10/19/2010
富山大学大学院講義
「遺伝情報制御学特論」

多様なクロマチン機能とエピジェネティクス

クロマチン制御のダイナミクスと可塑的システムの理解に向けたヒストン機能複合体解析

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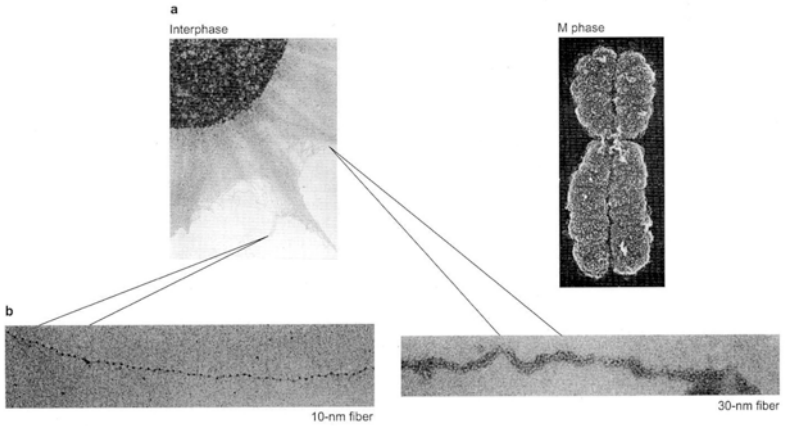
クロマチン構造の電顕写真



クイズ

以下のうちメンデル遺伝のものに○、非メンデル遺伝のものに×を付よ。

( ) A型とB型の血液型の両親から生まれた子供の血液型
( ) ミトコンドリア遺伝子
( ) 指の指紋
( ) スイートピーの花の色
( ) BSEに代表されるプリオン病の伝搬
( ) 三毛猫の模様

エピジェネティクス

遺伝暗号（Genetic Code): DNA上の塩基配列に刻み込まれた情報

Geneticsに対するEpigenetics: Epi-は「上」、「さらに」という意味の接頭語。

もともとは発生学で用いられた造語で、後生的な形質の変化のメカニズムを指す。現在は、DNA配列の変化を伴わないで細胞分裂以降も継承される情報を指す。

DNAメチル化やクロマチン制御を介すると考えられる。

エピジェネティック制御の例
ゲノミックインプリンティング
X染色体不活性化

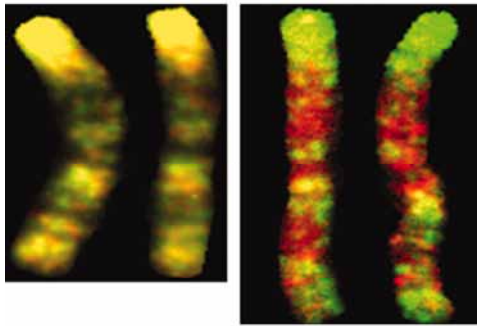
Epigenetic differences arise during the lifetime of monozygotic twins

Mario F. Fraga*, Esteban Ballestar*, Maria F. Paz*, Santiago Ropero*, Fernando Setien*, Maria L. Ballestar†, Damia Heine-Suñer‡, Juan C. Cigudosa§, Miguel Urioste¶, Javier Benitez¶, Manuel Boix-Chornet†, Abel Sanchez-Aguilera†, Charlotte Ling||, Emma Carlsson||, Pernille Poulsen**, Allan Vaag**, Zarko Stephan††, Tim D. Spector††, Yue-Zhong Wu‡‡, Christoph Plass‡‡, and Manel Esteller*§§

10604–10609 | PNAS | July 26, 2005 | vol. 102 | no. 30

1番染色体上のDNAメチル化のパターン

3才双子　　50才双子



X染色体不活性化

Dosage Compensation:量的補正
　雌において一方のX染色体がランダムに不活性化

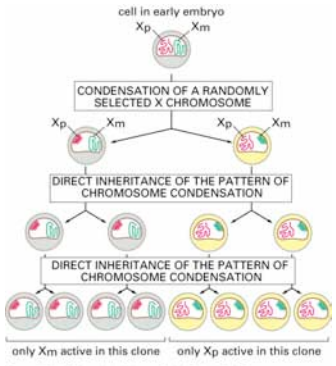


Figure 7–77. Molecular Biology of the Cell, 4th Edition.

