previous lecture

Euchromatin and Heterochromatin have distinguishing features:

1. histone post-translational modifications (PTM) = histone code
2. histone variants
3. proteins recognizing histone modifications and modifying enzymes
4. DNA Cytosine Methylation at CpG islands

During development, a progressive selection of genes to be expressed and genes to be silenced occurs.

Each cell type establishes its own profile of heterochromatic and euchromatic partition of the genome.

We see this quite clearly by following development using DNA microarray analysis of gene expression

The question now is:

How is the heterochromatic or euchromatic status transmitted to daughter cells during DNA replication and cell division ?

Epigenetic determinants

Chromatin in pluripotent embryonic stem cells and differentiation

Eran Meshorer and Tom Misteli

Abstract | Embryonic stem (ES) cells are unique in that they are pluripotent and have the ability to self-renew. The molecular mechanisms that underlie these two fundamental properties are largely unknown. We discuss how unique properties of chromatin in ES cells contribute to the maintenance of pluripotency and the determination of differentiation properties.

ES cells

neuronal progenitor cells

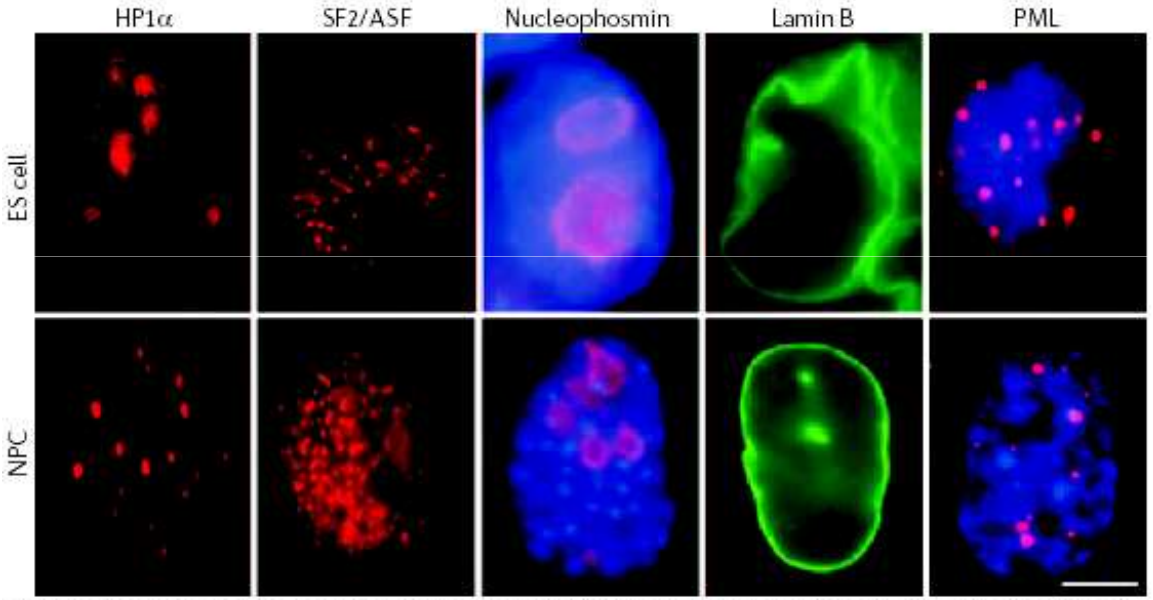


Figure 1 | **Nuclear architecture in ES cells and differentiating ES-derived cells.** Nuclear domains in an undifferentiated embryonic stem (ES) cell (top) and a differentiating ES-derived neuronal progenitor cell (NPC, bottom). From left to right: heterochromatin, as detected with an anti-HP1α antibody, is confined to fewer and larger foci in ES cells compared with NPCs; nuclear speckles, as detected with an anti-SF2/ASF antibody, appear as small, dispersed foci in ES cells and become more conspicuous in NPCs; nucleoli, as identified with an anti-nucleophosmin antibody, appear larger in ES cells compared with NPCs; the ill-defined nuclear lamina in ES cells, stained with an anti-lamin B antibody, becomes round and distinct in NPCs; promyelocytic leukaemia (PML) bodies labelled with an anti-PML antibody show similar patterns in ES cells and NPCs. DAPI, blue. Scale bar, 5 μm.

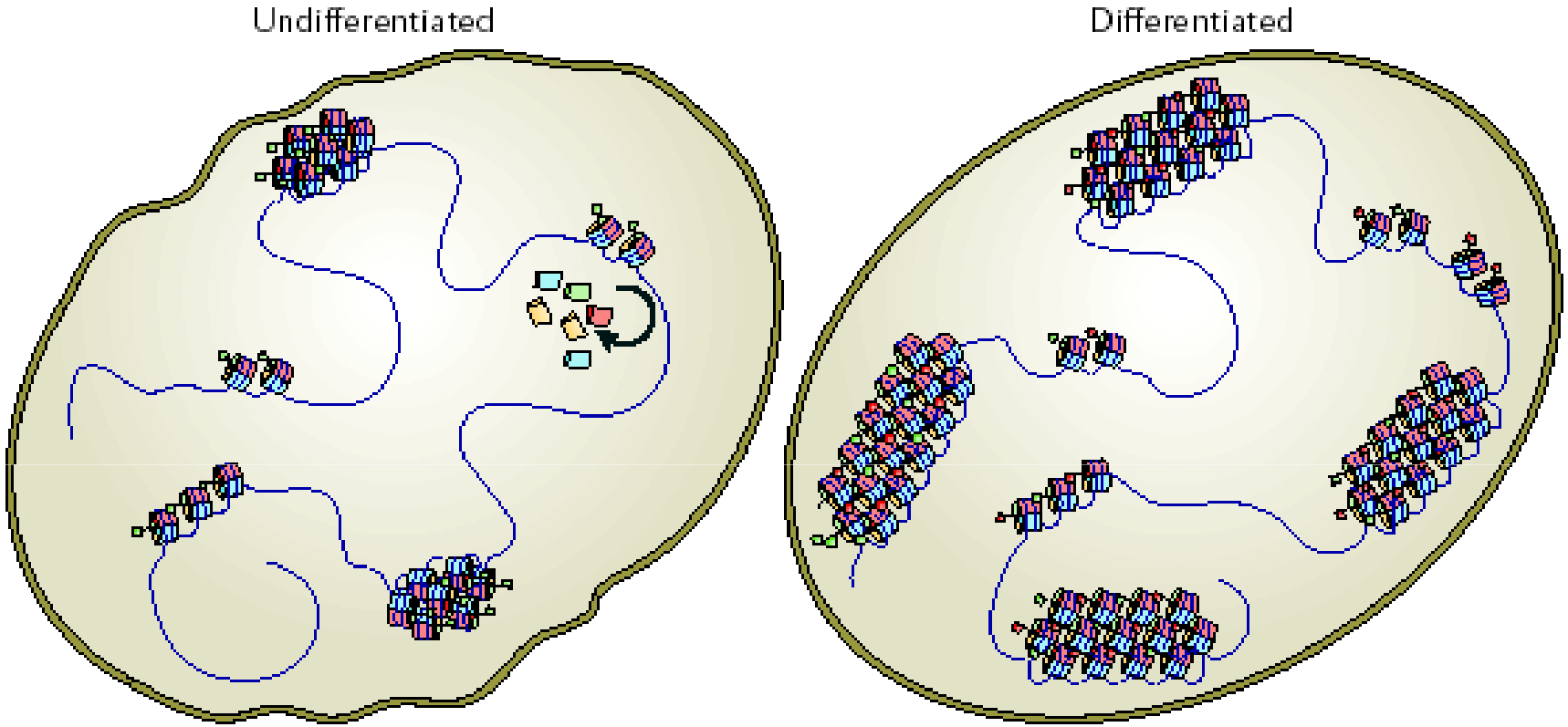


Figure 2 | **Chromatin during ES-cell differentiation.** In pluripotent embryonic stem (ES) cells (left), chromatin is globally decondensed, enriched in active histone marks (green circular tags), and contains a fraction of loosely bound architectural chromatin proteins. As cells differentiate (right), regions of condensed heterochromatin form, silencing histone marks (red circular tags) accumulate, and structural chromatin proteins become more stably associated with chromatin.

NATURE|Vol 447|24 May 2007|doi:10.1038/nature05918 INSIGHT REVIEW

Stability and flexibility of epigenetic gene regulation in mammalian development

Wolf Reik[1]

During development, cells start in a pluripotent state, from which they can differentiate into many cell types, and progressively develop a narrower potential. Their gene-expression programmes become more defined, restricted and, potentially, 'locked in'. Pluripotent stem cells express genes that encode a set of core transcription factors, while genes that are required later in development are repressed by histone marks, which confer short-term, and therefore flexible, epigenetic silencing. By contrast, the methylation of DNA confers long-term epigenetic silencing of particular sequences — transposons, imprinted genes and pluripotency-associated genes — in somatic cells. Long-term silencing can be reprogrammed by demethylation of DNA, and this process might involve DNA repair. It is not known whether any of the epigenetic marks has a primary role in determining cell and lineage commitment during development.

Nature 2007, 447: 425-432.

REVIEW

Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Cell Biology

Chromatin as a potential carrier of heritable information

Paul D Kaufman[1] and Oliver J Rando[2]

Current Opinion in Cell Biology 2010, 22:284–290

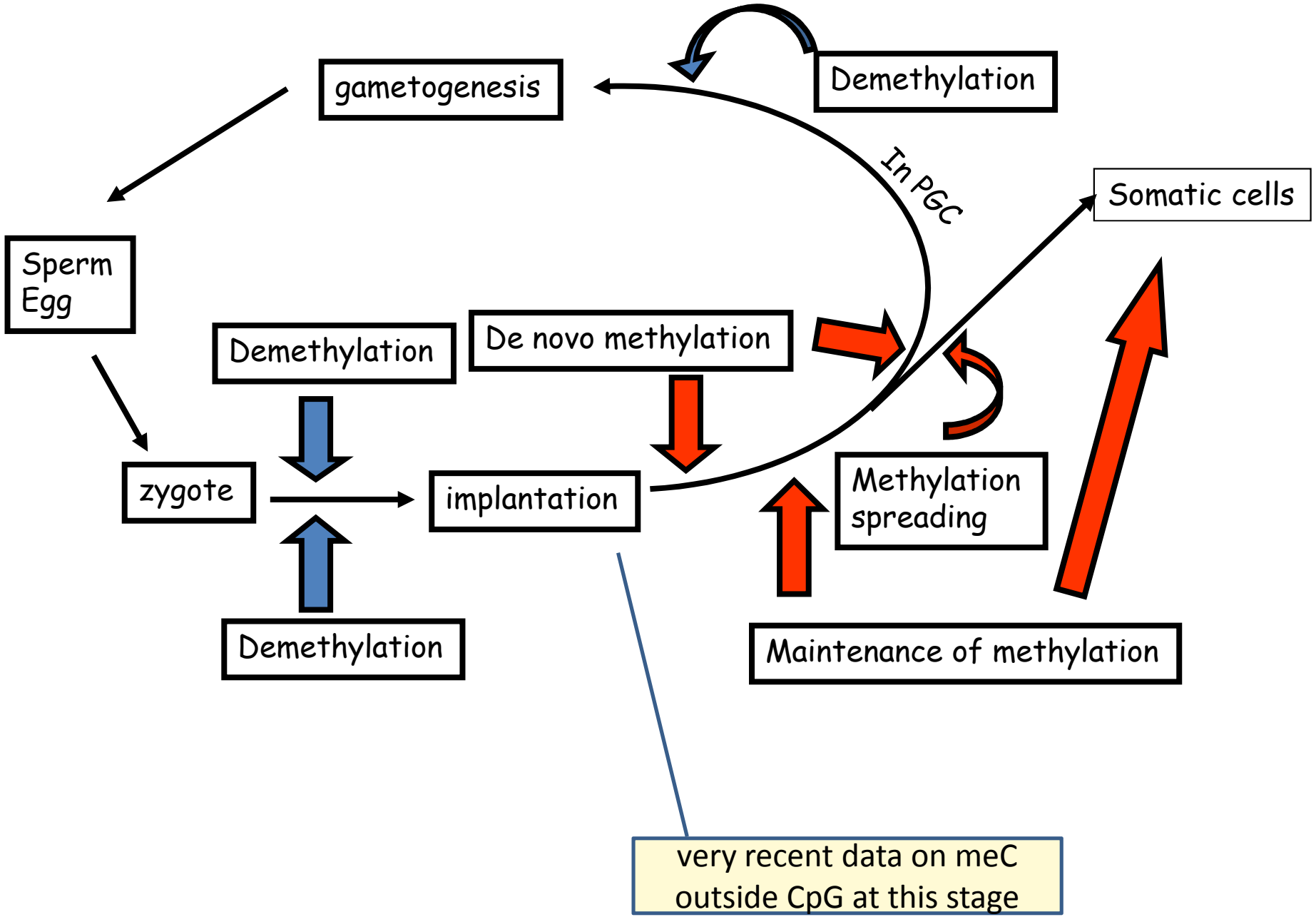
Organisms with the same genome can inherit information in addition to that encoded in the DNA sequence — this is known as epigenetic inheritance. Epigenetic inheritance is responsible for many of the phenotypic differences between different cell types in multicellular organisms. Work by many investigators over the past decades has suggested that a great deal of epigenetic information might be carried in the pattern of post-translational modifications of the histone proteins, although this is not as well established as many believe. For example, it is unclear whether and how the histones, which are displaced from the chromosome during passage of the replication fork and are often exchanged from the DNA template at other times, carry information from one cellular generation to the next. Here, we briefly review the evidence that some chromatin states are indeed heritable, and then focus on the mechanistic challenges that remain in order to understand how this inheritance can be achieved.

REVIEW

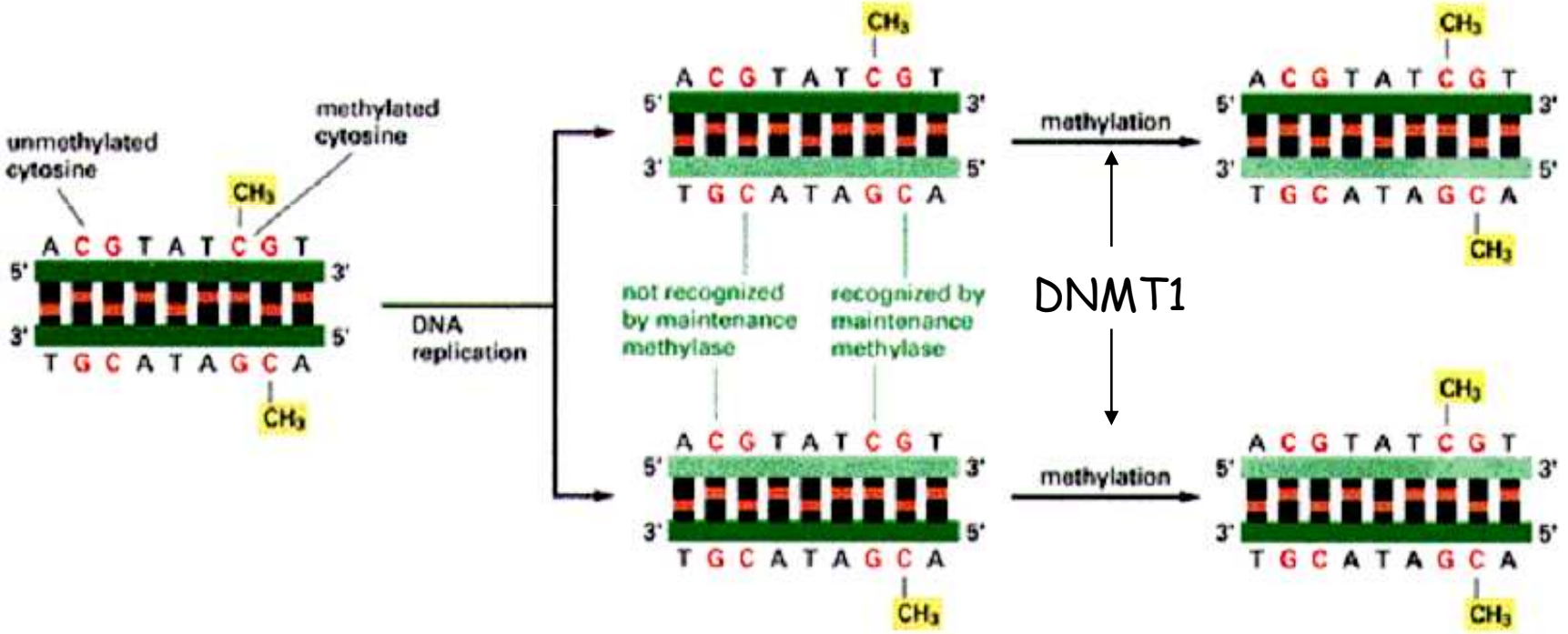
Epigenetic inheritance

Because many definitions of the word 'epigenetic' can now be found in the literature, it is important to start this review by pointing out that we use the term in the Holliday [1] sense — traits that are *mitotically heritable* without a change in DNA sequence are called epigenetically heritable.

DNA CpG methylation in plants and mammales provides a simple paradig how an epigentic mark it is established and mainitained, i.e. how it can be transmitted in somatic cells.


gametogenesis
Demethylation
In PGC
Somatic cells
Sperm
Egg
Demethylation
De novo methylation
zygote
implantation
Methylation
spreading
Demethylation
Maintenance of methylation
very recent data on meC
outside CpG at this stage

The DNMT1 methyl transferase act to maintain the methylation profiles when cells divide, as follows:



Why are methylated genes silenced ?

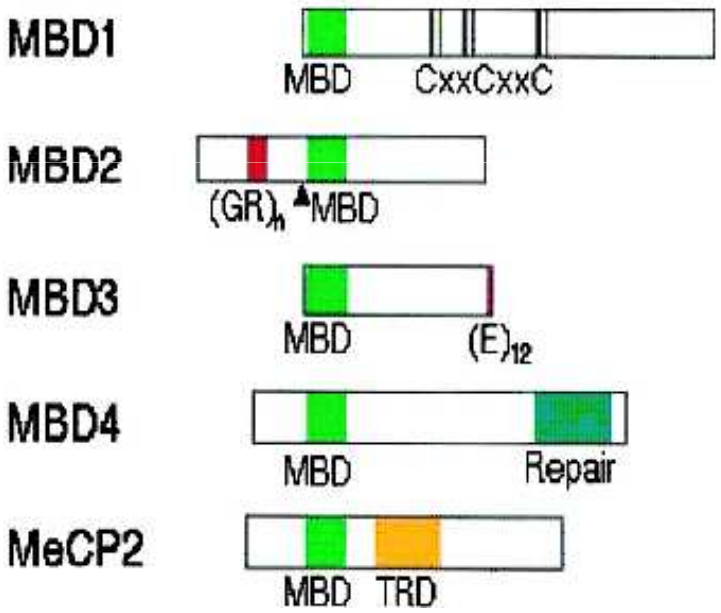
Usually CpG methylation does not limit access of transcription factors to DNA (there are few exceptions).

Is there any link between DNA CpG methylation and histone modifications typical of heterochromatin ?

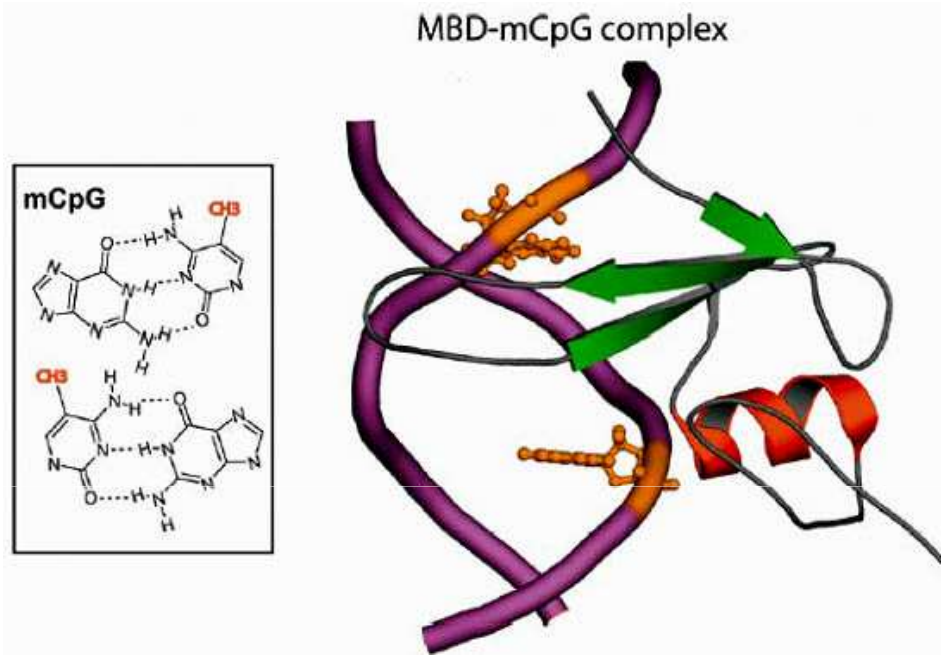
The answer is: YES !

There are several links between DNA CpG methylation and histone de-acetylation and H3-K9 methylation

There are several Methyl CpG binding proteins in eukaryotes, which deserve different functions. The protein involved in heterochromatinization is most likely MeCP2 that possesses both a Methyl CpG Binding Domain (MBD) and a transcriptional repression domain (TRD).

