Biologia Cellulare Molecolare Avanzata 2.2

"Functional & regulatory genomics" module

1. **Complexity of eukaryotic genomes.**
2. **Basic concepts of gene transcription and regulation**
3. **Transcriptomes**
4. **Coding, noncoding and alternative splicing**

5. **Chromatin as the dynamic environment of genomes.**
6. **Functional states of chromatin and chromosome territories**
7. **Epigenetics and gene imprinting**

8. **Mechanisms and pathways of gene control**
9. **Evolution and control of alternative splicing**
10. **RNA elements that regulate RNA fate and life**
11. **Other noncoding RNAs and primordial RNA functions**

http://biologia.i-learn.unito.it/

http://www.personalweb.unito.it/michele.debortoli

Michele De Bortoli
Professor of Molecular Biology
University of Turin

Heterochromatin structure and properties

Constitutive heterochromatin
(Mat locus in yeast)
Telomeric
Centromeric
2° X-chromosome in female
Cell-type specific

1. HC organizators

Very often, constitutive **heterochromatin** (HC) is formed on repetitive DNA sequences.

A characteristic feature of the DNA sequence of constitutive HC found in **pericentromeric** regions is that it is generally composed of long stretches of satellite repeats.

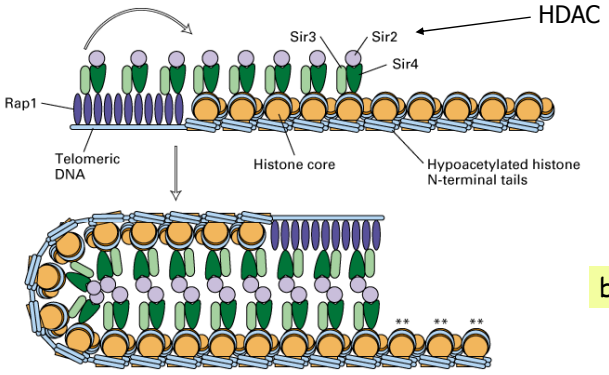
The lengths of the repeats vary widely between species: from the 5- to 7-bp repeats of *Drosophila* to the 1950-bp repeat found in a HC island in *Arabidopsis*.

The predominant satellite in human pericentromeric HC is the 171-bp a-satellite repeat, which shows significant variation between chromosomes.

Mouse pericentromeric heterochromatin is made up largely of the pancentromeric 234-bp -satellite repeat.

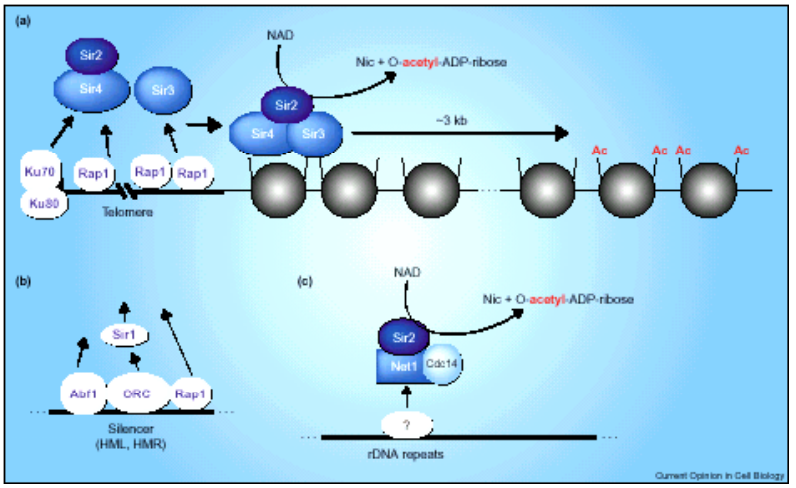
Experimental: Tandem repeats of transgenes are often HC. This depends on the presence of CG-rich prokaryotic sequences in the transgene, e.g. the lacZ gene. The sequence of repeats is also probably significant.

Telomeres are formed by microsatellite repetitive sequences



replicazione dei cromosomi lineari
struttura dei telomeri
telomerasi

budding yeast



Enzymatic activities of Sir2 and chromatin silencing
Danesh Moazed
Current Opinion in Cell Biology 2001, 13:232–238

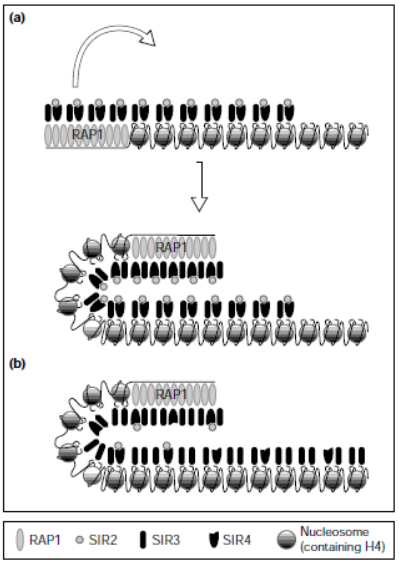
Genetic analysis in yeast for trans-gene suppression at telomeres and HMR mating locus identified several genes that, if expressed a little more or less, affect the extent of a phenomenon known how “Positional Effect Variegation” (PEV) that depends on the extent of the HC regions.