XIC: X inactivation center
Xist: X inactive specific transcript
　　17kb noncoding RNA
Histone methylation

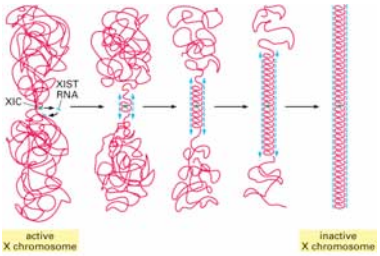


Figure 7–78. Molecular Biology of the Cell, 4th Edition.

Chromatin Structure: A Repeating Unit of Histones and DNA

Chromatin structure is based on a repeating unit of eight histone molecules and about 200 DNA base pairs.

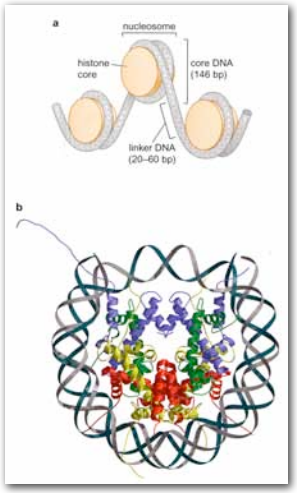
SCIENCE (1974) 184, 868

Roger D. Kornberg

Roger Kornberg
The Nobel Prize in Chemistry 2006
"for his studies of the molecular basis of eukaryotic transcription"

Arthur Kornberg
The Nobel Prize in Physiology or Medicine 1959
with Severo Ochoa
"for their discovery of the mechanisms in the biological synthesis of ribonucleic acid and deoxyribonucleic acid"

ヌクレオソーム



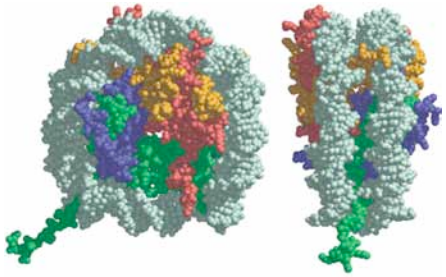


Figure 4–25. Molecular Biology of the Cell, 4th Edition.

ヒストン蛋白質

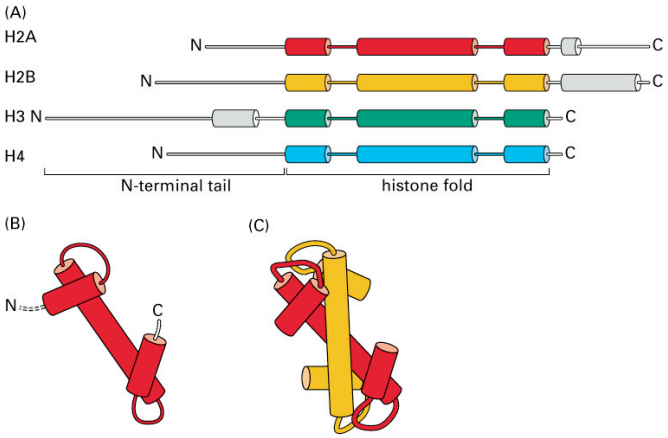


Figure 4–26. Molecular Biology of the Cell, 4th Edition.

