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REVIEW

Yeast Telosome Complex: Components and Their Functions

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Abstract—Telomeres are multi-component DNA–protein complexes protecting linear chromosome termini against degradation and fusion. Telomeres and their components are involved in many essential processes: control of cell division number, regulation of transcription, reparation. They are necessary for correct chromosome segregation during cell division. Telomere protein content and DNA length are under control of different factors, such as components of replication and reparation machinery and cell division cycle kinases. Here we discuss the participation of components of yeast telomere (telosome complex) in different cellular processes, telomeric DNA length regulation pathways, and some issues of telosome evolution.

Key words: telomere, telomerase, telosome complex, evolution, Rap1p, hRap1, KU, SIR, RIF, Cdc13p, Est1p, Tbf1p, TRF

Telomeres are DNA–protein complexes located at the ends of the linear eukaryotic chromosomes. They are involved in various essential cellular processes. Telomeres protect DNA termini against degradation and fusion. They are required for completeness of telomeric DNA replication. Telomeres are also involved in the control of cell division number (Hayflick's limit) and cell aging and in formation of nuclear architectonics and regulation of transcription of adjacent genes (TPE, telomere positioning effect or silencing) [1, 2].

Each replication of linear chromosomes is accompanied by shortening of their ends, representing telomeric DNA; this is compensated by addition of G-rich telomerase strand (Fig. 1) and synthesis of the complimentary strand catalyzed by DNA-polymerase α [3]. Budding yeast *Saccharomyces cerevisiae* telomeric DNA is characterized by irregular primary structure (Fig. 1a); this is explained by the abortive mode of telomerase functioning, i.e., complex dissociation before copying the template stretch of telomerase RNA [4].

Mutations in genes encoding telomere-binding proteins influence the length of telomeric DNA and its stability; they also change telomere localization, etc. However, such effects also appear in response to mutations in genes encoding protein regulators that do not represent components of the telosome complex [5]. This class of telomere-binding proteins includes proteins that bind to telomere via contacts with DNA (Cdc13p, Rap1p, yKu70, yKu80, Tel2p, Est1p, Tbf1p) or due to protein–protein interactions (Rif1p, Rif2p, Sir2p, Sir3p, Sir4p, Stn1p, Ten1p) (Fig. 1b, table). *In vitro* telomere-binding ability is usually evaluated by binding to telomeric DNA or interaction with some known telomere-binding protein; this is detected by methods of co-immune precipitation (CoIP) and/or by a bi-hybrid system. The most reliable tests include demonstration of co-localization of a protein under study and DNA *in vivo* by immunofluorescence microscopy, specific co-immune precipitation of telomeric DNA with this protein, and also its interaction with telomeric DNA in a mono-hybrid system *in vivo* [6].

Telomere structure varies depending on cell cycle [7], cell age, and external effects [8]. The telomere dynamics has two aspects: change in length of telomeric DNA and its localization and change in telomere protein composition. The latter plays the major role in the dynamics of the telomere complex because telomere-binding proteins regulate processing of telomeric DNA. The proteins trigger signaling cascades of the DNA repair system, formation of telomeric loops, and regulation of gene transcription in subtelomeric loci; they also control involvement of telomeric DNA in recombination processes, etc. The length of telomeres functions as a biological "clock", counting number of cell divisions [9], and influences gene transcription in the subtelomeric region [10].

According to number of structural elements recognized in telomeric DNA, all telomere-binding proteins can be subdivided into four classes: proteins interacting with subtelomeric region of DNA, proteins interacting

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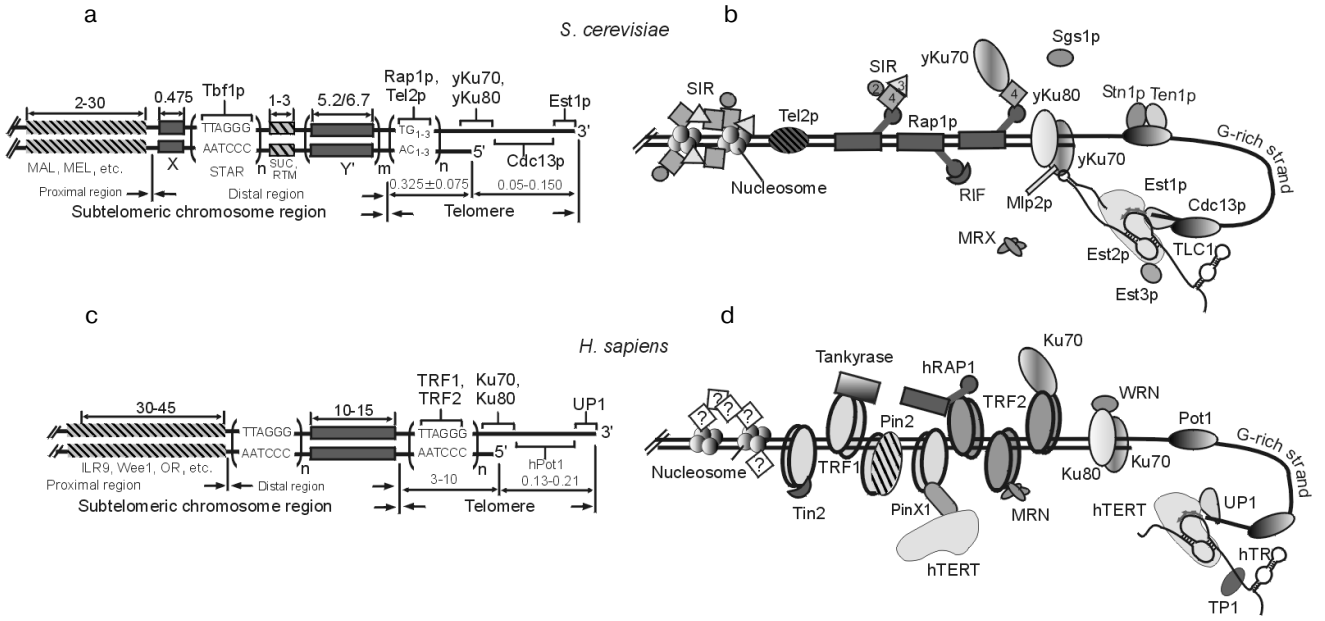

Fig. 1. a) *S. cerevisiae* telomeric DNA is given as a double strand site with primary structure $[TG_{1\text{-}3}/AC_{1\text{-}3}]_n$ overhang of G-rich single strand 3′-end. The distal (external) site of subtelomeric region contains X and Y′ elements; their combination may vary from chromosome to chromosome. The proximal (internal) site of the subtelomeric region contains carbohydrate metabolism genes (SUC, MAL, MEL, etc.), and also $[TTAGGG]_n$ repeats similar to mammalian telomeric DNA sequence [118] (m = 0, ..., 4; n has arbitrary value). b) Model of yeast telomere telosome structure at the moment of 3′-end G-rich chain elongation by telomerase (the same state is marked in Fig. 5 by a hexagonal asterisk). Yeast factors Rap1p, Tel2p, Rif1,2p, SIR are bound to double strand telomeric DNA. Factors Cdc13p, Stn1p, Ten1p, and also telomerase complex [33], which consist of Est2p, TLC1, Est1p, and Est3p, are associated with single strand telomere. Heterodimer yKU70/80 interacting with Sir4p is bound to the border between single and double strand telomeric DNA. KU complex also interacts with telomerase RNA TLC1. c) Distal part of subtelomeric region of a chromosome contains specific nucleotide sequence (of 10-15 kb), separated from the proximal part by $[TTAGGG]_n$ repeats. The proximal part of subtelomeric region of human (as well as yeast) chromosome contains various genes and pseudogenes (ILR9, Wee, OR, etc.) [119, 133]. d) In the case of human telosome complex TRF1, TRF2 [120], Pin2 [121], Tin2 [122], hRap1, MRN complex (Mre11–Rad50–Nbs1), PinX1, and tankirase [123] are associated with double strand site. Factors bound to telomere single strand site include Pot1, UP1 and also telomerase complex [124] containing hTERT, hTR, TP1 [125]. KU complex interacting with WRN factor is located at the border of single and double strand telomeric DNA. The subtelomeric site of a human chromosome is characterized by the existence of telomere positioning effect; however, factors responsible for its maintenance have not been found [131]. For homologs of human MRN complex and WRN factor in yeast cells the interaction with telosome complex components has not been demonstrated, and so they are shown separately. Numbers show length of DNA (in kb).

with double strand site of telomere, proteins interacting with border of double and single stranded sites, proteins interacting with single stranded site including 3′-end of G-rich strand of telomeric DNA (Fig. 1a). Each group of these proteins has structural characteristic features and plays a specific role in various cellular mechanisms and functioning of the telosome complex (table).

