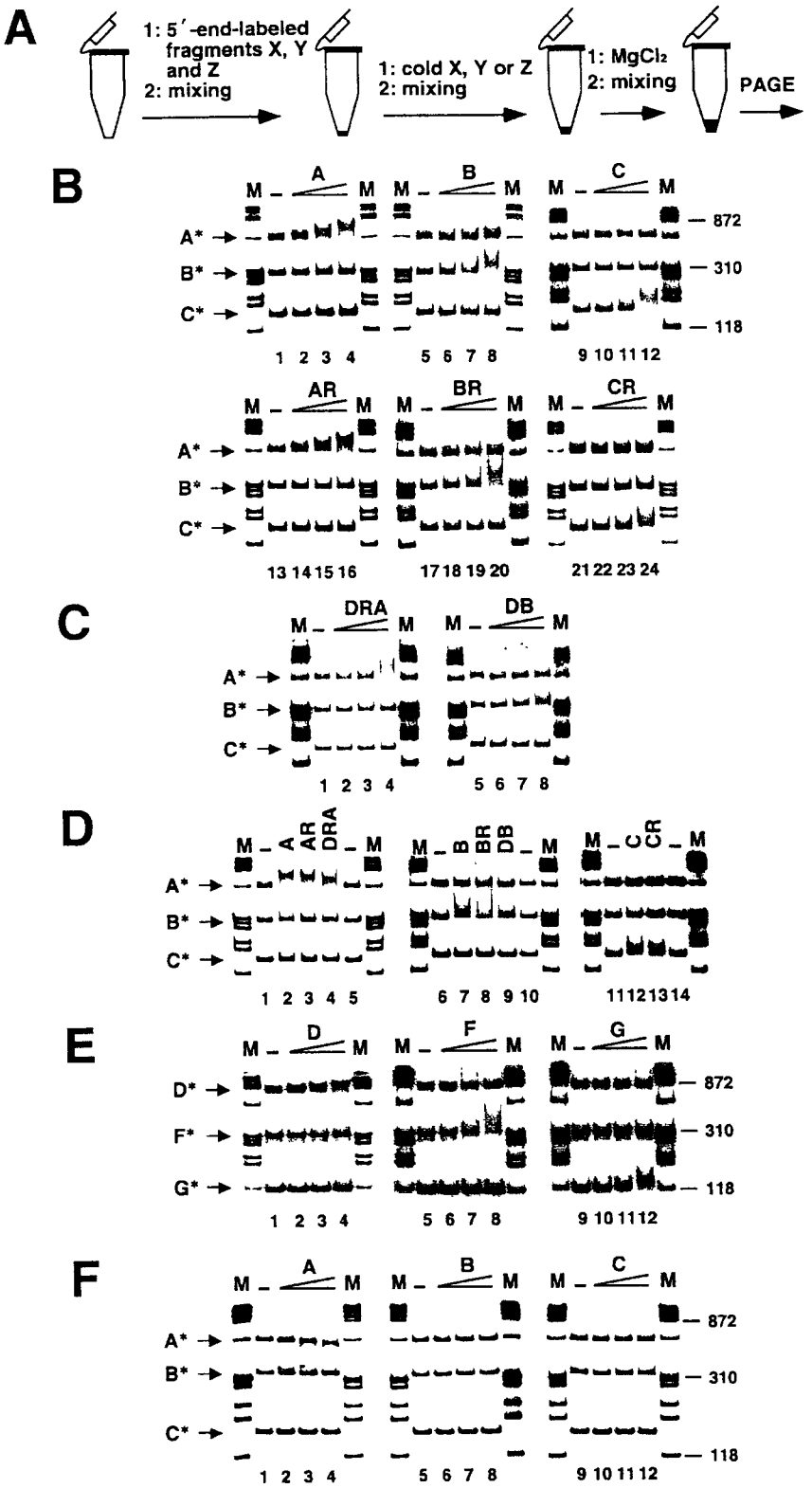


FIGURE 1: Selective interaction between dsDNA molecules as detected by electrophoresis. (A) Mixing procedure of DNA fragments. X, Y, and Z symbolize DNA fragments. Sample solutions each contained 1 mM $MgCl_2$, and electrophoreses were carried out at 10 °C. (B) Preferential interaction between identical or quasi-identical DNA molecules in the mixture of DNA fragments. The radiolabeled fragments (600 pM each) are denoted with anasterisk. Concentrations of added cold DNAs were as follows: lanes 1, 5, 9, 13, 17, and 21, no cold DNA added; lanes 2, 6, 10, 14, 18, and 22, 1 nM cold DNA added; lanes 3, 7, 11, 15, 19, and 23, 6 nM cold DNA added; and lanes 4, 8, 12, 16, 20, and 24, 30 nM cold DNA added. M lanes contained markers (mixture of *Hae*III digest of phage ϕX174 DNA and the 1748 bp *Hin*cII−*Sca*I fragment of pUC19). (C) Interaction between nonidentical DNA molecules with approximately the same length: lanes 1 and 5, no cold DNA added; lanes 2 and 6, 1 nM cold DNA added; lanes 3 and 7, 6 nM cold DNA added; and lanes 4 and 8, 30 nM cold DNA added. (D) Demonstration of the superiority of the sameness in the selective interaction: lanes 1, 5, 6, 10, 11, and 14, no cold DNA added; and the other lanes, 10 nM cold DNA added. (E) Effect of DNA conformation and property on the interaction: lanes 1, 5, and 9, no cold DNA added; lanes 2, 6, and 10, 1 nM cold DNA added; lanes 3, 7, and 11, 6 nM cold DNA added; and lanes 4, 8, and 12, 30 nM cold DNA added. (F) A control experiment conducted in the absence of $MgCl_2$.

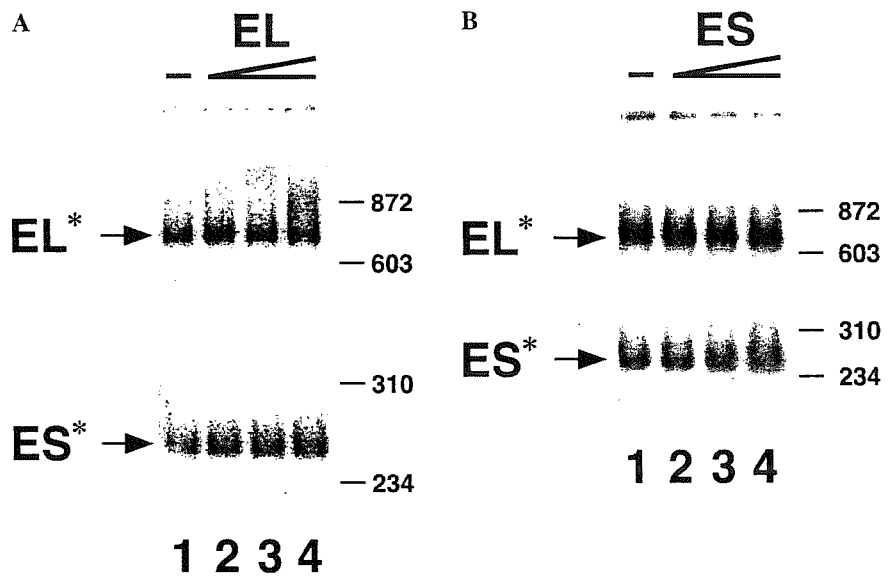


FIGURE 2: Electrophoretic detection of assembled DNA molecules with blunt ends. Cold fragment EL (743 bp) or ES (277 bp) was added to the mixture of radiolabeled fragments EL and ES (600 pM each) according to the procedure shown in Figure 1A. In panel A, the electrophoresis was carried out at 7.7 V/cm and 5 °C in the presence of 20 mM $MgCl_2$. In panel B, using a 4% polyacrylamide gel, the electrophoresis was carried out at 5 °C in the presence of 25 mM $MgCl_2$. The other conditions were the same as those used in Figure 1. In each panel, concentrations of the added cold fragment were as follows: lane 1, no cold DNA added; lane 2, 1 nM cold DNA added; lane 3, 6 nM cold DNA added; and lane 4, 30 nM cold DNA added.

DNA molecules in a sample solution; i.e., only a single species was contained in the samples in the previous study, while multiple species were contained in the samples in this study. We can reinterpret the phenomenon observed in the previous study as follows. DNA first self-assembled in the presence of $MgCl_2$; then, the assemblies were stabilized by end-to-end base pairings between constituent molecules.

To substantiate this hypothesis, we need to prove that DNA molecules can self-assemble irrespective of the form of fragment ends. Thus, we scrutinized the electrophoretic behavior of a mixture of blunt-ended DNA fragments. By increasing the concentration of surrounding Mg^{2+} ions, we could detect a selective (or preferred) interaction between the EL molecules in the mixture of fragments EL and ES (Figure 2A, lanes 3 and 4). When a rectangle, formed by the bandwidth of EL* (the long side) and its half-length (the short side), was placed on the EL* in lane 1, it contained most of the radioactivity of the fragment. Therefore, we quantified the smear in the upstream of this rectangle. The increases of the smear were 1.8-fold in lane 2, 1.3-fold in lane 3, and 6.4-fold in lane 4. We also analyzed lanes 1−4 in Figure 1B with the same method. The obtained values were 7.2-fold in lane 2, 23.8-fold in lane 3, and 41.2-fold in lane 4. Thus, the smear (band-shift rather than smear in Figure 1B) was quantified as being much greater in the case of the DNA fragments with a cohesive end. Using a gel with a lower polyacrylamide concentration (4%) and a further increasing $MgCl_2$ concentration (25 mM), a trace of the selective interaction between ES molecules was also narrowly detectable (Figure 2B, lane 4). Thus, the same phenomenon as that shown in Figure 1 could be shown by using the mixture of blunt-ended DNA fragments. However, the assemblies of blunt-ended DNA fragments and especially those of short DNA fragments seem to be fragile. The data from this experiment also suggest that the electrophoretic condition used in the previous study, in which shorter DNA fragments (~140−180 bp) were electrophoresed at a lower concentration of $MgCl_2$ (1 or 10 mM) by using 7.5% polyacrylamide gels (*12*), was not suitable for the elctrophoretic detection of assemblies of blunt-ended DNA molecules. Accordingly, we conclude that self-assembly of DNA molecules occurs in a Mg^{2+}-containing solution irrespective of the form of the fragment ends.

Kinetics of DNA Self-Assembly. The preparation and loading of multiple samples for electrophoresis normally take several minutes. To investigate the kinetics of self-recognition, we performed ligation reactions. A DNA concentration of 10 nM allowed interaction between nonidentical molecules A* and DRA and also between B* and DB (Figure 1D). In contrast, a concentration of 1 nM allowed, although only to a very limited extent, only the interaction between identical molecules (Figure 1B; broadening of bands A* and B* was observed in lanes 2 and 6, respectively). Thus, the ligation reaction was performed under conditions similar to this so preferential interactions could be detected. For this experiment, we used fragments A and C. As shown in Figure 3, the reactions were very inefficient (lanes 3−5 and 8−10). However, the results were largely consistent with those obtained in the electrophoretic experiments; i.e., although molecules A−C were generated in detectable amounts, greater amounts of both A−A and C−C molecules were also detected. Digestions of the ligation products with *Mlu*I or *Hinc*II showed that they were ligated at *Mlu*I ends (not shown). The electrophoretic assay thus appears to be more selective than the ligation assay. One possible reason for this is that the ligation assay traps the more transient interactions that would be too labile to be detected by electrophoresis.

AFM Images of the Assembled Molecules. The self-assembly of DNA fragments was confirmed by AFM. The representative images are shown in Figure 4. Initially, fragment A which has 631 bp (~210 nm) was used in the observation. Some thick DNA fibers with a height of >1 nm were observed when a 10 nM DNA solution was used (Figure 4A,D). Since the height of a single dsDNA observed

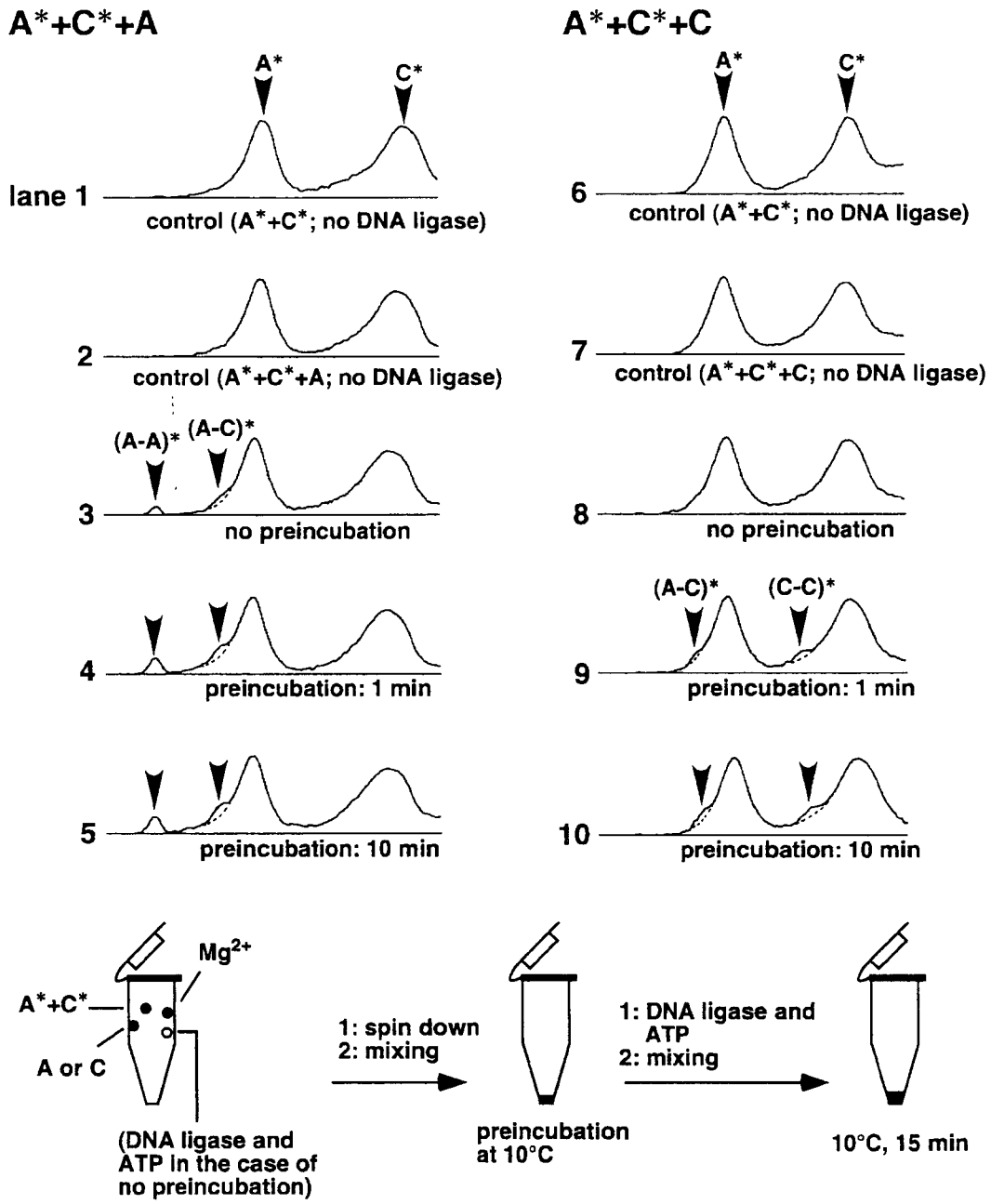


FIGURE 3: Time required for the selective interaction as measured by DNA ligation. Experimental conditions are summarized at the bottom. Ligation products were separated on 1% agarose gels, and densitometric analyses were performed. Shown are the results. A and C represent fragments A and C, respectively, and A* and C* represent 5′-end-labeled fragments A and C, respectively. The positions of fragments are denoted with arrowheads. (A-A)*, (C-C)*, and (A-C)* represent ligated molecules. The dashed lines in parts 3−5 were drawn on the basis of the line in part 2, and those in parts 9 and 10 were based on the line in part 7.

by AFM in air was reported to be less than 1 nm (*17*), these thick DNA fibers were possibly made of multiple (several) dsDNA molecules. Most of them have an approximate length of 200 nm, which is almost the same as the length of fragment A, indicating that very neat bunches of fragment A formed. Interestingly, a Y-shaped fiber, which indicates a decomposing (or forming) bunch, is observed near the center of the upper half in Figure 4A. Furthermore, in several bunches, one end is thicker than the other. The bulged ends may have been caused by the end-to-end base pairings, for such base pairings should require bowed ends of DNA molecules. We can also observe the thick and long fibers of an approximate length of 400 nm, which are considered to be dimers of the bunched dsDNAs. They were presumably formed through end-to-end base pairings between constituent fragments in any two DNA bunches. Fragment EL of 743 bp (~250 nm) that has two blunt ends also formed thick fibers (Figure 4B,E), confirming that the DNA self-assembly is independent of the form of the fragment ends. In contrast, when a 1 nM DNA solution was subjected to AFM observation, as shown panels C and F of Figure 4, the heights of DNA fibers were usually less than 1 nm, indicating that each fiber was a single dsDNA.

Because of the narrow observation area, the observed DNA type (bunched or single) was sometimes "region specific"; for example, even on the mica of Figure 4A, some areas exclusively contained thick DNAs assumed to be the bunched forms, and some areas had only fine DNAs (single molecules). However, the ratio of thick DNAs to fine DNAs in the 10 fmol/μL solution was much higher than that

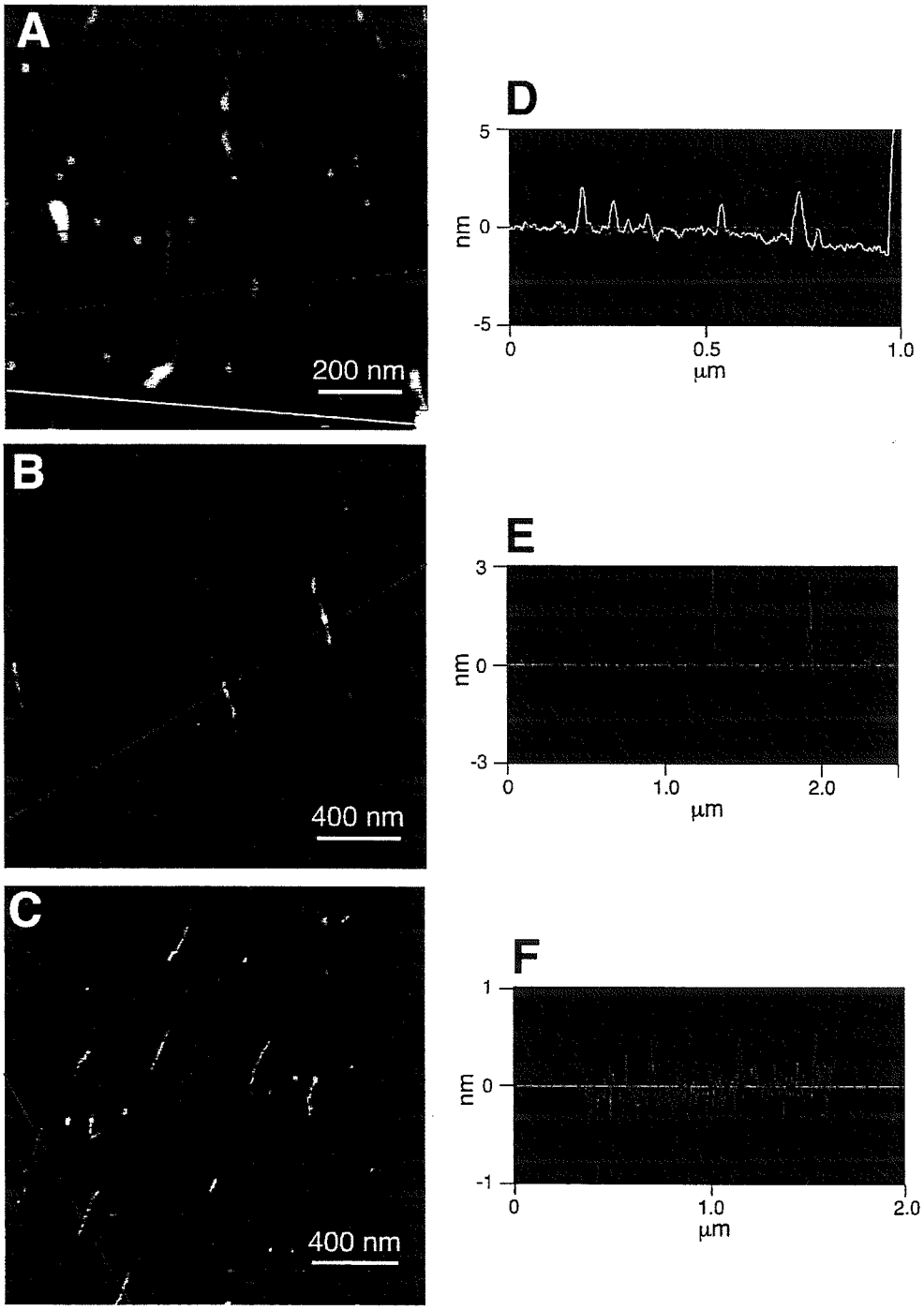


FIGURE 4: AFM observation of DNA at high and low concentrations. (A) Topographic image of fragment A at a high concentration (10 fmol/μL). The scan range was 1 × 1 μm^2. The height range was 5 nm. (B) Topographic image of fragment EL at a high concentration (10 fmol/μL). The scan range was 2 × 2 μm^2. The height range was 3 nm. (C) Topographic image of fragment A at a low concentration (1 fmol/μL). The scan range was 2 × 2 μm^2. The height range was 1 nm. (D) Section profiles along the colored lines (red, green, and white) in panel A. (E) Section profiles along the red and green lines in panel B. (F) Section profiles along the red and green lines in panel C.

in the 1 fmol/μL solution. Furthermore, the image in which almost all DNAs were bunched was only obtained when we used a 10 fmol/μL solution of fragment A (having a cohesive end). Thus, the microscopic fields shown in Figure 4 were chosen as the representative images under the respective conditions.

DISCUSSION

This study clearly demonstrates that the self-assembly of DNA molecules occurs at nanomolar concentrations in the presence of physiological concentrations of $MgCl_2$, irrespective of the form of fragment ends. The self-assemblies seem to be considerably stabilized when the molecules can base pair between the ends. The mechanism underlying the phenomenon in Figure 1 can be explained as follows; at first, the identical DNA molecules self-assemble with the aid of Mg^{2+} ions; second, end-to-end base pairings form between the fragments in each assembly, and finally, the base pairings stabilize DNA assemblies to such an extent that they resist decomposition caused by the electrophoretic friction. If the

self-assembly had not occurred, all combinations of base-paired DNA fragments should have been detected in Figure 1.

A Putative Mechanism of DNA Self-Assembly and Its Biological Relevance. What is the molecular basis of self-recognition? Our results indicate that this phenomenon most strongly favors identical lengths and sequences. Furthermore, this phenomenon needs magnesium ions, which is clearly shown in Figure 1. Magnesium ions normally alleviate electrostatic repulsive interactions between phosphates, thereby both stabilizing the sugar−phosphate backbone of DNA (*19*) and allowing the close approach of DNA duplexes. The favored sites for direct coordination to magnesium are the phosphate oxygen and N7 and O6 of guanosine (*20*, *21*). This kind of coordination may be possible in intermolecular interactions. If intermolecular phosphate coordination were preferred over intramolecular coordination, the assembly of DNA molecules of equal length would be favored. Nevertheless, such a phenomenon would be insufficient to explain the dependence on DNA sequence. However, even in the presence of magnesium ions, the DNA duplex is labile on a millisecond time scale, allowing local bubble formation as well as base flipping (*22*). One possibility is that the magnesium-dependent alignment of DNA molecules of identical sequence and length could be stabilized by such perturbations occurring simultaneously (and transiently) at identical positions in the sequence. These transient perturbations would tend to be suppressed at higher magnesium concentrations, and under these conditions, the interactions would exhibit a lower specificity. Indeed, in the electrophoretic analysis at higher magnesium concentrations, we also detected a slight retardation of band B* in the presence of 30 nM cold fragment A (not shown), indicating that the molecules with different sizes began to assemble under the condition. Thus, the self-assembly of DNA without contamination of different DNA species seems to occur in a limited range of magnesium and DNA concentrations. Interestingly, the concentration required for DNA self-assembly is far below that for polymerization of flagellin (*5*).

Do the fragment ends play a role in interfragment interactions? As shown in Figures 2 and 4 (B and E), the end structure of DNA is irrelevant to the self-assembly per se. Furthermore, the following point strengthens this conclusion. If the end-to-end interaction between cohesive ends functioned to trigger the molecular assembly, we would have expected to observe nonbunched dimer molecules that were simply composed of two dsDNA molecules. However, we did not observe such molecules in the atomic force microscope.

Large structures may most efficiently be built by repetition of smaller ones. The attractive force working between the same sequences may be used in various biological processes, including DNA. For example, it may be used, in part, to construct centromeres and telomeres by folding repetitive DNA sequences. Furthermore, the force may be used in DNA recombination between homologous sequences and synapsis in meiosis.

ACKNOWLEDGMENT

We acknowledge the contributions of Mitsunori Morita and Junko Ohyama.

