

Chapter 2

Subnuclear Architecture of Telomeres and Subtelomeres in Yeast

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Abstract Subtelomeres, upstream telomeres, have a very dynamic spatial positioning along the cell cycle. During G1 phase of the mitotic cell growth, subtelomere localisation close to the nuclear periphery results from the so-called Rabl chromosome configuration found in budding yeasts. In this chromosome configuration, centromeres are found clustered at one pole of the cell and chromosome arms lag behind. Subtelomere anchoring to the nuclear envelope relies on partly redundant molecular pathways, involving nuclear envelope components and structural composition of chromosome ends themselves. Subtelomere positioning also depends on chromosome arm length. Characteristic yeast subtelomere clustering thus results from chromosome arm length and location of subtelomeres close to the nuclear edge. During cell cycle progression, subtelomeres dynamics varies and subtelomeres localize towards the nuclear interior. During meiosis, distinct subtelomere positioning result from different spatial regulations. Dynamic spatial positioning of subtelomeres emerges as an important feature for chromosome end regulation and function.

2.1 Introduction

The closed mitosis, found in many fungi and in the yeast *Saccharomyces cerevisiae*, implies some basic rules in chromosome end positioning. During closed mitosis, the nuclear envelope never disassembles and in *S. cerevisiae* the mitotic organizing centre, i.e. the spindle pole body (SPB), sits in the double membrane of the nuclear envelope all along the cell cycle. Rigid microtubules

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emanate from the SPB, and a single nuclear microtubule binds to each of the kinetochores of the 16 chromosomes of *S. cerevisiae*. All chromosomes are thus attached to the centrosome by their centromere—composed by one to three nucleosomes in budding yeast (Lawrimore et al. 2011)—implying a spatial arrangement named Rabl configuration, after Carl Rabl (1885). This arrangement, which results from the maintenance of chromosome orientation after telophase at the end of mitotic cell division, is in part due to rapid cell cycle division, chromosome attachment by their centromere to the SPB and telomeres anchoring at the nuclear envelope. A rather Rabl-like chromosome configuration was however initially depicted in yeast because centromere clustering is not only a consequence of the anaphase movement of centromeres and the telomere–centromere polarization is moderately relaxed (Jin et al. 2000).

Telomeres correspond to the most distal part of linear chromosomes; subtelomeres, as it is implicit by their denomination, encompass the region immediately upstream to telomeric extremities. The structural definition of subtelomeres, described in detail in Chap. 3, remains a challenge since no clear barrier exists to distinguish a subtelomere from a non-subtelomeric “central” domain. Yet a consensus in the field emerges which refers to subtelomeres as large chromosomal regions in which few non-essential genes, separated by long AT-rich intergenic regions are found. These genes in addition often belong to similar structural and functional gene families involved in adaptive processes. Evolutionary studies in most eukaryotic species demonstrate the large number of chromosomal rearrangements that happen in these domains and converge to the idea that subtelomeres may perform in a function of gene reservoir (next chapters). Understanding spatial positioning of these regions is therefore particularly relevant for its possible role in chromosome end regulation.

2.2 Yeast Chromosome Ends: A Structural Definition of Telomeres and Subtelomeres

2.2.1 Yeast Telomere Structure

An important structural distinction exists between telomeres and subtelomeres. Telomeres are repeated nucleoprotein TG-rich regions in which no genes are encoded. In particular, telomeres permit to distinguish a chromosome extremity from a double-strand break (DSB). The protective role of telomeres in chromosome degradation, fusion or recombination events has pushed important studies for telomere–protein identification. Various techniques of biochemical fractionation including development of proteomics of isolated chromatin fragments (PICH) help to delineate the particular composition of this unusual structural chromatin cap (Wright et al. 1992; Dejardin and Kingston 2009). Indeed, telomere chromatin is not predominantly formed by nucleosomes, but rather by non-histone

proteins including chromatin assembly factors, replication, repair and telomere components (Enomoto et al. 1997). In addition to ~300-bp double-stranded TG₁₋₃ repeats, yeast telomeric DNA shows a 12–14 bases 3' overhang of a G-rich strand, the G-tail. In vitro, G-tails can form G-quadruplexes that are four-stranded DNA structures between four-stacked Guanines connected through stable non Watson–Crick-based associations (Sundquist and Klug 1989). Because G-quadruplexes are stable, DNA helicases, like Sgs1 of the RecQ family or Pif1, are thought to resolve G quartets by trapping single-stranded G-tails (Paeschke et al. 2011; Huppert 2010). In vivo G-quadruplexes are potentially present at telomeres, but also elsewhere in the genome (Capra et al. 2010). They could play a positive role in telomere stability, but their role is yet incompletely solved and it remains to determine to what extent these structures are present at each telomere (Smith et al. 2011).