Histone code

ヒストンコード(Histone Code): ヒストンテールのアセチル化が遺伝子発現制御に重要であることは古くから知られていたが、ヒストン上の特異的な部位の化学修飾、およびその組み合わせが暗号(Code)を構成するという仮説としてDavid Allisらによって提唱された。ヒストンコードは、そのバリエーションの豊富さから、それらを読む様々な機能因子によってクロマチン構造変換を介した複雑な遺伝子発現制御を可能にしていることを想像させる。また、ヒストンコードは一過的な役割を担うだけではなく、その状態を細胞分裂以降も継承するエピジェネティック情報としても機能する例が多く報告されている。今や、DNA上の遺伝暗号（Genetic Code）に対するエピジェネティックコードの重要な一つであり、ヒストンが情報を担うという“概念”としてこの言葉を使ってよいのではないだろう

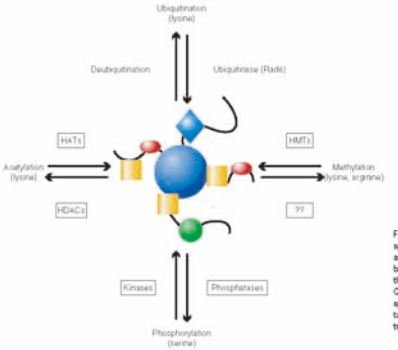


Figure 1 Enzyme families that modify histone tails on the nucleosome surface. Levels of specific histone modifications are maintained by the balanced activities of modifying and de-modifying enzymes. Levels of any particular modification will rise or fall as the balance between the activities of these two sets of enzymes is shifted by changes in their intracellular distribution, their targeting to chromatin or the action of inhibitors. Chromatin can certainly be demethylated, but whether this is through the action of a specific histone demethylase or by replacement or other mechanisms, remains uncertain. HAT, Histone acetyltransferase; HDAC, Histone deacetylase; HMT, Histone methyltransferase.

ヒストンアセチル化と転写制御

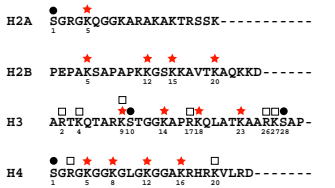
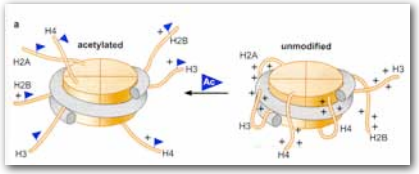


図2. ヒストンテールの化学修飾
コアヒストンのN末端のアミノ酸配列と化学修飾（アセチル化:★、メチル化:□、リン酸化:●）を受ける部位を示す。



HAT(Histone acetyltransferase)の発見
　Gcn5, p300/CBP = 既知のコアクチベーター

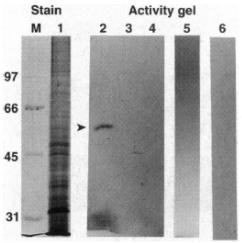
HDAC(Histone Deacetylase)の発見
　Sin3, NuRD, SIR2 complexes

Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 6364–6368, July 1995
Cell Biology

An activity gel assay detects a single, catalytically active histone acetyltransferase subunit in *Tetrahymena* macronuclei

(acetylation/chromatin)

JAMES E. BROWNELL AND C. DAVID ALLIS*
Department of Biology, Syracuse University, Syracuse, NY 13244



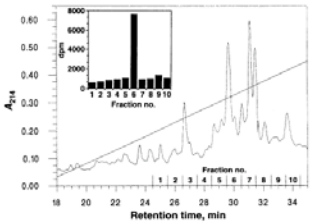
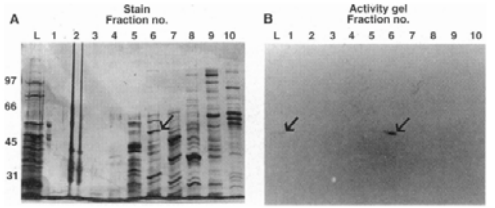


FIG. 4. Enrichment of histone acetyltransferase. Partially purified



Cell, Vol. 84, 843–851, March 22, 1996, Copyright ©1996 by Cell Press

Tetrahymena Histone Acetyltransferase A: A Homolog to Yeast Gcn5p Linking Histone Acetylation to Gene Activation