SITE OF DOUBLE STRANDED TELOMERIC DNA

Rap1p (Repressor-Activator Protein 1) is the main protein interacting with the double stranded site of telomeric DNA [11, 12] (Fig. 1b). Recognition sites for this protein are distributed over the whole genome, but specific binding of Rap1p occurs mainly at the sites located: 1) on telomeres; 2) at promoter regions of certain genes; 3) in HMR/L locus controlling expression of sex pheromones α and *a* [13]. Homologs of Rap1p found in human cells and also in *Schizosaccharomyces pombe* cannot bind to DNA and they are localized within telomeric DNA region due to protein–protein interactions, with TRF2 [14] (Fig. 1d) and Taz1 [15] factors, respectively (Fig. 6b).

Regulation of transcription is one of the major functions of Rap1p (Fig. 2) [16]. It activates transcription of genes sensitive to growth conditions. For example, Rap1p activates transcription of genes encoding ribosomal proteins and enzymes of glucose metabolism via interaction with regulator proteins Gcr1p and Gcr2p [17] (Fig. 2a). In the case of some genes encoding enzymes involved in amino acid and purine biosynthesis Rap1p also provides binding of direct transcription activator, Gcn4p, due to formation of a non-nucleosome structure in the promot-

Functions of telomere-binding proteins of *S. cerevisiae* and protein-orthologs and analogs from other organisms (H, *Homo sapiens*; M, *Mus musculus*; Sp, *Schizosaccharomyces pombe*; CE, *Caenorhabditis elegans*; ON, *Oxytricha nova*)

Telomeric DNA site	*S. cerevisiae* protein	DNA binding	Protein–protein interactions	Functions	Functional analog in cells of other organisms	Homolog/ ortholog in cells of other organisms
1	2	3	4	5	6	7
Double strand site	Rap1p [11]	+	Rif1p, Rif2p, Sir3p, Sir4p	telomere loop formation	p53 [129], TRF2 (h)	hRap1, spRap1 [15]
				activation of telomere elongation *de novo*	—	
				telomerase inhibition (RIF)	hRap1 [14]	
				repression of transcription (SIR)	—	
				activation of transcription (Gcr1p, Gcr2p, Gcn4p)	—	
	Rif1p [32]	—	Rap1p, Rif2p	telomerase inhibition	Tin2 [122]	spRif1 [15]
				TPE attenuation	—	
				inhibition of recombination	TRF1, TRF2 (h) [120]	
				formation of telomere cap	TRF1, TRF2 (h)	
	Rif2p [62]	—	Rap1p, Rif1p	see functions of Rif1p	see Rif1p analogs	—
	Sir3p [53]	—	Sir4p, Sir3, Rap1p, Zds1p [49]	repression of transcription	—	
				initiation of heterochromatin formation	—	—
	Sir4p [53]	—	Rap1p, Sir3p, Sir4p, Sif2p [48], Sir2p	repression of transcription	—	—
				initiation of heterochromatin formation	—	
	Sir2p [45]	—	Sir4p, Sir2p	NAD-dependent histone deacetylase	mSir2α [130], hSIRT1 [104]	mSir2α, hSIRT1
				regulation of number of cell divisions (ERC)	—	
				regulation of mitosis (Cdc14) and meiosis (Pch2p)	—	

Table (Contd.)

1	2	3	4	5	6	7
	Tel2p [40]	+		activation of telomeric DNA elongation (Tel1p)	Clk-2 (ce) [114]	Clk-2 (ce)
Border between single and double strand sites	yKu70 (Hdf1p) [64]	+	yKu80, Sir4p, Mlp2p [81]	involvement in NHEJ process	Ku70 (m, h) [64]	Ku70 (m, h)
				telomerase binding (TLC1)	—	
				regulation of telomeric DNA degradation/synthesis cycle	—	
				TPE regulation (Sir4p)	—	
				formation of telomere cap	—	
	yKu80 [64] (Hdf2p)	+	yKu70	see functions of yKu70	Ku80 (m, h) [64]	Ku80 (m, h)
Single strand site and 3′-end of G-rich chain	Cdc13 [84] (Est4p)	+	Stn1p, Cdc17p, Est1p, Ten1p	formation of telomere cap	Pot1 (sp, h) [115, 44], OnTBP	Pot1 (sp, h), αOnTBP
				regulation of telomeric DNA degradation/synthesis cycle	—	
				binding of DNA polymerase α	—	
				telomerase binding	UP1 (m, h)	
	Ten1p [86]	—	Cdc13p, Stn1p	telomerase inhibition	OnTBP [113]	—
				formation of telomere cap	Pot1 (sp, h), OnTBP	
	Stn1p [85]	—	Cdc13p, Ten1p	see functions of Ten1p	see Ten1p analogs	—
	Est1p [99]	+	Cdc13p	structural component of telomerase	—	—
				binding of telomerase catalytic component	UP1 (m, h) [116]	
Subtelomeric region	Tbf1p [29]	+	—	limitation of heterochromatin extension (STAR component)	—	—

