10

Telomere Position Effect: Silencing Near the End

Michelle A. Mondoux and Virginia A. Zakian

Department of Molecular Biology, Lewis Thomas Laboratories
Princeton University
Princeton, New Jersey 08544-1014

HETEROCHROMATIC SILENCING IS DISTINCT FROM TRANSCRIPTIONAL GENE REPRESSION

The organization of genomes into heterochromatic and euchromatic regions is a global method of gene regulation, in contrast to single-gene transcriptional control. Originally defined cytologically in higher organisms as dark-staining chromatin, heterochromatin is now characterized by a collection of molecular markers including repetitive DNA, regular nucleosome spacing, low gene density, late S phase replication, and histone tail modifications including hypoacetylation (for review, see Henikoff 2000; Richards and Elgin 2002). Heterochromatin is often found at centromeric and telomeric loci, and active genes that are translocated to heterochromatin are heritably silenced, often in a mosaic or variegated pattern from cell to cell. This variegation is thought to reflect a stochastic heterochromatin assembly at a formerly euchromatic locus. Termed "facultative heterochromatin," these genes are silent in only a subset of the cells, as opposed to "constitutive heterochromatin," where silencing is stable in all cells (for review, see Richards and Elgin 2002).

Heterochromatic transcriptional silencing is distinct from transcriptional repression seen at individual promoters that occurs at particular times in the cell cycle or during organismal development. Unlike the mechanisms of repression at specific promoters, heterochromatic silencing is generally promoter-nonspecific, such that almost all promoters are silenced by heterochromatin. Heterochromatin constitutes a repressive environment for gene expression over a large distance, in contrast to

gene-specific transcriptional repression that occurs over the relatively short range of the individual gene. Certain histone modifications associated with reduced transcription are found in both heterochromatic silencing and gene-specific transcriptional repression (see, e.g., Snowden et al. 2002; Kouskouti et al. 2004; Peinado et al. 2004). Although these histone modifications are shared, heterochromatin silencing and gene-specific repression are distinct in terms of specificity, timing, and regulation.

Telomere position effect (TPE) is one heterochromatic silencing phenomenon. TPE refers to the silencing of genes near telomeres and is classically defined as a continuous spread of heterochromatin from the telomere inward, although discontinuous heterochromatin formation has also been observed at telomeres in different organisms. TPE has been observed in diverse organisms, including baker's yeast (*Saccharomyces cerevisiae*), fission yeast (*Schizosaccharomyces pombe*), *Drosophila melanogaster*, the sleeping sickness parasite *Trypanosoma brucei*, the malaria parasite *Plasmodium falciparum*, plants, and humans (Hazelrigg et al. 1984; Levis et al. 1985; Gottschling et al. 1990; Matzke et al. 1994; Nimmo et al. 1994; Horn and Cross 1995; Scherf et al. 1998; Baur et al. 2001). Although first discovered in *Drosophila*, TPE is best understood in the yeast *S. cerevisiae*. Therefore, we use budding yeast as a paradigm, but we also discuss mechanisms of silencing in other organisms. We review how common silencing mechanisms, including subtelomeric structure, histone modifications, and nuclear localization, contribute to TPE. Finally, we discuss the potential biological roles of TPE.

THE DISCOVERY OF TELOMERE POSITION EFFECT

TPE was first observed 20 years ago in *D. melanogaster* (Gehring et al. 1984; Hazelrigg et al. 1984; Levis et al. 1985). Position effects resulting in the inactivation of genes had already been observed in flies as a result of natural or X-ray-induced chromosomal rearrangements that brought genes close to centromeric heterochromatin. This positional silencing is termed position effect variegation (PEV). The advent of P-element transposon DNA transformation allowed genes to be introduced into many distinct sites. When a P element carrying the *white* gene is introduced into the genome, it usually produces a red (wild-type) eye color. However, some fly stocks are obtained where the eyes have a mutant, mosaic phenotype (Hazelrigg et al. 1984). In these flies, the *white* gene is inserted near centromeric heterochromatin or near an autosomal telomere (Fig. 1A). This mosaic phenotype is not due to mutations in the *white*

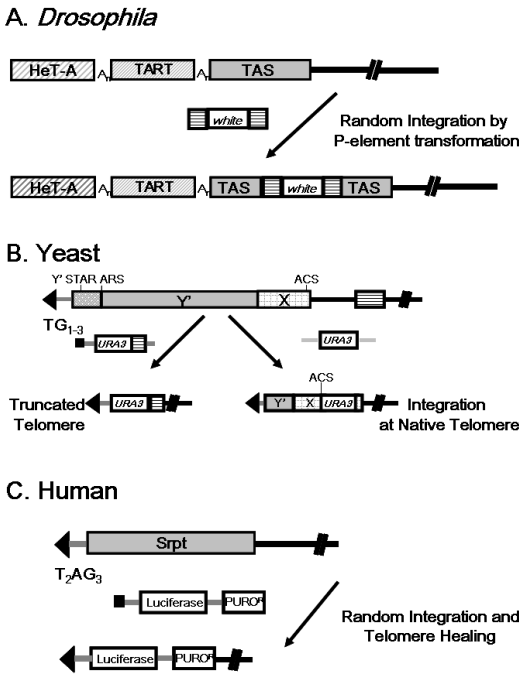

Figure 1. Telomere structure and TPE analysis in (*A*) *Drosophila*, (*B*) yeast, and (*C*) humans. (*A*) *Drosophila* telomeres consist of tandem arrays of the 6-kb *HeT-A* transposable element and the 12-kb *TART* transposable element. The number and arrangement of transposable elements vary from telomere to telomere. Proximal to the telomere is the subtelomeric telomere-associated sequence (TAS) array, tandem repeats that also vary in sequence and number between telomeres. TPE is assayed in *Drosophila* via P-element transformation of the *white* gene, which integrates randomly into the genome. P elements containing *white* that integrate within or adjacent to the TAS array are subject to TPE. (*B*) Natural yeast telomeres bear middle repetitive elements. The X element is heterogeneous, ranging from 0.3 to 3.75 kb in size. The "core X" element, containing an ARS consensus sequence (ACS), is 300–500 bp and found at most telomeres. The X element may also contain subtelomeric repeats (STRs) A–D in variable number and arrangement. The Y′ element is more conserved, with two classes of Y′ elements—long (6.7 kb) and short (5.2 kb). A given telomere may have 0–4 copies of Y′. TPE can be assayed at truncated or native yeast telomeres. Truncated telomeres are created via integration of a reporter gene and telomere seed at an upstream segment of unique DNA, eliminating the subtelomeric X and Y′ elements. TPE has also been assayed at native telomeres by integrating a reporter at the X-ACS site. (*C*) Human telomeres have highly variable subtelomeric repeats (Srpts). These repeats range from 1 kb to more than 200 kb in size. Some repeats are present at only one telomere and others are shared between several telomeres. TPE can be assayed in human cells via the random integration of a luciferase gene and telomere seed. When these integrations occur near a telomere, a new "healed" telomere is formed, analogous to the truncation events used to study yeast TPE.

gene, as mobilizing the P element produces flies with wild-type eye color (Levis et al. 1985).

Drosophila telomeres, which consist of transposable elements, are distinct from most other eukaryotic telomeres, which consist of highly repetitive telomerase-generated DNA (reviewed in Chapter 14). However, TPE is widespread among eukaryotes and is not unique to the unusual *Drosophila* telomeres. TPE was also discovered serendipitously in *S. cerevisiae* (Gottschling et al. 1990). In this case, a marker gene, *URA3*, was introduced next to a single telomere so that this unique sequence tag could be used to study the chromatin structure of an individual telomere (Fig. 1B). *URA3,* along with an adjacent seed of $TG_{1\text{-}3}$ telomeric DNA, integrated at *ADH4*, the gene closest to the VII-L telomere. This integration event deletes the terminal 15 kb of the chromosome, including the subtelomeric middle repetitive X and Y′ elements; a new telomere is formed at the $TG_{1\text{-}3}$ seed. As a result, the *URA3* transcription start site is positioned ~1.1 kb from the newly formed telomere. As expected, the cells bearing the telomeric *URA3* gene grow in the absence of uracil, indicating that *URA3* is expressed. Unexpectedly, however, 20–60% of the cells grew in the presence of 5-fluoro-orotic acid (5-FOA), a drug toxic to cells expressing *URA3* (Gottschling et al. 1990). As in flies, this effect is not due to mutation or inactivation of *URA3*. In fact, TPE at this truncated yeast telomere is reversible, as the FOA-grown colonies can be restreaked to plates lacking uracil, where *URA3* expression is required for growth. TPE was shown to function at multiple promoters, because expression of *ADE2* is also repressed when it is placed at the telomere. Red (*ade2*$^-$) and white (*ade2*$^+$) sectored colonies result, indicating that both the silent and the expressed states are stable through multiple cell cycles (Fig. 2). TPE is not specific to telomere VII-L: It can occur at other truncated telomeres as well as at some natural telomeres that bear the subtelomeric X and Y′ repeats (Gottschling et al. 1990; Renauld et al. 1993).

The *ADE2* reporter offers a visual demonstration of the variegated, stochastic nature of TPE at truncated telomeres. Because a strain with *ADE2* at a truncated telomere produces largely red (*ade2*$^-$) or largely white (*ade2*$^+$) colonies, both the silent and expressed states are stable through multiple cell divisions. However, red colonies contain white sectors and white colonies contain red sectors, indicating that cells retain the ability to switch expression states (Fig. 2). The regulation of expression state switching differs from telomere to telomere. This difference has been observed using a *URA3-GFP* fusion as a TPE reporter at both truncated and native telomeres (E. Louis, pers. comm.). At native telomeres, all cells in the culture seem to have a characteristic expression level for *GFP,*

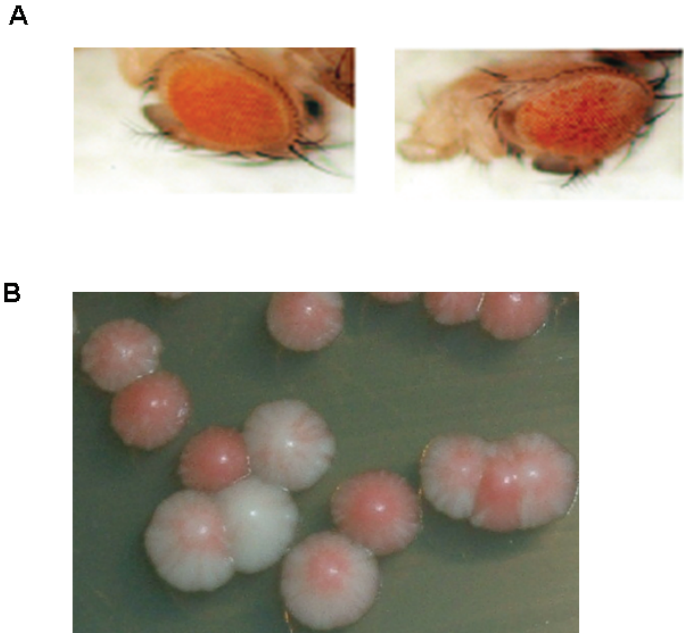

Figure 2. Variegation in (*A*) *Drosophila* and (*B*) yeast TPE. (*A*) TPE can be metastable at *Drosophila* telomeres. Complete expression of the *white* gene results in a red eye color; complete silencing results in a white eye color. TPE can be variegated, such that some cells maintain silencing and others lose silencing at the telomere, resulting in a mosaic phenotype, shown here at telomeres 3R (*left*) and the long arm of chromosome 4 (*right*). (*B*) Variegation at yeast telomeres. When *ADE2* is integrated at truncated telomere VII-L, cells appear red when the gene is silenced and white when the gene is expressed (Gottschling et al. 1990). The presence of sectored colonies indicates that although silencing is metastable, both silencing states are stable through multiple cell cycles. (*A*, Reprinted, with permission, from Shanower et al. 2005 [©GSA].)

which can be low or high depending on the telomere being assayed. At truncated telomeres, each cell in a culture can display a distinct expression level, from essentially no *GFP* expression to high *GFP* expression, even though each cell has the same reporter at the same truncated telomere.

Early attempts to detect TPE in human cells were unsuccessful, leading to early doubts as to whether this form of epigenetic silencing occurs in mammals (Sprung et al. 1996; Ofir et al. 1999). Ultimately, TPE was observed in human cells by constructing a linear plasmid containing the gene for puromycin resistance and a luciferase reporter gene adjacent to a 1.6-kb telomere repeat seed and transfecting the plasmid into telomerase-positive HeLa cells (Baur et al. 2001). When this construct integrates

into the chromosome, a new telomere is formed at the telomere seed, analogous to the strains first used to observe TPE at truncated yeast telomeres (Fig. 1C). TPE in humans (hTPE) has many of the molecular hallmarks observed for yeast TPE, including variegation, length dependence, and a role for histone deacetylation, all of which are discussed later in this chapter (Baur et al. 2001, 2004; Koering et al. 2002).

TRANS-ACTING FACTORS THAT MEDIATE TPE

Yeast TPE was discovered by placing *URA3* next to the truncated left telomere of chromosome VII (Gottschling et al. 1990). Almost all of the early analyses to determine requirements and features of TPE were done with this chromosome. However, as are discussed later in this chapter, the TPE properties of different telomeres are not necessarily the same. In this section, requirements for silencing are based mainly on analysis of the truncated VII-L telomere.

Yeast TPE requires the double-stranded $TG_{1\text{-}3}$ binding protein Rap1p (repressor activator protein 1), the end-binding heterodimeric Ku complex, and the multiprotein Sir2-4 (silent information regulator) complex, which does not bind DNA but is brought to telomeric chromatin via protein–protein interactions (for review, see Huang 2002; Tham and Zakian 2002; Perrod and Gasser 2003). Deleting any one of the Sir proteins or either Ku subunit essentially eliminates TPE. Likewise, deleting the carboxy-terminal domain of Rap1p eliminates TPE without impairing the essential function of Rap1p (Kyrion et al. 1993). All of these proteins likely act directly to affect TPE, as each is telomere bound in vivo (Conrad et al. 1990; Bourns et al. 1998; Gravel et al. 1998). Rap1p, Sir proteins, and Ku all have roles in processes other than TPE, including telomere length regulation, transcriptional repression at the silent mating type loci, rDNA silencing, and DNA repair (Ivy et al. 1986; Lustig et al. 1990; Mages et al. 1996; Porter et al. 1996; Smith and Boeke 1997). In addition to Rap1p, the Ku complex, and the Sir complex, there are more than 50 yeast genes that affect TPE. Many of these genes have relatively modest effects on the level of TPE, and probably many of them do not act directly. Table 1 presents a list of genes that affect TPE in *S. cerevisiae*. Not all of these genes are discussed in this chapter.

Based on the number of binding sites per telomere, Rap1p is thought to be present in ~10–20 copies at most yeast telomeres (Gilson et al. 1993). Sir4p and Sir3p both bind Rap1p, and mutations that eliminate this binding reduce TPE (Moretti et al. 1994; Moretti and Shore 2001). Sir4p also interacts with Ku (Tsukamoto et al. 1997). In the absence of Ku,

Table 1. Genes involved in Sir-mediated telomere position effect in *Saccharomyces cerevisiae*

Gene	Protein function	TPE phenotype in deletion mutant[a]
RAP1	binds to duplex $C_{1\text{-}3}A$ telomere repeats; binds Sir4p, Rif1p, and Rif2p; essential for role as transcription factor	carboxy-terminal deletion eliminates[b] TPE (Kyrion et al. 1993)
SIR2	histone deacetylase; binds Sir4p; required for *HM* and rDNA silencing	eliminated (Aparicio et al. 1991)
SIR3	spreading protein of silencing complex; binds Sir4p and histones H3 and H4; required for *HM* silencing	eliminated (Aparicio et al. 1991)
SIR4	binds Sir2p, Sir3p, Esc1p, and histones H3 and H4; required for *HM* silencing	eliminated (Aparicio et al. 1991)
YKU70 *YKU80*	as complex, promotes telomere lengthening via interaction with *TLC1*; required for peripheral localization of some telomeres; DNA repair	eliminated (Boulton and Jackson 1998)
RIF1 *RIF2*	compete with Sirs for Rap1p binding at telomere; antagonize telomere lengthening	increased (Kyrion et al. 1993)
SIR1	binds ORC; required for establishment of *HM* silencing	reduced at native telomere XI-L (Pryde and Louis 1999)
ORC2 *ORC5*	essential subunits of the origin recognition complex	partial loss-of-function alleles reduce TPE (Fox et al. 1997)
MCM5	essential helicase; component of MCM complex that primes origins of DNA replication	reduced at native telomeres[c] (Dziak et al. 2003)
ABF1	essential transcription factor; required for *HM* silencing; binds some ARSs	binding site mutation reduces TPE at native telomere XI-L (Pryde and Louis 1999)
ESC1	binds Sir4p; required for peripheral localization of some telomeres	reduced (Andrulis et al. 2002)
ESC8	binds Sir2p	reduced (Cuperus and Shore 2002)
HHT1 *HHT2*	histone H3; binds Sir3p and Sir4p	amino-terminal deletions eliminate TPE (Mann and Grunstein 1992; Thompson et al. 1994)
HHF1 *HHF2*	histone H4; binds Sir3p and Sir4p	amino-terminal deletions eliminate TPE (Aparicio et al. 1991; Mann and Grunstein 1992; Thompson et al. 1994)

(*Continued*)

Table 1. (*continued*)

Gene	Protein function	TPE phenotype in deletion mutant[a]
HTA1 *HTA2*	histone H2A	amino-terminal deletions reduce TPE (Wyatt et al. 2003)
HTZ1	histone-variant H2AZ; prevents spread of silent heterochromatin	increased at native telomeres[c] (Meneghini et al. 2003)
SAS2	histone H4K16 acetyltransferase; global histone acetylation	eliminated (Reifsnyder et al. 1996); increased at native telomere VI-R[c] (Kimura et al. 2002; Suka et al. 2002)
SAS4 *SAS5*	components of Sas2p complex	eliminated (Xu et al. 1999)
DOT1	histone H3K79 methyltransferase	reduced; overexpression also reduces TPE (Singer et al. 1998)
UBP10	ubiquitin-specific protease; may regulate silencing by acting on Sir4p	reduced; overexpression also reduces TPE (Singer et al. 1998)
DOT5	nuclear thiol peroxidase	overexpression reduces TPE (Singer et al. 1998)
CAC1 *CAC2* *CAC3*	subunits of CAF-I chromatin assembly complex; contribute to localization of Rap1p	reduced (Enomoto et al. 1997; Kaufman et al. 1997; Monson et al. 1997)
HIR1 *HIR2*	corepressors of histone gene transcription	increased; eliminated in concert with *cacΔ* (Kaufman et al. 1998)
NAT1 *ARD1*	subunit of amino-terminal acetyltransferase NatA	eliminated (Aparicio et al. 1991)
RAD6	E2 ubiquitin conjugating enzyme	eliminated (Huang et al. 1997)
BRE1	E3 ubiquitin ligase for Rad6p; required for the ubiquitination of histone H2B	eliminated (Wood et al. 2003)
HAT1 *HAT2* *HIF1*	subunits of the HAT-B histone acetyltransferase complex that acetylates free histone H4K12	reduced in concert with H3K14R mutation (Kelly al. 2000; Poveda et al. 2004)
ASF1	histone chaperone/chromatin assembly factor	overexpression reduces TPE (Le et al. 1997)
RPD3 *SAP30* *SIN3*	histone deacetylase complex	increased (Rundlett et al. 1996; Sun and Hampsey 1999)
SHG1 *SDC1* *SWD1*	components of the COMPASS complex required for H3K4 methylation	eliminated or reduced (Nislow et al. 1997; Krogan et al. 2003)

Table 1. (*continued*)