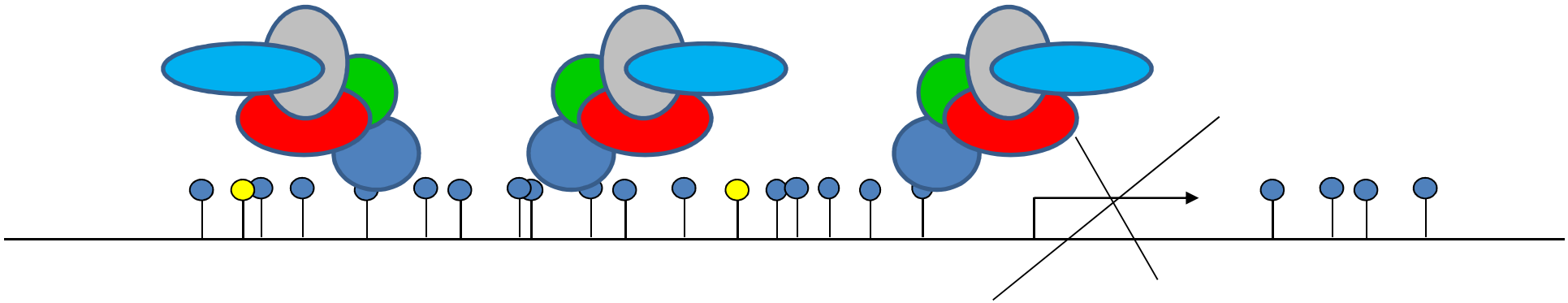


≈100 aa



The structure of Methyl Binding Domain (MBD) in complex with methyl CpG (orange)

CpG methylation is physically linked to campaction of chromatin through the MeCP2 protein and other MBD proteins that bind meCpG and make part of large protein complexes containing Histone Deacetylases (HDAC), Histone methyltransferases (HMT) etc... to the site.



Me Me

…ATTTA**CG**TATGTGAA**CG**TT**CG**ATCATC**CG**TTTATCACTGTATG…

Vol 462 | 19 November 2009 | doi:10.1038/nature08514 nature

Human DNA methylomes at base resolution show widespread epigenomic differences

Ryan Lister[1]*, Mattia Pelizzola[1]*, Robert H. Dowen[1], R. David Hawkins[2], Gary Hon[2], Julian Tonti-Filippini[4], Joseph R. Nery[1], Leonard Lee[2], Zhen Ye[2], Que-Minh Ngo[2], Lee Edsall[2], Jessica Antosiewicz-Bourget[5,6], Ron Stewart[5,6], Victor Ruotti[5,6], A. Harvey Millar[4], James A. Thomson[5,6,7,8], Bing Ren[2,3] & Joseph R. Ecker[1]

DNA cytosine methylation is a central epigenetic modification that has essential roles in cellular processes including genome regulation, development and disease. Here we present the first genome-wide, single-base-resolution maps of methylated cytosines in a mammalian genome, from both human embryonic stem cells and fetal fibroblasts, along with comparative analysis of messenger RNA and small RNA components of the transcriptome, several histone modifications, and sites of DNA–protein interaction for several key regulatory factors. Widespread differences were identified in the composition and patterning of cytosine methylation between the two genomes. Nearly one-quarter of all methylation identified in embryonic stem cells was in a non-CG context, suggesting that embryonic stem cells may use different methylation mechanisms to affect gene regulation. Methylation in non-CG contexts showed enrichment in gene bodies and depletion in protein binding sites and enhancers. Non-CG methylation disappeared upon induced differentiation of the embryonic stem cells, and was restored in induced pluripotent stem cells. We identified hundreds of differentially methylated regions proximal to genes involved in pluripotency and differentiation, and widespread reduced methylation levels in fibroblasts associated with lower transcriptional activity. These reference epigenomes provide a foundation for future studies exploring this key epigenetic modification in human disease and development.

EPIGENOMICS

Methylation matters

Dirk Schübeler

Genome-wide maps of methylated cytosine bases at single-base-pair resolution in human cells reveal distinct differences between cell types. These maps provide a starting point to decode the function of this enigmatic mark.

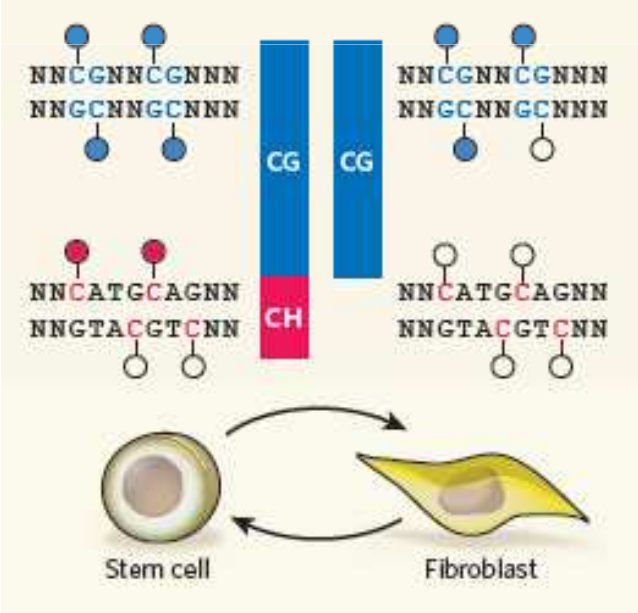


Figure 1 | DNA methylation patterns differ between stem cells and differentiated cells[1]. In stem cells, regions of DNA with CpG methylation (blue) are mostly uniformly methylated, whereas this modification is more heterogeneous in fibroblasts. Non-CpG methylation (red), which occurs primarily at CA nucleotides, is detected only in stem cells, yet is asymmetric and more scarce and patchy than CpG methylation. If fibroblasts are converted to induced pluripotent stem cells they regain non-CpG methylation. Filled circles, methylated cytosines; unfilled circles, unmethylated cytosines. H stands for A, C or T; N stands for any nucleotide.

CpG methylation partecipate in epigenetic inheritance but is not the (unique) (principal) mechanism

First, this is true in plants and mammalians, but other organism do not use CpG methylation to this purpose or do not use CpG methylation at all.

Second, heterochromatic CpG hypermethylation is characteristic of promoter CpG islands but many heterochromatic regions have no CpG island

The other modifications that we know and correlate with active/inactive status are histone PTMs, however....

Whereas DNMT1 activity easily explains how CpG methylation profiles are maintained at DNA replication,

....we need a mechanisms explaining how histone PTMs are “replicated” together with DNA replication

Chromatin structure and the inheritance of epigenetic information

Raphaël Margueron and Danny Reinberg

Abstract | Although it is widely accepted that the regulation of the chromatin landscape is pivotal to conveying the epigenetic program, it is still unclear how a defined chromatin domain is reproduced following DNA replication and transmitted from one cell generation to the next. Here, we review the multiple mechanisms that potentially affect the inheritance of epigenetic information in somatic cells. We consider models of how histones might be recycled following replication, and discuss the importance of positive-feedback loops, long-range gene interactions and the complex network of *trans*-acting factors in the transmission of chromatin states.

Review

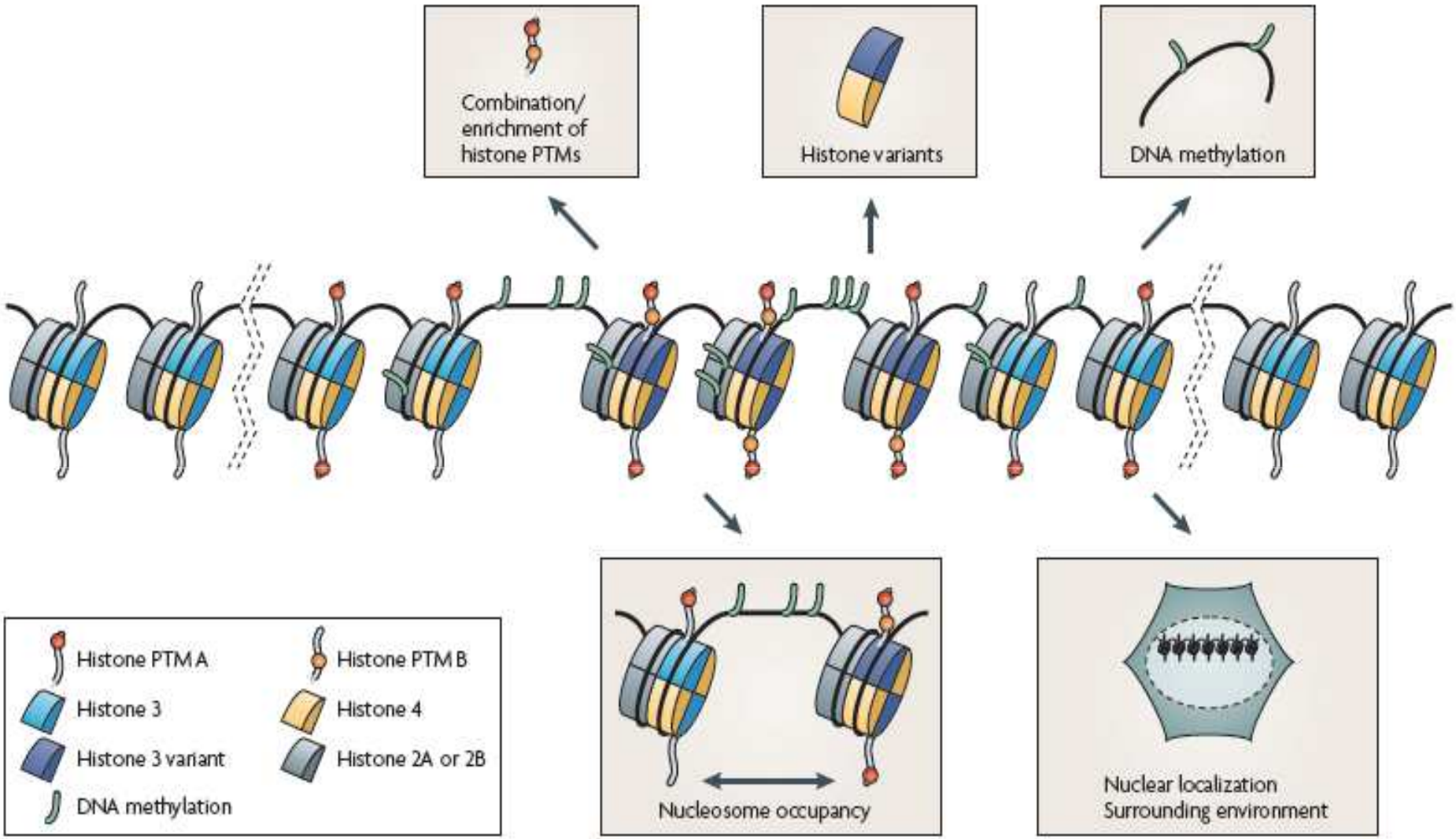


Figure 1 | **Characteristics of a chromatin domain**. Schematic depicting modifications that define different chromatin domains. The range of factors that can contribute to the characteristics of a domain are shown in the shaded boxes. The dashed lines represent the separation between two adjacent domains. PTM, post-translational modification.

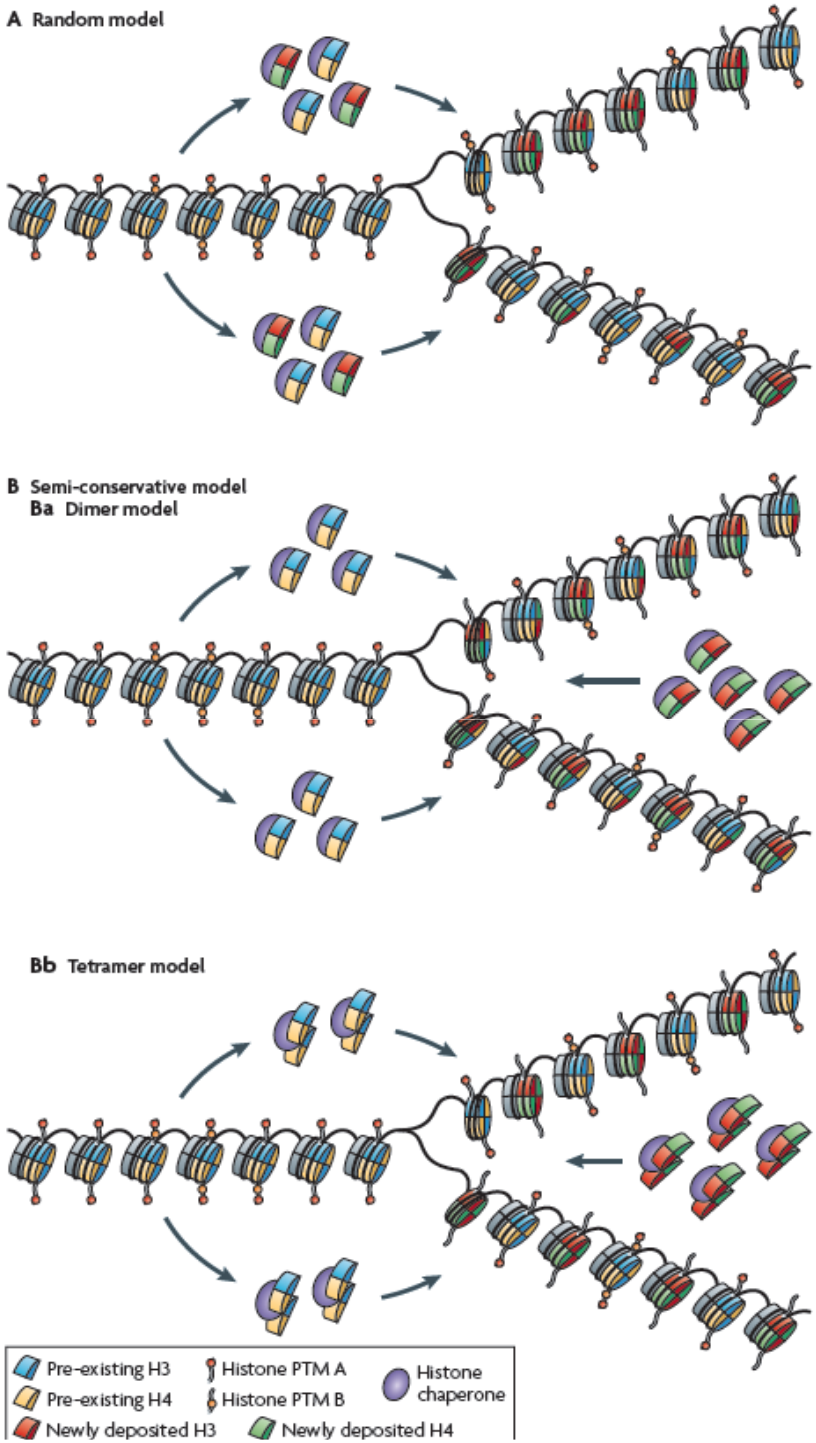


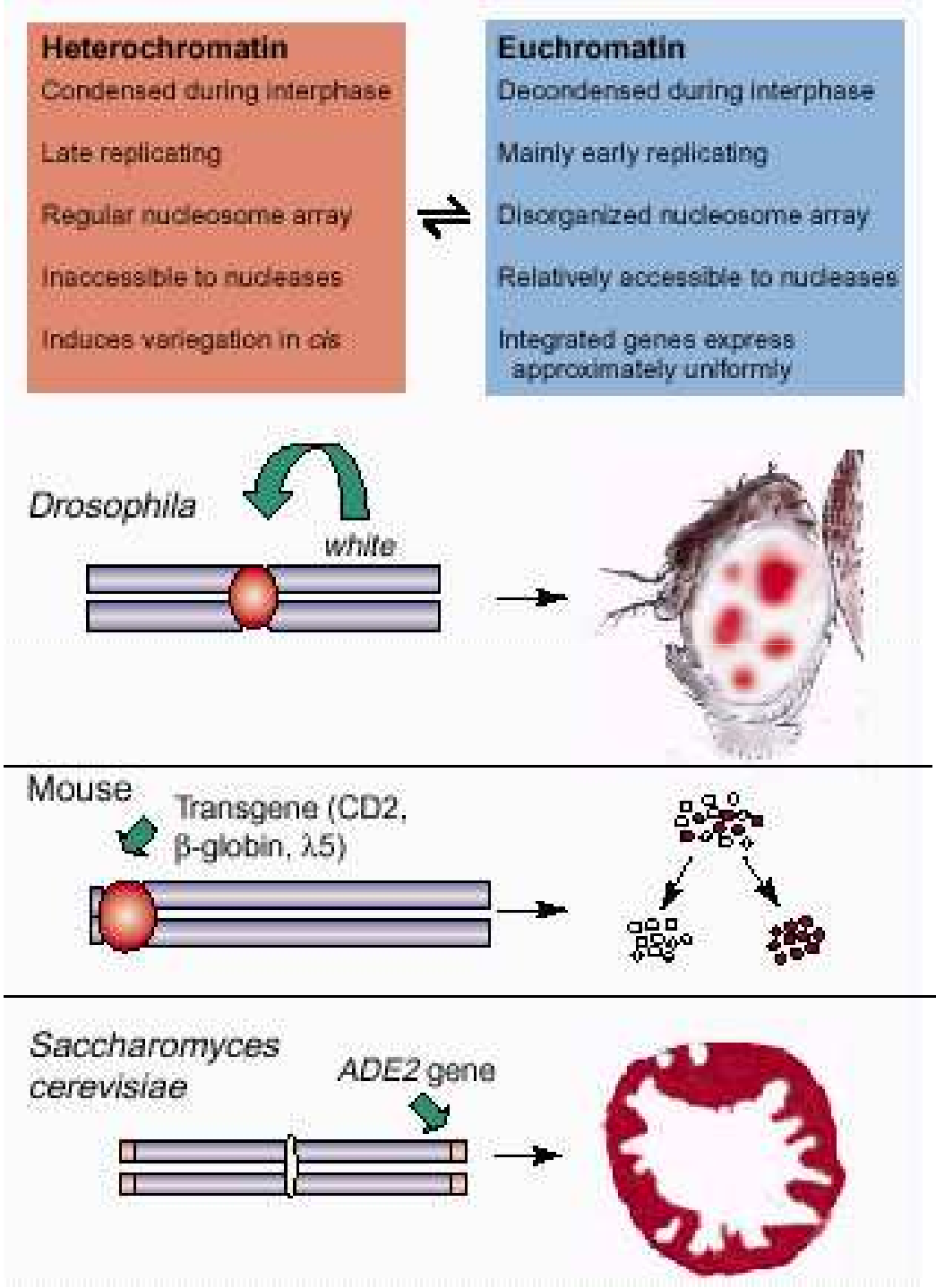
Figure 3 | Models of histone deposition during replication. Here, we illustrate the means by which old and new histones might be deposited following replication.
A | Random model of histone segregation. In this model, we do not discriminate between dimer or tetramer deposition. The histones segregate randomly between the leading and lagging replicated DNA strands.

B | Semi-conservative model of histone segregation.
Ba | the scheme is based on the assumption that the histone 3 (H3)–H4 tetramer is divided during DNA replication and the parental H3–H4 histones segregate as dimers onto the newly replicated DNA strands. The parental histones associate with naive dimers to reconstitute the tetramer.
Bb | This scheme posits that parental H3–H4 histones segregate as tetramers, resulting in the joint deposition of recycled histones and newly deposited naive histones. PTM, post-translational modification.

A very old discovery of Genetics , “PEV” (positional effect variegation),

illustrated a very interesting property of Heterochromatin:

“spreading” or “diffusion”

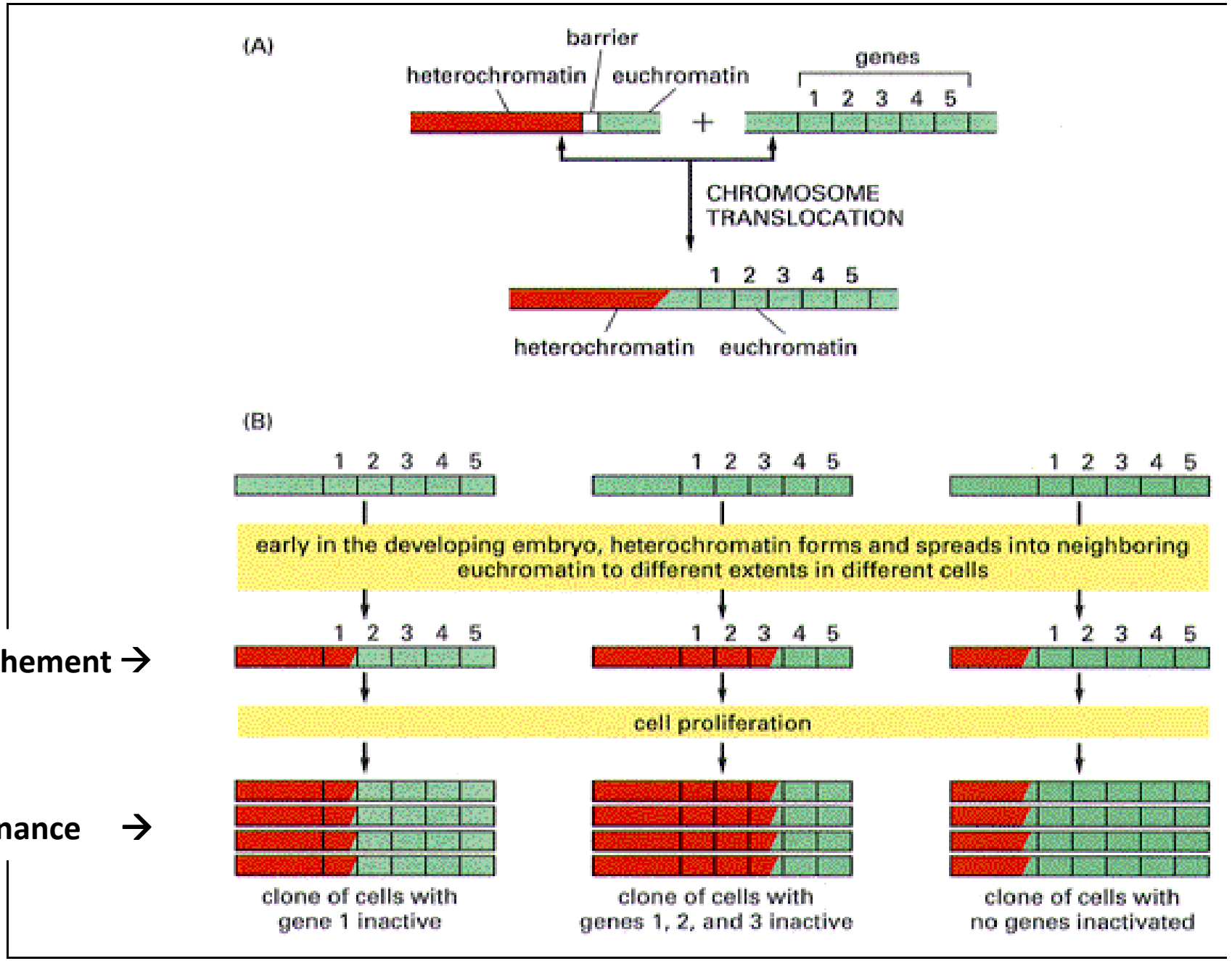


PEV = Positional Effect Variegation
Silencing effects of heterochromatin in different organisms.