James E. Brownell,* Jianxin Zhou,* Tamara Ranalli,*
Ryuji Kobayashi,† Diane G. Edmondson,‡
Sharon Y. Roth,‡ and C. David Allis*
*Department of Biology
University of Rochester
Rochester, New York 14627

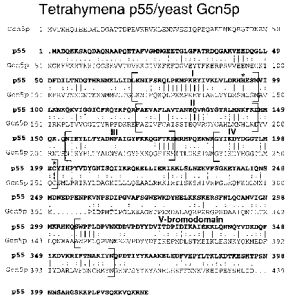


Figure 3. Yeast Gcn5p Is Highly Homologous to Tetrahymena p55

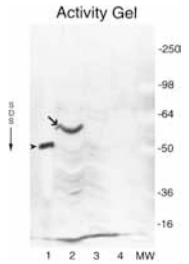


Figure 4. Gcn5p Has HAT Activity in the Gel Activity Assay

(A) Samples corresponding to macronuclear extracts (lane 1) or SDS-generated whole cell extracts of recombinant Gcn5p from either induced (lane 2) or uninduced cells (lane 3) or from induced cells containing the expression vector lacking the *GCN5* gene insert (vector only, lane 4) were electrophoresed in an 8% SDS–

A Mammalian Histone Deacetylase Related to the Yeast Transcriptional Regulator Rpd3p

Jack Taunton, Christian A. Hassig, Stuart L. Schreiber*

Trapoxin is a microbially derived cyclotetrapeptide that inhibits histone deacetylation in vivo and causes mammalian cells to arrest in the cell cycle. A trapoxin affinity matrix was used to isolate two nuclear proteins that copurified with histone deacetylase activity. Both proteins were identified by peptide microsequencing, and a complementary DNA encoding the histone deacetylase catalytic subunit (HD1) was cloned from a human Jurkat T cell library. As the predicted protein is very similar to the yeast transcriptional regulator Rpd3p, these results support a role for histone deacetylase as a key regulator of eukaryotic transcription.

408 SCIENCE • VOL. 272 • 19 APRIL 1996

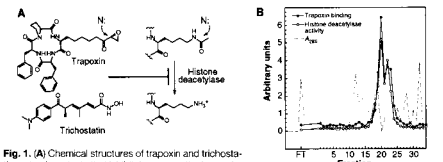
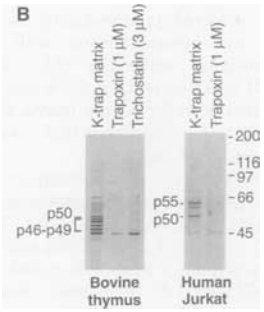
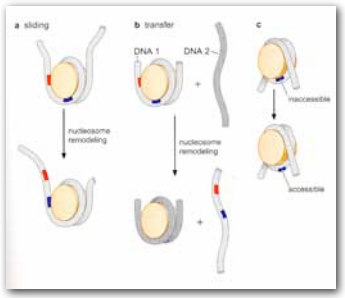
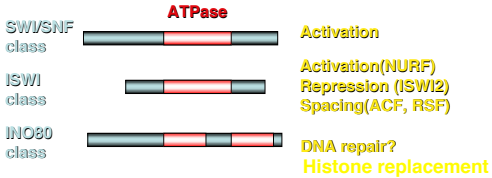


Fig. 1. (A) Chemical structures of trapoxin and trichostatin, natural products that inhibit the enzymatic deacetylation of lysine residues near the NH_2-terminus of histones. The epoxyketone side chain of trapoxin is approximately isosteric with *N*-acetyl lysine and likely alkylates an active site nucleophile. (B) Copurification of trapoxin binding and histone deacetylase activities.