SIR2
SIR3
SIR4 (silencing information regulators)
RAP1 (repressor activator protein 1)
H3
H4 (N-terminal tails)

RAP1 is the only one that binds DNA elements ($C_{1\text{-}3}A$ repeats)
RAP-1 C-terminal domain fused to GAL4 DBD can silence GAL4 element-containing genes.
In two-hybrid system, RAP-1 C-terminal domain interacts with SIR3 and SIR4

SIR3 is the limiting factor for chromatin spreading, i.e. increases the distance at which a trans-gene can be silenced.

SIR3 and SIR4 interact with H3 and H4 N-terminal domain in vitro.



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

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.

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.

Why does Sir2 utilize a high-energy substrate as NAD as the acceptor of acetyl moieties? Most other HDAC just use water!

Sir2 may represent a “sensor” of the metabolic status

Overexpression of Sir2 extends the lifespan in yeast and Caenorabditis elegans

Deletion of Sir2 gene consistently shortens lifespan in yeast

family Sirtuins (wikipedia)

Increased dosage of a *sir-2* gene extends lifespan in *Caenorhabditis elegans*

Heidi A. Tissenbaum & Leonard Guarente

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

In *Caenorhabditis elegans*, mutations that reduce the activity of an insulin-like receptor (*daf-2*)[1] or a phosphatidylinositol-3-OH kinase (*age-1*)[2] favour entry into the dauer state during larval development[3] and extend lifespan in adults[3–6]. Downregulation of this pathway activates a forkhead transcription factor (*daf-16*)[7,8], which may regulate targets that promote dauer formation in larvae and stress resistance and longevity in adults[9]. In yeast, the *SIR2* gene determines the lifespan of mother cells, and adding an extra copy of *SIR2* extends lifespan[10]. Sir2 mediates chromatin silencing through a histone deacetylase activity that depends on NAD (nicotinamide adenine dinucleotide) as a cofactor[11–13]. We have surveyed the lifespan of *C. elegans* strains containing duplications of chromosomal regions. Here we report that a duplication containing *sir-2.1*—the *C. elegans* gene most homologous to yeast *SIR2*—confers a lifespan that is extended by up to 50%. Genetic analysis indicates that the *sir-2.1* transgene functions upstream of *daf-16* in the insulin-like signalling pathway. Our findings suggest that Sir2 proteins may couple longevity to nutrient availability in many eukaryotic organisms.

CMLS, Cell. Mol. Life Sci. 60 (2003) 2295–2302
1420-682X/03/112295-08
DOI 10.1007/s00018-003-3245-y

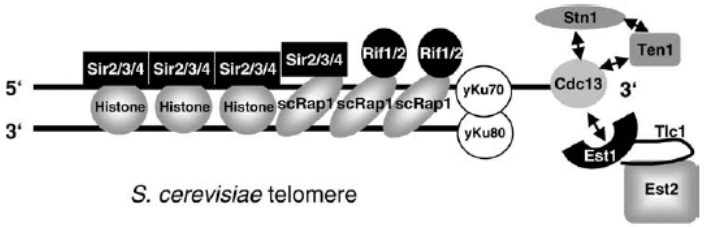
CMLS Cellular and Molecular Life Sciences

Composition and conservation of the telomeric complex

J. Kanoh* and F. Ishikawa

Graduate School of Biostudies, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502 (Japan), Fax: +81 75 753 4197, e-mail: jkanoh@lif.kyoto-u.ac.jp

Abstract. The telomere is composed of telomeric DNA and telomere-associated proteins. Recently, many telomere-associated proteins have been identified, and various telomere functions have been uncovered. In budding yeast, scRap1 binds directly to telomeric DNA, and other telomere regulators (Sir proteins and Rif proteins) are recruited to the telomeres by interacting with scRap1. Cdc13 binds to the most distal end of the chromosome and recruits telomerase to the telomeres. In fission yeast and humans, TTAGGG repeat binding factor (TRF) family proteins bind directly to telomeric DNA, and Rap1 proteins and other telomere regulators are recruited to the telomeres by interacting with the TRF family proteins. Both organisms have Pot1 proteins at the most distal end of the telomere instead of a budding-yeast Cdc13-like protein. Therefore, fission yeast and humans have in part common telomeric compositions that differ from that of budding yeast, a result that suggests budding yeast has lost some telomere components during the course of evolution.



Telomere-associated proteins in budding yeast. Arrows indicate physical interactions.