REFERENCES

1. Fraenkel-Conrat, H., and Williams, R. C. (1955) Reconstitution of active tobacco mosaic virus from its inactive protein and nucleic acid components, *Proc. Natl. Acad. Sci. U.S.A. 41*, 690−698.
2. Oosawa, F., and Asakura, S. (1975) *Thermodynamics of the polymerization of protein*, Academic Press, London.
3. Kushner, D. J. (1969) Self-assembly of biological structures, *Bacteriol. Rev. 33*, 302−345.
4. Horvath, I., Kiraly, C., and Szerb, J. (1949) Action of cardiac glycosides on the polymerization of actin, *Nature 164*, 792.
5. Asakura, S. (1968) A kinetic study of *in vitro* polymerization of flagellin, *J. Mol. Biol. 35*, 237−239.
6. Miki-Noumura, T., and Mori, H. (1972) Polymerization of tubulin: The linear polymer and its side-by-side aggregates, *J. Mechanochem. Cell Motil. 1*, 175−188.
7. Miyahara, M., and Noda, H. (1977) Self-assembly of myosin *in vitro* caused by rapid dilution: Effects of hydrogen ion, potassium chloride, and protein concentrations, *J. Biochem. 81*, 285−295.
8. Suda, T., Mishima, Y., Asakura, H., and Kominami, R. (1995) Formation of a parallel-stranded DNA homoduplex by d(GGA) repeat oligonucleotides, *Nucleic Acids Res. 23*, 3771−3777.
9. Liu, H., Matsugami, A., Katahira, M., and Uesugi, S. (2002) A dimeric RNA quadruplex architecture comprised of two G:G(:A):G:G(:A) hexads, G:G:G:G tetrads and UUUU loops, *J. Mol. Biol. 322*, 955−970.
10. Van Dyke, M. W. (2005) Do DNA triple helices or quadruplexes have a role in transcription?, in *DNA Conformation and Transcription* (Ohyama, T., Ed.) pp 105−126, Landes Bioscience, Georgetown, TX, and Springer, New York.
11. Timsit, Y., and Moras, D. (1992) Crystallization of DNA, *Methods Enzymol. 211*, 409−429.
12. Tagashira, H., Morita, M., and Ohyama, T. (2002) Multimerization of restriction fragments by magnesium-mediated stable base pairing between overhangs: A cause of electrophoretic mobility shift, *Biochemistry 41*, 12217−12223.
13. Ohyama, T. (1996) Bent DNA in the human adenovirus type 2 E1A enhancer is an architectural element for transcription stimulation, *J. Biol. Chem. 271*, 27823−27828.
14. Nishikawa, J., Amano, M., Tanaka, S., Kishi, H., Fukue, Y., Hirota, Y., Yoda, K., and Ohyama, T. (2003) Left-handedly curved DNA regulates accessibility to cis-DNA elements in chromatin, *Nucleic Acids Res. 31*, 6651−6662.
15. Ohyama, T., Tsujibayashi, H., Tagashira, H., Inano, K., Ueda, T., Hirota, Y., and Hashimoto, K. (1998) Suppression of electrophoretic anomaly of bent DNA segments by the structural property that causes rapid migration, *Nucleic Acids Res. 26*, 4811−4817.
16. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Plainview, NY.
17. Sasou, M., Sugiyama, S., Yoshino, T., and Ohtani, T. (2003) Molecular flat mica surface silanized with methyltrimethoxysilane for fixing and straightening DNA, *Langmuir 19*, 9845−9849.
18. Matsugami, A., Tani, K., Ouhashi, K., Uesugi, S., Morita, M., Ohyama, T., and Katahira, M. (2006) Structural property of DNA which migrates fast in gel, as deduced by CD spectroscopy, *Nucleosides, Nucleotides, Nucleic Acids 25*, 417−425.
19. Cowan, J. A. (1995) *The Biological Chemistry of Magnesium*, VCH Publishers, New York.
20. Ho, P. S., Frederick, C. A., Quingley, G. J., van der Marel, G. A., van Boom, J. H., Wang, A. H., and Rich, A. (1985) G·T wobble base-pairing in Z-DNA at 1.0 Å atomic resolution: The crystal structure of d(CGCGTG), *EMBO J. 4*, 3617−3623.
21. Gessner, R. V., Quingley, G. J., van der Marel, G. A., van Boom, J. H., and Rich, A. (1985) Structural basis for stabilization of Z-DNA by cobalt hexaammine and magnesium cations, *Biochemistry 24*, 237−240.
22. Travers, A. A. (2005) DNA dynamics: Bubble 'n' flip for DNA cyclisation? *Curr. Biol. 15*, R377−R379.

BI061539Y

Chromatin structure formed on a eukaryotic promoter activated by a left-handed superhelical bent DNA of 180 bp

Noriyuki SUMIDA
Haruyuki SONOBE
Takashi OHYAMA

Reprinted from
Journal of Advanced Science
Vol.19, No.1&2, 2007

SAS, Society of Advanced Science

Paper

Chromatin structure formed on a eukaryotic promoter activated by a left-handed superhelical bent DNA of 180 bp

180 塩基対の左巻き超らせん様ベント DNA により活性化されたプロモーターのクロマチン構造

Noriyuki SUMIDA and Haruyuki SONOBE

Department of Biology, Faculty of Science and Engineering, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan.

Takashi OHYAMA*

Department of Biology, Faculty of Education and Integrated Arts and Sciences, Major in Integrative Bioscience and Biomedical Engineering, Graduate School of Science and Engineering, Waseda University, 1-6-1 Nishi-Waseda, Shinjuku-ku, Tokyo 169-8050, Japan.

**Corresponding author E-mail: ohyama@waseda.jp, Tel: +81 3 5286 1520, Fax: +81 3 3207 9694*

(Received 4, October 2006 Accepted 16, November 2006)

We have shown previously that a left-handed curved DNA segment of 180 bp (T20) can activate eukaryotic transcription. Here, the local chromatin structure around the promoter was investigated in a HeLa cell line HLB10/T20 harboring the T20-containing reporter and in its control cell line HLB10 in which T20 was deleted from the same reporter locus. An analysis of translational positioning of nucleosomes indicated that the nucleosome density in the region spanning from about -500 to +200 relative to the transcription start site (+1) was lower in HLB10/T20 cells than in HLB10 cells. The results of the chromatin analyses also indicated that the histone core may slide within the T20 region, and the ability of T20 to capture and reposition histones may increase the accessibility of both the TATA box and other *cis*-DNA elements. These effects seem to be responsible for facilitation of transcription by T20.

Keywords : Curved DNA, Transcription regulation, Chromatin, Nucleosome positioning

I. INTRODUCTION

Curved DNA structures often occur in or around origins of DNA replication,[1-6] promoters and enhancers, and DNA recombination loci,[7-8] irrespective of the origin of the DNA. This suggests that DNA curvature is important in many basic genetic processes (for reviews, see refs. 9-12). In bacterial promoters, curved DNA structures have a range of functions: facilitating RNA polymerase binding to the promoter, promoting the transition from closed to open promoter complexes, and enhancing binding of transcription factors.[13]

Curved DNA structures are also likely to function in eukaryotic transcription in several ways. These include acting as a conformational signal for transcription factor binding; juxtaposition of the basal machinery with effector domains on upstream-bound factors; regulation of transcription in association with transcription-factor induced bending of DNA; and organization of local chromatin structure to increase the accessibility of *cis*-DNA elements.[14]

We have shown that a 36-bp curved DNA with a close structural resemblance to part of a negative supercoil is able to preferentially bind a histone core. Furthermore, this curved DNA can activate eukaryotic promoters when it is introduced upstream of a promoter at an appropriate distance and with proper spatial positioning.[15] Mechanistically, this curved DNA (referred to as T4 in the present study; where T indicates a dT•dA tract and the numeral indicates the number of tracts) facilitates nucleosome formation and the TATA box is thereby positioned in the linker DNA with its minor groove facing outwards; we note that this finding explains why curved DNA is often located near transcriptional control regions.

We have also shown that a left-handed curved DNA segment of 180 bp (referred to as T20) can activate transcription from the herpes simplex virus (HSV) thymidine kinase (*tk*) promoter by about 70-fold compared to a control segment, using a transient transfection assay in COS-7 cells.[16] The T20 segment was also able to activate transcription from the human adenovirus type 2 *E1A* promoter with an 18-fold increase in the same assay system, and also activated transcription from the *tk* promoter on episomes in COS-7 cells.[16] In addition, T20 was found to activate transcription in all of five stable transformants of HeLa cells, irrespective of the locus.[16] Here, we have investigated the chromatin structure formed on or around the T20 segment in the nucleus, in order to examine the mechanism of transcriptional activation induced by the T20 segment.

II. METHODS AND MATERIALS

II-A. Cell culture and nuclei preparation

HeLa cells were grown according to the standard method. Nuclei were prepared as described previously.[15]

II-B. Chromatin digestion

For digestion of chromatin with MNase, aliquots of 100 μl of nuclei suspension (1×10^7 nuclei/ml for indirect end-labeling analysis and 4×10^7 nuclei/ml for analysis of nucleosomal arrays) were pre-incubated at 25°C for 1 min. Micrococcal nuclease (MNase, Worthington Biochemical) was added at 1 U or 2 U for indirect end-labeling analysis, and at 2 U, 5 U and 8 U for analysis of nucleosomal arrays. Digestion was performed at 25°C for 5 min. For indirect end-labeling analysis, naked DNA was digested in the same way using 0.1 U or 0.2 U of MNase. The subsequent steps were carried out as described previously.[15]

For digestion of chromatin with DNase I, aliquots of 100 μl of nuclei suspension (1×10^7 nuclei/ml) were pre-incubated at 37°C for 1 min and subsequently 0.14 U, 0.6 U or 2.4 U of DNase I (TaKaRa Bio) was added. Digestion was then performed at 37°C for 2 min. Naked DNA was digested in the same way using 0.1 U, 0.2 U or 0.3 U of DNase I. The subsequent steps were carried out as described previously.[15]

II-C. Preparation of a G-ladder

Genomic DNA (3μg) was treated with Dimethyl sulfate and piperidine to prepare a G-ladder.[17]

II-D. Ligation-mediated PCR

Ligation-mediated PCR (LM-PCR) was performed to amplify the nuclease-digested DNA fragments.[18] For the indirect end-labeling analysis, 3μg of MNase digests were phosphorylated and ligated with linker DNA. The resulting products were digested with *Bsr*GI and amplified by PCR with oligo A (5'-GCGGTGACCCGGGAGATCTGAATTC-3') and oligonucleotide 5'-TCGACTGAAATCCCTGGTAATCCGT-3'using the following PCR conditions: 95°C for 5 min; 20 cycles at 95°C for 1 min, 65°C for 2 min and 72°C for 3 min; and 72°C for 10 min.

To detect DNase I cleavage sites, primer extension reactions were carried out with the following primers: 5'-TCTGCGTGTTCGAATTCGCC-3' for analysis of the T20 region; and 5'-GTCTTCCATTTTACCAACAG-3' for analysis of the *tk* promoter. The PCR conditions were as follows: 95°C for 5 min, 58°C for 30 min and 76°C for 10 min. After ligation with linker DNA and subsequent purification, PCRs were performed with the following sets of primers; oligoA and 5'-CCAATGACAAGACGCTGGGC-3' to detect signals in the T20 region; and oligoA and

5'-TAAGGCCATGACAACCATTT-3' to detect signals in the *tk* promoter region. The PCRs were carried out under the following conditions: 95°C for 5 min; 20 cycles at 95°C for 1 min, 60°C or 58°C for 2 min and 72°C for 3 min; and 72°C for 10 min.

II-E. Analysis of cutting sites

MNase cleavage sites were detected as follows. Purified DNA was electrophoresed on a 1.2% agarose gel in Tris-borate-EDTA buffer and then transferred onto nylon membranes. For detection of nucleosomal arrays, the membrane was hybridized with a 203-bp probe specific for T20 that spanned from -367 to -164 relative to the transcription start site, or with a 97-bp probe specific for the *tk* promoter that spanned from -81 to +16. In indirect end-labeling analysis, the membrane was hybridized with a 336-bp probe that spanned from the *Bsr*GI site to position +217. The probes were labeled with [α-^{32}P]dCTP (3000Ci/mmol) by random priming.

DNase I cleavage sites were detected by the PCR-based primer extension method,[15] using a (5'-^{32}P)-labeled primer 5'-AAGACGCTGGGCGGGGTTTGTGTC-3' for detection of cleavage sites in the T20 region or a 5'-GGAATGCCAAGCTTACTTAG-3' primer for detection of cleavage sites in the *tk* promoter region. The PCR conditions were as follows: 95°C for 5 min; 13 cycles at 95°C for 1 min, 65°C or 60°C for 2 min, and 72°C for 3 min; and 72°C for 10 min. All samples were purified and resolved in 6% polyacrylamide-7M urea gels.

III. RESULTS

To investigate the underlying mechanism of T20-mediated activation of transcription,[16] we studied the chromatin structure formed on and around T20 in the nucleus. Initially, nuclei were isolated from a HeLa cell line HLB10/T20 containing a single copy of a reporter gene within its genome (Fig. 1). MNase digestion of the nuclei was then performed; this enzyme digests the linker regions between nucleosomes. A Southern blot of the digest was probed using fragments corresponding to the T20-containing region and the promoter DNA(Fig.2).

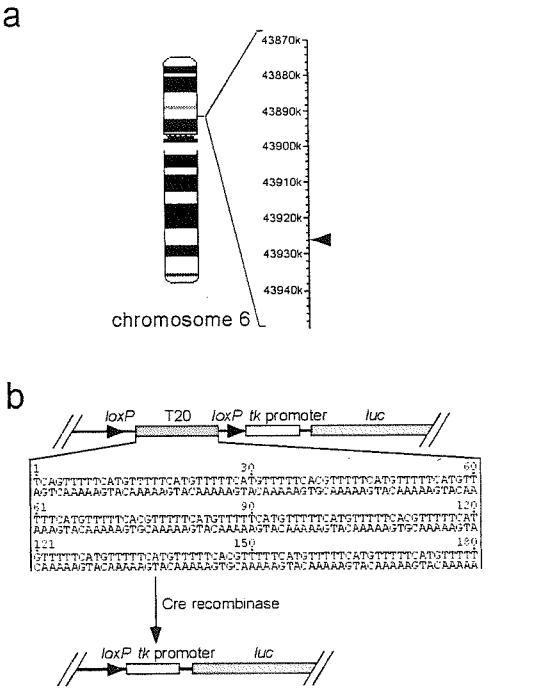


Fig. 1. Locus of the T20-containing reporter gene in the genome of HLB10/T20 (a) and the structure of the reporter gene (b).

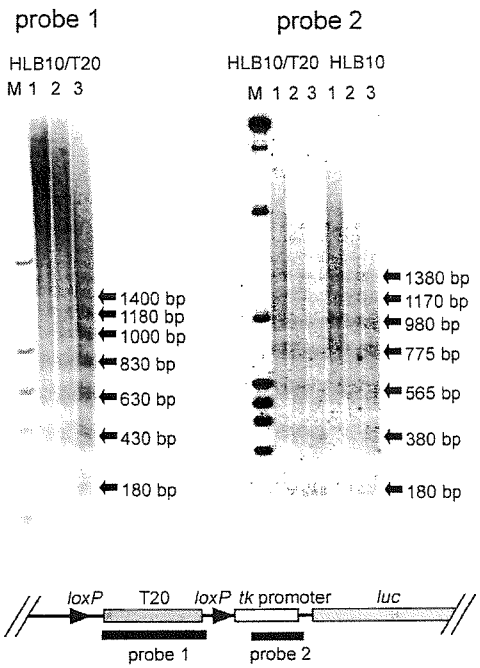


Fig. 2. MNase digests of HLB10/T20 chromatin and HLB10 chromatin using probes for T20 and promoter DNA. DNA fragments spanning from -367 to -164 and from -81 to +16 relative to the transcription start site (+1) were used as probes: lane 1, 20 U/ml; lane 2, 50 U/ml; lane 3, 80 U/ml. 'M' indicates the marker lane.

As shown in the figure, both probes detected a canonical ladder pattern for chromatin digestion with MNase; i.e., probe 1 detected regularly spaced DNA bands of approximately 180, 430, 630, 830, 1000, 1180 and 1400 bp, and probe 2 detected bands of 180, 380, 565, 775, 980, 1170 and 1380 bp. The distance between these bands ranged from 170 to 250 bp, consistent with the presence of nucleosomal arrays. This result indicates that nucleosomes were formed on the region spanning from T20 and the *tk* promoter. A similar analysis of the control cell line HLB10, in which the T20 segment was deleted from the reporter locus of HLB10/T20, showed that nucleosomes were also formed in the promoter region (Fig. 2).

Translational and rotational positioning of nucleosomes plays an important role in transcription.[19-22] Thus, we analyzed translational positioning (sequence-specific localization) of the nucleosomes using indirect end-labeling analysis.[23,24] Bands observed at -430, -370, -65, -35, +1, +85, +150 and +210 in digestion of naked DNA disappeared or became fainter in digestion of chromatin from the HLB10/T20, indicating that these sites were protected from MNase cleavage in the chromatin (Fig. 3). On the other hand, bands at -410, -300, -250, -130, -75, -20, +50, +105 and +180 either newly emerged or became clearer in digestion of HLB10/T20 chromatin, indicating that these sites were more susceptible to MNase cleavage in chromatin.

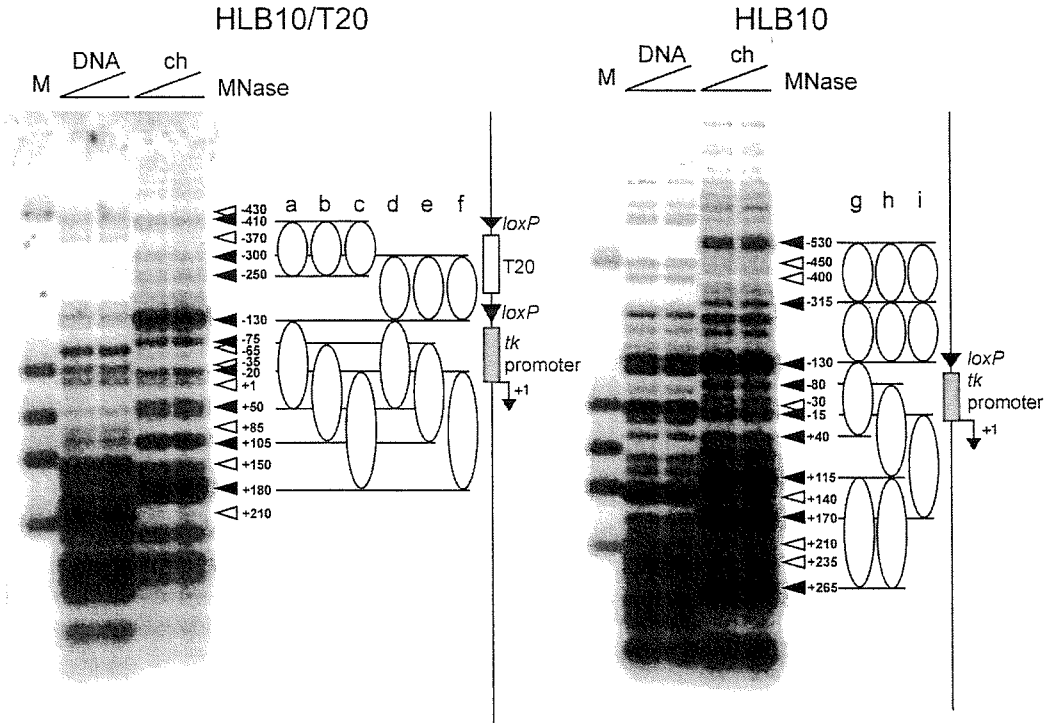


Fig. 3. Analysis of translational positions of nucleosomes formed on the reporter locus. Nuclei of HLB10/T20 cells and HLB10 cells were subjected to MNase digestion. After deproteinization, the genomic DNA was digested with *Bsr*GI and MNase-sensitive DNA sequences were detected using indirect end-labeling analysis. The relative extent of MNase digestion is indicated on the top of the autoradiogram. 'DNA', 'Ch' and 'M' indicate naked DNA, chromatin DNA and size marker lanes, respectively. Distinct cleavage sites and protected sites in chromatin are indicated with black and white arrowheads, respectively, and the sites are numbered relative to the transcription start site. Possible nucleosomal arrays are shown schematically on the right of each autoradiogram.

Although a region from –410 to -130 in naked DNA was protected from MNase digestion (this was presumably due to the intrinsic nature of the T20 segment), the bands described above suggest that the regions between -410 and -250, -300 and -130, -130 and +50, -75 and +105, and -20 and +180 harbored translationally positioned nucleosomes. Thus, as illustrated in Fig. 3, six possible alignments of nucleosomes (a-f) were assumed, with each nucleosomal array containing two nucleosomes with a wide spacing. Using the same procedure, the position of nucleosomes on the genome of HLB10 cells was analyzed. Bands at -530, -315, -130, -80, -15, +40, +115, +170 and +265 seem to reflect the linker centers and these data suggest three possible alignments (g-i); therefore, in the control cells, three to four nucleosomes appear to be deposited in the region of interest.

Using the same nuclei fraction, we then investigated the rotational orientation of the region from T20 to the *tk* promoter. DNase I digestion of chromatin from the HLB10/T20 nuclei gave a specific "10-bp ladder" cleavage pattern (with rung spacing of 9-21 bp) in the region spanning from the upstream end of T20 to the CCAAT box (Fig. 4).

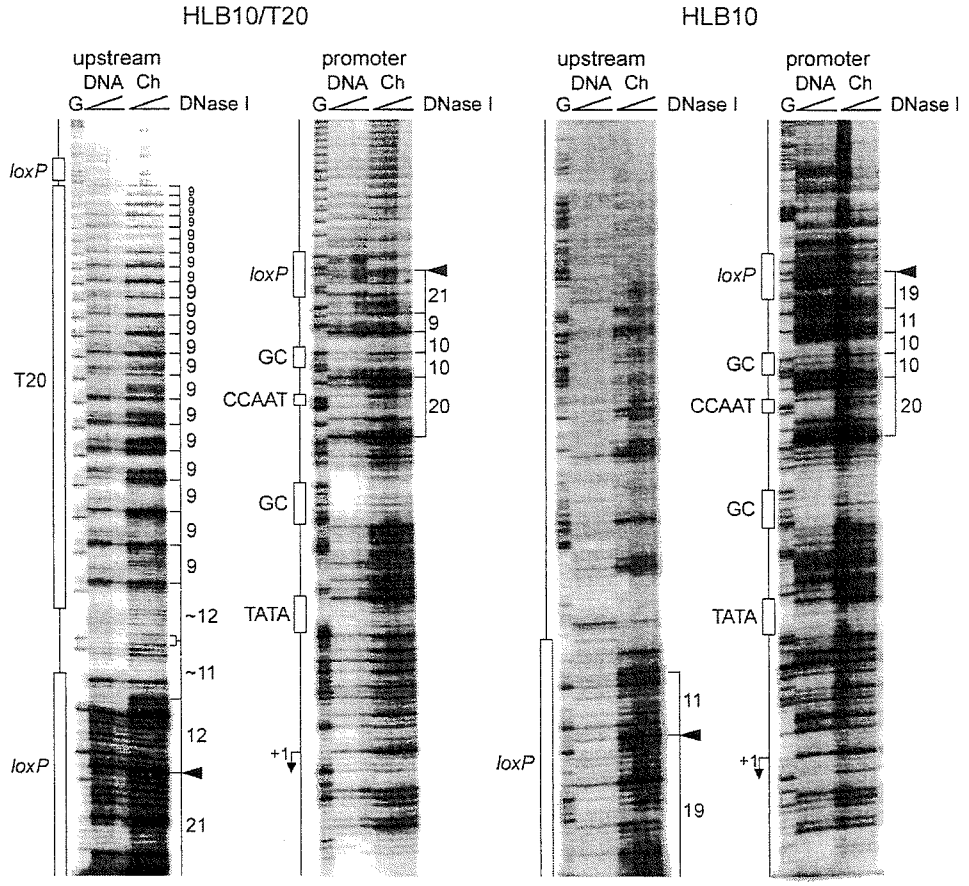


Fig. 4. Rotational positioning of the DNA on the surface of the histone core. Nuclei of HLB10/T20 cells and HLB10 cells were subjected to DNase I digestion. After deproteinization, cleavage sites were detected by linear amplification with a radiolabeled primer and a thermostable DNA polymerase. 'DNA' and 'Ch' indicate DNase I cleavage sites in naked DNA and chromatin DNA, respectively. A "G-ladder" was obtained according to the conventional method (METHODS AND MATERIALS) using part of the sample recovered from DNase I-untreated nuclei. Chromatin-specific DNase I cleavage sites are indicated on the right of each autoradiogram. The distances between the cleavage sites (bp) are indicated for gaps between 8 and 12 bp, or near-multiples of 10 bp. The small filled triangles on the right of each autoradiogram indicate the overlap points of signals.

This indicates that this region was positioned on the surface of a histone core. Interestingly, in the T20 region, the positions and intensities of the cleavage signals were almost the same for DNase I cleavage of naked DNA and chromatin, indicating that the conformation of T20 was maintained in the chromatin context. In contrast, DNase I digestion of HLB10 nuclei resulted in a very short ladder corresponding only to the region from the unique *loxP* site to the CCAAT box.

IV. DISCUSSION

Investigation of the chromatin structure formed on a T20-flanked *tk* promoter was performed to determine the basis for T20 activation of transcription. This region was found to harbor nucleosomes in HLB10/T20 cells (Fig. 2). An analysis of translational positioning of nucleosomes indicated that in each alignment two nucleosomes were implicated in the case of HLB10/T20cells, while three or four nucleosomes were implicated in HLB10 cells (Fig. 3). Thus, the nucleosomes density in the region from about -500 to +200 was lower in HLB10/T20 cells than in HLB10 cells. Based on the data, the accessibility of the TATA box was presumably higher in HLB10/T20 cells, and this may be one reason why the promoter activity is higher in HLB10/T20 cells than in HLB10 cells. In the next paragraph, we address how the T20 segment may contribute to organization of the chromatin structure in HLB10/T20 cells.

