CROMATINA

Estructura y función

3° clase
2009

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HETEROCROMATINA Y SILENCIAMIENTO DE LA EXPRESION GENICA

HETEROCROMATINA: forma más común de silenciamiento génico

Qué: - Forma altamente condensada. Se replica tardíamente; se mantiene condensada en interfase. Baja tasa de recombinación. Pocos genes codificadores de proteínas.

Dónde: - telómeros y centrómeros de cromosomas

- otras regiones del genoma

Ej. - loci de determinación del tipo sexual en *S. cerevisiae*

- hasta 50% genoma de mamíferos

Cómo: - Asociado a repetidos (tandems) de secuencias cortas

- Asociada a determinadas modificaciones de las histonas y a ADN metilado

- Efecto de posición; puede propagarse

- Rol de RNAi y transcripción de ADN heterocromático

Funciones:

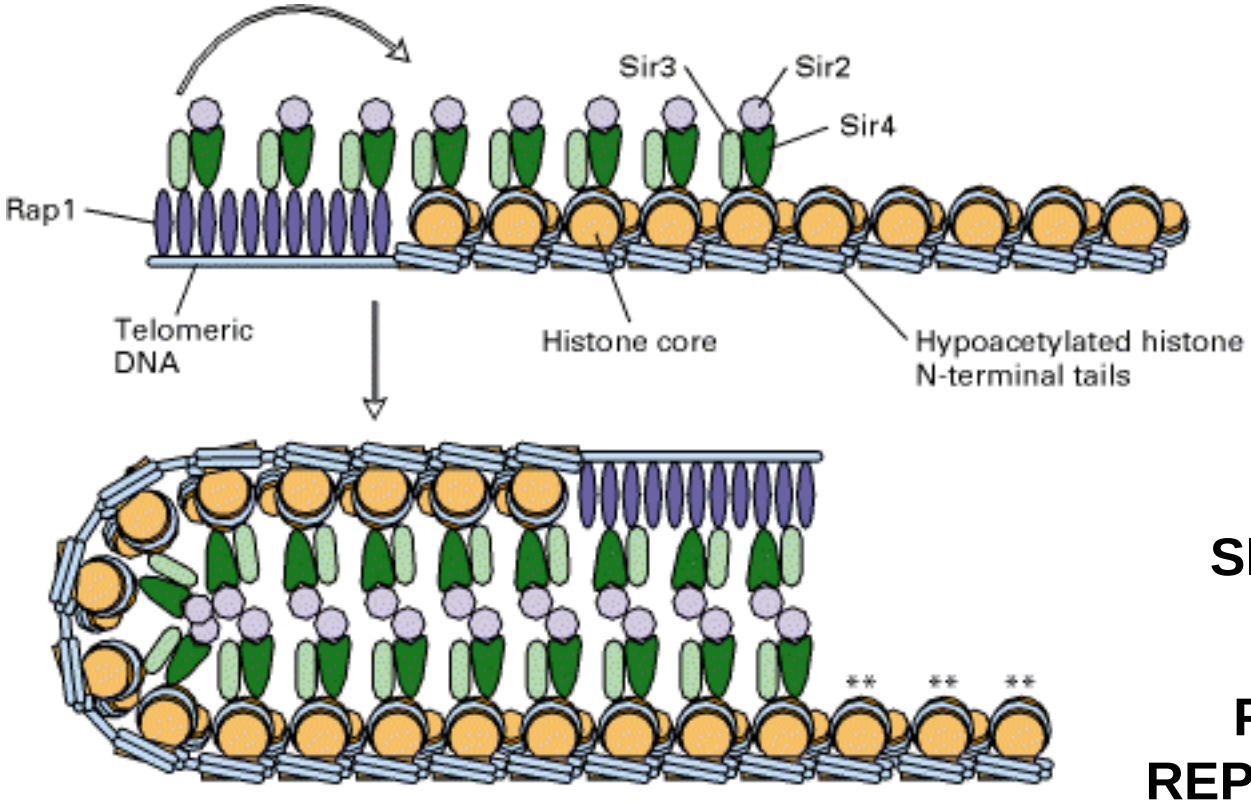
- **Mantenimiento de telómeros y centrómeros de cromosomas**
- **Regulación de la expresión génica; herencia epigenética**
- **Protección del genoma**

TELÓMERO

- “Sellan” y estabilizan los extremos de los cromosomas lineales

- Secuencias cortas repetidas Ej. 5´TTAGGG 3’ (humano)

- Gran parte monocatenario formando una estructura especial, resistente a nucleasas



