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Multiple Functions of Yeast Telomeric Heterochromatin

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Summary

Heterochromatin-like structure at yeast telomeres is involved in the regulation of several important biological processes. Telomeres facilitate end replication, stabilize chromosomes and display many features of higher eukaryotes heterochromatin. In eukaryotes telomeres exert position effect variegation on genes located in their immediate vicinity, a phenomenon called transcriptional silencing. Many examples of silencing are known in yeast, among which the *HM* mating type loci. In all cases the establishement and maintenance of silencing is essentially achieved through the organization of chromatin of higher order structure, involving many protein factors. The Sir proteins and Rap1p are the main components of this compact and inaccessible structure, and are usually found concentrated at a defined number of loci at the nuclear periphery. A large body of experimental evidences has lead to the definition of a tridimensional model that takes into account all the interactions among the Sir proteins, Rap1p and other additional factors. In spite of the compactness of heterochromatin, though, the Sir protein complex seems to be able to relocate to other loci in several instances, suggesting the existence of subnuclear compartments where differential regulation events may take place. In addition to the regulation of silencing, the Sir proteins, Rap1p and Rap1p interacting factors are involved in different ways in the regulation of telomeric organization, controlling the number of $(C_{1\text{-}3}A)_n$ repeats and the number and type of subtelomeric middle-repetitive elements present at each chromosome end. These mechanisms may involve either telomerase or recombination-mediated pathways. Few cases of naturally occurring telomeric silencing have been described, and the possible biological significance of this phenomenon still awaits clarification. Currently, the number of genes somehow connected with the »on« and »off« state of telomere-linked genes is increasing, shading light on the variety of cellular processes affected by the integrity and stability of telomeric heterochromatin.

Key words: telomeres, silencing, heterochromatin, *Saccharomyces cerevisiae*

Introduction

All eukaryotic genomes are organized into linear chromosomes. The problem of the replication of the ends raises the question of how the integrity of these linear chromosomes is preserved. In fact, genetic information can be potentially lost at each round of replication, due to the limitations of conventional polymerases.

The discovery of specialized protein-DNA structures called telomeres has shed light on this long-standing question. Telomeres were first characterized in ciliated protozoans such as *Tetrahymena thermophyla* (*1*) and *Oxytricha* (*2*). Since these first discoveries a large amount of reports has elucidated the nature of telomeres as a universal feature of eukaryotic organisms, from yeast to mammals and plants (*3*).

The structural leit-motiv of telomeres is a tandem array of simple, G-rich sequence DNA (*3*) with the ex-

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ception of *Drosophila melanogaster* chromosome ends, that are composed of transposable elements (*4*). The efficient replication of these capping sequences is a task for a specialized reverse transcriptase, called telomerase, that can add de novo telomeric repeat sequences onto the ends of chromosomes (*5,6*). By means of this activity telomeres prevent end-to-end fusions and protect the genetic material from degradation and inappropriate recombination.

Telomeric sequences are always associated with specific binding proteins, such as Rap1p in *Saccharomyces cerevisiae* (*7*) and Trf2p in humans (*8*). In addition to proteins that directly bind the telomeric DNA, a number of studies have revealed that telomeres are actually covered with a complex aggregate of proteins, which confer to them the characteristics of heterochromatin. This phenomenon was intensively studied in *S. cerevisiae*, an organism in which the chromosome ends are composed of approximately 300bp of $(C_{1\text{-}3}\text{-}A)_n$ repeats (*3*). In addition to the repeats, the immediately subtelomeric regions carry middle repetitive sequence DNA of two types, called X and Y′ elements which can vary in number at each chromosome end (*9,10*). In *S. cerevisiae* an articulate interplay among telomere-interacting factors organizes a structure that, like heterochromatin in the higher eukaryotes, is resistant to nucleases, late replicating and enriched in hypoacetylated histones (*11,12*).

The detailed analysis of the structure and function of telomeric heterochromatin components in *S. cerevisiae* has revealed a profound involvement of telomeres in the regulation of many cellular processes, from the counting and maintenance of repeat length (*13–16*), to transcriptional silencing (*17–19*), DNA repair, cell growth and aging (reviewed in *20*).

