

Brief Report

The Nuclear Envelope and Spindle Pole Body-Associated Mps3 Protein Bind Telomere Regulators and Function in Telomere Clustering

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ABSTRACT

It has long been posited that the nuclear envelope is a key regulator of both the spatial organization of chromatin and gene transcription. Mps3p is an integral nuclear envelope membrane protein with a single trans-membrane domain that is essential for spindle pole body duplication. More recently, Mps3p was shown to associate with the cohesion establishment factor Ctf7p and found to be critical for cohesion establishment. Here, we provide new evidence that the nuclear envelope, via Mps3p, plays a pivotal role in telomere foci formation. Results from in vitro pull-downs and in vivo coprecipitations also show that Mps3p associates with the telomerase-assembly component Est1p. Moreover, pair-wise combinations of *mps3*, *est1* or *ctf7* alleles all produce conditional lethality. Findings that Mps3p and the nuclear envelope recruit/sequester soluble chromatin metabolism factors such as Ctf7p and Est1p describe, at the molecular level, a new mechanism of nuclear envelope-dependent chromatin regulation.

INTRODUCTION

The nuclear envelope, a defining feature of eukaryotes, protects genomic DNA from cytosolic activities and provides for regulated transport of transcription and DNA replication factors into the nucleus and RNA out of the nucleus. As opposed to human cells in which the nuclear envelope is dismantled each and every cell cycle, budding yeast undergo a closed mitosis - placing additional burdens of coordinating nuclear division with both chromosome segregation and cytoplasm division. More recent evidence revealed that the yeast nuclear envelope also plays key regulatory mechanisms in both the spatial organization of chromatin within the nucleus and gene transcription.^{1,2} In the first case, telomeres cluster in defined combinations and these clusters become tethered to nuclear envelope domains.³⁻⁵ In the second case, silenced chromosomal loci coalesce to the nuclear periphery - although transcription repression does not require nuclear attachment per se.^{2,4,6} While the general role of the nuclear envelope in all of these processes is now well documented, an integral membrane protein that facilitates telomere clustering to the nuclear envelope remains elusive.

Mps3p is a single trans-membrane protein that is distributed throughout the nuclear envelope at relatively low levels but concentrated near spindle pole bodies embedded within the nuclear envelope.⁷ Mps3p recruits and subsequently inserts Spc42p into the spindle pole body and is required for spindle pole body duplication.^{8,9} A recent report reveals that Mps3p and the nuclear envelope directly function in chromatin metabolism. For instance, Mps3p is critical for proper sister chromatid cohesion and interacts both physically and genetically with the soluble cohesion establishment factor Ctf7p—an essential and highly conserved protein required for sister chromatid pairing during S-phase.¹⁰⁻¹⁴ Based on these findings and previous reports that Ctf7p associates with DNA replication factors,^{12,13,15} one model is that Mps3p recruits Ctf7p to the nuclear envelope—potentially activating Ctf7p before its release to the DNA replication fork to establish sister chromatid pairing.¹⁰ Note that other spindle pole body components such as Kar3p and Bim1p were previously identified as functioning in sister chromatid cohesion.^{16,17}

The finding that Mps3p plays a critical role in sister chromatid cohesion raises the possibility that the nuclear envelope may serve similar regulatory functions in other DNA metabolism pathways. Based on findings that proximity to the nuclear membrane is correlated with telomere clustering and transcription repression, a reasonable hypothesis is that Mps3p might regulate telomere metabolism. In yeast, telomerase is regulated in part by Est1p, which recruits telomerase to the telomere.^{18,19} Here, we provide new

evidence that the Est1p telomerase-assembly component associates with Mps3p—possibly to recruit Est1p to the nuclear envelope and sequester it away from the telomere.