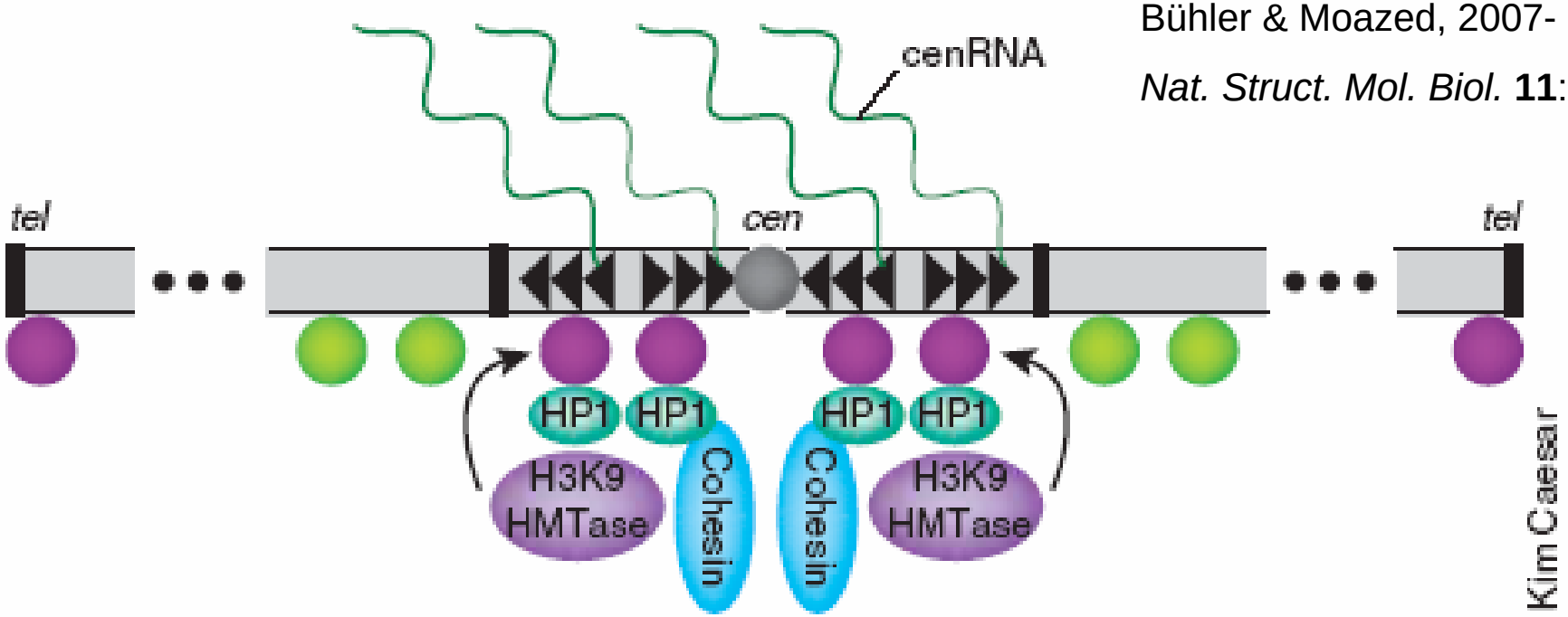
SIR: “silent infromation regulator”

FORMAN COMPLEJO REPRESOR, que se propaga

CENTRÓMERO

Bühler & Moazed, 2007-

Nat. Struct. Mol. Biol. **11**: 1017



Triángulos: secuencias repetitivas

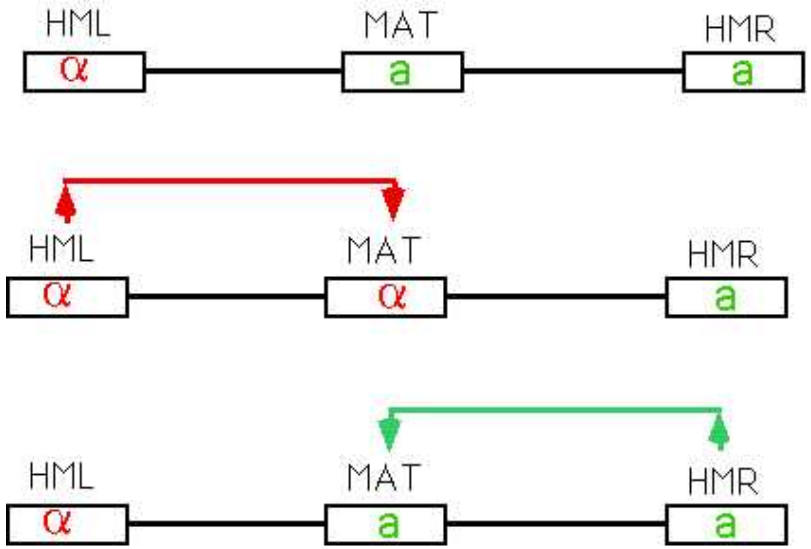
Círculos morados: histonas metiladas (ej. H3K9) e hipoacetiladas