At each replication cycle, a specific addition of the 3'G-rich overhang is required to avoid telomere shortening that will inevitably lead to cell ageing and cell death. G-tail addition happens through a specialized telomerase complex, which in *S. cerevisiae* is constitutively expressed and composed by Est1, Est2—the reverse transcriptase catalytic subunit—and the integral RNA component *TLCl*. G-tail formation also involves Rap1, the essential repressor/activator protein 1, found both at ~5 % of polII-driven ribosomal promoters and at repeated double-stranded telomeric DNA, the heterotrimer CST (Cdc13/Stn1/Ten1) that binds the single-stranded G-rich overhang, the Ku heterodimer, the MRX complex (Mre11/Rad50/Xrs2) and many other proteins whose role is yet unclear (Lieb et al. 2001). All these components are critical for a positive or negative access to the telomerase. For instance, Rap1 establishes a negative feedback loop on telomere elongation, by recruiting two factors, Rif1 and Rif2 through its C-terminal domain (Levy and Blackburn 2004; Marcand et al. 1999). Increased binding of Rif1 and Rif2 inhibits Tel1 (ATM) binding at longer telomeres (Hirano et al. 2009). Since Tel1 is also required for telomerase recruitment, extension of long telomeres is less efficient (Goudsouzian et al. 2006). Notably, telomere repeats are not homogeneous among telomeres inside a single cell, and telomerase does not act on every telomere in each cell cycle (Teixeira et al. 2004). The number of repeats and therefore of Rap1 molecules bound to it (see below) might intervene to convert telomeres from closed non-extendible state to open-extendible configurations which in turn influence telomere function (McEachern and Blackburn 1995; Teixeira et al. 2004). For instance, 100–125 repeat length increases telomerase processivity; ~30 bp are not enough for Rap1 to inhibit non-homologous end joining (NHEJ) which ends in telomere fusion (Marcand et al. 1999). The dynamic interplay of Rap1 with each telomere repeat element together with the inherent dynamics of each telomere is likely to influence the regulation of telomere function and localization.

Interestingly, telomeres are transcribed into specific transcripts, referred to as telomeric repeat-containing RNA (TERRA). In metazoans where TERRA have been discovered, these molecules are heterogeneous in size, are exclusively nuclear and colocalize with telomeres (Azzalin et al. 2007; Schoeftner and Blasco

2008). In budding yeast, TERRA are also produced. These molecules, larger than the telomere repeats, contain subtelomeric-derived sequences and are degraded by the 5' to 3' RNA exonuclease Rat1. In *rat1-1* mutants, RNA accumulation is linked to telomere shortening leading to the interesting possibility that DNA/RNA or TERRA/TLC1 hybrids inhibit telomerase (Luke et al. 2008). Telomere length regulation occurs hence through multiple pathways.

2.2.2 Yeast Subtelomere Structure and the Sir Proteins

Subtelomeric sequences are also repeated in different complex forms. They contain X elements, subtelomeric repeats (STR) and long tandem Y' repeats which could origin from transposable elements (Fourel et al. 1999). As described in detail in Chap. 3, one to four copies of tandem Y' sequences flanked by TG1-3 repeats are present on two-thirds of yeast S288c subtelomeres. S288c is the first fully assembled and sequenced *S. cerevisiae* strain (Goffeau et al. 1996). These elements are however highly variable between strains and species (Liti et al. 2009). For instance, in W303 strain, a recent cross between S288c and other recent lineages, the exact number of Y' sequences remains partly unknown because of the difficulty to assemble these repeats (Liti et al. 2009). The inherent variability of subtelomeres and of Y' sequences, often used as a probe for cytological subtelomere position studies, hence predicts some changeability between the different *S. cerevisiae* backgrounds analysed.

Subtelomeres, contrary to telomeres, show a nucleosome composition. In addition to subtelomeric histones however, a number of additional chromatin modifiers, including the silencing insulator (Sir) proteins, are specifically enriched in there (Rusche et al. 2003). Sir proteins were initially discovered as responsible for silencing of *HML* and *HMR* mating-type cassettes each located at one subtelomere of chromosome 3 (Rine and Herskowitz 1987), but Sir-mediated silencing is also found at some other subtelomeres and at the rDNA (Pryde and Louis 1999; Smith et al. 1998). Sir proteins are recruited to silencer sequences at the mating-type loci, while at subtelomeres Sir recruitment occurs through Rap1 binding to double-stranded telomeric repeats and ORC and Abf1 binding to the core X element (Pryde and Louis 1999). Sir3 binds deacetylated amino-terminal residues of histones H3 and H4, and cooperative binding between histone deacetylase Sir2, Sir3 and Sir4 is thought to enable Sir spreading through silenced regions (Hecht et al. 1995). Interestingly, Sir3 overproduction can lead to subtelomeric clustering independently of Sir3 function in silencing, pointing to the architectural role of this protein (see below and Ruault et al. 2011). Note that Rap1, as well as yKu, is also found by chromatin immunoprecipitation experiments not only at double-stranded telomeric repeats, but also in several kb of subtelomeric sequences, suggesting that the terminal region of the chromosome may fold into internal subtelomeric sequences (de Bruin et al. 2000; Marvin et al. 2009a).

Only Sir2 is required for silencing of polII-driven genes inserted into the rDNA (Pryde and Louis 1999; Smith et al. 1998). At subtelomeres, all Sir proteins, except Sir1, are recruited to silence similar reporter insertions. These reporters

have allowed to distinguish silencing occurring at the so-called truncated ends, in which subtelomeric sequences are deleted and silencing at native ends, in which all subtelomeric sequences are maintained (Gottschling et al. 1990; Pryde and Louis 1999). In the first case, Sir spreading can encompass several kb, while Sir proteins occupancy at native ends is limited to regions of 1–2 kb peaking at X elements and avoiding Y' sequences (Pryde and Louis 1999). These last data are in agreement with recent kinetic localization mapping by deep sequencing of an overexpressed version of Sir3. A rapid binding covers ~2 kb (i.e. 6 to 10 nucleosomes) around silencers and a slower binding is constrained to subtelomeric PAU genes and highly transcribed euchromatic sites, suggesting that Sir spreading is rather spatially limited and Sir3 recruitment not restricted to subtelomeres (Lynch and Rusche 2009; Radman-Livaja et al. 2011). This experimental evidence is coherent with the extremely variable silencing detected among native ends (Pryde and Louis 1999). Again, it points to a role for Sir proteins distinct to silencing which could rather be architectural, for instance to avoid recombination with internal sequences (Marvin et al. 2009b; Pryde and Louis 1999).