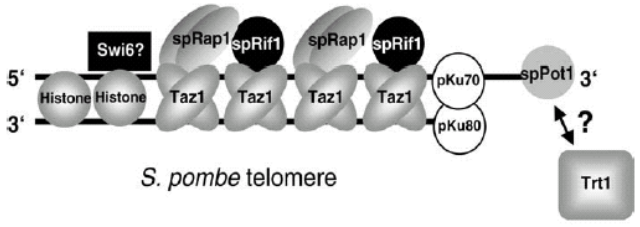


Figure 2. Telomere-associated proteins in fission yeast. Localization of Swi6 is speculative. spPot1 may regulate the access of telomerase (Trt11) to the telomeres.

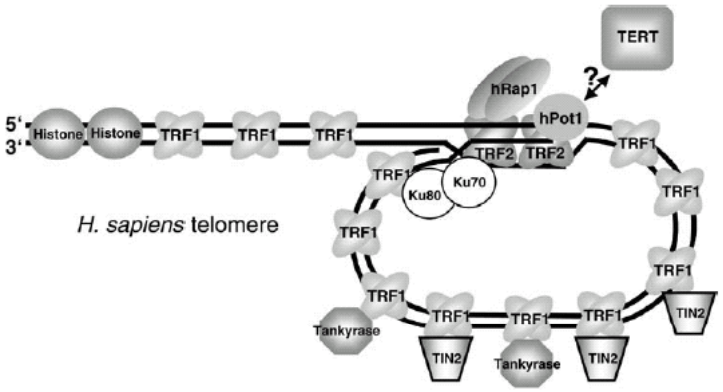
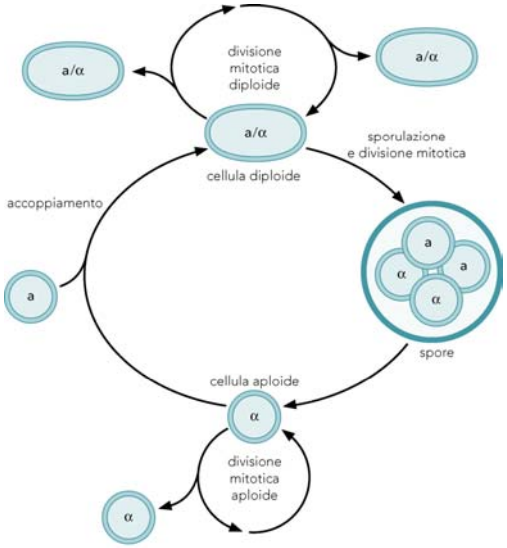


Figure 3. Telomere-associated proteins in human. Relationship between hPot1 and telomerase is not known.

One important question is how heterochromatin (HC) is excluded form adjacent loci.

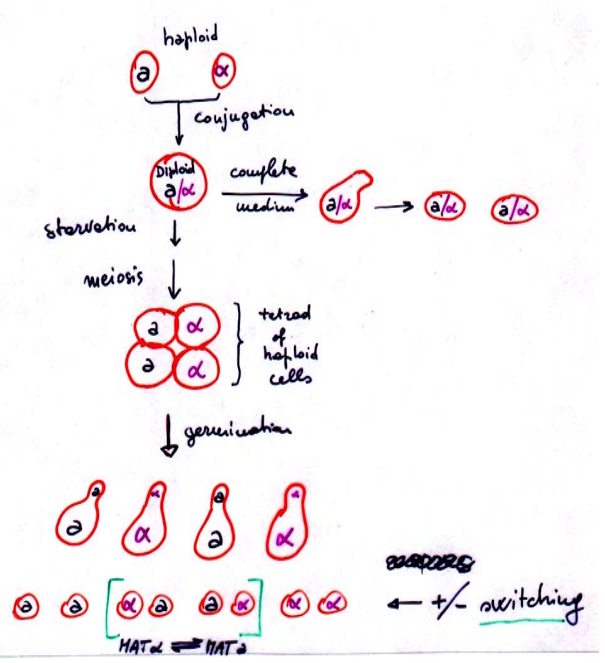
Studies on MAT locus in budding yeast (S. cerevisiae) has revealed the concept of insulators or HC boundaries.