Translocation of the Drosophila *white* gene close to the centromere results in mosaic (variegated) expression in the eye.

Insertion of transgenes into peri-centromeric regions in mice gives expression in a proportion of cells from expressing tissues. Expression is clonally stable through multiple cell divisions but also undergoes stochastic switching between the active and inactive states.

Insertion of the Ade2 gene into the telomeric region of Saccharomyces cerevisiae causes metastable silencing of the gene resulting in sectored colonies containing white (expressing) and red (nonexpressing) cells.

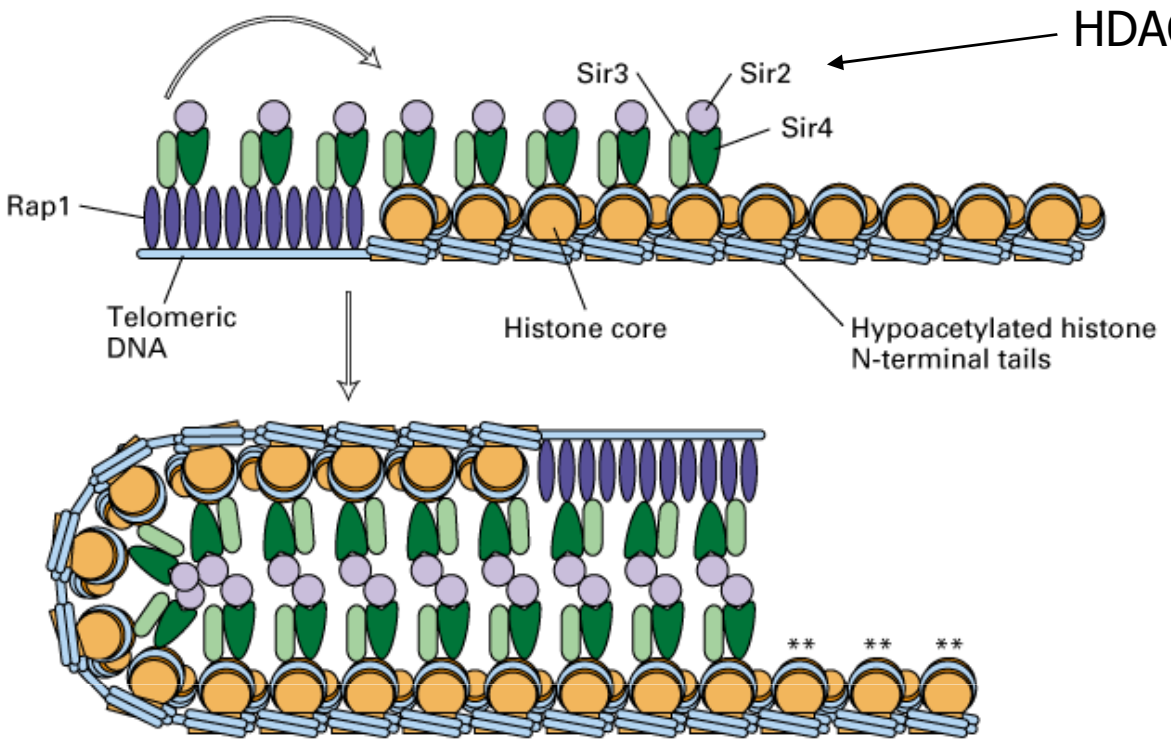


Establishement →

Maintenance →

Mechanisms operating at centromeres, telomeres ad other heterochromatic loci are similar, but not identical.

Nonetheless, PEV is seen in all these contexts



replicazione dei cromosomi lineari

struttura dei telomeri

telomerasi

budding yeast

Model for the formation of telomeric heterochromatin. Black lines wrapped around nucleosomes represent DNA.

(a) Core telomeric heterochromatin in wild-type cells containing only a single genomic copy of SIR3. It is proposed that the RAP1-containing telosome folds back onto subtelomeric regions. In this manner, RAP1–SIR–histone interactions are all required for stability of the complex.

(b) Upon SIR3 overexpression, telomere position effect and the presence of SIR3 is extended up to some 16–20 kb from the telomere. SIR3 overexpression causes loss of some SIR4 and most SIR2 from the complex. Due to the interdependence of RAP1–SIR3–H4 interactions, and because all three SIR proteins are required for extension of heterochromatin by SIR3, it is proposed that the complex necessary for the initiation of heterochromatin formation requires RAP1, the SIR proteins and H4.

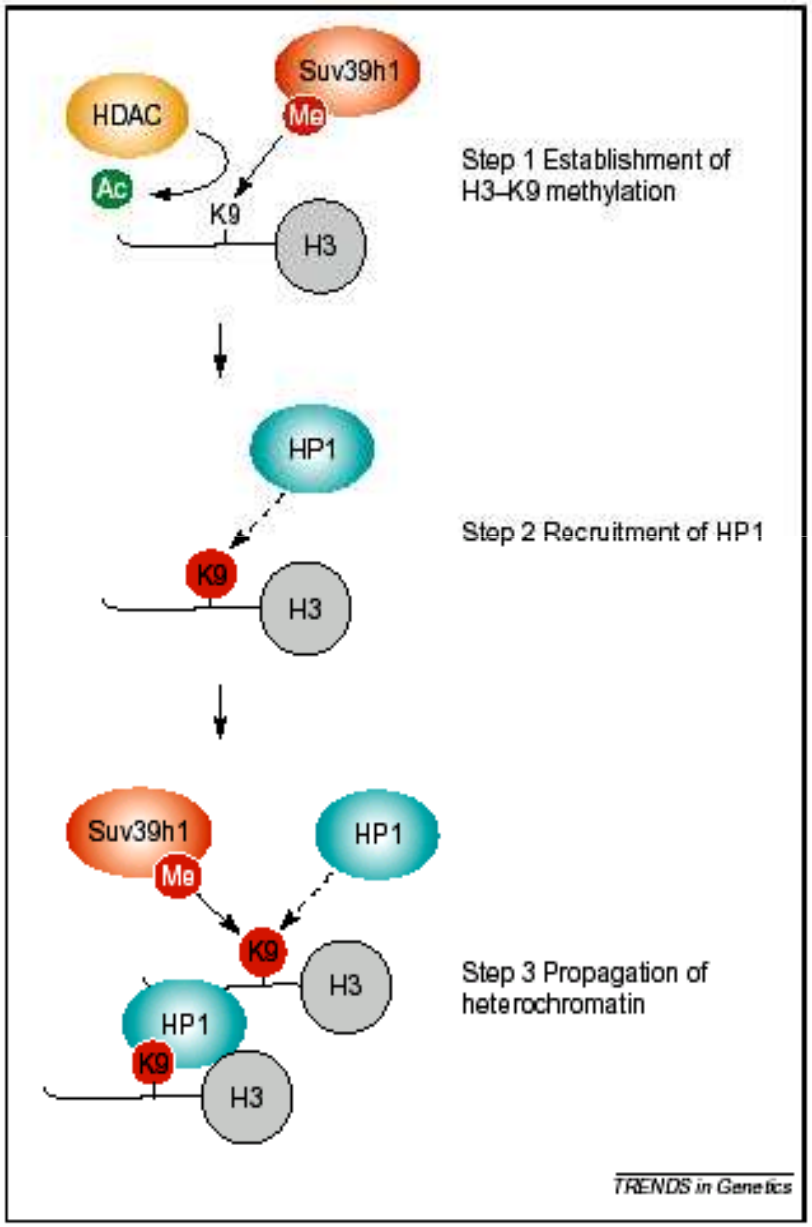
How does heterochromain “spread” ?

The simplest model is that proteins that modify histones (and CpG) show reciprocal **interaction**, so that one modification of a specific position in histones can bring about other coordinated modifications, either at other positions in histones making part of the same nucleosome, or in adjacent nucleosomes, or both.

Two principles:
1. Extensive network of protein-protein interaction between HAT, HDAC, HMT, demethylases, DNMT, and modification-binding proteins, such as HP-1.
This has been verified by c o-immunoprecipitation and re-ChIP

2. Modular structure of chromatin-interacting proteins, leading to double- or triple-function in individual proteins.

Example: interaction between HDAC, Suv39H1 and HP1



Active genes have HATs locally that acetylate many histone positions, especially H3-K9.

Acetylation of H3-K9 prevents H3-K9 methylation; thus, **step one** involves the deacetylation of H3-K9 by specific HDACs, and the subsequent methylation by a histone lysine acetyltransferase (HKMT), such as Suv39H1.

In **step two**, HP1 selectively recognizes methylated H3-K9 through its **chromodomain**

Step three involves propagation of heterochromatin through HP1 recruitment of Suv39H1 via protein association. Suv38H1 is one methyltransferase specific for H3-K9

Once nearby H3-K9 sites are methylated, additional heterochromatin-associated protein 1 (HP1) molecules recognize this mark through its chromo-domain. HP1 then recruits Suv39H1 through protein interactions recruiting further H3-K9 methylation activity.

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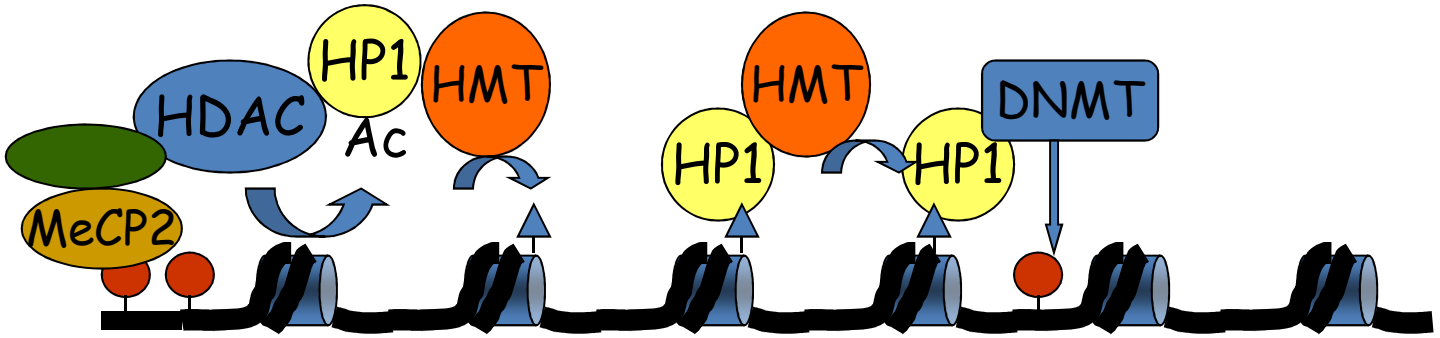
A model for heterochromatin establishment and propagation



Ac – acetyl groups

- methyl lysine 9 (H3)

- methyl CpG

The epigenetic code is read by multiprotein complexes that use

a defined set of “modules” or “domains” to recognize CpG methylations, histone modifications

and

a defined set of enzymes to introduce further modifications and/or maintain the chromatic status.

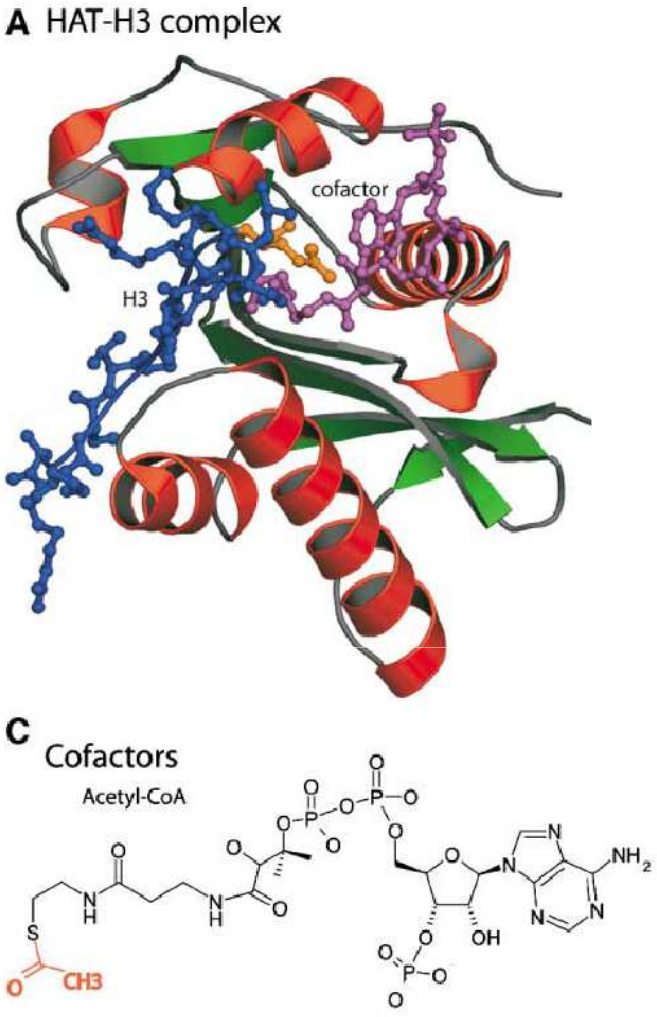


Figure 5. Lysine Acetylation and Deacetylation

(A) A HAT domain is shown in complex with a substrate H4 tail (blue) and its cofactor (purple) (Clements et al., 2003). The substrate lysine is shown in orange.

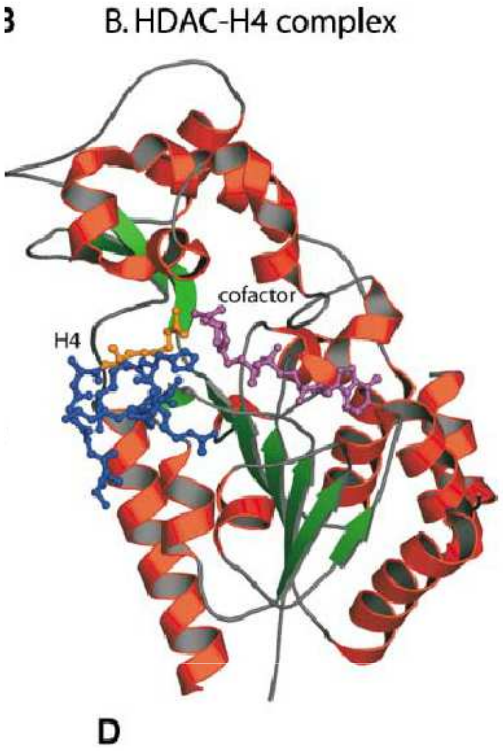


Figure 5. Lysine Acetylation and Deacetylation

(B) The structure of yeast Hst2 deacetylase (Sir2 homolog) in complex with H4 substrate (blue) and cofactor (purple) (Zhao et al., 2003). The substrate acetyllysine is shown in orange.

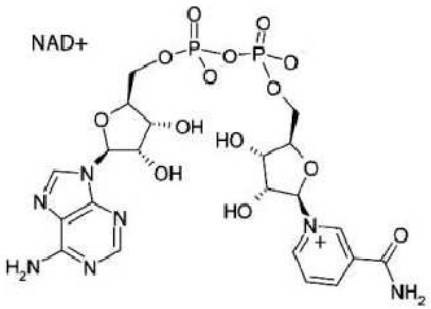
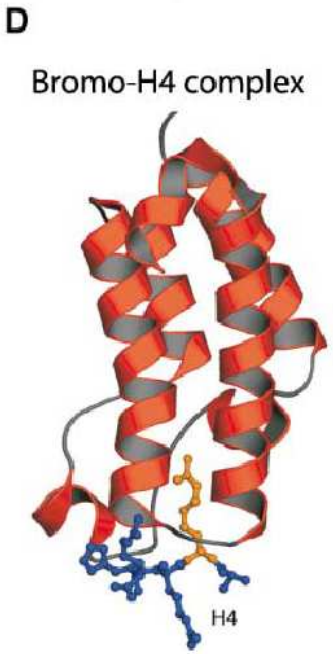


Figure 5. Lysine Acetylation and Deacetylation

(D) The bromodomain of Gcn5 in complex with the H4 tail (blue) with acetyllysine 16 (orange) (Owen et al., 2000).



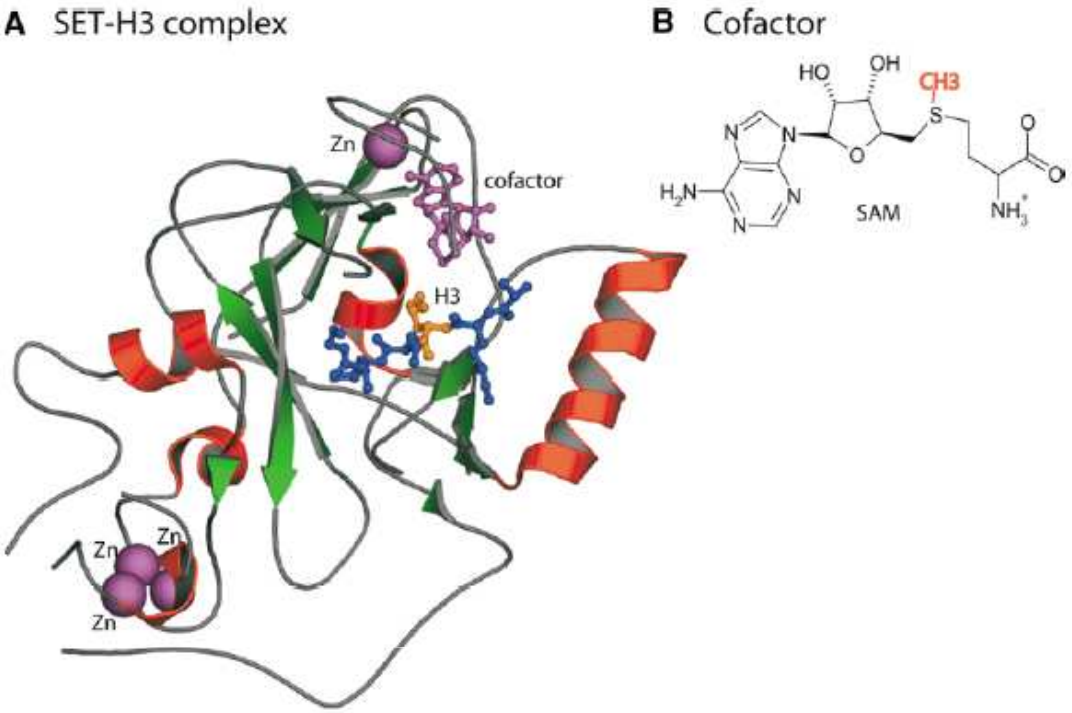


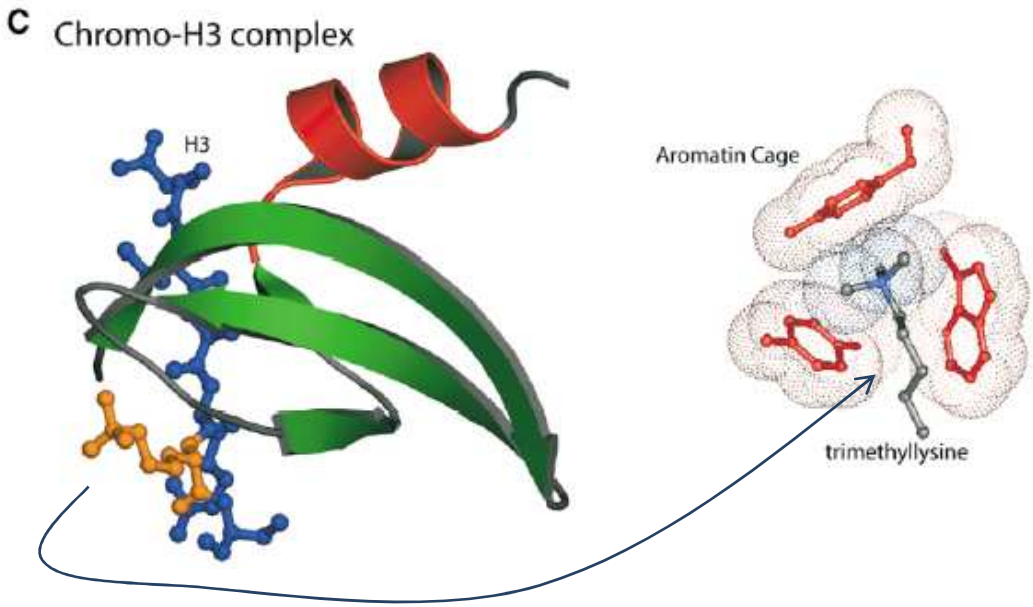
Figure 6. Lysine Methylation

(A) The structure of DIM5, a homolog of SUV39H1, which methylates lysine 9 (orange) in H3, is shown in complex with H3 tail (blue), cofactor (purple), and four zinc ions (pink) (Zhang et al., 2003). The zinc ions stabilize the pre-SET and post-SET regions, both of which are important for catalytic function.