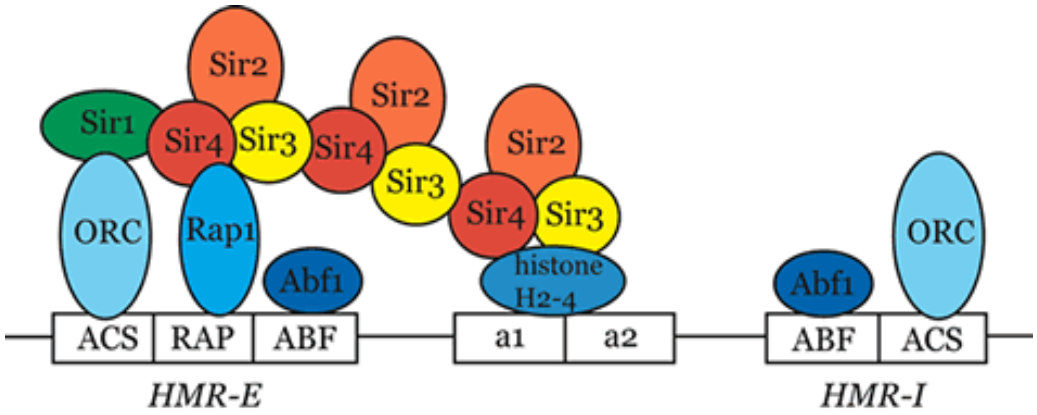
HP1- proteínas con cromodominio

H3K9 histone metil tranferase

Círculos verdes: histonas metiladas (H3K4) e hiperacetiladas

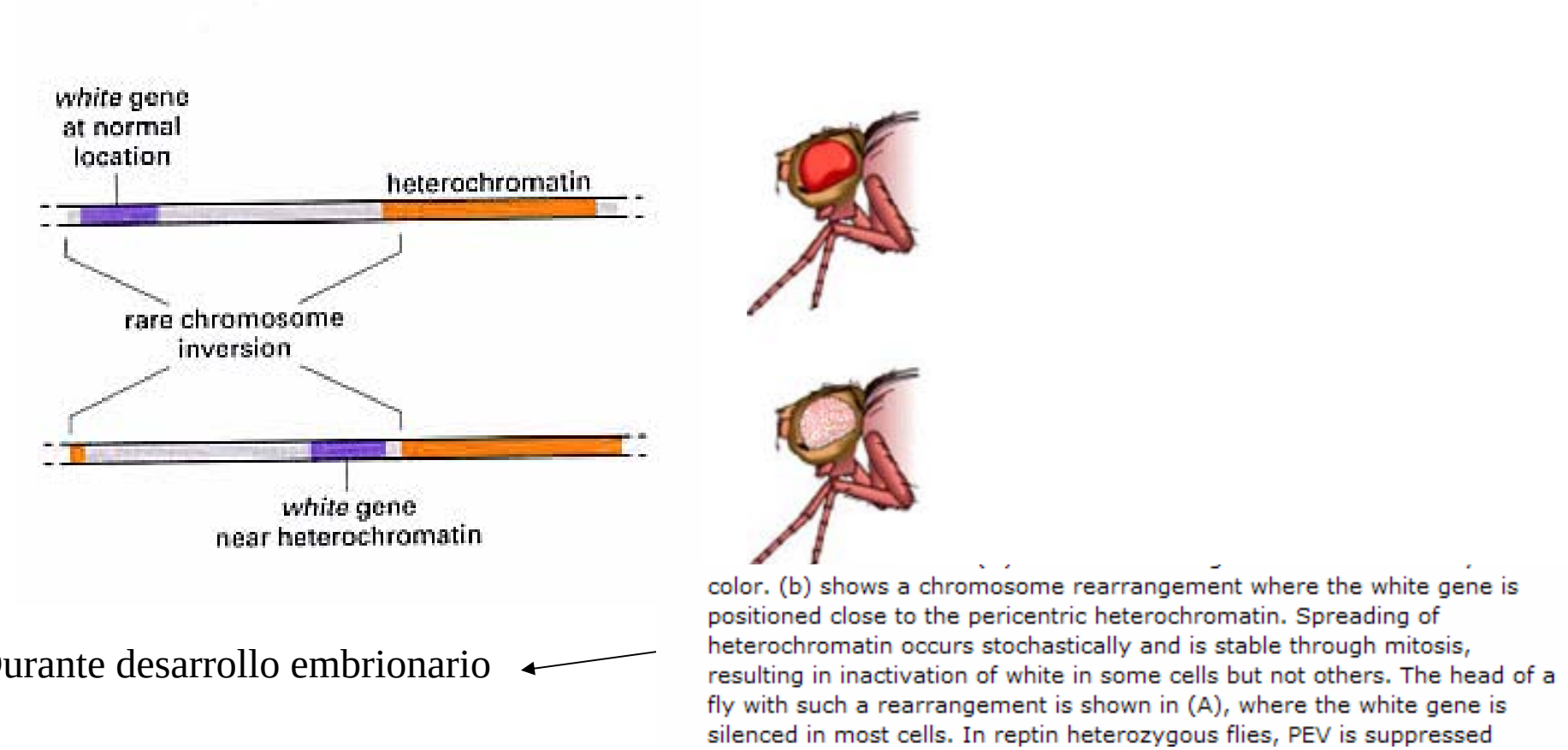
Loci de tipo sexual de *S.cerevisiae*





The a-information at *HMR* is flanked by the *HMR-E* and the *HMR-I* silencers.
Rap1 and Abf1 are regulatory proteins that function as transcriptional activators in other positions in the genome beside their role in silencing.
ORC, Rap1 and Abf1 serve as a platform for binding of the Sir-proteins to *HMR-E*.
Deletion of Sir1 only causes a weak silencing defect, whereas deletion of *SIR2-4* leads to complete derepression. Through the interaction with ORC, Sir1 facilitates the binding of Sir2, Sir3 and Sir4 to the silencer. Hereby, Sir3 and Sir4 interact directly with Rap 1 and the N-termini of histone H3 and H4. Sir2 further improves efficient spreading of the Sir-proteins throughout *HMR* via its NAD+-dependent deacetylation activity.

La heterocromatinización y sus efectos pueden propagarse por una distancia variable: VARIEGACIÓN



color. (b) shows a chromosome rearrangement where the white gene is positioned close to the pericentric heterochromatin. Spreading of heterochromatin occurs stochastically and is stable through mitosis, resulting in inactivation of white in some cells but not others. The head of a fly with such a rearrangement is shown in (A), where the white gene is silenced in most cells. In reptin heterozygous flies, PEV is suppressed

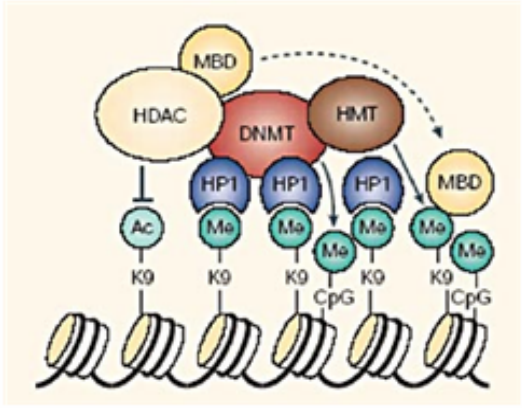
Durante desarrollo embrionario ←

Los estados de la cromatina pueden heredarse: herencia epigenética

Otra forma de silenciamiento: metilación del ADN

- Grandes regiones del ADN de mamíferos estan metiladas y son heterocromáticas

-Proteínas que reconocen ADN metilado (ej MeCP2, methyl-CpG binding proteins) reclutan HDACs y HMTs