We recently showed that a 36-bp curved DNA segment (T4) that mimics part of a left-handed superhelical structure can attract a histone core and activate eukaryotic promoters when introduced upstream of the promoter at an appropriate distance and with proper spatial positioning.[15] T20 may behave in a similar manner but have a more pronounced effect, since the T20 segment of 180 bp is much longer than T4. Indeed, T20 seems to have a high affinity for histone cores: i.e., two translational nucleosome locations were identified in the T20 region (Fig. 3). In addition, a specific "10-bp ladder" cleavage pattern much longer than ~150 bp, the canonical length of usual 10-bp ladders, was detected in the region from the upstream end of T20 to the CCAAT box (Fig. 4). The ladder length of ~290 bp suggests that a long DNA region was rotationally positioned on the surface of histone cores that were not translationally positioned on the region. In other words, these results suggest a dynamic feature of the relevant nucleosomes; i.e., the histone core may slide within the T20 segment, with a slight preference for the two indicated positions. These putative histone-capturing and -sliding properties of T20 may function statically and dynamically to increase the accessibility of the TATA box and other *cis*-DNA elements in the promoter, thereby facilitating transcription.

ACKNOWLEDGEMENTS

This study was supported in part by JSPS and MEXT research grants to T.O.

REFERENCES

[1] K. Zahn and FR. Blattner, *Science* 236, 416-422 (1987).
[2] GZ. Hertz, MR. Young and JE. Mertz, *J. Virol.* 61, 2322-2325 (1987).
[3] JS. Williams, TT. Eckdahl and JN. Anderson, *Mol. Cell. Biol.* 8, 2763-2769 (1988).
[4] C. Du, RP. Sanzgiri, WL. Shaiu, JK. Choi, Z. Hou, RM. Benbow and DL. Dobbs, *Nucleic Acids Res.* 23, 1766-1774 (1995).
[5] P. Polaczek, K. Kwan, DA. Liberies and JL. Campbell, *Mol. Microbiol.* 26, 261-275 (1997).
[6] T. Kusakabe, Y. Sugimoto, Y. Hirota, S. Tone, Y. Kawaguchi, K. Koga and T. Ohyama, *Mol. Biol. Rep.* 27, 13-19 (2000).
[7] E. Milot, A. Belmaaza, JC. Wallenburg, N. Gusew, WE. Bradley and P. Chartrand, *EMBO J.* 11, 5063-5070 (1992).
[8] A. Mazin, E. Milot, R. Devoret and P. Chartrand, *Mol. Gen. Genet.* 244, 435-438 (1994).
[9] A. Travers, *Nature* 341, 184-185 (1989).
[10] PJ. Hagerman, *Annu. Rev. Biochem.* 59, 755-781 (1990).
[11] J. Perez-Martin, F. Rojo and V. de Lorenzo, *Microbiol. Rev.* 58, 268-290 (1994).
[12] T. Ohyama, *Bioessays.* 23, 708-715 (2001).

[13]M. Asayama and T. Ohyama, Curved DNA and Prokaryotic Promoters: A Mechanism for Activation of Transcription, in *DNA Conformation and Transcription,* (T. Ohyama, ed.), 37-46, Landes Bioscience, Texas. (2005).

[14]T. Ohyama, Curved DNA and Transcription in Eukaryotes, in *DNA Conformation and Transcription,* (T. Ohyama, ed.), 66-74, Landes Bioscience, Texas. (2005).

[15]J. Nishikawa, M. Amano, Y. Fukue, S. Tanaka, H. Kishi, Y. Hirota, K. Yoda and T. Ohyama, *Nucleic Acids Res.* 31, 6651-6662 (2003).

[16]N. Sumida, J. Nishikawa, H. Kishi, M. Amano, T. Furuya, H. Sonobe and T. Ohyama, *FEBS J. to be published*

[17]AD. Riggs, J. Singer-Sam and GP. Pfeifer, In vivo footprint and chromatin analysis by LMPCR, in *Chromatin*, (H. Gould, ed.), 79-110, Oxford University Press, New York. (1998).

[18]GP. Pfeifer, HH. Chen, J. Komura and AD. Riggs, *Methods Enzymol.* 304, 548-571 (1999).

[19]RT. Simpson, *Nature* 343, 387-389 (1990).

[20]Q. Li and Ö. Wrange, *Genes Dev.* 7, 2471-2482 (1993).

[21]Q. Li and Ö. Wrange, *Mol. Cell. Biol.* 15, 4375-4384 (1995).

[22]J. Wong, Q. Li, BZ. Levi, YB. Shi and AP. Wolffe, *EMBO J.* 16, 7130-7145 (1997).

[23]SA. Nedospasov and GP. Georgiev, *Biochem. Biophys. Res. Commun.* 92, 532-539 (1980).

[24]C. Wu, *Nature* 286, 854-860 (1980).

Available online at www.sciencedirect.com
ScienceDirect
Archives of Biochemistry and Biophysics 461 (2007) 7–12
ABB
www.elsevier.com/locate/yabbi

The location of the left-handedly curved DNA sequence affects exogenous DNA expression *in vivo*

Hiroyuki Kamiya [a,b,*], Satoki Fukunaga [a], Takashi Ohyama [c], Hideyoshi Harashima [a,b]

[a] *Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan*
[b] *CREST, Japan Science and Technology, Japan*
[c] *Faculty of Education and Integrated Arts and Sciences, Major in Integrative Bioscience and Biomedical Engineering, Graduate School of Science and Engineering, Waseda University, 1-6-1, Nishi-Waseda, Shinjuku-ku, Tokyo 169-8050, Japan*

Received 15 November 2006, and in revised form 25 January 2007
Available online 8 March 2007

Abstract

The intranuclear disposition of a plasmid is extremely important for transgene expression. The effects of a left-handedly curved sequence with high histone affinity on plasmid expression were examined *in vivo*. A naked luciferase-plasmid was delivered into mouse liver by a hydrodynamics-based injection, and the luciferase activities were quantitated at various time points. The location of the left-handedly curved sequence determined the transgene expression, without affecting the amount of intranuclear exogenous DNA. The plasmid containing the curved sequence at the location that results in the exposure of the TATA box out of the nucleosome core showed the highest expression. These results suggest that sequences with high histone affinity could control transgene expression from plasmids *in vivo*.

Keywords: Left-handedly curved DNA; Histones; Exogenous DNA; Intranuclear disposition; Hydrodynamics-based injection

Plasmid DNAs are used for transgene expression with nonviral vectors in gene therapy and biotechnology, in either a naked or complexed form. Nonviral vectors are quite attractive, due to their excellent safety profile [1–5]. However, their low transgene expression efficiency, in comparison to that of viral vectors, is a major problem. To overcome this problem, control of intracellular DNA trafficking, particularly nuclear entry, has been studied in many laboratories.

In addition, the intranuclear disposition of the delivered plasmid is also a factor in efficient transgene expression [5]. The intranuclear disposition is also related to the transient transgene expression from the plasmid. Recently, the intranuclear disposition of a plasmid delivered into mouse liver by a hydrodynamics-based injection was examined [6]. The major reason for the transient transgene expression is the dramatic decrease in the expression efficiency from one copy of the exogenous DNA over time, and this phenomenon proceeds without promoter methylation. The decrease in the expression efficiency was also observed in cultured cells [7]. These results suggest that histones, which have an important function in chromosomal gene regulation, are involved in the phenomenon, since nucleosomes are formed on nonintegrated plasmids [8]. The binding of histones limits the access of transcriptional factors to their recognition sites in the plasmid, and thus, the binding mode of histones to the plasmid would affect transgene expression and its decrease. In addition, histone modifications could cause the decrease. Therefore, the interaction(s) between the plasmid and the histones are quite important for efficient transgene expression, and are one of the keys for controlling the intranuclear disposition.

We focused on DNA sequences that affect the histone binding. Previously, Nishikawa et al. reported that $[CATGTTTTT]_4$ (left-handedly curved DNA), with the

* Corresponding author. Address: Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan. Fax: +81 11 706 4879.
E-mail address: hirokam@pharm.hokudai.ac.jp (H. Kamiya).

0003-9861/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2007.02.012

appropriate combination of distance and spatial positioning, could activate a eukaryotic promoter when delivered in a naked form into cultured COS-7 cells [9]. They also showed that histones bound to the left-handedly curved DNA, and that the TATA box was exposed out of the nucleosome core when the curved DNA was located at appropriate positions [9]. This finding prompted us to examine the effects of this left-handedly curved sequence *in vivo*. In this study, we investigated the *in vivo* effects of this sequence on plasmids when delivered in a naked form into mouse liver. We found that the position of the left-handedly curved sequence affected the transgene expression by one order of magnitude, without altering the amount of the exogenous DNAs, suggesting the different accessibility of transcriptional factors to the plasmids *in vivo*. Similar results were observed when plasmids complexed with cationic lipids were delivered into mouse liver. These results indicate that the left-handedly curved sequence on plasmids also determined transgene expression *in vivo*, and that controlled interactions between the plasmid and the histones are important for the intranuclear disposition.

Materials and methods

Materials

Oligodeoxyribonucleotides were purchased from Sigma Genosys Japan (Ishikari, Japan) in purified forms. The pLHC4/TLN–6, pLHC4/TLN–16, pLHC4/TLN+47, and pST0/TLN–7 plasmids, containing the thymidine kinase (*tk*) promoter and the luciferase gene (Fig. 1) [9], were amplified in *Escherichia coli* strain DH5α and purified with a Qiagen (Hilden, Germany) EndoFree Plasmid Mega kit.

Hydrodynamics-based injection

Plasmid DNA (20 μg in 2 ml of saline) was injected into the tail vein of male 6-week-old Balb/c mice within 5 s [10,11]. The livers were harvested from the injected mice at various time points, and the luciferase activity and the amount of the exogenous DNA were measured, as described below.

Luciferase activity

Livers were minced with scissors and homogenized completely in lysis buffer (100 mM Tris–HCl, 2 mM EDTA, 0.1% Triton X-100, pH 7.8). After centrifugation at 13,000 *g* for 10 min at 4 °C, the supernatant was examined for luciferase activity, using a Luciferase Assay Systems kit (Promega, Madison, Wisconsin, USA).

Isolation of nuclear DNA and quantitative PCR

Livers were homogenized in phosphate-buffered saline (PBS). After centrifugation at 2500*g* for 5 min at 4 °C, the pellet was washed three times with PBS. The pellet was resuspended in DNA lysis buffer (100 mM Tris–HCl, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% (w/v) IGEPAL-CA630, pH 7.4) [12]. After centrifugation at 1400*g* for 5 min at 4 °C, the pellet was washed three times with DNA lysis Buffer. The intranuclear DNA was extracted with the SepaGene reagent (Sanko Jun-yaku, Tokyo, Japan).

Quantitative PCR (Q-PCR) was performed using an ABI 7500 real-time PCR system, and SYBR-Green chemistry (Applera, Norwalk, Connecticut, USA). A 100-ng portion of the recovered DNA was analyzed by Q-PCR. The luciferase gene in the mouse liver was detected using the following primers: Luc (+), 5′-GGTCCTATGATTATGTCCGGT TATG; Luc (−), 5′-ATGTAGCCATCCATCCTTGTCAAT.

Hydrodynamics-based injection of lipoplex

Plasmid DNA (50 μg) was mixed with 216.3 μl of Lipofectin Reagent (Invitrogen, Carlsbad, California, USA) in Hepes-buffered glucose (10 mM Hepes, 277.5 mM glucose, pH 7.4), and the mixture was incubated

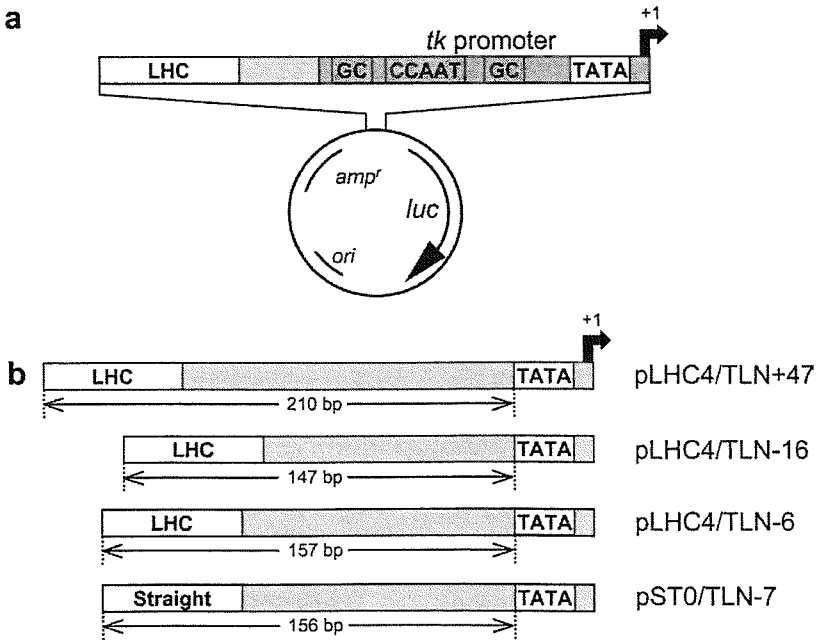


Fig. 1. Luciferase-plasmids used in this study. (a) Structure of the plasmid containing the left-handedly curved sequence and the *tk* promoter. LHC, left-handedly curved sequence; GC, GC box; CCAAT, CCAAT box; TATA, TATA box; *luc*, luciferase gene; *amp*^{r}, *E. coli* ampicillin resistance gene; *ori*, *E. coli* replication origin. (b) Distances between the 5′-end of the TATA box and the 5′-end of the left-handedly curved sequence. The pST0/TLN–7 plasmid containing straight sequence is also shown.

at room temperature for 15 min. Hepes-buffered glucose was then added to the mixture to a total volume of 2 ml. The DNA–lipid complex was injected as described above.

Statistical analysis

Statistical significance was examined by the Mann–Whitney test. Levels of $P < 0.05$ were considered to be significant.

Results

Luciferase-plasmids containing the sequence with high histone affinity

Previously, Nishikawa et al. compared luciferase expression from various plasmids containing the left-handedly curved sequence ($[CATGTTTTT]_4$) in the upstream region of the *tk* promoter, when they were electroporated into simian COS-7 cells [9]. The expression depended on the distance and the spatial positioning of the curved sequence. The luciferase expression was enhanced when the curved sequence was located at appropriate positions, due to nucleosome formation at the curved sequence, resulting in the exposure of the TATA box in the linker region. The pLHC4/TLN–6 and pLHC4/TLN–16 plasmids were the best and second best ones in the above experiments, using COS-7 cells (Fig. 1). In contrast, the expression from the pLHC4/TLN+47 plasmid was the least efficient among the plasmids containing the left-handedly curved sequence, possibly due to the binding of the TATA box to the nucleosome adjacent to that formed at the curved sequence. We examined pLHC4/TLN–6, pLHC4/TLN–16, and pLHC4/TLN+47 as model DNAs in this *in vivo* study. We also examined the pST0/TLN–7 plasmid containing the straight instead of left-handedly curved sequence as a control (Fig. 1).

Effects of the left-handedly curved sequence on gene expression from plasmids delivered in a naked form

A rapid, high-volume injection method (hydrodynamics-based administration) [10,11] was used for the delivery of naked plasmids to the livers of male Balb/c mice. This method enables the delivery of the plasmid into the nuclei of the liver, without the aid of cationic compounds, which could potentially affect the quantitation of the intranuclear exogenous DNA. The livers were harvested after 8, 24, 48, and 72 h, and the luciferase activities were measured. The amounts of exogenous DNA at the same time points were examined by Q-PCR after isolation of the nuclei.

At 8 h after injection, the luciferase expression from pLHC4/TLN–6 and pLHC4/TLN–16 seemed to be higher than that from pLHC4/TLN+47, although the difference was statistically insignificant, due to data variations, as often reported for hydrodynamics-based administrations (Fig. 2a) [10,11]. At 24 and 48 h, the luciferase activity was significantly higher for pLHC4/TLN–6 than for pLHC4/TLN+47 (Fig. 2b and c). The expression from pLHC4/TLN–16 was comparable to that from pLHC4/TLN–6 at 8 and 24 h, but was ~1/2 of that from pLHC4/TLN–6 at 48 h (Fig. 2a–c). Importantly, the luciferase expression from pLHC4/TLN–6 was ~10-fold more efficient than that from pLHC4/TLN+47 and pLHC4/TLN–16 at 72 h. Thus, the expression levels were in the order of pLHC4/TLN–6 > pLHC4/TLN–16 > pLHC4/TLN+47 at the time points examined. These results are similar to those obtained with COS-7 cells [9]. The luciferase expression from pST0/TLN–7 was significantly lower than that from pLHC4/TLN–6 at 24 and 48 h, and seemed lower at 72 h (Fig. 2b–d).

We then quantitated the amounts of the exogenous DNAs by Q-PCR (Fig. 2e–h). The amounts of the DNAs were broadly equal at each time point, although the data are variable possibly due to variations of introduction efficiency of plasmid DNA by the hydrodynamics-based administrations, and we could observe no tendency for the amounts of the DNAs. These results suggest that the location of the left-handedly curved sequence did not affect the amounts of plasmids in the nucleus.

Effects of the left-handedly curved sequence on decrease in expression

We then focused on the time courses of the luciferase expression and the amounts of exogenous DNA. As shown in Fig. 2a–d, the transgene expression was transient, and the luciferase activity decreased over time. The luciferase activities at 72 h were hundreds-fold less than those at 24 h for pLHC4/TLN–16 and pLHC4/TLN+47 (Table 1). In contrast, the decrease in the expression was smaller (1/56) for pLHC4/TLN–6. On the other hand, the amounts of these DNAs in the nuclei decreased similarly (Table 1). The amounts of the DNAs at 72 h were 12- to 20-fold less than those at 24 h. Thus, the decrease in the expression was one order of magnitude more rapid than the decrease in the amount of exogenous DNA for pLHC4/TLN–16 and pLHC4/TLN+47.

Ochiai et al. found that the transgene expression efficiency per copy of intranuclear plasmid rapidly decreased when the plasmid was delivered by the hydrodynamics-based administration method [6]. We calculated the expression efficiencies for the plasmids used in this study. The luciferase activities at 24 and 72 h were divided by the amounts of the exogenous DNAs at the same time points. As shown in Table 1, the expression efficiencies, the ratios of the luciferase activities, and the amounts of the exogenous DNA decreased from 24 to 72 h. The expression efficiencies at 72 h were 1/24, 1/15, and 1/19 of those at 24 h for pLHC4/TLN–16, pLHC4/TLN+47, and pST0/TLN–7, respectively. Thus, the expression from the plasmid was suppressed over time. In the case of the luciferase-plasmid containing the cytomegalovirus promoter, the expression efficiency at 72 h was 1/32 of that at 24 h [6]. In contrast, the expression efficiency at 72 h was only 1/2 of that at 24 h, in the case of pLHC4/TLN–6. This result suggests that the inactivation of pLHC4/TLN–6 proceeded more slowly than that of other plasmids.

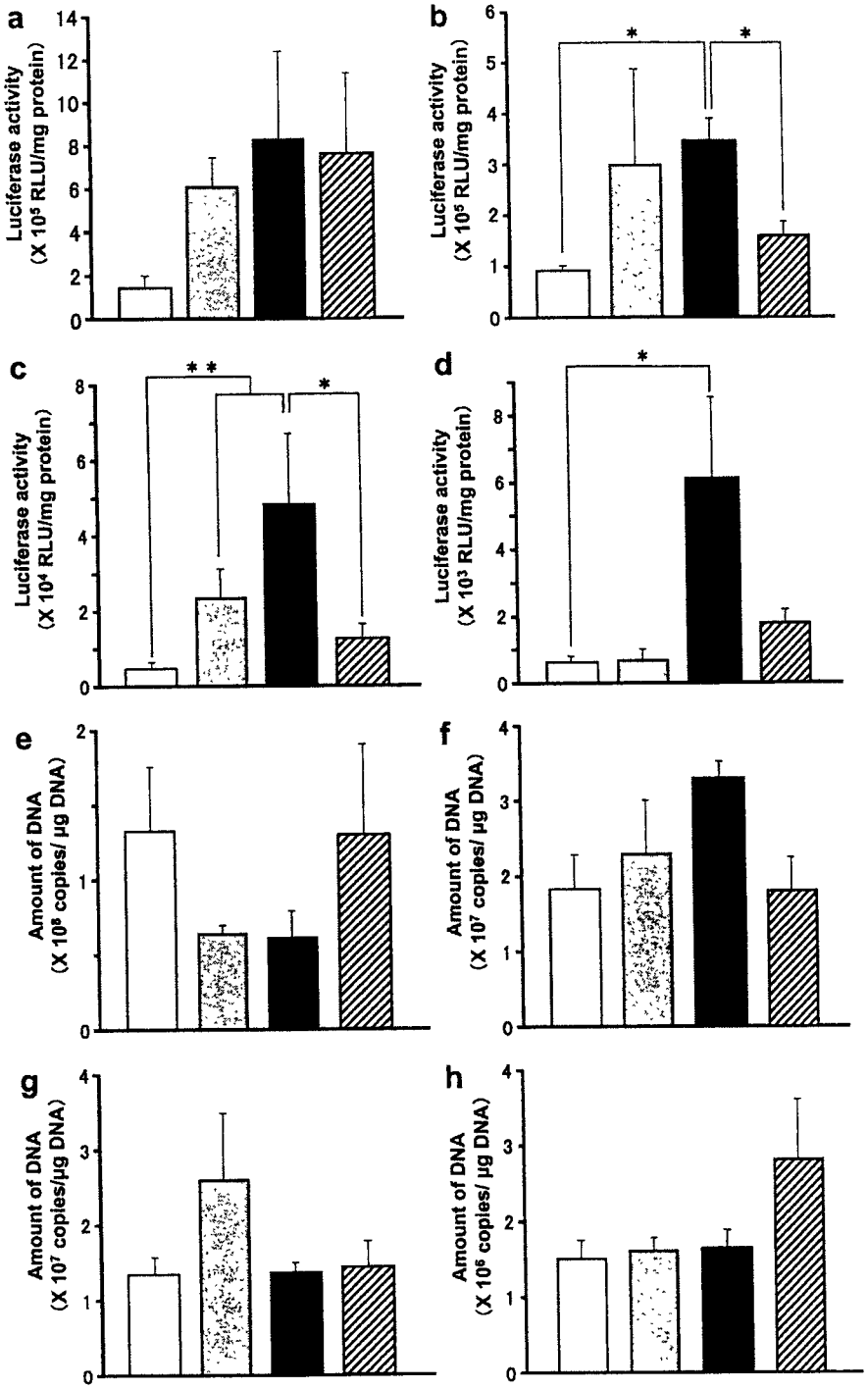


Fig. 2. (a–d) Expression of the luciferase gene and (e–h) amounts of luciferase-DNA in the nucleus, examined by hydrodynamics-based injection of the naked plasmid. Plasmid DNA (20 μg) was injected into the tail vein of male Balb/c mice (6-weeks old). The livers were harvested, and the luciferase activities and the amounts of the exogenous DNA were measured. The values represent the averages of at least three separate experiments. (a,e) 8 h; (b,f) 24 h; (c,g) 48 h; (d,h) 72 h. White columns, pLHC4/TLN+47; gray columns, pLHC4/TLN–16; black columns, pLHC4/TLN–6; hatched columns, pST0/TLN–7. Bars indicate SE (standard error) ($^{*}P < 0.05$ and $^{**}P < 0.01$).

Luciferase gene expression from plasmids delivered as complexes with cationic lipids

Next, we delivered the plasmids as complexes with cationic lipids into mouse liver. The hydrodynamics-based administration was also used in this case. Plasmids (50 μg) were mixed with Lipofectin reagent, and the luciferase expression in liver was monitored at 48 h after injection.

As shown in Fig. 3a, the luciferase expression from pLHC4/TLN–6 was higher than that from the other plasmids at 48 h. In contrast, the amounts of the exogenous DNAs were broadly equal (Fig. 3b). Thus, the effects of the sequence

Table 1
Decrease of luciferase activity and exogenous DNA from 24 to 72 h after administration[a]

Plasmid	Luciferase	Exogenous DNA	Ratio (luc/exogenous DNA)[b]
pLHC4/TLN+47	0.0070	0.0812	0.067
pLHC4/TLN–16	0.0023	0.0695	0.042
pLHC4/TLN–6	0.0177	0.0493	0.476
pST0/TLN–7	0.0121	0.1533	0.052

[a] The luciferase activity and the amount of exogenous DNA at 72 h after administration were divided by those at 24 h.

[b] The luciferase activities at 24 and 72 h were divided by the amounts of exogenous DNA at the same time points. The quotient at 72 h was divided by that at 24 h.

with high histone affinity were similar between the cases of lipoplexes and plasmid DNAs delivered in the naked form.

Discussion

The objective of this study was to examine the effects of the left-handedly curved sequence, with high histone affinity, on transgene expression from plasmids *in vivo*. Naked plasmids containing the sequence at different positions relative to the TATA box were delivered into mouse liver. As in the previous report using cultured cells [9], pLHC4/TLN–6 produced the luciferase protein most efficiently (Fig. 2a–d). The luciferase gene was expressed in the order of pLHC4/TLN–6 > pLHC4/TLN–16 > pLHC4/TLN+47 at every time point. Nishikawa et al. showed that a nucleosome was generated on the left-handedly curved sequence, and that the TATA box was located in the linker region for pLHC4/TLN–6 [9]. In the case of pLHC4/TLN–16, the TATA box was located at the edge of the nucleosome, making the access of the TATA box binding protein more difficult. The results obtained in this study also agreed with the findings of Nishikawa et al. Thus, the binding of histones to the left-handedly curved sequence seemed to affect transgene expression from plasmid DNA in the mouse liver. In addition, these results indicated that the location of the left-handedly curved sequence affected the transgene expression in nondividing cells, such as mouse hepatocytes, as well as in dividing COS-7 cells.

Alternatively, the effects of the left-handedly curved sequence in mouse hepatocytes might be attributed to binding of transcription factor(s) to the sequence. If the binding of the factor(s) influences transcription efficiency depending on distance from the TATA box, the results shown above could be obtained. In addition, the left-handedly curved sequence bends the DNA and this might affect the luciferase expression.