2.2.3 *Sir Structural Properties: In Vitro Consequences*

The chromatin architectural Sir3 protein provides one of the key structural properties of the subtelomeric Sir complex. The structure of the amino-terminal bromo-associated homology (BAH) domain is known (Hou et al. 2006). Even more, the crystal structure of a BAH-mutated version is now solved at 3 Å resolution in complex with a nucleosome (Armache et al. 2011). Crystal shows that BAH significantly binds each of the four core histone proteins (Armache et al. 2011). Although the BAH domain itself has weak self-associating properties, extensive BAH binding to nucleosome might contribute to the compaction properties of the full-length Sir3 protein. Indeed, Sir3 alone can compact nucleosomal arrays in vitro and high levels of Sir3 proteins promote oligomeric structure formation between individual nucleosomal arrays and Sir3 oligomers (McBryant et al. 2008). Furthermore, in vitro reconstitution of the Sir complex shows that in these conditions, Sir2, Sir3 and Sir4 maintain a 1:1:1 stoichiometry (Martino et al. 2009). Sedimentation analyses of this reconstituted holocomplex with chromatin estimate that one SIR complex binds two nucleosomes, arguing for a binding between two neighbouring nucleosomes (Martino et al. 2009).

It is interesting to note that in vitro Rap1 binds telomeric repeats at a frequency of 1 per 18 bp, corresponding in theory to 14 to 20 Rap1-binding sites per 250–350-bp-long telomere (Gilson et al. 1993). However, due to the intrinsic heterogeneity of *S. cerevisiae* telomeres, the precise number of Rap1 molecules remains uncertain. It has been recently shown that in solution, Rap1 binds multiple sites as a monomer and can bind multiple sites with a repeat array without obvious cooperativity and regardless of binding site affinity (Williams et al. 2010). Interestingly, a correlation exists between the ability of telomeric repeats

to regulate telomere length and in vitro Rap1 affinity to its binding sites (Williams et al. 2010). Relationship between in vitro and in vivo experiments is lacking, but these data suggest that multiple binding sites for Rap1 do not by themselves promote formation of an energetically favourable complex (Williams et al. 2010).

2.2.4 Dynamic Assembly of Subtelomeric and Telomeric Proteins

In normal conditions, telomere length changes rapidly among cells and in a few generations, suggesting that assembly and disassembly of telomeric and subtelomeric proteins is a dynamic process all along the cell cycle and even during each phase of the cell cycle. For instance, only 2 to 3 telomeres are bound by telomerase per cell in S phase (Teixeira et al. 2004). A number of telomeric or subtelomeric proteins like Rap1, Sir3, Sir4, Rif1 and Rif2 change their occupancy over the cell cycle. Lower at the G2/M phase, occupancy increases at G1 or S phase (Laroche et al. 2000). The C-terminal Rap1 domain interacts with Rif1, Rif2 and Sir proteins (Wotton and Shore 1997). Rap1 multiple partners raise the possibility that these proteins compete for Rap1 binding either along the cell cycle or even between each subtelomere. Consistently these data point to the variability that exists not only in a cell population but also between different chromosome ends in a single cell, lighting the variable position of distinct chromosome ends. Yet, as it is now discussed, rules for subtelomere positioning in the nuclear space are shared by many of them and are probably driven by the intrinsic property of the chromatin fibre.

2.3 Subtelomeres: Intranuclear Positioning and Relative Interactions

2.3.1 How to Define Subtelomere Position in the Nuclear Space

Deciphering telomere and subtelomere positions relative to the nuclear space has been essentially tackled by examining dedicated subtelomeric and/or telomeric DNA sequences or proteins, in fixed or living cells. Relative subtelomeric positions have furthermore benefited from recent outstanding progresses performed to capture frequent interactions between DNA segments, namely the chromosome conformation capture 3C derivatives (Dekker et al. 2002). The technique exists in many variations but can easily be understood as a process based on the cross-linking of DNA followed by restriction enzyme cutting and intramolecular ligation with an adaptor that allows the purification of the circularized DNA. By sequencing the DNA circles, inter- and intrachromosomal interactions existing in the nuclei at the moment of fixation can thus be identified. As this method requires

a high number of cells, it is possible to determine the statistical significance of given interactions. These frequencies are currently being used to model chromosome configurations through polymer physics-based models (Kalhor et al. 2012; Lieberman-Aiden et al. 2009). Using Hi-C, one model for 3D organization of the budding yeast genome, in an asynchronous population has been proposed, validating previous knowledge about subtelomere behaviour (Duan et al. 2010; Zimmer and Raue 2011). A close Hi-C method has similarly been applied to the 3 chromosomes that fission yeast contains. Close spatial proximity between both chromosome ends of chromosomes 2 and 3 is uncovered, chromosome 1 being particular as both of its subtelomeres carry rDNA sequences (Tanizawa et al. 2010).