クロマチンリモデリング因子





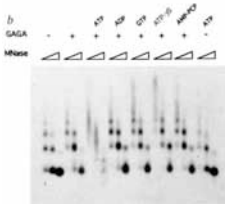
ARTICLES

ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor

Toshio Tsukiyama, Peter B. Becker* & Carl Wu†

Genetic control elements are usually situated in local regions of chromatin that are hypersensitive to structural probes such as DNase I. We have reconstructed the chromatin structure of the *hsp70* promoter using an *in vitro* nucleosome assembly system. Binding of the GAGA transcription factor on existing nucleosomes leads to nucleosome disruption, DNase I hypersensitivity at the TATA box and heat-shock elements, and rearrangement of adjacent nucleosomes. ATP hydrolysis facilitates this process, suggesting that an energy-dependent pathway is involved in chromatin remodelling.

NATURE · VOL 367 · 10 FEBRUARY 1994 525

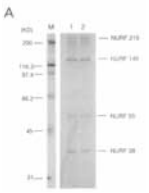


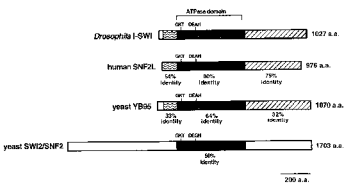
Cell, Vol. 83, 1011–1020, December 15, 1995, Copyright © 1995 by Cell Press

Purification and Properties of an ATP-Dependent Nucleosome Remodeling Factor

Cell, Vol. 83, 1021–1026, December 15, 1995, Copyright © 1995 by Cell Press

ISWI, a Member of the *SWI2/SNF2* ATPase Family, Encodes the 140 kDa Subunit of the Nucleosome Remodeling Factor





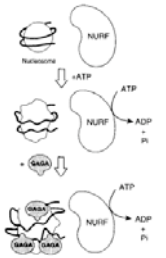


Figure 7. Model for the Mechanism of Action of NURF on a Single Nucleosome

転写活性化におけるHATと クロマチンリモデリング因子の作用

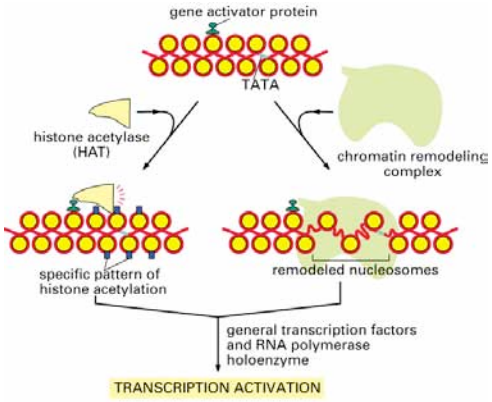


Figure 7–45. Molecular Biology of the Cell, 4th Edition.

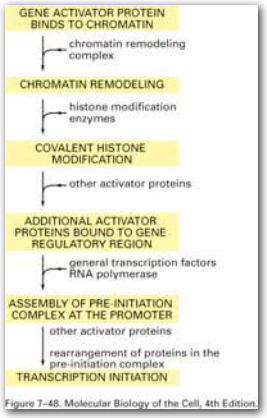
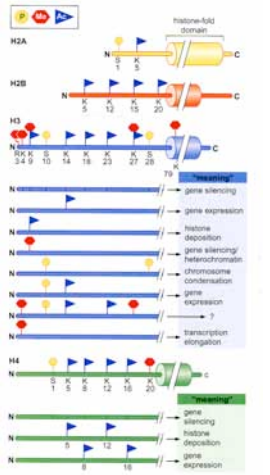
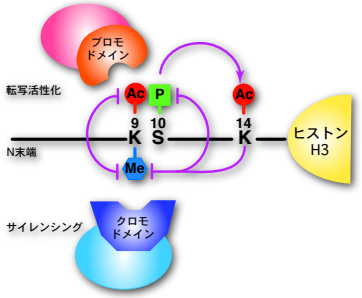


Figure 7–48. Molecular Biology of the Cell, 4th Edition.