The position effects of the left-handedly curved sequence were observed at 8 h after the injection (Fig. 2a). The results may suggest that histones bound to the plasmids at least in part at 8 h after their introduction. When we consider that the amount of the luciferase protein reflects the transcription efficiency at earlier time points, the binding of histones to plasmids may occur at an early stage after the administration. However, analysis on the histone binding is necessary for correct interpretation.

Liposomes are expected to be one of the vehicles for plasmids that will be used clinically in the near future. Liposomes have also been used as nonviral vectors in biological experiments. Thus, we examined the effects of the curved sequence on the expression from plasmids delivered in the lipoplex form *in vivo*. In general, transgenes are expressed mainly in the lung by the intravenous administration of lipoplexes, depending on the lipid composition [13–16]. Alternatively, the spleen is a transgene-expressing organ in the case of Lipofectin [17]. In this study, the hydrodynamics-based administration was also used for plasmid–Lipofectin complexes, since we could deliver the complexes into the mouse liver, the same tissue examined for the naked plasmids. The luciferase gene was expressed in the order of pLHC4/TLN–6 > pLHC4/TLN–16 > pLHC4/TLN+47 as naked plasmids (Fig. 3). Thus, the sequence with high histone affinity affected the expression similarly for naked plasmids and lipoplexes. This result suggests that the histones bound to the plasmids delivered in the complexed form. We presently do not know the actual pathway(s) by which the DNA-lipid complexes are converted to the

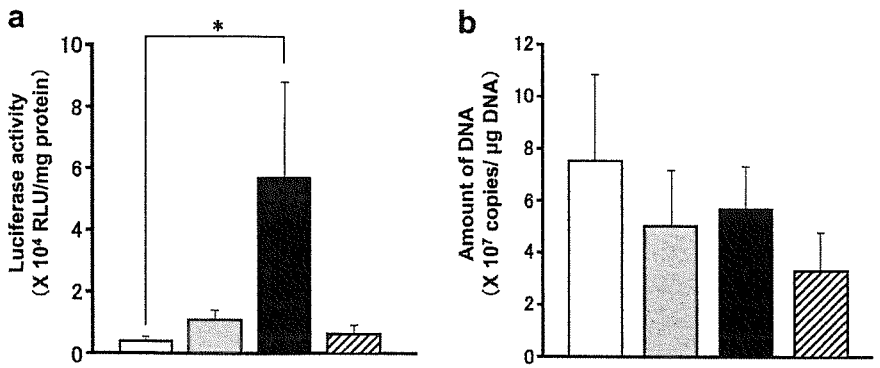


Fig. 3. Expression of the luciferase gene, examined by hydrodynamics-based injection of plasmid complexed with cationic lipids. Plasmid DNA (50 μg) complexed with Lipofectin (charge ratio = 1:1) was injected into the tail vein of male Balb/c mice (6-weeks old). The livers were harvested, and the luciferase activity was measured. The values represent the averages of at least three separate experiments. White columns, pLHC4/TLN+47; gray columns, pLHC4/TLN–16; black columns, pLHC4/TLN–6; hatched columns, pST0/TLN–7. Bars indicate SE (standard error) (*$P < 0.05$).

DNA–histone complexes in the mouse liver. In cultured cells, it is possible that the lipids are replaced directly by histones [18].

In conclusion, the left-handedly curved sequence at the appropriate position could enhance the *in vivo* expression of transgene delivered in naked and complexed forms. The 'controlled intranuclear disposition' of the delivered DNA is quite important for achieving practical gene therapy and efficient transfection [5]. Properly controlled interaction(s) of the plasmid with histones by the introduction of functional DNA sequence(s) would allow transcriptional factors to recognize their binding sites in the plasmid. The left-handedly curved sequence is one of the candidates of the functional sequences to control nucleosome formation on plasmids and transgene expression *in vivo*.

Acknowledgments

This work was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Japan Society for the Promotion of Science.

References

[1] R.I. Mahato, Y. Takakura, M. Hashida, Crit. Rev. Ther. Drug Carrier Syst. 14 (1997) 133–172.
[2] A.P. Rolland, Crit. Rev. Ther. Drug Carrier Syst. 15 (1998) 143–198.
[3] H. Kamiya, H. Tsuchiya, J. Yamazaki, H. Harashima, Adv. Drug Del. Rev. 52 (2001) 153–164.
[4] T. Niidome, L. Huang, Gene Ther. 9 (2002) 1647–1652.
[5] H. Kamiya, H. Akita, H. Harashima, Drug Discovery Today 8 (2003) 990–996.
[6] H. Ochiai, H. Harashima, H. Kamiya, FEBS Lett. 580 (2006) 918–922.
[7] H. Ochiai, H. Harashima, H. Kamiya, Biol. Pharm. Bull. 29 (2006) 1294–1296.
[8] R. Reeves, C.M. Gorman, B. Howard, Nucleic Acids Res. 13 (1985) 3599–3615.
[9] J. Nishikawa, M. Amano, Y. Fukue, S. Tanaka, H. Kishi, Y. Hirota, K. Yoda, T. Ohyama, Nucleic Acids Res. 31 (2003) 6651–6662.
[10] G. Zhang, V. Budker, J.A. Wolff, Hum. Gene Ther. 10 (1999) 1735–1737.
[11] F. Liu, Y. Song, D. Liu, Gene Ther. 6 (1999) 1258–1266.
[12] R. Tachibana, H. Harashima, N. Ide, S. Ukistu, Y. Ohta, N. Suzuki, H. Kikuchi, Y. Shinohara, H. Kiwada, Pharm. Res. 19 (2002) 377–381.
[13] S. Li, L. Huang, Gene Ther. 4 (1997) 891–900.
[14] J.W. McLean, E.A. Fox, P. Baluk, P.B. Bolton, A. Haskell, R. Pearlman, G. Thurston, E.Y. Umemoto, D.M. McDonald, Am. J. Physiol. Heart Circ. Physiol. 273 (1997) 387–404.
[15] M. McClarrinon, L. Gilkey, V. Watral, B. Fox, C. Bullock, L. Fradkin, D. Liggitt, L. Roche, L.B. Bussey, E. Fox, C. Gorman, DNA Cell Biol. 18 (1999) 533–547.
[16] L.G. Barron, K.B. Meyer, F.C. Szoka Jr., Hum. Gene Ther. 9 (1998) 315–323.
[17] H. Ochiai, H. Harashima, H. Kamiya, Biol. Pharm. Bull. 28 (2005) 2019–2022.
[18] H. Akita, R. Ito, H. Kamiya, K. Kogure, H. Harashima, J. Gene Med. in press, doi: 10.1002/jgm.1010.

Transworld Research Network
37/661 (2), Fort P.O., Trivandrum-695 023, Kerala, India

DNA Structure, Chromatin and Gene Expression, 2006: 71-84 ISBN: 81-7895-228-9
Editors: Ryoiti Kiyama and Mitsuhiro Shimizu

6

Genetic information carried in DNA conformation and properties

**Takashi Ohyama[1], Noriyuki Sumida[2], Jun-ichi Tanase[1]
Shotaro Inoue[2] and Takahiro Okabe[1]**
[1]Department of Biology, Faculty of Education and Integrated Arts and Sciences, Waseda University, 1-6-1 Nishi-Waseda, Shinjuku-ku Tokyo 169-8050, Japan; [2]Department of Biology, Faculty of Science and Engineering, Konan University, 8-9-1 Okamoto, Higashinada-ku Kobe 658-8501, Japan

Abstract

The conformation and mechanical properties of DNA provide additional structural and functional dimensions to chromatin organization and gene expression. The reason why transcriptional regulatory regions often contain curved DNA structures has

Correspondence/Reprint request: Dr. Takashi Ohyama, Department of Biology, Faculty of Education and Integrated Arts and Sciences, Major in Integrative Bioscience and Biomedical Engineering, Graduate School of Science and Engineering, Waseda University, 1-6-1 Nishi-Waseda, Shinjuku-ku, Tokyo 169-8050, Japan
E-mail: ohyama@waseda.jp

been roughly understood. They seem to construct chromatin structures that leave target elements exposed, permitting activators to recognize them and bind. Concerning the mechanical properties of DNA, a surprising finding was recently made for eukaryotic class II gene promoters. They have common mechanical properties irrespective of the promoter type. These properties are suggested to function as general markers in promoter recognition by transcription factors. Here, we discuss genetic information carried in DNA conformation and properties.

Introduction

Owing to the remarkable progress in genome science, nowadays, we have easy access to genome sequence databases for various organisms. However, we are still far from a clear understanding of how genomic DNA is packaged into a nucleus without entanglement, how chromatin structure is faithfully inherited from mother to daughter cells, how the differential expression of genes is enabled in a given cell type, and how transcriptional activators find their target sites embedded in the highly compacted genomic DNA. The aim of this review is to get a clue to these questions.

Multifarious DNA structures are found in the genome. Their implication in DNA packaging and gene expression has long been argued. In the meantime, much circumstantial evidence and several lines of direct evidence have been presented [1]. Among these structures, the biological role of curved DNA structures has been most intensively studied, for they often occur in or around origins of DNA replication [2-7], promoters and enhancers [8-11] and DNA recombination loci [12, 13], irrespective of the origin of the DNA (for reviews, see refs. 1, 14-17). At first, this review will focus on the genetic role of curved DNA structures.

Flexibility (rigidity) and deformability are representative mechanical properties of DNA. They are also considered to play important roles in DNA packaging into the nucleus and DNA recognition by sequence-specific DNA binding proteins [18]. Recently, we found interesting mechanical properties of eukaryotic promoters, which will be described in the second part of this review.

I. DNA conformation as a source of genetic information

I.1. Curved DNA and DNA packaging in chromatin

Both intrinsic DNA curvature and anisotropic DNA bendability influence the formation, stability and positioning of nucleosomes [19-41]. It seems thermodynamically favorable to form nucleosomes on DNA sequences that are already appropriately curved. With this logic, Crick and Klug hypothesized the presence of curved DNA structures to explain the mechanism underlying

DNA packaging into nucleosomes, which dates back to 1975 (seven years before the finding of naturally occurring curved DNA structure) [42]. This was the first reference to the intrinsically curved conformation of DNA. Later, it was revealed that indeed, nucleosomes often preferentially associate with curved DNA fragments [25, 27-29, 34, 36, 37, 40]. Nucleosome formation is a genome-wide phenomenon. Therefore, if curved DNA is implicated in nucleosome-mediated DNA packaging into the nucleus, the curved DNA loci should frequently occur on eukaryotic genomes. Indeed, they frequently occur.

The repetitive DNA sequences, including satellite DNAs, very often contain one or more curved DNA structures [43-50]. Satellite DNA sequences are universally associated with regions of constitutive heterochromatin and comprise anywhere from a few percent to > 50% of mammalian genomes [51, 52]. Interestingly, repeatedly occurring curved DNA sites are not restricted to satellite DNA, but are also reported for human ε-, Gγ-Aγ-ψβ-, δ-, and β-globin, c-*myc*, and immunoglobulin heavy chain μ loci, and in mouse β^{major}-globin locus [53-56]. Furthermore, considering that most findings of naturally occurring curved DNA structures have been based on detection of retarded migration in gel electrophoresis and that this phenomenon can be suppressed by another structural property that causes rapid migration [49], there seem to be many more curved DNA loci on eukaryotic genomes than expected. Thus, curved DNA structures seem to be implicated in the genome-wide nucleosome-mediated DNA packaging.

I.2. Why curved DNA structures frequently occur in control regions of transcription

Curved DNA structures and nucleosome positioning

As described above, curved DNA structures are presumably implicated in nucleosome formation. Then, how can we explain the reason why curved DNA structures often occur in transcriptional control regions irrespective of the promoter type (Table 1)? One hypothesis is that they may regulate positioning of nucleosomes in these regions so as to allow the binding of transcription factors [17, 57]. It is known that positioning of nucleosomes on a DNA sequence plays an important role in controlling the access of specific DNA-binding proteins to regulatory DNA elements [58-63]. Since the target DNA elements could become accessible by making the relevant region free of nucleosomes, or by exposing the target element toward the environment on a nucleosome (Fig. 1), curved DNAs may play these functions.

In making the regulatory region free of nucleosomes, the easiest way is presumably to make the DNA dissimilar to the negative supercoil. The adenylate kinase gene promoter of *Saccharomyces cerevisiae* may be an example of this. This promoter has a curved DNA of this type and is free of nucleosomes [64]. In addition, in the yeast *GAL80* promoter, an intrinsic DNA

Table 1. Curved DNA-containing promoters, enhancers and regulatory sequences[a].

Gene	Class	Origin	Locus
rRNA	I	human	promoter
rRNA	I	*Rattus norvegicus*	promoter
rRNA	I	mouse	promoter
rRNA	I	*Xenopus laevis*	promoter
rRNA	I	*Xenopus borealis*	promoter
rRNA	I	*Drosophila melanogaster*	promoter
rRNA	I	*Paracentrotus lividus*	promoter
rRNA	I	*Tetrahymena pyriformis*	promoter
rRNA	I	*Dictyostelium discoideum*	promoter
rRNA	I	*Arabidopsis thaliana*	promoter
rRNA	I	*Arabidopsis thaliana*	around position -270
rRNA	I	*Pisum sativum*	promoter
rRNA	I	*Triticum aestivum*	promoter
rRNA	I	*Zea mays*	promoter
rRNA	I	*Physarum polycephalum*	both sides of transcription start site
rRNA	I	*Physarum polycephalum*	promoter
β-actin	II	human	promoter
cdc2	II	human	promoter
c-myc	II	human	promoter
E2F1	II	human	E2F binding site
erythropoietin receptor	II	human	promoter
ε-globin	II	human	promoter
Gγ-globin	II	human	promoter
Aγ-globin	II	human	promoter
ψβ-globin	II	human	promoter
δ-globin	II	human	promoter
β-globin	II	human	promoter
IFNβ	II	human	enhancer
β^{major}-globin	II	mouse	promoter
A2 vitellogenin	II	*Xenopus*	upstream regulatory region
BhC4-1	II	*Bradysia hygida*	promoter
AaH I' toxin	II	*Androctonus australis*	promoter
DNA polymerase δ	II	*Plasmodium falciparum*	promoter
E1A	II	human adenovirus type 2	enhancer
E1A	II	human adenovirus type 5	enhancer
E2	II	adenovirus	E2F binding site
E6-E7	II	human papillomavirus type 16	E2 protein binding site
IE94	II	simian cytomegalovirus	upstream of the enhancer
rbcS-3A	II	pea	light responsive elements
rbcS-3.6	II	pea	light responsive elements
MF α 1	II	*Saccharomyces cerevisiae*	upstream activation site
STE3	II	*Saccharomyces cerevisiae*	upstream activation site
GAL1-10	II	yeast	promoter
GAL80	II	yeast	promoter
AKY2	II	*Saccharomyces cerevisiae*	promoter

[a]Reports on the TATA box conformation are not involved. Reproduced with permission from ref. 1,

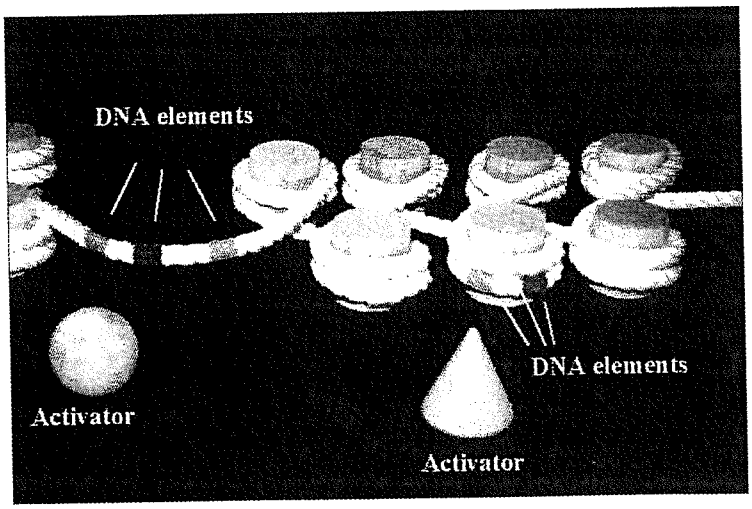


Figure 1. Two possible mechanisms to make a chromatin structure that permits activator binding: either by inhibiting nucleosome assembly on the target element, or by putting the target element on a nucleosome and displaying it toward the environment.

curvature seems to exclude nucleosome formation on the UAS_{GAL80} [65]. In contrast, high nucleosome-forming ability of neighboring DNA can also make the DNA elements free of nucleosomes. For example, the yeast *GAL1* promoter, which has two strong DNA curvatures [65], is incorporated into a nucleosome (referred to as nucleosome B) under inactivated (non-inducing) conditions. The UAS_G, which is the element involved in the induction of transcription, is located in the non-nucleosomal region just upstream of the nucleosome B.

If the helical axis of DNA intrinsically resembles the negatively supercoiled DNA seen on a nucleosome, it would recruit core histones easily like the case of the *GAL1* promoter. Furthermore, if a *cis*-DNA element is involved in, or is located near, this curved region, rotational position of the element on the histone core (or even in linker DNA region in some cases) would be restricted by the DNA curvature. When its recognition site is displayed toward the environment by the curvature, the recognition step would be facilitated. An example is seen in the nucleosome structure formed on the long terminal repeat of the mouse mammary tumor virus (MMTV-LTR). In this case, a left-handedly curved DNA seems to play the role described above [17, 66]. Four glucocorticoid receptor recognition elements (GREs) are located on the surface of a positioned nucleosome, and the major grooves of two GREs are exposed towards the environment [58]. These sites can be recognized by the receptor (a zinc finger protein), which initiates transcription. A synthetic left-handedly curved DNA was also found able to generate the effect of this type. When it was linked to the herpes simplex virus thymidine kinase promoter at a specific rotational phase and distance, it attracted a nucleosome and the TATA box was thereby left in the linker DNA with its minor groove facing outwards [63]. As the result, the segment activated the promoter approximately 10-fold in COS-7 cells. Neither planar DNA curvature, nor right-handedly curved DNA, nor straight DNA, had this effect [63]. How easily the DNA can be incorporated into nucleosomes seems to be determined by similarity or dissimilarity between a given DNA and the negatively supercoiled DNA seen on a nucleosome.

The other effects generated by DNA curvatures

Histone modifications such as acetylation, methylation and phosphorylation are implicated in transcription activation and gene silencing [67-72]. The major sites of these modifications are N-terminal tail domains. Interestingly, interactions between N-terminal domains and intrinsic DNA curvature could influence nucleosome positioning and stability [73, 74]. Furthermore, it is known that interactions between intrinsically curved DNA and the histone tails stabilizes the formation of nucleosomes by ca. 250 cal/mol [74]. Thus, curved DNA structures may also influence local chromatin through their interaction with histone N-terminal tail domains.

Curved DNA may deform nucleosomes to allow transcription factors to bind to their target DNA elements on them. An interesting result was obtained in an experiment that used the TG motif [$(A/T)_3NN(G/C)_3NN$] and a curved DNA segment. The repeatedly occurring TG motifs are anisotropically flexible and have a high nucleosome-forming ability [26]. Blomquist *et al.* constructed DNA fragments composed of the TG-motifs and the binding site for the NF-1 with an A_5 tract on both sides, and examined the NF-1 binding affinity to its target sequence on the nucleosome [75]. When nucleosomes were reconstituted on the fragments, the NF-1 binding affinity was higher when the flanking A-tracts were out-of-phase with the TG-motifs, than when they were in-phase.

In conclusion, curved DNA structures can organize local chromatin so as to allow the DNA binding proteins to bind to their target sites easily. Mechanically, they can enhance the accessibility of *cis*-DNA elements in chromatin by exposing them to the milieu while on the nucleosome or by preventing nucleosome formation. Thus, the curved DNA conformations seem to be a source of the genetic information that is used to package genomic DNA into chromatin and especially to reproduce functional chromatin in daughter cells.

I.3. Effects of the other unusual DNA structures on local chromatin

Poly(dA•dT) sequences and nucleosome positioning

In eukaryotic genomes, there are more $(dA•dT)_n$ sequences present than would be expected if the DNA sequence were random, while in prokaryotes, no difference is observed between actual and expected occurrences [76]. These sequences are rigid and adopt a unique DNA conformation [77-79]. Also, $(dA•dT)_n$-rich sequences, where several $(dA•dT)_n$ sequences are connected by other short sequences, have frequently been found in or around eukaryotic promoter regions. In *HIS3*, *PET56, DED1*, *ARG4*, and *URA3,* the $(dA•dT)_n$-rich sequences act as upstream promoter elements [80-82].

In some cases, the poly(dA•dT) sequences are incorporated into nucleosomes, with either the original conformation, or with an altered conformation [83-86], while in other cases they are not incorporated [85, 87]. Therefore, the effect of poly(dA•dT) sequences may be essentially the same as the proposed effect of curved DNA. Interestingly, it was reported that the HMG-I(Y) family of "high mobility group" proteins can preferentially bind to certain types of poly(dA•dT) sequences on the surface of nucleosomes and alter the local setting of DNA on the nucleosomes [88]. The poly(dA•dT) sequences may also function as a signal to introduce structural changes into nucleosomes.

Effects of Z-DNA, triplex DNA and cruciform DNA

Eukaryotic gene promoters sometimes contain, or are flanked by, Z-DNA forming sequences. In the transcription of the human *CSF1* (colony-stimulating factor 1 gene), Z-DNA seems to be implicated in gene activation coupled with chromatin remodeling: i.e., BAF (the SWI/SNF-like BRG1-associated factor)-induced Z-DNA formation at the *CSF1* promoter stabilizes an open chromatin structure [89]. However, we do not yet know whether Z-DNA can regulate nucleosome position. Triple-stranded DNA seems unable to be accommodated within nucleosomes [90, 91]. Nucleosome assembly and triplex formation are presumably competing processes. Cruciform structures are located mainly on internucleosomal DNA [92], perhaps because they cannot associate with histone cores [93]. Eukaryotes may use triplex structures and cruciforms to form open chromatin structures.

II. Genetic information carried in mechanical properties of DNA

Key genetic processes, such as DNA replication, transcription and recombination are regulated by DNA-protein interactions. However, how protein factors find their target sites efficiently is not yet understood. Our recent study strongly suggested that mechanical properties of promoter DNA sequences are implicated in the target-site selection [94, 95]. This section discusses the mechanical properties of DNA as a source of information.

It is generally known that the TATA box, the initiator (Inr) element and the downstream promoter element (DPE) are core promoter elements of the class II genes that are transcribed by RNA polymerase II. The TATA box is usually located ~25-31bp upstream of the transcription start site (+1) and has a consensus sequence TATAWAW (W, A or T) [96, 97]. The Inr is located around the transcription start site, with consensus sequence PyPyANWPyPy (Py, pyrimidine; N, any nucleotide) [98, 99], and the DPE, which has a consensus PuGWCGTG (Pu, purine) sequence, is centered at ~+30 [100]. There is also a group of class II gene promoters that comprise G/C-rich sequences and contain multiple GGGCGG sequences for the transcription factor Sp1 [101, 102]. We have heretofore believed that many eukaryotic class II gene promoters have either a TATA box, Inr or G/C-rich sequence, or a combination of them. However, in human and mouse promoter sequences in the EPD (eukaryotic promoter database), which is a non-redundant collection of eukaryotic class II gene promoters [103, 104], the population of promoters that contain a canonical TATA box was found to be small in each case (the analysis was performed using EPD releases 76 and 78, which contained 1871 human and 196 mouse class II gene promoters) [94]. Concretely, the TATA-only, TATA- and Inr-containing, or TATA-, Inr- and DPE-containing human

promoters accounted for only 6, 1 and 0 %, respectively. Screening of the database using TATAWA resulted in only slight increases in these populations. Since the population of TATA-containing promoters in yeast is also small [105], the population of eukaryotic promoters that contain a TATA box seems to be small. Also found was that the populations of Inr-containing human and mouse promoters are very small [94]. Although the GC box-containing human promoters accounted for ~20% [95], it was clarified that more than 50% of the human class II gene promoters do not contain any elements. Based on these data, the population of 'core-less' promoters seems to be high in all eukaryotes.

Then, how are core-less promoters recognized as promoters? Interestingly, they were revealed to have characteristic mechanical properties: i.e., (i) the region around the transcription start site contains a distinctively flexible sequence and a considerably rigid sequence, compared with other parts of the promoter region, (ii) the upstream region of the transcription start site is slightly more rigid than the downstream region, and (iii) the region around position -25 is relatively rigid (Fig. 2). The GC box-only promoters have the same profile [95]. The 'signal' described in (i) was not observed in the further upstream region spanning from -151 to -498. More interestingly, it was clarified that the TATA box-containing promoters and the Inr-containing promoters have common characteristic mechanical properties and these are strikingly similar to the properties of the core-less and GC box-only promoters: i.e., both of the TATA box and Inr comprise distinctively flexible and rigid sequences when compared with other parts of the promoter region and DNA region upstream of the TATA box or the Inr sequence is more rigid than region downstream of each element (Fig. 3).

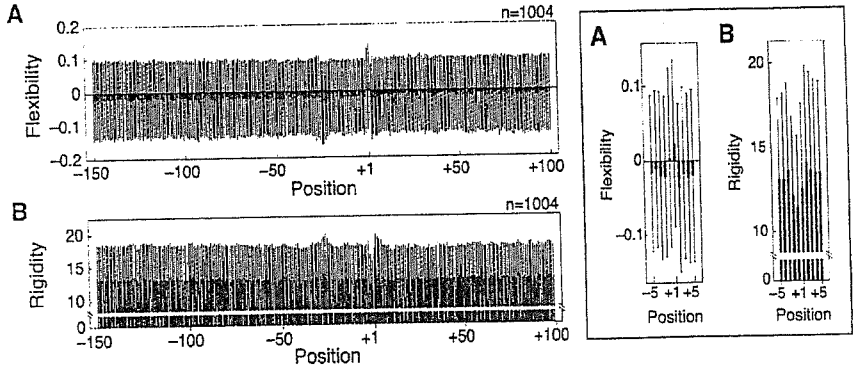


Figure 2. Average flexibility profiles of human core-less promoters, as calculated from DNase I-derived flexibility parameters [108] (**A**) or from the tetranucleotide potential energy surface model [109] (**B**). Values are shown as means ± SD. Lower values correspond to more flexible sequences in (**B**). Flexibility is plotted against the position of the center nucleotide (**A**) or the second nucleotide (**B**). The data around the transcription start site are magnified on the right. Reproduced with permission from ref. 95, ©2005 Oxford University Press.