Telomeric Transcriptional Silencing in Yeast

The stable inactivation of gene activity brought about by a novel chromosomal environment is a common phenomenon in higher eukaryotes. The first example of »position effect« came from genetic studies in *Drosophila* (see *21* for a review), when it was observed that gene inactivation occurs following rearrangements that place the gene near a heterochromatic region of the genome. Position effects and epigenetic control of gene expression are frequent events in *Drosophila* (*22–24*).

In *S. cerevisiae* the ability of exerting position effects is a characteristic of telomeres and of the silent mating type loci. Telomeric position effect (TPE) was first discovered when engineered chromosomes containing reporter genes such as *URA3* and *ADE2* were shown to be transcriptionally repressed (*25*). In both cases functional assays were developed that allowed the characterization and quantitation of the repressed state. The first assay takes advantage of the capability of *ura3* strains to survive treatment with the drug 5-fluoroorotic acid (5-FOA). In such strains a certain percentage of cells carrying the *URA3* gene in proximity of chromosome VII-L telomere was able to form colonies on 5-FOA containing medium (5-FOA^R), reflecting the transcriptional repression of *URA3* in that fraction of cells. This variegated behaviour was confirmed by means of the second assay, based on the ability of cells mutated in *ADE2* to form colonies of different colors. When the *ADE2* gene was located near the VII-L telomere in an *ade2* strain, the cells gave rise to red-white sectored colonies. The number and size of red and white patches within individual colonies, as well as the possibility for cells containing telomere-linked *URA3* of still growing on medium lacking uracil, suggested that the repressed and derepressed states are inherited in a semi-stable fashion.

The Repressor Activator Protein (RAP1)

Transcriptional silencing also occurs naturally and is stably maintained at *HM* loci, controlling the mating type switching in *S. cerevisiae*. Several studies have revealed that both types of silencing are brought about and regulated by the same set of genes, coding for a number of chromatin components (*17*). One of the central elements in the modulation of silencing is the Rap1 protein, whose DNA binding site is present both on silencers at the *HM* loci and on telomeric sequences. The detailed genetic analysis of mutants in the Rap1p allowed D. Shore and collegues to define a region at the C-terminal domain which is involved in silencing, since proteins lacking this domain induce transcription of silent *HM* loci (*26,27*). Likewise, C-terminal truncation of Rap1p results in dramatic loss of TPE (*18*), suggesting that this domain is an absolute requirement for telomeric repression. It is noteworthy that Rap1p is a key player in the regulation of telomere length. A typical yeast telomere is about 300bp long, but this tract can increase and decrease spontaneously due to the opposite effects of replication and telomerase activity. Therefore a heterogeneity is generated at each cell division (*28*), that is under a complex genetic control. The telomeric sequences have been demonstrated to bind Rap1p in a regular fashion (*7*) and several mutations in the Rap1p C-terminus have been shown to cause either loss of repeats or conspicuous elongation of telomeres (*29,30*). As mentioned above, some of these mutants also show loss of telomeric position effect and significant impairment of *HM* silencing. A particular class of mutants, called $rap1^t$, results in a 10-fold elongation of $(C_{1\text{-}3}A)_n$ tracts (*30*) and a considerable increase of the frequency of 5-FOA^R colonies from cells carrying *URA3*-marked V-R and VII-L telomeres. However, loss of TPE is not correlated with the increased distance of the reporter gene from the end of the chromosome since TPE is actually improved by telomere elongation in otherwise wild-type cells (*18*). This suggests that repression at telomeres in $rap1^t$ mutants is most likely linked to the corresponding loss of a function related to the establishment of repression. The requirement for the formation and maintenance of a closed chromatin structure was revealed by early experiments showing the increased accessibility of dam methylase to subtelomeric chromatin in $rap1^t$ cells (*18*).

The Silent Information Regulators (SIR)

The current models for the structure of telomeric heterochromatin followed from a number of studies focused on the genetic analysis of the regulators of telomeric repression. From early work by Gottschling and collegues, it became very soon clear that many of the factors involved in the transcriptional repression of