MATERIALS AND METHODS

In vitro GST pull-downs. *GST-CTF7* and *GST-MPS3* expression in *Escherichia coli* cells and pull-downs from yeast lysate cells were performed as previously described.¹³ Briefly, yeast cells containing Mps3p-13MYC, Mcd1p-MYC or Est1p-MYC (a generous gift of V. Zakian) were spheroplasted in 100T Zymolyase (Seikagaku), lysed by swelling and mechanical disruption (20 mM HEPES-HCl (pH 7.5), 5 mM MgCl₂, and protease inhibitors) and centrifuged at 9500 rpm for 45 min (model JA-20; Beckman). The supernatant was removed, and the insoluble chromatin pellet extracted with lysis buffer containing 1 M NaCl before recentrifugation. The salt-extracted supernatant was harvested and divided into equal aliquots, one of which was precipitated with trichloroacetic acid and resuspended in Laemmli buffer. The other aliquots were each diluted 10-fold in lysis buffer (to reduce the salt concentration) prior to incubation with one of the bead matrices (glutathione-Sepharose beads or beads coupled to GST, GST-Ctf7p, GST-Mcd1p, GST-Pds5p, or GST-Mps3p). Incubations were performed at 4°C for 2 h. The treated beads were washed several times before bound proteins were removed using SDS-containing solubilization buffer. During Western blot analyses, the MYC epitope was visualized using polyclonal anti-c-MYC A14 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), goat anti-rabbit horseradish peroxidase (Bio-Rad), and ECL-Plus (Amersham Biosciences).

Telomere clustering and anchoring. Log phase wild type, *mps3-2*, *mps3-3*, and *ctf7-203* cells grown at 23°C were arrested in S phase (Hydroxyurea). Cells were then washed and placed in fresh rich media supplemented with alpha-factor and shifted to 37°C to produce G₁ arrested cultures. Aliquots of cells taken two hours post addition of alpha factor were analyzed for DNA content (BD Biosciences FACSCAN). For Rap1p detection, cells were fixed with formaldehyde and polyclonal anti-Rap1 (a generous gift of D. Shore) and goat anti-rabbit Alexa 488 (Molecular Probes, Inc., Eugene, OR) were used for Rap1p localization. All cells were visualized using a Zeiss confocal microscope 63x and images acquired in single-track mode at 488nm at 10% power. 3D projection stacks were typically 10–14 slices of 0.2 μm. Number of cells counted for wild type n = 44, *ctf7-203* n = 66, *mps3-3* n = 45, *mps3-2* n = 16. Telomere anchoring was performed similar to the clustering assay. The nuclear envelope was detected using the monoclonal antibody 306 (a generous gift of Laura Davis) and goat anti-mouse Alexa 568 (Molecular Probes, Inc., Eugene, OR). Cells were visualized using a Nikon E800.

RESULTS

Mps3p associates with Est1p: A telomere length regulator. High through-put 2-hybrid assays previously identified an interaction between Mps3p and Est1p. However, this association remained unconfirmed nor was a physiological role for this interaction explored.^{20,21} To pursue the possibility that the nuclear envelope functions in telomere length regulation as well as in cohesion, we first tested for Mps3p-Est1p in vitro interactions using GST pull-downs. GST-Mps3p was bacterially expressed and coupled to glutathione-sepharose beads. Lysates from yeast cells expressing Est1-12MYCp as the sole source of Est1p function were incubated with sepharose beads or beads coupled to either GST or GST-Mps3p. After copious

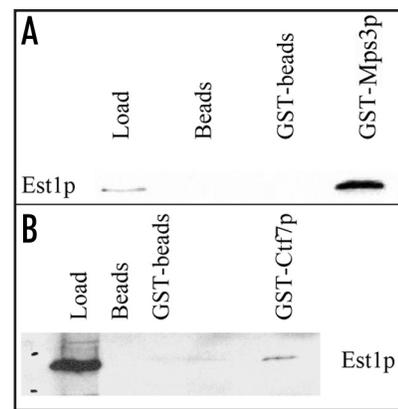


Figure 1. Est1p binding partners. (A) Est1p interacts in vitro with the nuclear envelope integral protein Mps3p. Extracts from cells expressing Est1p-MYC (Load) were used to test for interactions with glutathione-Sepharose beads (Beads) or beads coupled to either bacterially expressed glutathione S-transferase (GST) or GST-Mps3p (GST-Mps3p). (B) Est1p also associates with Ctf7p. Extracts from cells expressing Est1p-MYC (Load) were used to test for interactions with glutathione-Sepharose beads (Beads) or beads coupled to either bacterially expressed glutathione S-transferase (GST), or GST-Ctf7p (GST-Ctf7p).