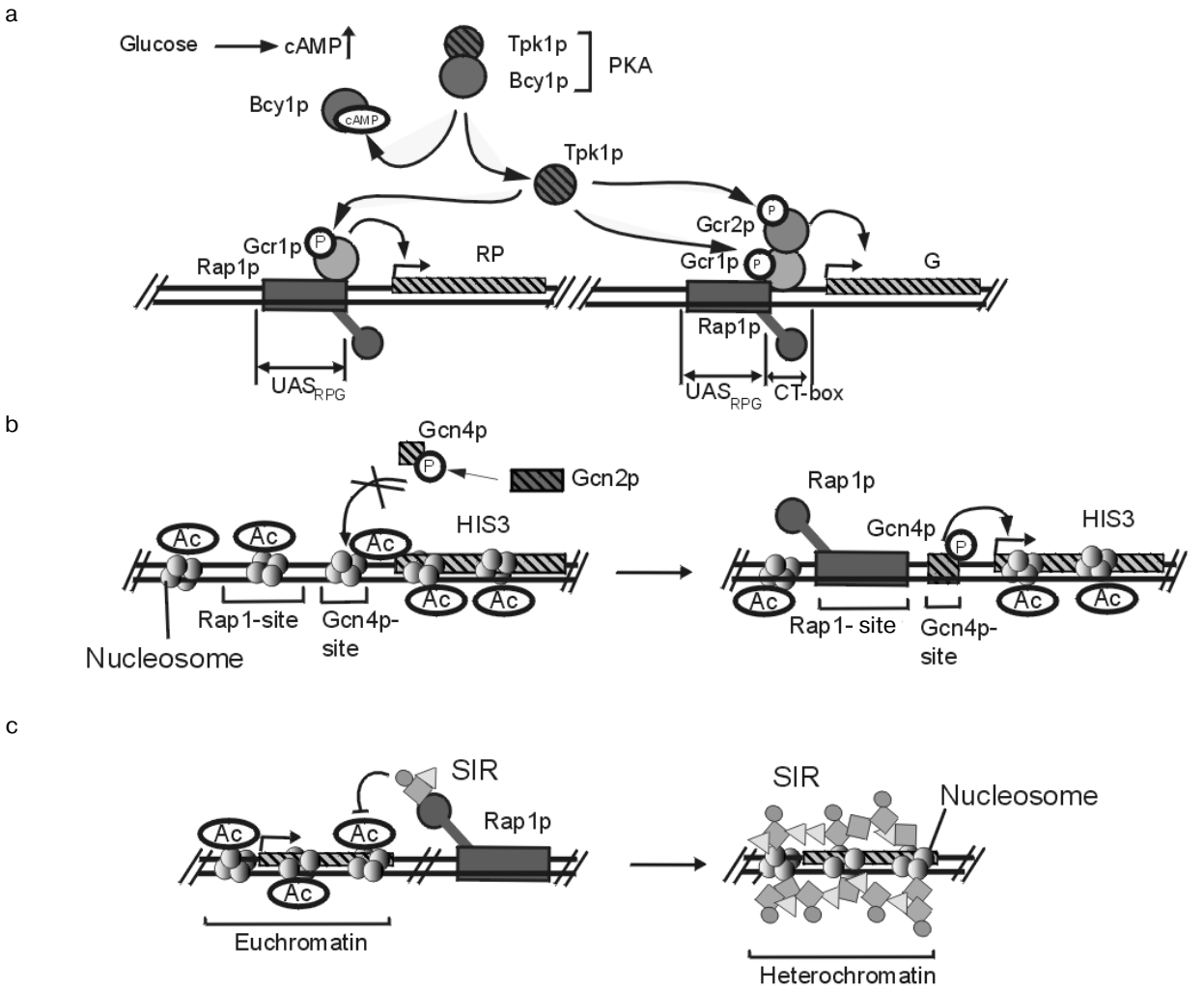

Fig. 2. Involvement of Rap1p protein in regulation of transcription. a) Rap1p binds to the regulatory site UAS_{RPG} and activates transcription of genes encoding most ribosomal proteins (RP) and proteins involved in glucose (G) metabolism (PGK1, DDC1, ADH, etc.). In the first case, Rap1p-dependent activation of transcription involves Gcr1p; activation of transcription of carbohydrate metabolism genes requires Gcr1p and Gcr2p proteins and Gcr1p specifically binds of a CT-box of DNA. b) Forming nucleosome-free sites of DNA (opened nucleosome structure), Rap1p promotes binding of transcription activators in the case of genes encoding proteins involved in amino acid biosynthesis (e.g., Gcn4p is the activator of the HIS3 gene). Thus, Rap1p is involved in activation of transcription of genes, which are expressed depending on conditions of cell growth. Increase in extracellular glucose level causes increase in intracellular cAMP concentration in the cell and activation of protein kinase A (PKA). cAMP binding causes impairment of inhibitory subunit Bcy1 with catalytic TPK subunit, and this results in PKA activation. c) Rap1p promotes repression of transcription of genes located in the subtelomeric region and also around internal silencers due to change of chromatin structure induced by SIR-complex interacting with Rap1p.

er region [18] (Fig. 2b). Activation of Gcr1p and Gcr2p, and also Gcn4p requires their phosphorylation catalyzed by cAMP-dependent protein kinase A (PKA) [19] and Gcn2p kinase [20], respectively.

If a gene is localized in the subtelomeric region or it is controlled, internal silencers Rap1p acts as repressor of its transcription (Fig. 2c). Pheromone α (Yα) and *a* (Y*a*) encoding genes located in the "silenced" site of HM locus [21] (Fig. 3a) may serve as an example of Rap1p repressor function. This protein also represses transcription of its own gene *RAP1*, required for regulation of this protein in the cell [22]. Rap1p realizes its repressor effect via facilitation of binding of silencing SIR (Silence Information Regulator) factors to promoter region of the regulatory gene [23]. In this case, involvement of Rap1p in regulation of transcription stems from the other important function of this protein, maintenance of specific localization of heterochromatin (Fig. 3).

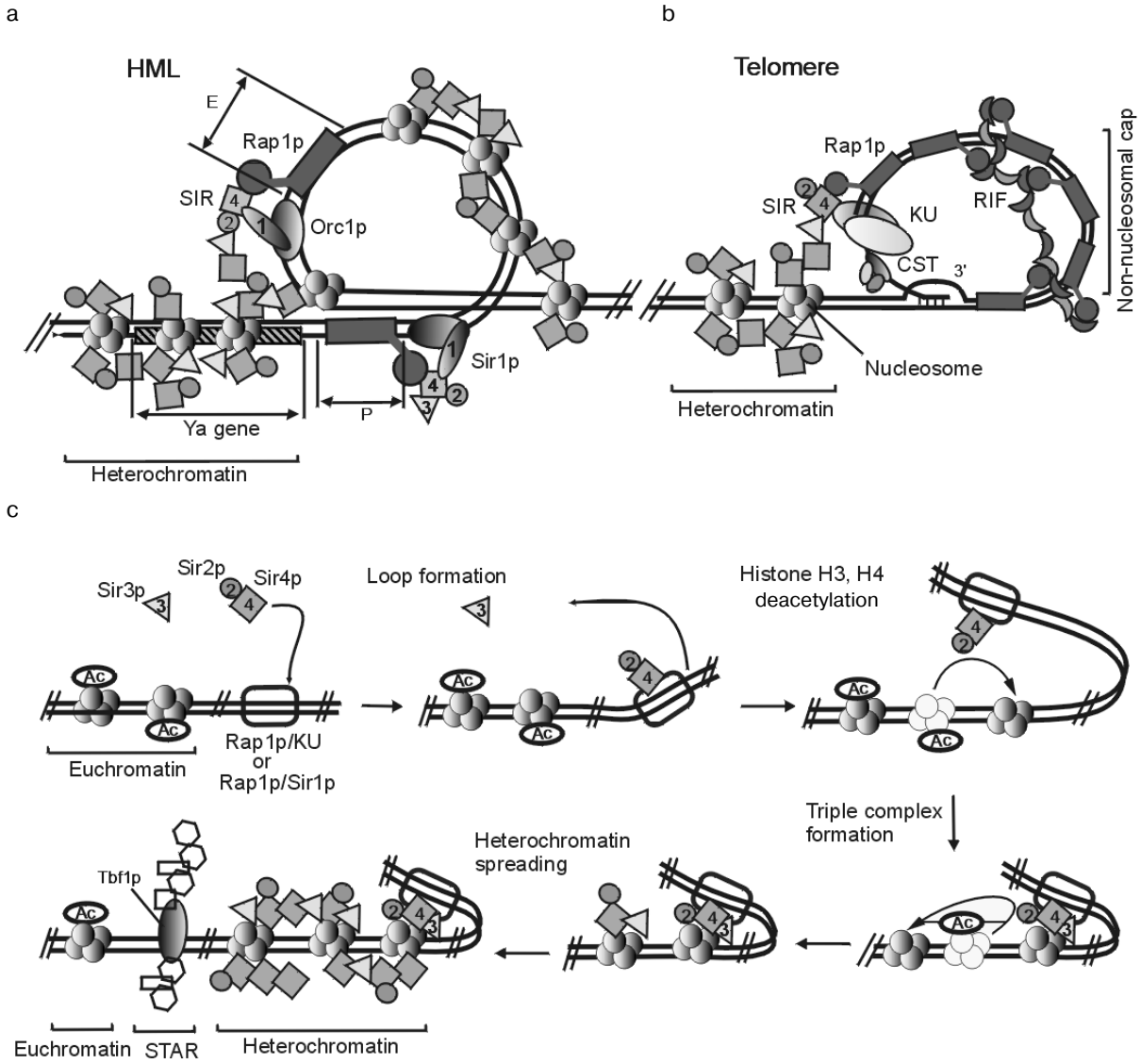

Fig. 3. a) The HML site of locus of pairing type regulation is an example of the inner loop, which is formed due to interaction of SIR proteins with Orc1p, Rap1p, and histones H3 and H4. SIR-mediated contacts of Rap1p bound to silencers E and I and promoter P [35] (not shown) also contribute to loop formation. b) Telomere loop (t-loop) is formed due to interaction of SIR with KU, Rap1p, and histones H3 and H4. Integration of 3′-end of G-rich chain into double strand DNA and Watson–Crick pairing to C-rich chain may also contribute to t-loop formation. It should be noted that experimental confirmation of such integration has been obtained only for mammalian telomeres (see [132] for more details). The figure shows completely formed cap (which is also marked by two hexagonal asterisks in Fig. 5). c) Scheme of heterochromatin formation. According to the modern model, formation of internal and telomere loops is required for initiation of heterochromatin formation, which occurs in several steps. Initially, silencing factors Sir4p and Sir2p bind to non-nucleosome sites of chromosome (silencers, Rap1p/Sir1p; telomeres, Rap1p/KU), then formation of loop structure occurs. This brings together SIR and nucleosome sites of euchromatin. Subsequent steps include histone deacetylation by Sir2p, which provides binding of H3 and H4 with Sir4p and Sir3p, formation of ternary complex SIR–Rap1p–H3/4, deacetylation of histones of adjacent nucleosomes, and progressive spreading of SIR proteins, inducing transformation of euchromatin sites into heterochromatin.