telomere-linked genes were the same responsible for the silencing of the *HM* loci (*17*). In particular, the Silent Information Regulators, the *SIR2*, *3* and *4* genes are required for complete silencing at both locations, while *SIR1* plays a role specifically at the mating type loci. At the same time these investigators also found that deletions or mutations at the N-terminal end of the histone H4 genes (*HHF1* and *HHF2*) relieve transcriptional silencing at telomeres. Moreover, genetic evidences pointed to a direct interaction of Sir3p with the histone H4 N-terminal tail, interaction that appeared to be one of the essential requirements for the maintenance of the silent state at *HML* and *HMLa* (*31*). Sir3p is therefore a central element in the chromatin-mediated silencing, it is present in equimolar amounts with Sir4p, but it is the only component that is limiting for the propagation of silencing. The subtelomeric silencing domain usually extends up to 3kbs from the end, but it can be extended up to 20kbs by overexpression of Sir3p. This propagation is presumably achieved through the interaction with the histone tails (*19*). More recently Sir3p was analyzed in detail and it was shown that while overexpression of its N-terminal region (Sir3N) enhances TPE, ectopic expression of the C-terminal domain (Sir3C) causes derepression of silencing. Moreover, increasing the amount of Sir3N counteracts the derepression caused by Sir4p overexpression (*32*). This may reflect the notion that the N and C-terminal portions of Sir3p interact with different sets of proteins and achieve regulation of silencing through different pathways (*33*). In addition to this, a specific role in the initiation of silencing was attributed to Sir3p, whose 144 C-terminal amino acids seem to be both necessary and sufficient for the establishment of the repressed state (*34*).

Also the other silencing factors Sir2p and Sir4p have been extensively studied and shown by two-hybrid assays to interact with Rap1p to form a complex containing also Sir3p (*35*). None of the Sir proteins binds DNA directly, but the precise interaction with Rap1p allows them to build up a structure whose architecture is still the object of extensive studies (see below).

Competition for silencing factors

One interesting aspect of the telomeric silencing is that it stands in apparent competition with silencing at other loci, *i.e. HM*. This follows from the observation that a class of Rap1p mutants, designated *rap1^s*, while abolishing repression at *HMR* (when the E silencer is weakened by deletion of the A site), actually improves silencing at telomeres. These mutants also have elongated telomeres, which suggests that they may compete for silencing factors present in limiting amount (*36*), principally Sir4p, which becomes actually concentrated at a telomeric location.

This concept of the competition among different loci for the silencing factors is gaining increasing interest as several examples of this type of competition have been correlated with important cellular processes. For example *sir4-42* mutants have been isolated, which exhibit extended life span (*37*). The delayed aging phenotype was correlated with loss of transcriptional silencing and relocation of Sir3p and Sir4p to the nucleolus (*38*,*39*). These studies point to the importance of the Sir proteins in the overall life cycle and to the necessity for the cell to balance the amounts of the silencing factors with respect to each other.

Telomeric Silencing in a Natural Context and its Biological Role

In spite of the large amount of research devoted to the regulation, maintenance and inheritance of telomeric silencing, its biological significance remains an open question.

Silencing in a natural context

Very few instances of naturally occurring telomeric silencing have been reported. The first example of silencing in a natural context is that of the Ty5-1 retrotransposon, located 1.8Kb from the left telomere of *S. cerevisiae* chromosome III (*40*). This inactive member of the Ty5 family of retrotransposon was shown by our work (*41*) to be subjected to transcriptional silencing. In fact, this element is not transcribed in wild type strains, but it can be active in either *sir3* or H4 N-terminal deletion strains, indicating that in wild type strains the Ty5-1 element is maintained in a transcriptionally repressed state in a way that is dependent on the heterochromatin components, like all the other examples of silencing described so far. The behaviour of this particular retrotransposon, along with data collected by the group of D. Voytas, allowed us to suggest a possible biological role for the silencing of retrotransposons. Voytas and his group discovered that the Ty5 family is often associated with telomeres and with the silent mating locus *HMR* (*42*), and that these are sites of preferential integration in a transposition assay (*43*), suggesting that they may have been selected by the Ty5 elements to turn off their own expression. Indeed, variegated and semi-stable silencing could represent a useful transposition regulatory mechanism for Ty5 elements, in order to limit their tendency to cause mutagenic damage to the cell population. Furthermore, it was found that relieving silencing at *HMR-E* by weakening the silencer or by loss of Sir3p and Sir4p, decreases the frequency of integration (*44*,*45*) and randomizes the Ty5 distribution patterns. These data imply that it is the silent chromatin, with its particular Sir-dependent organization, that directs transposition events, and that Ty5 is sensitive to the state of chromatin.