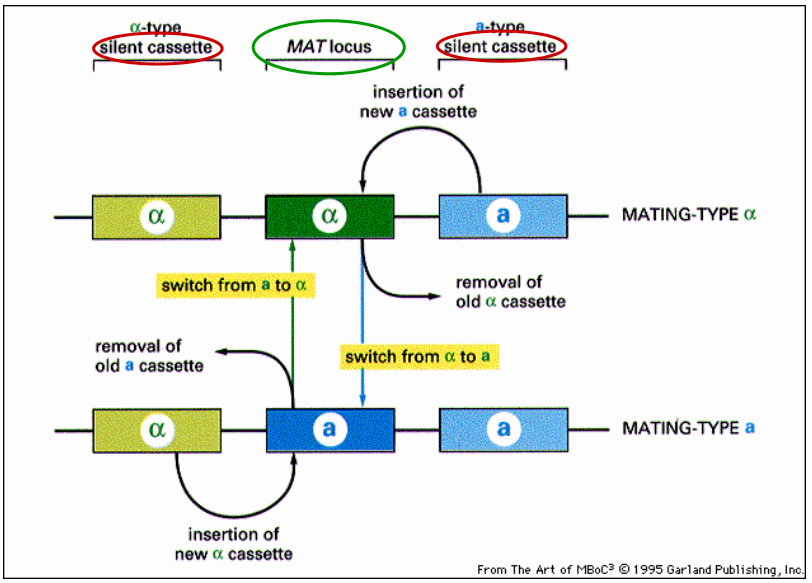


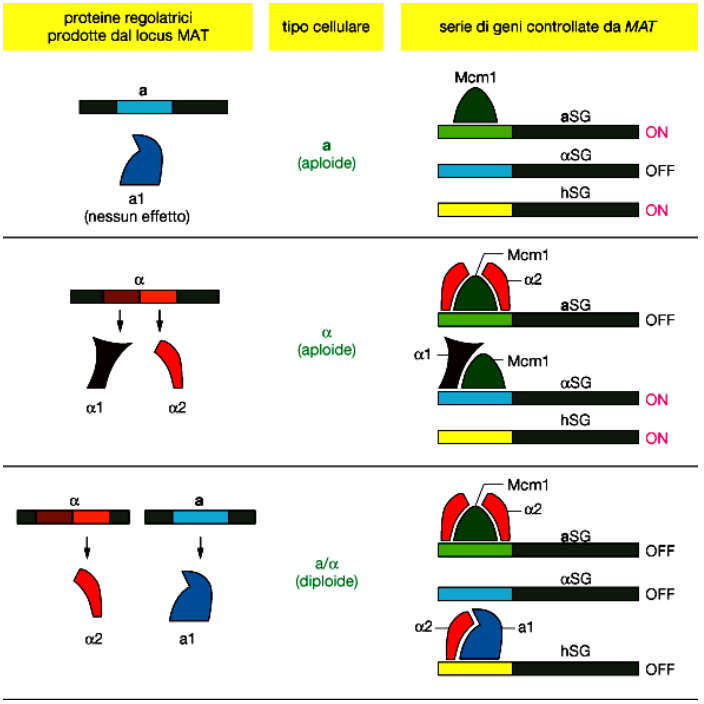
Saccaromyces cerevisiae
(budding yeast)

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

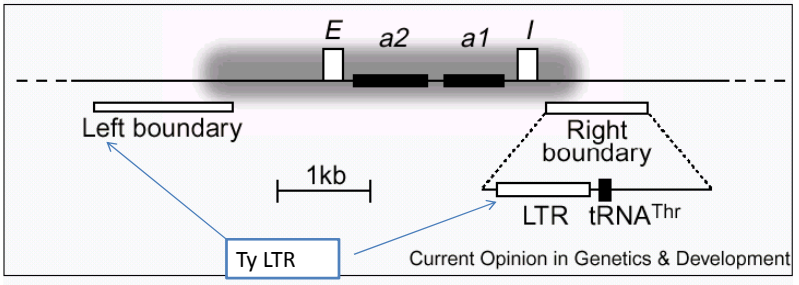
Very easy, since homologous recombination is favoured in yeasts and allows HC elements (i.e. HC organizers, silencers, boundaries, etc.) or reporters to be placed at will, in any genomic locus.



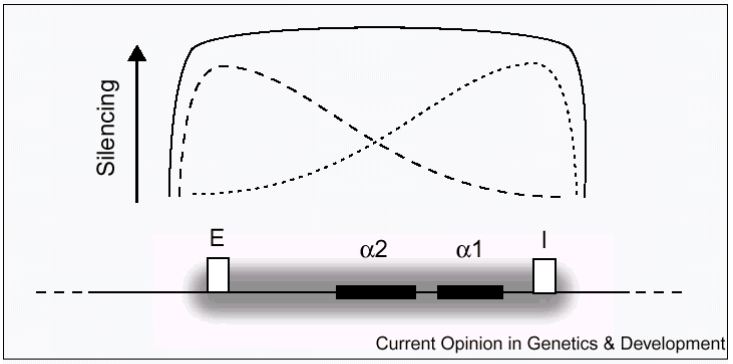

α-type
silent cassette
MAT locus
a-type
silent cassette
insertion of
new a cassette
α
α
a
MATING-TYPE α
switch from a to α
removal of
old α cassette
removal of
old a cassette
switch from α to a
α
a
a
MATING-TYPE a
insertion of
new α cassette
From The Art of MBoC³ © 1995 Garland Publishing, Inc.


proteine regolatrici prodotte dal locus MAT
tipo cellulare
serie di geni controllate da MAT
a
a1
(nessun effetto)
a
(aploide)
Mcm1
aSG
ON
αSG
OFF
hSG
ON
α
α1
α2
α
(aploide)
Mcm1
α2
aSG
OFF
α1
Mcm1
αSG
ON
hSG
ON
α
a
α2
a1
a/α
(diploide)
Mcm1
α2
aSG
OFF
αSG
OFF
α2
a1
hSG
OFF