washing, tightly bound proteins were eluted and analyzed by Western blot. The results reveal that Est1p specifically bound to GST-Mps3p but not to beads alone or beads coupled to GST (Fig. 1A), documenting a physical interaction between a telomere length regulator and an integral nuclear membrane protein. In a reciprocal study, we found that GST-Est1p efficiently associates with Mps3p but fails to pull down mutant *mps3-3p* (Supplemental Fig. S1), suggesting that Est1p-Mps3p association is physiologically relevant.

Given the pair-wise interactions between Mps3p and Est1p and between Mps3p and Ctf7p,¹⁰ we also tested for an interaction between Est1p and Ctf7p. GST-Ctf7p was bacterially expressed and coupled to glutathione-sepharose beads prior to coincubation with lysates from yeast cells expressing Est1-12MYCp. As before, Western blot analyses were performed on eluted proteins after copious washing of the bead matrices. The results reveal that Est1p specifically bound to GST-Ctf7p but not to beads alone nor beads coupled to GST (Fig. 1B). In combination with a prior study that Ctf7p binds to Mps3p,¹⁰ we document here novel complexes minimally comprised of Mps3p-Est1p, Est1p-Ctf7p and Ctf7p-Mps3p or a single complex comprised of Mps3p-Ctf7p-Est1p.

Pair-wise combinations of Mps3, Ctf7 and Est1 alleles are conditionally lethal. To test whether the above protein associations were of physiological relevance, *mps3* cells were crossed into *est1* null cells, the diploids sporulated and double mutant spores identified. Independent crosses using both *mps3-3* and *mps3-5* strains produced viable double mutant spores at expected frequencies. To test whether the double mutant strains exhibited growth characteristics different from the single mutants, *mps3-3 est1* and *mps3-5 est1* double mutant strains, in addition to the single mutants and wildtype cells, were plated in a dilution series on YPD-rich medium and grown at 23°C, 32°C, and 37°C (Fig. 2A). At 23°C, most strains showed robust growth, except for *est1 mps3-5* double mutant cells which exhibited decreased growth kinetics even at this temperature. At 32°C, wildtype and single mutant strains remained viable. In contrast, independent isolates of *mps3 est1* double mutants were inviable at 32°. At 37°C, all strains harboring temperature sensitive *mps3* alleles were inviable.