As far as functional genes are concerned, the question of their silencing is still open. Endogenous telomeric genes, such as members of the *SUC* and *MAL* families, are not regulated by the *SIR* genes (*46*). More recently *SIR*-dependent silencing has been reported at the YFR057w open reading frame, located in the immediate vicinity of the right telomere of chromosome VI (*47*). Silencing though does not appear to be a general feature of all telomerically located ORFs, as other two of them (YPR201w on chromosome XVIR and YER188w on chromosome VR) are not silenced. The fact that YFR057w is on ORF of unknown function limits the possibility to further speculate on the biological significance of silencing.

An important aspect of silencing is that it is influenced by the presence or absence of subtelomeric associated sequences, such as X and Y′ elements. The telomere proximal portion of Y′ elements and the STR component of X elements have been shown to possess antisilencing properties, while the X-core sequences act as protosilencers (*48*). Pryde and Louis (*49*) have found in addition that two types of telomeres exist which differ in silencing strength, the first one with a strong capability to silence reporters up to 1.5kb from the protosilencer, the second with very weak silencing properties. The reason for this differential behaviour is not fully understood although it is reasonable that different combinations of sequences with opposite capacities exert a strong influence on silencing. It also emerges from these reports that antisilencing elements display the features of insulators and suggest that silencing can be propagated in a discontinuous manner.

A Model for Telomeric Heterochromatin Structure

As already mentioned, yeast telomeres display the features of heterochromatin. From a structural point of view they were first found to be late replicating, associated in foci at the nuclear periphery (*50*) and resistant to several nucleases and modifying enzymes (*51*). This nuclease resistant structure, called the telosome, was shown to be devoid of nucleosomes (*51*), and to contain telomeric repeats associated with Rap1p. More recently a specific one-hybrid system was developed that allowed the identification of other components of the telosome (*52*), *i.e.* the Sir proteins, Rif1p and Rif2p, and the telomere-limited Cdc13 protein. The telosome most likely represents the center of nucleation of the telomeric heterochromatin, being Rap1p the only silencing protein capable of binding specific sites on DNA. Rap1p was shown to interact with the Sir proteins (*34*) through its C-terminal domain, and subsequent work suggested that this interaction may also depend on histone H4 *in vivo.* In fact, mutations of the histone H4 acetylatable lysine 16, which prevents interaction with Sir3p in cell extracts, also prevents coimmunoprecipitation of Sir3p with Rap1p (*11*).

Nucleosomes in telomeres

Nucleosomes appear to be present in a more or less defined organization in the immediate subtelomeric regions (*51*), representing the connecting bridge between the telosome and the adjacent X and Y′ middle-repetitive elements. Nucleosomes therefore, and particularly histone H3 and H4, are the central components through which the Sir protein complex spreads into the chromosome and achieves transcriptional silencing. Nonetheless, their distribution and specific organization at natural telomeres was not clearly defined.

The chromatin organization of telomere LIII

We have described (*53*) the nucleosomal structure of *S. cerevisiae* LIII telomere, encompassing an X element and the Ty5-1 retrotransposon, which is, as detailed above, transcriptionally silent in a Sir3p and H4 dependent manner. Treating yeast *in vivo* with micrococcal nuclease and DNaseI we were able to describe the major architectural features of this chromosome end: *i)* the X element displays the presence of randomly positioned nucleosomes along with possible protein complexes associated with the ABF1 and ACS binding sites. This was revealed by the overall resistance of this region to nucleases; *ii)* the Ty5-1 element is organized into a regular array of translationally phased nucleosomes (*53*). Most interesting is the fact that this chromatin organization is dependent on the presence of Sir3p, since the entire nucleosome distribution and general accessibility to nucleases changes in *sir3* mutants, providing *in vivo* evidence for the interaction between Sir3p and nucleosomes.