(B) The cofactor S-adenosyl-L-methionine.

Figure 6. Lysine Methylation

(C) HP1 chromodomain in complex with H3 tail (blue) with methyllysine 9 (orange) (Jacobs and Khorasanizadeh, 2002). An aromatic cage recognizes the methyllysine moiety.



Principal characteristics of chromatin-interacting proteins

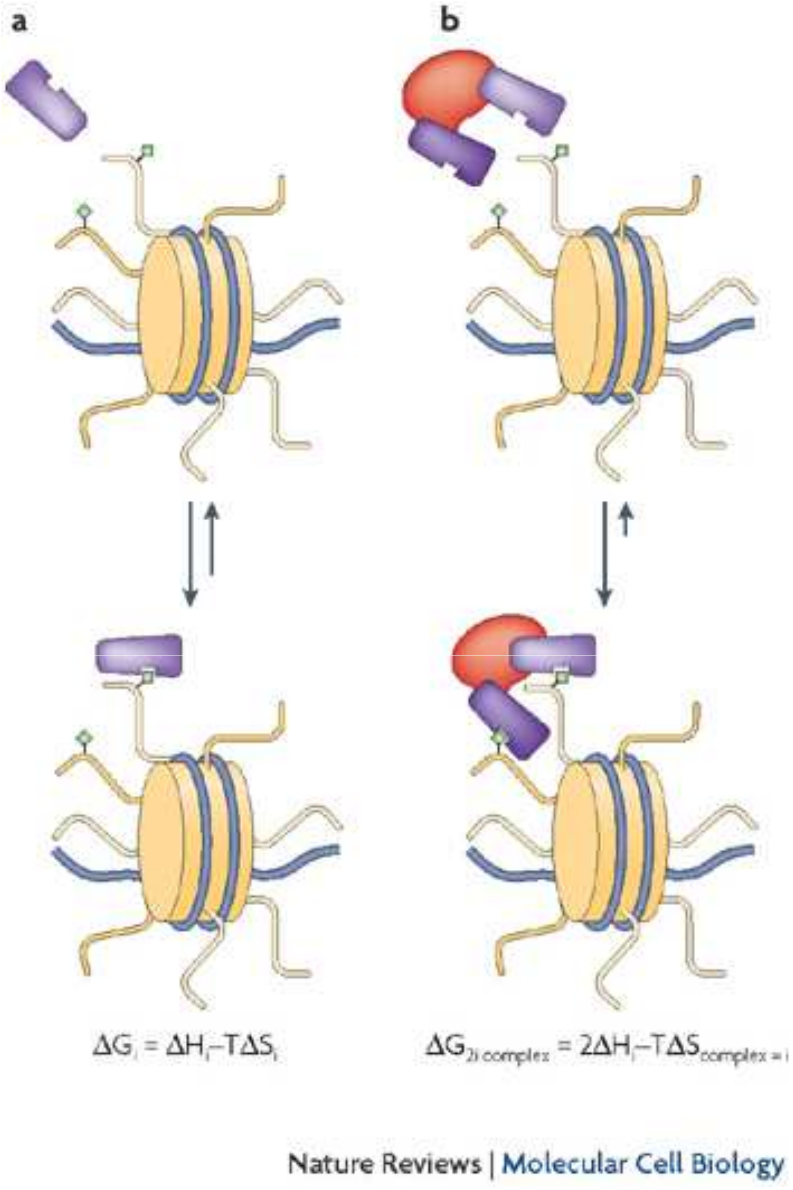
1. “Modules” or “domains” that recognize specific histone position / PMT are present in a plethora of chromatin metabolizing enzymes and proteins

2. These modular proteins are always present in megadalton, heteromeric and multifunctional complexes

Ruthenburg et al., 2007, Nat. Rev. Mol. Cell Biol. 8: 983.

Histone marks	Locus/chromatin state	Method
H3K4me2/3 + H4K16ac	Transcriptionally active homeotic genes	ChIP[122]
H3K4me2/3 + H3K9/14/18/23ac	Transcriptionally active chromatin	MS[125]
H3S10ph + H3K9/14ac	Mitogen-stimulated transcription	Ab[9,10] and MS[126]
H3R17me1/2a + H3K18ac	Oestrogen-stimulated transcription	Ab[127]
H4K5ac + H4K12ac	Pre-deposition	Ab and MS[128]
H3K4me3 + H3K27me3	'Bivalent domains' at key developmental genes	ChIP-chip, reChIP[22]
H3K9me3 + H3K27me3 + 5-MeC	Silent loci	ChIP, bisulphite sequencing[91,129], MS
H3K27me3 + H2AK119ub1	Silent homeotic genes	ChIP, ChIP-chip[41,89]
H3K9me3 + H4K20me3 + CpG 5-MeC	Heterochromatin	IF and nucleosomal CoIP[130], ChIP-seq[26] and bisulphite sequencing
H3K9me2/3 + H4K20me1 + H3K27me3 + CpG 5-MeC	Inactive X-chromosome	ChIP, ChIP-chip, reChIP[24], bisulphite sequencing

5-MeC, 5-methylcytosine; Ab, specific antibody in western blot; ac, acetyl; ChIP, chromatin immunoprecipitation; ChIP-chip, ChIP followed by amplification and microarray hybridization; ChIP-seq, ChIP followed by massively multiplexed sequencing; CoIP, co-immunoprecipitation; CpG, the DNA sequence that is often targeted for epigenetic 5-cytosine methylation; IF, immunofluorescence; me, methyl; MS, mass spectrometry; ph, phosphoryl; reChIP, ChIP with two sequential immunoprecipitations with different antibodies; Rme2a, asymmetric dimethylated Arg; ub, ubiquitin.



a | Monovalent association of a hypothetical effector module (purple) to a chromatin substrate (yellow tail with green diamond) is simplistically compared to a bivalent association of the same effector in a complex, representing the lowest order of multivalent interactions. The change in free energy G_i for the monovalent system undergoing binding is indicated by the change in enthalpy H_i minus the change in entropy S_i, scaled by the temperature (T). b | By tethering the two effector modules, the entropy term may be, to a first approximation, similar for each of the binding equilibria in panels a and b ($TS_{complex}$ TS_i). For our purposes, this example assumes that hydrogen-bonding electrostatic interactions dominate and desolvation is negligible, so that S for the system will be negative. Thus, the entropic penalty to binding is lessened approximately twofold by pre-organizing the effector domains in a complex ($TS_{complex}$ TS_i), while the enthalpy of the bivalent domain interaction is effectively double that of the monovalent case ($2H_i$, if enthalpic penalties due to the strain induced by bivalent binding are negligible). In this manner, the reduced net entropy loss for the binding process can be a significant determinant of free energy, especially in low-binding enthalpy regimes. Losses of entropy on the substrate side would be expected to be minimal due to the low intrinsic rotational and translational freedom of chromatin; however, conformational entropy losses here are assumed to be negligible for simplicity.

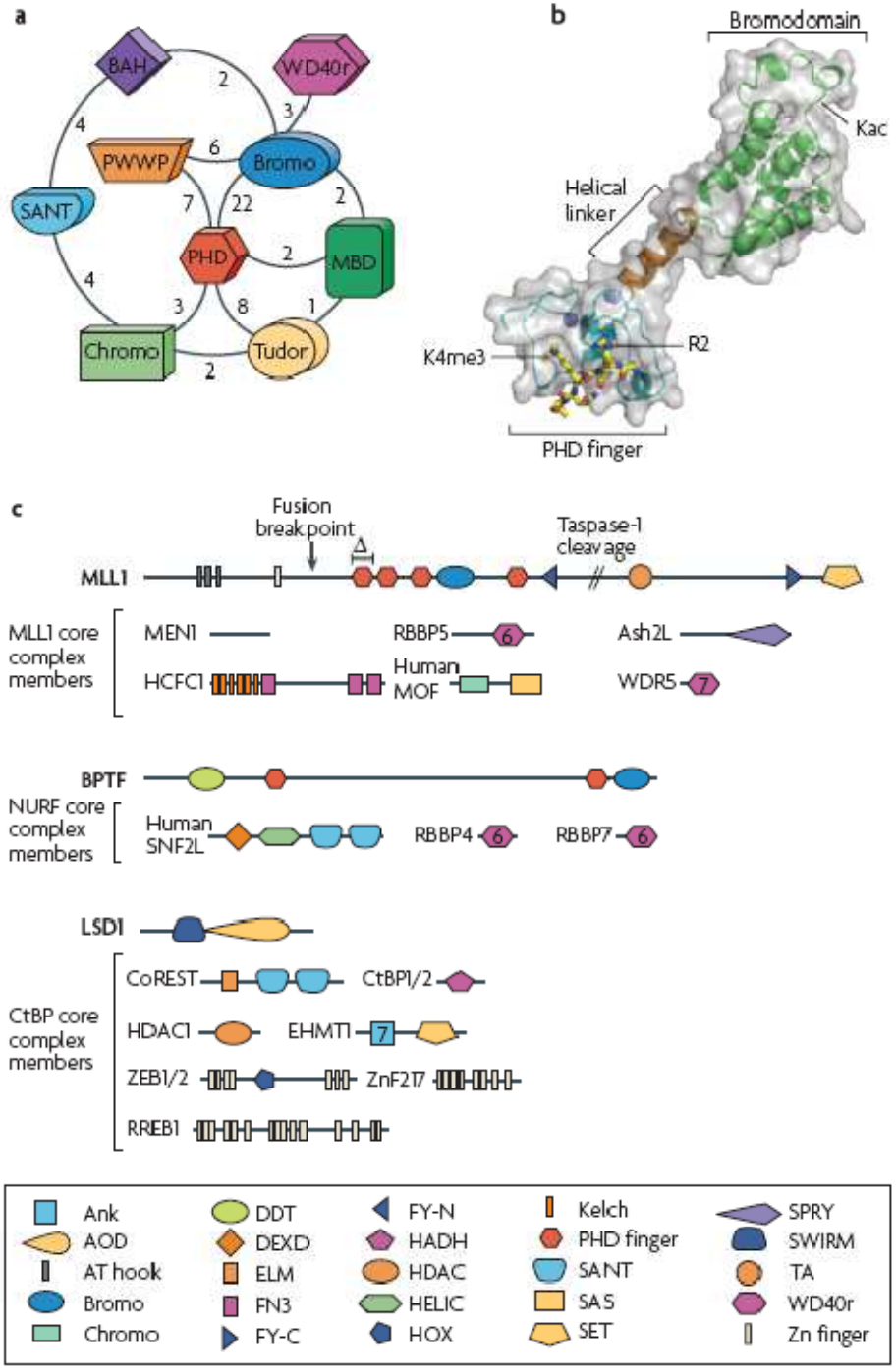
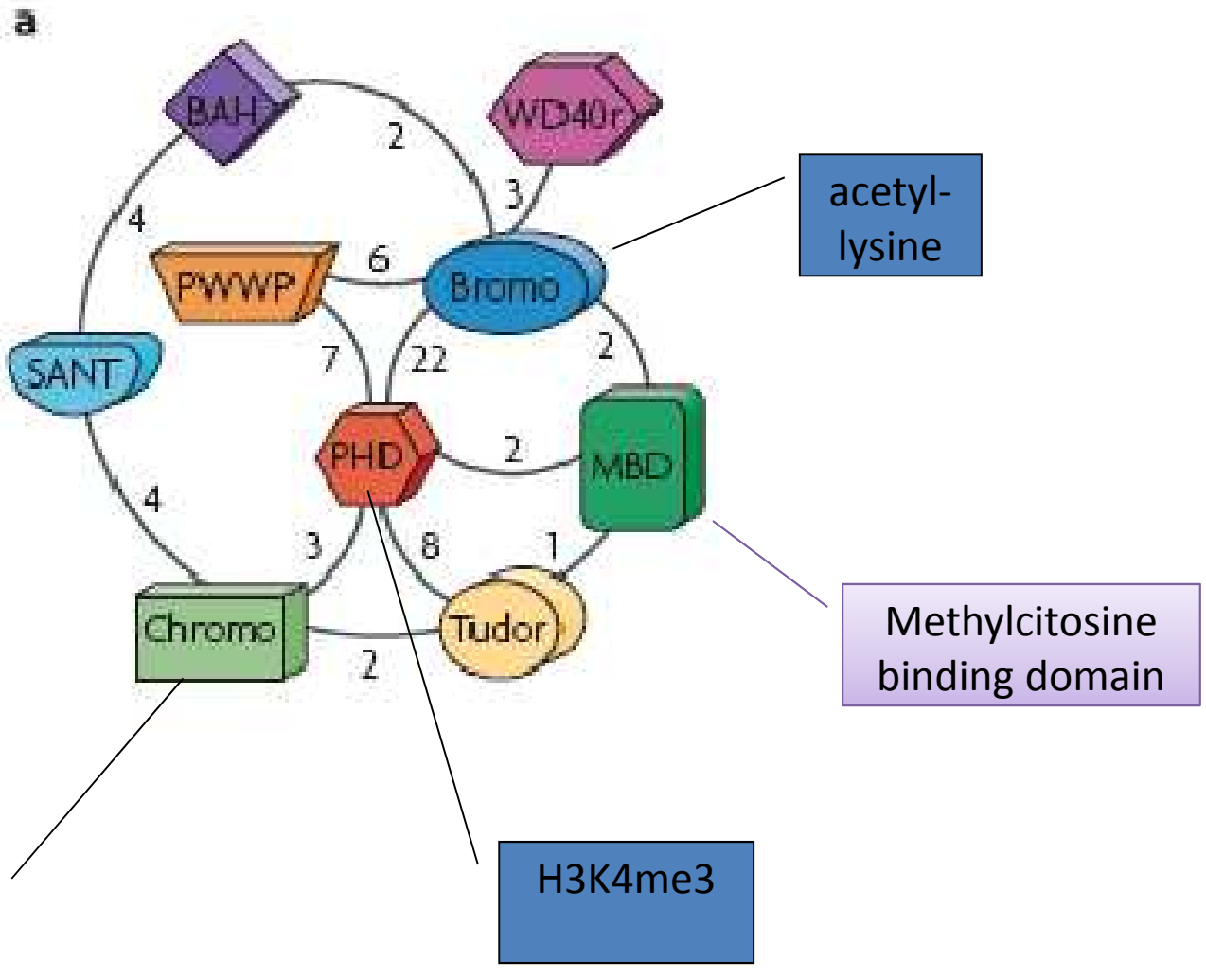


Figure 2 | **Polypeptides with many putative effector modules and representative complexes. a** | The coexistence of possible effector module domains within single polypeptides is depicted schematically, with the number of instances of linkage for any two domains within the human proteome listed near the line connecting them. The SMART database was used as the source of these linkages, and redundant entries were removed. **b** | A structurally characterized example of two linked effector domains is provided by the structure of a BPTF module that comprises a PHD finger, a helical linker and a bromodomain, with a trimethylated Lys4 of histone H3 (H3K4me3) peptide bound to the PHD finger[85]. The acetyl-Lys (Kac)-binding pocket on the bromodomain is shown, as well as residues R2 and K4me3 of the H3 peptide. **c** | Chromatin metabolism complexes, exemplified by the MLL1 (REF. 122), NURF[103,123] and CtBP[11] core complexes, have multiple putative effector domains. The predicted domain structure of subunits of the complex members are shown as a linear arrangement from N to C terminus. Chromatin-associated domains, most of which are modification sensitive, are coloured as in panel **a**, and are shown with additional predicted domains given in the key. The portion of the MLL1 protein that is cleaved by taspase-1 to yield two functional fragments (MLL1-N and MLL1-C) is shown. A frequent breakpoint at which fusion partners are appended and a domain deletion (Δ) that causes certain leukaemias are also depicted on the MLL1 domain structure. Ash2L, Set1–Ash2 histone methyltransferase complex subunit; BAH, bromo-adjacent homology domain; BPTF, bromodomain PHD finger transcription factor; Bromo, bromodomain; Chromo, chromodomain; CoREST, corepressor to the RE1 silencing transcription factor; CtBP, C-terminal binding protein; EHMT1, euchromatic histone-Lys *N*-methyltransferase-1; HCFC1, host cell factor C1; HDAC1, histone deacetylase-1; LSD1, Lys-specific demethylase-1; MBD, methyl-CpG binding domain; MEN1, multiple endocrine neoplasia-1; MLL1, mixed lineage leukaemia; MOF, males absent on first histone acetyltransferase; NURF, nucleosome remodelling factor; PHD, plant homeodomain; PWWP, PWWP motif protein of the Royal superfamily; RBBP, retinoblastoma binding protein; RREB1, Ras responsive element binding protein-1; SNF2L, sucrose non-fermenting-2-like ATPase; WD40r, WD40 repeat; WDR5, WD repeat domain-5; ZEB1/2, zinc finger E-box binding homeobox-1/2; ZnF217, zinc finger protein-217.


a
BAH
WD40r
PWWP
Bromo
SANT
PHD
MBD
Chromo
Tudor
2
3
4
6
7
22
2
2
4
3
8
1
2
acetyl-lysine
Methylcitosine binding domain
methyl-lysine
H3K4me3

"spreading".... indefinitely ?

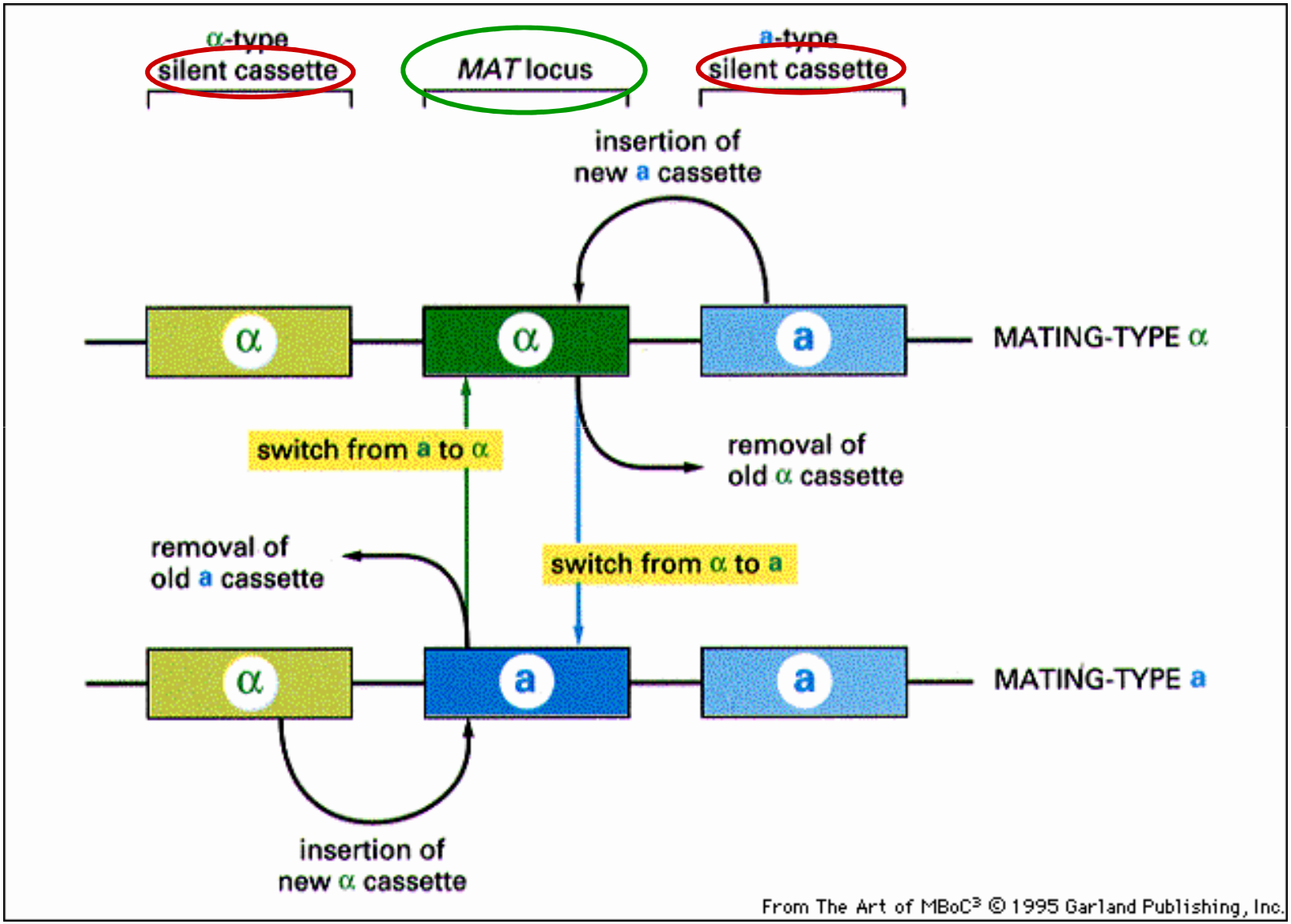
Elements that promote heterochromatin are called "silencers"