Yet, it is still difficult to correlate frequencies with real distances. These distances can be reached by single-cell observation of dedicated differently labelled loci. A popular system consists to insert a number of bacterial operator sequences, namely LacO or TetO sequences, respectively, 42 bp and 14 bp long, at chosen positions. Subsequent binding by the respective repressors LacI and TetR fused to different variants of the green fluorescent protein allows locus labelling and detection of a single spot with a good signal to noise ratio over the nucleoplasmic background (Robinet et al. 1996; Straight et al. 1997). Recently, a new generation of fluorescent repressor/repeats operators has been constructed allowing three-colour detection (Lassadi, I and Bystricky K, personal communication). Repressors bind their targets with a certain affinity; for instance, LacI binds its target with a $K_d \sim 10^{-11}$ mol/l and TetR with a $K_d \sim 5 \times 10^{-9}$ mol/l—for comparison, streptavidin binds biotin with a $K_d \sim 10^{-15}$ mol/l (Falcon and Matthews 1999). Therefore, it can be questioned whether this binding can affect global and/or local chromosome biology. Cell cycling remains unchanged in the presence of different repeat insertions bound or not bound by their respective repressors, arguing for unchanged chromosome replication and segregation (Belmont 2001). If the LacI hinge region is mutated, LacI-binding affinity decreases by 500-fold in vitro, i.e. the range of TetR constant affinity (Falcon and Matthews 1999). In vivo, this mutation relieves cooperative Sir4 recruitment observed at silencers when lacO arrays are inserted nearby (Dubarry et al. 2011). In the presence of silencers, it might therefore be prudent to verify expression behaviour of neighbouring genes after lacO repeats insertions. Yet, tetO and lacO insertions have been successfully used to localize many subtelomeres, recapitulating initial observations with subtelomeric probes on fixed cells (Gotta et al. 1996; Hediger et al. 2002b).

To localize subtelomeres towards the nuclear periphery in living cells, a functional GFP fusion of nuclear pore protein Nup49 is often chosen as a marker of the nuclear envelope. Most of the subsequent images captured are then based on 3D imaging, with a number of z stacks whose optimum is derived from the Nyquist criterion (which itself depends on the microscope used). After focal plane selection, close to the equatorial Z stack, spot 2D localization towards the nuclear envelope is defined. Gasser's lab has taken advantage of the focal plane to define three zones of similar surface and determine fluorescent spot distribution in either one of the three zones. By counting \sim hundred cells, the subtelomere is determined as being peripheral if preferentially found in the outmost zone, or randomly located

if equally distributed between the three zones (33.3 % of the cells in each zone (Hediger et al. 2002b)).

Recently, to overcome limitations due to 2D localization, it has been proposed to detect a second nuclear landmark, the nucleolus. In yeast, the nucleolus forms a stable crescent structure at one pole of the cell, opposite to the spindle pole body during interphase, allowing for segmentation and barycentre definition (Berger et al. 2008). This additional landmark allows defining an axis joining the two centres of mass of the nucleoplasm and the nucleolus (Berger et al. 2008). Orientated alignment of thousands of nuclei through this axis results in dimensional probability maps of a given chromosomal locus (Berger et al. 2008; Therizols et al. 2010). As discussed in the Chap. 3, the number of studies based on either methodology has localized subtelomeres inside the nucleoplasmic space.

2.3.2 Subtelomere Position in the Nuclear Space

Subtelomere positioning depends on various aspects, i.e. chromosome arm length, cell cycle stage and telomere replication state. Subtelomere positioning also varies between cell cycle stages during both vegetative and meiotic growth (see Sect. 2.2.4). As structural chromosome end variability anticipates, each chromosome end has a particular behaviour that can in addition depend on the strain background (Table 2.1).

Rap1 detection shows a limited number of spots at proximity of the nuclear envelope when observed in G1 and S phases (Gotta et al. 1996; Hiraga et al. 2008; Klein et al. 1992; Palladino et al. 1993; Schober et al. 2008), suggesting that if Rap1 signal reflects chromosome ends, they tend to cluster. After S phase, Rap1 signal is delocalized and subtelomeres randomly position in the nucleoplasm (Laroche et al. 2000). Individual labelling of subtelomeres also detects subtelomeres close to the nuclear envelope during almost the entire cell cycle, except during the G2/M phase (Bystricky et al. 2005; Hediger et al. 2002b, 2008; Schober et al. 2008; Therizols et al. 2010).

Moreover, two-dimensional probability maps of number of individually labelled subtelomeres show that they are non-randomly situated at the nuclear periphery depending on the size of their corresponding chromosome arm (Therizols et al. 2010). Interestingly, these probability maps based on the nucleolus as nuclear landmark are similar when the SPB is chosen as a reference (unpublished results). Subtelomeres on shorter chromosome arms are close to the SPB with a gradually increasing distance to the SPB corresponding to increasing chromosome arm size. Furthermore, the volume of the nucleolus influences subtelomere position. This region is largely avoided by all subtelomeres. Increase in chromosome arm length does not influence the exclusion of subtelomeres in the nucleolar area. Moreover, reducing the nucleolar mass by rapamycin extends the space subtelomere occupy at nuclear periphery, suggesting that the nucleolus represents a physical barrier (Therizols et al. 2010).