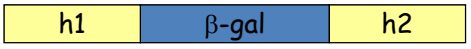
HMR mating locus in yeast has heterochromatic features,
e.g. is resistant to endonuclease digestion
and silences constructs placed within

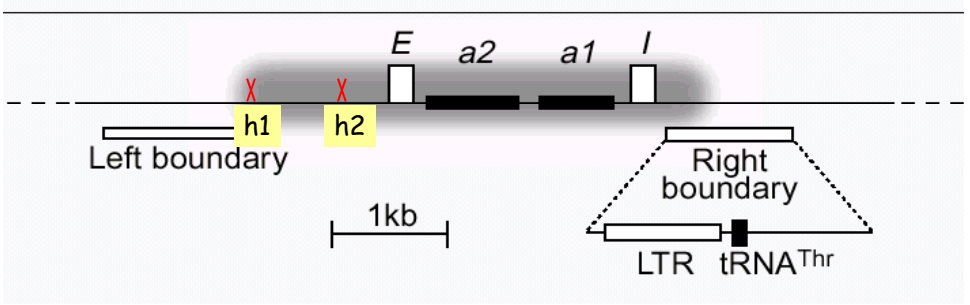


Organization of heterochromatin barriers surrounding *HMR*. The *HMR* locus is diagrammed, showing the location of the mating type genes *a1* and *a2*, the *E* and *I* heterochromatin organizing centers (silencers) and the left and right heterochromatin barriers as defined in [1••]. Background shading 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



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•].





Gene targeting by homologous recombination in yeast

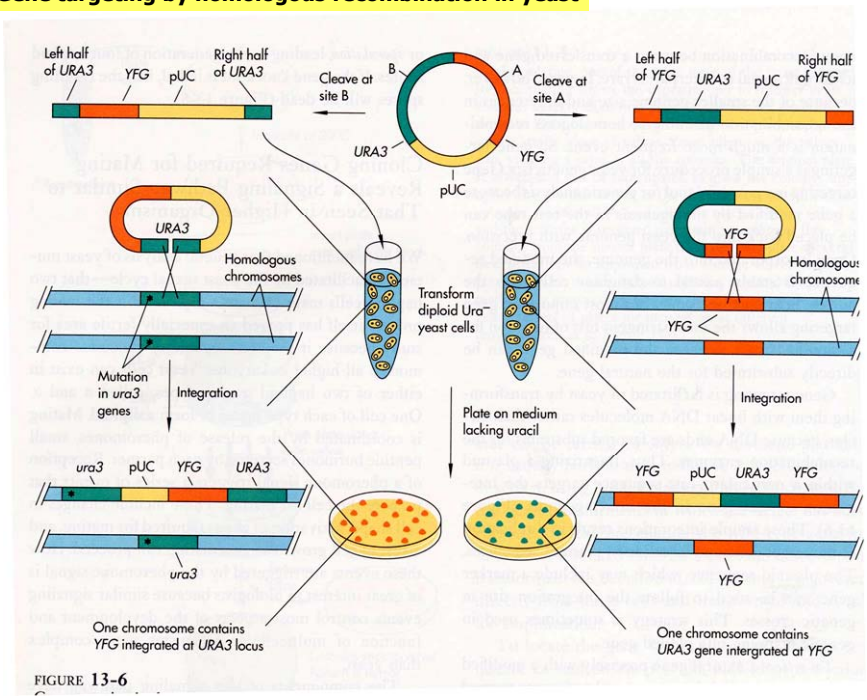
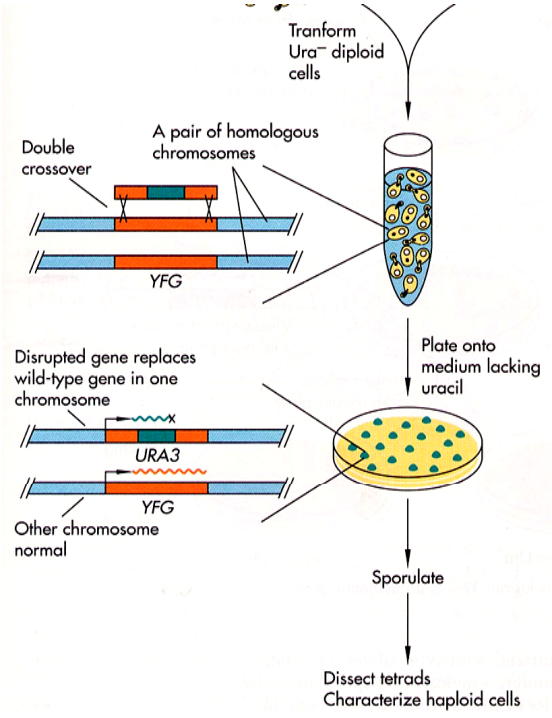