Gene	Protein function	TPE phenotype in deletion mutant[a]
SWD2		
SWD3		
SPP1		
BRE2		
SET1		
HST2	cytosolic member of Sir2 family of NAD^+-dependent deacetylases	overexpression eliminates TPE (Perrod et al. 2001)
HST3	members of Sir2 family of NAD^+ -dependent deacetylases	double mutant eliminates TPE (Brachmann et al. 1995)
HST4		
NPT1	nicotinate phosphoribosyltransferase; NAD^+ biosynthesis	eliminated (Sandmeier et al. 2002)
PNC1	pyrazinamidase and nicotinamidase; NAD^+ biosynthesis	reduced (Sandmeier et al. 2002)
SUM1	suppressor of *sir* mutations	*SUM1-1* allele increases TPE (Chi and Shore 1996)
PKC1	MAP kinase cascade; serine/threonine MAP kinase Slt2p phosphorylates Sir3p	*slt2Δ* eliminates TPE (Ray et al. 2003)
BCK1		
MKK1		
SLT2		
STE7	MAP kinase cascade that results in hyperphosphorylation of Sir3p	activated *STE11-4* allele increases TPE (Stone and Pillus 1996)
STE11		
STE12		
FUS3		
YAF9	component of NuA4 histone H4 acetyltransferase complex	increased at some native telomeres[c] (Zhang et al. 2004)
EPL1	essential component of NuA4 histone H4 acetyltransferase complex; homologous to *Drosophila* Enhancer of Polycomb	partial loss-of-function alleles eliminate or reduce TPE (Boudreault et al. 2003)
POL1	catalytic subunit of the DNA polymerase α-primase complex; essential	partial loss-of-function allele eliminates TPE (Adams Martin et al. 2000)
POL2	DNA polymerase II; essential	partial loss-of-function allele reduces TPE (Iida and Araki 2004)
DPB3	subunits of DNA polymerase II complex	reduced (Iida and Araki 2004)
DPB4		
RTF1	subunit of the RNA polymerase II-associated Paf1 complex	eliminated (Krogan et al. 2003; Ng et al. 2003)

(*Continued*)

Table 1. (*continued*)

Gene	Protein function	TPE phenotype in deletion mutant[a]
PCNA	essential protein; functions as the sliding clamp for DNA polymerase delta	partial loss-of-function alleles reduce TPE (Zhang et al. 2000)
SPT4	mediates activation and inhibition of transcription elongation; pre-mRNA processing; kinetochore function	eliminated (Crotti and Basrai 2004)
ISW2 *DLS1*	subunits of ISW2/yCHRAC chromatin accessibility complex	increased (Iida and Araki 2004)
RRM3	DNA helicase; promotes replication fork progression at nonhistone protein-DNA complexes	reduced (Ivessa et al. 2002)
MEC1	essential ATR kinase; checkpoint control; viability restored in *mec1Δ sml1Δ*	reduced in *mec1Δ sml1Δ* (Craven and Petes 2000)
SCS2	suppressor of choline sensitivity; regulation of *INO1*	reduced (Craven and Petes 2001)
MEC3	DNA repair; checkpoint control	increased (Corda et al. 1999)
MRC1	S-phase checkpoint protein required for DNA replication	reduced (Hu et al. 2001)
ELG1	Required for S-phase progression and telomere length regulation; forms an alternative replication factor C	increased (Smolikov et al. 2004)
GAL11	component of the RNA polymerase II holoenzyme; acts as target of activators and repressors	reduced (Suzuki and Nishizaw 1994)
SNF2	component of Swi/Snf complex; transcriptional regulator	eliminated (Dror and Winston 2004)
WTM1 *WTM2* *WTM3*	WD-repeat-containing transcriptional regulators	increased (Pemberton and Blobel 1997)
BDF1	promotes transcription initiation at TATA-containing promoters	increased at several native telomeres[c] (Ladurner et al. 2003)
PBP2 *HEK1*	RNA-binding proteins with similarity to mammalian heterogeneous nuclear RNP K protein	increased (Denisenko and Bomsztyk 2002)
UPF2	component of the nonsense-mediated mRNA decay pathway	reduced (Lew et al. 1998)
RPT4 *RPT6*	essential components of the 26S proteasome	eliminated in temperature sensitive mutants (Ezhkova and Tansey 2004)

Table 1. (*continued*)

Gene	Protein function	TPE phenotype in deletion mutant[a]
ZDS1 *ZDS2*	interact with Sir proteins and Rap1p by two-hybrid; involved in YAC stability	overexpression increases TPE (Roy and Runge 1999)
IFH1	essential protein; potential Cdc28p substrate	overexpression reduces TPE (Singer et al. 1998)

[a]Refers to reporter gene at truncated telomere VII-L and/or truncated telomere V-R unless otherwise noted; point mutations in some of these genes may also affect TPE.

[b]Eliminated: less than 1% TPE; increased: twofold or greater increase relative to wild type; reduced: twofold or greater decrease relative to wild type, but greater than 1%.

[c]In genome-wide study.

Sir4p binding to the VI-R telomere is reduced to ~40% of wild-type levels, and Sir complex binding to subtelomeric chromatin is lost (Martin et al. 1999; Luo et al. 2002). These data suggest that the Sir complex can be brought to the telomere by its interaction with either Rap1p or Ku. Unlike Sir2 or Sir3p, Sir4p is still telomere bound at both the VII-L and VI-R telomeres in the absence of the other Sir proteins (Bourns et al. 1998; Luo et al. 2002). Thus, silencing is thought to initiate by Sir4p binding to the telomere followed by recruitment of Sir2p and Sir3p. The Sir complex spreads into adjacent subtelomeric chromatin by its ability to interact with histone tails (Hecht et al. 1995; Strahl-Bolsinger et al. 1997). This spreading extends ~3 kb from the telomere. The enzymatic activity of Sir2p, which is a histone deacetylase (HDAC), is required for this spreading (Hoppe et al. 2002; interactions between Sir proteins and histones and the activity of Sir2p are discussed in more detail in the section TPE and Histone Modifications).

Telomere-binding proteins that mediate TPE have been identified in only some of the organisms where TPE has been observed. In *Drosophila*, HP1 (heterochromatin protein 1), first discovered for its role in PEV in centromeric heterochromatin, is also needed for TPE at the largely heterochromatic chromosome 4 (for review, see Wallrath and Elgin 1995; Mason et al. 2000). However, HP1 is not required for TPE at the other *Drosophila* telomeres. HP1 is conserved in many eukaryotes but is not found in baker's yeast. The HP1 homolog in *S. pombe,* Swi6, is involved in TPE and is believed to be the structural protein that mediates the spread of silent chromatin in a manner analogous to Sir3p in *S. cerevisiae* (Allshire et al. 1995; for review, see Huang 2002). The conservation of HP1 may account for the lack of Sir3p and Sir4p homologs in other eukaryotes. Other conserved telomere-binding proteins do not mediate TPE in other organisms. The Ku complex, for example, is required for

telomere maintenance in trypanosomes (Conway et al. 2002) and fission yeast (Manolis et al. 2001), and for telomere accessibility and length regulation in *Drosophila* (Melnikova et al. 2005), but there is no evidence that Ku has a role in TPE in these organisms. The yeast telomeric DNA-binding protein Rap1p, which recruits the Sir complex to telomeres, is conserved in fission yeast (spRap1) and humans (hRap1), although spRap1 and hRap1 do not bind directly to the telomeric DNA, but rather are recruited by other telomere-binding proteins (Li et al. 2000; Kanoh and Ishikawa 2001). In *S. pombe*, this mediator protein, Taz1p, is required for TPE, as is spRap1 (Nimmo et al. 1998; Park et al. 2002). hRap1's function in telomere length regulation is conserved from yeast to humans (Li et al. 2000). The roles of the hRap1 and its mediator protein, TRF2 (the duplex human $\underline{T}_2AG_3$ repeat binding factor) in human TPE are unknown. However, another human telomere-binding protein, TRF1, has been shown to negatively regulate TPE when overexpressed (Koering et al. 2002). It is unclear if TRF1 binding to the telomere inhibits TPE or if its overexpression titrates another factor away from the telomere that is itself necessary for human TPE.

THE ROLE OF TELOMERE LENGTH IN TPE

Telomere Length and TPE in Yeast: Length Is Only Part of the Story

Because Rap1p is a sequence-specific duplex DNA-binding protein that initiates TPE by recruiting the Sir complex, long telomeres should have higher TPE than short telomeres. Indeed, in otherwise wild-type yeast, a gene adjacent to a long telomere shows higher and more stable repression than a gene next to a short telomere (Kyrion et al. 1993). However, if telomeres are made longer by mutations that affect the establishment or maintenance of silencing, TPE is reduced. For example, *rap1*^{t} mutations eliminate a region in the carboxyl terminus of Rap1p that is necessary for both Sir complex and Rif1p/Rif2p binding (Rap1p interacting factor 1 and 2; Hardy et al. 1992; Moretti et al. 1994; Wotton and Shore 1997). Because Rif1p and Rif2p inhibit telomerase-mediated telomere lengthening (Teng et al. 2000), *rap1*^{t} cells have very long telomeres. However, the *rap1*^{t} mutation also prevents Sir binding, so, despite its long telomeres, this strain has no TPE (Kyrion et al. 1992, 1993). The Sir complex and the Rif proteins, which bind to the same portion of Rap1p, compete for Rap1p binding (Moretti et al. 1994; Wotton and Shore 1997). A *rif1Δ rif2Δ* strain, which has very long telomeres, also has elevated TPE (Kyrion et al. 1993). This higher level of silencing is due not only to more

Rap1p sites per telomere but also to reduced competition for Sir binding to telomeres.

Some of the mutations that eliminate TPE, such as *sir3Δ*, *sir4Δ*, and *yku70Δ* also cause telomere shortening. However, the *sir3Δ* and *sir4Δ* mutants have only modest effects on telomere length (Palladino et al. 1993), so it is unlikely that the complete loss of TPE in these strains is due to shortened telomeres. Certainly short telomeres are not sufficient to eliminate TPE: *tel1* cells have very short telomeres of <100 bp (Lustig and Petes 1986; Greenwell et al. 1995), yet TPE is normal in these cells (Gottschling et al. 1990; Runge and Zakian 1996).

Telomeres are also very short in *yku70Δ* cells. In this strain, Sir4p recruitment is impaired not only by the smaller number of Rap1p binding sites but also by the absence of the alternative Ku-mediated pathway for Sir4p recruitment. As predicted, Sir4p binding is reduced at telomeres and subtelomeric chromatin in *ykuΔ* strains (Martin et al. 1999; Luo et al. 2002). Ku does not appear to provide any direct activity that is essential for silencing, because in an otherwise wild-type *yku70* strain, a truncated VII-L telomere with a long telomeric repeat tract exhibits normal TPE (Mishra and Shore 1999). Moreover, relatively modest telomere lengthening (~100 bp) can restore telomere silencing to the truncated VII-L telomere in *yku70 rif1* strain, where the Sir complex does not have to compete with Rif1p for Rap1p binding. Thus, the loss of TPE in Ku-deficient cells is best explained by reduced Sir4p binding, caused partly by short telomeres and partly by loss of the Rap1-independent pathway for Sir4p recruitment. Ku has additional effects on telomeres and these might also influence TPE. For example, *ykuΔ* telomeres bear long single-strand G-tails throughout the cell cycle, not just in late S/G_2 phase, as seen in wild-type cells (Wellinger et al. 1993b; Gravel et al. 1998). Telomerase recruitment is also defective in the absence of Ku (Fisher et al. 2004), and some telomeres require Ku to target them to the nuclear periphery (Hediger et al. 2002b; discussed in detail in the section Nuclear Localization and TPE). It is not known if these changes contribute to the loss of silencing that is characteristic of Ku-deficient cells.

Telomere Length and TPE in Humans

Telomere length in human cells, as in yeast, follows the general trend of longer telomeres correlating with higher gene silencing. HeLa cell telomeres that are elongated from ~5 kb to ~14 kb by overexpression of the human telomerase reverse transcriptase (hTERT) display a twofold to tenfold increase in TPE at a luciferase reporter gene (Baur et al. 2001). As in

yeast, however, there is not a simple, absolute correlation between telomere length and TPE levels. A study of telomere reporter gene expression in a different cell type found no correlation between resting telomere length and TPE (Koering et al. 2002). It is possible that relatively small differences in telomere length are not sufficient to produce a detectable change in TPE, whereas large changes, such as the 9-kb extension that accompanies hTERT overexpression, are required for measurable effects.

Telomere Length in *Drosophila* Contributes Negatively to TPE

Telomere length in *Drosophila* also contributes to TPE; however, the trend is opposite to that in yeast and humans—in *Drosophila*, longer telomeres display decreased TPE compared to shorter telomeres (Mason et al. 2003). This inverse correlation is probably a result of the unique telomere structure in flies. Unlike most eukaryotes, whose telomeres are composed of simple repetitive, noncoding DNA, *Drosophila* telomeres are made up of *HeT-A* and *TART* transposable elements. A new transposable element, *TAHRE*, has also recently been found at *Drosophila* telomeres (Abad et al. 2004). One, two, or all of these elements can be present at a given telomere, although *HeT-A* seems to be the most abundant (Levis et al. 1993; Pardue and DeBaryshe 1999). The negative effect of the transposable elements on TPE may be due to their transcription, as the 3′ end of *HeT-A* has promoter activity when tested with a *lacZ* reporter (Danilevskaya et al. 1997). Internal to the transposable elements, *Drosophila* telomeres bear telomere-associated sequence (TAS) repeats, which are similar to the subtelomeric repeats found on yeast and human chromosomes. When TPE is monitored at telomeres with the same TAS array but with different numbers of telomeric transposable elements *in cis*, the shortest telomere has high TPE while the reporter gene is not repressed as much in the long telomere strain (Mason et al. 2003).

In contrast to the telomeric transposons, the *Drosophila* subtelomeric TAS elements act positively to promote TPE. Indeed, TPE is detected only when transgenes are inserted within or adjacent to TAS arrays (Karpen and Spradling 1992; Levis et al. 1993; Cryderman et al. 1999). Further evidence for the importance of TAS elements is that their deletion at telomere 2L greatly reduces TPE of a reporter gene on the other copy of 2L (Golubovsky et al. 2001). Moreover, a deficiency screen for dominant suppressors of TPE at 2L found several positive loci that mapped back to the 2L TAS (Mason et al. 2004). The ability of TAS elements to mediate silencing is not limited to the telomere. When the TAS array from telomere 2L, along with a reporter gene, is moved into a nontelomeric

site, the transgene is still silenced and its level of silencing is dependent on the length of the TAS array (Kurenova et al. 1998).

How the *Drosophila* TAS array mediates heterochromatin formation is unclear, as binding sites for *trans*-acting silencing proteins in the TAS elements have not been identified. Polycomb group proteins localize to telomeres, and there is a putative polycomb-response element (PRE) in the TAS on the left telomere of the X chromosome (Boivin et al. 2003). Direct binding of polycomb group proteins to the TAS has yet to be demonstrated. TAS elements may function by recruiting polycomb group or other yet to be identified silencing proteins. Alternatively, or in addition, silencing may be mediated by homolog pairing or nuclear localization. A role for higher-order structure in *Drosophila* TPE is suggested by the fact that deletions in the 2L TAS array have effects on TPE not only at telomere 2L but also at telomere 3R (Golubovsky et al. 2001).

TELOMERE IDENTITY AND EFFECTS ON TPE

Different Yeast Telomeres Have Different Subtelomeric Structures and Different TPE Phenotypes

Middle repetitive subtelomeric sequences are immediately proximal to the telomere repeats in many species, including yeast, *Drosophila*, and humans, and in several organisms have been shown to affect telomere length (Craven and Petes 1999; Figueiredo et al. 2002; Jacob et al. 2004). In yeast, there are two types of subtelomeric repeats, X and Y′ (for review, see Louis 1995). X elements are found at essentially all yeast telomeres and range in size from ~300 bp to 3 kb. Although X elements are quite heterogeneous, each contains a "core X" repeat, consisting of an ARS (autonomously replicating sequence) consensus sequence (ACS) and a binding site for the protein Abf1p, a transcription factor that, like Rap1p, also functions at the *HM* silencers (Diffley and Stillman 1989; Kurtz and Shore 1991; for review, see Haber 1998). Some X repeats also contain STR (subtelomeric repeat) elements A–D. STR elements have recognition sites for Reb1p, which also functions in RNA polymerase I gene transcription and termination (Morrow et al. 1989; Wang et al. 1990), and Tbf1p (T_2AG_3 binding factor), an essential protein of unknown function (Brigati et al. 1993) that can act as a boundary element in subtelomeric DNA (Fourel et al. 1999, 2001). The combination, number, and arrangement of STR elements vary from telomere to telomere.

In contrast to the ubiquitous X element, the Y′ element is found at only one-half to two-thirds of yeast telomeres. When present, Y′ is distal

to X and is found in up to four tandem copies (Chan and Tye 1983). Short tracts of $TG_{1\text{-}3}$ telomeric sequence are found between some tandem Y′ elements and at some X-Y′ junctions (Walmsley et al. 1984). Because internal $TG_{1\text{-}3}$ tracts can recruit Rap1p and other silencing proteins (Stavenhagen and Zakian 1994; Bourns et al. 1998), their presence may bolster TPE. Like X, Y′ contains an ARS that can bind ORC and possibly Abf1p. Y′ also contains sequences that counter the spread of TPE. The Y′ STAR (subtelomeric antisilencing repeat) element has additional Tbf1p and Reb1p binding sites and functions as a boundary element, able to block the spread of heterochromatin into adjacent DNA (Fourel et al. 1999). Y′ elements also contain two open reading frames (ORFs), one of which codes for a putative RNA helicase.

Although all yeast telomeres end in $TG_{1\text{-}3}$ repeats and there are only two classes of subtelomeric repeats, the X and Y′ elements are sufficiently diverse that each telomere is unique. Thus, the 32 yeast telomeres can be thought of as having a "barcode" that confers a distinct identity on each telomere. This barcode is not immutable, as the X and Y′ content of a given chromosome end varies among different strains (Zakian and Blanton 1988).

What is the effect of the different subtelomeric structures on TPE? Early TPE studies in yeast were done using reporter genes integrated immediately adjacent to the telomeric $TG_{1\text{-}3}$ tract, such that these truncated telomeres lack both the X and Y′ elements (Gottschling et al. 1990). Although there are no reports of a truncated telomere that lacks TPE, the level of silencing at different truncated telomeres can vary widely. For example, in the same strain background, silencing at truncated telomere V-R is about tenfold lower (~4%) than at truncated VII-L (~33%; Gottschling et al. 1990). The source of TPE differences at truncated telomeres is not known, but they might reflect differences in the identity and transcription rate of internal sequences, nuclear localization, telomere length, or specific telomere–telomere associations.

TPE at "native" telomeres has been studied in two ways: (1) inserting *URA3* or another reporter gene into the X-ACS, keeping the Y′ and X elements largely intact (Pryde and Louis 1999), and (2) observing expression levels of subtelomeric genes, either at individual unmodified telomeres (Vega-Palas et al. 1997, 2000) or on a genome-wide scale (Wyrick et al. 1999, 2001). Results from this second method are discussed below in the section Biological Functions of TPE.

Because individual telomeres have different subtelomeric structures that have binding sites for proteins that can promote or limit TPE, it is not surprising that the level of silencing at native telomeres varies considerably from telomere to telomere. In reporter gene assays, TPE is virtually

nonexistent (<1%) at many native telomeres, occurring at only 6 of the 17 "native" telomeres that have been tested by insertion of reporter genes (Pryde and Louis 1999; M.A. Mondoux and V.A. Zakian, unpubl.; E. Louis, pers. comm.). The X-only telomeres III-R and IV-L, which do not support TPE, also do not bind Rap1p by a chromatin immunoprecipitation assay (Lieb et al. 2001). This result was unexpected, as Rap1p was assumed to bind to all yeast telomeres and to be the major determinant of telomeric chromatin. No or even very low Rap1p binding is sufficient to explain the lack of TPE at these telomeres. In contrast, some native X-only telomeres have very high TPE, comparable to TPE levels at truncated telomeres, with silencing seen in ~30–60% of cells (Pryde and Louis 1999). TPE is even higher, occurring in essentially all cells, at the core X-only telomere VI-R (M.A. Mondoux and V.A. Zakian, unpubl.). Given that the X and Y′ content of a given telomere can vary from strain to strain, the TPE phenotype of a given chromosome end is probably also not fixed.