Table 2.1 Compilation of yeast subtelomeres positions studied in living cells. Subtelomeres are ranked by chromosome arm size

Chromosome arm	Length (kb) (S288c)	Strain	WT		Mutant		Method		References
			Asynchronous (%)	G1 S (%)	Asynchronous (%)	G1 S (%)	G2 (%)	G2 (%)	
9R	85	S288c	80						3D GM 12
8L	105	W303		50	Early S 60, late S 45				2D 1
			52			<i>mps3Δ75-150</i>	30		2D 1
			81			<i>ctf18</i>	50		2D 11
						<i>ctf8</i>	45		2D 11
						<i>dcc1</i>	52		2D 11
						<i>ku70</i>	45		2D 11
				50		<i>ku70</i>	33		2D 5
				61	58	<i>rtt109</i>		42 40	2D 10
				Early S 60, late S 35		<i>clb5/clb6</i>		Early S 60, late S 50	2D 4
3L	115	W303		43					
6R	122	W303		60	Early S 60, late S 35	35			2D 7
			45	50	49		<i>mps3Δ75-150</i>	25	2D 4
				55	65		<i>sir4</i>	50	2D 1
				58			<i>sir4</i>	57	2D 2
							<i>ku70</i>	30	2D 7
				50	58		<i>ku70</i>	45 60	2D 7
							<i>esc1</i>	40 42	2D 8
						<i>esc1/ku70</i>	37 38		2D 8

(continued)

Table 2.1 (continued)

Chromosome arm	Length (kb) (S288c)	Strain	WT		Mutant		Method		References
			Asynchronous (%)	G1 S (%)	Asynchronous (%)	G1 S (%)	G1 S (%)	G2 (%)	
				58	Early S 63, late S 48	<i>ku70</i>	30	Early S 40, late S 33	2D 9
						<i>mlp1/mlp2</i>	63	Early S 65, late S 52	2D 9
				65	65	<i>rtt109</i>	45	45	2D 10
			82			<i>ctf18</i>	55		2D 11
						<i>ctf8</i>	53		2D 11
						<i>dec1</i>	45		2D 11
		S288c	70			<i>ku70</i>	40		3D GM 12
6L	148			49		<i>str4</i>	25		2D 7
		S288c	80						2D 7
1L	150	S288c	70						3D GM 12
5L	150	S288c		60					3D GM 12
14R	157	S288c	70						2D 7
				71					3D GM 12
3R	200			51					2D 7
10R	310	S288c	70						2D 7
5R	430	S288c	80						3D GM 12
		W303		40					3D GM 12
		W303		35					2D 5
11L	440	S288c	65			<i>nup145</i>	55		2D 7
						<i>nup84</i>	50		2D 7
									3D 6
									3D 6

(continued)

Table 2.1 (continued)

Chromosome arm	Length (kb) (S288c)	Strain	WT		Mutant		Method		References			
			Asynchronous (%)	G1 S (%)	G2 (%)	Asynchronous (%)	G1 S (%)	G2 (%)				
7L 14L	492	W303	80	55 50 54 52 59 65	Early S 63, late S 53	<i>nup133</i>	45		3D	6		
						<i>nup120</i>	45		3D	6		
	628	W303				<i>ku70</i>	45		3D	6		
						<i>sir4</i>	40		3D	6		
						<i>ku70sir4</i>	45		3D	6		
						<i>ku70hup145</i>	40		3D	6		
						<i>esc1</i>	50		3D	6		
									3D GM	12		
									2D	5		
									2D	5		
									2D	7		
									2D	9		
	63						45	45	2D	1		
						<i>mps3Δ75–150</i>	30		2D	3		
						<i>mps3Δ75–150</i>		50		2D	3	
						<i>mps3Δ75–150/heh1</i>		45	43	2D	3	
						<i>esc1/heh1</i>			45	2D	3	
						<i>heh1</i>			58	60	2D	3
						<i>lrs4</i>	38	45	40	2D	3	
						<i>nur1</i>			60	2D	3	
						<i>ku80</i>			33	2D	3	
						<i>ku80/lrs4</i>			33	2D	3	
						<i>esc1/lrs4</i>			33	2D	3	
						<i>rif1</i>	70			2D	3	
						<i>lrs4/rif1</i>	50			2D	3	

(continued)

Table 2.1 (continued)

Chromosome arm	Length (kb) (S288c)	Strain	WT		Mutant			Method		References		
			Asynchronous (%)	G1 (%)	S (%)	G2 (%)	Asynchronous (%)	G1 (%)	S (%)		G2 (%)	
	84			60	60		45		40	52	2D	11
						<i>ctf18</i>					2D	11
						<i>ctf8</i>	53				2D	11
						<i>dcc1</i>	58				2D	11
						<i>ku70</i>	58				2D	11
				50	Early S 50, late S 40	35		50	Early S 70, late S 40	35	2D	4
						<i>clb5/clb6</i>		50	Early S 50, late S 50	35	2D	4
						<i>ku70</i>		70	Early S 70, late S 80	70	2D	4
				50	58			50	60		2D	8
						<i>esc1</i>					2D	8
						<i>ku70</i>		40	62		2D	8
						<i>ku70/esc1</i>		40	38		2D	8
				65	67	<i>sir4</i>		45	57		2D	10
						<i>ku70</i>		55	57		2D	10
						<i>ctf18</i>		42	53		2D	10
						<i>pgd1</i>		37	45		2D	10
						<i>rrm3</i>		45	37		2D	10
						<i>elp4</i>		48	63		2D	10
						<i>tel1</i>		45	73		2D	10
						<i>asf1</i>		68	43		2D	10

(continued)

(continued)