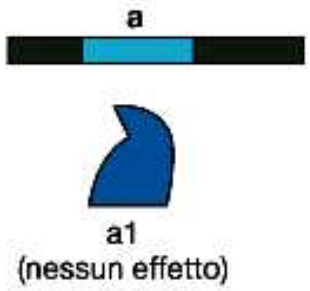
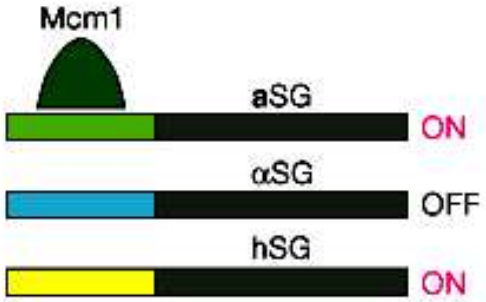
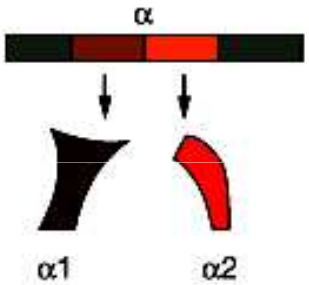
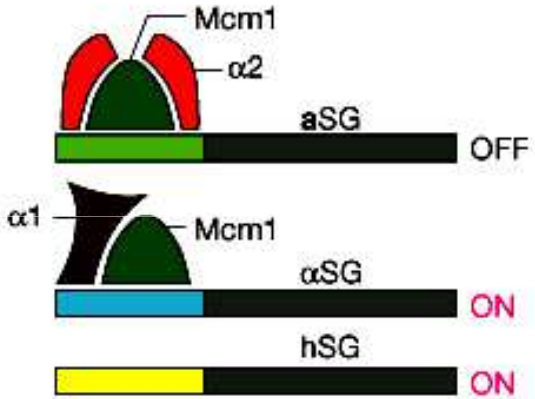
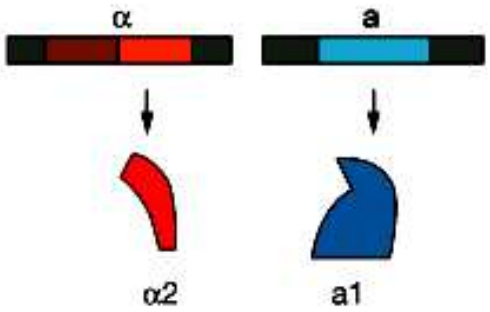
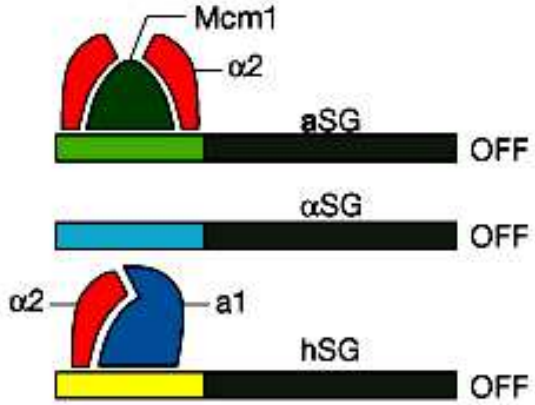
Elements that stop heterochromatin spreading are called "insulators" or "boundaries"

Boundaries of heterochromatin are cis-elements regulated by trans-factors

Chromosomal boundaries in S. cerevisiae
Xin Bi* and James R Broach †
Current Opinion in Genetics & Development 2001, 11:199–204

HMR mating locus in yeast has heterochromatic features, e.g. is resistant to endonuclease digestion and **silences constructs placed within**

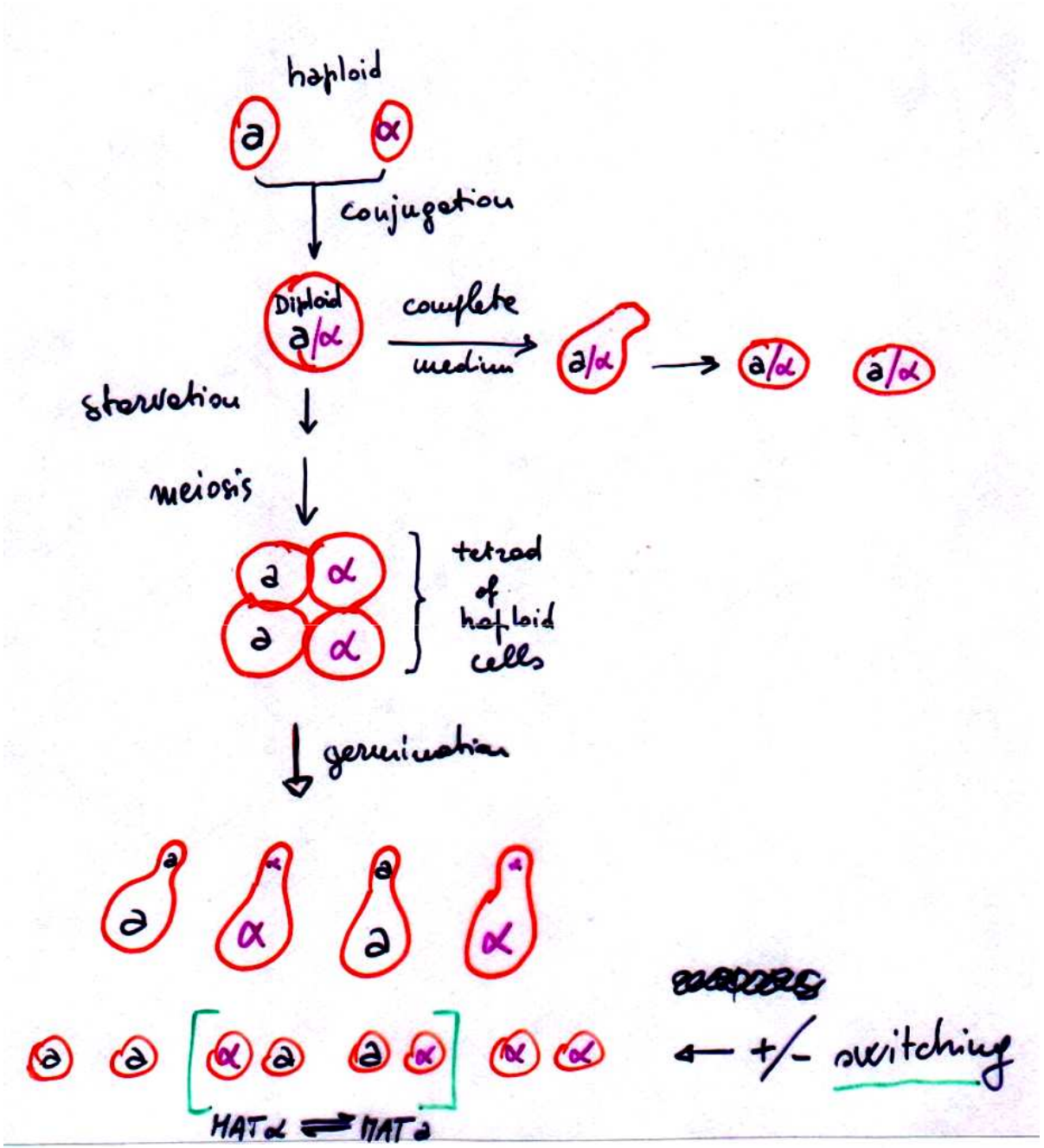


proteine regolatrici prodotte dal locus MAT	tipo cellulare	serie di geni controllate da *MAT*
	a (aploide)	
	α (aploide)	
	a/α (diploide)	

Saccaromyces cerevisiae
(budding yeast)

Most studies on HC made in yeasts (S. cerevisiae / S. pombe)

Very easy, since homologous recombination is favoured in yeasts and allows HC elements (i.e. HC organizers, silencers, boundaries, etc.) or reporters to be placed at will, in any genomic locus.



Gene targeting by homologous recombination in yeast

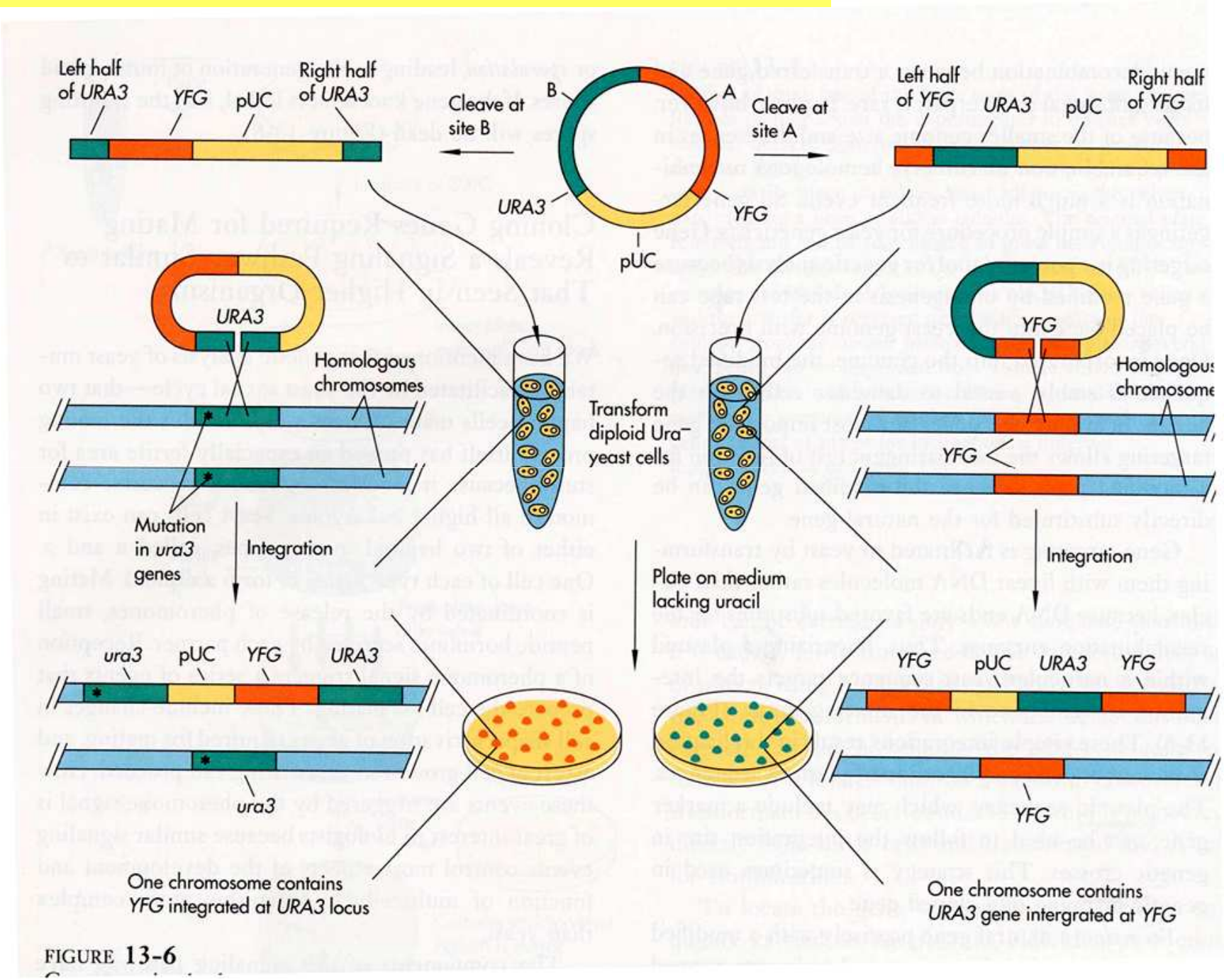
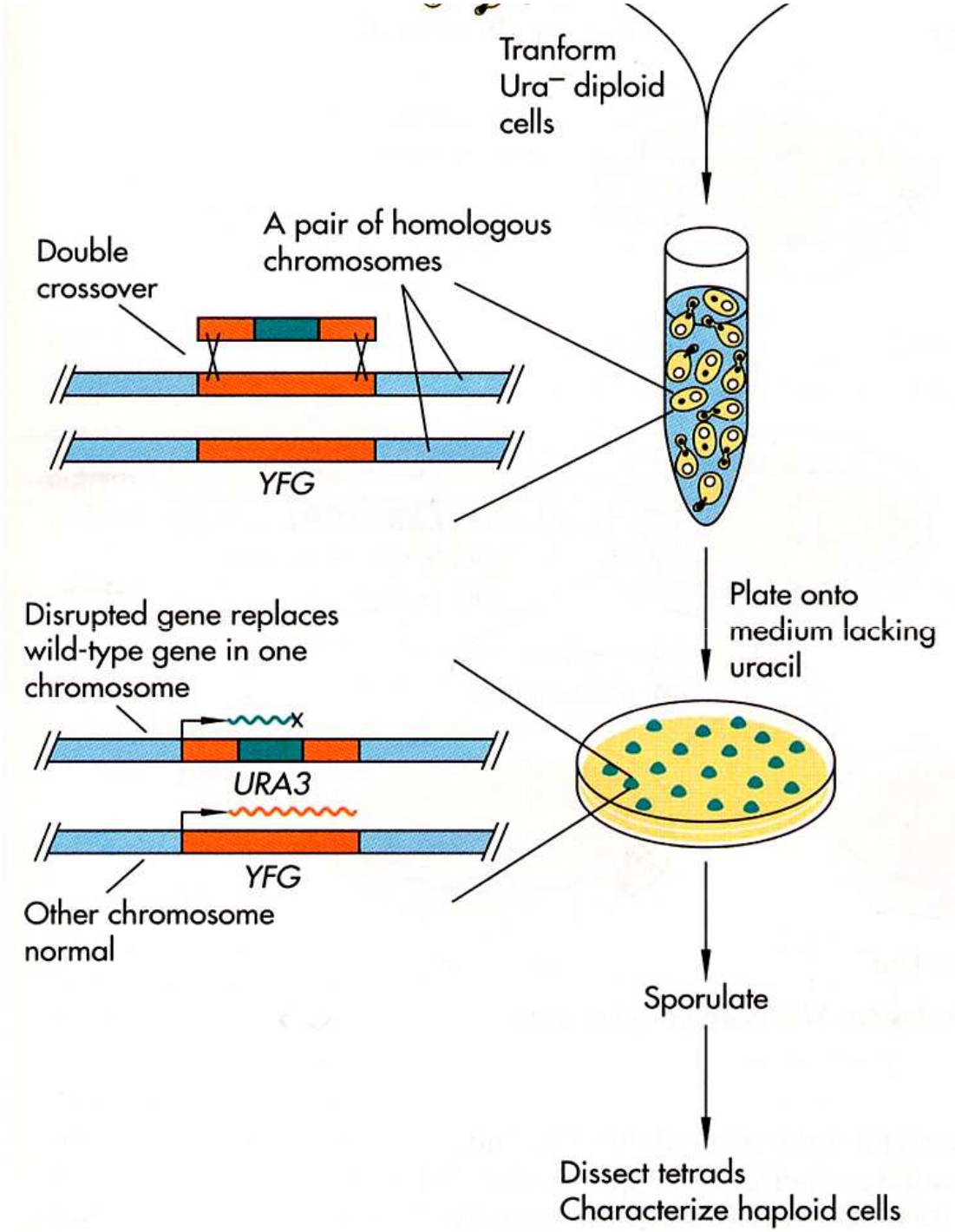


FIGURE 13-6



Replacement of a gene with a version of the same gene that lacks important parts or carryes lethal mutations, abolishes the function of the gene.

The gene is now called "knock-out".

Since the yeast is diploid, his genotype will be YFG +/-
If aploid are produced and mutants isolated, a diploid YFG -/- mutants can be isolated.

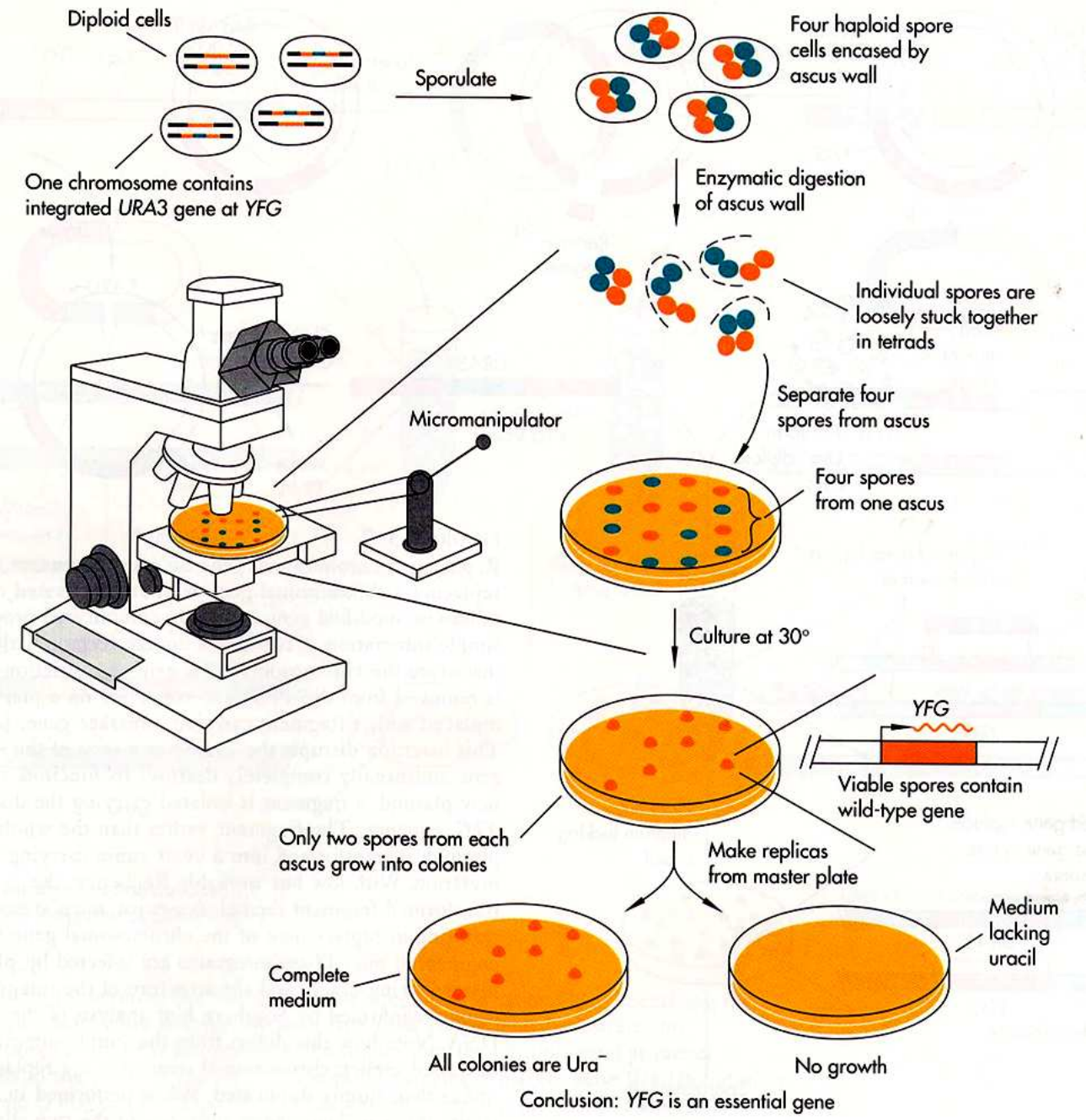
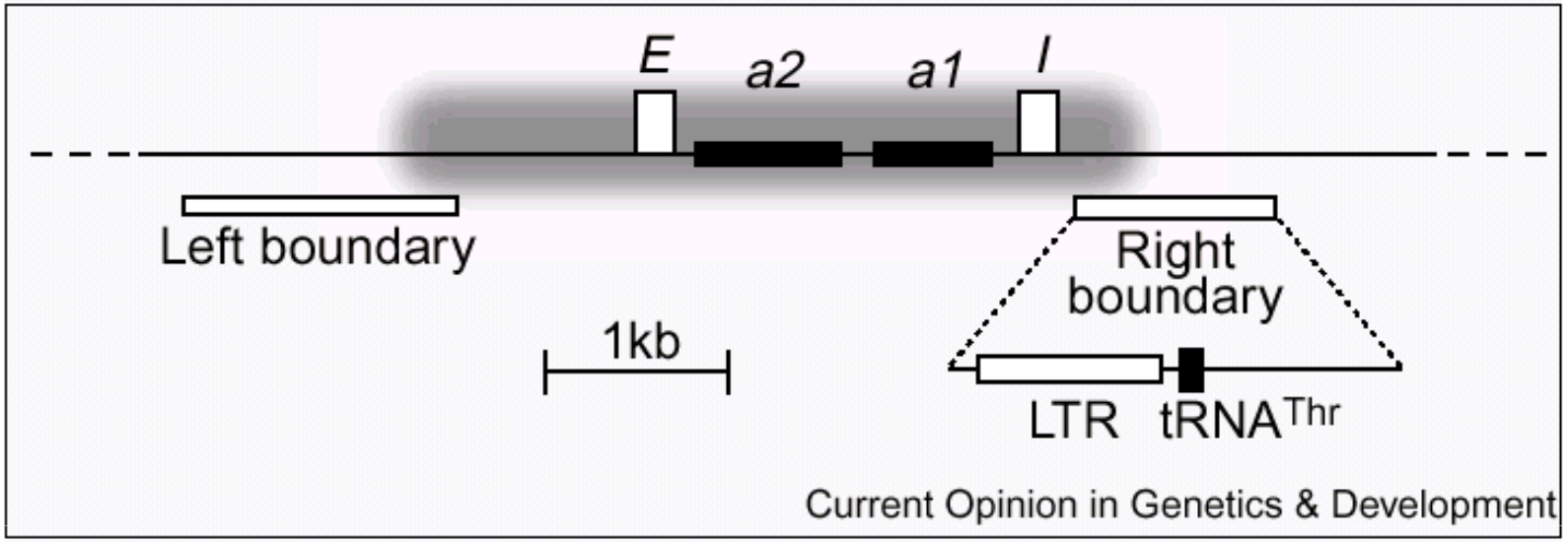
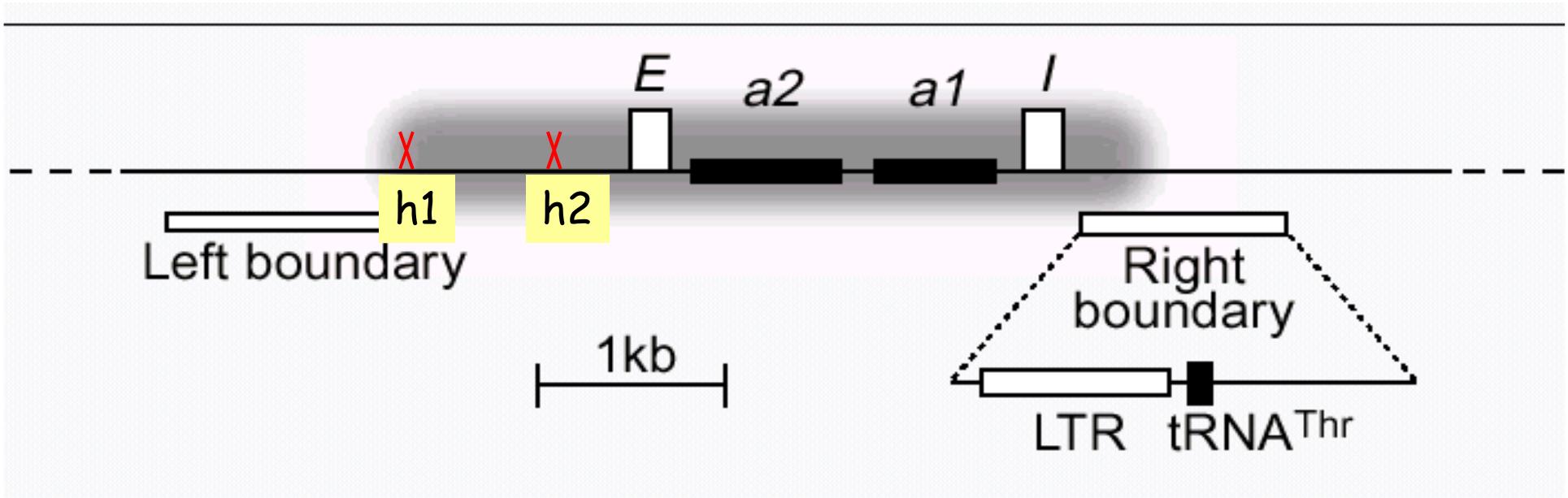