In addition to TPE levels, requirements for silencing proteins are different at different telomeres. Sir1p, which acts in the establishment of silencing at the yeast silent mating type loci (Pillus and Rine 1989), is not necessary for TPE at truncated telomeres (Aparicio et al. 1991). Nevertheless, if Sir1p is tethered to the truncated VII-L telomere, silencing increases (Chien et al. 1993). In contrast, *sir1Δ* reduces TPE at the native telomere XI-L by approximately twofold (Fourel et al. 1999; Pryde and Louis 1999). Although the ACS sites located in core X elements (X-ACS) do not seem to be active as origins of replication, the X-ACS does bind the origin recognition complex (ORC; Wyrick et al. 2001). Mutation of the X-ACS reduces TPE at telomere XI-L ~100-fold (Pryde and Louis 1999). Sir1p could play a role in native telomere silencing by binding to ORC at the X telomeres, just as it does at the silent mating type loci (Foss et al. 1993; Triolo and Sternglanz 1996). Indeed, the core X element can improve silencing at a weakened *HML* locus that lacks most of the *HML-E* silencer (Lebrun et al. 2001). The core X element also contains a binding site for Abf1p, another protein necessary for *HM* silencing. Mutation of the Abf1p-binding site at telomere XI-L reduces TPE at this telomere by approximately tenfold (Pryde and Louis 1999). Clearly, the subtelomeric DNA, which recruits different *trans*-acting factors to different telomeres, makes a strong contribution to the silencing profile of individual telomeres.

Unlike the X element, which can have positive effects on TPE, the Y′ element has antisilencing properties. These effects are observed when Y′s are located between the telomere and a reporter gene, or between an *HML* silencer and a reporter gene, consistent with their functioning as heterochromatin boundaries (Fourel et al. 1999, 2001). Although Reb1p

is a transcriptional activator at other sites in the genome (Wang et al. 1990), in subtelomeric DNA, Reb1p acts as a boundary protein as does Tbf1p. These two Y′ binding proteins are sufficient for the Y′ boundary activity, as multiple binding sites for either protein recapitulate the Y′ STAR boundary activity at truncated telomere VII-L (Fourel et al. 1999).

At truncated telomere VII-L, TPE can be detected inward from the telomere, but TPE levels decrease exponentially with distance from the telomere (Gottschling et al. 1990). In contrast, at native telomeres, TPE does not decrease in a regular manner as a function of distance from the telomere. Rather, at native ends, there are zones of transcriptional repression that are punctuated by regions of normal gene expression (Fourel et al. 1999; Pryde and Louis 1999). The discontinuous nature of silencing at native ends is a consequence of the subtelomeric X and Y′ elements, which have binding sites for proteins that promote TPE and for boundary proteins that limit heterochromatin spread.

The unique identities of native telomeres in terms of sequence content and *trans*-acting binding proteins make it difficult to generalize telomere behavior from the study of one or even a few telomeres. This issue is especially troublesome when several different telomere phenotypes are being monitored, such as TPE, telomere length, or telomere position within the nucleus. Given the diversity from telomere to telomere, even between different truncated telomeres, it is critical that all behaviors be measured at the same telomere. These considerations are not unique to yeast, because, as described below, the subtelomeric regions of *Drosophila* and human chromosomes are also diverse.

Drosophila Subtelomeric Structure Also Leads to Different TPE Requirements and Phenotypes

Each of the eight *Drosophila* telomeres is distinguished by the length and sequence of its TAS array, with different telomeres bearing ~10 to ~20 kb of these repeats. The 2L and 3L TAS arrays have a highly similar sequence composition (Abad et al. 2004), but they are not similar to the XL, 2R, or 3R telomeres by the criterion of in situ hybridization (Mechler et al. 1985; Walter et al. 1995). The presence or absence of a TAS array on a particular chromosome end can vary by *Drosophila* stock. These TAS differences may explain differences in the requirements for TPE at different telomeres. For example, TPE on the long arm of the fourth chromosome, which is largely heterochromatic and has a much shorter distance from the centromere to the telomere, requires many of the classic suppressors of position effect variegation (*Su(var)*s), including HP1

(Wallrath and Elgin 1995). In contrast, neither telomere on the second or third chromosomes requires the *Su(var)*s or HP1 for TPE. Rather, TPE at these telomeres is sensitive to mutations in the Polycomb group genes, which do not affect TPE at chromosome 4.

Given the small size of the fourth chromosome, the TPE phenotype of chromosome 4 telomeres may be influenced by their proximity to pericentric heterochromatin. When the telomeric region of the *Drosophila* fourth chromosome is translocated to telomere 2L or 2R, there is a dramatic loss of TPE at the newly formed 2-4 hybrid telomere. This residual TPE is still regulated by the chromosome 4 TPE modifier HP1, not by the chromosome 2 modifier *Su(z)2* (Cryderman et al. 1999). This continued dependence on HP1 at this new location, far from a centromere, argues that genetic dependencies for TPE at chromosome 4 telomeres are inherent to them. When the 2R telomere is translocated to chromosome 4, there is no change in its TPE level, and the 2R transgene is still regulated by the chromosome 2 modifiers (Cryderman et al. 1999). Thus, the local TAS environment dictates the requirements for TPE, although chromosomal context also plays a role.

Even when *Drosophila* telomeres share requirements for TPE, their response to mutations in those genes can vary. For example, *grappa*, the H3K79 methyltransferase (*DOT1* in yeast), is an essential gene in flies that is required for TPE but not for pericentric PEV (Shanower et al. 2005). Three partial loss-of-function alleles of *grappa* have recently been identified that eliminate TPE at all *Drosophila* telomeres except 2L, which is only weakly affected by these alleles. Other alleles with differential effects on TPE were also identified, including gpp^{94A}, which reduces TPE at 3R but has weak or no effects at the other telomeres tested (Shanower et al. 2005). Grappa is the first example of a protein that modulates TPE on the long arm of the fourth chromosome that does not affect PEV. The lack of a role for *grappa* in PEV is further evidence that silencing at the chromosome 4 telomeres is a form of TPE, not an extension of PEV.

Human Subtelomeric Repeats and Links to Genetic Diseases

Human subtelomeric repeats are less well-studied than in yeast or *Drosophila* and represent one of the last frontiers in the human genome project, as their highly repetitive nature makes them difficult to sequence and assemble. Human subtelomeric repeats seem to have undergone evolutionarily recent duplications and deletions, as their number and chromosomal location can vary in different individuals (for review, see Mefford and Trask 2002). A recent sequencing effort (Riethman et al.

2004) found that human subtelomeric repeats range from 1 kb to more than 200 kb in size. Some of these subtelomeric repeats (Srpts) are present at only one telomere, whereas others are common to several telomeres (Riethman et al. 2004). Another component of human subtelomeric DNA, the X region, is a restriction-endonuclease-resistant 2–4-kb segment. The length of the X region has been calculated by comparing apparent telomere lengths on gels and quantitative fluorescent in situ hybridization (Hultdin et al. 1998; Steinert et al. 2004). The size of the X region varies, even at a specific telomere, as a direct function of telomere length (Steinert et al. 2004). The cause of the restriction-endonuclease refractory nature of the X region is unknown, but it does not seem to be due to DNA methylation (Steinert et al. 2004).

One rare example of a well-studied human subtelomeric repeat is the 3.3-kb D4Z4 repeat. This element is of particular interest because of its linkage to facioscapulohumeral muscular dystrophy (FSHD). In the human population, D4Z4 is present in varying numbers at telomeres 4q and 10q. FSHD patients have only 1–10 copies of the repeat at the subtelomere of one of the chromosome 4 homologs. In contrast, unaffected individuals have 11–150 copies of the repeat at both homologs (van Deutekom et al. 1993; Lemmers et al. 2001). Severity of the disease seems to correlate with decreased numbers of D4Z4 repeats. Portions of the D4Z4 repeat are highly homologous to known heterochromatic repeats on other chromosomes (1q, 21p, and 22p; Meneveri et al. 1993; Hewitt et al. 1994). An attractive model, then, is that as the D4Z4 repeat number decreases, there is a loss of TPE and increased expression at the nearby FSHD candidate genes that result in the disease state. In support of this model, three genes upstream of the D4Z4 repeats are inappropriately expressed in muscle samples from affected individuals, with the biggest effect at the gene closest to the telomere (Gabellini et al. 2002). Further analysis showed that there is a D4Z4 binding element (DBE) that binds a multiprotein repressor complex. The DBE is capable of acting as a silencer, with multiple copies of the DBE resulting in greater repression of the reporter gene (Gabellini et al. 2002). However, using a different quantitation technique, a second group did not observe increased expression in 4q subtelomeric genes in FSHD patients (Jiang et al. 2003). In addition, the second group argued that the 4q region was not heterochromatic based on histone H4 acetylation levels. This group proposes a "looping model," as opposed to a heterochromatin spreading model, as the mechanism for D4Z4's role in FSHD. These models are not necessarily mutually exclusive, as telomeres are known to loop in mammals, ciliates, trypanosomes, and yeast (Griffith et al. 1999; Murti and Prescott 1999; de Bruin et al. 2000; Muñoz-Jordan et al. 2001). This looping could

bring silencing factors into closer proximity to the target genes. In this way, the subtelomeric repeats could play a role in silencing that is distinct from the classic definition of TPE that resembles the looping model for boundary activity (for review, see Schedl and Broach 2003).

TPE AND HISTONE MODIFICATIONS

Early Evidence That Chromatin Structure Determines TPE Status

The early cytological studies that defined heterochromatin identified the telomeres and centromeres as regions associated with condensed, darkly staining DNA. What "heterochromatin" meant in molecular terms, and how this influenced gene expression, was unknown. Although a correlation between increased histone acetylation and increased transcription was first observed 40 years ago (Allfrey et al. 1964), histone modifications and their effects on heterochromatin have only recently been explored.

Chromatin was first implicated in yeast gene silencing at the silent mating type loci, *HML* and *HMR*. Point mutations in the amino-terminal "tail" of histone H4 eliminate silencing at *HML* (Johnson et al. 1990; Megee et al. 1990; Park and Szostak 1990). Three of the six mutations that affect *HML* silencing, H4-K16Q, H4-K16G, and H4-R17G, also do not support TPE at truncated telomere VII-L (Aparicio et al. 1991). Alleles of *SIR3* were identified that partially suppress the *HM* silencing defect of histone H4 point mutants (Johnson et al. 1990). However, these *sir3* alleles do not restore TPE in cells with the same H4 mutations (Aparicio et al. 1991). This finding is one of several that indicates that the requirements for silencing at the *HM* loci and telomeres are similar but not identical. Further mutational analysis defined domains of both histones H3 and H4 that are completely contained within the amino-terminal tails of these histones that are essential for TPE, but not for cell viability (Mann and Grunstein 1992; Thompson et al. 1994). Amino-terminal deletions of H2B have no effect on either *HM* silencing or TPE (Kayne et al. 1988; Thompson et al. 1994). However, amino-terminal deletions of H2A have recently been shown to reduce TPE (Wyatt et al. 2003). Both the H3 and H4 tails contain several highly conserved lysine residues. Because lysines are subject to posttranslational acetylation, and acetylation had already been linked to transcriptional activity, the individual H3 lysines were mutated either to arginine to mimic the unacetylated state or to glycine or glutamine to mimic the acetylated state. Some of the lysine mutants that mimic acetylation, like K16Q, eliminate TPE (Aparicio et al. 1991; Thompson et al. 1994). Other lysine residues have

no phenotype when mutated alone, but mutating several of these lysine residues affects TPE (Thompson et al. 1994).

In biochemical experiments, the H3 and H4 amino termini interact with Sir3p and Sir4p but not with Rap1p or Sir2p (Hecht et al. 1995). These interactions are lost when the tail domains of H3 and H4, known to be necessary in vivo for TPE, are deleted. Furthermore, specific point mutations known to eliminate TPE, like H4-K16Q, also disrupt the interactions between the Sir proteins and the histone tails (Hecht et al. 1995). Amino-terminal deletions of histones H3 or H4 or the H4-K16Q mutation also eliminate the foci of silencing proteins at the nuclear periphery (Hecht et al. 1995). Thus, the requirement of the H3 and H4 amino-terminal tails for silencing can be explained by their role in Sir protein recruitment.

Although Sir3p and Sir4p binding to histone tails is thought to play a structural role that makes subtelomeric chromatin less accessible to the transcription apparatus, Sir2p plays an enzymatic role in the formation of telomeric heterochromatin. Overexpression of *SIR2* reduces histone acetylation in vivo, leading to the suggestion that Sir2p could be an HDAC (Braunstein et al. 1993). Subsequently, several biochemical studies showed that Sir2p is an NAD^+-dependent lysine deacetylase (Tanny et al. 1999; Imai et al. 2000; Landry et al. 2000; Smith et al. 2000). The HDAC activity of Sir2p is required for TPE, *HM* silencing, and rDNA silencing (for review, see Gasser and Cockell 2001; Moazed 2001). After being recruited to the telomere by Sir4p, Sir2p can deacetylate adjacent histone lysine residues, creating binding sites for the structural heterochromatin component Sir3p. The repetition of this process is thought to propagate heterochromatin toward the centromere, with the spreading being limited by the concentration of Sir3p (Renauld et al. 1993).

In addition to the formation of heterochromatin, the balance of HDAC and histone acetyltransferase (HAT) activities is also important in setting up boundaries between heterochromatin and euchromatin. At telomeres, the HAT that counteracts Sir2p activity is Sas2p (something about silencing 2). Sas2p, a HAT required for the global acetylation of H4-K16, is also involved in boundary formation at *HMR* (Reifsnyder et al. 1996; Ehrenhofer-Murray et al. 1997; Kimura et al. 2002; Suka et al. 2002). In a wild-type cell, there is a gradient of acetylated H4-K16 from hyperacetylated at internal chromosomal regions to hypoacetylated at telomeric regions (Kimura et al. 2002). This gradient of acetylation corresponds to an inverse gradient of increasing Sir3p binding to chromatin (Kimura et al. 2002). In a *sas2Δ* strain, Sir3p spreads further from the telomere, increasing the span of Sir3-mediated heterochromatin from ~3 kb at telomere VI-R to ~15 kb, and concomitantly reducing the span

of H4 lysine acetylation (Kimura et al. 2002; Suka et al. 2002). This change in chromatin state is correlated with a significant reduction in the transcription level of subtelomeric ORFs below that seen in wild-type cells (Kimura et al. 2002; Suka et al. 2002). This reduction is Sir3p dependent, as a *sas2Δ sir3Δ* strain has wild-type expression levels of the subtelomeric ORFs (Kimura et al. 2002; Suka et al. 2002).

Histone Modifications Other Than Acetylation That Affect Yeast TPE

In addition to histone acetylation, there are other, more recently identified, posttranslational histone modifications that affect TPE, including methylation, phosphorylation, ubiquitination, and ADP-ribosylation (for review, see Berger 2002; Richards and Elgin 2002). These individual histone tail modifications act in a combinatorial manner to mediate gene expression or silencing. For example, histone H3-S10 phosphorylation stimulates the acetylation of H3-K9, leading to gene activation (Lo et al. 2000). This connection is just one of many examples that led to the "histone code" hypothesis, which proposes that different patterns of histone tail modifications lead to distinct binding patterns for both activator and repressor proteins. These proteins, in turn, facilitate the formation of a euchromatic or heterochromatic DNA structure that is permissive or restrictive for gene expression (for review, see Strahl and Allis 2000; Rice and Allis 2001; Khan and Hampsey 2002; Richards and Elgin 2002).

The histone code model is useful for thinking about the role of Dot1p, a protein of previously unknown function that is necessary for TPE. *DOT1* was originally identified in a screen for high-copy disruptors of telomeric silencing (Singer et al. 1998). *DOT1* overexpression eliminates or greatly reduces TPE at truncated telomeres VII-L and V-R and also decreases *HM* and rDNA silencing (Singer et al. 1998). Intriguingly, *dot1Δ* also eliminates TPE and *HM* silencing but has no effect on rDNA silencing (Singer et al. 1998). *dot1Δ* mutants also fail to form wild-type foci of silencing proteins and demonstrate a decrease in Sir2p and Sir3p association with subtelomeric DNA (San-Segundo and Roeder 2000; Ng et al. 2002; van Leeuwen et al. 2002).

How could the same protein function to relieve silencing both when deleted and on overexpression? At the time of the initial screen, Dot1p showed no sequence similarity to any known proteins. Using a computational approach, the Dot1p secondary structure was shown to match the structure of known S-adenosyl-L-methionine methyltransferases (SAM-MTs; Dlakic 2001). This in silico experiment was validated by

subsequent biochemical and genetic analyses that demonstrate that Dot1p is indeed a histone methyltransferase that methylates ~90% of the H3-K79 residues (Briggs et al. 2002; Lacoste et al. 2002; Ng et al. 2002; van Leeuwen et al. 2002). These data led to a model whereby methylation of H3-K79 prevents Sir complex binding. In this model, in wild-type cells, methylation is absent at the heterochromatic subtelomeric regions. In *dot1Δ* cells, TPE is disrupted because the resulting lack of H3-K79 methylation in nontelomeric regions allows the Sir complex to bind chromatin away from the telomere, resulting in decreased Sir binding at the telomeres. In a *DOT1* overexpression strain, TPE is disrupted because the H3-K79 methylation extends into the subtelomeric heterochromatin, preventing Sir complex binding to this region.

Unlike most of the other posttranslational histone modifications that have been identified, histone H3-K79 is located in the nucleosome core, not on an amino-terminal tail. The methylation of H3-K79 is dependent on the ubiquitination of histone H2B, suggesting that the histone code also extends beyond the tails (Briggs et al. 2002). Recently, a series of ten amino acids within the core region, including K79, that are necessary for TPE at truncated telomere VII-L and for *HM* silencing was identified (Thompson et al. 2003). Modification of the core domain is also important for heterochromatin formation in higher eukaryotes, as H3-K79 seems to be methylated by Dot1p homologs in flies and humans (Feng et al. 2002; Schubeler et al. 2004; Shanower et al. 2005). The *Drosophila DOT1* homolog, *grappa*, is also necessary for TPE (Shanower et al. 2005).

NUCLEAR LOCALIZATION AND TPE

Some or all telomeres are localized to the nuclear periphery in yeasts, flies, humans, and in the pathogenic protozoa *T. brucei* and *P. falciparum*. Localization of the protozoan telomeres is discussed later in this chapter in the section on Biological Functions of TPE.

Peripheral Localization of Yeast Telomeres and TPE

Localization within the nucleus has long been thought to contribute to heterochromatin formation and gene silencing. Specifically, the nuclear periphery seems to be a region of the nucleus that is conducive to silencing. For example, the human inactive X chromosome, which localizes to the nuclear periphery (Bourgeois et al. 1985), and the *Drosophila* chromocenter, a cluster of the pericentric heterochromatin from all three autosomes, is found at the nuclear periphery (Mathog et al. 1984;

Hochstrasser and Sedat 1987). Perhaps the most compelling experiment arguing that this peripheral localization has functional significance comes from yeast, where tethering a weakened *HMR* silencer lacking its ORC- and Rap1p-binding sites to the nuclear periphery increases the fraction of cells that exhibit silencing (Andrulis et al. 1998).