Chromatin subdivision into transcriptionally active (euchromatin) and inactive (heterochromatin) regions is an essential aspect of eukaryotic genome organization [24]. The genome of budding yeast *S. cerevisiae* is characterized by the existence of transcriptional silencing sites. These include a region of ribosomal operon repeats (RDN1), locus of regulation of pairing type, and subtelomeric genome regions. Transcription silencing also appears within the site of double strand break (DSB) of chromosomal DNA [25] (Fig. 4).

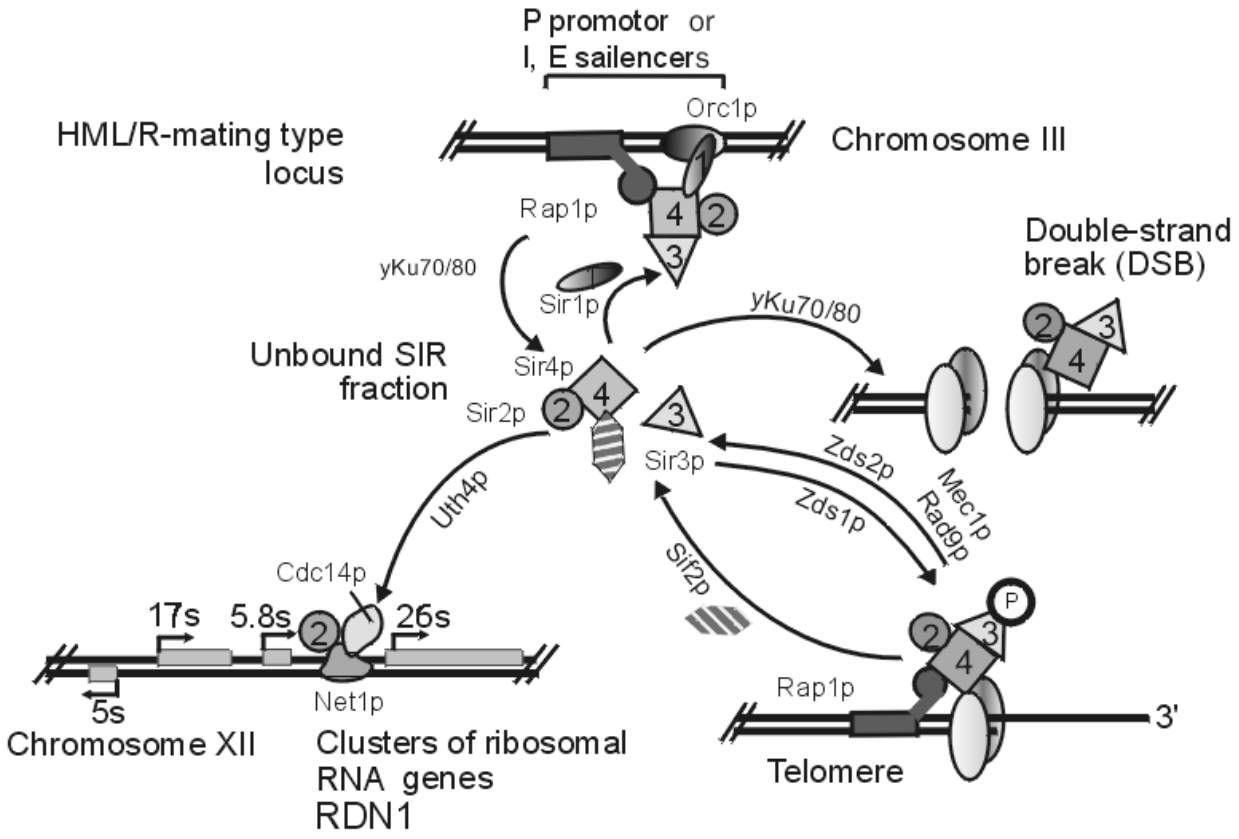

Fig. 4. Composition and regulation of localization of SIR complex on various sites of heterochromatin. See explanations in the text.

Rap1p initiates heterochromatin formation during interaction with SIR proteins [26] (Fig. 3c). Multisubunit protein complexes, known as bordering elements, prevent heterochromatin extension. Such bordering element may be formed in so-called subtelomeric STAR region (**S**ub-**T**elomeric **A**nti-silencing **R**egion) resulting in an obstacle for telomere heterochromatin extension and also in other genome regions for limitation of internal silencer effects [27] (Fig. 3c).

According to the modern hypothesis, bordering element is formed by Reb1p proteins (recognition site is located at X-element of subtelomeric chromosome region) and Tbf1p (TTAGGG repeat binding factor 1) [28] (Fig. 1a). The latter belongs to the family of "telobox" proteins, including some other telomere-binding factors [29].

Besides involvement in regulation of transcription and formation of heterochromatin structure, Rap1p is also involved in telomere formation *de novo* and activation of telomerase extension of telomeric DNA during its critical shortening [30, 31]. This was demonstrated using development of artificially shortened ("critical") telomere on the yeast chromosome by means of recombination between terminal region of chromosome and YAC (**Y**east **A**rtificial **C**hromosome) site, denominated as the telomere grain. Telomerase-dependent extension of the critical telomere occurs only in the presence of Rap1p-binding sites near telomere repeats.

Nevertheless, the role of Rap1p in the activation of telomerase extension of telomeres remains poorly understood. Negative regulation of the telomere extension by Rap1p is known in more detail; this effect is realized via Rap1p interaction with RIF (**R**ap1p-**I**nteracting **F**actor) proteins [32], which form a protective cap. The group of RIF proteins is considered in the final part of this section.

In human (hRap1) [14] and *S. pombe* (spRap1) cells, telomerase inhibition is the main function of Rap1p homologs. In the case of *S. pombe*, spRap1 is also involved in telomere positioning effect; it is also necessary for telomere clustering [15].

Rap1p is involved in formation of loop structures of yeast chromosomes. Organization of genes in the form of the loop domain promotes concerted regulation for the genes responsible for manifestation of the same function. Electron microscopy revealed that Rap1p is required for loop structure formation of the "silencing" site of *in vitro* pairing type HML/R regulatory locus [35] (Fig. 3a). The formation of loop structure in this region promotes its

transition in heterochromatin state via a mechanism involving SIR complex (see the final part of this section).

During certain stages of the cell cycle, telomeric DNA also forms loops known as T-loop; they share some similarity with D-loops formed in the process of recombination [36]. In the case of yeast, telomere loop formation requires SIR and Rap1p proteins [37] (Figs. 3b and 5a), whereas in the case of mammalian cells this process depends on the presence of TRF2 protein [38] (Fig. 1d). According to Egorov's hypothesis, another mammalian protein, TRF1, responsible for interaction of telomeric DNA with nuclear matrix, may also participate in formation of telomere macrostructure [39]. The formation of telomere loops of mammalian chromosomes occurs during integration of protruded 3′-end of telomeric DNA in the double stranded DNA site (Fig. 3b). This involves telomere repeat pairing at 3′-end of G-rich strand with the repeats of complementary (C-rich) strand of the double strand site of telomeric DNA. T-loops are required for telomere 3′-end hiding from various components of telomeric DNA synthesis/degradation machinery and also from various repairing systems of the cell. In the case of yeast cells the interaction of SIR proteins with KU, Rap1p, and histones H3 and H4 makes a major contribution to the loop formation. Formation of telomere loops is also important for initiation of telomere heterochromatin formation [40].

Thus, it is reasonable to suggest that the formation of loop structure represents a universal mechanism of heterochromatin formation both at the inner genome sites and in the telomere region (Fig. 3c).

Protein Tel2p is the other yeast protein associated with the double strand site of telomeric DNA [41]. This protein can bind directly telomeric DNA and it recognizes a consensus sequence that is similar to the Rap1p-recognition site. According to a current model, the process of telomeric DNA shortening is characterized by a certain moment when the number of molecules of Rap1p/RIF complex is not sufficient for the protective cap formation. This is accompanied by the loss of the "cooperative binding effect" for Rap1p/RIF complex and Tel2p can compete with Rap1p for binding at telomeric DNA. Binding of Tel2p to DNA activates Tel1p kinase, which triggers mechanism of degradation/extension of telomeric DNA [42] (Fig. 5).

SIR proteins. SIR complex of proteins cannot bind to DNA directly; it interacts with histones H3, H4, and Rap1p and Orc1p (a component of **O**rigin **R**ecognition **C**omplex) proteins. The composition of SIR protein complex has characteristic features at each of the silencing sites (Fig. 4). For example, transcription silencing within the region of ribosomal genes requires only SIR protein Sir2p, whereas formation of telomeric heterochromatin involves not only Sir2p, but also Sir3p and Sir4p.