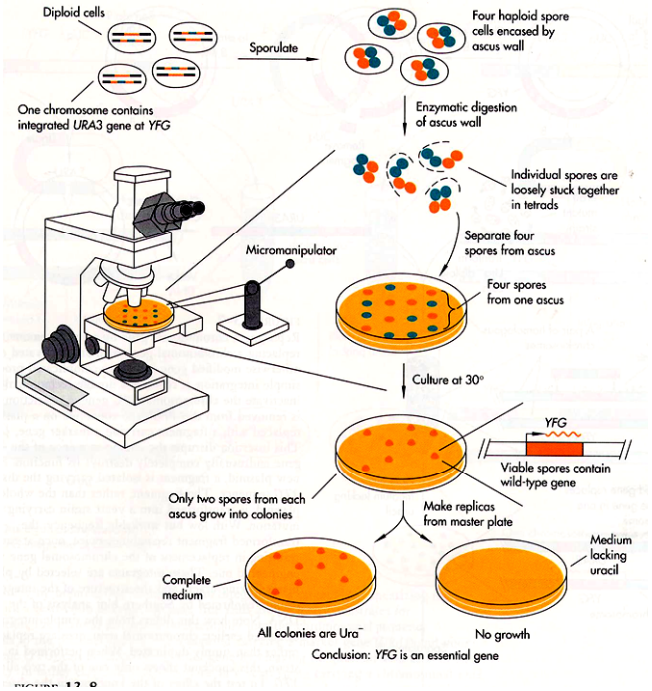
FIGURE 13-6



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.



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

Pericentromeric chromatin in fission yeast (S. pombe) reveals a role for RNA interference in HC maintenance

SCIENCE VOL 297 13 SEPTEMBER 2002

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.

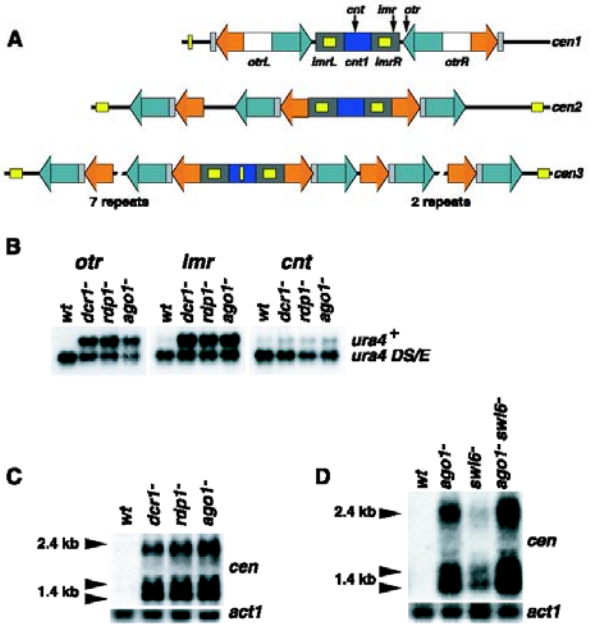


Fig. 1. Centromeric silencing is relieved in *ago1*⁻, *dcr1*⁻, and *rdp1*⁻ mutant strains compared to wild type. Diagram of the three *S. pombe* centromeres (**A**) including locations of *ura4*⁺ transgenes as well as outermost (*otr*), innermost (*imr*), and central (*cnt*) centromeric regions (*24*, *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 transcribed 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).

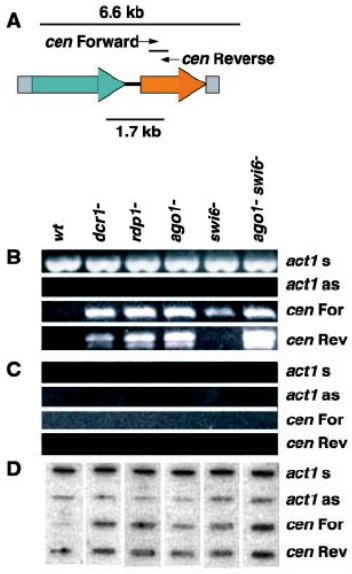
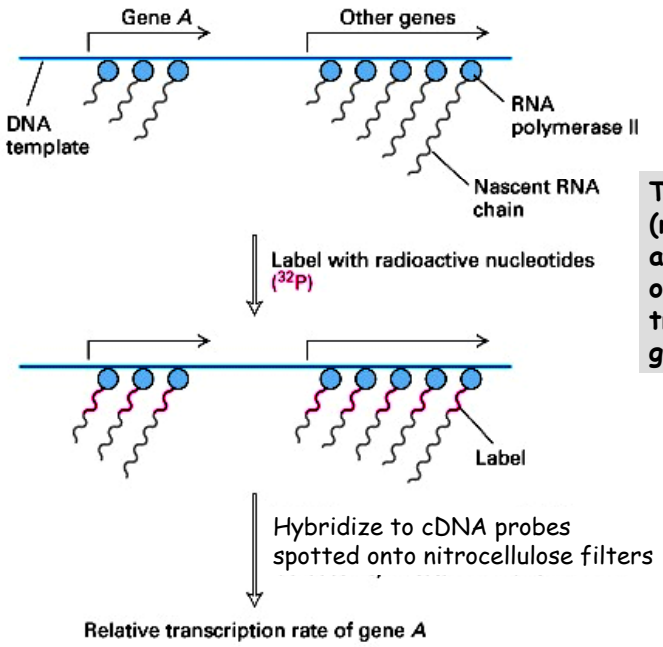