Diploid cells
One chromosome contains integrated *URA3* gene at *YFG*
Sporulate
Four haploid spore cells encased by ascus wall
Enzymatic digestion of ascus wall
Individual spores are loosely stuck together in tetrads
Separate four spores from ascus
Micromanipulator
Four spores from one ascus
Culture at 30°
YFG
Viable spores contain wild-type gene
Only two spores from each ascus grow into colonies
Make replicas from master plate
Medium lacking uracil
Complete medium
All colonies are Ura⁻
No growth
Conclusion: *YFG* is an essential gene

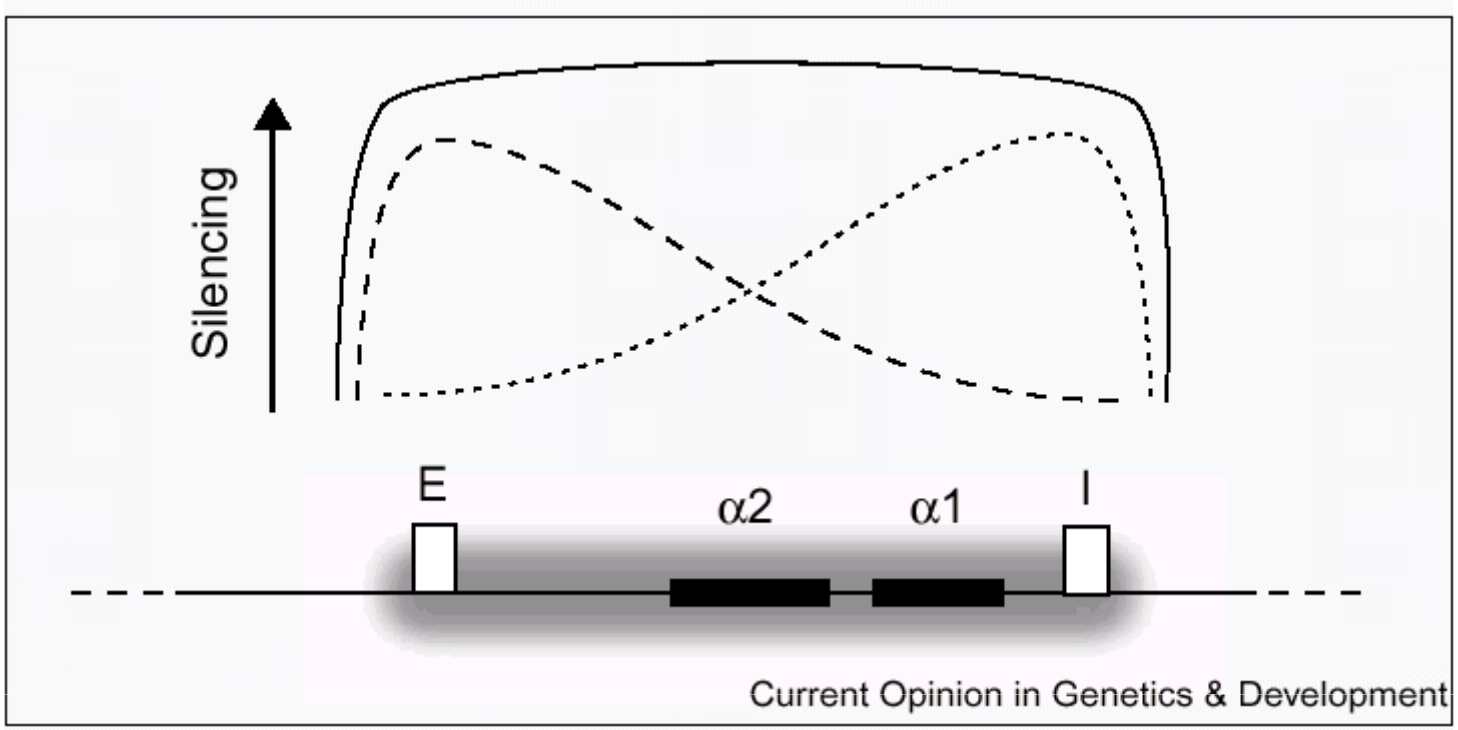
FIGURE 13.8



Organization of heterochromatin barriers surrounding *HMR*. The *HMR* locus is diagrammed, showing the location of the mating type genes *a1* and *a2*, the *E* and *I* heterochromatin organizing centers (silencers) and the left and right heterochromatin barriers as defined in [1••]. Background shading indicates the extent of the repressed domain. An expansion of the right barrier shows the location of a Ty1 LTR and the gene for $tRNA^{Thr}$. Earlier evidence suggested that both these elements contribute to barrier activity [1••] but more recent data indicate that the $tRNA^{Thr}$ gene is necessary and sufficient for full barrier activity

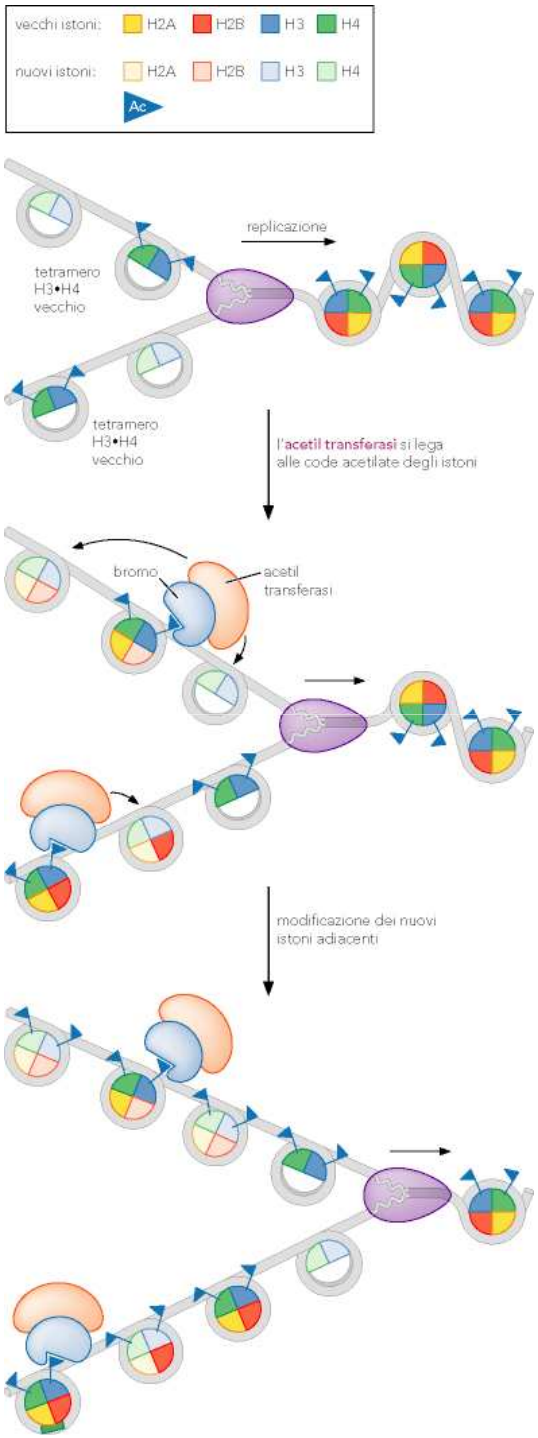

h1
β-gal
h2


E
a2
a1
I
h1
h2
Left boundary
1kb
Right
boundary
LTR
tRNA Thr



Domain organization by directional initiation of heterochromatin. The *HML* locus is diagrammed, showing the location of the mating type genes, α*1* and α*2*, and the *E* and *I* heterochromatin organizing centers (silencers). Background shading indicates the extent of the repressed domain. Repression emanating from *E* is represented as a dashed line, whereas that emanating from *I* is represented as a dotted line. The sum of the effects of the two organizing centers, shown as a solid line, results in uniformly high repression between the two centers with repression dropping off sharply outside the domain. This model emerges from studies on the domain organization of HML presented in [35•].

Combination of spreading properties and “border” elements make epigenetic inheritance “local”.



A model explaining how HC is epigenetically transmitted to daughter DNA molecules.

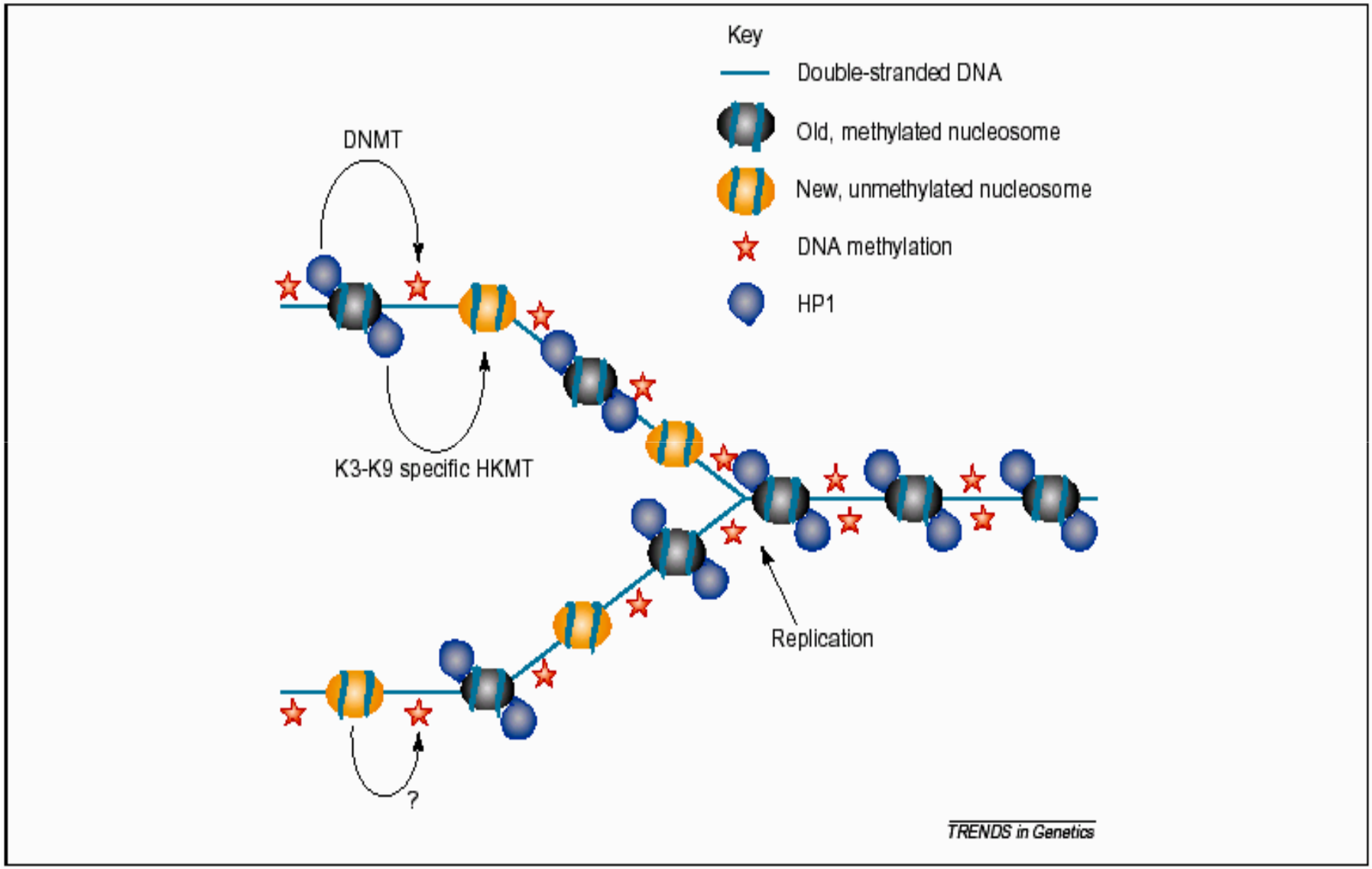


Figure 4. The functional relationship between histone and DNA methylation during replication. After DNA replication, methylated and unmethylated nucleosomes are deposited on the newly synthesized DNA. Heterochromatin-associated protein 1 (HP1) associates with lysine 9 of histone 3 (H3-K9)-specific HKMTs and maintenance DNA methyltransferases, perhaps bridging histone and DNA methylation and retaining the methylation patterns established before DNA replication. Additional undiscovered mechanisms probably exist that propagate both histone and DNA methylation through DNA replication, possibly in a concerted manner.

imprinting

Some genes show a very interesting properties:

.

1. They are expressed only from 1 allele, and their expression depends on whether they are inherited from either the mother or the father.
2. One allele is DNA CpG hypermethylated in a specific control region
3. The methylation is made in germinal cells

This is called germinal epigenetic inheritance (to distingiush from somatic epigenetic inheritance discussed above).

We say these genes are **imprinted**

and the phenomenon is known **as genomic imprinting**

Genomic imprinting was discovered twenty years ago, as an outcome of nuclear transplantation experiments in the mouse.

About 80-90 genes are known to be imprinted in mice and humans, and imprinting is conserved in ruminant species as well.

Many imprinted genes are involved in foetal development and growth, and some influence behaviour.

What is the biological role of imprinting ?

It represents possibly a form of sexual predominance, where a character from one of the partners is programmed to prevail upon the one from the other partner

Usually grouped into Large Chromosomal Domains (LCD)

Transgenic studies have identified sequence elements in these domains that are essential for the imprinted gene expression:

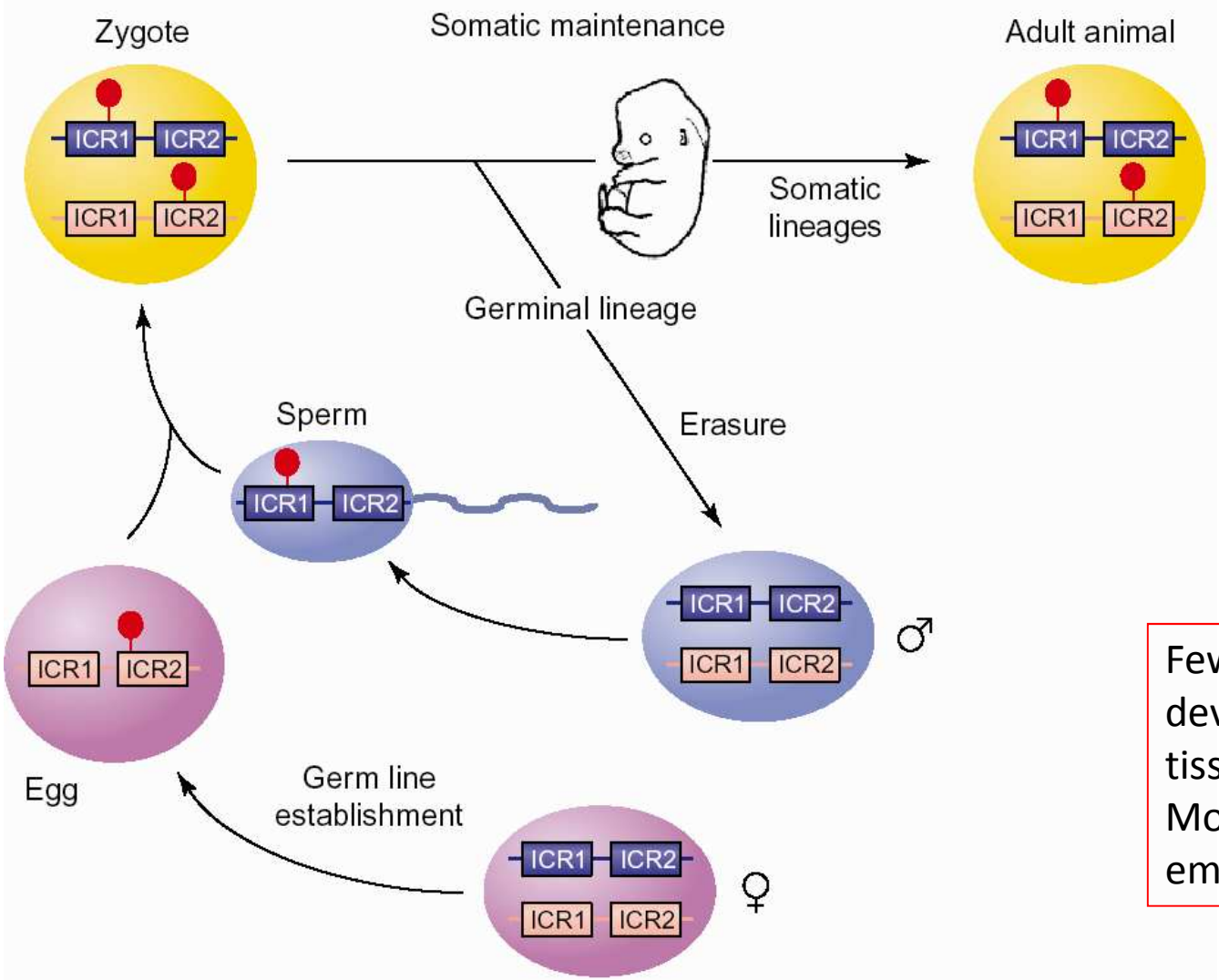
the ICRs - imprinting control regions

- regulated by epigenetic modifications
- up to several kilobases in length
- rich in CpG dinucleotides (many correspond to CpG islands)
- CpG DNA methylation on one of the two parental alleles
  (DMR=differentially methylated region).

At most ICRs, the allelic methylation originates from the egg.
At only a few, it is established during spermatogenesis.

Following fertilisation, allelic methylation marks are maintained throughout development and they mediate imprinted expression.

Parental imprints are established during oogenesis, or spermatogenesis, at sequence elements that control the imprinted expression (ICRs). After fertilisation of the egg by the sperm, these imprints are maintained throughout development. DNA methylation (lollypop) is the most consistent hallmark of imprints. Two examples of ICRs are depicted: ICR with paternally-derived (ICR1); ICR with maternally-derived DNA methylation (ICR2).