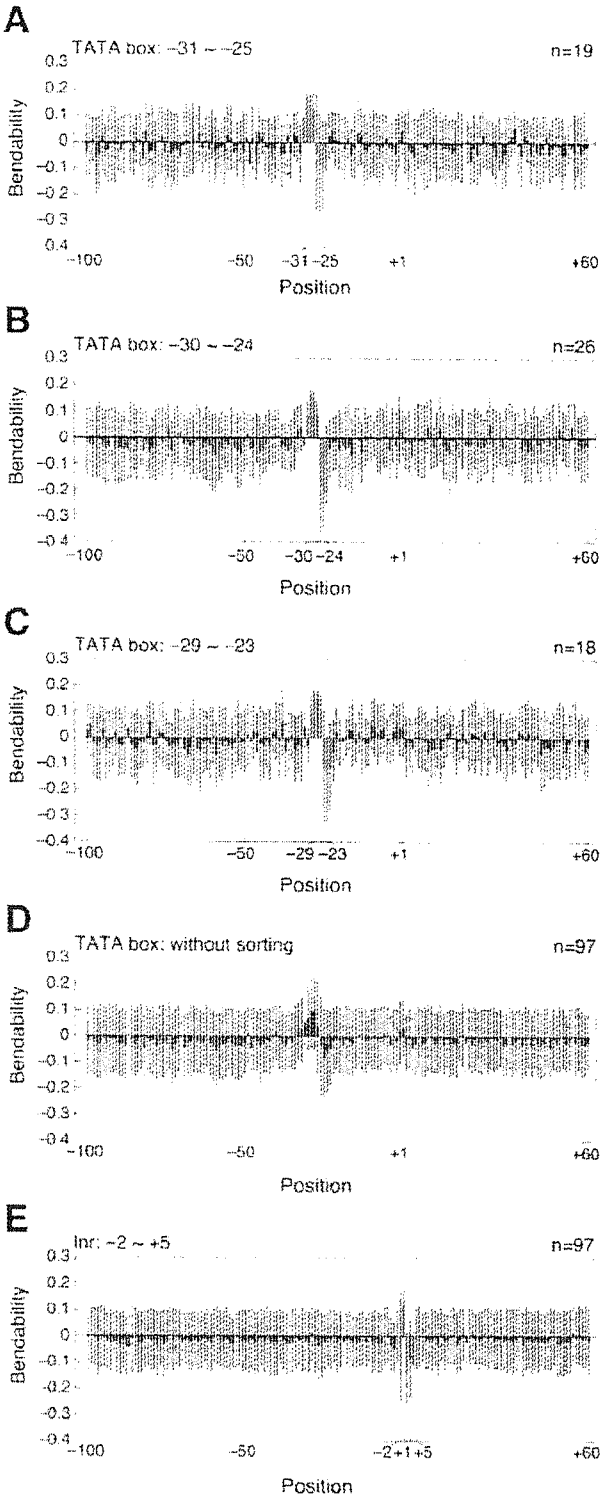


Figure 3. Average flexibility profiles of human promoters containing either a TATA box or an Inr sequence only. DNase I-derived flexibility parameters [108] were used in the calculation. The flexibilities of TATA box and Inr sequences are colored red (A–C) and blue (E), respectively. Reproduced with permission from ref. 94, ©2004 Oxford University Press.

The mechanical properties of the class II gene promoters may function as general markers in recognition by transcription factors. The difference in flexibility between upstream and downstream regions may be recognized at first as regional features. Then, transcription factors may find their target sites by searching the short sequence comprising of unusually rigid and flexible segments.

Conclusions

The 'genetic information' is generally defined as "the information contained in a sequence of nucleotide bases in a nucleic acid molecule" [106] or "the information carried in a sequence of nucleotides in a molecule of DNA or RNA" [107]. However, as we discussed in this review, DNA conformation and properties also carry genetic information that are used for the construction of functional chromatin and presumably for the target-site selection. To understand such information is one of the next frontiers in the biological science.

Acknowledgements

The author would like to acknowledge the contributions of Jun-ichi Nishikawa, Yoshiro Fukue and Junko Ohyama. The studies reported from my laboratory were supported in part by JSPS and MEXT research grants to TO.

References

1. Ohyama, T. (Ed.), 2005, DNA Conformation and Transcription, Springer, New York, and Landes Bioscience, Texas.
2. Zahn, K., and Blattner, F.R. 1987, Science, 236, 416-422.
3. Hertz, G.Z., Young, M.R., and Mertz, J.E. 1987, J. Virol., 61, 2322-2325.
4. Williams, J.S., Eckdahl, T.T., and Anderson, J.N. 1988, Mol. Cell. Biol., 8, 2763-2769.
5. Du, C., Sanzgiri, R.P., Shaiu, W.L., Choi, J.K., Hou, Z., Benbow, R.M., and Dobbs, D.L. 1995, Nucl. Acids Res., 23, 1766-1774.
6. Polaczek, P., Kwan, K., Liberies, D.A., and Campbell, J.L. 1997, Mol. Microbiol., 26, 261-275.
7. Kusakabe, T., Sugimoto, Y., Hirota, Y., Tone, S., Kawaguchi, Y., Koga, K., and Ohyama, T. 2000, Mol. Biol. Rep., 27, 13-19.
8. Ohyama, T., and Hashimoto, S. 1989, Nucl. Acids Res., 17, 3845-3853.
9. Ohyama, T., Nagumo, M., Hirota, Y., and Sakuma, S. 1992, Nucl. Acids Res., 20, 1617-1622.
10. Hirota, Y., and Ohyama, T. 1995, J. Mol. Biol., 254, 566-578.
11. Ohyama, T. 1996, J. Biol. Chem., 271, 27823-27828.
12. Milot, E., Belmaaza, A., Wallenburg, J.C., Gusew, N., Bradley, W.E.C., and Chartrand, P. 1992, EMBO J., 11, 5063-5070.

13. Mazin, A., Milot, E., Devoret, R., and Chartrand, P. 1994, Mol. Gen. Genet., 244, 435-438.
14. Travers, A. 1989, Nature, 341, 184-185.
15. Hagerman, P.J. 1990, Annu. Rev. Biochem., 59, 755-781.
16. Pérez-Martín, J., Rojo, F., and De Lorenzo, V. 1994, Microbiol. Rev., 58, 268-290.
17. Ohyama, T. 2001, Bioessays, 23, 708-715.
18. Dlakic, M., Ussery, D.M., and Brunak, S. 2005, DNA Conformation and Transcription, T. Ohyama (Ed.), Springer, New York, and Landes Bioscience, Texas, 189-202.
19. Zhurkin, V.B., Lysov, Y.P., and Ivanov, V.I. 1979, Nucl. Acids Res., 6, 1081-1096.
20. Trifonov, E.N., and Sussman, J.L. 1980, Proc. Natl. Acad. Sci. U. S. A., 77, 3816-3820.
21. Dickerson, R.E., Kopka, M.L., and Pjura, P. 1983, Proc. Natl. Acad. Sci. U. S. A., 80, 7099-7103.
22. Drew, H.R., and Travers, A.A. 1985, J. Mol. Biol., 186, 773-790.
23. Zhurkin, V.B. 1985, J. Biomol. Struct. Dyn., 2, 785-804.
24. Satchwell, S.C., Drew, H.R., and Travers, A.A. 1986, J. Mol. Biol., 191, 659-675.
25. Pennings, S., Muyldermans, S., Meersseman, G., and Wyns, L. 1989, J. Mol. Biol., 207, 183-192.
26. Shrader, T.E., and Crothers, D.M. 1989, Proc. Natl. Acad. Sci. U. S. A., 86, 7418-7422.
27. Wolffe, A.P., and Drew, H.R. 1989, Proc. Natl. Acad. Sci. U. S. A., 86, 9817-9821.
28. Costanzo, G., Di Mauro, E., Salina, G., and Negri, R. 1990, J. Mol. Biol., 216, 363-374.
29. Shrader, T.E., and Crothers, D.M. 1990, J. Mol. Biol., 216, 69-84.
30. Ioshikhes, I., Bolshoy, A., and Trifonov, E.N. 1992, J. Biomol. Struct. Dyn., 9, 1111-1117.
31. Patterton, H-G., and Simpson, R.T. 1995, Nucl. Acids Res., 23, 4170-4179.
32. Sivolob, A.V., and Khrapunov, S.N. 1995, J. Mol. Biol., 247, 918-931.
33. Baldi, P., Brunak, S., Chauvin, Y., and Krogh, A. 1996, J. Mol. Biol., 263, 503-510.
34. De Santis, P., Kropp, B., Leoni, L., Sampaolese, B., and Savino, M. 1996, Biophys. Chem., 62, 47-61.
35. Ioshikhes, I., Bolshoy, A., Derenshteyn, K., Borodovsky, M., and Trifonov, E.N. 1996, J. Mol. Biol., 262, 129-139.
36. Widlund, H.R., Cao, H., Simonsson, S., Magnusson, E., Simonsson, T., Nielsen, P.E., Kahn, J.D., Crothers, D.M., and Kubista, M. 1997, J. Mol. Biol., 267, 807-817.
37. Fitzgerald, D.J., and Anderson, J.N. 1998, Nucl. Acids Res., 26, 2526-2535.
38. Olson, W.K., Gorin, A.A., Lu, X.J., Hock, L.M., and Zhurkin, V.B. 1998, Proc. Natl. Acad. Sci. U. S. A., 95, 11163-11168.
39. Anselmi, C., Bocchinfuso, G., De Santis, P., Savino, M., and Scipioni, A. 1999, J. Mol. Biol., 286, 1293-1301.
40. Widlund, H.R., Kuduvalli, P.N., Bengtsson, M., Cao, H., Tullius, T.D., and Kubista, M. 1999, J. Biol. Chem., 274, 31847-31852.

41. Roychoudhury, M., Sitlani, A., Lapham, J., and Crothers, D.M. 2000, Proc. Natl. Acad. Sci. U. S. A., 97, 13608-13613.
42. Crick, F.H., and Klug, A. 1975, Nature, 255, 530-533.
43. Radic, M.Z., Lundgren, K., and Hamkalo, B.A. 1987, Cell, 50, 1101-1108.
44. Benfante, R., Landsberger, N., Tubiello, G., and Badaracco, G. 1989, Nucl. Acids Res., 1989, 17, 8273-8282.
45. Martínez-Balbás, A., Rodríguez-Campos, A., García-Ramírez, M., Sainz, J., Carrera, P., Aymami, J., and Azorín, F. 1990, Biochemistry, 29, 2342-2348.
46. Pasero, P., Sjakste, N., Blettry, C., Got, C., and Marilley, M. 1993, Nucl. Acids Res., 21, 4703-4710.
47. Fitzgerald, D.J., Dryden, G.L., Bronson, E.C., Williams, J.S., and Anderson, J.N. 1994, J. Biol. Chem., 269, 21303-21314.
48. Královics, R., Fajkus, J., Kovarík, A., and Bezdek, M. 1995, J. Biomol. Struct. Dyn., 12, 1103-1119.
49. Ohyama, T., Tsujibayashi, H., Tagashira, H., Inano, K., Ueda, T., Hirota, Y., and Hashimoto, K. 1998, Nucl. Acids Res., 26, 4811-4817.
50. Ohyama, T. 2005, DNA Conformation and Transcription, T. Ohyama (Ed.), Springer, New York, and Landes Bioscience, Texas, 177-188 .
51. John, B., and Miklos, G.L.G. 1979, Int. Rev. Cytol., 58, 1-114.
52. Singer, M.F. 1982, Int. Rev. Cytol., 76, 67-112.
53. Wada-Kiyama, Y., and Kiyama, R. 1994, J. Biol. Chem., 269, 22238-22244.
54. Wada-Kiyama, Y., and Kiyama, R. 1995, J. Biol. Chem., 270, 12439-12445.
55. Wada-Kiyama, Y., and Kiyama, R. 1996, Mol. Cell. Biol., 16, 5664-5673.
56. Ohki, R., Hirota, M., Oishi, M., and Kiyama, R. 1998, Nucl. Acids Res., 26, 3026-3033.
57. Ohyama, T. Nuclear Dynamics, K. Nagata, and K. Takeyasu (Eds.), Springer-Verlag, Tokyo, in press.
58. Piña, B., Brüggemeier, U., and Beato, M. 1990, Cell, 60, 719-731.
59. Schild, C., Claret, F-X., Wahli, W., and Wolffe, A.P. 1993, EMBO J., 12, 423-433.
60. Imbalzano, A.N., Kwon, H., Green, M.R., and Kingston, R.E. 1994, Nature, 370, 481-485.
61. Godde, J.S., Nakatani, Y., and Wolffe, A.P. 1995, Nucl. Acids Res., 23, 4557-4564.
62. Wong, J., Li, Q., Levi, B-Z., Shi, Y.B., and Wolffe, A.P. 1997, EMBO J., 16, 7130-7145.
63. Nishikawa, J., Amano, M., Fukue, Y., Tanaka, S., Kishi, H., Hirota, Y., Yoda, K., and Ohyama, T. 2003, Nucl. Acids Res., 31, 6651-6662.
64. Angermayr, M., Oechsner, U., Gregor, K., Schroth, G.P., and Bandlow, W. 2002, Nucl. Acids Res., 30, 4199-4207.
65. Bash, R.C., Vargason, J.M., Cornejo, S., Ho, P.S., and Lohr, D. 2001, J. Biol. Chem., 276, 861-866.
66. Piña, B., Barettino, D., Truss, M., and Beato, M. 1990, J. Mol. Biol. 216, 975-990.
67. Imhof, A., and Wolffe, A.P. 1998, Curr. Biol., 8, R422-R424.
68. Turner, B.M. 2000, Bioessays, 22, 836-845.
69. Jenuwein, T. 2001, Trends Cell Biol., 11, 266-273.
70. Geiman, T.M., and Robertson, K.D. 2002, J. Cell. Biochem., 87, 117-125.
71. Turner, B.M. 2002, Cell, 111, 285-291.

72. Carrozza, M.J., Utley, R.T., Workman, J.L., and Côté, J. 2003, Trends Genet., 19, 321-329.
73. Kropp, B., Leoni, L., Sampaolese, B., and Savino, M. 1995, FEBS Lett., 364, 17-22.
74. Widlund, H.R., Vitolo, J.M., Thiriet, C., and Hayes, J.J. 2000, Biochemistry, 39, 3835-3841.
75. Blomquist, P., Belikov, S., and Wrange, Ö. 1999, Nucl. Acids Res., 27, 517-525.
76. Dechering, K.J., Cuelenaere, K., Konings, R.N., and Leunissen, J.A. 1998, Nucl. Acids Res., 26, 4056-4062.
77. Alexeev, D.G., Lipanov, A.A., and Skuratovskii, I.Y. 1987, Nature, 325, 821-823.
78. Nelson, H.C., Finch, J.T., Luisi, B.F., and Klug, A. 1987, Nature, 330, 221-226.
79. Park, H.S., Arnott, S., Chandrasekaran, R., Millane, R.P., and Campagnari, F. 1987, J. Mol. Biol., 197, 513-523.
80. Struhl, K. 1985, Proc. Natl. Acad. Sci. U. S. A., 82, 8419-8423.
81. Roy, A., Exinger, F., and Losson, R. 1990, Mol. Cell. Biol., 10, 5257-5270.
82. Thiry-Blaise, L.M., and Loppes, R. 1990, Mol. Gen. Genet., 223, 474-480.
83. Zhu, Z., and Thiele, D.J. 1996, Cell, 87, 459-470.
84. Schieferstein, U., and Thoma, F. 1996, Biochemistry, 35, 7705-7714.
85. Shimizu, M., Mori, T., Sakurai, T., and Shindo, H. 2000, EMBO J., 19, 3358-3365.
86. Koch, K.A., and Thiele, D.J. 1999, J. Biol. Chem., 274, 23752-23760.
87. Suter, B., Schnappauf, G., and Thoma, F. 2000, Nucl. Acids Res., 28, 4083-4089.
88. Reeves, R., and Wolffe, A.P. 1996, Biochemistry, 35, 5063-5074.
89. Liu, R., Liu, H., Chen, X., Kirby, M., Brown, P.O., and Zhao, K. 2001, Cell, 106, 309-318.
90. Westin, L., Blomquist, P., Milligan, J.F., and Wrange, Ö. 1995, Nucl. Acids Res., 23, 2184-2191.
91. Espinás, M.L., Jiménez-García, E., Martínez-Balbás, Á., and Azorín, F. 1996, J. Biol. Chem., 271, 31807-31812.
92. Battistoni, A., Leoni, L., Sampaolese, B., and Savino, M. 1988, Biochim. Biophys. Acta., 950, 161-171.
93. Nobile, C., Nickol, J., and Martin, R.G. 1986, Mol. Cell. Biol., 6, 2916-2922.
94. Fukue, Y., Sumida, N., Nishikawa, J., and Ohyama, T. 2004, Nucl. Acids Res. 32, 5834-5840.
95. Fukue, Y., Sumida, N., Tanase, J., and Ohyama, T. 2005, Nucl. Acids Res. 33, 3821-3827.
96. Breathnach, R., and Chambon, P. 1981, Annu. Rev. Biochem., 50, 349-383.
97. Carey, M., and Smale, S.T. 2000, Transcriptional regulation in eukaryotes: concepts, strategies, and techniques, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
98. Javahery, R., Khachi, A., Lo, K., Zenzie-Gregory, B., and Smale, S.T. 1994, Mol. Cell. Biol., 14, 116-127.
99. Smale, S.T., Jain, A., Kaufmann, J., Emami, K.H., Lo, K., and Garraway, I.P. 1998, Cold Spring Harb. Symp. Quant. Biol., 63, 21-31.
100. Burke, T.W., and Kadonaga, J.T. 1997, Genes Dev., 11, 3020-3031.
101. Kadonaga, J.T., Courey, A.J., Ladika, J., and Tjian, R. 1988, Science, 242, 1566-1570.

102. Mitchell, P.J., and Tjian, R. 1989, Science, 245, 371-378.
103. Périer, R.C., Junier, T., and Bucher, P. 1998, Nucl. Acids Res., 26, 353-357.
104. Schmid, C.D., Praz, V., Delorenzi, M., Périer, R., and Bucher, P. 2004, Nucl. Acids Res., 32, D82-D85.
105. Basehoar, A.D., Zanton, S.J., and Pugh, B.F. 2004, Cell, 116, 699-709.
106. King, R.C., and Stansfield, W.D. 1997, A Dictionary of Genetics (5th ed.), Oxford University Press, Inc., New York.
107. Smith, A.D., Datts, S.P., et al. (Eds.), 1997, Oxford Dictionary of Biochemistry and Molecular Biology, Oxford University Press, Inc., New York.
108. Brukner, I., Sánchez, R., Suck, D., and Pongor, S. 1995, EMBO J., 14, 1812-1818.
109. Packer, M.J., Dauncey, M.P., and Hunter, C.A. 2000, J. Mol. Biol., 295, 85-103.

Reprint from

K. Nagata, K. Takeyasu (Eds.)

Nuclear Dynamics

Molecular Biology and Visualization of the Nucleus

Springer

10-1. Regulation of Chromatin Structure by Curved DNA: How Activator Binding Sites Become Accessible

Takashi Ohyama

Department of Biology, Faculty of Education and Integrated Arts and Sciences, Major in Integrative Bioscience and Biomedical Engineering, Graduate School of Science and Engineering, Waseda University, 1-6-1, Nishi-Waseda, Shinjuku-ku, Tokyo 169-8050, Japan

10-1.1 Introduction

A single somatic cell of humans contains DNA fibers of a total length of approximately 2 m, which are compacted, without entanglement, into the nucleus of approximately 1×10^{-5} m in diameter. To greater or lesser degrees, all organisms compact their DNA. Biologically important DNA regions, such as the origins of DNA replication, regulatory regions of transcription, and recombination loci, must all be compacted. The tightly constrained DNA, however, presents the appropriate environment for replication, transcription, and recombination to take place.

In eukaryotes, the DNA fiber is packaged into chromatin. In the last decade, much progress has been made in understanding how transcription is initiated in chromatin (Workman and Kingston 1998; Peterson and Workman 2000; Aalfs and Kingston 2000; Vignali et al. 2000; Wu and Grunstein 2000; Becker and Horz 2002; Narlikar et al. 2002). In the first step, transcription activators bind to their target DNA elements and recruit chromatin remodeling or modifying activities, to alter chromatin structure. We know that activators can bind to their targets, even when those DNA elements are adjacent to nucleosomes, or actually within nucleosomes (Almer et al. 1986; Archer et al. 1992; Zhu and Thiele 1996; Wolffe 1998; Nishikawa et al. 2003). Until recently, however, it was unclear what structures of chromatin allow activator binding, or how those structures are constructed. Several recent studies have shed light on the significance of curved DNA structures for this (Blomquist et al. 1999; Ohyama 2001; Nishikawa et al. 2003). This essay describes some recent advances.

10-1.2 Three-Dimensional Architecture of Naturally Occurring Curved DNA Structures

A DNA bend can be generated either by an exterior force such as a protein binding, or by the nucleotide sequence per se. The former is called (protein-) induced DNA bend or simply DNA bending; and the latter is called curved DNA, bent DNA, or intrinsic DNA curvature. This essay concentrates on the latter. Generally, regularly distributed runs of adenines or thymines (A-tracts or T-tracts), with a periodicity of one run per helical repeat, form bent DNA structures (Trifonov and Sussman 1980; Marini et al. 1982; Wu and Crothers 1984; Hagerman 1986; Diekmann 1986; Ulanovsky and Trifonov 1987; Koo and Crothers 1988; Barbic et al. 2003). The periodicity of the tracts relative to the helical repeat length of DNA (about 10.5 bp) determines the DNA's three-dimensional (3D) architecture (Calladine et al. 1988). When the A- or T-tracts occur with a periodicity almost equal to 10.5 bp, the helical trajectory of DNA becomes planar (Fig. 1), that is to say, a flat curved structure (plane curve or 2D curve) is formed. However, if the tract periodicity is other than this, then a 3D curved structure is formed, like a corkscrew, with either a right- or a left-handed writhe. When the periodicity is less than 10.5 bp (e.g., 9 or 10 bp), DNA adopts a left-handed curved structure, and when the periodicity is larger than 10.5 bp (e.g., 11 or 12 bp), it adopts a right-handed curved structure (Calladine et al. 1988; Hirota and Ohyama 1995; Brukner et al. 1997; Nishikawa et al. 2003). These are sometimes called "space curves." Periodicities of 5–7 bp result in a nearly straight (actually a zigzag) trajectory of the helical axis. Naturally occurring DNA adopts various 3D shapes by combining these basic structures.

10-1.3 Curved DNA and Packaging of Genomes into Chromatin

The structure and mechanical properties of DNA influence the formation, stability and positioning of nucleosomes (Zhurkin et al. 1979; Trifonov and Sussman 1980; Satchwell et al. 1986; Shrader and Crothers 1989; Ioshikhes et al. 1992; Sivolob and Khrapunov 1995; Ioshikhes et al. 1996; Olson et al. 1998; Fitzgerald and Anderson 1998). DNA has to be bent around the histone core, and it therefore seems thermodynamically favorable to form a nucleosome on a DNA sequence that is already appropriately curved (Drew and Travers 1985; Zhurkin 1985; Anselmi et al. 1999). Indeed, nucleosomes often preferentially associate with curved DNA

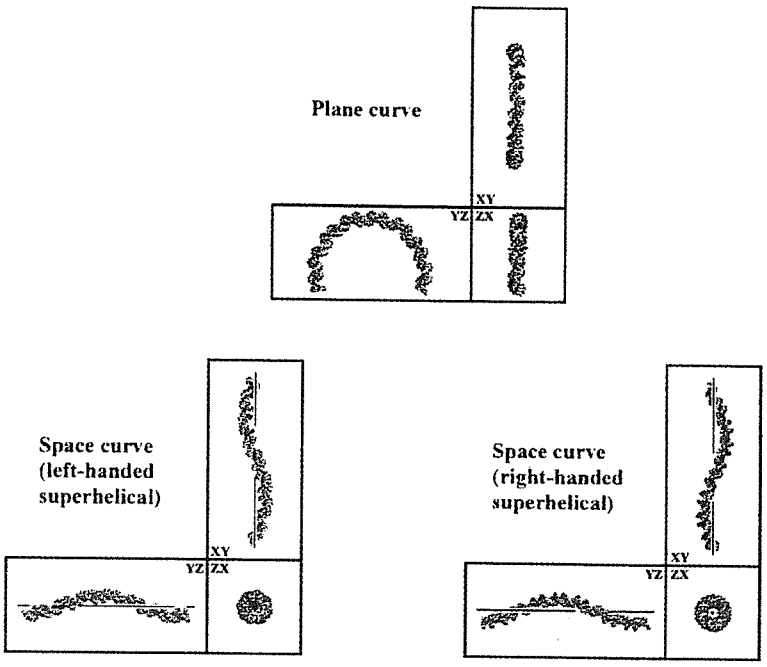


Fig. 1. Intrinsic DNA curvatures with superhelical and circular conformations. The figure shows the structures formed by the nucleotide sequences $(A_5CATG)_8$ (*left*), $(A_5CAGTCA_5CAGTCG)_4$ (*top*), and $(A_5CAGTCAG)_7$ (*right*). They were drawn by a combination of DIAMOD (Dlakic and Harrington 1998) and RASMOL (Sayle and Milner-White 1995).

fragments (Pennings et al. 1989; Costanzo et al. 1990; De Santis et al. 1996; Widlund et al. 1997). By screening a library of DNA fragments from nucleosome cores from the mouse, Widlund et al. (1997) showed that among the fragments that form the most stable nucleosomes, a curved DNA structure is the most common feature.