ヒストン修飾とヒストンコード仮説





ヒストンコード：ヒストン上に刻まれた情報
複雑なヒストン修飾のバリエーション
認識する多様な蛋白質

↓

エピジェネティック情報

PEV(Position Effect Variegation)

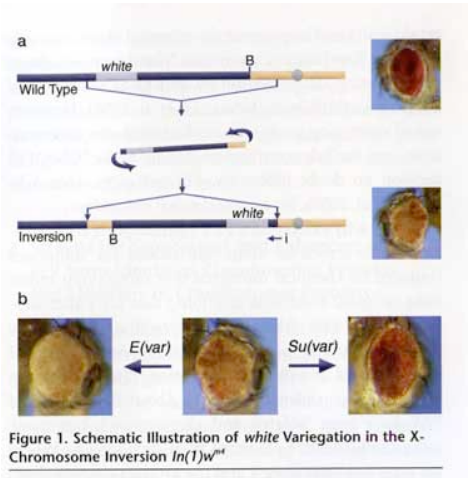


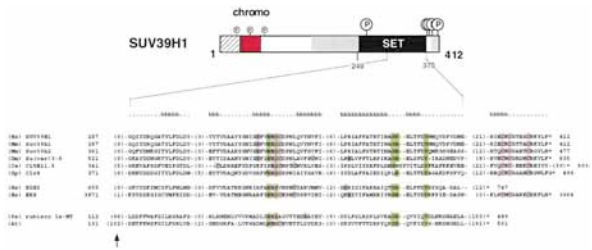
Figure 1. Schematic Illustration of *white* Variegation in the X-Chromosome Inversion *In(1)w*^{m4}

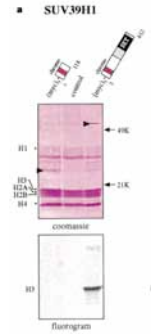
NATURE | VOL 406 | 10 AUGUST 2000 | www.nature.com

Regulation of chromatin structure by site-specific histone H3 methyltransferases

Stephen Rea*, Frank Eisenhaber*, Dónal O'Carroll*, Brian D. Strahl†, Zu-Wen Sun†, Manfred Schmid*, Susanne Opravil*, Karl Mechtler*, Chris P. Ponting‡, C. David Allis† & Thomas Jenuwein*

* Research Institute of Molecular Pathology (IMP), The Vienna Biocenter, Dr. Bohrgasse 7, A-1030 Vienna, Austria
† Department of Biochemistry and Molecular Genetics, University of Virginia Health Science Center, Charlottesville, Virginia 22908, USA
‡ MRC, Functional Genetics Unit, Department of Human Anatomy and Genetics, University of Oxford, OX1 3QX, UK



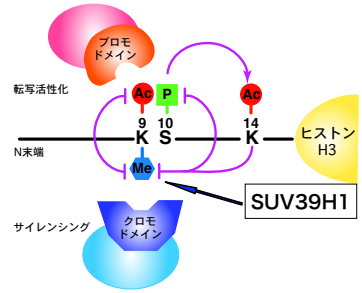


ヒストンのメチル化とサイレンシング

ヘテロクロマチン形成に関与するSUV39H1

↓

H3-K9 メチル化酵素



HP1(heterochromatin protein 1)

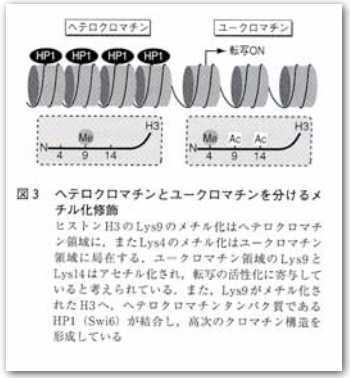
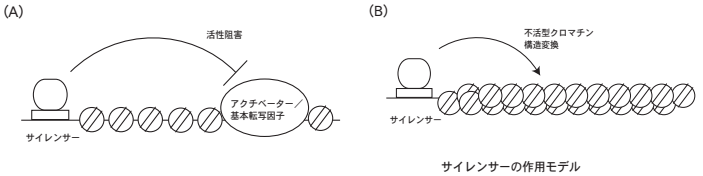