Telomere heterochromatin determines the effect of telomeric silencing or TPE. This effect is characterized by decreased transcription of genes located in subtelomeric regions of chromosomes (Figs. 1a and 1c) and by the increase in DNA resistance to nucleases and components of the recombinant machinery [43].

According to the current model, initiation of heterochromatin formation requires formation of a telomeric loop (t-loop), which spatially brings together three components: N-terminal parts of H3/H4 histones, C-terminus of Rap1p, and SIR complex (Figs. 3a and 3b). Successful extension of heterochromatin also requires deacetylation of N-termini of H3/H4 histones at the silencing site [23]. This process is carried out by Sir2p, which functions as an NAD-dependent histone deacetylase [45, 46] (Fig. 3c). The other region-selective deacetylases Hos1p and Hos3p deacylate histones located in the region of ribosomal genes, whereas Hda1p plays the role in HAST (Hda1p affected subtelomere) subtelomeric regions [47].

Several factors are involved in regulation of redistribution of SIR proteins among various sites of the chromosome (Fig. 4). Evidently, redistribution of SIR proteins from one site of chromosomal DNA to another requires transition of components of this complex into a form that is not associated with chromatin [48]. This involves such proteins as ZDS (**Z**illion **D**ifferent **S**creens), KU, and Sif2p (**S**ir4p **I**nteracting **F**actor). It was shown that the group of ZDS proteins regulates redistribution of Sir3p. Zds1p promotes Sir3p phosphorylation; this increases Sir3p association with telomeres. Zds2p dephosphorylates Sir3p and promotes accumulation of this factor at non-telomeric loci (rDNA and HML/R) [49]. Sir1p promoting redistribution of SIR complex to the region of HML/R silencers is involved in regulation of localization of Sir3p and Sir4p [50]. (The latter is also necessary for silencing.) In this region, binding with DNA occurs via Orc1p protein [51].

Positioning of Sir2p in the region of rDNA requires Uth4p factor whereas Net1p protein provides Sir2p binding to DNA [52]. In the region of ribosomal operon repeats Sir2p promotes heterochromatin formation; interacting with Cdc14p kinase, it is involved in regulation of the cell cycle [53]. In this genome region Sir2p also antagonizes the aging process in yeast cells. Recombination of repeated sequences in this region results in the appearance of circular DNA molecules containing fragments of ribosomal operons, ERC (**E**xtrach**R**omosome **C**ircle). Accumulation of critical amounts of ERC causes the transition of the yeast cell into the senescent state (cell aging). Since Sir2p maintains the region of rDNA in heterochromatin state, characterized by low level of recombinant processes, it is negative regulator of the cell aging process [34].

RIF proteins. These proteins [32] belong to the class of proteins localized on the telomere due to tight protein–protein interactions [6] (table).

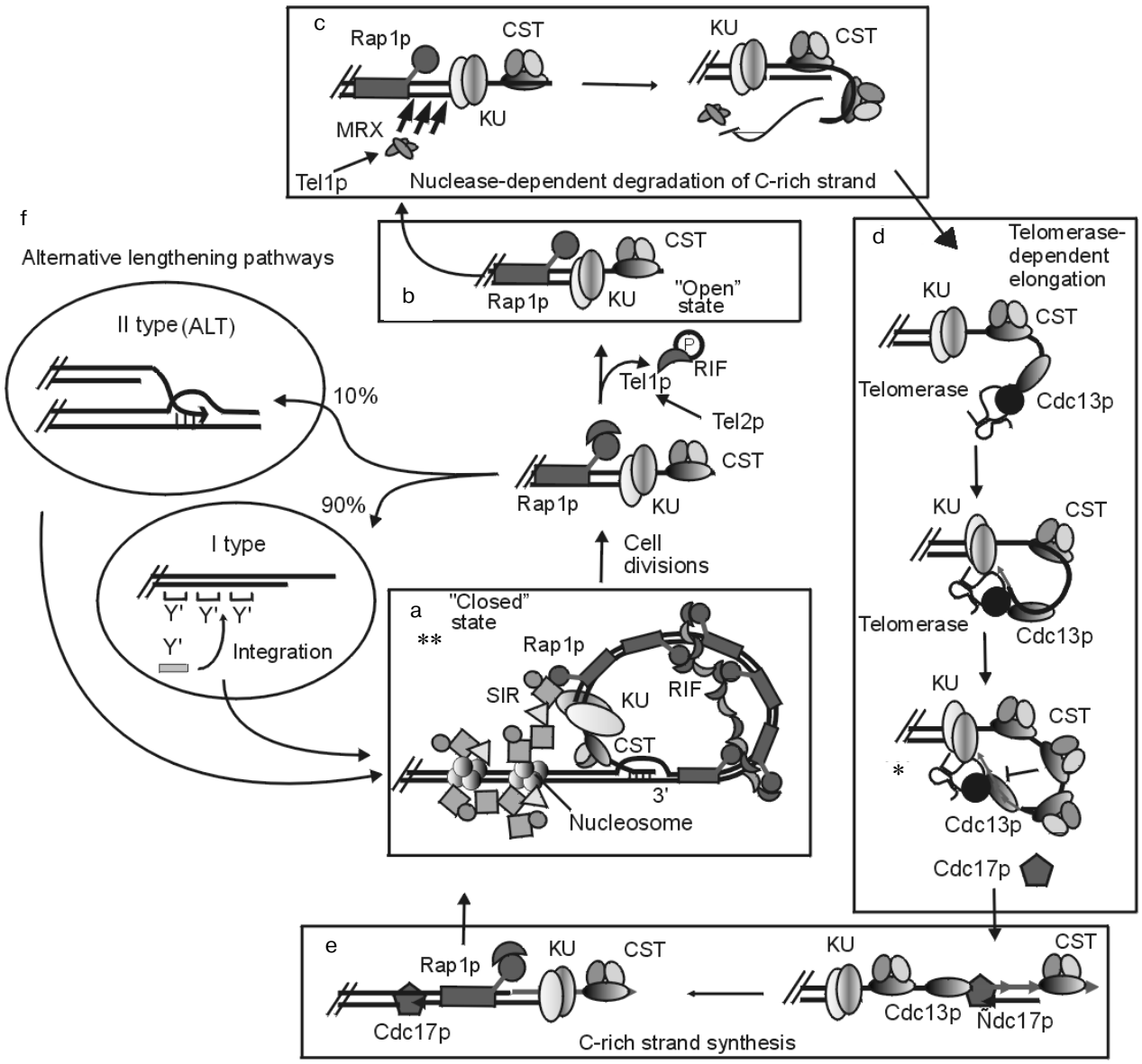

Fig. 5. Telomeric DNA degradation/synthesis cycle. a) Telomeric DNA is inaccessible for processing due to the protective cap formed by Rap1p-RIF proteins and also by factors that bind to telomere single strand site ("closed state"). b) During cell divisions telomeric DNA shortens and this results in the loss of the protective cap. This promotes access of Tel2p and Tel1p kinase activator to telomeric DNA. Tel1p phosphorylates remaining DNA bound components of the protective cap accompanied by their dissociation (indirect conformation of this hypothesis is in [126, 127]) and activates MRX complex. Telosome complex transforms into "opened state". c) Stage of nuclease degradation. Complex MRX catalyzes hydrolysis of C-rich chain of telomeric DNA and this results in the increase in CST proteins associated with single strand site of telomeric DNA. The critical concentration of CST leads to inactivation of MRX and transition to the stage of telomerase elongation. d) Stage of telomerase elongation. Due to interaction with Cdc13p, telomere complex associated with the 3′-end of G-rich chain of telomeric DNA may be activated by forming contact between telomerase RNA and KU complex. Elongation of telomeric DNA 3′-end by telomerase is accompanied by the increase in CST proteins associated with single strand site of telomeric DNA. Factors Stn1p and Ten1p promote dissociation of telomerase complex due to impairment in Cdc13p binding to Est1p subunit of telomerase complex. e) At the final stage DNA polymerases α and δ synthesize telomere C-rich chain. f) Alternative pathways for telomeric DNA elongation. * Telosome complex at the moment of telomere elongation of G-rich chain (compare with Fig. 1). ** Telosome complex with completely formed cap (compare with Fig. 3).