This suggests that curved DNA structures may frequently occur on eukaryotic genomes in order to package them. Indeed, the repeating units of satellite DNA sequences frequently contain one or more curved DNA structures (Martinez-Balbas et al. 1990; Pasero et al. 1993; Fitzgerald et al. 1994). The satellites are universally associated with regions of constitutive heterochromatin, and comprise anywhere from a few percent to >50% of mammalian genomes (John and Miklos 1979; Singer 1982). Also, curved DNA sites occur repeatedly in human ε-, Gγ-Aγ-ψβ-, δ-, and β-globin, c-*myc*, and immunoglobulin heavy chain μ loci, and in mouse β^{major}-globin

locus (Wada-Kiyama and Kiyama 1994, 1995, 1996; Ohki et al. 1998). Most of these findings have been based on retardation of DNA fragments during electrophoresis in non-denaturing polyacrylamide gels. Fragments that migrate at normal speed are not usually thought to contain a curved DNA structure, but an "unseen DNA curvature" was found in one such DNA fragment, in which another structural property, that caused rapid migration, had suppressed the effect of the curved DNA (Ohyama et al. 1998). Thus, there could be many more curved DNA structures in the genome than we have previously thought.

10-1.4 Curved DNA Is Often Located in the Control Regions of Transcription

In both prokaryotic and eukaryotic genomes, curved DNA occurs frequently in regions that control transcription (Ohyama and Hashimoto 1989; Ohyama et al. 1992; Ohyama 1996; Ohyama 2001; Asayama et al. 2002; and references therein). In the class II genes of eukaryotes, both TATA-box-containing and TATA-box-less promoters often contain this structure. Moreover, curved DNA may be common to all class I gene promoters (Marilley and Pasero 1996). Despite the many reports of curved DNA in promoters, the role of curved DNA is not fully understood. As described above, nucleosomes often preferentially associate with curved DNA fragments. However, it is generally thought that if nucleosomes assemble over a promoter region, they would inhibit access and/or assembly of transcription factors. How do eukaryotes circumvent this problem? They appear to use the DNA curvature cleverly.

10-1.5 Chromatin Structure That is Permissive to the Activator Binding

Structures That Encourage Nucleosome Formation

To make target DNA elements accessible, there are logically two options: expose the region toward the environment on the surface of a nucleosome; or make the region free of nucleosomes (Fig. 2). As argued above, it seems thermodynamically favorable to incorporate into a nucleosome a DNA sequence that is already appropriately curved. Thus the

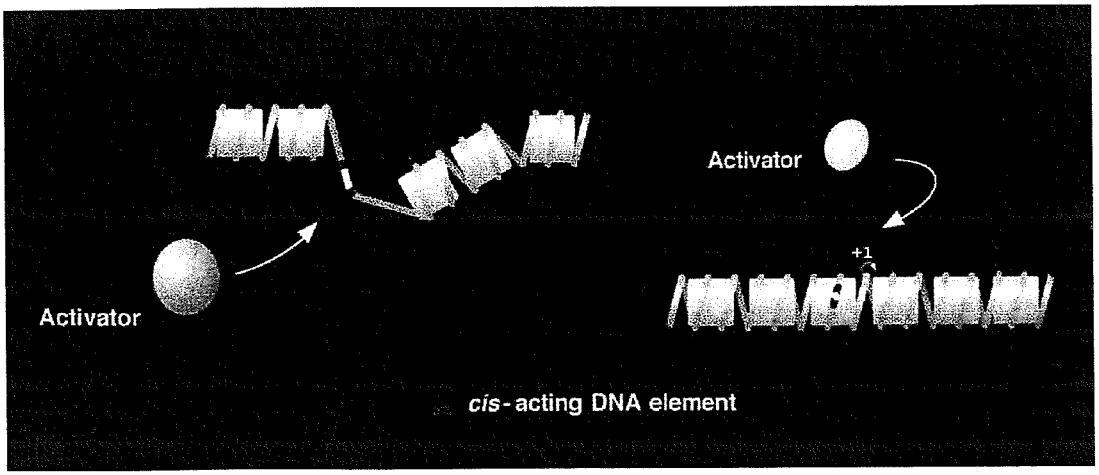


Fig. 2. A chromatin structure that permits activator binding can be formed in two ways: by inhibiting nucleosome assembly on the target element; or by putting the target element on a nucleosome, and displaying it towards the environment.

first option could occur with DNA that has an intrinsic conformation similar to the writhing of DNA in nucleosomes, namely, when it mimics left-handed (negative) supercoils. The second option could occur when the DNA's intrinsic structure is different from this.

Unusual DNA Structures That Inhibit Nucleosome Formation

In *Saccharomyces cerevisiae*, the adenylate kinase gene promoter has a curved DNA structure which is dissimilar to the negative supercoil. This promoter was shown to be free of nucleosomes (Angermayr et al. 2002). Also, in the yeast *GAL80* promoter, intrinsic DNA curvature close to the upstream activator sequence (UAS_{GAL80}) may play the same role. Nucleosomes are not formed on this curved DNA, or on UAS_{GAL80} (Bash et al. 2001). Other unusual DNA structures such as poly (dA•dT) sequences, triple-stranded DNAs, and cruciform DNAs, may also keep DNA free of nucleosomes. Using in vivo UV photo-footprinting and DNA repair by photolyase, Suter et al. (2000) demonstrated that poly (dA•dT) sequences in yeast promoters such as *HIS3*, *URA3*, and *ILV1* were not folded into nucleosomes. Formation of triple-stranded DNA and nucleosome assembly are competing processes. For example, Espinas et al. (1996) studied in vitro assembly of mononucleosomes onto 180 bp DNA fragments containing $(GA•TC)_{22}$, or onto 190 bp fragments with $(GA•TC)_{10}$. Nucleosome assembly was strongly inhibited when triple-stranded DNA was formed at

the $(GA•TC)_n$ site, while the formation of triple-stranded DNA was inhibited when the $(GA•TC)_n$ site was incorporated into a nucleosome. The $(GA•TC)_n$ sequences themselves had no influence on nucleosome formation. Similarly, it is known that cruciform structures are unable to associate with core histones, and are located mainly on inter-nucleosomal DNA (Battistoni et al. 1988).

Left-Handedly Curved DNA Can Expose *cis*-DNA Elements in Nucleosomes

When curved DNA structures mimic part of the negative supercoils seen in nucleosomes, they seem to be very effective in recruiting histone cores. Even though the target DNA elements of activators are incorporated into nucleosomes, if they can display their recognition sites on the surface of the nucleosomes, recognition would be facilitated (Ohyama 2001). Curved DNA is implicated in the formation of such structures, and the nucleosome structure formed on the long terminal repeat of the mouse mammary tumor virus (MMTV-LTR) is a good example. Four recognition elements (GRE1–4) of the glucocorticoid receptor, a zinc finger protein, are located within a positioned nucleosome. Two GREs expose their major grooves towards the environment on the surface of this nucleosome, and are recognized by the receptor (Pina et al.1990a; Fletcher et al. 2000; and references therein). An early study revealed the presence of a curved DNA structure between GRE2 and GRE3, and proposed that this may determine the rotational setting of the nucleosomal DNA (Pina et al. 1990b). This curved DNA has a left-handed curved trajectory (Ohyama 2001).

Recently, by creating 35 reporter constructs with the herpes simplex virus thymidine kinase (HSV *tk*) promoter, we studied the relationship between the geometry of DNA upstream of the promoter, nucleosome positioning, and promoter activity (Nishikawa et al. 2003). Left-handed curved, right-handed curved, planar, zigzag, and straight DNA segments were studied. A left-handed curved DNA of about 40 bp activated transcription by about 10-fold when it was linked to the promoter at a specific rotational phase and distance. The other DNA conformations did not have this effect. Transcription was activated by the following mechanism: the histone core was attracted by the left-handed curved DNA; the TATA box was thereby left in the linker DNA with its minor groove facing outwards; this structure enhanced accessibility of the TATA box; and presumably the enhanced interaction between the TATA box and the TFIID activated transcription (Fig. 3).

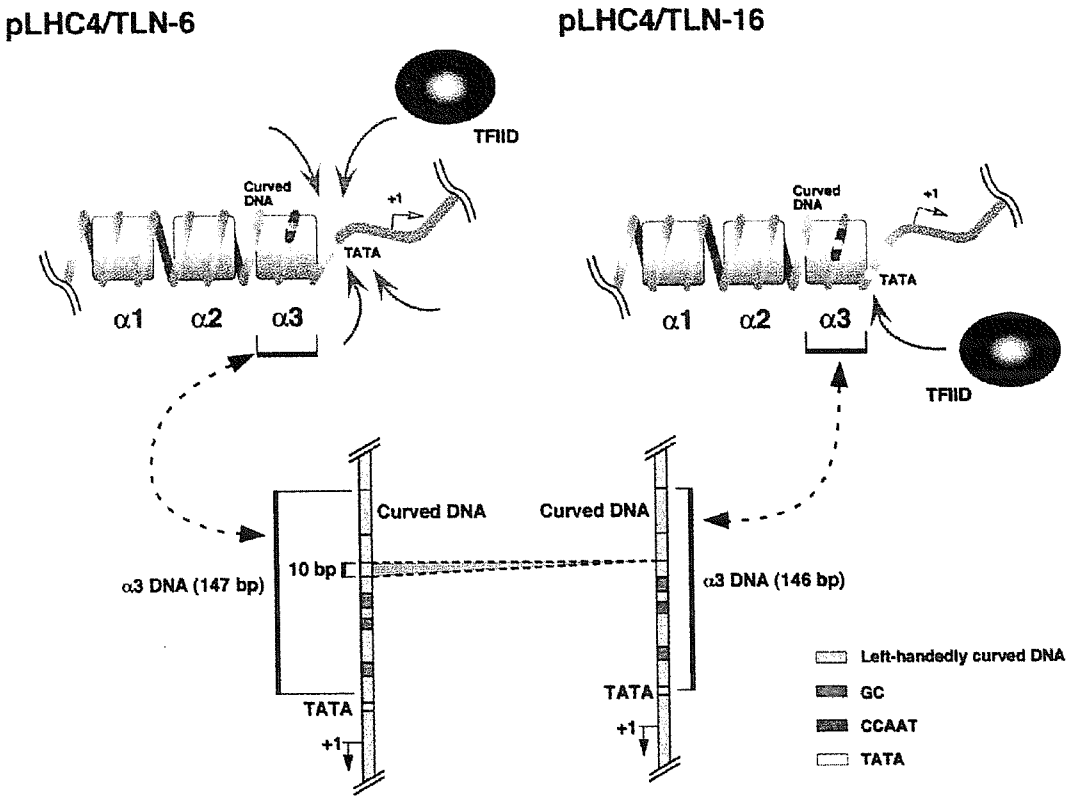


Fig. 3. A left-handed curved DNA structure that is appropriately introduced upstream of a promoter can activate transcription, by modulating local chromatin structure. The figure shows an example using the HSV *tk* promoter as a test system. Curved DNA can attract histone octamers, and depending on the distance between it and the TATA box, can position the box either in the linker DNA region (*pLHC4/TLN-6*) or at the edge of the nucleosome (*pLHC4/TLN-16*) with its minor groove facing outwards. Both make the box more accessible to transcription factors, and activate transcription, although the first structure is more active than the second. The symbols *α1*, *α2*, and *α3* are nucleosomes formed on the promoter region. Reprinted, with permission, from Nishikawa et al. 2003.

Another Role of DNA Curvature

Even when DNA geometry is dissimilar to the negative supercoils on histone octamers, neighboring DNA sequences may allow a nucleosome to form. In this case, however, the nucleosome structure may be altered. This mechanism also seems to construct nucleosomes that permit activators to

bind to DNA. Using the repeated $(A/T)_3NN(G/C)_3NN$ motifs (TG-motifs) that are anisotropically flexible and have a high nucleosome-forming ability (Shrader and Crothers 1989), Blomquist et al. (1999) constructed DNA fragments composed of the TG-motifs and the binding site for the nuclear factor 1 (NF-1) with an A_5-tract on both sides. They then reconstituted nucleosomes on these DNA fragments, and studied NF-1 binding affinity. Binding affinity was more elevated when the flanking A-tracts were positioned out-of-phase with the TG-motifs than when the tracts were in-phase. The formation of altered nucleosome structures, and the enhanced accessibility of a *cis*-DNA element on a nucleosome, have also been reported for a poly (dA•dT) sequence (Zhu and Thiele 1996).

10-1.6 Concluding Remarks

In eukaryotic genomes, many DNA structures are present. Among them, curved DNA structures play an important role in nucleosome formation, stability, and positioning. The reason why curved DNA is frequently located in transcriptional control regions is presumably that it constructs chromatin structures that leave target elements exposed, permitting activators to recognize them and bind. Right-handed curved DNA seems to inhibit nucleosome formation, which is similar to its putative role in prokaryotes, inhibiting assembly of nucleoid structuring proteins (Ohyama 2001). In prokaryotes, the DNA located in the promoter regions frequently has a right-handed curved path of helical axis (Travers 1990; Ohyama et al. 1992; Asayama et al. 1999), which helps open promoter complexes to form (Hirota and Ohyama 1995), and could inhibit association of nucleoid proteins (the effects of left-handed curved DNA structures have not yet been clarified). In eukaryotic chromatin, left-handed curved DNAs ensure the accessibility of target sites of activators, by regulating the rotational setting of neighboring DNA on the surface of histone octamers, or in the close vicinity of the nucleosomes. Although this essay has highlighted the role of DNA curvature in the organization of chromatin infrastructure, DNA curvature may also play other roles, after local chromatin structure has been remodeled.

Acknowledgments

The author would like to acknowledge the contributions of Mr. J. Nishikawa, Mr. Y. Fukue, and Ms. J. Ohyama. This work was supported in part

by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

References

Aalfs JD, Kingston RE (2000) What does "chromatin remodeling" mean? Trends Biochem Sci 25:548–555

Almer A, Rudolph H, Hinnen A, Horz W (1986) Removal of positioned nucleosomes from the yeast *PHO5* promoter upon PHO5 induction releases additional upstream activating DNA elements. EMBO J 5:2689–2696

Angermayr M, Oechsner U, Gregor K, Schroth GP, Bandlow W (2002) Transcription initiation in vivo without classical transactivators: DNA kinks flanking the core promoter of the housekeeping yeast adenylate kinase gene, *AKY2*, position nucleosomes and constitutively activate transcription. Nucleic Acids Res 30:4199–4207

Anselmi C, Bocchinfuso G, De Santis P, Savino M, Scipioni A (1999) Dual role of DNA intrinsic curvature and flexibility in determining nucleosome stability. J Mol Biol 286:1293–1301

Archer TK, Lefebvre P, Wolford RG, Hager GL (1992) Transcription factor loading on the MMTV promoter: a bimodal mechanism for promoter activation. Science 255:1573–1576

Asayama M, Hayasaka Y, Kabasawa M, Shirai M, Ohyama T (1999) An intrinsic DNA curvature found in the cyanobacterium *Microcystis aeruginosa* K-81 affects the promoter activity of *rpoD1* encoding a principal sigma factor. J Biochem (Tokyo) 125:460–468

Asayama M, Kato H, Shibato J, Shirai M, Ohyama T (2002) The curved DNA structure in the 5′-upstream region of the light-responsive genes: its universality, binding factor and function for cyanobacterial *psbA* transcription. Nucleic Acids Res 30:4658–4666

Barbic A, Zimmer DP, Crothers DM (2003) Structural origins of adenine-tract bending. Proc Natl Acad Sci USA 100:2369–2373

Bash RC, Vargason JM, Cornejo S, Ho PS, Lohr D (2001) Intrinsically bent DNA in the promoter regions of the yeast *GAL1-10* and *GAL80* genes. J Biol Chem 276:861–866

Battistoni A, Leoni L, Sampaolese B, Savino M (1988) Kinetic persistence of cruciform structures in reconstituted minichromosomes. Biochim Biophys Acta 950:161–171

Becker PB, Horz W (2002) ATP-dependent nucleosome remodeling. Annu Rev Biochem 271:247–273

Blomquist P, Belikov S, Wrange O (1999) Increased nuclear factor 1 binding to its nucleosomal site mediated by sequence-dependent DNA structure. Nucleic Acids Res 27:517–525

Brukner I, Belmaaza A, Chartrand P (1997) Differential behavior of curved DNA upon untwisting. Proc Natl Acad Sci USA 94:403–406

Calladine CR, Drew HR, McCall MJ (1988) The intrinsic curvature of DNA in solution. J Mol Biol 201:127–137

Costanzo G, di Mauro E, Salina G, Negri R (1990) Attraction, phasing and neighbour effects of histone octamers on curved DNA. J Mol Biol 216:363–374

De Santis P, Kropp B, Leoni L, Sampaolese B, Savino M (1996) Influence of DNA superstructural features and histones aminoterminal domains on mononucleosome and dinucleosome positioning. Biophys Chem 62:47–61

Diekmann S (1986) Sequence specificity of curved DNA. FEBS Lett 195:53–56

Dlakic M, Harrington RE (1998) DIAMOD: display and modeling of DNA bending. Bioinformatics 14:326–331

Drew HR, Travers AA (1985) DNA bending and its relation to nucleosome positioning. J Mol Biol 186:773–790

Espinas ML, Jimenez-Garcia E, Martinez-Balbas A, Azorin F (1996) Formation of triple-stranded DNA at $d(GA{\bullet}TC)_n$ sequences prevents nucleosome assembly and is hindered by nucleosomes. J Biol Chem 271:31807–31812

Fitzgerald DJ, Anderson JN (1998) Unique translational positioning of nucleosomes on synthetic DNAs. Nucleic Acids Res 26:2526–2535

Fitzgerald DJ, Dryden GL, Bronson EC, Williams JS, Anderson JN (1994) Conserved patterns of bending in satellite and nucleosome positioning DNA. J Biol Chem 269:21303–21314

Fletcher TM, Ryu B-W, Baumann CT, Warren BS, Fragoso G, John S, Hager GL (2000) Structure and dynamic properties of a glucocorticoid receptor-induced chromatin transition. Mol Cell Biol 20:6466–6475

Hagerman PJ (1986) Sequence-directed curvature of DNA. Nature 321:449–450

Hirota Y, Ohyama T (1995) Adjacent upstream superhelical writhe influences an *Escherichia coli* promoter as measured by in vivo strength and in vitro open complex formation. J Mol Biol 254:566–578

Ioshikhes I, Bolshoy A, Trifonov EN (1992) Preferred positions of AA and TT dinucleotides in aligned nucleosomal DNA sequences. J Biomol Struct Dyn 9:1111–1117

Ioshikhes I, Bolshoy A, Derenshteyn K, Borodovsky M, Trifonov EN (1996) Nucleosome DNA sequence pattern revealed by multiple alignment of experimentally mapped sequences. J Mol Biol 262:129–139

John B, Miklos GLG (1979) Functional aspects of satellite DNA and heterochromatin. Int Rev Cytol 58:1–114.

Koo HS, Crothers DM (1988) Calibration of DNA curvature and a unified description of sequence-directed bending. Proc Natl Acad Sci USA 85:1763–1767

Marilley M, Pasero P (1996) Common DNA structural features exhibited by eukaryotic ribosomal gene promoters. Nucleic Acids Res; 24:2204–2211

Marini JC, Levene SD, Crothers DM, Englund PT (1982) Bent helical structure in kinetoplast DNA. Proc Natl Acad Sci USA 79:7664–7668

Martinez-Balbas A, Rodriguez-Campos A, Garcia-Ramirez M, Sainz J, Carrera P, Aymami J, Azorin F (1990) Satelite DNAs contain sequences that induced curvature. Biochemistry 29:2342–234

Narlikar GJ, Fan HY, Kingston RE (2002) Cooperation between complexes that regulate chromatin structure and transcription. Cell 108:475–487

Nishikawa J, Amano M, Fukue Y, Tanaka S, Kishi H, Hirota Y, Yoda K, Ohyama T (2003) Left-handedly curved DNA regulates accessibility to *cis*-DNA elements in chromatin. Nucleic Acids Res 31:6651–6662

Ohki R, Hirota M, Oishi M, Kiyama R (1998) Conservation and continuity of periodic bent DNA in genomic rearrangements between the c-*myc* and immunoglobulin heavy chain μ loci. Nucleic Acids Res 26:3026–3033

Ohyama T (1996) Bent DNA in the human adenovirus type 2 E1A enhancer is an architectural element for transcription stimulation. J Biol Chem 271:27823–27828

Ohyama T (2001) Intrinsic DNA bends: an organizer of local chromatin structure for transcription. BioEssays 23:708–715

Ohyama T, Hashimoto S (1989) Upstream half of adenovirus type 2 enhancer adopts a curved DNA conformation. Nucleic Acids Res 17:3845–3853

Ohyama T, Nagumo M, Hirota Y, Sakuma S (1992) Alteration of the curved helical structure located in the upstream region of the β-lactamase promoter of plasmid pUC19 and its effect on transcription. Nucleic Acids Res 20:1617–1622

Ohyama T, Tsujibayashi H, Tagashira H, Inano K, Ueda T, Hirota Y, Hashimoto K (1998) Suppression of electrophoretic anomaly of bent DNA segments by the structural property that causes rapid migration. Nucleic Acids Res 26:4811–4817

Olson WK, Gorin AA, Lu XJ, Hock LM, Zhurkin VB (1998) DNA sequence-dependent deformability deduced from protein-DNA crystal complexes. Proc Natl Acad Sci USA 95:11163–11168

Pasero P, Sjakste N, Blettry C, Got C, Marilley M (1993) Long-range organization and sequence-directed curvature of *Xenopus laevis* satellite 1 DNA. Nucleic Acids Res 21:4703–4710

Pennings S, Muyldermans S, Meersseman G, Wyns L (1989) Formation, stability and core histone positioning of nucleosomes reassembled on bent and other nucleosome-derived DNA. J Mol Biol 207:183–192

Peterson CL, Workman JL (2000) Promoter targeting and chromatin remodeling by the SWI/SNF complex. Curr Opin Genet Dev 10:187–192

Pina B, Bruggemeier U, Beato M (1990a) Nucleosome positioning modulates accessibility of regulatory proteins to the mouse mammary tumor virus promoter. Cell 60:719–731

Pina B, Barettino D, Truss M, Beato M (1990b) Structural features of a regulatory nucleosome. J Mol Biol 216:975–990

Satchwell SC, Drew HR, Travers AA (1986) Sequence periodicities in chicken nucleosome core DNA. J Mol Biol 191:659–675

Sayle RA, Milner-White EJ (1995) RASMOL: biomolecular graphics for all. Trends Biochem Sci 20:374–376

Shrader TE, Crothers DM (1989) Artificial nucleosome positioning sequences. Proc Natl Acad Sci USA 86:7418–7422

Singer MF (1982) Highly repeated sequences in mammalian genomes. Int Rev Cytol 76:67–112

Sivolob AV, Khrapunov SN (1995) Translational positioning of nucleosomes on DNA: the role of sequence-dependent isotropic DNA bending stiffness. J Mol Biol 247:918–931

Suter B, Schnappauf G, Thoma F (2000) Poly(dA•dT) sequences exist as rigid DNA structures in nucleosome-free yeast promoters in vivo. Nucleic Acids Res 28:4083–4089

Travers AA (1990) Why bend DNA? Cell 60:177–180

Trifonov EN, Sussman JL (1980) The pitch of chromatin DNA is reflected in its nucleotide sequence. Proc Natl Acad Sci USA 77:3816–3820

Ulanovsky LE, Trifonov EN (1987) Estimation of wedge components in curved DNA. Nature 326:720–722

Vignali M, Hassan AH, Neely KE, Workman JL (2000) ATP-dependent chromatin-remodeling complexes. Mol Cell Biol 20:1899–1910

Wada-Kiyama Y, Kiyama R (1994) Periodicity of DNA bend sites in human ε-globin gene region. Possibility of sequence-directed nucleosome phasing. J Biol Chem 269:22238–22244

Wada-Kiyama Y, Kiyama R (1995) Conservation and periodicity of DNA bend sites in the human β-globin gene locus. J Biol Chem 270:12439–12445

Wada-Kiyama Y, Kiyama R (1996) An intrachromosomal repeating unit based on DNA bending. Mol Cell Biol 16:5664–5673

Widlund HR, Cao H, Simonsson S, Magnusson E, Simonsson T, Nielsen PE, Kahn JD, Crothers DM, Kubista M (1997) Identification and characterization of genomic nucleosome-positioning sequences. J Mol Biol 267:807–817

Wolffe AP (1998) Chromatin: structure and function 3rd edn. Academic Press, London

Workman JL, Kingston RE (1998) Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu Rev Biochem 67:545–579

Wu HM, Crothers DM (1984) The locus of sequence-directed and protein-induced DNA bending. Nature 308:509–513

Wu J, Grunstein M (2000) 25 years after the nucleosome model: chromatin modifications. Trends Biochem Sci 25:619–623

Zhu Z, Thiele DJ (1996) A specialized nucleosome modulates transcription factor access to a *C. glabrata* metal responsive promoter. Cell 87:459–470

Zhurkin VB (1985) Sequence-dependent bending of DNA and phasing of nucleosomes. J Biomol Struct Dyn 2:785–804

Zhurkin VB, Lysov YP, Ivanov VI (1979) Anisotropic flexibility of DNA and the nucleosomal structure. Nucleic Acids Res 6:1081–1096