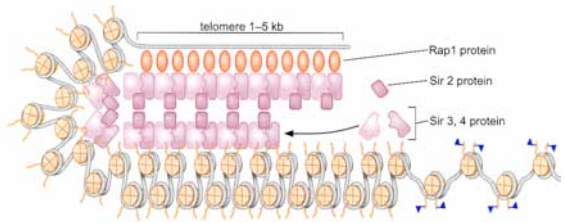
図3 ヘテロクロマチンとユークロマチンを分けるメチル化修飾
ヒストンH3のLys9のメチル化はヘテロクロマチン領域に，またLys4のメチル化はユークロマチン領域に局在する．ユークロマチン領域のLys9とLys14はアセチル化され，転写の活性化に寄与していると考えられている．また，Lys9がメチル化されたH3へ，ヘテロクロマチンタンパク質であるHP1（Swi6）が結合し，高次のクロマチン構造を形成している

遺伝子サイレンシング



サイレンサーの作用モデル

出芽酵母テロメアのヘテロクロマチン化



DNAメチル化とヒストンメチル化

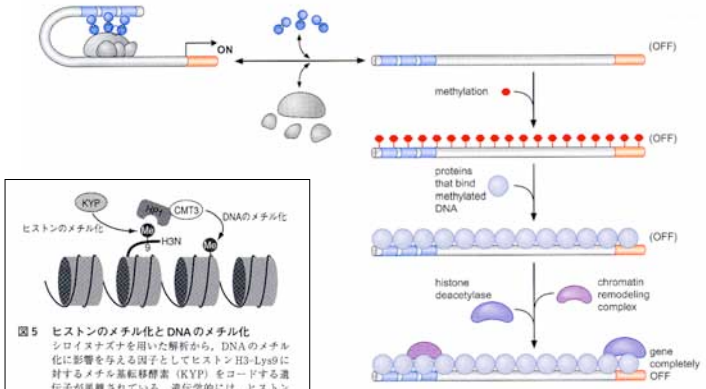
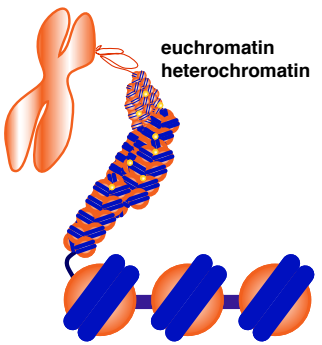


図5 ヒストンのメチル化とDNAのメチル化
シロイヌナズナを用いた解析から，DNAのメチル化に影響を与える因子としてヒストンH3-Lys9に対するメチル基転移酵素（KYP）をコードする遺伝子が単離されている．遺伝学的には，ヒストンのメチル化修飾はDNAのメチル化の上流に位置し，実際にDNAをメチル化するCMT3が，HP1との相互作用を通じてLys9がメチル化されたヒストンテールに結合することが明らかにされている（文献25より改変）

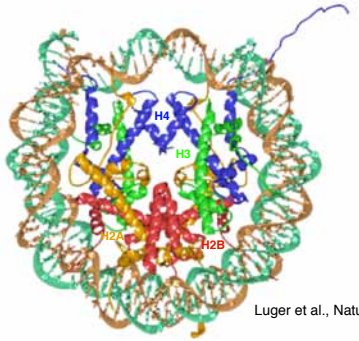
Chromatin Structures

Mitotic Chromosome



euchromatin
heterochromatin

Nucleosomes



Luger et al., Nature (1997)

Nucleosome Core: H2A, H2B, H3, H4

Histone modifications (Acetylation, Methylation, Phosphorylation, etc)
Histone variants (H2A.Z, H3.3, CENP-A, etc)
Non histone proteins (HP-1, HMGs, etc)

Histone Modifiers (HAT, HDAC, HMT, etc)
ATP-dependent Remodelers (SWI/SNF, etc)
Histone chaperones Laskey et al., Nature (1978)