Rif1p and Rif2p are negative regulators of the length of telomeric DNA. According to the current hypothesis, protein–protein interactions between RIF proteins form the cap protecting telomere from attack by telomerase and other factors involved in degradation/synthesis of telomeric DNA. An alternative mechanism of the negative regulation consists of inhibition of the telomerase complex by specific factors. In human cells, this includes PinX1 protein, which binds to the catalytic subunit of telomerase (hTERT) and inhibit its activity [54]. In yeast cells such interaction has not been recognized. Moreover, yeast Gno1p protein (homolog of PinX1) is not involved in telomeric DNA elongation [55].

Cap limits access to DNA for recombination factors involved in telomeric DNA elongation via alternative mechanism (Fig. 5f) [56]. In telomerase-negative yeast mutants, two alternative Rap52p-dependent pathways for telomeric DNA elongation exist. They lead to the appearance of two cell types overcoming Hayflick's limit (maximal number of division of the cells with inactive telomerase) [57, 58]. One mechanism (type I) is responsible for multiple integration of the retrotransposon-like element Y′, located in the subtelomeric region of certain chromosomes; this element encodes helicase Y′Help (Y′**Hel**icase **P**rotein I) [59]. The integration process requires Rad54,55,57p factors and especially Rad51p. The latter is a RecA-like protein essential for heterochromatin DNA recombination. The other mechanism (type II), ALT, consists of Rad50p-dependent recombination, leading to elongation of $G_{1\text{-}3}T$ repeats due to homologous recombination with other telomeres [60] or circular fragments of telomeric DNA [61]. It should be noted that Rad50p is also a component of MRX complex, which includes Mre11p, Rad50p, and Xrs2p. This complex is involved in processing of DSB and telomeric DNA.

RIF proteins are also involved in regulation of the telomere positioning effect. The latter is realized by RIF proteins via telomerase inhibition, leading to telomeric DNA shortening, and, consequently, to reduction in the number of silencing factors bound to it [62]. According to another mechanism, RIF proteins compete with SIR proteins for binding at the C-terminus of Rap1p and, consequently, they impair the process of heterochromatin formation [63].

BORDER BETWEEN DOUBLE AND SINGLE STRANDED SITES OF TELOMERIC DNA

The border between double and single stranded sites of yeast telomeric DNA is the site for binding of conservative proteins yKU70 (Hdf1p) and yKU80 (Hdf2p) (Fig. 1, table). These factors and their homologs found in higher eukaryotes are components of cell machinery repairing double strand breaks (DSB) by the mechanism of non-homologous end joining (NHEJ). In mammalian cells, KU proteins are essential for the process of V(D)J-recombination [64]; they also regulate transcription of some operons [65].

In NHEJ machinery, KU proteins are responsible for recognition of a double strand break and binding of reparation factors, related to the system of processing and ligation (Mre11p/Rad50p/Xrs2p, Dnl4p/Lif1p in *S. cerevisiae* and Mre11/Rad50/Nbs1, DNAPKcs/XRCC4/DNA, Lig4 in *Homo sapiens*). Heterodimer KU also promotes binding of SIR proteins, forming heterochromatin sites around DSB. This prevents homologous recombination and transcription of adjacent genes and facilitates the reparation process [66].

Apart from their C-terminal domains, yKu70 and yKu80 share similar tertiary structure. These proteins form symmetrical circular molecules surrounding the DNA duplex [67]. The circular structure of the heterodimer KU determines its binding at the border of a double strand break as well as at the border between single and double stranded DNA in the telomere region.

The C-terminal domains of the subunits forming the KU heterodimer have different functions. In the case of mammalian Ku80, the C-terminal domain is responsible for binding and activation of DNAPKcs kinase [68], whereas in Ku70 the C-terminus represents a DNA-binding domain of HELH (**H**elix-**E**xtended **L**oop-**H**elix) type [69]. In fact, Ku70 can bind to internal sites of DNA irrespectively to Ku80. Ku70 binding sites include the promoter region of U1snRNA gene, site of LTR-promoter of MMTV (**M**ouse **M**ammary **T**umor **V**irus), and HIV-1 (**H**uman **I**mmunodeficiency **V**irus) LTR. KU complex binds to the site of NRE1 (**N**egative **R**egulatory **E**lement) [70], and DNAPKcs associated with KU activates Oct1 and Oct2 proteins, which are inhibitors of Tat factor. The latter is essential for transcription of promoter in the region of LTR and, consequently, for HIV-1 [71]. Thus, KU complex is involved in regulation of transcription.

KU proteins are positioned on telomeres due to binding to the border of double and single stranded sites. It is suggested that these proteins may bind to double strand region of telomeric DNA due to protein–protein interactions between TRF2-Ku70 [72] (in mammals) and Sir4p-yKu70 [73] (in yeast).

KU factors play an important role as components of the yeast telosome complex.

Together with CST proteins binding to the single stranded site of telomere and RIF proteins interacting with Rap1p, KU factors are involved in formation of the telomere cap that protects telomeric DNA against degradation and fusion [74].

KU factors also control nuclease processing of telomeric DNA, which is necessary for subsequent telomerase elongation of telomeres. In mammalian cells, Ku80 interacts with WRN (Werner syndrome) protein, possessing helicase and 3′-exonuclease activities [75]. This factor

is suggested to be involved in processing of both DSB and telomeric DNA. Although in yeast cells such interaction has not been recognized, nevertheless, the homolog of WRN protein, Sgs1p helicase functions in subtelomeric and telomeric regions of DNA; it is essential for replication and recombinant elongation of telomeres [76]. In yeast cells KU protein also controls nuclease processing. Telomeres of yeast cells deficient in yKu70/80 were shown to possess extended single stranded sites of G-rich strand of telomeric DNA during all stages of the cell cycle, whereas in wild type cells such sites were observed only at S-phase [77].

KU proteins prevent recognition of telomeric DNA by NHEJ systems such as DSB. The latter function may be realized via specific contacts formed by KU heterodimer with proteins of telosome complex. This results in loss of DNAPKcs binding.

The interaction between KU proteins and RNA is an interesting alternative model. In some mammalian cells, some RNA may compete with DNAPKcs for binding sites on Ku80 [78]. It was previously shown that in yeast cells the hairpin (288-355) of telomerase RNA TLC1 interacts with heterodimer KU [79]. Taking into consideration the data in the literature [78, 79], it is possible to suggest that in the case of yeast cells interaction of yKu70/yKu80 with RNA also results in dissociation of the signal factor similarly to mammalian DNAPKcs. So, involvement of telomerase RNA in this process may explain why the terminal part of telomeric DNA is not recognized by DSB reparation system as a double strand break.

The KU proteins are also essential for localization of yeast telomeres on the periphery of the nucleus [80]. It was shown that yKU70 interacts with the nuclear pore component Mlp2p [81]. Telomere localization is closely related to the transcription process. Deletion of yKu70 is accompanied by their delocalization [82] and increased repression of transcription in inner chromosome sites [83]. This stems from redistribution of silencing factors from telomeric DNA to non-telomeric sites due to loss of yKU70 with Sir4p silencing factor [73]. Such interaction increases binding of Sir4p with the C-terminus of Rap1p protein and promotes displacement of negative telomere regulators of Rif1,2p telomerase, which bind to Rap1p at the same site. Formation of contact between Sir4p and Rap1p leads to stabilization of telomere heterochromatin and enriches it by SIR factors due to inner sites [63].

Thus, yeast KU heterodimer is involved in regulation of telosome complex components, which bind to double strand (SIR) and single strand (Cdc13p) telomeric DNA. It is possible that KU proteins, which bind at the border of double and single strand sites, represent one of the systems responsible for concerted functioning of components of double and single strand telomere sites. The cyclic degradation/synthesis of telomeric DNA is one of the most important processes required for such regulation. Critical shortening of double strand site telomeric DNA triggers mechanisms of degradation of C-rich chain, appearance of single strand G-rich site, and activation of specific factors, first of all telomerase (Fig. 5). This cycle is considered in more details in the next section.

THE SITE OF SINGLE STRAND TELOMERIC DNA

Several CST proteins bind the single strand site of yeast telomeric DNA. These include Cdc13p [84], Stn1p [85], and Ten1p [86]. The OB motif (Oligonucleotide/Oligosaccharide Binding) of Cdc13p is responsible for complex binding to telomeric DNA [87]. The gene encoding this protein was detected among the group of EST genes (**E**ver **S**horter **T**elomeres). Mutations in these genes cause progressive telomere shortening and cell aging (senescence) [88].