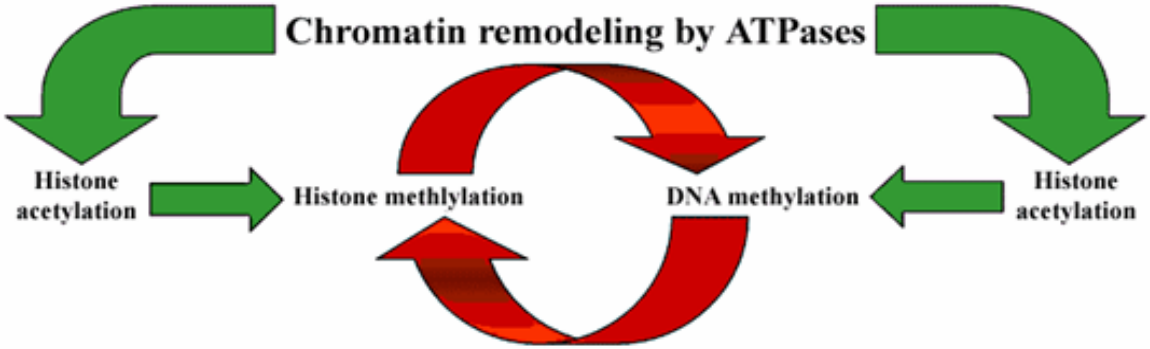


Cooperative and self-reinforcing organization of the chromatin & DNA-modifying machinery responsible for gene silencing in normal & malignant cells

The DNA Methylation Machinery Interacts with the Histone Modification Machinery

DNMTs interacts with:

- Histone deacetylases (HDACs)
- Histone methylases (HMTases)
- ATP-dependent chromatin remodelers
- Chromatin structural proteins (HP1 family)



ESTOS "ESTADOS" DE EXPRESION GENICA PUEDEN HEREDARSE: EPIGENETICA

La metilación de dinucleótidos CpG recluta factores de unión específicos implicados en el silenciamiento génico

Ej. – “apagado” de genes embrionarios: al momento del nacimiento se da una fuerte oleada de metilación que lleva al silenciamiento de genes

- impronta genética (“imprinting” o imprimación) : genes donde solo uno de los alelos, el heredados del padre o el heredado de la madre, es expresado. El otro se silencia por metilación del ADN

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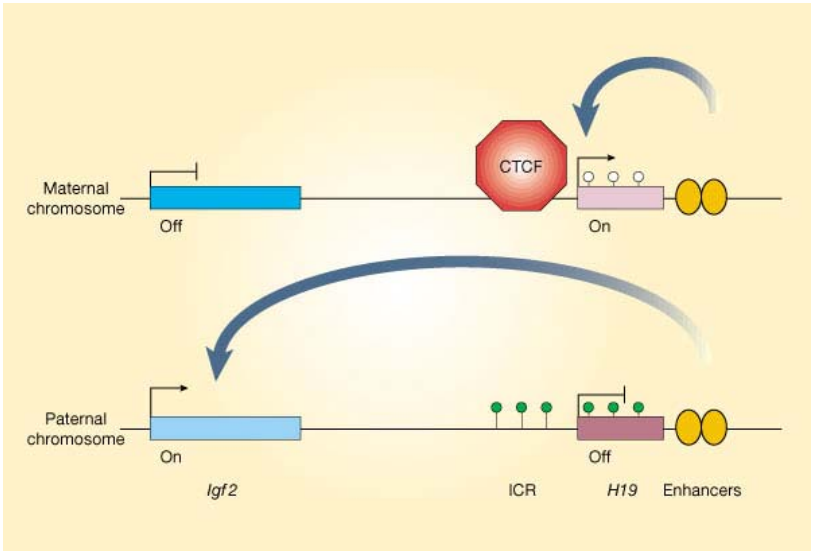
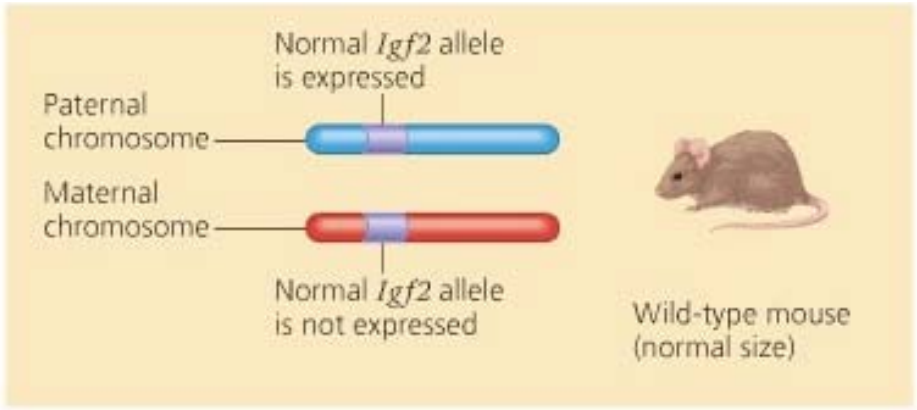
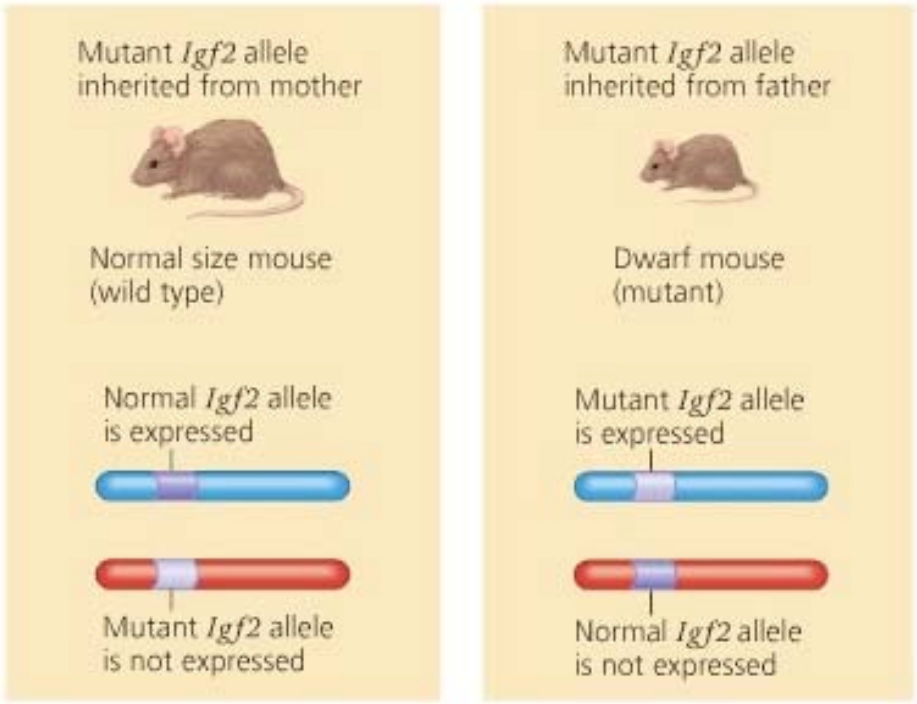