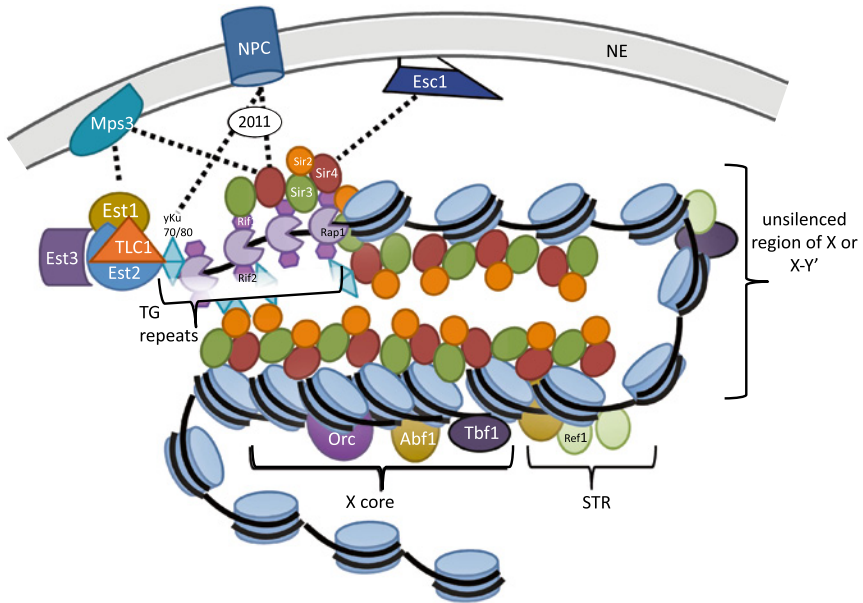


Fig. 2.1 Model for telomere and subtelomere nuclear architecture and links with molecular components of the nuclear periphery (*dashed lines*)

2.3.3 How Subtelomeres are Anchored at the Nuclear Periphery

A number of molecular pathways, partly redundant, have been discovered which are responsible for the transient anchoring of subtelomeres to the nuclear envelope (Fig. 2.1). They involve both nuclear envelope components and structural composition of chromosome ends.

Initially, two pathways, Sir4 and Ku dependent, were shown to mediate tethering of telomeres to the nuclear envelope (Hediger et al. 2002b; Laroche et al. 1998; Taddei et al. 2004; Tham et al. 2001; Therizols et al. 2006). These two telomeric proteins are evidently not integral membrane proteins, but they do transiently associate with nuclear envelope partners, favouring telomere positioning. Sir4 binds to Esc1, to Mps3, but also to Ku80 (Ribes-Zamora et al. 2007; Taddei et al. 2004). Esc1 (establishes silent chromatin) is a non-abundant acidic protein found in patches on the nuclear membrane distinct from nuclear pores and is thought to associate with the nuclear envelope through post-translational modification of its carboxy terminus (Andrulis et al. 2002; Lewis et al. 2007; Taddei et al. 2004). Esc1 deletion or yku70 mutant on their own has a modest effect on subtelomere 14L (L stands for left, R for right) tethering but when combined, they lead to its defective localization (Taddei et al. 2004). Mps3 is a Sad1/UNC-84 (SUN) domain integral membrane protein essential in SPB duplication. However, simultaneous deletion of *MPS3* and the gene encoding the nuclear pore protein Pom152 and/or Nup157

renders Mps3 redundant even in SPB duplication, implying that changes in the lipid environment of the nuclear membrane can alleviate the lack of the nuclear membrane protein Mps3 (Friederichs et al. 2011; Witkin et al. 2010). The deletion of the nucleoplasmic N-terminal domain of Mps3 allows disconnecting Mps3 from its anchoring function without disturbing its influence on the SPB (Bupp et al. 2007). Ku70 mediates telomeric peripheral anchoring (Laroche et al. 1998). A Ku70 interaction partner could be the nuclear pore complex (Galy et al. 2000; Feuerbach et al. 2002), in agreement with the observation when components of nuclear pore complex are mutated, the Nup84 complex or peripheral Mlp1/2 proteins, subtelomeres are displaced towards the nucleoplasm (Galy et al. 2000; Therizols et al. 2006). Yet the role of Mlp1/2 proteins in subtelomere tethering has been called into question (Hediger et al. 2002a). The fact that Mps3 was recently shown to bind not only to Sir4 but also to the yKu70/yKu80 heterodimer and Est1 (Bupp et al. 2007; Chan et al. 2011; Schober et al. 2009) and the fact that Sir4 was also shown to interact with to the cohibin complex (Lrs4/Csm1) through the integral proteins Heh1/Nur1 (Chan et al. 2011) indicate the intricate multiplicity and dependence of these different pathways. The observation that localization of different subtelomeres is differently compromised according to different mutated contexts additionally points to the variable dependency of each of the 32 chromosome ends towards the nuclear envelope, which is shown Table 2.1.

Chromosome end positioning is cell cycle regulated, mostly peripheral in G1/S phase, and it is displaced towards the nucleoplasm G2/M. In G1, Sir4 seems to be predominant in telomere anchoring in W303, but not in S288c (Table 2.1, Hediger et al. 2002b; Hiraga et al. 2006; Tham et al. 2001). In S phase, Mps3 seems to play a prominent role together with the telomerase and the Ku complex (Bupp et al. 2007; Schober et al. 2009). However, the lack of clear correlation between telomere length and localization behaviour and the fact that different cell cycle specificities are observed between *mre11* and *tell* mutants (see also Sect. 2.1.1), suggest that telomerase might have a role in telomere anchoring distinct to telomere length regulation (Hiraga et al. 2008). Yet, S-specific events are important for telomere localization. Telomere dislodgement is delayed when S phase is delayed, suggesting that anchoring is probably dependent on DNA replication. Once DNA is fully replicated, telomeres are released (Ebrahimi and Donaldson 2008; Ferreira et al. 2011). This is coherent with the finding that Ctf18-RFC, a subunit of the replication factor required for sister chromatid cohesion, is required for subtelomere positioning (Hiraga et al. 2006). Moreover, the number of proteins involved in chromatin structure, particularly during DNA replication, is shown to be important in telomere positioning (Hiraga et al. 2008). Asf1, the histone chaperone that stimulates acetylation of K56 of newly synthesized histone H3, is one of them (Hiraga et al. 2008).