Based on the 2.8 Å crystal structure of the nucleosome it was suggested that the N-terminal tail of histone H4 can be involved in interparticle contacts through its aminoacidic residues 16–24. It is relevant that these residues represent the domain of interaction with Sir3p, leading to the hypothesis that in the higher order heterochromatin structure the internucleosomal contacts may be replaced by the binding of Sir3p (*54*, *55*). In this light we found that the structural alteration in the LIII nucleosomal distribution of *sir3* strains is indistinguishable from chromatin changes in this region caused by deletion of the N-terminal tail of histone H4 (*56*). Most notably, nucleosomes seem to undergo displacement, and to be positioned in quite different locations, suggesting that in the presence of a staple-like function provided by the Sir3p-H4 interaction, nucleosomes are constrained in certain positions related to the heterochromatin configuration. These data strongly support the notion that the establishment *in vivo* of repressive heterochromatin structures at LIII, and most likely in general (*33*), requires the recruitment of Sir3p through interaction with the N-terminus of histone H4. Indeed, *in vivo* crosslinking experiments and immunoprecipitation of Sir3p show that Sir3p co-immunoprecipitates Sir4p, Rap1p and histones from cellular extracts, showing that these proteins are structural components of heterochromatin (*57*). Furthermore these experiments show that Sir3p spreads into adjacent chromatin when overexpressed (Fig. 1).

The foldback model

This and the detailed analysis of all the heterochromatin components by chromatin immunoprecipitation allowed M. Grunstein and collegues to propose a structural model of telomeric heterochromatin in which the Sir proteins spread along the surface of the chromosome interacting with each other and with histones. The complex is also helped in the spreading by the folding back of the chromosome end onto the subtelomeric region (*12*,*58*). Again the central role of Sir3p is pointed out by the fact that Sir2p and Sir4p do not spread equally, and actually they seem to be lost from telomere proximal regions, with Sir2p spreading even more weakly than Sir4p. Since Sir2p and Sir4p are also required for the extension of heterochromatin, it is possible that they are essential for the initiation of extended heterochromatin (*58*).