(a) Homozygote. A mouse homozygous for the wild-type *Igf2* allele is normal sized. Only the paternal allele of this gene is expressed.



(b) Heterozygotes. Matings between wild-type mice and those homozygous for the recessive mutant *Igf2* allele produce heterozygous offspring. The mutant phenotype is seen only when the father contributed the mutant allele because the maternal allele is not expressed.

Todas las marcas epigenéticas que acabamos de ver variarán en respuesta a pautas vinculadas con programas de desarrollo o metabólicos: niveles hormonales, dieta, exposición a drogas, etc.

ERRORES EN LAS MARCAS EPIGENETICAS PUEDEN LLEVAR A CONDICIONES PATOLOGICAS

Box 1: Normal cellular functions regulated in part by epigenetic mechanisms and molecular abnormalities caused by epigenetic errors

Normal functions	
• Correct organization of chromatin	Controls active and inactive states of embryonic and somatic cells
• Specific DNA methylation and histone modifications	Controls gene- and tissue-specific epigenetic patterns
• Silencing repetitive elements	Ensures that chromatin order and proper gene expression patterns are maintained
• Genomic imprinting	Is essential for development
• X chromosome inactivation	Balances gene expression between males and females
Abnormalities	
• DNA hypermethylation	Results in chromatin condensation and silencing of tumour suppressor and other genes
• DNA hypomethylation	Activates oncogenes, results in chromosomal instability, activates transposons
• Mutations at methylated cytosines	Results in inappropriate gene expression
• Imprinting defects	Results in loss of parental identity

Epigenetics and human disease: translating basic biology into clinical applications

David Rodenhiser, Mellissa Mann

Table 1: Associations between epigenetic modifications and human diseases and conditions

Disease/condition	Gene	Biological process
Cancer		
Bladder	Multiple genes	Hypermethylation[20]
Brain (glioma)	*RASSF1A*	Hypermethylation[28,29]
Brain (glioblast)	*MGMT*	Hypermethylation[30]
Breast	*BRCA1*	Hypermethylation[31]
Breast	Multiple genes	Hypermethylation[32,33]
Cervix	*p16*	Hypermethylation[34]
Colon	Multiple genes	Hypermethylation[20]
Colorectal	L1 repeats	Hypomethylation[35]
Esophagus	*CDH1*	Hypermethylation[20]
Head/neck	*p16, MGMT*	Hypermethylation[20]
Kidney	*TIMP-3*	Hypermethylation[20]
Leukemia	p15	Hypermethylation[20]
Liver	Multiple genes	Hypermethylation[36]
Lung	*p16, p73*	Hypermethylation[20]
Lymphoma	*DAPK*	Hypermethylation[20]
Myeloma	*DAPK*	Hypermethylation[37]
Ovary	*BRCA1*	Hypermethylation[38]
Ovary	*Sat2*	Hypomethylation[39]
Pancreas	*APC*	Hypermethylation[20]
Pancreas	Multiple genes	Hypomethylation[40]
Prostate	*BRCA2*	Hypermethylation[20,41]
Rhabdomyosarcoma	*PAX3*	Hypermethylation[42]
Neurologic		
Schizophrenia	*RELN*	Hypermethylation[46,47]
Bipolar disorder	*11p?*	Unknown[48]
Memory formation	Multiple genes	Hypo-, hypermethylation[49]
Lupus	Retroviral DNA	Hypomethylation[50]
Cardiovascular		
Atherosclerosis	Multiple genes	Hypo-, hypermethylation[19,5]
Homocysteinemia	Multiple genes	Hypomethylation[52]
Vascular endothelium	*eNOS*	Hypomethylation[53]
Imprinting and pediatric syndromes		
PWS or AS	15q11-q13	Imprinting[54]
BWS	11p15	Imprinting[55]
SRS	Chromosome 7	Imprinting[56]
UPD14	14q23-q32	Imprinting[57]
PHP, AHO, MAS	20q13.2	Imprinting[58]
Rett syndrome	*MECP2*	Mutation[59]
ICF syndrome	*DNMT3B*	Mutation[60]
ATRX	*ATRX*	Chromatin structure[61]
FraX	Triplet repeat	Silencing[62]
FSHD	3.3 kb repeat	Chromatin structure[63]
Reproductive		
Ovarian teratoma	No paternal genome	Imprinting[64]
CHM	No maternal genome	Imprinting[65]

Los perfiles epigenéticos de los gemelos idénticos difieren más a edades más avanzadas