PNE
PROTEIN,
NUCLEIC ACID
AND ENZYME
蛋白質核酸酵素

別刷

「蛋白質 核酸 酵素」編集部
共立出版株式会社
〒112-8700　東京都文京区小日向 4-6-19
Tel.03-3947-2515　FAX 03-3944-8182
E-mail：pne@kyoritsu-pub.co.jp
http://www.kyoritsu-pub.co.jp/

DNAの高次構造と物理的特性に印された遺伝情報

Genetic information carried in DNA conformation and physical properties

大山 隆

DNAの塩基配列と構造・特性とはコインの表と裏のように切っても切れない関係にある．したがって，同じ塩基配列をもつDNAが同じ構造や特性をもつことは当然としても，意外なことに，異なった塩基配列をもつDNAが同じような高次構造をとったり似たような物理的特性をもったりする場合がある．近年，ある特定のDNA高次構造がクロマチン内でヌクレオソームの配置を決める情報を担っていることが明らかになってきた．くわえて，最近，プロモーター配列の物理的特性に遺伝情報らしきものが発見された．本稿では，DNAの高次構造と物理的特性が担う遺伝情報について概説する．

Key words ●遺伝情報 ●遺伝子発現 ●DNA高次構造 ●クロマチン ●ヌクレオソームポジショニング

はじめに

遺伝情報とは，生物が自己と同じものを複製するために次世代に伝える情報である．そしていまや，“遺伝情報は核酸塩基の配列として符号化されている”という概念が定着している．確かに遺伝暗号は塩基配列に“記”され，遺伝子発現にかかわる遺伝情報の多くも特定の塩基配列が担っている．それでは，遺伝情報は塩基配列にしか記されていないのだろうか．答えは“No”である．近年，DNAの高次構造や特性にも情報が“印”されていることが明らかになってきた．エピジェネティクスの時代をむかえ，今日，このような高次の遺伝情報*1が注目を集めている．

DNA高次構造の生物学的意義については，1980年代から1990年代中ごろにかけて，さかんに研究された．この時代に，ベントDNA（bent DNA）構造に原核生物の遺伝子転写を調節する機能があることが解明された[1-3]．そののち，研究はクロマチンの構築や機能とのかかわりを解明する方向にむかい，この10年のあいだに，ヌクレオソームの形成や配置（ポジショニング）におけるDNA高次構造の役割がしだいに明らかになってきた[4-6]．一方，DNAの物理的特性は，分子生物学や分子遺伝学の分野ではこれまでほとんど研究の対象とされず，関心も低かった．しかし，今世紀に入って，真核生物のプロモーターが共通の機械的特性をもつことと，その特性が転写に利用されていることが明らかになったことで[7,8]，にわかに関心が高まってきた．

クロマチンには，ヌクレオソームが正確に配置されている領域とランダムに配置されている領域，さらに，ヌクレオソームが配置されていない領域とがある．DNAの高次構造や物理的特性には，主として，ヌクレオソームの配置に関する情報，すなわち，クロマチンの基盤構造に関する情報が印されていると推察される．本稿では，DNAの自己集合現象の発見というトピックスも含め，真核生物のゲノムDNAに印された高次の遺伝情報について概説する．

Takashi Ohyama
早稲田大学教育・総合科学学術院 理学科生物学専修
E-mail：ohyama@waseda.jp

I DNAの形にかくされた遺伝情報

1. ゲノムDNAを彩るさまざまな構造

WatsonとCrickはB型DNAの構造を解いた．B型という限定的な用語から想像できるように，DNAには非B型構造も存在する．B型DNAに比べ太短い構造をとるA型DNAと，左巻きの二重らせん構造をとるZ型DNAは有名である．このA型，Z型のほかにも，C型，D型，T型とよばれるDNA構造が知られている．さらに，コンフォメー

(a)

(b)

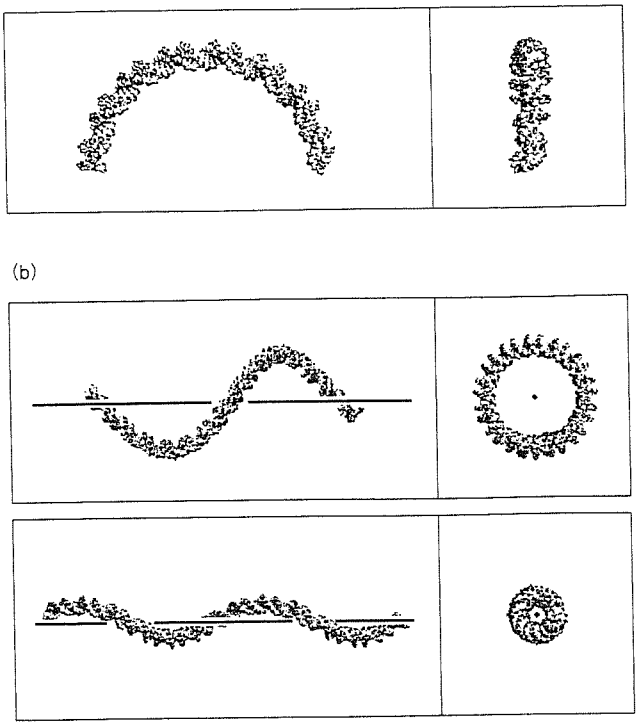


図1　ベントDNAの例

(a) 2次元的にきれいに曲がった，平面的ベントDNA．
(b) 3次元的にきれいにねじれた，スーパーコイル様ベントDNA．負のスーパーコイルに似た構造をとったもの(上)と，正のスーパーコイルに似た構造をとったもの(下)．

ションが大きく異なる構造も存在する．たとえば，ベントDNA，三重鎖DNA，四重鎖DNA，十字架構造，などである．ベントDNAは曲がったらせん軸をもつDNA，三重鎖DNAと四重鎖DNAはそれぞれ3本または4本のヌクレオチド鎖からなるDNA，十字架構造は直交するらせん軸をもつDNAである[9]．このほか，ポリ(dA·dT)も硬くて副溝が狭いという特徴をもっている[10]．ベントDNA，Z型DNA，三重鎖DNA，ポリ(dA·dT)，十字架構造については，数多くの研究が行なわれ，なかにはその存在理由が明らかになってきたものもある．遺伝情報の観点から，各構造の意義を以下に解説する．

2. ベントDNAとクロマチン

A. 構造概説

ベントDNAは一般に，塩基配列中にA・Tのトラクト*2がB型DNAのらせん周期である約10.5 bpに近い周期で規則正しく分布しているような場合に形成される．そして，周期がほぼ10.5 bpの場合には平面的に曲がった構造(plane curve)が形成されるが，10.5 bpよりも少ない塩基対数で反復する場合には左巻きスーパーコイルのようにねじれた構造になり，10.5 bpよりも多い塩基対数での反復では右巻きスーパーコイル状の構造となる(図1)．3次元的に曲がった構造は，しばしばスペースカーブ(space curve)とよばれる．配列の出現周期が10.5 bpから離れるにしたがって，ベントDNAの形状は直線に近づいていく[2]．ベントDNAは非変性ポリアクリルアミドゲルを用いた電気泳動で，分子サイズから予想される速度より低速で泳動されるという大きな特徴をもつ．

B. ゲノム上のヌクレオソームの配置を決める情報

真核生物の場合，ゲノムDNAはクロマチンを形成して細胞核に収納されている．クロマチンの基本単位はヌクレオソームである．ヌクレオソームを形成する際には，DNAはコアヒストン(ヒストン8量体)のまわりに巻きつかなければならない．したがって，すでに曲がっているDNAのうえでヌクレオソームを形成するほうが熱力学的に有利であると考えられる．実際に，コアヒストンがベントDNAに高い親和性を示すという報告が数多くある．たとえば，ヌクレオソームに含まれるDNAのライブラリーを作製し安定なヌクレオソームをつくるDNAをスクリーニングしたところ，どれもベント構造をもっていたという報告がある[11]．ベントDNAは，クロマチンの基盤構造を構築するための基本的なランドマークになっている可能性がある．

ベントDNAがヌクレオソームを介したゲノムの折りたたみに寄与しているのであれば，ゲノム内に高頻度に存在するはずである．はたして，そのような領域がいくつか知られている．ひとつは，ベントDNAを単位配列内にもつ，反復配列の領域である(表1)．サテライトDNAはおもに恒常的ヘテロクロマチン領域に局在する反復配列で，哺乳動物の場合，ゲノムの数%から数十%をしめる．サテライトDNA領域ではベントDNAを介してヌクレオソームが規則的に配置され，それを基盤としてヘテロクロマチンが構築されているのかもしれない．興味深いことに，“反復ベント構造”はサテライトDNA以外にも存在する．木山らは，ヒトのβグロビン遺伝子座，c-*myc*遺伝子座，免疫グロブリンH鎖遺

＊1　**高次の遺伝情報**：塩基配列に記された遺伝情報と区別するため，本稿では，DNAの高次構造や特性に印された遺伝情報をこうよぶことにする．
＊2　**トラクト**：同じ塩基対が連続した小領域のこと．ベントDNAでは，(A·T)が3対から6対連続する場合が多い．

伝子領域，マウスβ^{major}グロビン遺伝子座などに，反復ベント構造を見いだしている[12]．

筆者らは，10年ほどまえに，プリン塩基が連続した配列を多数もつDNAは，その分子量から予測されるより速く泳動されることを発見した．分子内にこのような特性をもつ領域と，ベント構造（泳動速度を低下させる）をとった領域とが共存すると，両者の効果が相殺されて電気泳動によりベントDNAの存在を検出できないことがある．ウシサテライトⅠDNAの一部の領域はその一例で，ベントDNAが存在するにもかかわらず，ほぼ正常な速さで泳動される[13]．従来，正常に泳動されるDNAにはベント構造は存在しないと考えられていたことから，筆者は，ベントDNAが存在するにもかかわらず見逃されてしまったケースが過去に多くあったのではないかと想像している．また，サテライトDNAには必ずベントDNAが存在するのではないか，さらに，ベントDNAはゲノム全体にわたって高頻度に存在するのではないか，と推察している．なお，最近，ウシサテライトⅠDNAの泳動速度を高める領域は，部分的にA型DNA様のコンフォメーションをとっていることが明らかになった[14]．

表1　単位配列内にベントDNAをもつ反復配列の例[4]

生物種	反復配列の種類
サル	サテライト
ウシ（*Bos taurus*）	サテライト
ラット（*Rattus norvegicus*）	サテライト
マウス	サテライト
ニワトリ（*Gallus gallus*）	サテライト
ハト（*Columba risoria*）	サテライト
ハト（*Columba livia*）	サテライト
コモドオオトカゲ（*Varanus komodoensis*）	サテライト
オオトカゲ（*Varanus dumereliaddi*）	サテライト
ボア（*Boa constrictor*）*	サテライト
カエル（*Xenopus laevis*）	サテライト
オートミールネマトーダ（*Panagrellus redivirus*）	サテライト
シュリンプ（*Artemia franciscana*）	*Alu* Ⅰファミリー
タバコ	高度反復配列

* おもにアメリカ大陸に分布する大型のヘビ

C. 転写制御領域のクロマチン構造

ベントDNAは転写制御領域に高頻度にみつかる[15,16]（表2）．多くの場合，転写はDNA結合性の転写制御因子がクロマチン内の標的配列に結合することではじまる．したがって，クロマチンは転写制御因子が標的配列に結合できるような構造をとる必要がある．そしてそのためには，つぎのどちらかのメカニズムが必要である．①標的配列上にヌクレオソームを形成させない，または，②ヌクレオソーム内で標的配列内の制御因子結合部位をヒストン側ではなく環境側（外側）にむける．筆者は，そのどちらの機構にもベントDNAが深くかかわっているという仮説を2001年に提唱した[17]（図2）．のちの研究で，この仮説が正しいことが明らかになってきた．

まず，①の機構について解説する．すでに述べたように，コアヒストンはベントDNAに高い親和性を示す．しかし，

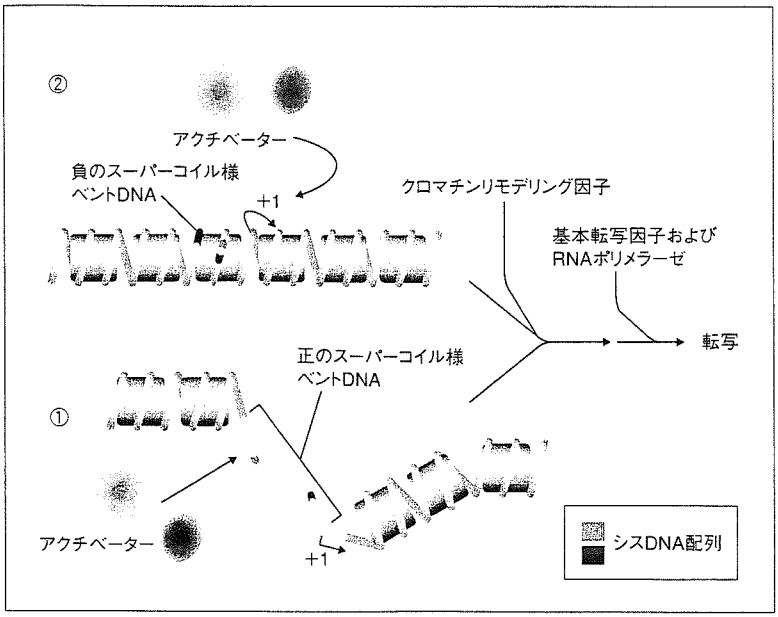


図2　転写制御領域のクロマチン構築におけるベントDNAの役割[17]

筆者の仮説を模式的に示した．

表2　ベントDNA構造を含むプロモーター，エンハンサー，および，そのほかの制御配列[16]

遺伝子	生物種	部位
β-アクチン	ヒト	プロモーター
cdc2	ヒト	プロモーター
c-myc	ヒト	プロモーター
E2F1	ヒト	E2F結合部位
エリスロポエチンレセプター	ヒト	プロモーター
β-グロビン	ヒト	プロモーター
IFN β	ヒト	エンハンサー
β^{major}-グロビン	マウス	プロモーター
A2ビテロゲニン	アフリカツメガエル	上流制御領域
DNAポリメラーゼδ	マラリア病原虫	プロモーター
EIA	ヒトアデノウイルス2型	エンハンサー
E6-E7	ヒトパピローマウイルス16型	E2結合部位
IE94	サルサイトメガロウイルス	エンハンサー上流
rbcS-3A	エンドウ	光応答配列
rbcS-3.6	エンドウ	光応答配列
MFα1	出芽酵母	上流活性化配列
STE3	出芽酵母	上流活性化配列
GAL1-10	出芽酵母	プロモーター
GAL80	出芽酵母	プロモーター
AKY2	出芽酵母	プロモーター

これまでの報告の一部（しかも，クラスⅡ遺伝子に関するものだけ）しか示していないことに注意

すべてのベントDNAに対して親和性が高いわけではない．さきには述べなかったが，ベントDNAの形がコアヒストンとの親和性を決定する重要な要因になる．ヌクレオソーム内でDNAはコアヒストンに左巻きに（つまり，負のスーパーコイル構造をとって）巻きついている．したがって，ベントDNAが負のスーパーコイルに似た構造をとっていれば，コアヒストンはこのベントDNAに高い親和性をもつことが予想される．一方，ベントDNAが負のスーパーコイルに似ていない場合は，その領域にはヌクレオソームが形成されないかされにくいと想像される．出芽酵母のアデニル酸キナーゼ遺伝子のプロモーターには，後者のタイプのベントDNAが存在する．そして，その領域にはヌクレオソームが形成されないこともわかっている[18]．*GAL80*プロモーターの場合も，上流活性化配列（upstream activation sequence；UAS）の近傍に存在するベントDNAがそこでのヌクレオソームの形成を阻害しているらしい[19]．DNaseⅠ高感受性の部位（クロマチン内でDNAが"裸"になっている部位）が，しばしば転写制御領域内にみられることはよく知られている．上述の例のように，ベントDNAによってこのような部位がつくられているケースは少なくないと推察される．

①の機構は，標的配列が存在する領域をヌクレオソームのない状態にする機構で，標的配列への転写制御因子のアクセスを容易にする機構としては考えやすい．つぎに，②の機構，すなわち，ヌクレオソーム内で特定のDNA配列を環境側にむける機構について考える．この機構では，負のスーパーコイル構造に似たベントDNAが主役になる．このような形をとったベントDNAの近傍に標的DNA配列が存在すると，その配列の上または近傍にヌクレオソームが形成されやすくなるだけでなく，ヌクレオソームのなか（または，その近傍）で標的DNA配列の回転的位相*3が規定されると考えられる．そしてそのとき，その配列が環境側にむけられれば，DNA結合性の転写制御因子との相互作用が容易になって転写開始の効率が上がると考えられる．

マウス乳癌ウイルスのLTR（long terminal repeat）で起こる転写には，この機構が使われている可能性が高い．このLTR上には数個のヌクレオソームがポジショニングし，そのひとつは，4つのグルココルチコイドレセプター結合配

＊3　**回転的位相**：DNAをらせん軸のまわりに回転させた場合の配置・向き．つまり，どの面がどの方向をむいているかということ．

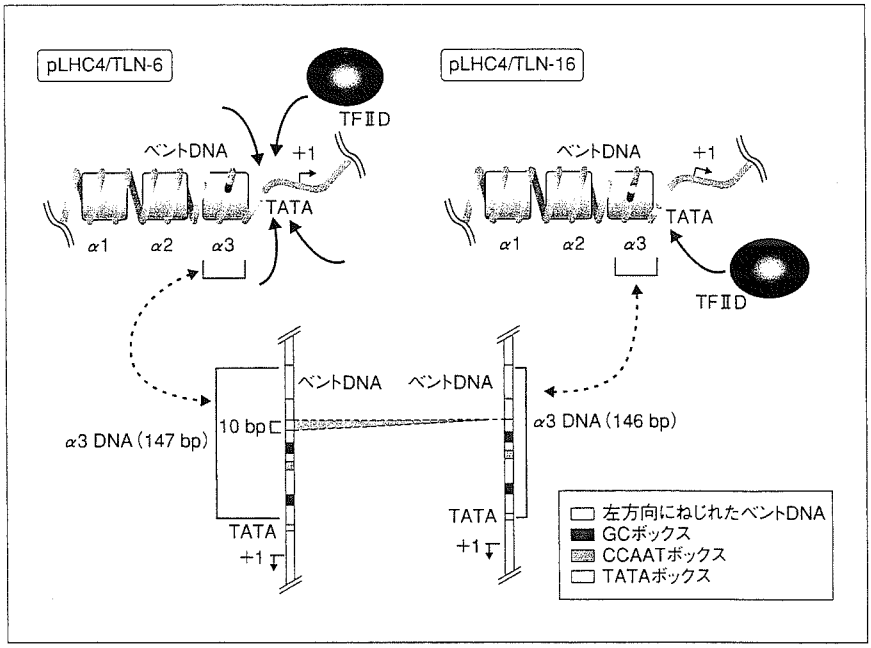


図3　LHC型ベントDNAはクロマチンを局所的に規定できる[22]

LHC型ベントDNAはコアヒストンに対する親和性が高い．したがって，プロモーターとの相対的配置が適当な場合，転写制御配列をヌクレオソーム内またはその近傍で環境側にむけることができ，転写に寄与することができる．ここで示したpLHC4/TLN-6とpLHC4/TLN-16の場合，LHC型ベントDNAによりTATAボックスの副溝が露出したことで，転写が活性化した（両者のあいだではpLHC4/TLN-6のほうが高活性）．この実験には，単純ヘルペスウイルスのチミジンキナーゼプロモーターを用いている．α1，α2，α3，はポジショニングしたヌクレオソーム．

列と左向きにねじれたベントDNA[17]の上に形成される．そして，このヌクレオソーム内では2つのグルココルチコイドレセプター結合配列の主溝が環境側にむけられており，これらにジンクフィンガーモチーフをもつグルココルチコイドレセプターが結合すると転写が開始される[20]．この系では，ベントDNAは2つのグルココルチコイドレセプター結合配列をヒストン上で環境側にむける役割を担っていると考えられる[21]．

さらに筆者らは，負のスーパーコイル様構造をとったベントDNA（LHC型ベントDNA＊4）がこのような機構に関与していることを実験的に証明した．まず，このLHC型ベントDNAをプロモーター上流の特定の位置に特定の回転的位相をとらせて配置すると，転写が活性化することを確認した[22,23]．解析の結果，この活性化は，ベントDNAがコアヒストンをひきつけることで，TATAボックスがリンカーDNA内に位置するようになることと，そこで，TATAボックスの副溝が環境側にむけられることで起こることが明らかになった（図3）．

このほか，ヌクレオソームDNAがいびつに曲がった構造をとった場合にも，ヌクレオソーム内の標的DNA配列に転写因子が結合しやすくなることが報告されている[24]．以上から明らかなように，転写制御領域に存在するベントDNAの多くは，その領域のクロマチン基盤構造を規定する役割（言い換えれば，情報）を担っていると考えられる．

3．ポリ（dA･dT）とクロマチン

A．ゲノムにおけるポリ（dA･dT）配列の出現頻度

ゲノムにおける$(dA･dT)_n$の出現頻度を，塩基配列の組合せから単純に予測される出現頻度と比較すると，ヒト，線虫，シロイヌナズナ，出芽酵母では前者のほうが有意に高いが，大腸菌，結核菌では両者に差はないことが明らかになっている[25]．また，興味深いことに，$(dA･dT)_n$はベントDNAと同様，真核生物のプロモーター領域に多くみられる．ただし，必ずしもひとつづきの配列が多いというわけではなく，ほかの短いヌクレオチド対で分断されたものも多い．たとえば，出芽酵母では，*HIS3*遺伝子，*PET56*遺伝子，*DED1*遺伝子，*CBS2*遺伝子，*ARG4*遺伝子，*URA3*遺伝子，*ADH2*遺伝子のプロモーター領域にそのような配列がある．そして，*HIS3*遺伝子，*PET56*遺伝子，*DED1*遺伝子，*ARG4*遺伝子，*URA3*遺伝子の場合には，$(dA･dT)_n$が実際に転写制御配列として機能している[26-28]．

B．ポリ（dA･dT）とヌクレオソームポジショニング

ポリ（dA･dT）は硬いという特性をもつため，その上にヌクレオソームは形成されにくいと想像されるが，この点に関してはまだはっきりしていない．*in vitro*でのヌクレオソーム再構成の結果も，細胞内ヌクレオソームの解析結果も，

＊4　LHC型：left-handedly curvedに由来する．

2つに分かれている．たとえば，$(dT\cdot dA)_{14}(dA\cdot dT)_{11}$がヌクレオソームの形成に阻害的にはたらいたという報告もあれば，一方で，$(dA\cdot dT)_n$（nは32，34，41）をもつヒトゲノムDNAの断片や，$(dT\cdot dA)_6$と$(dT\cdot dA)_5$（2トラクト）と$(dT\cdot dA)_9$を含む出芽酵母の*DED1*遺伝子プロモーターにヌクレオソームが形成されたという報告もある[4]．このような違いは，ポリ（dA·dT）の長さ，数，まわりの配列などによって生じるのかもしれない．

C. ポリ（dA·dT）がもつ情報

ポリ（dA·dT）はベントDNAと同様，転写制御領域のクロマチン構造を規定する情報を担っていると考えられる．オープンクロマチン（緩んだ構造のクロマチン）の形成に関する事例としては，ポリ（dA·dT）の導入によりヌクレオソームの形成が阻害され転写が活性化したという報告がある[29]．ポリ（dA·dT）がヌクレオソーム内またはその近傍のDNA配列の回転的位相を規定するという報告はまだないが，ベントDNAの場合と同様，そのような機構により転写に寄与している可能性はある．このほか，転写との関連では，ポリ（dA·dT）がHMG-I（Y）（非ヒストン核蛋白質の主要成分として知られるHMG蛋白質のひとつ）を介したクロマチンの構造変換に関与している可能性が指摘されている．たとえば，HMG-I（Y）はヌクレオソーム内の特定のポリ（dA·dT）に選択的に結合し，コアヒストン上のDNAの局所的な配置を変えるという報告がある[30]．いずれにせよ，ヌクレオソームに取り込まれるポリ（dA·dT）と取り込まれないポリ（dA·dT）の違いなど，まだ不明な点が多い．

4. クロマチンとZ型DNA，三重鎖DNA，十字架構造

細胞内のDNAは，原核生物・真核生物をとわず，通常，負のスーパーコイル構造をとっている．負のスーパーコイルはDNAトポロジーの一形態で，リンキング数の不足（つまり，二重らせんの巻き不足）により生じ，DNA複製，転写，組換えなど，2本鎖DNAの解離を必要とする過程に寄与できる．真核生物の場合，一般に，負のスーパーコイルはヌクレオソームに内包されてしまうため，単独（裸の状態）で長時間にわたり存在することはあまりない．しかし，ヌクレオソームからコアヒストンが外れた場合や，転写中のRNAポリメラーゼの後方などには，一過的に生じる．そして，負のスーパーコイルの密度が高いと，Z型DNA，三重鎖DNA，十字架構造などが形成されやすくなる．

生体内にZ型DNAが存在するかどうかは長いあいだ不明であった．しかし，10年ほどまえに，ADAR1というアデノシンデアミナーゼの一種がZ型DNAに特異的な結合ドメインをもっていることがわかり，生体内でもこのような構造が実際に存在すると考えられるようになった[31]．Z型DNA形成に適した塩基配列をもつ領域（プリン塩基とピリミジン塩基が交互に存在する領域）はゲノムのいたるところに存在する．さらに興味深いことに，ベントDNAやポリ（dA·dT）と同様に，Z型DNAを形成する可能性のある配列は，転写開始点近傍にしばしば存在する．