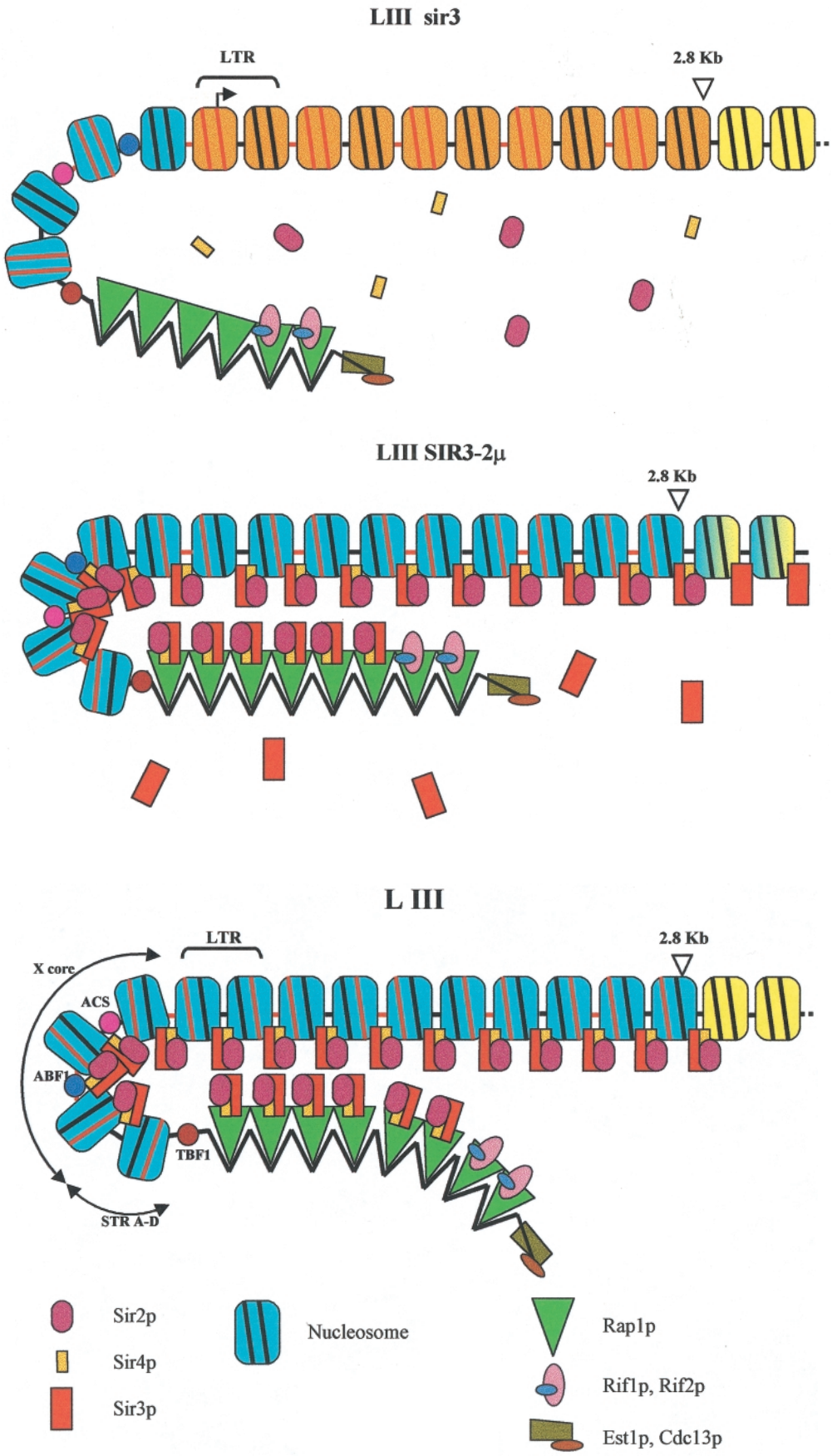

Fig. 1. The foldback model of telomeric heterochromatin. A possible view of chromosome LIII in wild type, *sir3* and *SIR3* overexpressing backgrounds

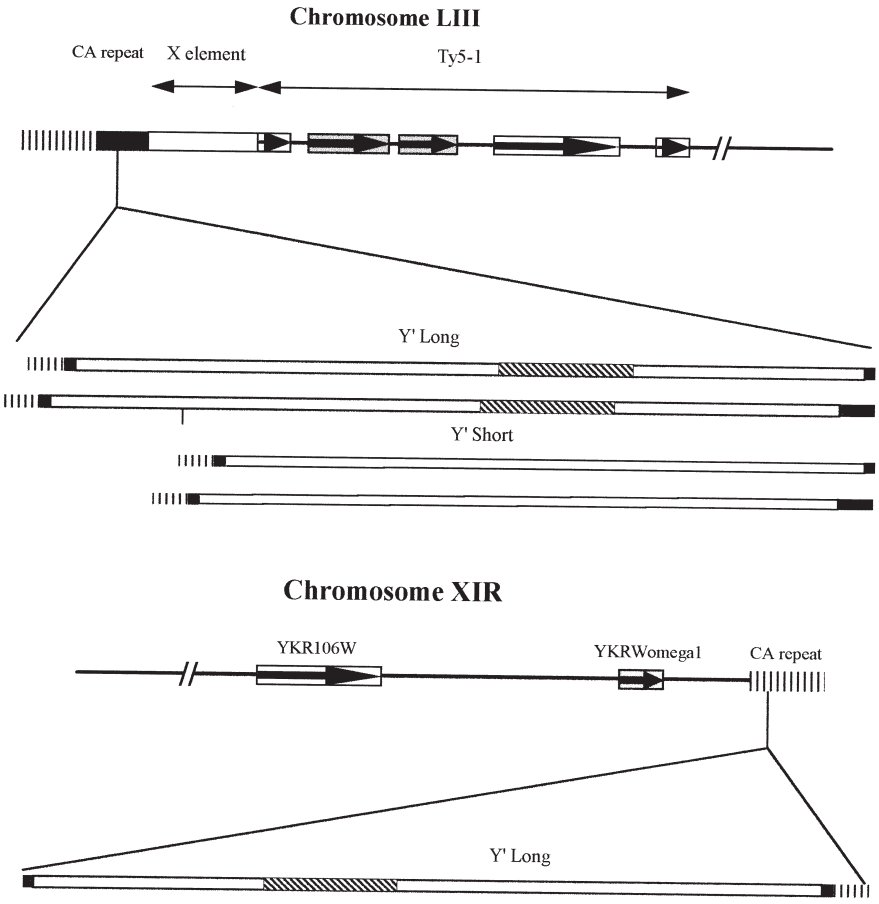

Fig. 2. Schematic summary of the Y′ insertions detected at chromosomes LIII and RXI. Y′ elements of two types, Long and Short, are found at these and several other extremities upon imbalance of two main heterochromatin components, Sir3p and histone H4

As with higher eukaryotes, yeast heterochromatin contains hypoacetylated histones, especially H4 which is hypoacetylated at lysines 5, 8 and 16. The only lysine residue of H4 to be acetylated in heterochromatin is K12. Being this acetylation pattern also conserved in *Drosophila* (*59*), this characteristic is likely to be a hallmark of heterochromatin. It is noteworthy that acetylation of K12 does not disrupt silencing, while acetylation of K16, preventing the interaction between H4 and Sir3p, derepresses *HML* and telomeric silencing. Furthermore, K16 euchromatic sites is mostly acetylated (*59*,*12*), suggesting that patterns of acetylation may be actively kept to define subdomains of eu- and heterochromatin, with different consequences on gene expression.