According to the current model, CST group proteins form a protective cap at the telomere ends (Figs. 3b and 5a); this prevents their degradation and effects various systems of double strand break repair and also the system of detection of DNA breaks [89].

The CST complex controls degradation/synthesis processes of telomeric DNA and its coordination with the cell cycle [86, 90]. Control of telomeric DNA processing falls into two interrelated problems: 1) control of C-rich telomere strand nuclease hydrolysis [91], and 2) control of elongation of 3′-end of G-rich overhang of the telomere strand [84].

The initial stage of the degradation/synthesis cycle includes hydrolysis of C-rich telomeric DNA. According to the current model, the mechanism of degradation of telomeric DNA complementary chain involves MRX complex (Mre11p-Rad50p-Xrs2p) [42] (see above). This protein complex is involved in double strand break formation during meiosis, homologous recombination, and DSB processing [92]. A similar complex in mammalian cells (Mre11-Rad50-Nbs1) interacts with TRF1 (Fig. 1) [93]. So, it is reasonable to suggest that this complex may represent a telosome component, although MRX association with any components of the telosome complex has not been found in yeast.

The MRX complex includes Mre11p protein [94], which can function as endo- and exonuclease, hydrolyzing one of the strands of a DNA duplex in the direction 3′→5′, Rad50p (binding to DNA in an ATP-dependent manner), and helicase Xrs2p. It is suggested that Rad50p is responsible for MRX binding to telomeric DNA, Mre11p introduces break into the C-rich telomere chain and hydrolyzes it in the 3′→5′ direction. Helicase activity of Xrs2p protein, which is activated via phosphorylation by kinase Tel1p [95, 96], facilitates this process. Cdc13p and other CST proteins bind at the single strand

site of telomeric DNA [97]. Increase of the length of single strand site results in binding of more CST molecules at the single strand site of the telomere. This inhibits MRX complex. Thus, MRX exonuclease activity is regulated by CST complex via a negative feedback mechanism (Fig. 5c).

When telomeric DNA reaches optimal length of single strand, it becomes ready for the next stage of degradation/synthesis cycle of telomeric DNA: binding of telomerase complex (Fig. 5d). The optimal length of the single strand site obviously allows a loop to form, providing interaction of KU proteins with the telomerase complex [79] and the telomerase complex with telomeric DNA [98].

Good evidence exists that yeast telomerase complex consists of reverse transcriptase subunit Est2p and RNA molecule (TLC1), representing a template for the reverse transcription. Telomere complex also includes additional subunits Est1p [99] and Est3p [100] (Fig. 1b). The amino acid sequence of Est1p contains a potential RNA-recognizing motif (RRM) [101]. Together with Est2p, this protein forms contacts with telomere RNA around the template site; this indicates its possible involvement in formation of the active site of telomerase [102].

Besides telomere RNA, Est1p also interacts with the 3′-terminal region of telomeric DNA [99]. However, *in vivo* Est1p may effectively bind to telomeric DNA only in the complex with Cdc13p. Thus, according to the current model, Est1p plays a role of a messenger subunit that is responsible for binding of telomerase complex with Cdc13p [103].

Est1p protein has to compete with Stn1p (negative telomerase regulator) for Cdc13p binding [85]. Thus, CST proteins not only determine telomere complex binding with telomeric DNA, but also regulate the elongation degree of 3′-end overhang of G-rich chain. This process may also be regulated via a negative feedback mechanism: excessive elongation of G-rich chain by telomerase leads to binding of critical amounts of CST complex molecules. Cooperative binding of these molecules to Stn1p may displace Est1p and the telomerase complex from its binding site on Cdc13p (Fig. 5d). The other mechanism of limitation of telomerase processivity may include displacement of the telomere complex by DNA polymerase α [105] catalyzing synthesis of the C-rich chain of telomeric DNA.

The role of Cdc13p and Cdc13p-associated proteins in the regulation of telomeric DNA is not limited by simple telomerase binding: this protein apparently regulates properties of the telomerase complex. This suggestion is supported by the fact that mutations in the *CDC13* gene did not influence Cdc13p binding with DNA or Est1p, but they cause both shortening [106] and elongation [107] of telomeric DNA.

The final stage of degradation/synthesis of telomeric DNA consists of re-synthesis of lagging C-rich strand of telomeric DNA catalyzed by DNA polymerases α (Cdc17p) [108] and possibly δ [109, 110] (Fig. 5e). In this process, Cdc13p is responsible for Cdc17p binding to telomeric DNA and coordination of its operation with telomerase complex functioning [105]. Impairment of such coordination results in uncontrolled telomere elongation by telomerase and increase in telomeric DNA length.

Telomere biogenesis involves the other yeast polymerase, DNA polymerase ε. This enzyme is suggested to be involved in synthesis of leading G-rich strand of telomeric DNA during replication. Mutations in the catalytic domain of this polymerase cause total slowing of DNA replication, particularly incomplete replication of the telomere region; this results in rapid telomere shortening and cell transition into senescence [111].

Several other components of the replication machinery are also located within the telomeric DNA region. These include primase Pri2p associated with DNA polymerase α and Dna2p (factor responsible for Okazaki fragment processing); the latter is localized in the region of telomeric DNA due to interaction with Sir3p [112].

SOME EVOLUTIONARY ASPECTS OF TELOMERE BINDING PROTEINS

In this review we have considered the main components of the telosome complex of *S. cerevisiae* and their involvement in various cell processes. However, study of any model system may be useful only if the results obtained using such system may be applicable to studies of other systems, especially higher mammals.

The components of yeast telosome complex, regulatory factors, and also factors involved in telomeric DNA degradation/synthesis cycle may represent highly conservative proteins such as KU, Sir2p, Rad50p, Mre11p, Tel1p, Mec1p, and Est2p; some other essential components do not share homology with proteins from other species. Nevertheless, models of evolutionary development have been proposed for some of them (Fig. 6). These models can be used to analyze relations between proteins characterized by distinct primary structure and different functions.

Such a situation is typical for proteins that bind to double strand site of telomeres of budding yeast (*S. cerevisiae*), dividing yeast (*S. pombe*), and mammals.

Factors TRF1 and TRF2 bind to the site of mammalian telomeric DNA (Fig. 1d). They play an important role in protection of chromosome termini from fusion and degradation, regulation of telomeric DNA degradation/synthesis cycle, and formation of telomere loops. TRF2 is also needed for hRap1 protein positioning in the telomere region; Taz1 binding to double strand telomeric DNA of dividing *S. pombe* is needed for spRap1 binding.

Thus, *S. cerevisiae* Rap1p protein, which can directly bind to DNA, represents rather a unique case. This