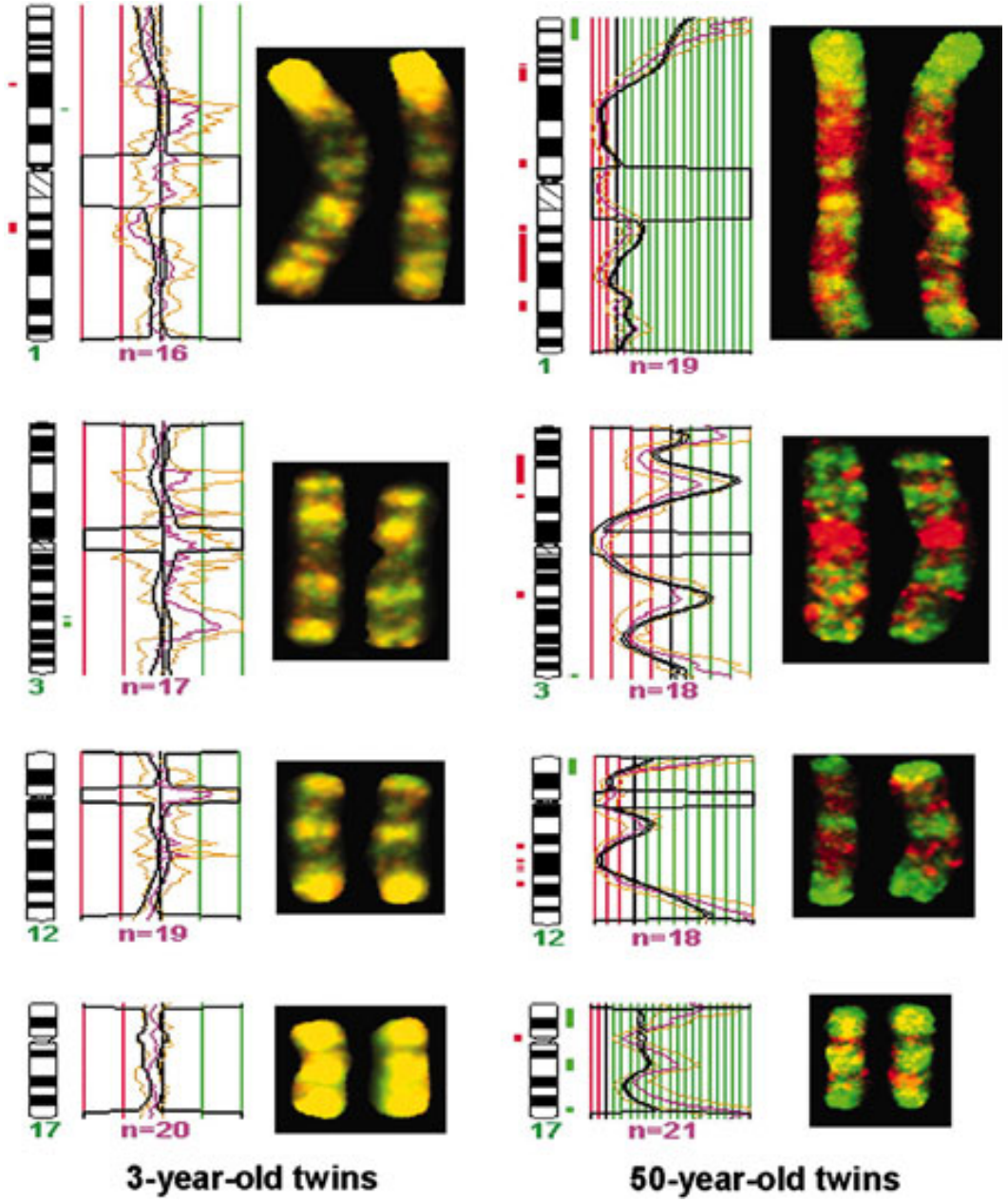


Fig. 3. Mapping chromosomal regions with differential DNA methylation in MZ twins by using comparative genomic hybridization for methylated DNA. Competitive hybridization onto normal metaphase chromosomes of the AIMS products generated from 3- and 50-year-old twin pairs. Examples of the hybridization of chromosomes 1, 3, 12, and 17 are displayed. The 50-year-old twin pair shows abundant changes in the pattern of DNA methylation observed by the presence of green and red signals that indicate hypermethylation and hypomethylation events, whereas the 3-year-old twins have a very similar distribution of DNA methylation indicated by the presence of the yellow color obtained by equal amounts of the green and red dyes. Significant DNA methylation changes are indicated as thick red and green blocks in the ideograms.

Epigenetic differences arise during the lifetime of monozygotic twins

Fraga et al.

PNAS | July 26, 2005 | vol. 102 | no. 30 | 10605

En *S. pombe* el establecimiento de la heterocromatina parece involucrar el mecanismo de RNAi