Telomeric Heterochromatin and the Existence of Nuclear Compartments

The tridimensional complexity of telomeric heterochromatin structure is increased by the location of telomeres at the nuclear periphery. Immunofluorescence of Rap1p, Sir3p and Sir4p allowed the precise localization of these proteins to a limited number of loci, associated with the nuclear envelope (*50*). Combining immunofluorescence with *in situ* hybridization techniques allowed the colocalization of these proteins with signals of subtelomeric repeats, suggesting that telomeric DNA is actually clustered at these loci (*60*). This clustering is likely to create a localized pool of Sir proteins at the nuclear periphery, favoring the formation of repressed chromatin, thus supporting the notion that existence of subnuclear compartments may influence gene expression. By targeting a reporter gene flanked by complete *HML-E* and *HML-I* silencers to a variety of chromosomal locations, Maillet and collegues were able to show that proximity to telomeric repeats is necessary for repression of the reporter (*61*). Furthermore, tethering a reporter gene flanked by a weak silencer to the nuclear envelope is sufficient to promote Sir-mediated repression of the reporter, inserted at *HMR* (*62*).

It was also suggested that telomeric heterochromatin may serve as a reservoir of Sir proteins and other factors that can be released upon specific physiological signaling (*63*) The Ku complex is a heterodimer involved in the repair of Double Strand Breaks (DBS) and appears to be required for the maintenance of the telomeric repeat length. Deletion of either HDF1 and HDF2, the genes encoding the yKu subunits, disrupts the perinuclear positioning of telomeres and derepresses telomeric silencing. In addition, the Sir proteins are relocated, along with the yKu complex itself, at sites of DSB in response to DNA damage. These evidences highligth the functional relevance of maintaining nuclear compart-

ments with concentration gradients of limiting silencing factors.

Heterochromatin and the Structural Organization of Yeast Telomeres

Telomerase-based size control

Maintenance of telomere length and structure is a complex phenomenon, being influenced by a wide number of genes and by the balancing of shortening and lengthening processes involving multiple factors. In yeast telomere length is usually kept within a narrow-sized distribution, which is also strain-specific and heterogeneous among chromosomes (*28*). It has been proposed that a length-sensitive mechanism exists that allows discrimination of even small differences in the number of telomeric repeats. Rap1p is the sensor of this mechanism based on its regular binding to telomeric repeats every 18bp, through its C-terminal domain (*64*). A defined and constant number of Rap1p molecules is kept bound at telomeres. Upon degradation or incomplete replication, the loss of one or more Rap1p molecules triggers elongation, most likely through intermediate target proteins like Stn1p and Cdc13p which are able to communicate with telomerase (*65*). Telomerase itself is obviously responsible for the maintenance of repeat tract length, as mutations of *EST2* and *TLC1*, encoding the catalytic subunit and the RNA component respectively, result in the progressive loss of sequences (*66*). In addition, mutations of other factors, such as the Rap1 Interacting Factors 1 and 2 (Rif1p and Rif2p) have an influence on telomerase activity and appear to negatively regulate it by binding to Rap1p (*15*,*16*). Telomerase activity has also been shown to be restricted to S phase (*67*), linking the maintenance of telomeres to the cell cycle progression.

Recombinational control of telomere length

In addition to the telomerase-mediated size control mechanisms described above it seems that yeast cells have more than one way to preserve the integrity of chromosome ends, exploiting alternative pathways based on recombination. The first evidence for this phenomenon came from the finding that *est1* mutants (in which an important subunit of telomerase is affected) display a lethal phenotype due to progressive shortening of telomeres (*68*). Survivors can arise in this genetic background on account of *RAD52*-dependent recombination events leading to amplification or aquisition of subtelomeric Y′ elements or deletion derivatives of them (*69*).