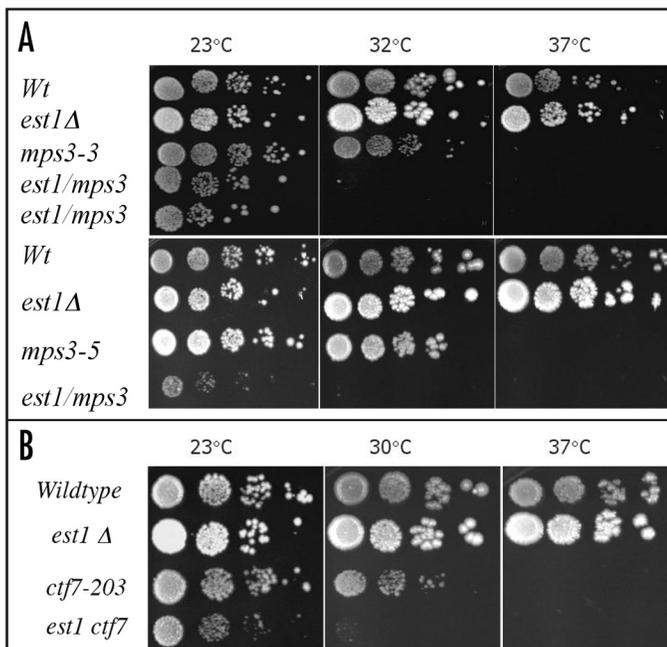


Figure 2. *MPS3*, *EST1* and *CTF7* genetically interact. (A) *mps3 est1* double mutant cells exhibit conditional synthetic lethality. Wild type, single mutants *mps3-3*, *mps3-5*, and *est1Δ* and double mutant *mps3-3 est1Δ* and *mps3-5 est1Δ* cells were grown on YPD rich medium at 23°C, 32°C and 37°C. *est1Δ* combined with either *mps3-3* (two independent double mutant strains shown in upper panel) or *mps3-5* (shown in lower panel) are inviable at 32°C. (B) *ctf7-203 est1Δ* double mutant cells exhibit conditional synthetic lethality. Wild type, single mutants *ctf7-203*, and *est1Δ* and double mutant *ctf7-203 est1Δ* cells were grown on YPD rich medium at 23°C, 30°C and 37°C. *ctf7-203 est1Δ* double mutant cells are inviable at 30°C.

We also crossed the *est1* deletion into *ctf7-203* cells previously reported to genetically interact with *mps3* alleles. Viable double mutant spores were recovered at the expected frequencies (Table 1) and a similar dilution series was performed for the *ctf7-203 est1* double mutant cells and control strains (Fig. 2B). At both 23°C and 30°C, wild type and *est1* single mutants showed robust growth while *ctf7* mutant strains exhibited a moderate growth reduction at 30°C. However, *ctf7 est1* double mutant strains were completely inviable at 30°C and exhibited a significant growth defect even at 23°C. In fact, each pair-wise combination of *mps3*, *est1* and *ctf7* alleles exhibit conditional synthetic lethality,¹⁰ providing indirect evidence for a tripartite complex.

Mps3p plays a role in telomere clustering. Telomeres reproducibly cluster into a discrete number of foci proximal to the nuclear periphery, raising the possibility that Mps3p may be critical for foci formation. To test this model, wildtype and *mps3-3* log phase cells were synchronized at the permissive temperature in S-phase using medium supplemented with hydroxyurea. Given that Ctf7p also associates with Mps3p, we included *ctf7-203* mutant cells in our analyses. The synchronized cultures were washed and shifted to 37°C in the presence of alpha factor to produce cells synchronously arrested in G₁. This regimen allows for both Mps3p and Ctf7p inactivation (in mutant cells) during that portion of the cell cycle when each performs their respective essential function: Mps3p in G₁ and Ctf7p during S-phase. Important to this regimen, telomeres cluster in G₁,³ allowing us to test for clustering defects within the mutant strains. Upon G₁ arrest, cells were fixed and telomere clusters visualized using an antibody directed against the telomere-associated protein

Table 1 **Analyses spore recovery for *ctf7* crossed to *est1* mutant strains**

	Observed	Expected
Wildtype	25	24
<i>ctf7-203</i>	17	18
<i>est1 Δ</i>	22	24
Inviability	13	12
<i>ctf7, est1</i>	18	18
Total spores	96	96

No synthetic lethality or meiotic defects were observed during *ctf7* and *est1* crosses such that the expected frequency of *ctf7 est1* double mutant spores was obtained. Similar results were obtained for *mps3 est1* cross (data not shown).

Rap1p. Telomere cluster foci were quantified from several independent experiments based on maximum projections of images obtained using a confocal microscope (Fig. 3A). Wild type cells contained 4–6 telomere clusters—in strong agreement with previous reports.³ *ctf7-203* exhibited similar numbers to wild type cells indicating that Ctf7p does not play a role in telomere clustering. In contrast, *mps3-3* cells exhibited a significantly higher number of telomere clusters such that 32% of the cells had more than six telomere clusters, compared to 9% in wild type and 4% in *ctf7-203* cells (Fig. 3B). No obvious differences were detected in nuclear envelope structure using antibodies directed against the FG repeat domains of nucleoporins (data not shown). The finding that *mps3* mutant cells exhibit increased numbers of telomere foci provides novel evidence that the nuclear envelope is a direct substrate for telomere clustering mechanisms.