Sumoylation might be critical in understanding how temporal regulation of these different pathways happens. For instance, Ku80 and Sir4 are found to be sumoylated, and their sumoylation occurs through the PIAS-like SUMO E3 ligase Siz2 (Ferreira et al. 2011). In *siz2Δ* mutants, telomeres are randomly positioned in both G1 and S phases. The *siz2Δ* mutant phenotype can be antagonized by deletion of the Pif1 helicase, probably through the increase in telomere-bound

telomerase. Since only telomeres that are elongated are detached from the nuclear periphery, sumoylation acts as a negative regulator of telomere length, probably through sumoylation of many telomere-bound proteins, as it was shown for Cdc13 (Ferreira et al. 2011; Hang et al. 2011). Interestingly, Cdc13 sumoylation is cell cycle regulated (Hang et al. 2011). On the other hand, the Slx5/Slx8 SUMO-dependent ubiquitin ligase is found at the nuclear pore complex in interaction with the Nup84 complex; the SUMO-protease Ulp1 is also associated with the NPC (Collins et al. 2007; Palancade et al. 2007). Modification of the sumoylated status of proteins that come in association with the NPC, including nuclear pore proteins themselves, might participate in the regulation of telomere positioning as it was proposed to regulate DNA break repair (Nagai et al. 2008; Therizols et al. 2006).

The strength of telomere anchoring to the nuclear periphery is also controlled by the structural composition of the subtelomeric region, i.e. Y' elements or the STR repeats. Different tethering capacities are indeed related to the presence or absence of Y' elements (Hediger et al. 2002b, 2006; Hediger and Gasser 2006; Tham et al. 2001). Upon STR repeat deletion, anchoring efficiency of weakly attached telomeres increases. This could be attributed to the absence of the STR-repeat-binding proteins Reb1 and Tbf1 (Hediger et al. 2002b; Lieb et al. 2001). However, the increased anchoring cannot be explained by removal of a Sir4 spreading barrier, as there is no link between the abundance of Sir proteins and anchoring efficiency (Lieb et al. 2001). It rather seems that Reb1 and Tbf1 are implicated in the conformation of telomeres that influences the silencing and anchoring strength to the nuclear periphery (see Sect. 2.1.2).

2.3.4 Subtelomere Position During Meiosis

In contrast to the mitotic cell division, in which chromosomes are separated after DNA replication, meiosis proceeds in two subsequent division processes, meiosis I and II. During the first meiotic cell division, DNA is replicated in the diploid cell and homologous chromosomes undergo recombination, resulting in two genetically unique diploid cells. In meiosis II, the two daughter cells undergo a second division to produce four haploid cells, spores in yeast.

These unique cell cycle events, especially during meiosis I, also require distinctive telomere and subtelomere behaviour. At the beginning of meiotic prophase I, between the leptotene and zygotene transition, telomeres concentrate in a cluster at the centromeres, forming the so-called meiotic “bouquet” (Trelles-Sticken et al. 1999, 2005). The structure is transient but seems to be important for the subsequent recombination, as mutants that are defective for bouquet formation also show malfunctioning or altered steps in crossing over (Chua and Roeder 1997; Kosaka et al. 2008; Wanat et al. 2008). At early zygotene stage, the bouquet formation dissolves through rapid movements of dispersed telomeres (Conrad et al. 2008; Koszul et al. 2008).

The rapid telomere movement along the nuclear envelope in budding yeast meiosis is mediated through a complex consisting of Mps3, Ndj1 and Csm4.

In meiosis, Mps3 is connected to both the actin cytoskeleton by Csm4, a protein that sits in the outer nuclear membrane and to telomeres through Ndj1, a telomeric meiotic protein (Conrad et al. 1997, 2008). In support to an actin skeleton-led telomere movement, actin cable extension occurs at a speed similar to chromosome motion (ca. 0.3 $\mu\text{m/s}$; Yang and Pon 2002). Furthermore, actin cytoskeleton and Csm4 mutants do not impede telomere attachment to the nuclear periphery but rather impair directed rapid telomere movements (Conrad et al. 1997, 2008). Although Mps3 also connects telomeres to the nuclear membrane in interphase (see above), directed chromosome movements mediated by Mps3 have yet only been observed during meiotic prophase. The role of such a chromosome movement is still unclear, but it could help chromosome pairing resolution of meiotic crossovers (Conrad et al. 2008; Koszul et al. 2008; Sonntag Brown et al. 2011).

2.3.5 Subtelomeric Dynamics and Associations

Contrary to the rapid movements observed for telomeres during meiosis, telomeric movements observed during the G1 and S phases of the mitotic cycle are much slower. Moreover, the dynamic behaviour observed for subtelomere 6R during G1 is more constrained than centromere proximal autonomously replicating origin (ARS) during the same cell cycle phase, with a mean speed of 98 nm/s vs. 118 nm/s for the ARS (Heun et al. 2001). Each studied telomere had a distinct mobility, possibly depending on its chromosome arm size and compaction (Bystricky et al. 2005). Subtelomere loci generally occupy a confinement radius of 0.2–0.4 μm in the about 2- μm -diameter yeast nucleus, due to their attachment to the nuclear periphery (Bressan et al. 2004; Bystricky et al. 2005; Heun et al. 2001; Rosa et al. 2006). For comparison, a 16 kb artificially generated chromatin ring moves within a confinement radius of more than 0.8 μm (Gartenberg et al. 2004). Accordingly, the subtelomere confinement radius increases to 0.5 μm in *sir4* mutants and to more than 0.6 μm in *yKu70* deficient cells (Bystricky et al. 2005; Hediger et al. 2002b). Note that movement measurements by locus detection methods are restricted to G1 phase of the cell cycle, as the replication of DNA coincides with the presence of two fluorescent spots that can hardly be distinguished from one another.