Few genes expressed in developing and adult tissues
Most of them in extra-embryonic tissues

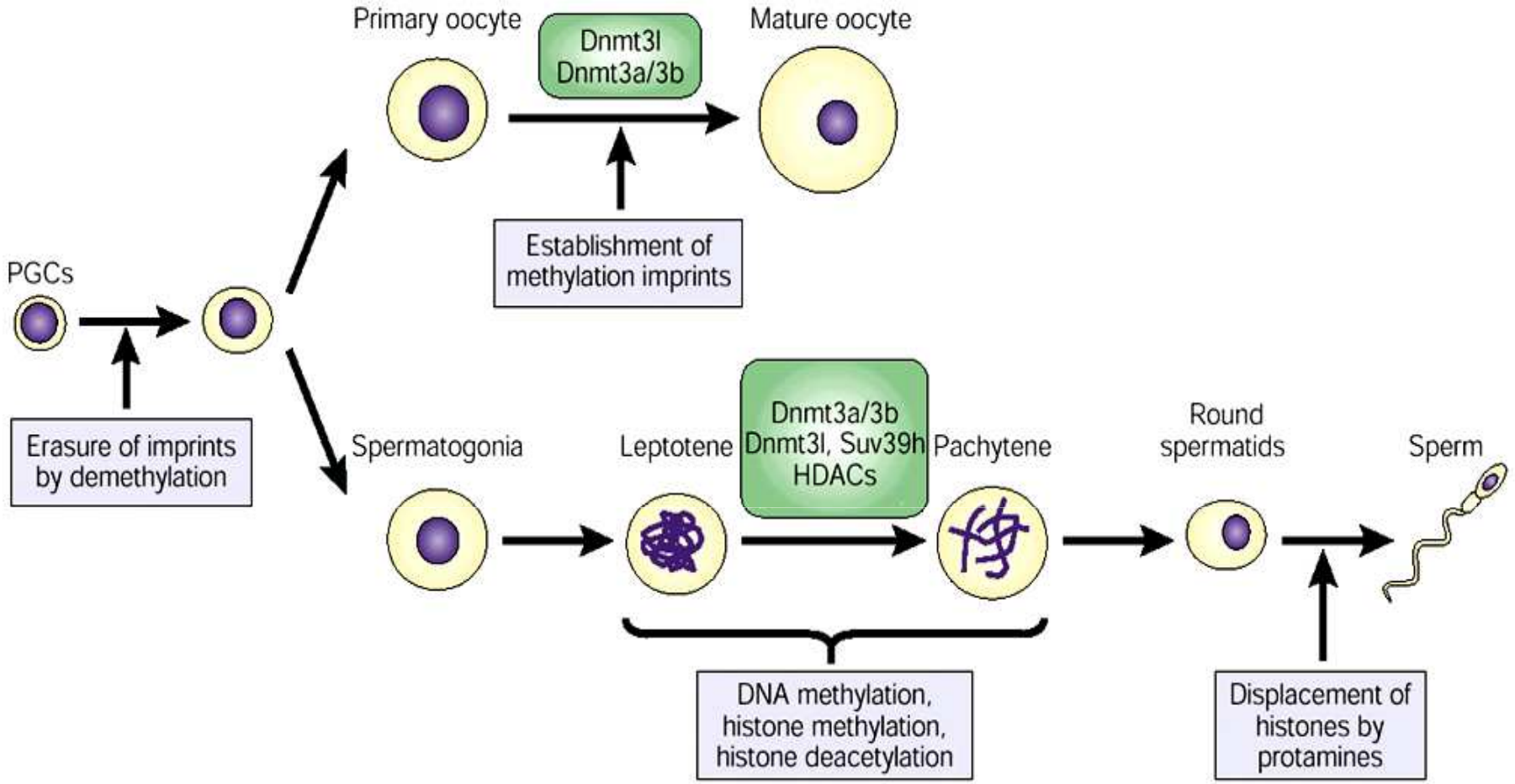


Figure 2 | **Epigenetic reprogramming during gametogenesis.** Primordial germ cells (PGCs) undergo demethylation at imprinted loci, which erases parental imprinting marks at around embryonic day 11.5–12.5 (REFS 50,51). The female PGCs develop to form primary oocytes. During oocyte growth and maturation, the maternal-specific genomic imprints are re-established through the *de novo* methylation activities of the methyltransferases Dnmt3a and Dnmt3b, and an associated protein Dnmt3l[52,53]. During spermatogenesis, several factors seem to function during the differentiation of the spermatocytes from the leptotene to pachytene stages of meiosis. During this period, histones are hypoacetylated, and the functions of Suv39h, Dnmt3a and Dnmt3l are essential[36,52,53,55]. The crucial stage when these factors function is not defined.

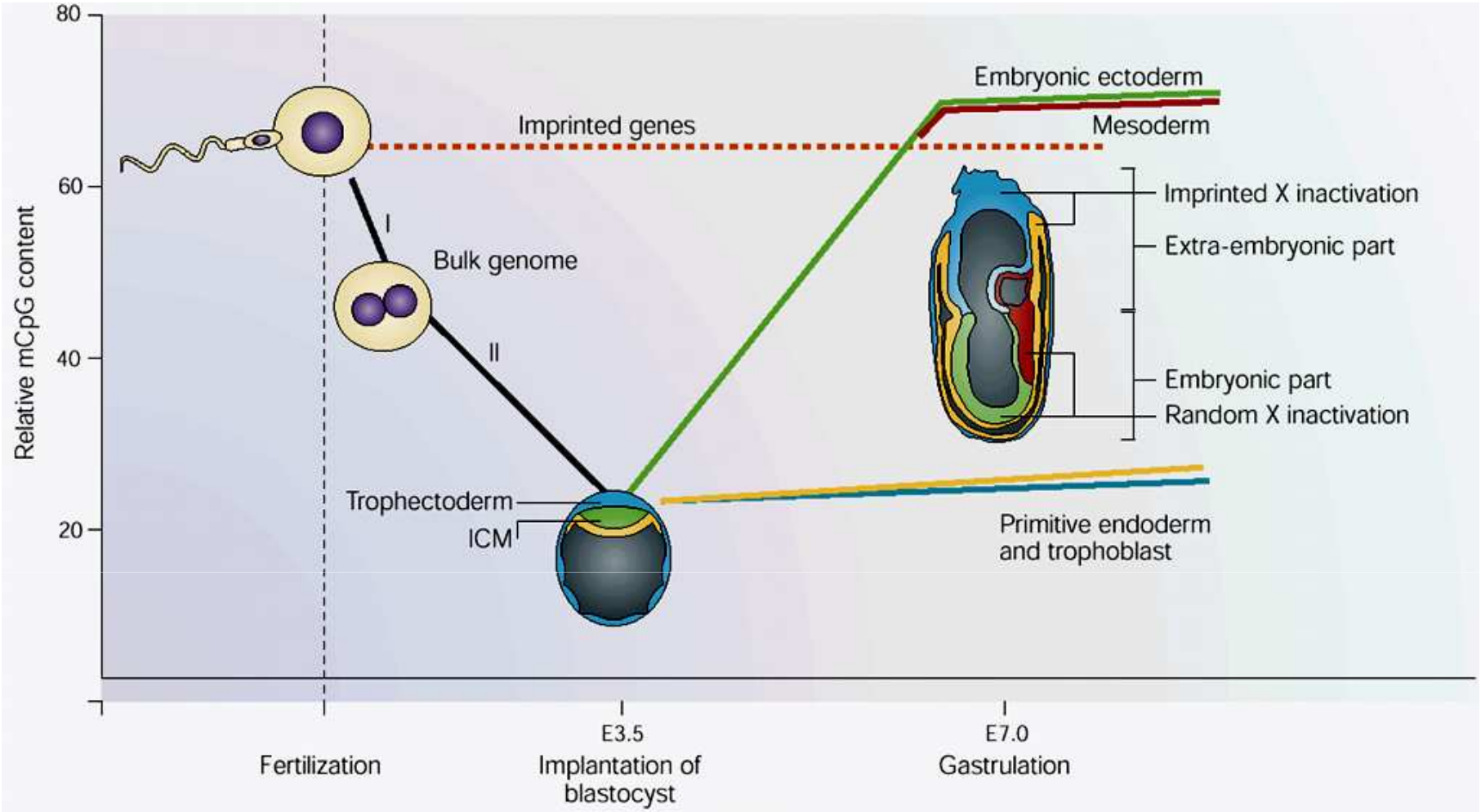
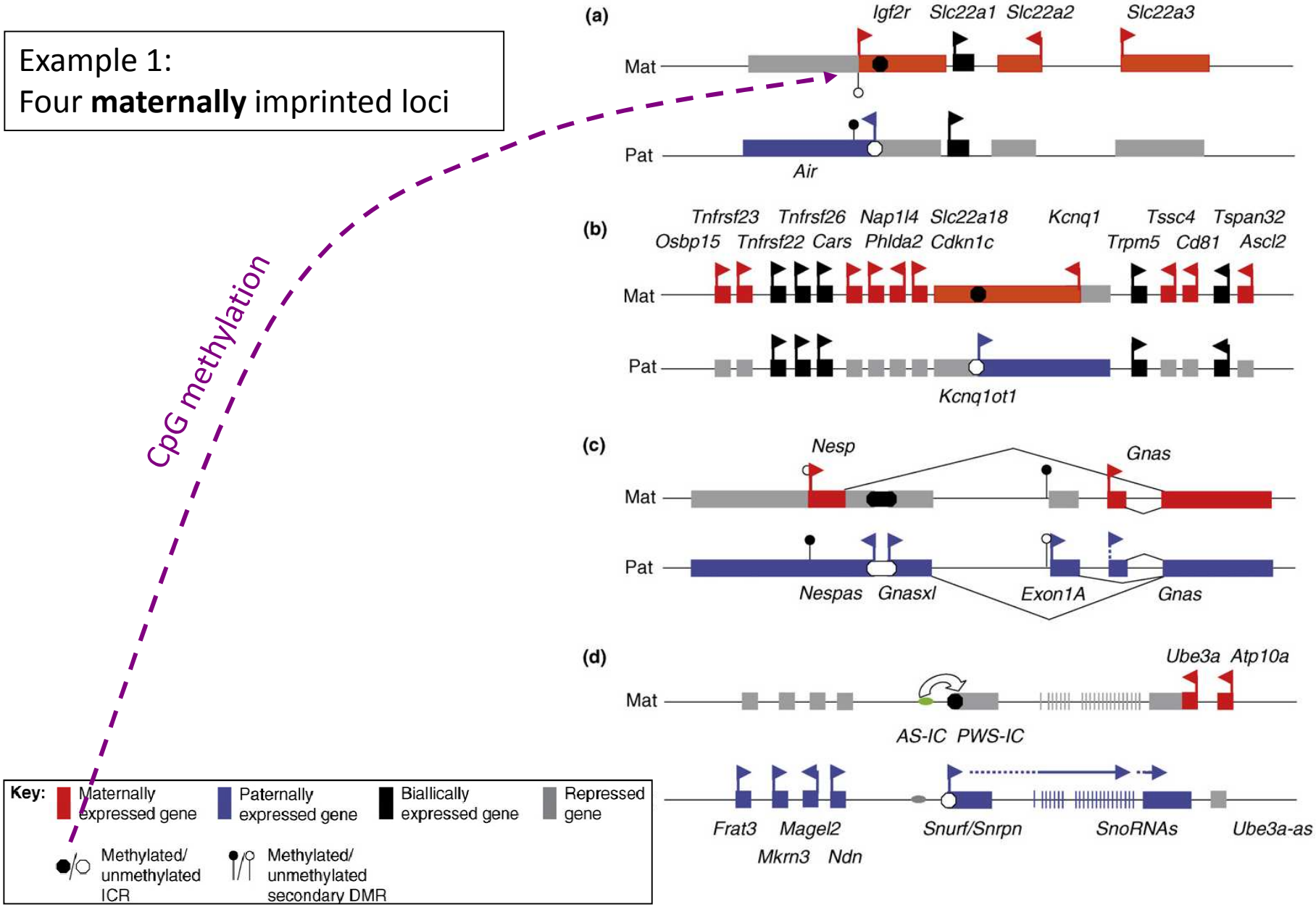


Figure 3 | **DNA-methylation reprogramming during early mouse development.** The methylation status of the bulk mouse genome, which consists of repeats and unique genes but excludes most CpG islands and imprinted regions, undergoes dynamic changes during early development[56–60]. After fertilization, the bulk genome undergoes demethylation through an active demethylation phase (I), followed by a passive demethylation phase (II). The methylation level of a blastocyst reaches the lowest point at embryonic day (E)3.5. After implantation, the bulk genome becomes hypermethylated in the embryonic ectoderm (green) and mesoderm (red) through active *de novo* methylation, whereas the genome of extra-embryonic cells, such as the primitive endoderm (yellow) and trophoblast (blue), remains hypomethylated. The parental methylation imprints in imprinted genes (orange) escape demethylation and *de novo* methylation. Interestingly, X inactivation is imprinted in the primitive endoderm (yellow) and the trophectoderm-derived cells (blue), whereas it is random in the embryonic tissues. ICM, inner cell mass.

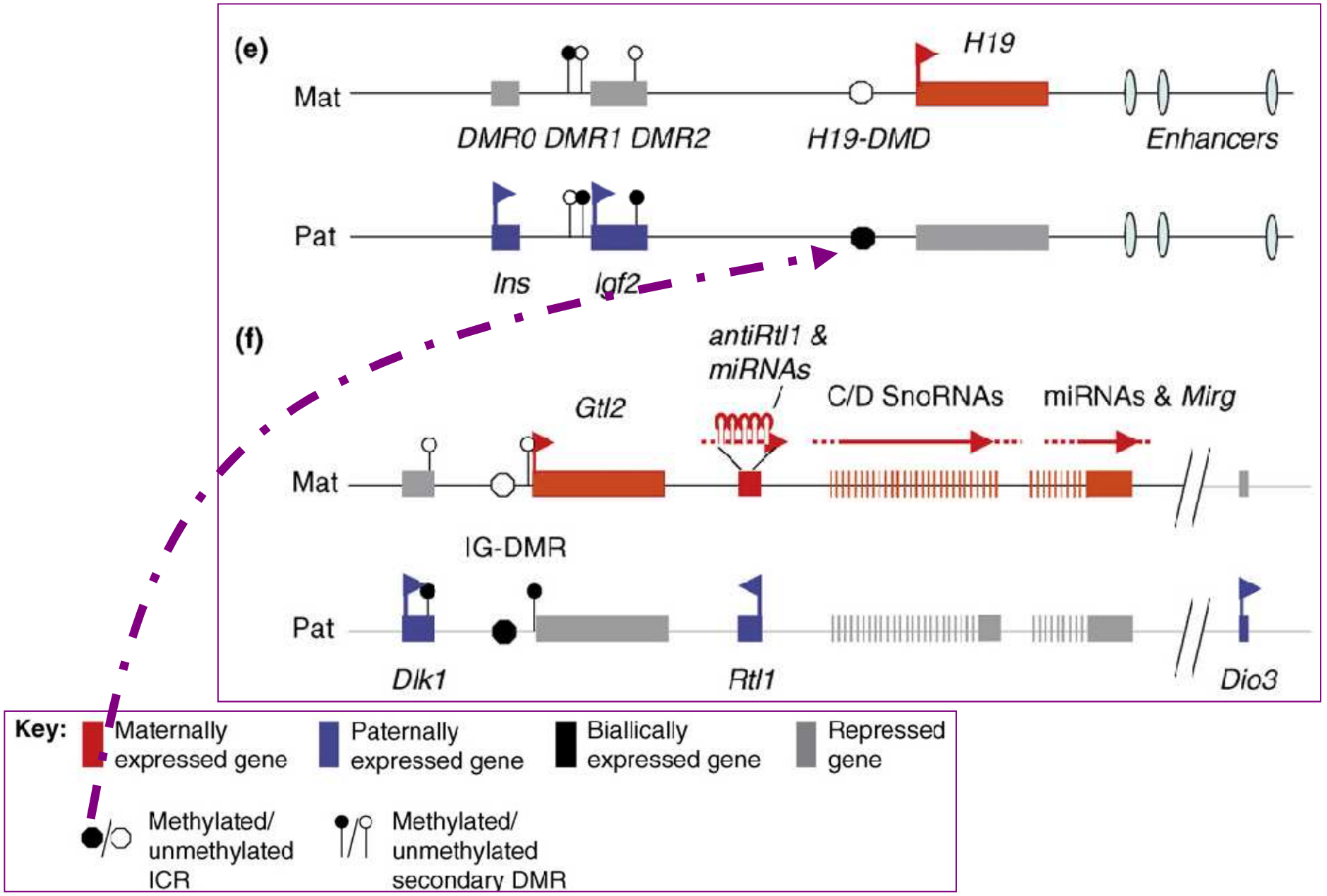
Loci showing imprinting are often very complex

Example 1:
Four **maternally** imprinted loci

CpG methylation



Example 2: Two paternally imprinted loci

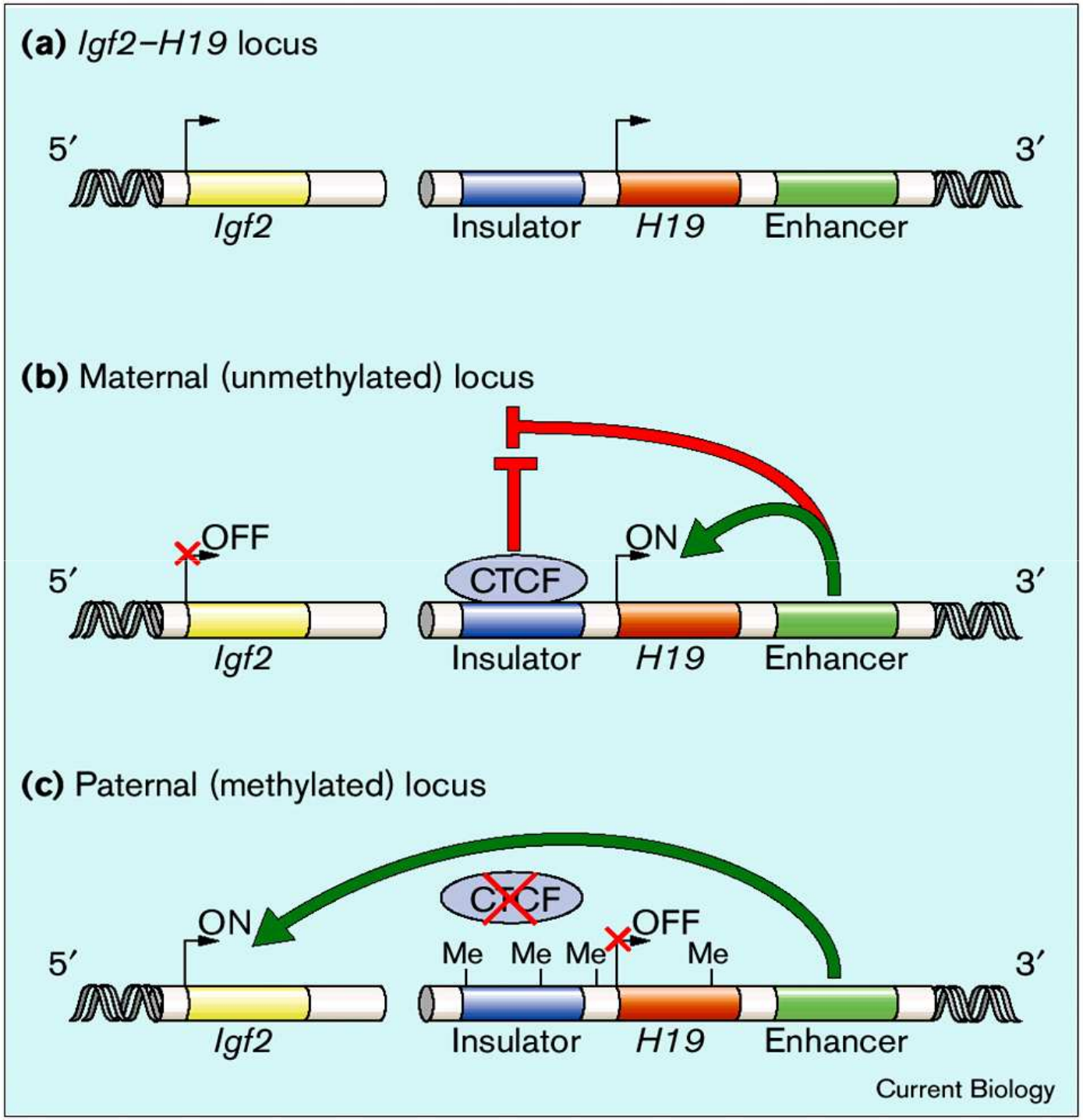


Mechanisms of imprinting:

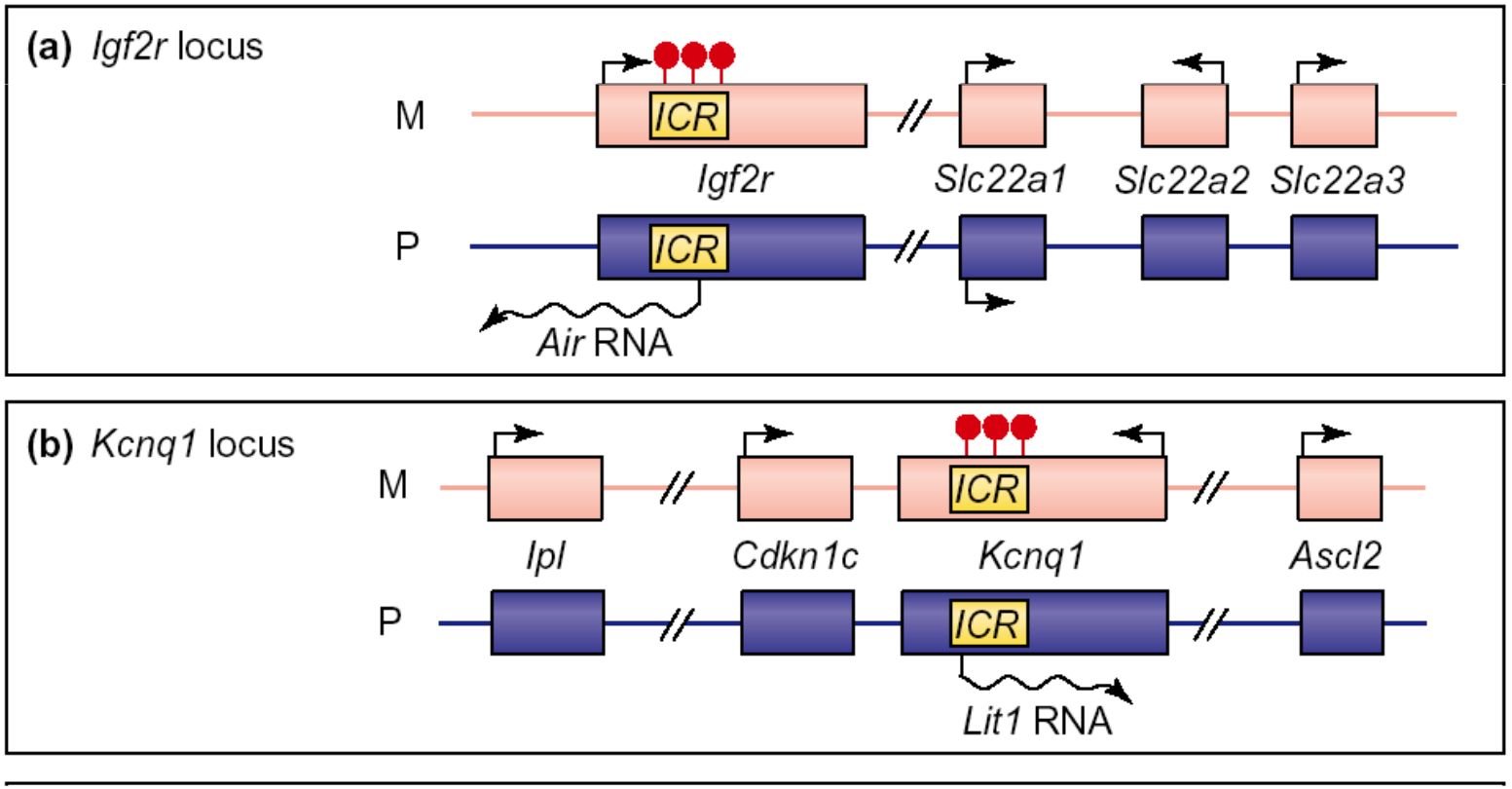
-The Insulators model of imprinting

-The Non-coding RNA model of imprinting

1° mechanism: insulators



2° mechanism: noncoding RNAs



Mutation Research 647 (2008) 77–85

Review

Review

Genomic imprinting mechanisms in mammals

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Insulators
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ABSTRACT

Genomic imprinting is a form of epigenetic gene regulation that results in expression from a single allele in a parent-of-origin-dependent manner. This form of monoallelic expression affects a small but growing number of genes and is essential to normal mammalian development. Despite extensive studies and some major breakthroughs regarding this intriguing phenomenon, we have not yet fully characterized the underlying molecular mechanisms of genomic imprinting. This is in part due to the complexity of the system in that the epigenetic markings required for proper imprinting must be established in the germline, maintained throughout development, and then erased before being re-established in the next generation's germline. Furthermore, imprinted gene expression is often tissue or stage-specific. It has also become clear that while imprinted loci across the genome seem to rely consistently on epigenetic markings of DNA methylation and/or histone modifications to discern parental alleles, the regulatory activities underlying these markings vary among loci. Here, we discuss different modes of imprinting regulation in mammals and how perturbations of these systems result in human disease. We focus on the mechanism of genomic imprinting mediated by insulators as is present at the *H19/Igf2* locus, and by non-coding RNA present at the *Igf2r* and *Kcnq1* loci. In addition to imprinting mechanisms at autosomal loci, what is known about imprinted X-chromosome inactivation and how it compares to autosomal imprinting is also discussed. Overall, this review summarizes many years of imprinting research, while pointing out exciting new discoveries that further elucidate the mechanism of genomic imprinting, and speculating on areas that require further investigation.

Regulation of Heterochromatic Silencing and Histone H3 Lysine-9 Methylation by RNAi

Thomas A. Volpe,[1] Catherine Kidner,[1] Ira M. Hall,[1,2] Grace Teng,[1,2] Shiv I. S. Grewal,[1]* Robert A. Martienssen[1]*

Eukaryotic heterochromatin is characterized by a high density of repeats and transposons, as well as by modified histones, and influences both gene expression and chromosome segregation. In the fission yeast *Schizosaccharomyces pombe*, we deleted the argonaute, dicer, and RNA-dependent RNA polymerase gene homologs, which encode part of the machinery responsible for RNA interference (RNAi). Deletion results in the aberrant accumulation of complementary transcripts from centromeric heterochromatic repeats. This is accompanied by transcriptional de-repression of transgenes integrated at the centromere, loss of histone H3 lysine-9 methylation, and impairment of centromere function. We propose that double-stranded RNA arising from centromeric repeats targets formation and maintenance of heterochromatin through RNAi.

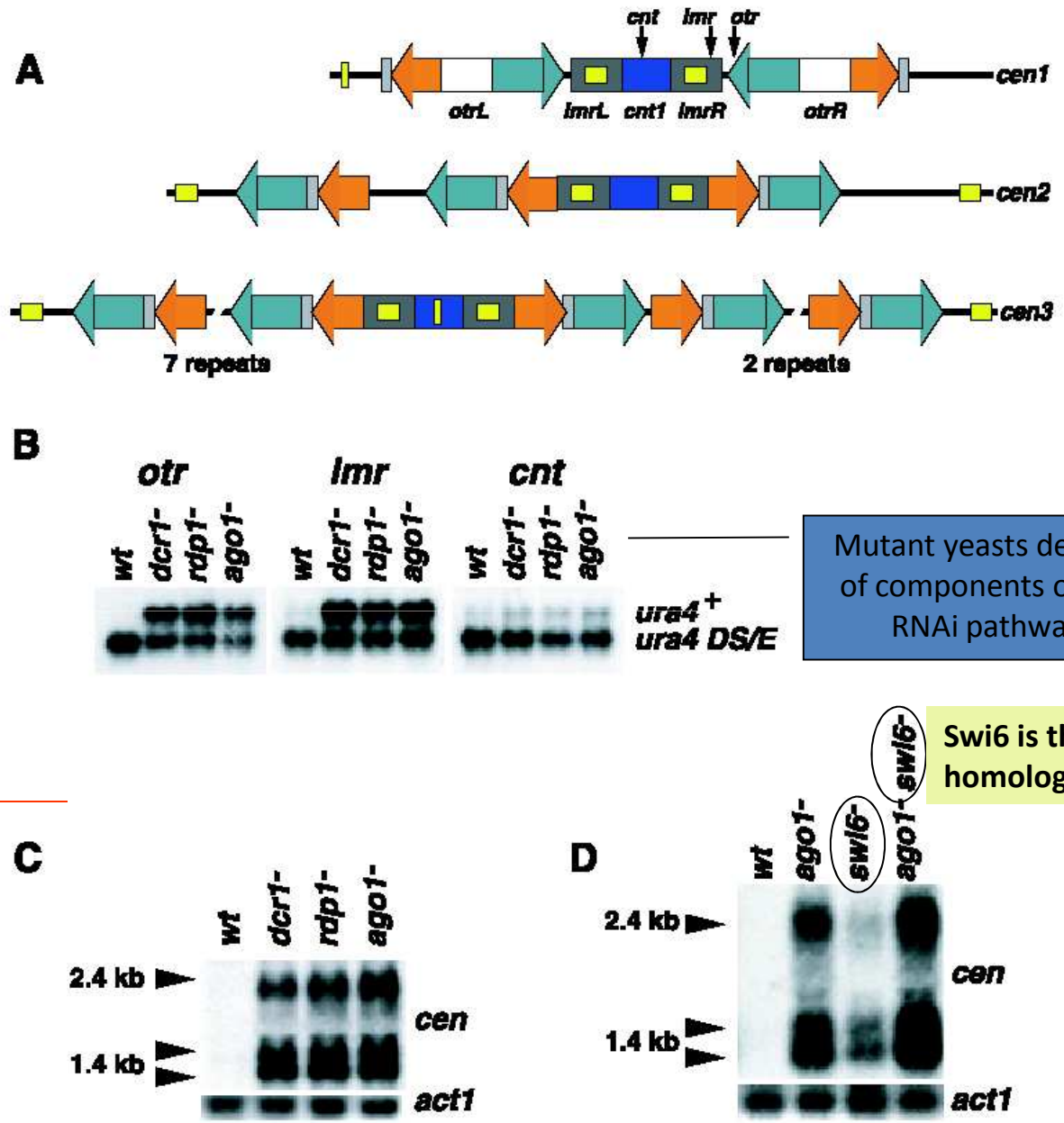

Fig. 1. Centromeric silencing is relieved in *ago1*$^-$, *dcr1*$^-$, and *rdp1*$^-$ mutant strains compared to wild type. Diagram of the three *S. pombe* centromeres (**A**) including locations of *ura4*$^+$ transgenes as well as outermost (*otr*), innermost (*imr*), and central (*cnt*) centromeric regions (*24*, *25*). Conserved *dg* (green) and *dh* (red) repeats are indicated as arrows. Regions containing one or more tRNA genes are indicated by yellow boxes. Northern analysis (**B**) of RNA ~~transcripts tran~~scribed from centromeric *ura4*$^+$ transgenes and a *ura4*$^+$ (*DS/E*) mini-gene located on the chromosome arm. Transcripts derived from centromeric repeats were detected by Northern blotting (**C** and **D**) using probes specific for *dg* centromeric repeats (Fig. 5).

RNAi-Mediated Targeting of Heterochromatin by the RITS Complex

André Verdel,[1] Songtao Jia,[2] Scott Gerber,[1,3] Tomoyasu Sugiyama,[2] Steven Gygi,[1,3] Shiv I. S. Grewal,[2]* Danesh Moazed[1]*

RNA interference (RNAi) is a widespread silencing mechanism that acts at both the posttranscriptional and transcriptional levels. Here, we describe the purification of an RNAi effector complex termed RITS (RNA-induced initiation of transcriptional gene silencing) that is required for heterochromatin assembly in fission yeast. The RITS complex contains Ago1 (the fission yeast *Argonaute* homolog), Chp1 (a heterochromatin-associated chromodomain protein), and Tas3 (a novel protein). In addition, the complex contains small RNAs that require the Dicer ribonuclease for their production. These small RNAs are homologous to centromeric repeats and are required for the localization of RITS to heterochromatic domains. The results suggest a mechanism for the role of the RNAi machinery and small RNAs in targeting of heterochromatin complexes and epigenetic gene silencing at specific chromosomal loci.

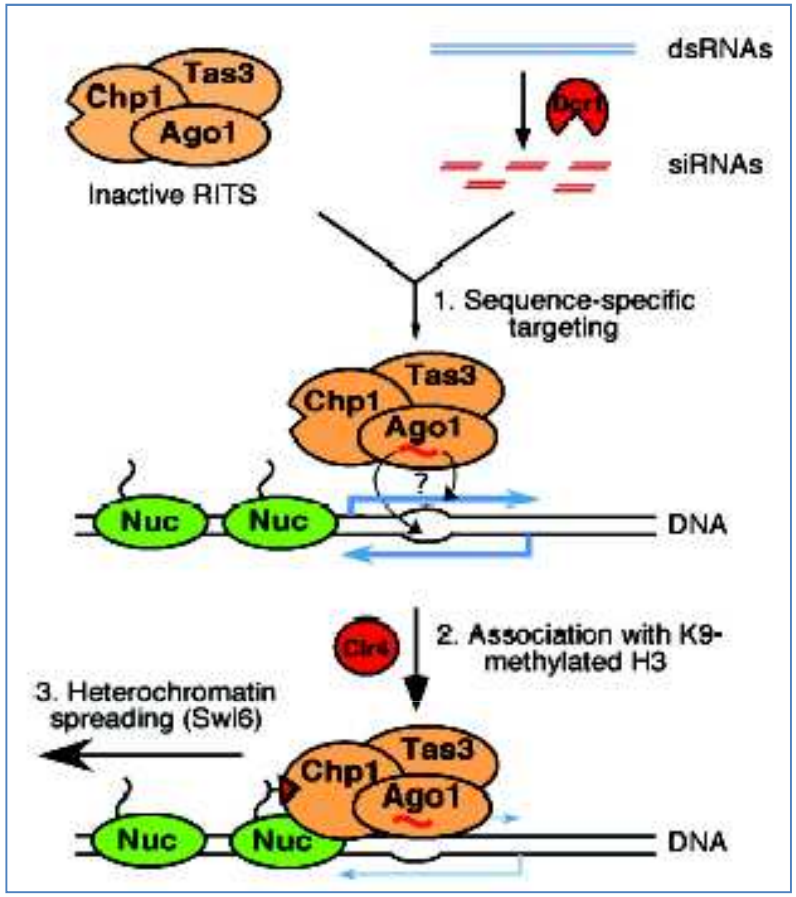

Fig. 5. A model for siRNA-dependent initiation of heterochromatin assembly by RITS. The RITS complex is programmed by Dcr1-produced siRNAs to target specific chromosome regions by sequence-specific interactions involving either siRNA-DNA or siRNA-nascent transcript (blue arrows) base pairing. Nuc, nucleosome; red triangle, K9-methylation on the amino terminus of histone H3. See text for further discussion and references.

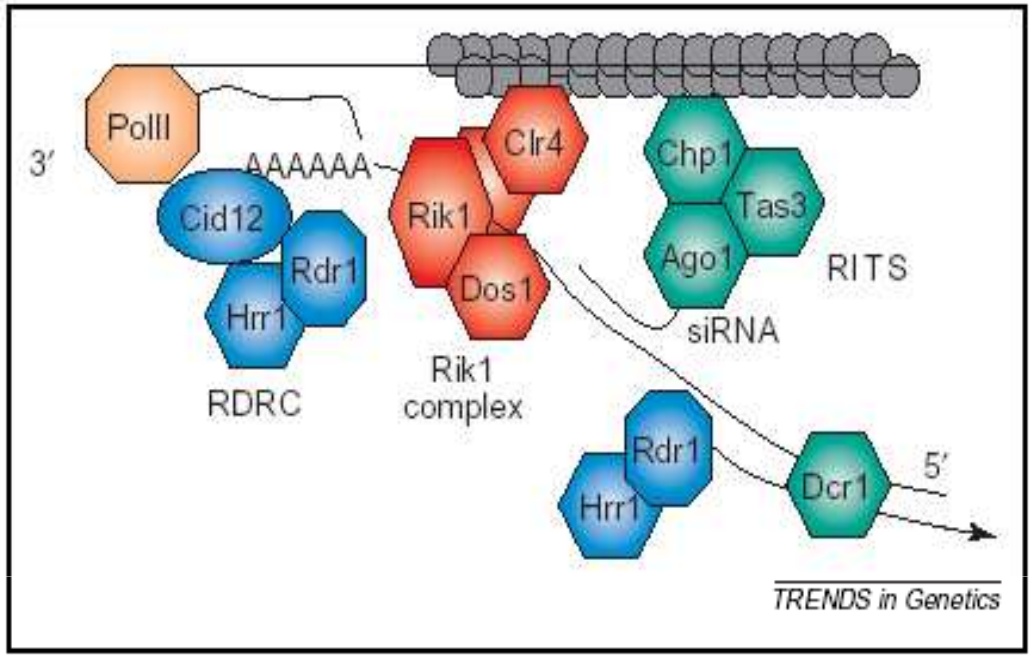

Figure 1. A model for RNAi-mediated histone modification. The four complexes required for both RNAi and histone modification are shown interacting with nascent transcripts from heterochromatic repeats. Transcription by DNA-dependent RNA polymerase II results in tethered transcripts that recruit the RNA-dependent RNA polymerase complex (RDRC) including Cid12, which might interact with centromeric transcripts by polyadenylation. siRNA loaded onto RNAi-mediated initiation of transcriptional silencing (RITS) could also recruit RdRP, which synthesizes second strand transcripts that are rapidly degraded by Dicer, providing a source of siRNA. RITS is bound to the chromosome via the chromodomain of Chp1, which binds H3K9me2 (black circles) and depends on the K9 methyltransferase Clr4. Clr4 is in turn recruited by the Rik1 complex, which might bind polyadenylation sites via similarity to the cleavage and polyadenylation specificity factor, CPSF.

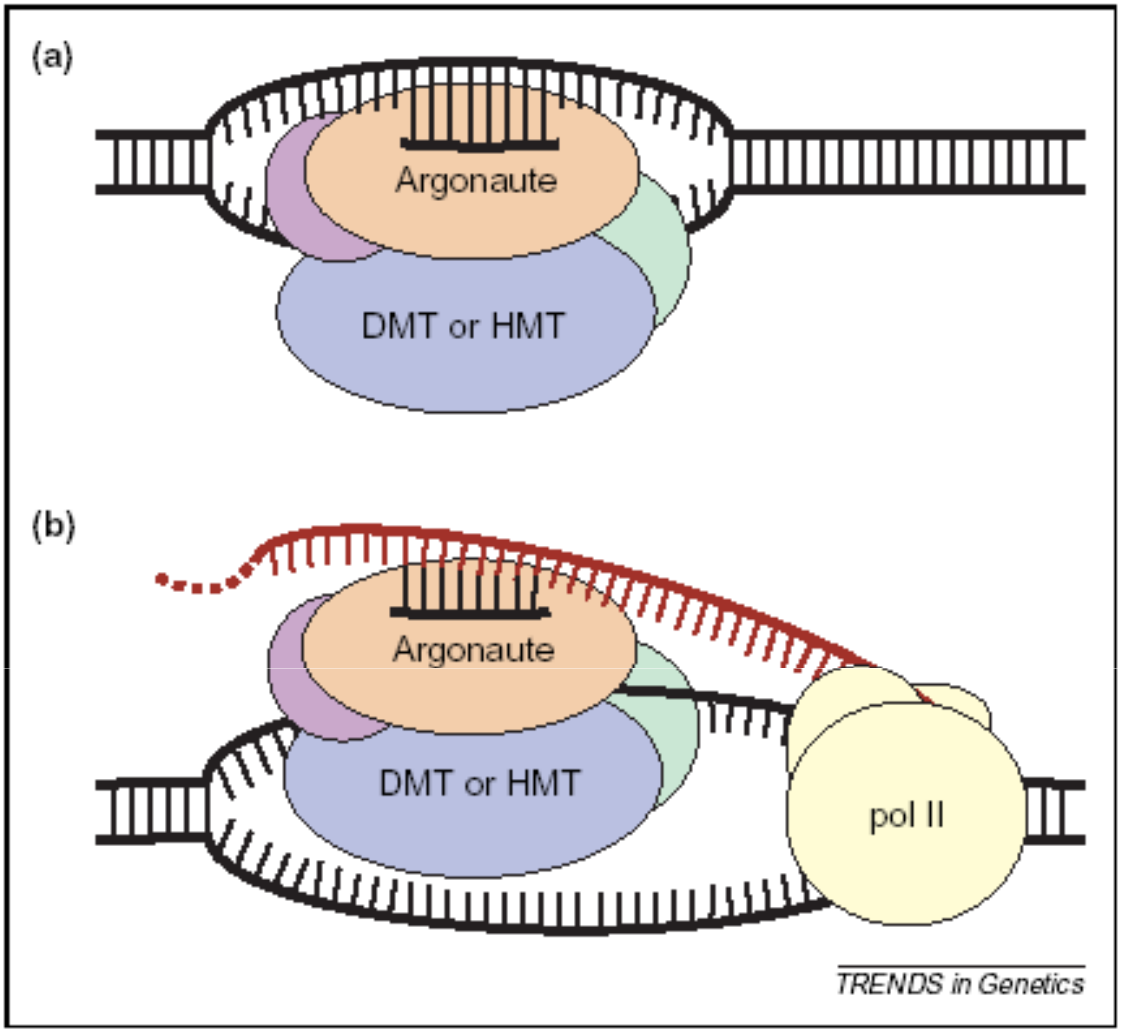

Figure 1. Models for siRNA-directed chromatin modification. DNA (DMT) or histone (HMT) methyltransferase activity is thought to be recruited to target loci by a RITS-like complex that includes an argonaute protein and an siRNA. Two potential mechanisms for target recognition are: **(a)** siRNA binding to target DNA; or **(b)** siRNA binding to nascent transcripts produced from target DNA by RNA polymerase II (pol II).

Two different hypothetical modes RITS can use ss-RNAi to target the gene it derives from

In the first model, a DNA-RNA hybrid is depicted and transcription is **not** required.

In the second model, transcription initiation is required, since the siRNA is thought to form a RNA-RNA hybrid with the nascent transcript.

Indeed, RNA Pol activity seems necessary to target the RITS complex to the locus, although a complete demontration is still lacking.

Interaction of RITS with several chromatin-modifying enzymes (Histone Methyl Transferases HMT, DNA Methyl Transferases DMT) was demonstrated using both co-immunoprecipitation experimetns and by showing binding of RITS together with HMTs and DMTs to the same regions of DNA, by chromatin immunoprecipitation (ChIP).

In S. pombe, the RdRP enzyme (not present in higher animals) is also localized to the centromere, thus suggesting a further amplification mechanism for centromeric siRNA.