In baker's yeast, many of the proteins required for TPE localize in foci at the nuclear periphery. Rap1p, Sir2, Sir3, Sir4p, and the Ku complex colocalize in three to six foci at the nuclear periphery (Klein et al. 1992; Palladino et al. 1993; Gotta et al. 1996; Laroche et al. 1998). Deleting any one of the Sir or Ku proteins or the carboxy-terminal Sir-interaction domain of Rap1p eliminates TPE and disperses these foci (Aparicio et al. 1991; Hecht et al. 1995; Laroche et al. 1998). Therefore, all of these key silencing proteins are required not only for TPE but also for the integrity of these foci. Whether the foci themselves contribute to TPE is not clear.

Yeast chromosomes are quite small and therefore not easily visualized by fluorescent hybridization (FISH) in intact nuclei, unless probes for multicopy sequences are used. Using FISH, ~70% of Y′ sequences localize to the foci of silencing proteins (Gotta et al. 1996). Deletion of the Sirs or Rap1p-C has no effect on the Y′ localization (Gotta et al. 1996), whereas deleting Ku leads to more Y′ foci, some of which are no longer at the nuclear periphery (Laroche et al. 1998). It is difficult to generalize about telomere behavior from FISH, especially in terms of TPE, as the Y′ signal detects only the subset of telomeres that have this element, most of which do not exhibit TPE (Pryde and Louis 1999). However, the position of individual telomeres can be determined by inserting a *lac* operator array (LacO) near a telomere and using *lac* repressor–green fluorescent protein (LacI-GFP) fusions, which bind the array in vivo, to visualize the position of the telomere (Fig. 3) (Robinett et al. 1996; Michaelis et al. 1997). The nuclear envelope can be detected in fixed cells using an antibody to a nuclear pore protein or in vivo by expressing a nuclear pore–GFP fusion protein. With the LacO visualization system, localization and silencing can be monitored at the same telomere in a population of cells.

This type of analysis was first performed in fixed cells for truncated telomere VII-L (Tham et al. 2001). As anticipated from the Y′ analysis, the truncated telomere VII-L is often at the periphery. However, even under conditions where TPE is high (80% repression), the telomere is at the periphery in only a subset of cells (66%). The fraction of telomeres at the periphery varies throughout the cell cycle, with the highest level of association being in G_1 phase. Nonetheless, even in G_1 phase, the truncated VII-L telomere is away from the periphery in many cells. Late in the cell cycle, the association with the periphery is low. Localization of the

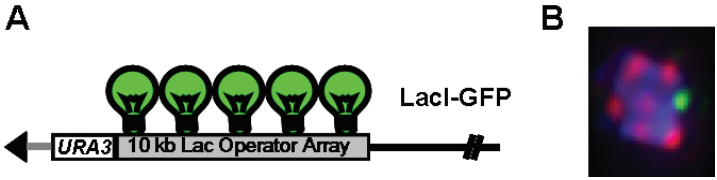

Figure 3. Visualization of single telomeres in the yeast nucleus. (*A*) Schematic of the system to measure TPE and nuclear localization of the truncated VII-L telomere. A *URA3* reporter gene is integrated adjacent to the telomere, along with a *lac* operator array, containing 256 copies of the LacO repeat (as in Tham et al. 2001). In the same strain, a fusion protein between the *lac* repressor and GFP is expressed. LacI-GFP binds to the LacO array, which can be visualized under the microscope as in *B*. (*B*) Visualization of the VII-L telomere and the Rap1p-silencing foci. In this haploid cell, the truncated VII-L telomere is visualized via the LacI-GFP protein. Antibody staining for Rap1p in the characteristic peripheral silencing foci (Gotta et al. 1996) is shown in *red*; DAPI is shown in *blue*. Projection of 3D deconvolution image taken under 100× power. (M.A. Mondoux, unpubl.)

truncated VII-L telomere is not reduced in *sir3Δ* or *yku70Δ* cells, even though TPE is eliminated at this telomere in these strains. Thus, neither Sir3p nor Ku is essential for localization of truncated telomere VII-L to the nuclear periphery.

The LacO assay has also been used to study the behavior of several "native" telomeres in living cells (Hediger et al. 2002b). As found for truncated VII-L, the X-only VI-R telomere, as well as the X-Y′ XIV-L and VIII-L telomeres, is at the periphery in some but not all cells (∼50–60%). As with VII-L, this fraction varies with position in the cell cycle, but not in exactly the same way for each telomere. Unlike truncated VII-L, localization of the VI-R and VIII-L telomeres is Ku dependent. Native VI-R also requires Sir proteins to localize to the periphery. In contrast, truncated VI-R, like truncated VII-L, does not require Ku to bind the periphery. Ku independence is not limited to truncated telomeres as the association of the XIV-L telomere is Ku-independent in S phase and only partially Ku-dependent in G_1 phase. This telomere also requires Sir4p for association with the periphery in G_1.

It is difficult to put the data on telomere localization into a simple coherent picture. What is clear (at least for the subset of telomeres that have been examined) is that telomeres are at the periphery in many, but by no means all, cells, and this association decreases late in the cell cycle. Different telomeres show different dependencies on Ku and Sir proteins for localization to the periphery. With the limited data available, it is impossible to attribute a given pattern of genetic dependencies to the

presence or absence of specific subtelomeric repeats. In this way, telomere behaviors in terms of TPE status and nuclear localization are similar. Different telomeres have different personalities.

Localization of a telomere to the periphery presumably requires at least two players, a telomere-bound protein and a protein associated with the nuclear periphery or nuclear envelope. From the data described above, the Sir complex, Ku complex, and perhaps other telomere-binding proteins can provide the telomere link. What provides the connection between telomeric chromatin and the nuclear periphery?

There are several candidates for the protein bridge between yeast telomeres and the nuclear envelope. Mlp1p (myosinlike protein 1) was identified in a screen to find nonnucleoporin proteins that associate with the nuclear envelope. Mlp1p and Mlp2p, a related protein, both localize to a filamentous structure that stretches from the inner nuclear envelope toward the nucleoplasm (Strambio-de-Castillia et al. 1999). Mlp2p also associates with the nucleoporin Nic96p (Kosova et al. 2000) and coimmunoprecipitates with yKu70p (Galy et al. 2000). These cell-biological approaches suggested a link between Mlps and telomere localization. However, these data are controversial. One group reported that the number of telomere foci detected by FISH increases, and some of the foci are less peripheral in *mlp1Δ mlp2Δ* cells (Galy et al. 2000), similar to the FISH results in *yku70Δ* cells (Laroche et al. 1998). However, the disruption in nuclear architecture that is seen in *mlp1Δ mlp2Δ* cells (Hediger et al. 2002a) makes it difficult to interpret these changes in Y′ foci. Moreover, using the LacO system, the localization of the X-only telomere VI-R is not altered in the absence of Mlp proteins (Hediger et al. 2002b).

The effects of Mlp proteins on TPE are also disputed. One group reported that *mlp1Δ mlp2Δ* cells show an ~1000-fold reduction in TPE at truncated telomere VII-L (Galy et al. 2000). This result is compromised by the fact that the wild-type strain in this study inexplicably showed poor growth on plates lacking uracil, a result not seen in earlier studies from multiple labs with the same truncated telomere. Two other groups found normal levels of TPE in *mlp1Δ mlp2Δ* cells, both at the truncated VII-L telomere and at the V-R telomere (Andrulis et al. 2002; Hediger et al. 2002a). This result was true in two strain backgrounds and using two reporter genes (Hediger et al. 2002a). Taken together, there is not compelling evidence that Mlp proteins function in TPE or telomere positioning. Mlp1p plays a role in export of unspliced mRNAs, so it is possible that under some conditions, TPE is affected indirectly in *mlp* mutants (Galy et al. 2004).

Are there other candidates for proteins that serve as the link between telomeres and the nuclear envelope? *ESC1* (establishes silent chromatin)

was identified in a screen for proteins that could mediate silencing when tethered to the truncated VII-L telomere in a *rap1ΔC* background, in which the mutated Rap1p cannot recruit the Sir complex (Andrulis et al. 2002). Because Esc1p localizes to the inner nuclear membrane (but not to the nuclear pores or the foci of silencing proteins), it is in the right place to be part of the telomere tethering apparatus (Andrulis et al. 2002; Taddei and Gasser 2004). Moreover, by two-hybrid analysis, Esc1p interacts with a portion of Sir4p known as the partitioning and anchoring domain (PAD; Andrulis et al. 2002; Taddei and Gasser 2004), a region that is able to promote partitioning of unstable ARS plasmids in mitosis (Ansari and Gartenberg 1997). Both the carboxyl terminus of Esc1p and the Sir4p PAD are capable of repositioning an internal chromosomal locus to the nuclear periphery when tethered to the locus via LexA, as expected if the two proteins cooperate to bring sequences to the nuclear envelope (Taddei and Gasser 2004).

Taken together, these data are consistent with a model in which telomere-bound Sir4p interacts with Esc1p at the inner nuclear membrane to hold telomeres at the periphery. However, the peripheral localization of both truncated telomere VI-R and the X-Y′ telomere XIV-L is not perturbed in an *esc1Δ* strain (Taddei and Gasser 2004). These telomeres are also localized to the nuclear periphery in a *yku70Δ* strain (Hediger et al. 2002b). Both of these telomeres show a random distribution within the nucleus in an *esc1Δ yku70Δ* double mutant or a *sir4Δ yku70Δ* double mutant (Taddei and Gasser 2004). Thus, at least for these two telomeres, there are two redundant telomere localization pathways, one mediated by Esc1p/Sir4p and the other by the Ku complex in concert with an as-yet-unidentified nuclear-membrane-associated protein. The presence of redundant targeting pathways can also explain the behavior of truncated VII-L, which is localized to the nuclear periphery in both Sir- and Ku-deficient cells (Tham et al. 2001).

It is tempting to speculate that the association of telomeres with the nuclear periphery promotes TPE by bringing telomeres into close proximity to the foci of silencing proteins. In addition (or alternatively), placement near the nuclear periphery may promote TPE in other ways. For example, constraining telomere mobility by an association with the nuclear periphery may make it more difficult for RNA polymerase to transcribe through a telomere-linked gene. Telomere positioning at the periphery is clearly not sufficient for TPE. For example, several telomeres remain at the periphery when their silencing is eliminated, as does the VII-L telomere in *sir3* and y*ku70* strains (Tham et al. 2001). Also, Y′ telomeres are often localized both to the nuclear periphery and to foci of silencing proteins (Palladino et al. 1993) despite their having very low or

no TPE (Pryde and Louis 1999). If telomere positioning is important for TPE, then mechanisms that eliminate tethering should also eliminate TPE. Indeed, both Ku and Sir4p are essential for TPE. However, the loss of TPE seen in their absence is not due to loss of telomere positioning, as truncated telomeres are still at the nuclear periphery in Ku- and Sir-deficient cells (Tham et al. 2001; Hediger et al. 2002b). What about Esc1p? The foci of silencing proteins are not perturbed, and TPE is only modestly reduced at truncated telomeres VII-L and VI-R in *esc1Δ* cells (Andrulis et al. 2002; Taddei and Gasser 2004). However, these telomeres are still associated with the nuclear periphery in the absence of Esc1p, presumably via the redundant Ku-mediated pathway. A critical test of the importance of tethering for TPE could be the identification of a telomere whose association with the periphery occurs solely by the Esc1/Sir4 pathway, but none of the small subset of telomeres that have been tested is tethered in this way.

Another way of gauging if TPE and peripheral localization are causally linked is to ask if the fraction of telomeres associated with the periphery decreases when TPE is low and increases when TPE is high. The truncated VII-L telomere has the same high level of association with the nuclear periphery (Tham et al. 2001) and with silencing foci (M.A. Mondoux and V.A. Zakian, unpubl.) when wild-type cells are grown in medium that requires expression of the telomere-linked *URA3* gene. Thus, even in silencing competent cells, where foci of silencing proteins are not disturbed, telomeres can be actively transcribed yet still show high association with the periphery and with foci of silencing proteins.

Another experiment tested telomere localization in cells where TPE is increased by deleting the histone deacetylase Rpd3p, which increases silencing ~25-fold at truncated telomere V-R and confers TPE on the otherwise TPE-deficient native X-Y′ V-R telomere (Rundlett et al. 1996). Because TPE is associated with hypoacetylated histones, the effects of Rpd3p on TPE are probably not due to its effects on subtelomeric chromatin. Rather, its HDAC activity may normally act to reduce expression of a gene that is needed for TPE. If the level of TPE at a given telomere correlates with telomere localization, then one expects that peripheral localization would increase in *rpd3Δ* cells. The results were mixed. The X-only VI-R telomere is more often at the nuclear periphery in mid-late S phase in *rpd3Δ* (more than 70%) compared to wild-type cells (~45%), but there is no change in the peripheral localization of telomere VI-R in G_1 or early S phase (Hediger et al. 2002b). Although the effects of Rpd3p on TPE at telomere VI-R were not determined in this study, in another study a reporter gene inserted at the native VI-R telomere was 100% silenced in wild-type cells (M.A. Mondoux and V.A. Zakian, unpubl.). Thus, it is difficult to

correlate telomere position with silencing. In considering the current data, there is still no compelling evidence that TPE and peripheral positioning are causally linked. Nonetheless, although a variety of genes and conditions have been tested, other than late in the cell cycle (Tham et al. 2001), there is no case where telomeres are away from the periphery and silenced. Thus, localization to the nuclear periphery may be necessary for TPE.

Nuclear Localization and TPE in *Drosophila*

As in yeast, *Drosophila* telomeres are localized to the nuclear periphery; however, the exact pattern of localization is not the same for each telomere. The chromocenter, which is itself at the periphery, is formed by the clustering of the centromeric regions of all the chromosomes. The telomeres of the X, second, and third chromosomes are also at the nuclear periphery but are positioned on the opposite side of the nucleus from the chromocenter (the so-called Rabl orientation; Mathog et al. 1984; Hochstrasser et al. 1986). The chromosome 4 telomeres colocalize with the chromocenter. At the periphery, the same telomeres are not always next to each other, and the arrangements of different telomeres can change during development (Dernburg et al. 1996).

As described in the earlier section on *Drosophila* subtelomeric structure, requirements for TPE on the fourth chromosome are different from the requirements for TPE on the second and third chromosomes. When the telomere of the fourth chromosome is translocated to the end of the right arm of chromosome 2, silencing is reduced at this telomere, but the protein requirements for TPE at this new telomere mimic those for TPE at chromosome 4 (Cryderman et al. 1999). However, the translocated fourth telomere shows a localization pattern typical of the 2R telomere. That is, the fourth telomere fused to the right arm of chromosome 2 localizes to the nuclear periphery opposite the chromocenter (Cryderman et al. 1999). Compared to the chromocenter, the nuclear periphery has a lower concentration of HP1 (Kellum et al. 1995), which probably explains why the translocated fourth telomere, whose TPE is HP1 dependent, shows lowered silencing (Kellum et al. 1995; Cryderman et al. 1999). The reciprocal translocation of the 2R telomere to the fourth chromosome also results in its relocation. In this case, the telomere relocates to the HP1-rich chromocenter, but shows no change in TPE levels (Cryderman et al. 1999). It is possible that the proteins required for TPE at 2R are evenly distributed throughout the nucleus, or are as abundant in the chromocenter as they are in 2R's normal location near the periphery, and therefore TPE at this hybrid telomere is not reduced. Although TPE requirements are mediated by

subtelomeric structure, localization seems to be mediated by chromosomal context as translocated telomeres retain their characteristic protein dependencies for TPE but lose their characteristic localization patterns. As in yeast, it is not clear if silencing requires localization to the nuclear periphery.

Peripheral Localization of a Particular Human Telomere May Play a Role in Silencing

Human telomeres are usually not localized to the nuclear periphery. Rather, telomeres are distributed throughout the nucleus in HeLa tissue culture cells, primary myoblasts, and fibroblasts (Luderus et al. 1996; Tam et al. 2004). Nonetheless, certain telomeres are specifically localized to heterochromatin at the nuclear and nucleolar peripheries. Although only 17% of telomeres localize to the nuclear periphery in primary fibroblasts, telomere 4q, which contains the D4Z4 repeats associated with FSHD, localizes with the nuclear periphery in primary fibroblasts in 65% of cells (Tam et al. 2004). This localization to heterochromatin compartments is even more pronounced (90%) in muscle precursor cells, the cell type where the disease is manifest (Tam et al. 2004). In addition, translocating the 4q tip onto the human active X chromosome significantly increases peripheral localization of the active X and decreases peripheral localization of the hybrid chromosome 4q (Tam et al. 2004).

Is there a link between D4Z4 subtelomeric structure, nuclear localization, and TPE? A plausible model is that as the D4Z4 subtelomeric repeats are lost, localization to the peripheral heterochromatin and thus TPE are reduced, resulting in expression of genes close to the telomere and the FSHD disease phenotype. To test this model, 4q localization was examined in heterozygous FSHD patient cell lines. Although there is a slight decrease in peripheral localization of the mutant allele compared to the normal allele, this difference is not statistically significant and the association of the mutant allele with the heterochromatin compartment remains high in all cell lines tested (Tam et al. 2004).

Mutant alleles all contain at least one copy of the D4Z4 repeat, which may be sufficient to direct localization of the telomere to the nuclear and nucleolar periphery. Telomere 10q also contains D4Z4 repeats, although the 10q repeats are not associated with FSHD. Telomere 10q also localizes to the nuclear periphery in ~65% of myoblasts (Tam et al. 2004). Thus, it is possible that the presence of the D4Z4 repeats mediates peripheral localization to heterochromatin, permitting and facilitating TPE at telomere 4q and, perhaps, at other telomeres. In this model, repeat loss leads to a loss of TPE through a mechanism other than loss of peripheral

localization, for example, loss of subtelomeric binding proteins that promote heterochromatin formation. An alternate hypothesis, given the controversial subtelomeric gene expression data, is that D4Z4 acts as an insulator to block the spread of heterochromatin at telomere 4q. In this model, the peripheral nuclear localization is a consequence of insulator or boundary activity. In both models, subtelomeric elements and the nuclear periphery play a role in human gene regulation.

TPE AND THE CELL CYCLE

It is well established that heterochromatic regions, such as the pericentric heterochromatin of *Drosophila* and the mammalian inactive X chromosome, are late replicating (for review, see Gilbert 2002). Yeast telomeres also replicate late in S phase (McCarroll and Fangman 1988; Raghuraman et al. 2001). Late replication of heterochromatin could be explained by two general models. First, active origins may not be present in heterochromatin, either because origins are absent or because the compact chromatin structure of the region makes existing origins inaccessible to the replication machinery. Alternatively, heterochromatin may contain active origins, but these origins may not fire until late S phase. In baker's yeast, the X and Y′ subtelomeric repeats contain origins that are active on plasmids (Chan and Tye 1983) but that are rarely activated in their normal chromosomal context (Dubey et al. 1991; Vujcic et al. 1999). However, these origins are functional in the chromosomal context, because if replication fork progression is slowed by mutations in replication factors, these origins fire, although their efficiency of use is still relatively low (Ivessa et al. 2002, 2003). Other origins, such as ARS501, which is ~25 kb from the chromosome end and is located within unique DNA, are active but late firing (Ferguson et al. 1991).

Reduced or late origin activation is conferred by proximity to the telomere, not by the sequence of the origin. For example, when the late-firing origin ARS501 is placed on a circular plasmid, it activates early (Ferguson and Fangman 1992). This change in origin timing is not due to its placement on a small plasmid because the same origin on a linear plasmid activates in late S phase. Likewise, if the normally early-firing origin ARS1 is placed in the subtelomeric region of chromosome V-R or the early-firing 2-μm ARS is placed on a linear plasmid, both are still active as origins, but they now fire in late S phase (Ferguson and Fangman 1992; Wellinger et al. 1993a,b). Thus, yeast telomeres exert a position effect on origin activation, in some cases inhibiting and in other cases delaying origin activation.