Subtelomeres move with a speed of 0.1–0.5 $\mu\text{m/s}$ meaning that regions preferably occupied by a particular chromosome end are largely overlapping (Heun et al. 2001; Marshall et al. 1997; Schober et al. 2008). Given that subtelomeres of different chromosome arm lengths show similar probability maps of their localization, it is not surprising to find that subtelomeres on similar chromosome arm sizes interact more frequently, although very transiently (Therizols et al. 2010). Hence, different subtelomere interactions detected in living cells as foci are direct consequences of preferred subtelomere localizations (Schober et al. 2008; Therizols et al. 2010; Zimmer and Fabre 2011).

Subtelomeres rarely exceed a distance of 0.3 μm from the nuclear envelope, in an oscillating movement suggesting reversible interactions with nuclear envelope components (Bystricky et al. 2005; Hediger et al. 2002b; Heun et al. 2001; Therizols et al. 2010). Large movements of over 0.5 μm in a 10.5s interval take place only once every 10 min in G1 phase for chromosome ends, in contrast to other chromosomal loci that come to about 10 large movements in the same time frame (Heun et al. 2001). These large telomere movements in G1 phase point towards a certain directionality, although nature or function of the force that could be responsible for them are missing. A higher temporal resolution is expected to give more information on the nature of these movements (Hajjoul et al. 2009). Yet, abolition of all movements by ATP depletion shows that chromosome movements are not only randomly caused by diffusion (Heun et al. 2001).

2.4 Conclusions on Functional Implications of Chromosome End Nuclear Architecture from the Repair Point of View

Double-strand break (DSB) repair efficiency in haploid yeast depends on the chromosomal region affected (Ricchetti et al. 2003). The closer the regions are to the chromosomal ends, the higher the repair efficiency rate. This can be explained by the fact that in yeast haploids G1 cells, central chromosome regions are repaired by NHEJ, while chromosomal ends can also be repaired by other mechanisms like telomere addition and break-induced replication (BIR) (Malkova et al. 1996; Sandell and Zakian 1993). Moreover, subtelomeres position close to the nuclear periphery is important, since mutations that release nuclear envelope tethering of subtelomere 11L, decrease repair efficiency of DSBs induced in there (Therizols et al. 2006). Persistent DNA DSBs are also shown to interact with Cdc13 and the telomerase component Est2. These proteins anchor the slowly repaired or unreparable DSBs to Mps3 at the nuclear periphery (Oza et al. 2009; Schober et al. 2009). The nuclear periphery thus appears to have evolved as a compartment that helps uptake of these specific DNA DSBs.

Following S phase, DNA is replicated, and therefore, two identical homologues exist for each chromosome allowing DNA repair by homologous recombination. After S phase, telomeres are no longer anchored to the nuclear periphery. May be a consequence of nuclear envelope positioning failure in S phase, a hyper-recombination among telomeres has been observed in mutants for the telomere length maintenance protein Tel1 (Schober et al. 2009). One reason why telomeres are no longer anchored at the nuclear envelope could be that more efficient repair mechanisms make telomere anchoring difficult.

Mechanisms for insulating chromosome ends could have been evolved in order to distinguish chromosomal ends from DSBs. For instance, in budding yeast, different pathways to prevent chromosome fusion inhibit NHEJ. They involve at least Rap1 and Rif2 (Marcand et al. 2008). Sir4 also inhibits NHEJ through an

unknown mechanism. Coexistence of several pathways is understandable to ensure that chromosome ends will never fuse, unless they are too short (Pardo and Marcand 2005). In agreement with such a negative role in telomere fusion, is the Ku70-mediated fold-back structure, depicted between telomeres and subtelomeres (Marvin et al. 2009b). Accordingly, telomeres are 1,000-fold less effective in DNA damage response activation than DSBs (Lydall 2009). These mechanisms might have evolved by taking advantage of the proteins enriched in there, as it is the case for Rap1 and Sir4. Therefore, nuclear architecture of telomeres appears to be a major pathway to ensure genome stability, by avoiding chromosome end fusion.

On the other hand, subtelomere positioning in a restricted domain at the nuclear periphery also ensures that breaks happening in there can efficiently be repaired with other neighbouring subtelomeres. This could explain why, from an evolutionary point of view, subtelomeres could behave as gene reservoirs. Their improved genome flexibility could also explain why linear chromosomes and therefore telomeres and subtelomeres might have been evolved. In fact, cells seem to be very adaptable with respect to chromosomal structure; telomeres do not seem to be neither specific to eukaryotes as there are examples of bacteria with linear chromosomes nor required to eukaryotes since yeast *S. pombe pot1* mutants survive with fused telomeres forming circular chromosomes (Baumann and Cech 2001; Jain et al. 2010).

Nuclear architecture of yeast telomeres and subtelomeres in a Rab1-like chromosome organization is a characteristic often associated with fast-dividing cells. Many yeast mutants show that telomere attachment to the nuclear envelope is not required for cell survival. It rather seems that the attachment helps to untangle and sort chromosomes for different chromosome interactions and therefore faster cell division, thus out competing less organized chromosome conformations.

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