Fig. 2. Centromeric *otr* transcripts are both transcriptionally and posttranscriptionally regulated. (**A**) A schematic diagram showing the direction of transcription of forward and reverse transcripts corresponding to the *dh* repeat. Strand-specific RT-PCR analysis was performed in the presence (**B**) or absence (**C**) of reverse transcriptase. Samples were incubated with primers from the *dh* repeat complementary to either the forward (*cen* For) or reverse (*cen* Rev) centromeric transcripts in first strand cDNA synthesis reaction (primer locations are summarized in Fig. 5). Both primers were present in subsequent cycles of PCR amplification after heat inactivation of the reverse transcriptase. Treatment of control reactions lacking reverse transcriptase (C) was identical except that these samples were not subjected to first strand synthesis. Strand-specific control reactions were also conducted using primers specific for *act1* sense (*act1* s) or *act1* antisense (*act1* as) transcripts. Strand-specific analysis of nascent RNA transcripts was performed by nuclear run-on assay (**D**). Radiolabeled nascent RNA purified from mutant and wild-type strains was hybridized to nylon membranes containing strand-specific probes made using the same primer pair as in (**A**). These probes recognized either forward (*cen* For) or reverse (*cen* Rev) centromeric transcripts. Control probes recognized either sense (*act1* s) or antisense (*act1* as) actin transcripts.



The nascent chain (run-on) assay allows measurement of the rate of transcription of a given gene

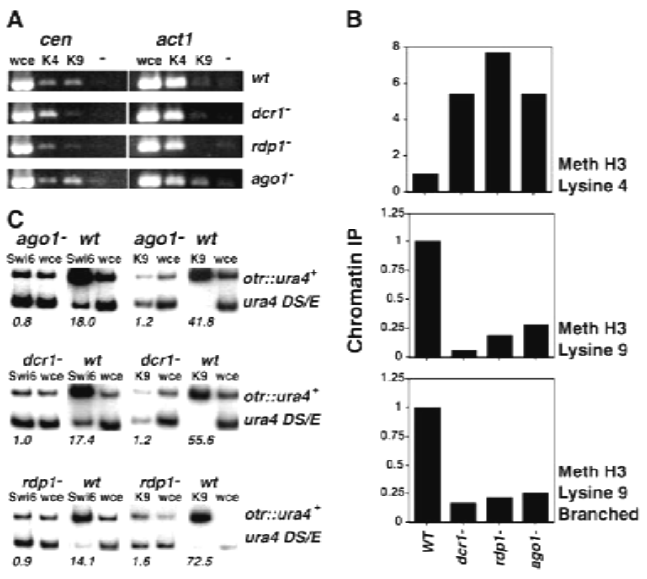


Fig. 3. Chromatin structure at centromeric repeats is altered in *ago1*⁻, *dcr1*⁻, and *rdp1*⁻ mutants. Chromatin immunoprecipitation (ChIP) was performed on extracts from formaldehyde fixed mutant or wild-type cells. DNA fragments purified from whole-cell extracts (wce) or co-precipitated with antibodies to K4 or K9 histone H3 were amplified by PCR using primers specific for centromeric *dg* repeats (Fig. 5) or *act1* (**A**). Quantitation was performed using quantitative PCR (**B**) and normalized to actin (K4) and mating type region (K9) controls, which were amplified from the same ChIPs in each genotype (*21*). DNA fragments from wce or co-precipitated with antibodies to Swi6 or K9 histone H3 were amplified by multiplex PCR using *ura4*-specific primers (**C**). These primers amplify both a *ura4*$^{+}$ transgene located in the *otr* of *cen1* (*otr*::*ura4*$^{+}$) and the *ura4 DS/E* minigene (*ura4 DS/E*) located on the chromosome arm. Relative levels were estimated using a FUJI phosphoimager and are indicated below each lane.

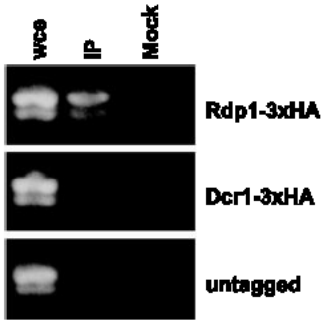


Fig. 4. Rdp1, but not Dcr1, is bound to centromeric chromatin. Chromatin immunoprecipitation (ChIP) was performed on extracts derived from triple-HA tagged Dcr1 or Rdp1 strains. DNA fragments from wce or co-precipitated with antibodies raised against the triple-HA tag were amplified by PCR using centromere-specific primers and compared to untagged strains. Mock reactions were identical except without the addition of primary antibody.