Are the telomere effects on replication exerted through the same heterochromatic chromatin structure that limits transcription? The Ku complex, which is critical for TPE, is also necessary for late replication of telomeric regions (Cosgrove et al. 2002). In a *yku70Δ* strain, origins that normally fire in late S phase, such as ARS501, fire much earlier in S phase. This effect on replication appears to be limited to telomeres as the absence of Ku does not affect replication timing in other parts of the chromosome. However, Ku's effects on replication timing are not easily explained by its TPE function because ARS501 fires only slightly earlier in a *sir3Δ* strain compared to wild type (Stevenson and Gottschling 1999; Cosgrove et al. 2002). Likewise, the origin near the III-L telomere, which is inactive in wild-type cells, is also inactive in *sir2*, *sir3*, or *sir4* cells, although this origin is used when replication fork progression is slowed (Ivessa et al. 2003). Thus, Ku's function in setting replication timing is unlikely to be due to its role in TPE.

Human telomeres replicate throughout S phase. One model for studying whether heterochromatin and replication timing are linked at human telomeres is a naturally occurring truncation of chromosome 22q that results in the deletion of 130 kb. The region of the truncation breakpoint, which is now closer to the telomere, is late replicating. In contrast, the same piece of DNA on the intact allele replicates in mid S phase (Ofir et al. 1999). This change in replication timing may be due to the deletion of active origins in that region. Nevertheless, late replication does not seem to be accompanied by imposition of a heterochromatic-like chromatin structure: DNase I sensitivity, methylation, and expression of the closest gene (~54 kb from the breakpoint) are indistinguishable at the mutant and wild-type alleles (Ofir et al. 1999). Taken together, the data from yeast and humans suggest that the telomere's effects on transcription and replication timing are separable phenomena.

Cell Cycle Requirements for the Establishment of Silencing

S phase progression plays a role in the establishment of heterochromatin. Silencing can be established at the *HM* loci in cells that have passed from G_1 to M phase, but not in cells that are arrested in early S phase. In contrast, derepression can occur in both cell populations (Miller and Nasmyth 1984). These results were interpreted as indicating that S-phase progression, and specifically DNA replication, are needed to establish the silent state. Several lines of evidence confirmed that S-phase progression is, in fact, crucial for establishment of silencing, but suggested that replication is not the critical event (Ehrenhofer-Murray et al. 1995; Fox et al. 1997). For

example, silencing can be established on a nonreplicating, plasmid-borne *HMR* locus, as long as cells carrying the plasmid progress through S phase (Kirchmaier and Rine 2001; Li et al. 2001). Moreover, because Sir protein recruitment and spreading do not occur robustly until G_2/M phase, the complete repression of the *HM* loci occurs after S phase (Lau et al. 2002).

How do cell cycle progression and DNA replication affect TPE? When *URA3* is adjacent to the truncated VII-L telomere, its transactivator, Ppr1p, has different effects on *URA3* expression at different points in the cell cycle. Ppr1p is able to activate transcription in cells that have been arrested at G_2/M, but cannot activate transcription in cells arrested in stationary phase, G_1, or early S phase (Aparicio and Gottschling 1994). The time at which a telomeric *URA3* gene can be activated corresponds to the time in the cell cycle when the VII-L telomere moves away from the nuclear periphery (Tham et al. 2001). Moreover, if the truncated VII-L telomere is not associated with the nuclear periphery, TPE can be eliminated efficiently in G_1 phase cells (Tham et al. 2001). These data suggest that although S-phase progression is usually required for telomeres to switch from a silent to an active transcription state, the event that allows switching is probably not DNA replication. Rather, the key event may be movement of the telomere away from the periphery, which occurs normally after DNA replication (Tham et al. 2001). Cell cycle control of TPE is not limited to baker's yeast, as TPE at the *P. falciparum* subtelomeric *var* genes is also regulated by cell cycle progression and is established during S phase (Deitsch et al. 2001).

BIOLOGICAL FUNCTIONS OF TPE

Yeast TPE as a Mechanism for Metabolism, Stress Response, and Adaptation

TPE was discovered serendipitously in both *Drosophila* and yeast when genes that are normally far from telomeres were positioned adjacent to a chromosome end and found to be transcriptionally repressed (Levis et al. 1985; Gottschling et al. 1990). Although there are considerable data that bear on the mechanism by which telomeres affect transcription, the in vivo importance of TPE has been more difficult to assess.

The early experiments on TPE in yeast demonstrated that it was regulated by many of the key genes needed for silencing at the silent mating type loci (Aparicio et al. 1991). Thus, one way to determine if TPE is a bona fide mechanism of transcriptional regulation is to ask if genes that are naturally near telomeres are expressed at low levels in a Sir-dependent

manner. As a further test, these telomere-linked genes can be moved to a nontelomeric site. If the gene's low-level expression at its telomeric site is due to TPE, then its transcription should increase at an internal site, even in a *SIR*-proficient strain.

Early attempts to address the question of the biological relevance of TPE examined the expression of individual, telomere-linked yeast genes by conventional methods. One study found that transcription of the Ty-5 transposon near the III-L telomere is low in wild-type cells, and this level increases in a *sir3* strain (Vega-Palas et al. 1997). Likewise, an ORF near the VI-R telomere is transcriptionally repressed and this repression is relieved in Sir-deficient strains (Vega-Palas et al. 2000). This same study found three other telomere-linked genes whose transcription is not increased when *SIR* genes are deleted.

The introduction of techniques for genome-wide transcription analysis made it possible to compare expression levels of all telomere-linked genes to that of the remainder of the genome. Consistent with TPE being a biologically relevant phenomenon, genes near telomeres are generally expressed at lower levels than nontelomeric genes (Wyrick et al. 1999). The 267 yeast genes that are located within 20 kb of a telomere are represented by an average of 0.5 RNA molecules per cell, a fivefold lower level than the average for nontelomeric genes. However, expression of only 20 of these genes is increased in the absence of Sir proteins. Similar effects on transcription are seen in *sir2*, *sir3*, and *sir4* strains. Most of the Sir-sensitive genes are within 8 kb of a telomere. Thus, by the classical definition, some yeast genes are regulated by TPE, but their number is relatively small, and they are very close to a chromosome end. Moreover, none of these genes has been moved to a nontelomeric site to determine if their Sir-mediated low-level transcription is telomere dependent. Without this test, it could be that genes that are expressed at low levels tend to accumulate near telomeres, but their low-level expression does not require telomere proximity.

Genome-wide transcription analysis is one of two ways used to assess TPE at "native" telomeres; the other method involves inserting the *URA3* reporter at the X-ACS in a manner that does not delete any of the telomeric repeats (see the section Telomere Identity and Effects on TPE and Fig. 1B for description). Both methods indicate that TPE functions at only a subset of "native" telomeres, but the two methods do not completely agree. Genome-wide analysis suggests that TPE regulates Sir-dependent gene expression at telomeres I-R, III-L, IV-R, V-L, VI-L, VI-R, VII-L, VIII-L, IX-R, X-R, XIII-R, XIV-L, XIV-R, XV-L, and XVI-R (Wyrick et al. 1999). Although not all of the telomeres have been tested

via insertion of *URA3* at the X-ACS, of those that have, four telomeres containing genes up-regulated in the absence of *SIR3* do not support TPE when *URA3* is inserted at the X-ACS (VI-L, X-R, XIV-L, and XV-L; Wyrick et al. 1999; E. Louis, pers. comm.). In these cases, the ORFs naturally affected by TPE are very close to or within the subtelomeric repeats and are thus closer to the telomere than the reporter gene inserted at the X-ACS. It is intriguing that these ORFs within the subtelomeric repeats, some of which are within Y′, may be naturally regulated by TPE, even though *URA3* reporter genes inserted within the Y′ repeat are not subject to TPE (Pryde and Louis 1999). Two telomeres that support TPE by the *URA3* integration assay do not contain any natural ORFs that are up-regulated in the absence of *SIR3* (II-R and XI-L; Wyrick et al. 1999; E. Louis, pers. comm.). In fact, one of the ORFs close to telomere II-R is actually down-regulated in the absence of *SIR3* (Wyrick et al. 1999). These results emphasize that the specific subtelomeric context has a strong impact on TPE. This DNA sequence and chromatin conformation context may not be identical at the same telomere in different strains.

The effects of other mutations suggest that there are also Sir-independent transcriptional repression mechanisms that act preferentially on telomere proximal regions. Depletion of histone H4 increases gene expression at ~15% of yeast genes, most of which are located near a telomere (Wyrick et al. 1999). Under these conditions, ~50% of the 267 genes that are within 20 kb of the telomeres are derepressed, many more than the number affected by Sir depletion. Likewise, genome-wide HDAC activity maps reveal that Hda1p, which specifically deacetylates lysines on H3 and H2B (Wu et al. 2001), plays a role in deacetylation of histones in repressed domains that are ~10–25 kb away from the telomeres (Robyr et al. 2002). These regions, which are termed HAST (Hda1-affected subtelomeric) domains are distinct from the immediately subtelomeric zones that are the targets of Sir-protein-mediated TPE. These experiments suggest that we should expand our definition of TPE to include genes whose transcription is reduced by proximity to the telomere in a Sir-independent, Hda1p-dependent fashion. Again, none of the HAST domain genes has been tested to see if they are expressed at higher levels at a nontelomeric site.

What can we learn about functions of Sir-dependent and Sir-independent TPE from the identities of the low-expression telomere-linked genes? Rapamycin treatment, pheromone treatment, nutrient starvation, and heat shock all lead to hyperphosphorylation of Sir3p, which is correlated with decreased TPE at truncated telomere VII-L (Stone and Pillus 1996; Ai et al. 2002). In concert with the loss of TPE, upon Sir3p

hyperphosphorylation, there is an up-regulation of at least some of the subtelomeric *PAU* genes, which encode cell wall proteins (Ai et al. 2002). Overexpression of the *PAU* genes confers partial rapamycin resistance (Ai et al. 2002). In addition to the *PAU* genes, there are several other subtelomeric genes, such as the *MAL* and *SUC* families, that regulate growth and stress response. Thus, in yeast, Sir-dependent TPE may be a mechanism for responding to nutrient deprivation and environmental stress.

HAST domains contain genes necessary for gluconeogenesis, growth in nonglucose carbon sources, and stress response, again genes that are not expressed under normal growth conditions (Robyr et al. 2002). For example, four of the five members of the *FLO* gene family are subtelomeric, located from 15 to 35 kb from chromosome ends. The four subtelomeric *FLO* genes are transcriptionally silent, whereas *FLO11*, which is ~50 kb from a telomere, is the only *FLO* gene expressed in the Σ1278b strain (Guo et al. 2000). When expressed, the subtelomeric *FLO* genes enhance cell-to-cell adherence, promote adhesion to surfaces, and promote pseudohyphal growth. Although expression of the subtelomeric *FLO* genes is not Sir2p-dependent, they are regulated by the Sir2p homologs Hst1p and Hst2p (homolog of Sir two). *FLO11*, which is further from a telomere than most HAST domain genes, is nonetheless regulated by Hda1p (Robyr et al. 2002; Halme et al. 2004).

In *S. pombe*, deletion of the Hda1p ortholog Clr3 results in the up-regulation of a large class of subtelomeric genes up to 50 kb from the telomere (Hansen et al. 2005). Many of these genes are involved in the response to nitrogen starvation. Cell wall proteins are affected by TPE in the yeast *Candida glabrata*, an opportunistic human pathogen. In *Candida*, the *EPA* genes, which encode cell wall proteins that regulate cell-to-cell adherence and surface adhesion, are located in two gene clusters adjacent to the telomeres. Although *EPA1* is expressed, the *EPA2-5* genes are transcriptionally silent in a Sir- and Rap-dependent manner (De Las Peñas et al. 2003). These data suggest that the organization of genes for stress and alternative growth strategies into subtelomeric domains is evolutionarily conserved.

Telomere Localization and Gene Expression in Eukaryotic Parasites

T. brucei is a single-celled protozoan that causes sleeping sickness. TPE exists in *Trypanosoma* because an active, nontelomeric promoter becomes transcriptionally inert when moved next to a telomere (Horn and Cross 1995). One mechanism this organism has evolved to evade the host immune system is a regular changing of its surface coat proteins, termed

antigenic variation (for review, see Borst and Ulbert 2001). In *T. brucei,* there are approximately 1000 VSG (variant surface glycoprotein) genes that encode the coat proteins. However, there are only approximately 20 sites from which these genes can be expressed, all of which are subtelomeric. In *T. brucei*, only 1 of the 20 subtelomeric VSG genes is active at a time and the rest are silent, prompting an attractive model in which TPE prevents VSG expression at all but one subtelomeric expression site. Thus, one very interesting question is to determine how the active subtelomeric expression site escapes TPE and how it differs from the inactive expression sites in the same cell (Chaves et al. 1999). By FISH analysis, the 20 subtelomeric expression sites are randomly distributed in the trypanosome nucleus, without any evident clustering or organizational pattern (Chaves et al. 1998; Navarro and Gull 2001). However, these experiments could not distinguish the position of the single active subtelomeric expression site from the inactive sites. Unlike most protein-coding genes, the VSGs are transcribed by RNA polymerase I (Shea et al. 1987; Brown et al. 1992). In *T. brucei* nuclei, RNA polymerase I is found in the nucleolus, as expected for its role in rDNA transcription. There is also a transcriptionally active, extranucleolar structure that contains RNA polymerase I that is not associated with rDNA transcription (Navarro and Gull 2001). Using the *lac* operator system to visualize different expression sites, the active expression site is observed to associate with this RNA polymerase I body, whereas a silent expression site does not (Navarro and Gull 2001). This nuclear structure was thus renamed the expression site body (ESB). A new model for antigenic variegation at subtelomeric genes, then, is that the active expression site associates with the ESB, and switching occurs not as a loss or gain of conventional TPE, but as a relocalization to or away from the ESB. It is also possible that the localization of the active ES with the ESB is a secondary consequence of its association with RNA polymerase I.

Antigenic variation also occurs in the malaria parasite *P. falciparum*, modulating the transcription of the subtelomeric *var* genes, whose products are expressed on the surface of the infected erythrocyte (for review, see Wahlgren et al. 1999). As in *Trypanosoma*, only one of the *var* genes is expressed at a time, suggesting that their expression might also be regulated by TPE. The 28 *Plasmodium* telomeres cluster into four to seven foci at the nuclear periphery in a manner that is affected by their subtelomeric DNA content (Freitas-Junior et al. 2000; Figueiredo et al. 2002). Telomeres lacking the subtelomeric TAS array fail to localize to telomere clusters, though they still localize to the nuclear periphery (Figueiredo et al. 2002). Telomere clustering may play a role in the frequent ectopic recombination

events between the *var* genes (Freitas-Junior et al. 2000; Figueiredo et al. 2002).

TPE and Aging in Yeast and Metazoan Cells

Because human telomeres shorten with age, it is possible that TPE is developmentally regulated in a manner that impacts the aging process. For example, as human telomeres shorten over time, the expression of TPE-regulated subtelomeric genes might change, leading to the expression of genes that either contribute to or combat the aging process. Alternatively, as telomeres shorten, key silencing proteins that are normally sequestered at the telomere might be freed, enabling them to silence nontelomeric genes that inhibit aging. Although attractive, there is currently no direct evidence to support a role for TPE in human aging. For example, no human genes that contribute to aging as we understand it are known to be located at telomeres and to increase expression over time.

There are, however, connections between the conserved Sir2 HDAC and aging in several organisms. Aging in baker's yeast is defined by how many times mother cells can bud to give rise to daughter cells. Using this metric, *sir2Δ* yeast have decreased life spans (~50%) and cells overexpressing Sir2p have increased life spans (30–40%; Kaeberlein et al. 1999; Roy and Runge 2000). Aging in *C. elegans* is defined by the decay of the nondividing cells of the soma. Despite this being a very different metric for aging, as in yeast, increased dosage of the *C. elegans sir-2.1* gene extends life span (Tissenbaum and Guarente 2001). However, there is no evidence in either organism that the effects of Sir2p on life span are connected to its role in TPE. Yeast Sir2p has multiple functions: It inhibits transcription not only at telomeres but also at the silent mating type loci and in the rDNA. Yeast Sir2p also inhibits activation of origins of DNA replication, both in the rDNA and elsewhere (Pasero et al. 2002; Pappas et al. 2004). The replication function of Sir2p has been implicated in aging through its role in preventing the accumulation of extrachromosomal rDNA circles, although the association of rDNA circles with aging has been challenged (Sinclair et al. 1997; Kaeberlein et al. 1999; Roy and Runge 2000; Falcon and Aris 2003). Sir2p's function as an NAD^+ deacetylase may link its role in aging to metabolism. Caloric restriction increases life span in yeast, worms, and humans (for review, see Tissenbaum and Guarente 2002). In yeast and worms, mutating *SIR2* is argued to eliminate the increased life span associated with caloric restriction (Lin et al. 2000, 2002). However, more recent evidence suggests that although caloric restriction and Sir2p both function in the regulation of yeast life

span, they act in independent pathways (Jiang et al. 2002; Kaeberlein et al. 2004).

Sir2p is the only one of the yeast Sir proteins that is conserved among diverse organisms (Brachmann et al. 1995). Moreover, there are multiple members of the Sir2 family in most organisms, including baker's yeast, which has four HST genes. Compounds that activate Sir2 homologs promote longevity in yeast, human cells, *Drosophila*, and *C. elegans* (Howitz et al. 2003; Wood et al. 2004). In mammalian cells, the effects of caloric restriction on life span are argued to be mediated by the closest of the Sir2p homologs, SIRT1 (Cohen et al. 2004). In addition, the human SIRT3 gene, which also encodes a NAD-dependent deacetylase, is located close to the 11p telomere. This region of the chromosome contains five genes that have been linked to aging (De Luca et al. 2001; Bonafe et al. 2002; Tan et al. 2002).

Sir2p and its homologs are not the only HDACs with correlations to aging. In yeast, the HDAC Rpd3p contributes negatively to longevity (Jiang et al. 2002). A partial reduction in Rpd3 levels also results in increased life span in flies (*Drosophila* Rpd3 is essential). Because *Drosophila* Sir2 expression is increased twofold in the *rpd3* mutants, the effects of Rpd3 on life span may be indirect (Rogina et al. 2002). In yeast, deleting the HDAC *HDA1*, required to inhibit transcription of HAST domain genes, has no effect by itself on longevity but its deletion acts synergistically with caloric restriction to increase life span (Jiang et al. 2002). Is the role of Sir2p and other histone deacetylases in aging related to their roles in TPE and Sir-independent subtelomeric gene regulation? The most likely link seems to be metabolism and stress response, as gene families involved in these processes are located in subtelomeric regions and are subject to TPE or Sir-independent regulation by acetylation. However, at this point, there is at best a tenuous connection between telomeric gene expression and aging.

CONCLUDING REMARKS

In diverse organisms, telomeres exert position effects on the expression of subtelomeric genes. TPE is classically defined in *S. cerevisiae* as the Sir-protein-mediated spread of heterochromatin from the telomere inward. However, there is recent evidence in yeast for Sir-independent transcriptional inhibition of genes that are near telomeres but outside of the Sir-associated domain. These larger subtelomeric domains are still regulated by histone modifications, but they are silenced via the Hda1p or Hst1/2p, not the Sir2p, HDAC. One model for TPE at human telomere 4q involves

the looping of the telomere to bring silencing factors in proximity to target genes, as opposed to a continuous spread of heterochromatin. We should therefore expand our definition of TPE to include all gene silencing that is mediated by the telomeric and subtelomeric repeats, not just that which is Sir-mediated or continuous.

Different species have evolved similar mechanisms to regulate TPE, including conserved or analogous telomere-binding proteins, telomere length regulation, nuclear localization, and cell cycle regulation. Although different species may use similar mechanisms to silence subtelomeric genes, different telomeres within a species can have differing abilities to support TPE, levels of TPE, and even requirements for TPE. Some, but not all, of these differences can be explained by the varied subtelomeric repeat elements found at different telomeres.