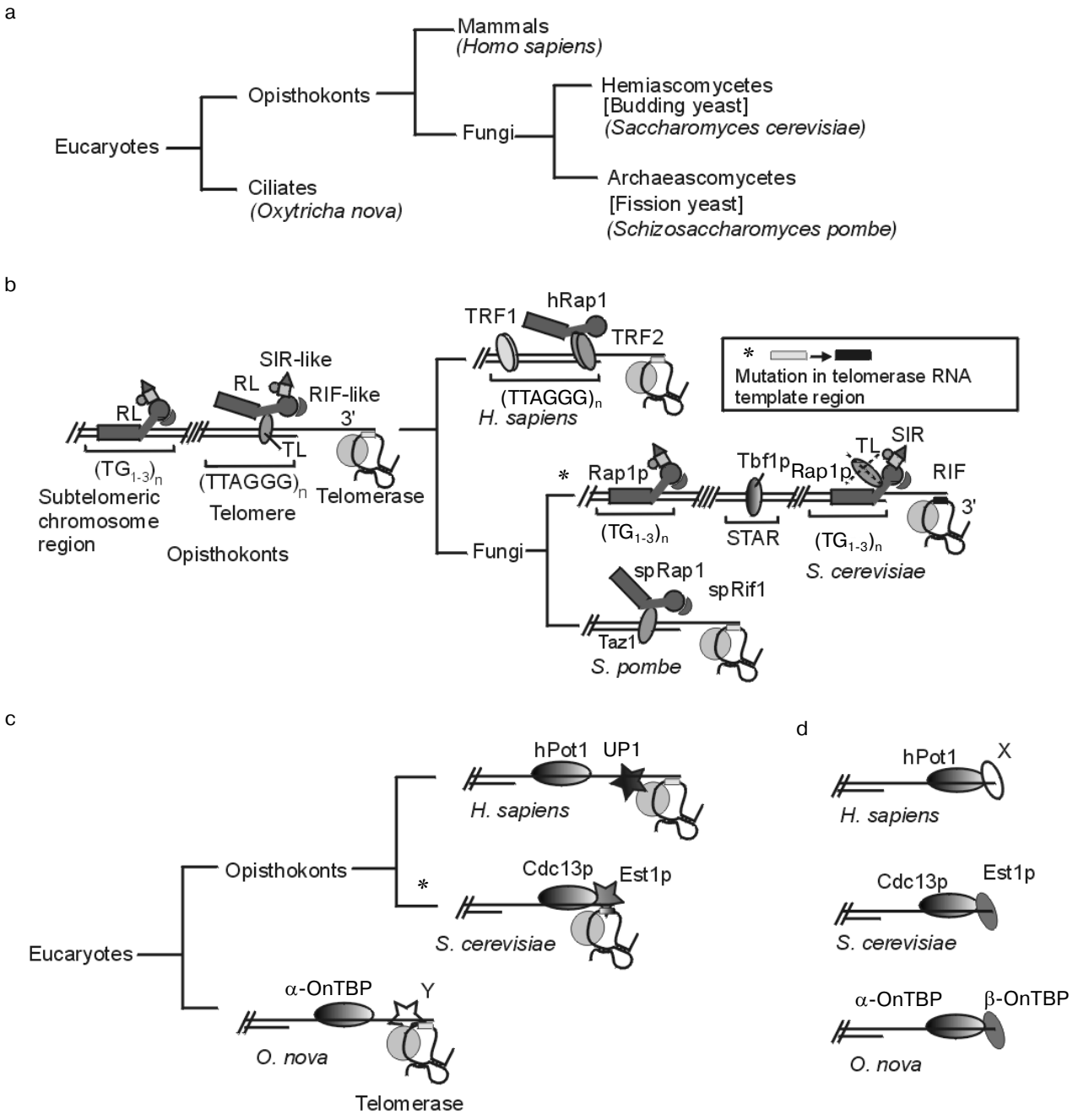

Fig. 6. a) Simplified phylogenetic tree demonstrating evolutionary interrelationships between organisms mentioned in the text [128]. b) Model of evolutionarily ancient TL (TRF-like) and RL (Rap-like proteins). Separation of Hemiascomicetes branch coincided with appearance of mutation in the template site of telomerase RNA (see explanations in the text). c) Model of evolutionary relations of telosome proteins which bind at the single strand site of telomeric DNA. UP1, Est1p, and also hypothetical protein Y from *O. nova* form a single protein family. d) Alternative model: Est1p belongs to the group of factors forming the protective cap at 3′-end of the G-rich chain. This group also includes β-subunit of OnTBP and hypothetical X protein of *H. sapiens*.

phenomenon can be explained in the evolutionary model of telomere proteins [14] (Fig. 6b). According to this model, at some stage of evolution telomere-binding TL (TRF-like) protein existed. This hypothetical protein was responsible for binding of RL (RAP-like) protein with the telomere. This RL protein could form a heterochromatin region within telomere ends and it also inhibited telomerase activity. RL protein also functioned as transcription

regulator: it bound to genome DNA by its own DNA-binding domain.

During evolution (under the influence of natural selection) the primary structure of telomeric DNA changed and this was accompanied by the loss of DNA binding by telosome complex proteins. However, it can be suggested that at a certain stage mutation in the template site of telomerase RNA led to such change of telomeric DNA primary structure, which resulted in appearance of a new telomere repeat, coinciding with the consensus sequence recognized by RL protein. Under these conditions, TL lost its ability to bind newly synthesized telomeric DNA but maintained its ability to interact with inner telomere region, resistant to replication degradation. This mutation was fixed in one of the evolutionary branches from which budding yeast *S. cerevisiae* originated. In these cells Rap1p represents a "descendant" of RL protein.

Subsequent evolution of organisms with conservative primary structure of telomeric DNA resulted in divergence of RL functions and this protein became the ancestor for transcription regulators and also for such proteins as hRap1 and spRap1, which preserved its regulatory function of telomeric DNA elongation. TL protein existing in *S. pombe* as Taz1 diverged into TRF1 and TRF2 during evolution. Thus, it acquired a property of fine regulation of the telosome complex dynamics. It is suggested that TL protein is the ancestor for yeast Tbf1p factor.

Phylogenetic analysis of primary structure of proteins which bind to the double strand site of telomeric DNA seems to support this hypothesis. According to results of this analysis, *S. pombe* Taz1 protein, human TRF1 and TRF2 proteins, and yeast Tbf1 protein can be pooled into the family of "telobox" proteins. These proteins are characterized by the existence of Myb-like DNA-recognizing an HTH (Helix-Turn-Helix) motif of R3 type [29]. At the same time, Rap1p is not related to this family and this underlines independence of the development protein precursors TL and RL.

S. cerevisiae Cdc13p and α-subunit of *Oxytricha nova* OnTBP complex are factors interacting with the single strand telomeric DNA region (Figs. 6c and 6d); it is suggested that they are related proteins [87]. OnTBP complex required for formation of the telomere cap is a heterodimer that consists of α- and β-subunits [113]. The former (as well as Cdc13p) contains OB (Oligosaccharide–Oligonucleotide Binding) structural motif. Pot1 protein found in *H. sapiens* (hPot1) and *S. pombe* also plays a protective function for telomeric DNA similar to that of Cdc13p and OnTBP. This factor shares some homology with OnTBP α-subunit. Thus, this protein may be also referred to the family of cap-forming factors which bind to the single strand site of telomeric DNA.

However, it should be noted that the telomere binding function typical for Cdc13p was not recognized in the case of OnTBP and Pot1. In mammalian cells, this is attributed to UP1 protein which is formed during proteolytic processing of hnRNPA1 splicing factor [116]. No data on telomerase binding factor(s) are available for *O. nova*.

We may only suggest that involvement of Cdc13p in telomerase binding, which occurs via an accessory Est1p subunit, compensated impairments in the structure of telomere complex that originated from changes in primary structure of telomeric DNA (see above). Within this model Est1p and UP1 proteins belong to the family of telomerase accessory subunits (Fig. 6c).

An alternative explanation of the existence of interaction between Cdc13p and Est1p factor follows the assumption that the latter originates from the group of cap-forming proteins (which also includes β-subunit of OnTBP). In this case Est1p involvement in telomerase binding is not its primary function in the telomere complex (Fig. 6d).

Recent years are characterized by intensification of studies on telomerase and telomerase complex due to employment of methods of detection and inhibition of telomerase activity for diagnostics and treatment of oncological diseases [117]. In the case of mammals, these are preferentially applied medical studies whereas studies of telosome and telomerase complexes of *S. cerevisiae* reveal fundamental principles of their functioning and regulation and also interaction with other systems of the cell. Such information is ultimately important for the search for new pathways for telomerase treatment and, consequently, this promotes studies of mammalian telomerase and telosome complex.

In this review we have considered components and some aspects of *S. cerevisiae* telosome complex. This organism provides a convenient and widely used model system. Much attention has been paid to recent data that appeared after the special issue of *Biochemistry* (Moscow) (No. 11, 1997) on telomeres and telomerase.

Telomeres share some properties with various DNA regions. For example, the double strand site of telomeric DNA typical for heterochromatin is the site for interaction with Rap1p and SIR proteins, and also with RIF proteins, which are specific only for this genome fragment.

KU proteins bind to the border between double and single strand telomeric DNA and also to DSB. These proteins have a specific function that consists of telomere complex binding.

Finally, the single strand G-rich site of telomeric DNA is a unique telomere structure, which is the target for binding of Cdc13p, Est1p, Stn1p, Ten1p, and also for telomerase complex. This less studied site of telomeric DNA is the most "mysterious" in terms of evolutionary interaction with protein factors. Many important points such as correspondence of composition of yeast and mammalian telomerase complexes remain to be clarified. Particularly, the function of Est3p subunit, which is unique for yeast telomerase, remains unknown.

Thus, we believe subsequent studies will focus on investigation of unique components and development of models for their evolution. Having reliable schemes describing development of all components of telosome complexes, it is possible to apply these models to higher mammals.

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