DISCUSSION

In this study we identified a new role for Mps3p, and thus a direct role for the nuclear envelope, in chromosome organization within the nucleus and in associating with soluble chromatin metabolism factors. Our physical data, coupled with 2-hybrid interactions, reveals that the integral nuclear membrane protein Mps3p binds Est1p, a regulator of telomere length.^{20,21} Importantly, cells harboring mutations in both *mps3* and *est1* exhibit strong conditional growth defects, consistent with the model that Mps3p-Est1p binding is of physiological relevance. Est1p is a nuclear protein that is expressed in late G₁ and then binds to telomeres in late S-phase to recruit telomerase to telomeres.²²⁻²⁴ Our data suggest that after expression in late G₁, Est1p may be recruited to the nuclear envelope by Mps3p and held there until late S-phase when it is released and in turn binds telomeres. However, numerous attempts to directly demonstrate a role for Est1p-Mps3p association in telomere length homeostasis were unsuccessful, indicating that length-based defects associated with diminished Est1p-Mps3p binding are either below the limit of detection or are transient and repairable by alternate pathways (see Supplemental Fig. S2).

Based on the physical, functional, and genetic data linking Mps3p, Est1p and Ctf7p—several models can be envisioned and two are briefly explored here. In the first model, the nuclear envelope protein Mps3p recruits soluble DNA metabolism proteins before they are released to the telomere (Est1p) or replication fork (Ctf7p). Within this recruitment model, we consider the consequence of Mps3p binding to both Est1p and Ctf7p. In one scenario, Mps3p may tether Est1p to the nuclear envelope and away from telomeres—limiting

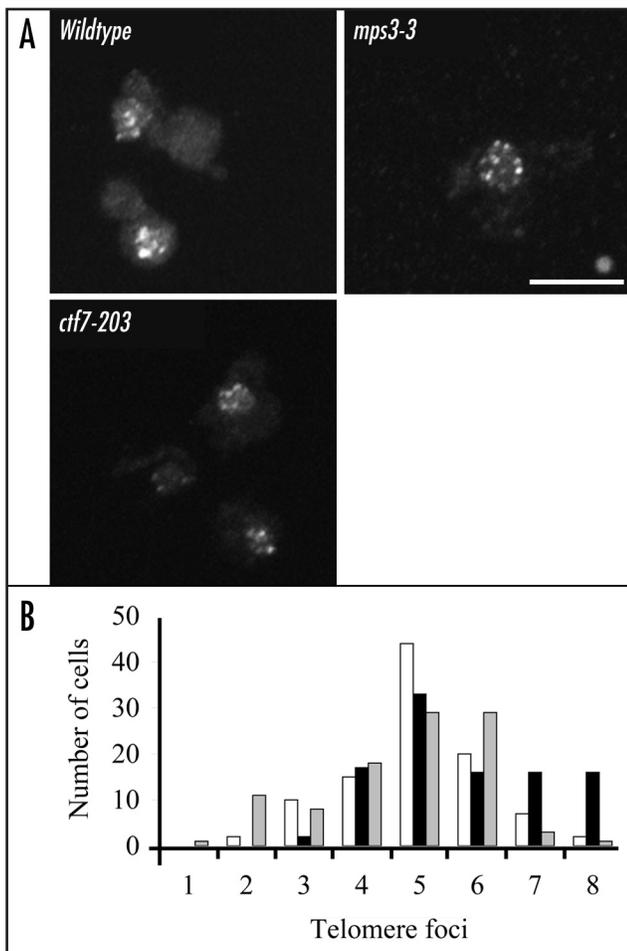


Figure 3. Visualization of telomere clustering in *mps3* and *ctf7-203* mutants. (A) 3-D maximum projections of G_1 arrested haploid cells. Rap1p staining (green) at the telomere indicates decreased telomere clusters in *mps3-3* cells, relative to wildtype and *ctf7* mutant cells. Scale bars = $5\mu\text{m}$. (B) Quantification of telomere clustering defects.