TPE is a biologically relevant phenomenon, regulating rarely used genes for growth and stress response in *S. cerevisiae* and *S. pombe*, cell adhesion in the opportunistic pathogen *C. glabrata*, immune system evasion in the parasites *T. brucei* and *P. falciparum*, and potentially a form of muscular dystrophy in humans. The study of telomere position effect thus not only serves as a model for epigenetic silencing and regulation, but also provides the potential for understanding wide-ranging biological problems, including genome organization, survival mechanisms, pathogenesis, and disease.

ACKNOWLEDGMENTS

We thank our colleagues who sent manuscripts that helped us write this paper. We also thank Ed Louis, Art Lustig, Jim Mason, Lorraine Pillus, Kurt Runge, and Woody Wright for their comments and the NIH for support of research in our laboratory.

REFERENCES

Abad J.P., De Pablos B., Osoegawa K., De Jong P.J., Martin-Gallardo A., and Villasante A. 2004. *TAHRE*, a novel telomeric retrotransposon from *Drosophila melanogaster*, reveals the origin of *Drosophila* telomeres. *Mol. Biol. Evol.* **21:** 1620–1624.

Adams Martin A., Dionne I., Wellinger R.J., and Holm C. 2000. The function of DNA polymerase α at telomeric G tails is important for telomere homeostasis. *Mol. Cell. Biol.* **20:** 786–796.

Ai W., Bertram P.G., Tsang C.K., Chan T.F., and Zheng X.F. 2002. Regulation of subtelomeric silencing during stress response. *Mol. Cell* **10:** 1295–1305.

Allfrey V.G., Faulkner R., and Mirsky A.E. 1964. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc. Natl. Acad. Sci.* **51:** 786–794.

Allshire R.C., Nimmo E.R., Ekwall K., Javerzat J.P., and Cranston G. 1995. Mutations derepressing silent centromeric domains in fission yeast disrupt chromosome segregation. *Genes Dev.* **9:** 218–233.

Andrulis E.D., Neiman A.M., Zappulla D.C., and Sternglanz R. 1998. Perinuclear localization of chromatin facilitates transcriptional silencing. *Nature* **394:** 592–595.

Andrulis E.D., Zappulla D.C., Ansari A., Perrod S., Laiosa C.V., Gartenberg M.R., and Sternglanz R. 2002. Esc1, a nuclear periphery protein required for Sir4-based plasmid anchoring and partitioning. *Mol. Cell. Biol.* **22:** 8292–8301.

Ansari A. and Gartenberg M.R. 1997. The yeast silent information regulator Sir4p anchors and partitions plasmids. *Mol. Cell. Biol.* **17:** 7061–7068.

Aparicio O.M. and Gottschling D.E. 1994. Overcoming telomeric silencing: A *trans*-activator competes to establish gene expression in a cell cycle-dependent way. *Genes Dev.* **8:** 1133–1146.

Aparicio O.M., Billington B.L., and Gottschling D.E. 1991. Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. *Cell* **66:** 1279–1287.

Baur J.A., Shay J.W., and Wright W.E. 2004. Spontaneous reactivation of a silent telomeric transgene in a human cell line. *Chromosoma* **112:** 240–246.

Baur J.A., Zou Y., Shay J.W., and Wright W.E. 2001. Telomere position effect in human cells. *Science* **292:** 2075–2077.

Berger S.L. 2002. Histone modifications in transcriptional regulation. *Curr. Opin. Genet. Dev.* **12:** 142–148.

Boivin A., Gally C., Netter S., Anxolabehere D., and Ronsseray S. 2003. Telomeric associated sequences of *Drosophila* recruit polycomb-group proteins *in vivo* and can induce pairing-sensitive repression. *Genetics* **164:** 195–208.

Bonafe M., Barbi C., Olivieri F., Yashin A., Andreev K.F., Vaupel J.W., De Benedictis G., Rose G., Carrieri G., Jazwinski S.M., and Franceschi C. 2002. An allele of HRAS1 3′variable number of tandem repeats is a frailty allele: Implication for an evolutionarily-conserved pathway involved in longevity. *Gene* **286:** 121–126.

Borst P. and Ulbert S. 2001. Control of VSG gene expression sites. *Mol. Biochem. Parasitol.* **114:** 17–27.

Boudreault A.A., Cronier D., Selleck W., Lacoste N., Utley R.T., Allard S., Savard J., Lane W.S., Tan S., and Cote J. 2003. Yeast enhancer of polycomb defines global Esa1-dependent acetylation of chromatin. *Genes Dev.* **17:** 1415–1428.

Boulton S.J. and Jackson S.P. 1998. Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *EMBO J.* **17:** 1819–1828.

Bourgeois C.A., Laquerriere F., Hemon D., Hubert J., and Bouteille M. 1985. New data on the in-situ position of the inactive X chromosome in the interphase nucleus of human fibroblasts. *Hum. Genet.* **69:** 122–129.

Bourns B.D., Alexander M.K., Smith A.M., and Zakian V.A. 1998. Sir proteins, Rif proteins and Cdc13p bind *Saccharomyces* telomeres *in vivo*. *Mol. Cell. Biol.* **18:** 5600–5608.

Brachmann C.B., Sherman J.M., Devine S.E., Cameron E.E., Pillus L., and Boeke J.D. 1995. The SIR2 gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. *Genes Dev.* **9:** 2888–2902.

Braunstein M., Rose A.B., Holmes S.G., Allis C.D., and Broach J.R. 1993. Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. *Genes Dev.* **7:** 592–604.

Brigati C., Kurtz S., Balderes D., Vidali G., and Shore D. 1993. An essential yeast gene encoding a TTAGGG repeat-binding protein. *Mol. Cell. Biol.* **13:** 1306–1314.

Briggs S.D., Xiao T., Sun Z.W., Caldwell J.A., Shabanowitz J., Hunt D.F., Allis C.D., and Strahl B.D. 2002. Gene silencing: *Trans*-histone regulatory pathway in chromatin. *Nature* **418:** 498.

Brown S.D., Huang J., and Van der Ploeg L.H. 1992. The promoter for the procyclic acidic repetitive protein (PARP) genes of *Trypanosoma brucei* shares features with RNA polymerase I promoters. *Mol. Cell. Biol.* **12:** 2644–2652.

Chan C.S.M. and Tye B.-K. 1983. Organization of DNA sequences and replication origins at yeast telomeres. *Cell* **33:** 563–573.

Chaves I., Rudenko G., Dirks-Mulder A., Cross M., and Borst P. 1999. Control of variant surface glycoprotein gene-expression sites in *Trypanosoma brucei*. *EMBO J.* **18:** 4846–4855.

Chaves I., Zomerdijk J., Dirks-Mulder A., Dirks R.W., Raap A.K., and Borst P. 1998. Subnuclear localization of the active variant surface glycoprotein gene expression site in *Trypanosoma brucei*. *Proc. Natl. Acad. Sci.* **95:** 12328–12333.

Chi M.H. and Shore D. 1996. SUM1-1, a dominant suppressor of SIR mutations in *Saccharomyces cerevisiae*, increases transcriptional silencing at telomeres and HM mating-type loci and decreases chromosome stability. *Mol. Cell. Biol.* **16:** 4281–4294.

Chien C.T., Buck S., Sternglanz R., and Shore D. 1993. Targeting of SIR1 protein establishes transcriptional silencing at *HM* loci and telomeres in yeast. *Cell* **75:** 531–541.

Cohen H.Y., Miller C., Bitterman K.J., Wall N.R., Hekking B., Kessler B., Howitz K.T., Gorospe M., de Cabo R., and Sinclair D.A. 2004. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* **305:** 390–392.

Conrad M.N., Wright J.H., Wolf A.J., and Zakian V.A. 1990. RAP1 protein interacts with yeast telomeres *in vivo*: Overproduction alters telomere structure and decreases chromosome stability. *Cell* **63:** 739–750.

Conway C., McCulloch R., Ginger M.L., Robinson N.P., Browitt A., and Barry J.D. 2002. Ku is important for telomere maintenance, but not for differential expression of telomeric VSG genes, in African trypanosomes. *J. Biol. Chem.* **277:** 21269–21277.

Corda Y., Schramke V., Longhese M.P., Smokvina T., Paciotti V., Brevet V., Gilson E., and Geli V. 1999. Interaction between Set1p and checkpoint protein Mec3p in DNA repair and telomere functions. *Nat. Genet.* **21:** 204–208.

Cosgrove A.J., Nieduszynski C.A., and Donaldson A.D. 2002. Ku complex controls the replication time of DNA in telomere regions. *Genes Dev.* **16:** 2485–2490.

Craven R.J. and Petes T.D. 1999. Dependence of the regulation of telomere length on the type of subtelomeric repeat in the yeast *Saccharomyces cerevisiae*. *Genetics* **152:** 1531–1541.

———. 2000. Involvement of the checkpoint protein Mec1p in silencing of gene expression at telomeres in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **20:** 2378–2384.

———. 2001. The *Saccharomyces cerevisiae* suppressor of choline sensitivity (SCS2) gene is a multicopy suppressor of mec1 telomeric silencing defects. *Genetics* **158:** 145–154.

Crotti L.B. and Basrai M.A. 2004. Functional roles for evolutionarily conserved Spt4p at centromeres and heterochromatin in *Saccharomyces cerevisiae*. *EMBO J.* **23:** 1804–1814.

Cryderman D.E., Morris E.J., Biessmann H., Elgin S.C., and Wallrath L.L. 1999. Silencing at *Drosophila* telomeres: Nuclear organization and chromatin structure play critical roles. *EMBO J.* **18:** 3724–3735.

Cuperus G. and Shore D. 2002. Restoration of silencing in *Saccharomyces cerevisiae* by tethering of a novel Sir2-interacting protein, Esc8. *Genetics* **162:** 633–645.

Danilevskaya O.N., Arkhipova I.R., Traverse K.L., and Pardue M.L. 1997. Promoting in tandem: The promoter for telomere transposon HeT-A and implications for the evolution of retroviral LTRs. *Cell* **88:** 647–655.

de Bruin D., Kantrow S.M., Liberatore R.A., and Zakian V.A. 2000. Telomere folding is required for the stable maintenance of telomere position effects in yeast. *Mol. Cell. Biol.* **20:** 7991–8000.

Deitsch K.W., Calderwood M.S., and Wellems T.E. 2001. Cooperative silencing elements in *var* genes. *Nature* **412:** 875–876.

De Las Peñas A., Pan S.J., Castano I., Alder J., Cregg R., and Cormack B.P. 2003. Virulence-related surface glycoproteins in the yeast pathogen *Candida glabrata* are encoded in subtelomeric clusters and subject to *RAP1*- and *SIR*-dependent transcriptional silencing. *Genes Dev.* **17:** 2245–2258.

De Luca M., Rose G., Bonafe M., Garasto S., Greco V., Weir B.S., Franceschi C., and De Benedictis G. 2001. Sex-specific longevity associations defined by Tyrosine Hydroxylase-Insulin-Insulin Growth Factor 2 haplotypes on the 11p15.5 chromosomal region. *Exp. Gerontol.* **36:** 1663–1671.

Denisenko O. and Bomsztyk K. 2002. Yeast hnRNP K-like genes are involved in regulation of the telomeric position effect and telomere length. *Mol. Cell. Biol.* **22:** 286–297.

Dernburg A.F., Broman K.W., Fung J.C., Marshall W.F., Philips J., Agard D.A., and Sedat J.W. 1996. Perturbation of nuclear architecture by long-distance chromosome interactions. *Cell* **85:** 745–759.

Diffley J.F. and Stillman B. 1989. Similarity between the transcriptional silencer binding proteins ABF1 and RAP1. *Science* **246:** 1034–1038.

Dlakic M. 2001. Chromatin silencing protein and pachytene checkpoint regulator Dot1p has a methyltransferase fold. *Trends Biochem. Sci.* **26:** 405–407.

Dror V. and Winston F. 2004. The Swi/Snf chromatin remodeling complex is required for ribosomal DNA and telomeric silencing in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **24:** 8227–8235.

Dubey D.D., Davis L.R., Greenfeder S.A., Ong L.Y., Zhu J.G., Broach J.R., Newlon C.S., and Huberman J.A. 1991. Evidence suggesting that the *ARS* elements associated with silencers of the yeast mating-type locus HML do not function as chromosomal DNA replication origins. *Mol. Cell. Biol.* **11:** 5346–5355.

Dziak R., Leishman D., Radovic M., Tye B.K., and Yankulov K. 2003. Evidence for a role of MCM (mini-chromosome maintenance)5 in transcriptional repression of sub-telomeric and *Ty*-proximal genes in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **278:** 27372–27381.

Ehrenhofer-Murray A.E., Rivier D.H., and Rine J. 1997. The role of Sas2, an acetyltransferase homologue of *Saccharomyces cerevisiae*, in silencing and ORC function. *Genetics* **145:** 923–934.

Ehrenhofer-Murray A.E., Gossen M., Pak D.T., Botchan M.R., and Rine J. 1995. Separation of origin recognition complex functions by cross-species complementation. *Science* **270:** 1671–1674.

Enomoto S., McCune-Zierath P.D., Gerami-Nejad M., Sanders M.A., and Berman J. 1997. *RLF2,* a subunit of yeast chromatin assembly-I, is required for telomeric chromatin function in vivo. *Genes Dev.* **11:** 358–370.

Ezhkova E. and Tansey W.P. 2004. Proteasomal ATPases link ubiquitylation of histone H2B to methylation of histone H3. *Mol. Cell* **13:** 435–442.

Falcon A.A. and Aris J.P. 2003. Plasmid accumulation reduces life span in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **278:** 41607–41617.

Feng Q., Wang H., Ng H.H., Erdjument-Bromage H., Tempst P., Struhl K., and Zhang Y. 2002. Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. *Curr. Biol.* **12:** 1052–1058.

Ferguson B.M. and Fangman W.L. 1992. A position effect on the time of replication origin activation in yeast. *Cell* **68:** 333–339.

Ferguson B.M., Brewer B.J., Reynolds A.E., and Fangman W.L. 1991. A yeast origin of replication is activated late in S phase. *Cell* **65:** 507–515.

Figueiredo L.M., Freitas-Junior L.H., Bottius E., Olivo-Marin J.C., and Scherf A. 2002. A central role for *Plasmodium falciparum* subtelomeric regions in spatial positioning and telomere length regulation. *EMBO J.* **21:** 815–824.

Fisher T.S., Taggart A.K.P., and Zakian V.A. 2004. Cell cycle-dependent regulation of yeast telomerase by Ku. *Nat. Struct. Mol. Biol.* **11:** 1198–1205.

Foss M., McNally F.J., Laurenson P., and Rine J. 1993. Origin recognition complex (ORC) in transcriptional silencing and DNA replication in *S. cerevisiae*. *Science* **262:** 1838–1844.

Fourel G., Revardel E., Koering C.E., and Gilson E. 1999. Cohabitation of insulators and silencing elements in yeast subtelomeric regions. *EMBO J.* **18:** 2522–2537.

Fourel G., Boscheron C., Revardel E., Lebrun E., Hu Y.F., Simmen K.C., Muller K., Li R., Mermod N., and Gilson E. 2001. An activation-independent role of transcription factors in insulator function. *EMBO Rep.* **2:** 124–132.

Fox C.A., Ehrenhofer-Murray A.E., Loo S., and Rine J. 1997. The origin recognition complex, *SIR1*, and the S phase requirement for silencing. *Science* **276:** 1547–1551.

Freitas-Junior L.H., Bottius E., Pirrit L.A., Deitsch K.W., Scheidig C., Guinet F., Nehrbass U., Wellems T.E., and Scherf A. 2000. Frequent ectopic recombination of virulence factor genes in telomeric chromosome clusters of *P. falciparum*. *Nature* **407:** 1018–1022.

Gabellini D., Green M.R., and Tupler R. 2002. Inappropriate gene activation in FSHD: A repressor complex binds a chromosomal repeat deleted in dystrophic muscle. *Cell* **110:** 339–348.

Galy V., Gadal O., Fromont-Racine M., Romano A., Jacquier A., and Nehrbass U. 2004. Nuclear retention of unspliced mRNAs in yeast is mediated by perinuclear Mlp1. *Cell* **116:** 63–73.

Galy V., Olivo-Marin J.C., Scherthan H., Doye V., Rascalou N., and Nehrbass U. 2000. Nuclear pore complexes in the organization of silent telomeric chromatin. *Nature* **403:** 108–112.

Gasser S.M. and Cockell M.M. 2001. The molecular biology of the SIR proteins. *Gene* **279:** 1–16.

Gehring W.J., Klemenz R., Weber U., and Kloter U. 1984. Functional analysis of the white+ gene of *Drosophila* by P-factor-mediated transformation. *EMBO J.* **3:** 2077–2085.

Gilbert D.M. 2002. Replication timing and transcriptional control: Beyond cause and effect. *Curr. Opin. Cell Biol.* **14:** 377–383.

Gilson E., Roberge M., Giraldo R., Rhodes D., and Gasser S.M. 1993. Distortion of the DNA double helix by RAP1 at silencers and multiple telomeric binding sites. *J. Mol. Biol.* **231:** 293–310.

Golubovsky M.D., Konev A.Y., Walter M.F., Biessmann H., and Mason J.M. 2001. Terminal retrotransposons activate a subtelomeric white transgene at the 2L telomere in *Drosophila*. *Genetics* **158:** 1111–1123.

Gotta M., Laroche T., Formenton A., Maillet L., Scherthan H., and Gasser S.M. 1996. The clustering of telomeres and colocalization with Rap1, Sir3, and Sir4 proteins in wild-type *Saccharomyces cerevisiae*. *J. Cell Biol.* **134:** 1349–1363.

Gottschling D.E., Aparicio O.M., Billington B.L., and Zakian V.A. 1990. Position effect at *S. cerevisiae* telomeres: Reversible repression of Pol II transcription. *Cell* **63:** 751–762.

Gravel S., Larrivee M., Labrecque P., and Wellinger R.J. 1998. Yeast Ku as a regulator of chromosomal DNA end structure. *Science* **280:** 741–744.

Greenwell P.W., Kronmal S.L., Porter S.E., Gassenhuber J., Obermaier B., and Petes T.D. 1995. *TEL1*, a gene involved in controlling telomere length in *S. cerevisiae*, is homologous to the human ataxia telangiectasia gene. *Cell* **82:** 823–829.

Griffith J.D., Comeau L., Rosenfield S., Stansel R.M., Bianchi A., Moss H., and de Lange T. 1999. Mammalian telomeres end in a large duplex loop. *Cell* **97:** 503–514.

Guo B., Styles C.A., Feng Q., and Fink G.R. 2000. A *Saccharomyces* gene family involved in invasive growth, cell-cell adhesion, and mating. *Proc. Natl. Acad. Sci.* **97:** 12158–12163.

Haber J.E. 1998. Mating-type gene switching in *Saccharomyces cerevisiae*. *Annu. Rev. Genet.* **32:** 561–599.

Halme A., Bumgarner S., Styles C., and Fink G.R. 2004. Genetic and epigenetic regulation of the FLO gene family generates cell-surface variation in yeast. *Cell* **116:** 405–415.

Hansen K.R., Burns G., Mata J., Volpe T.A., Martienssen R.A., Bahler J., and Thon G. 2005. Global effects on gene expression in fission yeast by silencing and RNA interference machineries. *Mol. Cell. Biol.* **25:** 590–601.

Hardy C.F., Sussel L., and Shore D. 1992. A RAP1-interacting protein involved in transcriptional silencing and telomere length regulation. *Genes Dev.* **6:** 801–814.

Hazelrigg T., Levis R., and Rubin G.M. 1984. Transformation of *white* locus DNA in *Drosophila*: Dosage compensation, *zeste* interaction, and position effects. *Cell* **36:** 469–481.