数年前，クロマチンリモデリングを介した遺伝子発現の活性化にZ型DNAがかかわっているという興味深い例が報告された[32]．ヒトの*CSF1*（*colony-stimulating factor 1*）遺伝子のプロモーターにはZ型DNAをつくりうるTGの反復配列がある．この反復配列は，BAF（BRG1-associated factor，SWI/SNF様蛋白質）が転写を活性化する際にZ型DNA構造をとってその活性化を助けていることが明らかになった[32]．Z型DNAの形成にともなって，負のスーパーコイルは部分的に解消される．*CSF1*遺伝子プロモーターにおいては，BAFにより誘起されるZ型DNAの形成が，ヌクレオソームの排除，言い換えれば，オープンクロマチンの形成を誘導しているのかもしれない．この報告は，プロモーター領域またはその近傍にZ型DNAがしばしば存在する理由を説明している可能性がある．

三重鎖DNAと十字架構造も，オープンクロマチンの形成に利用されている可能性がある．三重鎖DNAは，ポリプリン配列・ポリピリミジン配列が鏡像的に反復した領域で形成されうる．たとえば，5′-GGAGAGGAGAA-T-AAGAGGAGAGG-3′（片鎖のみ表記）のような領域である．一方，十字架構造は，逆方向反復配列が存在するところで形成されうる．これらの構造は，ともにコアヒストンに会合できないといわれている[4]．したがって，三重鎖DNAも十字架構造も，安定化された場合にはオープンクロマチン領域の維持に役立つと考えられるが，それらの生物学的意義はいまだ不明である．

II DNAの物理的特性にかくされた遺伝情報

1. DNAの機械的特性と遺伝情報

A. DNAの柔軟性

ゲノムDNAには，相対的に柔らかい領域もあれば硬い領域もある．さらに，異方的（anisotropic）に柔らかい領域も

ある．柔らかい領域とは，力を加えたときに曲がりやすい領域（つまり，曲げやすい領域）と考えてよい．硬い領域とはその逆の性質を示す領域をさす．そして，異方的に柔らかい領域とは，ある特定の方向には曲がりやすいが，それ以外の方向には曲がりにくい領域のことである．硬さ・柔らかさ（以後，柔軟性*5と表記する）は，DNAの物理的特性のひとつである機械的特性（mechanical property）の代表格である．DNAの柔軟性は，おもにゲノムを機能的に折りたたむために利用されているのではないかと想像されてきたが，その具体的な証拠はまだ得られていない．ゲノムの収納機構との関連はさだかではないが，つい最近，DNAのこの特性に遺伝情報らしきものがはじめてみつかった[7,8]．

B. DNAの柔軟性を解析する方法

いくつかのグループが，短い2本鎖DNAの相対的柔軟性を明らかにしている．たとえば，Brukner らは，3 bpの全DNA（32種類）の柔軟性を実験的に求め[33]，Packer らは，4 bpの全DNA（136種類）の柔軟性をエネルギー計算により求めて数値化した[34]．なお，DNAの柔軟性は塩基配列によって決まるが，両者の対応は一対一ではない．これは，複数のDNAが同じ柔軟性をもつ場合があるからである．

さて，われわれが議論したいのは，最小でも数十bpにわたる領域である．しかし，10 bpからなるDNAでさえ全部で524,800種類もあり，個々の柔軟性を実験的に求めることはもはや不可能である．数十bpから数百bp，あるいは，それ以上のサイズをもつDNAの柔軟性をどのように評価したらよいのだろうか．このような場合，たとえば，トリヌクレオチドまたはテトラヌクレオチドの単位で解析し，その結果をさらに分析するという方法をとる．たとえば，5′-AGCTTAAGCCG‥‥-3′という配列では，AGC，GCT，CTT，TTA，TAA，AAG，AGC，GCC，CCG，CG‥，G‥，‥‥，といった具合に順次，数値化し，そのままプロットするなり平均化するなりして評価する．

C. プロモーターは共通の機械的特性をもつ

EPD*6とよばれるRNAポリメラーゼⅡプロモーターのデータベースには，1871種のヒトプロモーターの塩基配列が登録・公開されている．筆者らは，数年前にその全配列を解析し，TATAボックス，イニシエーター，下流プロモーター配列（downstream promoter element；DPE）などのコアプロモーター配列をもたず，しかも，GCボックスももたないプロモーター（筆者らは，コアレスプロモーターとよんでいる）が50％以上も存在することに気づいた（表3）．マウスのプロモーター196種を調べた結果も，ほぼ同様であった．話が少し横道にそれるが，出芽酵母の場合もTATAボックスをもつプロモーターの割合は少ない[35]．したがって，真核生物全般の傾向として，TATAボックスをもつプロモーターは案外，少ないのかもしれない．ともあれ，このようなことが明らかになると，コアレスプロモーターは認識されるためにどのような方策をとっているのだろうか，という素朴な疑問が生じる．

表3　コア配列別にみたヒトのプロモーターの割合

コア配列*	割合（%）
TATAボックス	6.09
イニシエーター	9.30
下流プロモーター配列	0.43
TATAボックス＋イニシエーター	1.02
TATAボックス＋下流プロモーター配列	0.05
イニシエーター＋下流プロモーター配列	0.05
TATAボックス＋イニシエーター＋下流プロモーター配列	0
GCボックス	20.4
なし**	53.7

EPDに公開されているすべてのヒトクラスⅡ遺伝子プロモーター，1871種を分類した[7,8]

* コア配列としては，表中に示された配列だけをもつプロモーターを意味する

** 表中の配列をいっさいもたないプロモーターの意味．TATAボックスのコンセンサス配列はもたないがTATAというテトラヌクレオチド配列はもつ，といったプロモーターは，"TATAボックス"の範疇にも"なし"の範疇にも入れなかった．プロモーターの割合を合計しても100％にならないのは，おもにこの理由による

筆者らは，プロモーターの柔軟性を解析し，その答えらしきものをみつけた[7,8]．まず，解析結果を要約する．

①コアレスプロモーターの場合，転写開始点領域の数塩基対の柔軟性がきわめて特異で，転写開始点を境にして，上流側の数塩基対が際立って柔らかく，下流側の数塩基対は逆に際立って硬い（図4）．

②これとほぼ同じ特性をもつ小領域が，他のプロモーターにも存在する．コア配列としてTATAボックスだけ，あるいは，イニシエーターだけをもつプロモーターの場合は，

＊5　**柔軟性**：英語論文では単に"DNA flexibility"という表現が用いられる場合が多いので，本稿でもそれにならった．

＊6　**EPD**：eukaryotic promoter database．真核生物クラスⅡ遺伝子のプロモーターのデータベースで，重複がないことと，実験的に転写開始点が決められているプロモーターだけを集めていることを特徴としている．URLは**http://www.epd.isb-sib.ch/**．

その配列自体が同様の特異な特性をもつ．GCボックスだけをもつプロモーターの場合は，コアレスプロモーターの場合と同様に，転写開始点領域が特異な特性をもつ．

③上記のどのタイプのプロモーターも，特異領域の上流のDNAは下流のDNAより，わずかではあるが相対的に硬いDNAでできている（プロモーターによって多少，解析範囲が異なるが，コアレスプロモーターに関しては，上流側－500まで，下流側＋100までの解析結果）．

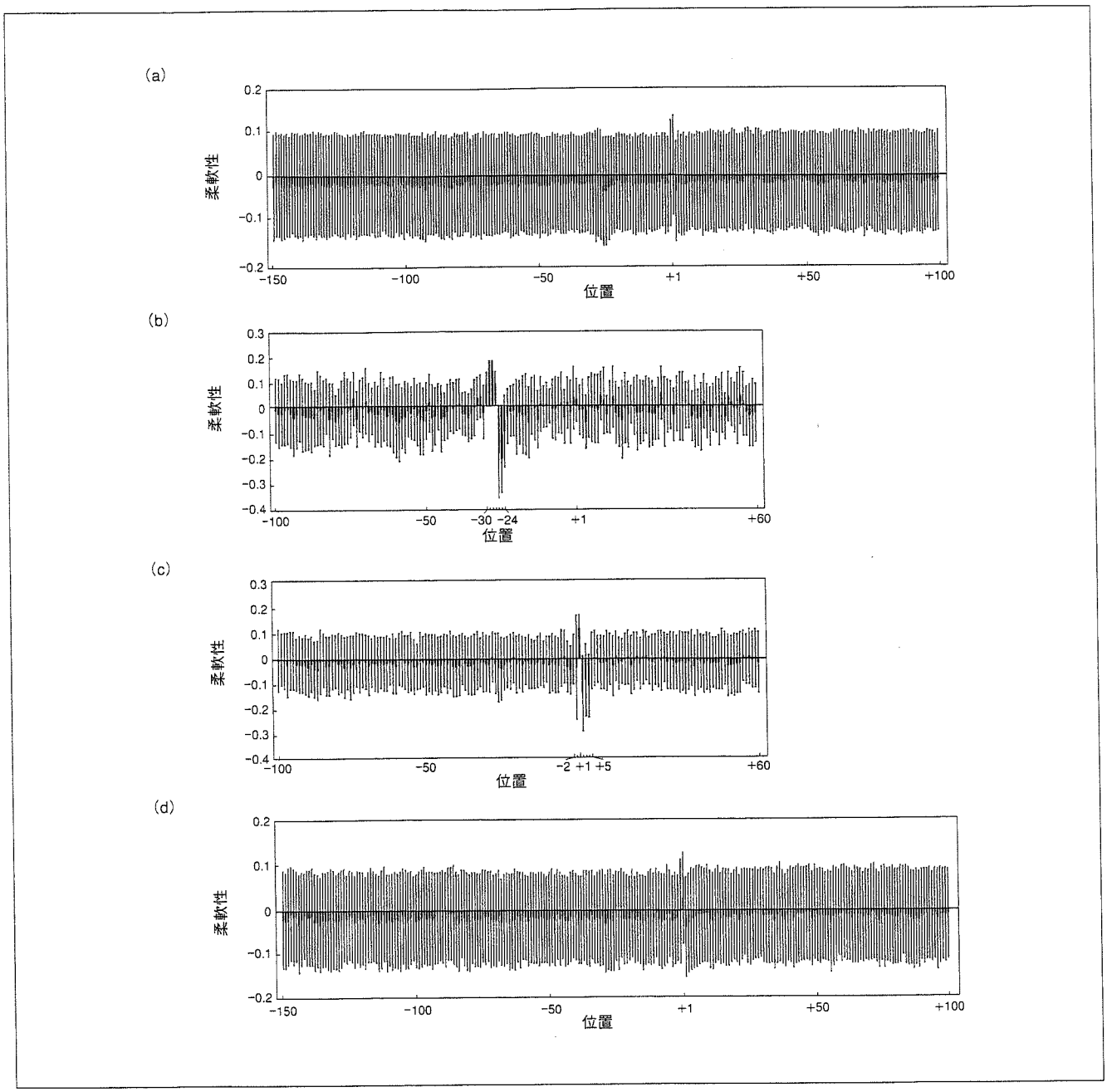


図4　ヒトクラスⅡ遺伝子プロモーターの機械的特性[7,8]

転写開始点から数えた位置と，その位置の平均的柔軟性（$\bar{x} \pm s$）の関係を示した．数値が大きいほど柔らかいことを意味する．

(a) コアレスプロモーター（$n = 1004$）．

(b) TATAボックスだけをもつプロモーター（$n = 26$）．TATAボックスの位置を赤色で示した．なお，－30～－24以外の位置にTATAボックスをもつプロモーターも，同じ関係を示した[7]．

(c) イニシエーターだけをもつプロモーター（$n = 97$）．イニシエーターの位置を青色で示した．

(d) GCボックスだけをもつプロモーター（$n = 382$）．

つまり，柔軟性という視点でみると，すべてのプロモーターが共通の特徴をもっていることが明らかになった．さらに筆者らは，この特徴を模した人工のDNAが，実際に転写を開始できることを明らかにした[8]．

この結果から，コアレスプロモーターはその柔軟性の特徴を認識シグナルにしている可能性が高いと推察される．しかし，今回の発見は，コアレスプロモーターの認識機構にとどまらず，すべてのプロモーターの認識機構に重要な示唆を与えているのかもしれない．いずれにせよ，プロモーター領域のDNAの機械的特性がなんらかの遺伝情報を担っていることはまちがいないと考えられる．

2．DNAの自己集合と遺伝情報

ある種の蛋白質は，自己集合して超分子構造を構築する．真核生物のアクチン，ミオシン，チューブリンや，原核生物のフラジェリンなどは有名な例である．ところがこれまで，2本鎖DNAに自己集合能があることは知られていなかった．筆者らは，最近，2本鎖DNAがMg^{2+}存在下で自己集合することを発見した[36]（図5）．この現象も，一種の遺伝情報として機能している可能性がある．

2本鎖DNAの自己集合現象は，数nM以上のDNAが存在する系に数百μMから数mM（すなわち，生理学的な濃度）のMg^{2+}が共存すると起こる．そしてこの現象は，DNAの混合溶液中でも起こる．つまり，系に含まれる，自己と同じDNAを“見分けて”集合するという現象が存在するのである[36]．原子間力顕微鏡のデータからは，集合体は平行に並んだ数分子のDNAからなることが示唆された（図5 b, c）．この現象は，同じ塩基配列をもつDNA分子のあいだに，なんらかの“引力”がはたらくことを示唆している．水和Mg^{2+}はグアニン（7位の窒素原子と，6位の炭素に結合した酸素原子）に配位することから，筆者は，Mg^{2+}がバインダーになって分子間のグアニンどうしを結びつけているのではないかと想像している[36,37]（ひとつの分子のなかで，同じ面に位置するグアニン残基たちと，他の分子の対応する残基たちを，それぞれ対向させて結びつけていると想像している）．

遺伝情報の観点からは，この“引力”は同一の配列をみつけるメカニズムまたは情報として利用されているのではないかと想像される．たとえば，相同組換えに利用されている可能性が考えられる．また，反復配列の折りたたみにも利用されているかもしれない．このほかにも，さまざまな現象に関与していることが想像される．

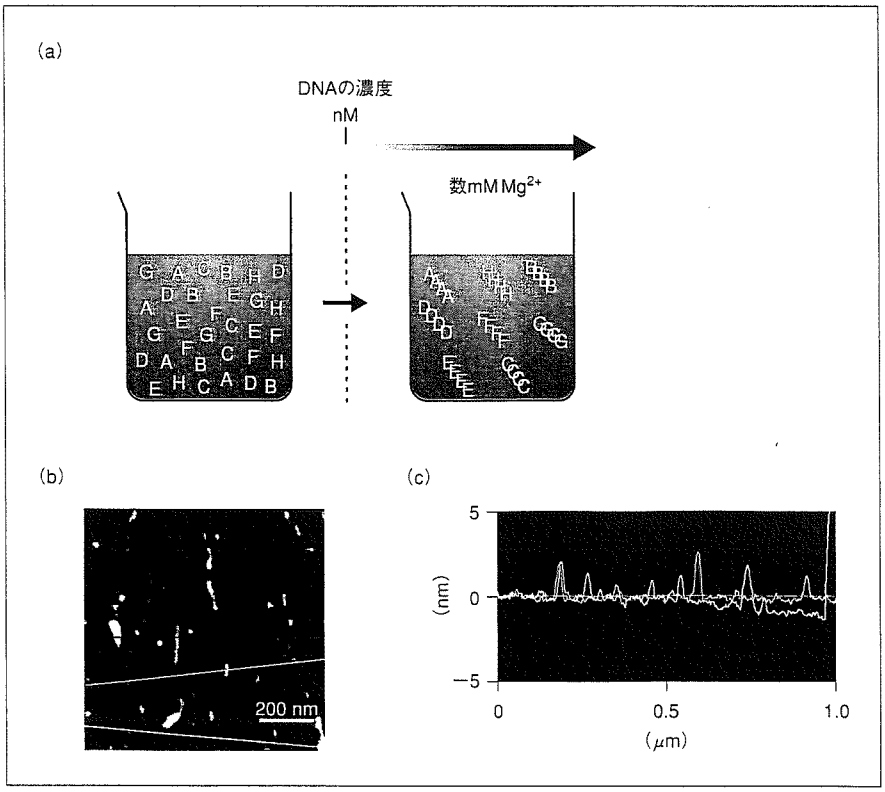


図5　2本鎖DNAの自己集合

(a) 概念図．DNA分子をアルファベットで示した．
(b) 原子間力顕微鏡像[36]．2.5 mMの$MgCl_2$を含む水溶液中，10 nMの濃度で自己集合したDNAを観察した．
(c) (b)において，赤色の線，緑色の線，白色の線で示した断面におけるDNAの厚み（セクションプロファイル）．2本鎖DNA単独で観測される厚みは1 nm以下である．したがって，この系では数分子の集合体が多いことがわかる．

III 高次遺伝情報とエピジェネティクス

1. DNA高次構造・機械的特性とエピジェネティクス

2価カチオンにより，まっすぐなDNAが曲がったり，ベントDNAの形が変わったりする現象があることは，一部の研究者のあいだではよく知られている[13]．細胞核内の局所的イオン濃度のわずかな違いや変動が，DNAの高次構造や機械的特性の変化を誘起し，それがクロマチン構造の変化をひき起こし，ひいては，遺伝子発現のパターンに変化をもたらすというメカニズムが存在していても不思議ではない．つまり，DNA高次構造や機械的特性と，細胞核内の局所的イオン環境が複合的に作用して，細胞系列に限定された遺伝子発現パターンを形成するひとつの要因になっている可能性がある．

2. DNAの自己集合とエピジェネティクス

クロマチンの凝縮・弛緩の程度がカチオンの濃度に依存して大きく変わることは昔から知られている[38]．DNAの自己集合もこの現象に関与している可能性がある．DNAの同一配列のあいだではたらく引力がヌクレオソームのあいだでも有効にはたらけば，その引力は縦列型・分散型をとわず，反復配列上のヌクレオソームどうしを近づける効果をもつことになる．この引力の強さはMg^{2+}の濃度により変動すると考えられるので，その濃度をパラメーターとしてクロマチン全体の凝縮の程度が変化し，それがエピジェネティクス現象のひとつの原動力になるという図式が考えられる（エピジェネティクスに関係しないまでも，サテライトDNAなどのうえにヘテロクロマチンを形成する際には効果的であろう）．DNAの構造とエピジェネティクスの関係では，従来，メチル化DNAだけが解析の対象となってきたが，上述の視点をもった研究の展開が新たな発見につながると思われる．いずれにせよ，このような力が存在することの生物学的意義は今後の研究に待たねばならないが，遺伝現象との関係は深いものと推察している．

おわりに

ベントDNAやポリ（dA·dT）などある種のDNA高次構造は，クロマチンの基盤構造を世代をこえて再現するため，すなわち，ヌクレオソームの基本的配置を決めるための情報の所在になっていると推察される．DNAの物理的特性も同様の役割をはたしていると想像されるが，まだ明確な証拠は得られていない．ただし，プロモーター領域の機械的特性に，転写に必要ななんらかの情報が印されていることは明らかになった．

ヌクレオソームの配置を決める情報はゲノムに記されている，という内容の論文が2006年に発表されたが[39]，筆者は，つきつめれば，くり返し述べたように，この情報はDNAの高次構造や物理的特性に印されていると考えている．筆者らは，最近，ヒトゲノム約30億bpの全長にわたるDNAの機械的特性（柔軟性）のマップを作成した．その解析には，今後，しばらくの時間を要すると思われるが，これまでに知られていなかった遺伝情報がみつかるのではないかと期待している．いずれにせよ，高次の遺伝情報を解明する研究をとおして，“生物はどのようにして遺伝子を機能的にクロマチンのなかに折りたたんでいるか”，“生物はどのようにしてクロマチンの構造を世代をこえて忠実に再現しているか”といった，古くからつづいた疑問が解けるときがいつかおとずれると予想している．さらに，このような研究により，新しい応用分野が拓かれることも期待できる．たとえば，遺伝子発現に有利なクロマチン構造を人為的に構築するクロマチン工学はその例で，実際に，ベントDNAやポリ（dA·dT）がこの技術に有用であることがすでに示されはじめている[23,29,40]．

Carl Sagan原作の映画『Contact』のラストシーンで，Jodie Foster演ずる主人公のエリーが子供たちに，“Are there other people out there in the universe?”と問われ，“Universe is a pretty big place. …. So, if just us, seems like awful waste of space.”と答えるシーンがある．高等生物のゲノムと遺伝情報の関係もこれと同じである．自然は塩基配列だけに情報を担わせるようなもったいないことはしていないだろう．

文　献

1) Hirota, Y., Ohyama, T.: *J. Mol. Biol.*, **254**, 566-578 (1995)
2) 大山 隆: 生化学, **70**, 1339-1344 (1998)
3) Asayama, M., Ohyama, T.: *in* DNA Conformation and Transcription (Ohyama, T. ed.), pp. 37-51, Springer, New York (2005)
4) Ohyama, T.: *in* DNA Conformation and Transcription (Ohyama, T. ed.), pp. 177-188, Springer, New York (2005)
5) Ohyama, T. *et al.*: *in* DNA Structure, Chromatin and Gene Expression (Kiyama, R., Shimizu, M. eds.), pp. 71-84, Transworld Research Network, Kerala (2006)

6) Ohyama, T.: *in* Nuclear Dynamics: Molecular Biology and Visualization of the Nucleus (Nagata, K., Takeyasu, K. eds.), pp. 227-238, Springer-Verlag, Tokyo (2007)
7) Fukue, Y., Sumida, N., Nishikawa, J., Ohyama, T.: *Nucleic Acids Res.*, **32**, 5834-5840 (2004)
8) Fukue, Y., Sumida, N., Tanase, J., Ohyama, T.: *Nucleic Acids Res.*, **33**, 3821-3827 (2005)
9) Potaman, V. N., Sinden, R. R.: *in* DNA Conformation and Transcription (Ohyama, T. ed.), pp. 3-17, Springer, New York (2005)
10) Nelson, H. C., Finch, J. T., Luisi, B. F., Klug, A.: *Nature*, **330**, 221-226 (1987)
11) Widlund, H. R. *et al.*: *J. Mol. Biol.*, **267**, 807-817 (1997)
12) Kiyama, R., Wada-Kiyama, Y.: *in* DNA Structure, Chromatin and Gene Expression (Kiyama, R., Shimizu, M. eds.), pp. 55-70, Transworld Research Network, Kerala (2006)
13) Ohyama, T. *et al.*: *Nucleic Acids Res.*, **26**, 4811-4817 (1998)
14) Matsugami, A. *et al.*: *Nucleosides Nucleotides Nucleic Acids*, **25**, 417-425 (2006)
15) Ohyama, T.: *J. Biol. Chem.*, **271**, 27823-27828 (1996)
16) Ohyama, T.: *in* DNA Conformation and Transcription (Ohyama, T. ed.), pp. 66-74, Springer, New York (2005)
17) Ohyama, T.: *BioEssays*, **23**, 708-715 (2001)
18) Angermayr, M. *et al.*: *Nucleic Acids Res.*, **30**, 4199-4207 (2002)
19) Bash, R. C. *et al.*: *J. Biol. Chem.*, **276**, 861-866 (2001)
20) Piña, B., Brüggemeier, U., Beato, M.: *Cell*, **60**, 719-731 (1990)
21) Piña, B., Barettino, D., Truss, M., Beato, M.: *J. Mol. Biol.*, **216**, 975-990 (1990)
22) Nishikawa, J. *et al.*: *Nucleic Acids Res.*, **31**, 6651-6662 (2003)
23) Sumida, N. *et al.*: *FEBS J.*, **273**, 5691-5702 (2006)
24) Blomquist, P., Belikov, S., Wrange, Ö.: *Nucleic Acids Res.*, **27**, 517-525 (1999)
25) Dechering, K. J., Cuelenaere, K., Konings, R. N., Leunissen, J. A.: *Nucleic Acids Res.*, **26**, 4056-4062 (1998)
26) Struhl, K.: *Proc. Natl. Acad. Sci. USA*, **82**, 8419-8423 (1985)
27) Roy, A., Exinger, F., Losson, R.: *Mol. Cell. Biol.*, **10**, 5257-5270 (1990)
28) Thiry-Blaise, L. M., Loppes, R.: *Mol. Gen. Genet.*, **223**, 474-480 (1990)
29) Morohashi, N. *et al.*: *Eukaryot. Cell*, **5**, 1925-1933 (2006)
30) Reeves, R., Wolffe, A. P.: *Biochemistry*, **35**, 5063-5074 (1996)
31) Herbert, A.: *in* DNA Conformation and Transcription (Ohyama, T. ed.), pp. 93-104, Springer, New York (2005)
32) Liu, R. *et al.*: *Cell*, **106**, 309-318 (2001)
33) Brukner, I., Sánchez, R., Suck, D., Pongor, S.: *EMBO J.*, **14**, 1812-1818 (1995)
34) Packer, M. J., Dauncey, M. P., Hunter, C. A.: *J. Mol. Biol.*, **295**, 85-103 (2000)
35) Basehoar, A. D., Zanton, S. J., Pugh, B. F.: *Cell*, **116**, 699-709 (2004)
36) Inoue, S., Sugiyama, S., Travers, A. A., Ohyama, T.: *Biochemistry*, **46**, 164-171 (2007)
37) Tagashira, H., Morita, M., Ohyama, T.: *Biochemistry*, **41**, 12217-12223 (2002)
38) Thoma, F., Koller, T. H., Klug, A.: *J. Cell Biol.*, **83**, 403-427 (1979)
39) Segal, E. *et al.*: *Nature*, **442**, 772-778 (2006)
40) Kamiya, H., Fukunaga, S., Ohyama, T., Harashima, H.: *Arch. Biochem. Biophys.*, **461**, 7-12 (2007)

大山 隆

略歴：1985年 名古屋大学大学院理学研究科博士後期課程 修了，理学博士，1986年 三菱化成生命科学研究所 特別研究員，1987年 明治乳業ヘルスサイエンス研究所 研究員（一時期，RITE研究員およびスイスETH細胞生物学研究所客員研究員），1994年 甲南大学理学部 助教授，同 教授を経て，2006年より早稲田大学教育・総合科学学術院 教授．

研究テーマ：細胞核における遺伝子の機能的収納機構の解明．