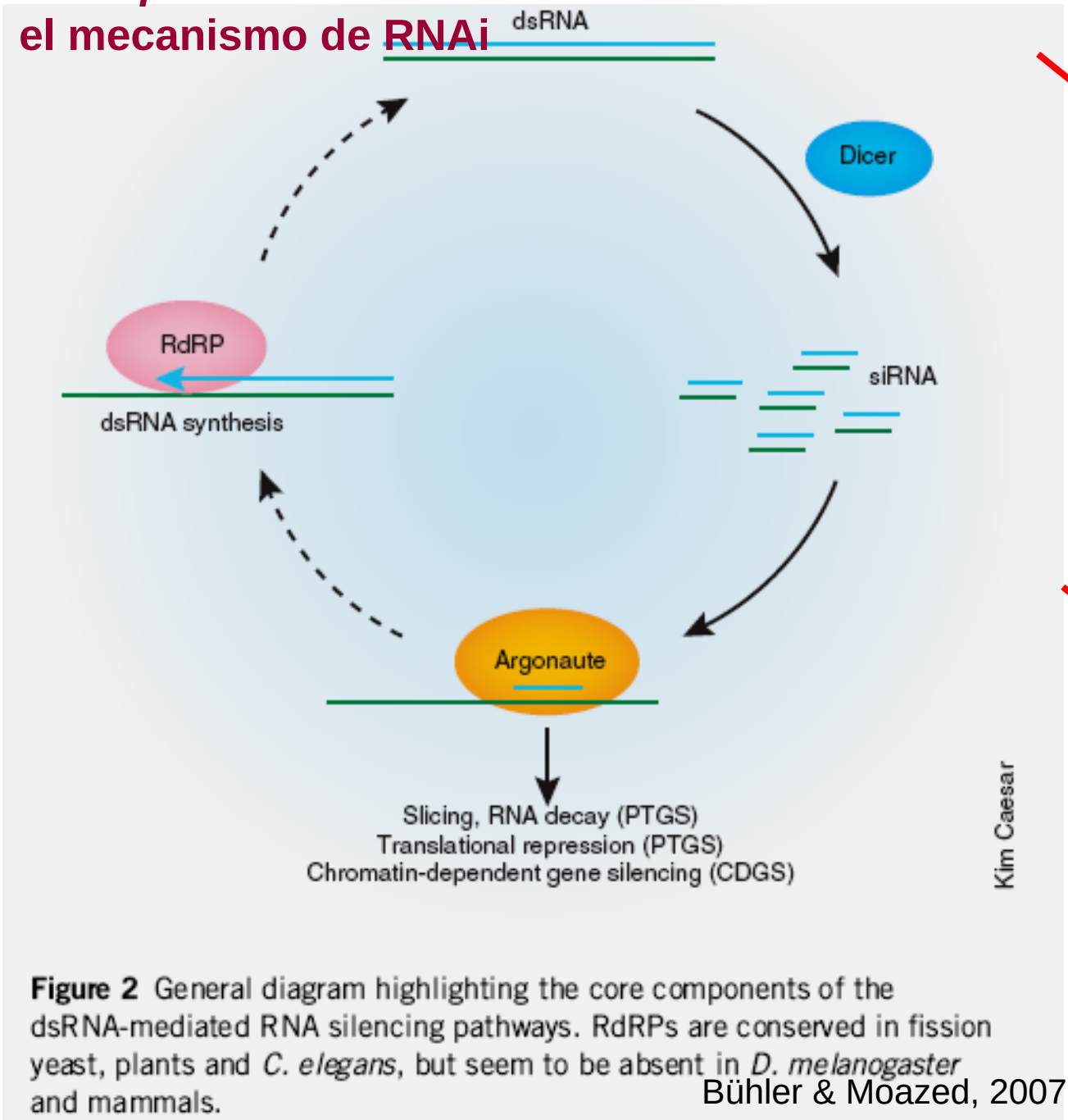


Figure 2 General diagram highlighting the core components of the dsRNA-mediated RNA silencing pathways. RdRPs are conserved in fission yeast, plants and *C. elegans*, but seem to be absent in *D. melanogaster* and mammals.

Dcr1

Ago1

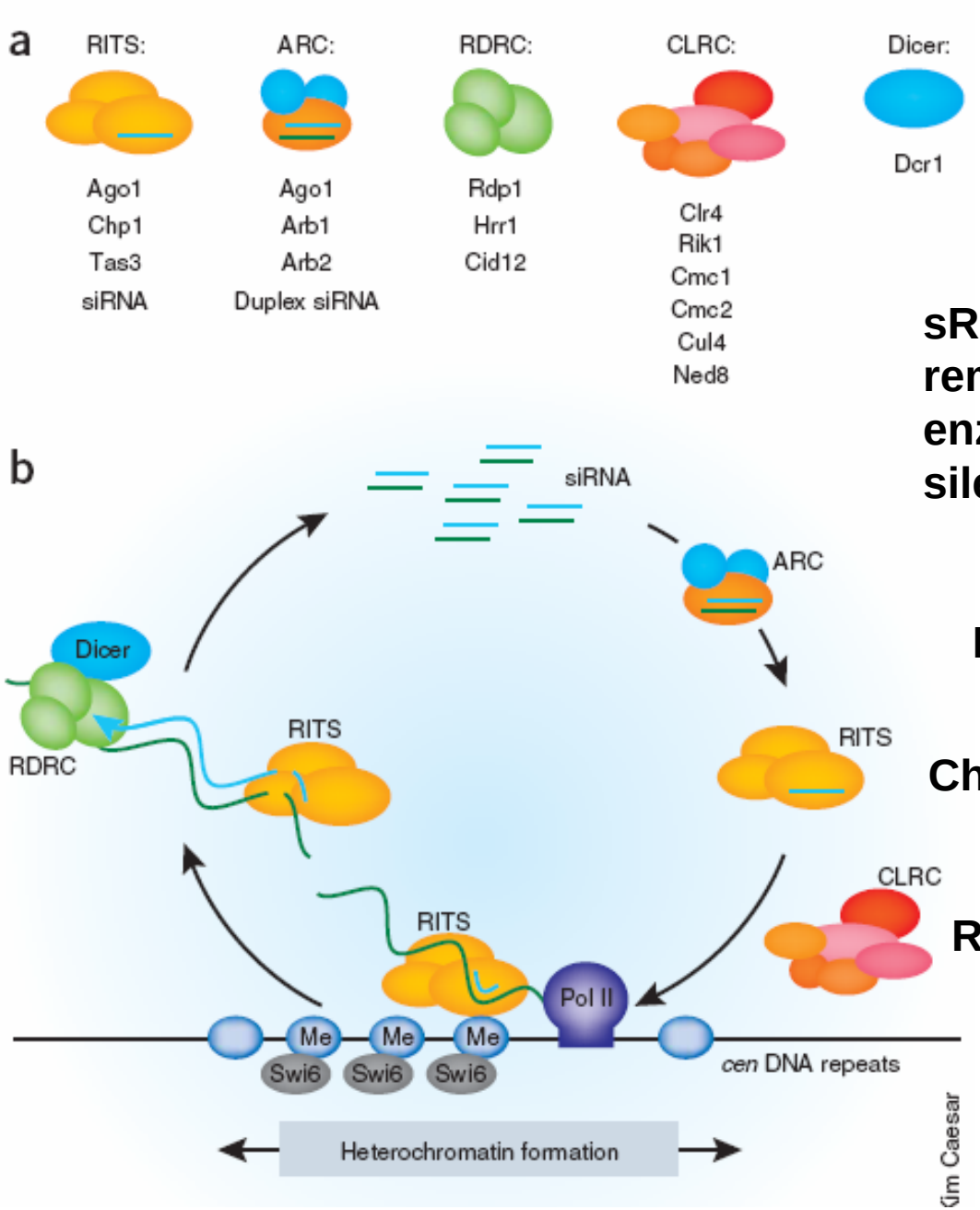
Rdp1

-H3K9me

-Localización de Swi6 (HP1)