Hecht A., Laroche T., Strahl-Bolsinger S., Gasser S.M., and Grunstein M. 1995. Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: A molecular model for the formation of heterochromatin in yeast. *Cell* **80:** 583–592.

Hediger F., Dubrana K., and Gasser S.M. 2002a. Myosin-like proteins 1 and 2 are not required for silencing or telomere anchoring, but act in the Tel1 pathway of telomere length control. *J. Struct. Biol.* **140:** 79–91.

Hediger F., Neumann F.R., Van Houwe G., Dubrana K., and Gasser S.M. 2002b. Live imaging of telomeres: yKu and Sir proteins define redundant telomere-anchoring pathways in yeast. *Curr. Biol.* **12:** 2076–2089.

Henikoff S. 2000. Heterochromatin function in complex genomes. *Biochim. Biophys. Acta.* **1470:** 1–8.

Hewitt J.E., Lyle R., Clark L.N., Valleley E.M., Wright T.J., Wijmenga C., van Deutekom J.C., Francis F., Sharpe P.T., Hofker M., et al. 1994. Analysis of the tandem repeat locus D4Z4 associated with facioscapulohumeral muscular dystrophy. *Hum. Mol. Genet.* **3:** 1287–1295.

Hochstrasser M. and Sedat J.W. 1987. Three-dimensional organization of *Drosophila melanogaster* interphase nuclei. I. Tissue-specific aspects of polytene nuclear architecture. *J. Cell Biol.* **104:** 1455–1470.

Hochstrasser M., Mathog D., Gruenbaum Y., Saumweber H., and Sedat J.W. 1986. Spatial organization of chromosomes in the salivary gland nuclei of *Drosophila melanogaster*. *J. Cell Biol.* **102:** 112–123.

Hoppe G.J., Tanny J.C., Rudner A.D., Gerber S.A., Danaie S., Gygi S.P., and Moazed D. 2002. Steps in assembly of silent chromatin in yeast: Sir3-independent binding of a

Sir2/Sir4 complex to silencers and role for Sir2-dependent deacetylation. *Mol. Cell. Biol.* **22:** 4167–4180.

Horn D. and Cross G.A. 1995. A developmentally regulated position effect at a telomeric locus in *Trypanosoma brucei*. *Cell* **83:** 555–561.

Howitz K.T., Bitterman K.J., Cohen H.Y., Lamming D.W., Lavu S., Wood J.G., Zipkin R.E., Chung P., Kisielewski A., Zhang L.L., Scherer B., and Sinclair D.A. 2003. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* **425:** 191–196.

Hu F., Alcasabas A.A., and Elledge S.J. 2001. Asf1 links Rad53 to control of chromatin assembly. *Genes Dev.* **15:** 1061–1066.

Huang H., Kahana A., Gottschling D.E., Prakash L., and Liebman S.W. 1997. The ubiquitin-conjugating enzyme Rad6 (Ubc2) is required for silencing in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **17:** 6693–6699.

Huang Y. 2002. Transcriptional silencing in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Nucleic Acids Res.* **30:** 1465–1482.

Hultdin M., Gronlund E., Norrback K., Eriksson-Lindstrom E., Just T., and Roos G. 1998. Telomere analysis by fluorescence *in situ* hybridization and flow cytometry. *Nucleic Acids Res.* **26:** 3651–3656.

Iida T. and Araki H. 2004. Noncompetitive counteractions of DNA polymerase ε and ISW2/yCHRAC for epigenetic inheritance of telomere position effect in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **24:** 217–227.

Imai S., Armstrong C.M., Kaeberlein M., and Guarente L. 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* **403:** 795–800.

Ivessa A.S., Lenzmeier B.A., Bessler J.B., Goudsouzian L.K., Schnakenberg S.L., and Zakian V.A. 2003. The *Saccharomyces cerevisiae* helicase Rrm3p facilitates replication past nonhistone protein-DNA complexes. *Mol. Cell* **12:** 1525–1536.

Ivessa A.S., Zhou J.-Q., Schulz V.P., Monson E.M., and Zakian V.A. 2002. *Saccharomyces* Rrm3p, a 5′ to 3′ DNA helicase that promotes replication fork progression through telomeric and sub-telomeric DNA. *Genes Dev.* **16:** 1383–1396.

Ivy J.M., Klar A.J.S., and Hicks J.B. 1986. Cloning and characterization of four *SIR* genes of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **6:** 688–702.

Jacob N.K., Stout A.R., and Price C.M. 2004. Modulation of telomere length dynamics by the subtelomeric region of tetrahymena telomeres. *Mol. Biol. Cell.* **15:** 3719–3728.

Jiang G., Yang F., van Overveld P.G., Vedanarayanan V., van der Maarel S., and Ehrlich M. 2003. Testing the position-effect variegation hypothesis for facioscapulohumeral muscular dystrophy by analysis of histone modification and gene expression in subtelomeric 4q. *Hum. Mol. Genet.* **12:** 2909–2921.

Jiang J.C., Wawryn J., Shantha Kumara H.M., and Jazwinski S.M. 2002. Distinct roles of processes modulated by histone deacetylases Rpd3p, Hda1p, and Sir2p in life extension by caloric restriction in yeast. *Exp. Gerontol.* **37:** 1023–1030.

Johnson L.M., Kayne P.S., Kahn E.S., and Grunstein M. 1990. Genetic evidence for an interaction between SIR3 and histone H4 in the repression of the silent mating loci in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **87:** 6286–6290.

Kaeberlein M., McVey M., and Guarente L. 1999. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev.* **13:** 2570–2580.

Kaeberlein M., Kirkland K.T., Fields S., and Kennedy B.K. 2004. Sir2-independent life span extension by calorie restriction in yeast. *PLoS Biol.* **2:** E296.

Kanoh J. and Ishikawa F. 2001. spRap1 and spRif1, recruited to telomeres by Taz1, are essential for telomere function in fission yeast. *Curr. Biol.* **11:** 1624–1630.

Karpen G.H. and Spradling A.C. 1992. Analysis of subtelomeric heterochromatin in the *Drosophila* minichromosome *Dp1187* by single P element insertional mutagenesis. *Genetics* **132:** 737–753.

Kaufman P.D., Cohen J.L., and Osley M.A. 1998. Hir proteins are required for position-dependent gene silencing in *Saccharomyces cerevisiae* in the absence of chromatin assembly factor I. *Mol. Cell. Biol.* **18:** 4793–4806.

Kaufman P.D., Kobayashi R., and Stillman B. 1997. Ultraviolet radiation sensitivity and reduction of telomeric silencing in *Saccharomyces cerevisiae* cells lacking chromatin assembly factor-I. *Genes Dev.* **11:** 345–357.

Kayne P.S., Kim U.-J., Han M., Mullen J., Yoshizaki F., and Grunstein M. 1988. Extremely conserved histone H4 N-terminus is dispensable for growth but essential for repressing the silent mating loci in yeast. *Cell* **55:** 27–39.

Kellum R., Raff J.W., and Alberts B.M. 1995. Heterochromatin protein 1 distribution during development and during the cell cycle in *Drosophila* embryos. *J. Cell Sci.* **108:** 1407–1418.

Kelly T.J., Qin S., Gottschling D.E., and Parthun M.R. 2000. Type B histone acetyltransferase Hat1p participates in telomeric silencing. *Mol. Cell. Biol.* **20:** 7051–7058.

Khan A.U. and Hampsey M. 2002. Connecting the DOTs: Covalent histone modifications and the formation of silent chromatin. *Trends Genet.* **18:** 387–389.

Kimura A., Umehara T., and Horikoshi M. 2002. Chromosomal gradient of histone acetylation established by Sas2p and Sir2p functions as a shield against gene silencing. *Nat. Genet.* **32:** 370–377.

Kirchmaier A.L. and Rine J. 2001. DNA replication-independent silencing in *S. cerevisiae*. *Science* **291:** 646–650.

Klein F., Laroche T., Cardenas M.E., Hofmann J.F., Schweizer D., and Gasser S.M. 1992. Localization of RAP1 and topoisomerase II in nucleic and meiotic chromosomes of yeast. *J. Cell. Biol.* **117:** 935–948.

Koering C.E., Pollice A., Zibella M.P., Bauwens S., Puisieux A., Brunori M., Brun C., Martins L., Sabatier L., Pulitzer J.F., and Gilson E. 2002. Human telomeric position effect is determined by chromosomal context and telomeric chromatin integrity. *EMBO Rep.* **3:** 1055–1061.

Kosova B., Pante N., Rollenhagen C., Podtelejnikov A., Mann M., Aebi U., and Hurt E. 2000. Mlp2p, a component of nuclear pore attached intranuclear filaments, associates with nic96p. *J. Biol. Chem.* **275:** 343–350.

Kouskouti A., Scheer E., Staub A., Tora L., and Talianidis I. 2004. Gene-specific modulation of TAF10 function by SET9-mediated methylation. *Mol. Cell* **14:** 175–182.

Krogan N.J., Dover J., Wood A., Schneider J., Heidt J., Boateng M.A., Dean K., Ryan O.W., Golshani A., Johnston M., Greenblatt J.F., and Shilatifard A. 2003. The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: Linking transcriptional elongation to histone methylation. *Mol. Cell* **11:** 721–729.

Kurenova E., Champion L., Biessmann H., and Mason J.M. 1998. Directional gene silencing induced by a complex subtelomeric satellite from *Drosophila*. *Chromosoma* **107:** 311–320.

Kurtz S. and Shore D. 1991. RAP1 protein activates and silences transcription of mating-type genes in yeast. *Genes Dev.* **5:** 616–628.

Kyrion G., Boakye K.A., and Lustig A.J. 1992. C-terminal truncation of RAP1 results in the deregulation of telomere size, stability, and function in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12:** 5159–5173.

Kyrion G., Liu K., Liu C., and Lustig A.J. 1993. RAP1 and telomere structure regulate telomere position effects in *Saccharomyces cerevisiae*. *Genes Dev.* **7:** 1146–1159.

Lacoste N., Utley R.T., Hunter J.M., Poirier G.G., and Cote J. 2002. Disruptor of telomeric silencing-1 is a chromatin-specific histone H3 methyltransferase. *J. Biol. Chem.* **277:** 30421–30424.

Ladurner A.G., Inouye C., Jain R., and Tjian R. 2003. Bromodomains mediate an acetyl-histone encoded antisilencing function at heterochromatin boundaries. *Mol. Cell* **11:** 365–376.

Landry J., Sutton A., Tafrov S.T., Heller R.C., Stebbins J., Pillus L., and Sternglanz R. 2000. The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *Proc. Natl. Acad. Sci.* **97:** 5807–5811.

Laroche T., Martin S.G., Gotta M., Gorham H.C., Pryde F.E., Louis E.J., and Gasser S.M. 1998. Mutation of yeast Ku genes disrupts the subnuclear organization of telomeres. *Curr. Biol.* **8:** 653–656.

Lau A., Blitzblau H., and Bell S.P. 2002. Cell-cycle control of the establishment of mating-type silencing in *S. cerevisiae*. *Genes Dev.* **16:** 2935–2945.

Le S., Davis C., Konopka J.B., and Sternglanz R. 1997. Two new S-phase-specific genes from *Saccharomyces cerevisiae*. *Yeast* **13:** 1029–1042.

Lebrun E., Revardel E., Boscheron C., Li R., Gilson E., and Fourel G. 2001. Protosilencers in *Saccharomyces cerevisiae* subtelomeric regions. *Genetics* **158:** 167–176.

Lemmers R.J.L., de Kievit P., van Geel M., van der Wielen M.J., Bakker E., Padberg G.W., Frants R.R., and van der Maarel S.M. 2001. Complete allele information in the diagnosis of facioscapulohumeral muscular dystrophy by triple DNA analysis. *Ann. Neurol.* **50:** 816–819.

Levis R., Hazelrigg T., and Rubin G.M. 1985. Effects of genomic position on the expression of transduced copies of the *white* gene of *Drosophila*. *Science* **229:** 558–561.

Levis R.W., Ganesan R., Houtchens K., Tolar L.A., and Sheen F.-M. 1993. Transposons in place of telomeric repeats at a *Drosophila* telomere. *Cell* **75:** 1083–1093.

Lew J.E., Enomoto S., and Berman J. 1998. Telomere length regulation and telomeric chromatin require the nonsense-mediated mRNA decay pathway. *Mol. Cell. Biol.* **18:** 6121–6130.

Li B., Oestreich S., and de Lange T. 2000. Identification of human Rap1: Implications for telomere evolution. *Cell* **101:** 471–483.

Li Y.C., Cheng T.H., and Gartenberg M.R. 2001. Establishment of transcriptional silencing in the absence of DNA replication. *Science* **291:** 650–653.

Lieb J.D., Liu X., Botstein D., and Brown P.O. 2001. Promoter-specific binding of Rap1 revealed by genome-wide maps of protein-DNA association. *Nat. Genet.* **28:** 327–334.

Lin S.J., Defossez P.A., and Guarente L. 2000. Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* **289:** 2126–2128.

Lin S.J., Kaeberlein M., Andalis A.A., Sturtz L.A., Defossez P.A., Culotta V.C., Fink G.R., and Guarente L. 2002. Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. *Nature* **418:** 344–348.

Lo W.S., Trievel R.C., Rojas J.R., Duggan L., Hsu J.Y., Allis C.D., Marmorstein R., and Berger S.L. 2000. Phosphorylation of serine 10 in histone H3 is functionally linked in vitro and in vivo to Gcn5-mediated acetylation at lysine 14. *Mol. Cell* **5:** 917–926.

Louis E.J. 1995. The chromosome ends of *Saccharomyces cerevisiae*. *Yeast* **11:** 1553–1574.

Luderus M.E., van Steensel B., Chong L., Sibon O.C., Cremers F.F., and de Lange T. 1996. Structure, subnuclear distribution, and nuclear matrix association of the mammalian telomeric complex. *J. Cell Biol.* **135:** 867–881.

Luo K., Vega-Palas M.A., and Grunstein M. 2002. Rap1-Sir4 binding independent of other Sir, yKu, or histone interactions initiates the assembly of telomeric heterochromatin in yeast. *Genes Dev.* **16:** 1528–1539.

Lustig A.J. and Petes T.D. 1986. Identification of yeast mutants with altered telomere structure. *Proc. Natl. Acad. Sci.* **83:** 1398–1402.

Lustig A.J., Kurtz S., and Shore D. 1990. Involvement of the silencer and UAS binding protein RAP1 in regulation of telomere length. *Science* **250:** 549–553.

Mages G.J., Feldmann H.M., and Winnacker E.L. 1996. Involvement of the *Saccharomyces cerevisiae* HDF1 gene in DNA double-strand break repair and recombination. *J. Biol. Chem.* **271:** 7910–7915.

Mann R.K. and Grunstein M. 1992. Histone H3 N-terminal mutations allow hyperactivation of the yeast GAL1 gene *in vivo*. *EMBO J.* **11:** 3297–3306.

Manolis K.G., Nimmo E.R., Hartsuiker E., Carr A.M., Jeggo P.A., and Allshire R.C. 2001. Novel functional requirements for non-homologous DNA end joining in *Schizosaccharomyces pombe*. *EMBO J.* **20:** 210–221.

Martin S.G., Laroche T., Suka N., Grunstein M., and Gasser S.M. 1999. Relocalization of telomeric Ku and SIR proteins in response to DNA strand breaks in yeast. *Cell* **97:** 621–633.

Mason J., Ransom J., and Konev A. 2004. A deficiency screen for dominant suppressors of telomeric silencing in *Drosophila*. *Genetics* **168:** 1353–1370.

Mason J.M., Konev A.Y., Golubovsky M.D., and Biessmann H. 2003. *Cis*- and *trans*-acting influences on telomeric position effect in *Drosophila melanogaster* detected with a subterminal transgene. *Genetics* **163:** 917–930.

Mason J.M., Haoudi A., Konev A.Y., Kurenova E., Walter M.F., and Biessmann H. 2000. Control of telomere elongation and telomeric silencing in *Drosophila melanogaster*. *Genetica* **109:** 61–70.

Mathog D., Hochstrasser M., Gruenbaum Y., Saumweber H., and Sedat J. 1984. Characteristic folding pattern of polytene chromosomes in *Drosophila* salivary gland nuclei. *Nature* **308:** 414–421.

Matzke M.A., Moscone E.A., Park Y.D., Papp I., Oberkofler H., Neuhuber F., and Matzke A.J. 1994. Inheritance and expression of a transgene insert in an aneuploid tobacco line. *Mol. Gen. Genet.* **245:** 471–485.

McCarroll R.M. and Fangman W.L. 1988. Time of replication of yeast centromeres and telomeres. *Cell* **54:** 505–513.

Mechler B.M., McGinnis W., and Gehring W.J. 1985. Molecular cloning of lethal(2)giant larvae, a recessive oncogene of *Drosophila melanogaster*. *EMBO J.* **4:** 1551–1557.

Mefford H.C. and Trask B.J. 2002. The complex structure and dynamic evolution of human subtelomeres. *Nat. Rev. Genet.* **3:** 91–102.

Megee P.C., Morgan B.A., Mittman B.A., and Smith M.M. 1990. Genetic analysis of histone H4: Essential role of lysines subject to reversible acetylation. *Science* **247:** 841–845.

Melnikova L., Biessmann H., and Georgiev P. 2005. The Ku protein complex is involved in length regulation of *Drosophila* telomeres. *Genetics* **170:** 221–235.

Meneghini M.D., Wu M., and Madhani H.D. 2003. Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. *Cell* **112:** 725–736.

Meneveri R., Agresti A., Marozzi A., Saccone S., Rocchi M., Archidiacono N., Corneo G., Della Valle G., and Ginelli E. 1993. Molecular organization and chromosomal location of human GC-rich heterochromatic blocks. *Gene* **123:** 227–234.

Michaelis C., Ciosk R., and Nasmyth K. 1997. Cohesins: Chromosomal proteins that prevent premature separation of sister chromatids. *Cell* **91:** 35–45.

Miller A.M. and Nasmyth K.A. 1984. Role of DNA replication in the repression of silent mating type loci in yeast. *Nature* **312:** 247–251.

Mishra K. and Shore D. 1999. Yeast Ku protein plays a direct role in telomeric silencing and counteracts inhibition by rif proteins. *Curr. Biol.* **9:** 1123–1126.

Moazed D. 2001. Enzymatic activities of Sir2 and chromatin silencing. *Curr. Opin. Cell Biol.* **13:** 232–238.

Monson E.K., de Bruin D., and Zakian V.A. 1997. The yeast Cacl protein is required for the stable inheritance of transcriptionally repressed chromatin at telomeres. *Proc. Natl. Acad. Sci.* **94:** 13081–13086.

Moretti P. and Shore D. 2001. Multiple interactions in Sir protein recruitment by Rap1p at silencers and telomeres in yeast. *Mol. Cell. Biol.* **21:** 8082–8094.

Moretti P., Freeman K., Coodly L., and Shore D. 1994. Evidence that a complex of SIR proteins interacts with the silencer and telomere-binding protein RAP1. *Genes Dev.* **8:** 2257–2269.

Morrow B.E., Johnson S.P., and Warner J.R. 1989. Proteins that bind to the yeast rDNA enhancer. *J. Biol. Chem.* **264:** 9061–9068.

Muñoz-Jordan J.L., Cross G.A., de Lange T., and Griffith J.D. 2001. t-loops at trypanosome telomeres. *EMBO J.* **20:** 579–588.

Murti K.G. and Prescott D.M. 1999. Telomeres of polytene chromosomes in a ciliated protozoan terminate in duplex DNA loops. *Proc. Natl. Acad. Sci.* **96:** 14436–14439.

Navarro M. and Gull K. 2001. A pol I transcriptional body associated with VSG mono-allelic expression in *Trypanosoma brucei*. *Nature* **414:** 759–763.