Est1p-dependent telomerase recruitment to the telomeres. In *mps3* mutant cells, Est1p is not sufficiently sequestered to the nuclear envelope and instead prematurely associates with telomeres. In turn, telomerase is then precociously recruited/activated at the telomeres. This model predicts that *mps3* mutants might result in abnormally long telomeres, however numerous experiments have yet to uncover a reproducible telomere lengthening effect (Supplemental Fig. S2). Mps3p may also act as a landing pad to pair Ctf7p with regulatory factors. In *mps3* mutant cells, Ctf7p may be only weakly activated, resulting in reduced cohesion function. In support of this model, Mps3p binds Ctf7p in vivo and *mps3* cells exhibit precocious sister chromatid separation.¹⁰⁻¹¹

In the second model, Mps3p directly links telomeres to the nuclear envelope. This model is in part supported by evidence that Mps3p is critical for efficient movement of telomeres into discrete clusters (this study). Previously, several groups showed telomere clustering and subnuclear organization of telomeres at the nuclear envelope during vegetative growth and meiosis in yeast.^{3,25} More recently, nuclear envelope proximity effects on telomere anchoring and silencing were reported for Esc1p.² However, none of these studies provide a direct link to the nuclear membrane. We tested the possibility that Mps3p might be the factor that promotes perinuclear anchoring of

silent chromatin,² but have thus far not found a tethering defect (Antoniacci L, Skibbens RV, unpublished results).

Our studies highlight the role of Mps3p to anchor soluble chromatin factors to the nuclear envelope. Recently, Jaspersen and colleagues identified Mps3p as a SUN domain protein.²⁶ The Mps3p SUN domain resides in the inter-membrane space bounded by the inner and outer nuclear envelope membranes. In that study, the SUN domain was shown to bind to the inter-membrane domain of Mps2p - tethering the spindle pole body to the spindle half-bridge.²⁶ SUN domain proteins are highly conserved through evolution and implicated in both nuclear positioning and karyogamy.²⁷ Thus, findings that the SUN domain Mps3 protein functions in nuclear envelope tethering is consistent with a model that Mps3p similarly tethers soluble chromatin metabolism factors and telomeres on the nucleoplasm side to intermembrane structures. We note that there is precedence that other nuclear transmembrane proteins such as Emerin/LBR and MAN1 play an analogous scaffold role in tethering gene repression and prereplication machinery to the nuclear envelope.²⁸ This story promises to become much more interesting as we continue to look to the periphery.

Note

Supplementary Material can be found at:

www.landesbioscience.com/supplement/antoniacciCC6-1-sup.pdf

References

- Hediger F, Neumann FR, Van Houwe G, Dubrana K, Gasser SM. Live imaging of telomeres: yKu and Sir proteins define redundant telomere-anchoring pathways in yeast. *Curr Biol* 2002; 12:2076-89.
- Taddei A, Hediger F, Neumann FR, Bauer C, Gasser SM. Separation of silencing from perinuclear anchoring functions in yeast Ku80, Sir4 and Esc1 proteins. *EMBO J* 2004; 23:1301-12.
- Gotta M, Laroche T, Formenton A, Mailet L, Schertan H, Gasser SM. The clustering of telomeres and colocalization with Rap1, Sir3, and Sir4 proteins in wild-type *Saccharomyces cerevisiae*. *J Cell Biol* 1996; 134:1349-63.
- Laroche T, Martin SG, Gotta M, Gorham HC, Pryde FE, Louis EJ, Gasser SM. Mutation of yeast Ku genes disrupts the subnuclear organization of telomeres. *Curr Biol* 1998; 8:653-6.
- Heun P, Laroche T, Shimad K, Furrer P, Gasser SM. Chromosome dynamics in the yeast interphase nucleus. *Science* 2001; 294:2181-6.
- Gartenberg MR, Neumann FR, Laroche T, Blaszyk M, Gasser SM. Sir-mediated repression can occur independently of chromosomal and subnuclear context. *Cell* 2004; 119:955-67.
- Jaspersen SL, Winey M. The budding yeast spindle pole body: Structure, duplication, and function. *Annu Rev Cell Dev Biol* 2004; 20:1-28.
- Jaspersen SL, Giddings Jr TH, Winey M. Mps3p is a novel component of the yeast spindle pole body that interacts with the yeast centrin homologue Cdc31p. *J Cell Biol* 2002; 159:945-56.
- Nishikawa S, Terazawa Y, Nakayama T, Hirata A, Makio T, Endo T. Nep98p is a component of the yeast spindle pole body and essential for nuclear division and fusion. *J Biol Chem* 2003; 278:9938-43.
- Antoniacci LM, Kenna MA, Uetz P, Fields S, Skibbens RV. The spindle pole body assembly component Mps3p/Nep98p functions in sister chromatid cohesion. *J Biol Chem* 2004; 279:49542-50.
- Antoniacci LM, Skibbens RV. Sister-chromatid telomere cohesion is nonredundant and resists both spindle forces and telomere motility. *Curr Biol* 2006; 16:902-6.
- Skibbens RV, Corson LB, Koshland D, Hieter P. Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. *Genes and Dev* 1999; 13:307-19.
- Kenna MA, Skibbens RV. Mechanical link between cohesion establishment and DNA replication: Ctf7p/Eco1p, a cohesion establishment factor, associates with three different replication factor C complexes. *Mol Cell Biol* 2003; 23:2999-3007.
- Toth A, Ciosk R, Uhlmann F, Galova M, Schleiffer A, Nasmyth K. Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. *Genes and Dev* 1999; 13:320-33.
- Skibbens RV. Unzipped and loaded: The role of DNA helicases and RFC clamp-loading complexes in sister chromatid cohesion. *J Cell Biol* 2005; 169:841-6.
- Warren CD, Eckley DM, Lee MS, Hanna JS, Hughes A, Peyser B, Jie C, Irizarry R, Spencer FA. S-phase checkpoint genes safeguard high-fidelity sister chromatid cohesion. *Mol Biol Cell* 2004; 15:1724-35.
- Mayer ML, Pot I, Chang M, Xu H, AneliunaV, Kwok T, Newitt R, Aebbersold R, Boone C, Brown GW, Hieter P. Identification of protein complexes required for efficient sister chromatid cohesion. *Mol Biol Cell* 2004; 15:1736-45.