-Silenciamiento en centrómeros

Bühler & Moazed, 2007-*Nat. Struct. Mol. Biol.* **11**: 1017



sRNAs se asocian con complejos remodeladores de comatina y llevan enzimas modificadoras a lugares a silenciar

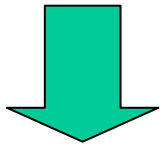
RITS: apreamiento de siRNAs con transcriptos nacientes

Chp1: chromodomain, reconoce H3K9

CLRC: metiltransferasa (DNA)

RDRC: dsRNA sintesis; establece el "loop"

IMPORTANTE: se requieren transcriptos de secuencias centroméricas y PolII



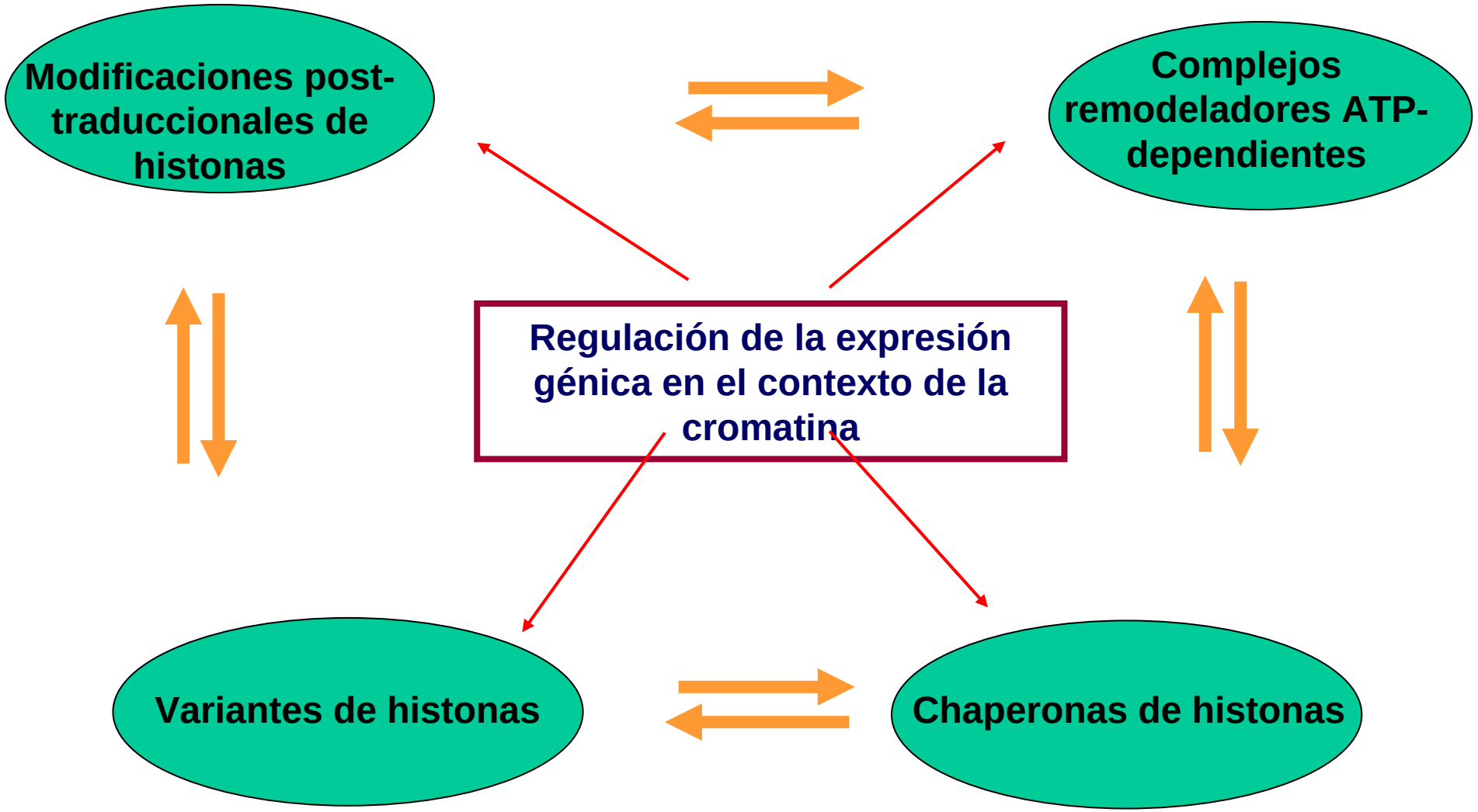
SILENCIAMIENTO

-Transcripción de ncRNAs de repetidos de ADN de heterocromatina, que son sustrato del ciclo de RNAi

¡!

-ncRNAs participan directamente en el establecimiento de la heterocromatina

-ncRNAs serian la forma de transmitir el estado de la crmoatina


Modificaciones post-traduccionales de histonas
Complejos remodeladores ATP-dependientes
Regulación de la expresión génica en el contexto de la cromatina
Variantes de histonas
Chaperonas de histonas

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