Ng H.H., Dole S., and Struhl K. 2003. The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. *J. Biol. Chem.* **278:** 33625–33628.

Ng H.H., Feng Q., Wang H., Erdjument-Bromage H., Tempst P., Zhang Y., and Struhl K. 2002. Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. *Genes Dev.* **16:** 1518–1527.

Nimmo E.R., Cranston G., and Allshire R.C. 1994. Telomere-associated chromosome breakage in fission yeast results in variegated expression of adjacent genes. *EMBO J.* **13:** 3801–3811.

Nimmo E.R., Pidoux A.L., Perry P.E., and Allshire R.C. 1998. Defective meiosis in telomere-silencing mutants of *Schizosaccharomyces pombe*. *Nature* **392:** 825–828.

Nislow C., Ray E., and Pillus L. 1997. *SET1*, a yeast member of the *trithorax* family, functions in transcriptional silencing and diverse cellular processes. *Mol. Biol. Cell* **8:** 2421–2436.

Ofir R., Wong A.C., McDermid H.E., Skorecki K.L., and Selig S. 1999. Position effect of human telomeric repeats on replication timing. *Proc. Natl. Acad. Sci.* **96:** 11434–11439.

Palladino F., Laroche T., Gilson E., Axelrod A., Pillus L., and Gasser S.M. 1993. SIR3 and SIR4 proteins are required for the positioning and integrity of yeast telomeres. *Cell* **75:** 543–555.

Pappas D.L., Jr., Frisch R., and Weinreich M. 2004. The NAD^+-dependent Sir2p histone deacetylase is a negative regulator of chromosomal DNA replication. *Genes Dev.* **18:** 769–781.

Pardue M.L. and DeBaryshe P.G. 1999. *Drosophila* telomeres: Two transposable elements with important roles in chromosomes. *Genetica* **107:** 189–196.

Park E.-C. and Szostak J.W. 1990. Point mutations in the yeast histone H4 gene prevent silencing of the silent mating type locus *HML*. *Mol. Cell. Biol.* **10:** 4932–4934.

Park M.J., Jang Y.K., Choi E.S., Kim H.S., and Park S.D. 2002. Fission yeast Rap1 homolog is a telomere-specific silencing factor and interacts with Taz1p. *Mol. Cell* **13:** 327–333.

Pasero P., Bensimon A., and Schwob E. 2002. Single-molecule analysis reveals clustering and epigenetic regulation of replication origins at the yeast rDNA locus. *Genes Dev.* **16:** 2479–2484.

Peinado H., Ballestar E., Esteller M., and Cano A. 2004. Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. *Mol. Cell. Biol.* **24:** 306–319.

Pemberton L.F. and Blobel G. 1997. Characterization of the Wtm proteins, a novel family of *Saccharomyces cerevisiae* transcriptional modulators with roles in meiotic regulation and silencing. *Mol. Cell. Biol.* **17:** 4830–4841.

Perrod S. and Gasser S.M. 2003. Long-range silencing and position effects at telomeres and centromeres: Parallels and differences. *Cell. Mol. Life Sci.* **60:** 2303–2318.

Perrod S., Cockell M.M., Laroche T., Renauld H., Ducrest A.L., Bonnard C., and Gasser S.M. 2001. A cytosolic NAD-dependent deacetylase, Hst2p, can modulate nucleolar and telomeric silencing in yeast. *EMBO J.* **20:** 197–209.

Pillus L. and Rine J. 1989. Epigenetic inheritance of transcriptional states in *S. cerevisiae*. *Cell* **59:** 637–647.

Porter S.E., Greenwell P.W., Ritchie K.B., and Petes T.D. 1996. The DNA-binding protein Hdf1p (a putative Ku homologue) is required for maintaining normal telomere length in *Saccharomyces cerevisiae*. *Nuc. Acids Res.* **24:** 582–585.

Poveda A., Pamblanco M., Tafrov S., Tordera V., Sternglanz R., and Sendra R. 2004. Hif1 is a component of yeast histone acetyltransferase B, a complex mainly localized in the nucleus. *J. Biol. Chem.* **279:** 16033–16043.

Pryde F.E. and Louis E.J. 1999. Limitations of silencing at native yeast telomeres. *EMBO J.* **18:** 2538–2550.

Raghuraman M.K., Winzeler E.A., Collingwood D., Hunt S., Wodicka L., Conway A., Lockhart D.J., Davis R.W., Brewer B.J., and Fangman W.L. 2001. Replication dynamics of the yeast genome. *Science* **294:** 115–121.

Ray A., Hector R.E., Roy N., Song J.H., Berkner K.L., and Runge K.W. 2003. Sir3p phosphorylation by the Slt2p pathway effects redistribution of silencing function and shortened lifespan. *Nat. Genet.* **33:** 522–526.

Reifsnyder C., Lowell J., Clarke A., and Pillus L. 1996. Yeast *SAS* silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. *Nat. Genet.* **14:** 42–49.

Renauld H., Aparicio O.M., Zierath P.D., Billington B.L., Chhablani S.K., and Gottschling D.E. 1993. Silent domains are assembled continuously from the telomere and are defined by promoter distance and strength, and by *SIR3* dosage. *Genes Dev.* **7:** 1133–1145.

Rice J.C. and Allis C.D. 2001. Histone methylation versus histone acetylation: New insights into epigenetic regulation. *Curr. Opin. Cell Biol.* **13:** 263–273.

Richards E.J. and Elgin S.C. 2002. Epigenetic codes for heterochromatin formation and silencing: Rounding up the usual suspects. *Cell* **108:** 489–500.

Riethman H., Ambrosini A., Castaneda C., Finklestein J., Hu X.L., Mudunuri U., Paul S., and Wei J. 2004. Mapping and initial analysis of human subtelomeric sequence assemblies. *Genome Res.* **14:** 18–28.

Robinett C.C., Straight A., Li G., Willhelm C., Sudlow G., Murray A., and Belmont A.S. 1996. In vivo localization of DNA sequences and visualization of large-scale chromatin organization using lac operator/repressor recognition. *J. Cell Biol.* **135:** 1685–1700.

Robyr D., Suka Y., Xenarios I., Kurdistani S.K., Wang A., Suka N., and Grunstein M. 2002. Microarray deacetylation maps determine genome-wide functions for yeast histone deacetylases. *Cell* **109:** 437–446.

Rogina B., Helfand S.L., and Frankel S. 2002. Longevity regulation by *Drosophila* Rpd3 deacetylase and caloric restriction. *Science* **298:** 1745.

Roy N. and Runge K.W. 1999. The ZDS1 and ZDS2 proteins require the Sir3p component of yeast silent chromatin to enhance the stability of short linear centromeric plasmids. *Chromosoma* **108:** 146–161.

———. 2000. Two paralogs involved in transcriptional silencing that antagonistically control yeast life span. *Curr. Biol.* **10:** 111–114.

Rundlett S.E., Carmen A.A., Kobayashi R., Bavykin S., Turner B.M., and Grunstein M. 1996. HDA1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription. *Proc. Natl. Acad. Sci.* **93:** 14503–14508.

Runge K.W. and Zakian V.A. 1996. *TEL2*, an essential gene required for telomere length regulation and telomere position effect in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **16:** 3094–3105.

Sandmeier J.J., Celic I., Boeke J.D., and Smith J.S. 2002. Telomeric and rDNA silencing in *Saccharomyces cerevisiae* are dependent on a nuclear NAD^+ salvage pathway. *Genetics* **160:** 877–889.

San-Segundo P.A. and Roeder G.S. 2000. Role for the silencing protein Dot1 in meiotic checkpoint control. *Mol. Biol. Cell* **11:** 3601–3615.

Schedl P. and Broach J.R. 2003. Making good neighbors: The right fence for the right job. *Nat. Struct. Biol.* **10:** 241–243.

Scherf A., Hernandez-Rivas R., Buffet P., Bottius E., Benatar C., Pouvelle B., Gysin J., and Lanzer M. 1998. Antigenic variation in malaria: *In situ* switching, relaxed and mutually exclusive transcription of *var* genes during intra-erythrocytic development in *Plasmodium falciparum*. *EMBO J.* **17:** 5418–5426.

Schubeler D., MacAlpine D.M., Scalzo D., Wirbelauer C., Kooperberg C., van Leeuwen F., Gottschling D.E., O′Neill L.P., Turner B.M., Delrow J., Bell S.P., and Groudine M. 2004. The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. *Genes Dev.* **18:** 1263–1271.

Shanower G.A., Muller M., Blanton J.L., Honti V., Gyurkovics H., and Schedl P. 2005. Characterization of the *grappa* gene, the *Drosophila* histone H3 lysine 79 methyltransferase. *Genetics* **169:** 173–184.

Shea C., Lee M.G., and Van der Ploeg L.H. 1987. VSG gene 118 is transcribed from a cotransposed pol I-like promoter. *Cell* **50:** 603–612.

Sinclair D.A., Mills K., and Guarente L. 1997. Accelerated aging and nucleolar fragmentation in yeast sgs1 mutants. *Science* **277:** 1313–1316.

Singer M.S., Kahana A., Wolf A.J., Meisinger L.L., Peterson S.E., Goggin C., Mahowald M., and Gottschling D.E. 1998. Identification of high-copy disruptors of telomeric silencing in *Saccharomyces cerevisiae*. *Genetics* **150:** 613–632.

Smith J.S. and Boeke J.D. 1997. An unusual form of transcriptional silencing in yeast ribosomal DNA. *Genes Dev.* **11:** 241–254.

Smith J.S., Brachmann C.B., Celic I., Kenna M.A., Muhammad S., Starai V.J., Avalos J.L., Escalante-Semerena J.C., Grubmeyer C., Wolberger C., and Boeke J.D. 2000. A phylogenetically conserved NAD^+-dependent protein deacetylase activity in the Sir2 protein family. *Proc. Natl. Acad. Sci.* **97:** 6658–6663.

Smolikov S., Mazor Y., and Krauskopf A. 2004. ELG1, a regulator of genome stability, has a role in telomere length regulation and in silencing. *Proc. Natl. Acad. Sci.* **101:** 1656–1661.

Snowden A.W., Gregory P.D., Case C.C., and Pabo C.O. 2002. Gene-specific targeting of H3K9 methylation is sufficient for initiating repression in vivo. *Curr. Biol.* **12:** 2159–2166.

Sprung C.N., Sabatier L., and Murnane J.P. 1996. Effect of telomere length on telomeric gene expression. *Nucleic Acids Res.* **24:** 4336–4340.

Stavenhagen J.B. and Zakian V.A. 1994. Internal tracts of telomeric DNA act as silencers in *Saccharomyces cerevisiae*. *Genes Dev.* **8:** 1411–1422.

Steinert S., Shay J.W., and Wright W.E. 2004. Modification of subtelomeric DNA. *Mol. Cell. Biol.* **24:** 4571–4580.

Stevenson J. and Gottschling D. 1999. Telomeric chromatin modulates replication timing near chromosome ends. *Genes Dev.* **15:** 146–151.

Stone E.M. and Pillus L. 1996. Activation of an MAP kinase cascade leads to Sir3p hyperphosphorylation and strengthens transcriptional silencing. *J. Cell Biol.* **135:** 571–583.

Strahl B.D. and Allis C.D. 2000. The language of covalent histone modifications. *Nature* **403:** 41–45.

Strahl-Bolsinger S., Hecht A., Luo K., and Grunstein M. 1997. SIR2 and SIR4 interactions differ in core and extended telomeric heterochromatin in yeast. *Genes Dev.* **11:** 83–93.

Strambio-de-Castillia C., Blobel G., and Rout M.P. 1999. Proteins connecting the nuclear pore complex with the nuclear interior. *J. Cell Biol.* **144:** 839–855.

Suka N., Luo K., and Grunstein M. 2002. Sir2p and Sas2p opposingly regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. *Nat. Genet.* **32:** 378–383.

Sun Z.W. and Hampsey M. 1999. A general requirement for the Sin3-Rpd3 histone deacetylase complex in regulating silencing in *Saccharomyces cerevisiae*. *Genetics* **152:** 921–932.

Suzuki Y. and Nishizaw M. 1994. The yeast GAL11 protein is involved in regulation of the structure and the position effect of telomeres. *Mol. Cell. Biol.* **14:** 3791–3799.

Taddei A. and Gasser S.M. 2004. Multiple pathways for telomere tethering: Functional implications of subnuclear position for heterochromatin formation. *Biochim. Biophys. Acta* **1677:** 120–128.

Tam R., Smith K.P., and Lawrence J.B. 2004. The 4q subtelomere harboring the FSHD locus is specifically anchored with peripheral heterochromatin unlike most human telomeres. *J. Cell. Biol.* **167:** 269–279.

Tan Q., Bellizzi D., Rose G., Garasto S., Franceschi C., Kruse T., Vaupel J.W., De Benedictis G., and Yashin A.I. 2002. The influences on human longevity by HUMTHO1.STR polymorphism (Tyrosine Hydroxylase gene). A relative risk approach. *Mech. Ageing Dev.* **123:** 1403–1410.

Tanny J.C., Dowd G.J., Huang J., Hilz H., and Moazed D. 1999. An enzymatic activity in the yeast Sir2 protein that is essential for gene silencing. *Cell* **99:** 735–745.

Teng S.-C., Chang J., McCowan B., and Zakian V.A. 2000. Telomerase-independent lengthening of yeast telomeres occurs by an abrupt Rad50p-dependent, Rif-inhibited recombinational process. *Mol. Cell.* **6:** 947–952.

Tham W.H. and Zakian V.A. 2002. Transcriptional silencing at *Saccharomyces* telomeres: Implications for other organisms. *Oncogene* **21:** 512–521.

Tham W.H., Wyithe J.S., Ferrigno P.K., Silver P.A., and Zakian V.A. 2001. Localization of yeast telomeres to the nuclear periphery is separable from transcriptional repression and telomere stability functions. *Mol. Cell* **8:** 189–199.

Thompson J.S., Ling X., and Grunstein M. 1994. Histone H3 amino terminus is required for telomeric and silent mating locus repression in yeast. *Nature* **369:** 245–247.

Thompson J.S., Snow M.L., Giles S., McPherson L.E., and Grunstein M. 2003. Identification of a functional domain within the essential core of histone H3 that is required for telomeric and HM silencing in *Saccharomyces cerevisiae*. *Genetics* **163:** 447–452.

Tissenbaum H.A. and Guarente L. 2001. Increased dosage of a *sir-2* gene extends lifespan in *Caenorhabditis elegans*. *Nature* **410:** 227–230.

———. 2002. Model organisms as a guide to mammalian aging. *Dev. Cell* **2:** 9–19.

Triolo T. and Sternglanz R. 1996. Role of interactions between the origin recognition complex and SIR1 in transcriptional silencing. *Nature* **381:** 251–253.

Tsukamoto Y., Kato J., and Ikeda H. 1997. Silencing factors participate in DNA repair and recombination in *Saccharomyces cerevisiae*. *Nature* **388:** 900–903.

van Deutekom J.C., Wijmenga C., van Tienhoven E.A., Gruter A.M., Hewitt J.E., Padberg G.W., van Ommen G.J., Hofker M.H., and Frants R.R. 1993. FSHD associated DNA rearrangements are due to deletions of integral copies of a 3.2 kb tandemly repeated unit. *Hum. Mol. Genet.* **2:** 2037–2042.

van Leeuwen F., Gafken P.R., and Gottschling D.E. 2002. Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell* **109:** 745–756.

Vega-Palas M.A., Martin-Figueroa E., and Florencio F.J. 2000. Telomeric silencing of a natural subtelomeric gene. *Mol. Gen. Genet.* **263:** 287–291.

Vega-Palas M.A., Venditti S., and Di Mauro E. 1997. Telomeric transcriptional silencing in a natural context. *Nat. Genet.* **15:** 232–233.

Vujcic M., Miller C.A., and Kowalski D. 1999. Activation of silent replication origins at autonomously replicating sequence elements near the HML locus in budding yeast. *Mol. Cell. Biol.* **19:** 6098–6109.

Wahlgren M., Fernandez V., Chen Q., Svard S., and Hagblom P. 1999. Waves of malarial *var*-iations. *Cell* **96:** 603–606.

Wallrath L.L. and Elgin S.C. 1995. Position effect variegation in *Drosophila* is associated with an altered chromatin structure. *Genes Dev.* **9:** 1263–1277.

Walmsley R.M., Chan C.S.M., Tye B.-K., and Petes T.D. 1984. Unusual DNA sequences associated with the ends of yeast chromosomes. *Nature* **310:** 157–160.

Walter M.F., Jang C., Kasravi B., Donath J., Mechler B.M., Mason J.M., and Biessmann H. 1995. DNA organization and polymorphism of a wild-type *Drosophila* telomere region. *Chromosoma* **104:** 229–241.

Wang H., Nicholson P.R., and Stillman D.J. 1990. Identification of a *Saccharomyces cerevisiae* DNA-binding protein involved in transcriptional regulation. *Mol. Cell. Biol.* **10:** 1743–1753.

Wellinger R.J., Wolf A.J., and Zakian V.A. 1993a. Origin activation and formation of single-strand $TG_{1\text{-}3}$ tails occur sequentially in late S phase on a yeast linear plasmid. *Mol. Cell. Biol.* **13:** 4057–4065.

———. 1993b. *Saccharomyces* telomeres acquire single-strand TG_{1-3} tails late in S phase. *Cell* **72:** 51–60.

Wood A., Krogan N.J., Dover J., Schneider J., Heidt J., Boateng M.A., Dean K., Golshani A., Zhang Y., Greenblatt J.F., Johnston M., and Shilatifard A. 2003. Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. *Mol. Cell* **11:** 267–274.

Wood J.G., Rogina B., Lavu S., Howitz K., Helfand S.L., Tatar M., and Sinclair D. 2004. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* **430:** 686–689.

Wotton D. and Shore D. 1997. A novel Rap1p-interacting factor, Rif2p, cooperates with Rif1p to regulate telomere length in *Saccharomyces cerevisiae*. *Genes Dev.* **11:** 748–760.

Wu J., Suka N., Carlson M., and Grunstein M. 2001. TUP1 utilizes histone H3/H2B-specific HDA1 deacetylase to repress gene activity in yeast. *Mol. Cell* **7:** 117–126.

Wyatt H.R., Liaw H., Green G.R., and Lustig A.J. 2003. Multiple roles for *Saccharomyces cerevisiae* histone H2A in telomere position effect, Spt phenotypes and double-strand-break repair. *Genetics* **164:** 47–64.

Wyrick J.J., Aparicio J.G., Chen T., Barnett J.D., Jennings E.G., Young R.A., Bell S.P., and Aparicio O.M. 2001. Genome-wide distribution of ORC and MCM proteins in *S. cerevisiae:* High-resolution mapping of replication origins. *Science* **294:** 2357–2360.

Wyrick J.J., Holstege F.C., Jennings E.G., Causton H.C., Shore D., Grunstein M., Lander E.S., and Young R.A. 1999. Chromosomal landscape of nucleosome-dependent gene expression and silencing in yeast. *Nature* **402:** 418–421.

Xu E.Y., Kim S., and Rivier D.H. 1999. SAS4 and SAS5 are locus-specific regulators of silencing in *Saccharomyces cerevisiae*. *Genetics* **153:** 25–33.

Zakian V.A. and Blanton H.M. 1988. Distribution of telomere-associated sequences on natural chromosomes of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **8:** 2257–2260.

Zhang H., Richardson D.O., Roberts D.N., Utley R., Erdjument-Bromage H., Tempst P., Cote J., and Cairns B.R. 2004. The Yaf9 component of the SWR1 and NuA4 complexes is required for proper gene expression, histone H4 acetylation, and Htz1 replacement near telomeres. *Mol. Cell. Biol.* **24:** 9424–9436.

Zhang Z., Shibahara K., and Stillman B. 2000. PCNA connects DNA replication to epigenetic inheritance in yeast. *Nature* **408:** 221–225.