18. Lundblad V. Telomere replication: An Est fest. *Curr Biol* 2003; 13:R439-41.
19. Smorgorzewska A, de Lange T. Regulation of telomerase by telomeric proteins. *Annu Rev Biochem* 2004; 73:177-208.
20. Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, Lockshon D, Narayan V, Srinivasan M, Pochart P, Qureshi-Emili A, Li Y, Godwin B, Conover D, Kalbfleisch T, Vijayadamar G, Yang M, Johnston M, Fields S, Rothberg JM. A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. *Nature* 2000; 403:623-7.
21. Hazbun TR, Malmstrom L, Anderson S, Graczyk BJ, Fox B, Riffle M, Sundin BA, Aranda JD, McDonald WH, Chiu CH, Snyderman BE, Muller EG, Fields S, Baker D, Yates IIIrd JR, Davis TN. Assigning function to yeast proteins by integration of technologies. *Mol Cell* 2003; 12:1353-65.
22. Evans SK, Lundblad V. The Est1 subunit of *Saccharomyces cerevisiae* telomerase makes multiple contributions to telomere length maintenance. *Genetics* 2002; 162:1101-15.
23. Taggart AK, Teng SC, Zakian VA. Est1p as a cell cycle-regulated activator of telomere-bound telomerase. *Science* 2002; 297:1023-6.
24. Takata H, Tanaka Y, Matsuura A. Late S phase-specific recruitment of Mre11 complex triggers hierarchical assembly of telomere replication proteins in *Saccharomyces cerevisiae*. *Mol Cell* 2005; 17:573-83.
25. Jin Y, Uzawa S, Cande WZ. Fission yeast mutants affecting telomere clustering and meiosis-specific spindle pole body integrity. *Genetics* 2002; 160:861-76.
26. Jaspersen SL, Martin AE, Glazko G, Giddings TH, Morgan G, Mushegian A, Winey M. The Sad1-UNC-84 homology domain in Mps3p interacts with Mps2p to connect the spindle pole body with the nuclear envelope. *J Cell Biol* 2006; 174:665-75.
27. Starr DA, Han M. ANChors away: An actin based mechanism of nuclear positioning. *J Cell Sci* 2003; 116:211-6.
28. Gruenbaum Y, Margalit A, Goldman RD, Shumaker DK, Wilson KL. The nuclear lamina comes of age. *Nat Rev Mol Cell Biol* 2005; 6:21-31.