Several evidences point to the involvement of the Sir proteins in the telomere length control mediated by recombination. A mechanism called Telomeric Rapid Deletion (TRD) seems capable of reducing elongated telomeres to wild type length through intrachromatid recombination favoured by telomere clustering and alignment. Sir3p seems to increase the rate of TRD, maybe facilitating alignment itself (*70*).

As mentioned above the Ku complex involved in the DSB repair is actually present along the telomeres, as shown by chromatin immunoprecipitation experiments (*71*). The protein yKu80 colocalizes with the Sir proteins and Rap1p (*63*) and yKu70p was shown to interact with Sir4p (*72*). Deletion of either two proteins causes loss of silencing and dramatic telomere shortening (*73*), linking an important process like illegitimate recombination to the regulation of telomere functions.

Gene dosage of heterochromatin components and telomere structure

We have recently discovered a phenomenon which adds more weight to the relevance of maintaining the integrity of heterochromatin in the regulation of telomeric structure and composition (*74*). Using a set of strains that vary widely in the *SIR3*/*HHF* ratio, we have observed complex clonal dynamics of repeat number variations and Y′ elements aquisition upon unbalancing the dosage of *SIR3* and *HHF* (Fig. 2). In particular, overexpression of Sir3p favours insertions of Y′ elements at chromosome LIII, which normally does not have one, with the rate of insertion increasing when only one of the two H4 encoding genes is present. In addition, this appears to be a general phenomenon, as insertions are also found at other telomeres in this genetic background. Restoration of the wild type dosage of *SIR3* and *HHF* restores the normal profile of chromosome ends. This highlights the fact that also the structure of subtelomeric regions is involved in the control of telomere organization, in addition to the telosome-mediated mechanisms described above. In the light of the well established interaction between Sir3p and H4 it is reasonable to think that excess amounts of Sir3p perturbs the normal interaction, especially if H4 is scarce. Moreover, it is known that overexpression of Sir3p causes removal of Sir4p and Sir2p from the proximal telomeric regions (*58*). Relevant structural changes may occur, that become somehow signals for telomere reshaping recombinational events.

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Mnogostruke funkcije telomernog heterokromatina kvasca

Sažetak

Struktura slična heterokromatinu u telomerama kvasca sudjeluje u regulaciji nekoliko važnih bioloških procesa. Telomeri olakšavaju replikaciju na krajevima, stabiliziraju kromosome i pokazuju mnoga svojstva heterokromatina viših eukariota. U eukariotskim stanicama položaj telomera na krajevima utječe na različite gene smještene u njihovoj neposrednoj blizini. Ta se pojava naziva utišavanje transkripcije. U kvascu su poznati mnogi primjeri utišavanja među kojima je i *HM* lokus za tip parenja. U svim slučajevima uspostaljanje i održavanje utišavanja u biti se postiže organizacijom viših strukturnih oblika kromatina, koje omogućavaju mnogi proteinski faktori. Sir proteini i Rap1p su glavne komponente ove zbijene i nepristupačne strukture, a uglavnom su nađeni koncentrirani na određenom broju lokusa na periferiji jezgre. Veliki je broj eksperimentalno dobivenih dokaza omogućio utvrđivanje trodimenzionalnog modela u kojem su uzete u obzir sve interakcije između Sir proteina, Rap1p i ostalih dodatnih faktora. Unatoč kompaktnosti heterokromatina, izgleda da se kompleks Sir proteina može premještati u druge lokuse, što upućuje na postojanje subnuklearnih odjeljaka gdje se događaju različite regulacijske pojave. Osim regulacije utišavanja, Sir proteini, Rap1p i faktori koji reagiraju s Rap1p sudjeluju na različite načine u regulaciji telomera, kontrolirajući broj odsječaka $(C_{1\text{-}3}A)_n$ što se ponavljaju, te broj i tip čestica koje se ne ponavljaju toliko često u telomeru na svakom kraju kromosoma. Ovi bi mehanizmi mogli obuhvatiti bilo telomerazu, bilo put ostvaren rekombinacijom. Opisano je nekoliko slučajeva prirodnih telomernih utišavanja, a moguće biološko značenje ove pojave još nije objašnjeno. Trenutačno se povećava broj gena koji su na neki način povezani s »uključivanjem« i »isključivanjem« gena vezanih uz telomere, razjašnjavajući brojne stanične procese na koje utječe integritet i stabilnost telomernog heterokromatina.