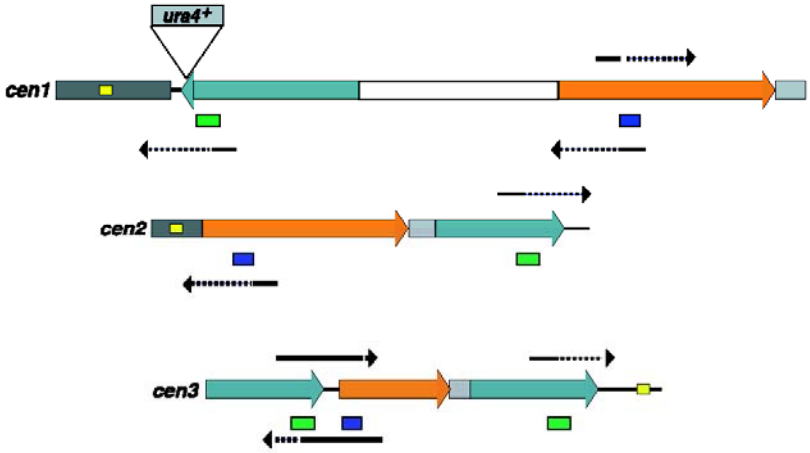


Fig. 5. One repeat unit from each of the three centromeres is shown (see Fig. 1). Transcripts from *ago1*⁻ mutant cells were identified by sequencing RT-PCR and RACE-PCR products, and those unique to each centromere are shown as thick arrows. Transcripts whose origin was ambiguous due to sequence identity between the centromeres are shown as thin arrows. Transcript length, determined by Northern blots (Fig. 1), allowed us to estimate the approximate position of the transcribed region (indicated by dashed lines). The PCR product that detected run-on *dh* repeat transcription toward the centromere in wild-type cells (Fig. 3) is indicated as a blue bar. The PCR product amplified in ChIP experiments with K9 and K4 antibodies (Fig. 3A) as well as with rdp-HA antibodies (Fig. 4) is indicated as a green bar underneath each *dg* repeat. A similar PCR product was used as the probe for the Northern blot shown in Fig. 1.

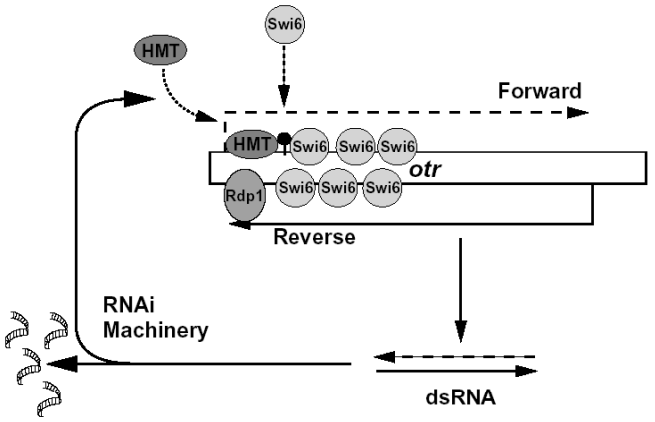


Fig. 6. The RNAi machinery is required for the initiation and maintenance of the heterochromatic state of centromeric repeats. Reverse strand centromeric transcription occurs in wild-type cells and is degraded posttranscriptionally by the RNAi machinery. Low-level transcription from the forward strand and/or amplification by Rdp1 results in generation of dsRNA, which is converted to siRNA by RNAi. Rdp1, bound to the chromatin, promotes targeting of histone modifications to specific sequences via siRNA, resulting in maintenance of the heterochromatic state (HMT, histone methyl transferase).

ELSEVIER

Available online at www.sciencedirect.com
ScienceDirect

Current Opinion in
Cell Biology

Boundaries. Boundaries... Boundaries???

Victoria V Lunyak

One way to modulate transcription is by partitioning the chromatin fiber within the nucleus into the active or inactive domains through the establishment of higher-order chromatin structure. Such subdivision of chromatin implies the existence of insulators and boundaries that delimit differentially regulated chromosomal loci. Recently published data on transcriptional interference from the repeated component of the genome fits the classic definition of insulator/boundary activity. This review discusses the phenomena of transcriptional interference and raises the question about functionality of genomic "junk" along with the need to stimulate a dialogue on how we would define the insulators and boundaries in the light of contemporary data. Rule 19 (a) (Boundaries) "Before the toss, the umpires shall agree the boundary of the field of play with both captains. The boundary shall, if possible, be marked along its whole length" Rules of Cricket

REVIEW

Current Opinion in Cell Biology 2008, **20**:281–287