Introduction

The three-dimensional organization of chromosomes within the nucleus of many organisms is nonrandom. Changes in chromosome position can have dramatic effects on gene expression by establishment of heritable, transcriptionally repressive subdomains. However, little is known about the integral membrane proteins that mediate telomere tethering at the nuclear envelope. Here, we find a previously unrecognized function for the *Saccharomyces cerevisiae* Sad1-UNC-84 domain protein Mps3 in regulating telomere positioning in mitotic cells. Our data demonstrate that the nucleoplasmic N-terminal acidic domain of Mps3 is not essential for viability. However, this acidic domain is necessary and sufficient for telomere tethering during S phase and the silencing of reporter constructs integrated at telomeres. We show that this is caused by the role of the Mps3 acidic domain in binding and localization of the silent information regulator protein Sir4 to the nuclear periphery. Thus, Mps3 functions as an integral membrane anchor for telomeres and is a novel nuclear receptor for the Sir4 pathway of telomere tethering and gene inactivation.

Telomere anchoring at the nuclear periphery requires the budding yeast Sad1-UNC-84 domain protein Mps3

Jennifer M. Bupp,1 Adriana E. Martin,1 Elizabeth S. Stensrud,1 and Sue L. Jaspersen1,2

1Stowers Institute for Medical Research, Kansas City, MO 64110
2Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS 66160

Positioning of telomeres at the nuclear periphery can have dramatic effects on gene expression by establishment of heritable, transcriptionally repressive subdomains. However, little is known about the integral membrane proteins that mediate telomere tethering at the nuclear envelope. Here, we find a previously unrecognized function for the *Saccharomyces cerevisiae* Sad1-UNC-84 domain protein Mps3 in regulating telomere positioning in mitotic cells. Our data demonstrate that the nucleoplasmic N-terminal acidic domain of Mps3 is not essential for viability. However, this acidic domain is necessary and sufficient for telomere tethering during S phase and the silencing of reporter constructs integrated at telomeres. We show that this is caused by the role of the Mps3 acidic domain in binding and localization of the silent information regulator protein Sir4 to the nuclear periphery. Thus, Mps3 functions as an integral membrane anchor for telomeres and is a novel nuclear receptor for the Sir4 pathway of telomere tethering and gene inactivation.
have been shown to bind to meiosis-specific telomere-binding proteins and play a role in the formation of the meiotic bouquet, a specialized clustering of telomeres at the nuclear envelope that occurs before meiosis I (Chikashige et al., 2006; Tang et al., 2006; Conrad et al., 2007; Ding et al., 2007; Penkner et al., 2007; Schmitt et al., 2007). The yeast SUN protein Mps3 has been shown to play a role in telomere clustering in mitotic cells (Antoniacci et al., 2007), suggesting that SUN proteins may also play a role in telomere tethering at the nuclear membrane during mitotic growth.

We generated mutants in the N-terminal acidic domain of Mps3 and found that this domain is not essential for viability or Mps3’s known function in spindle pole body (SPB) duplication (Jaspersen et al., 2002; Nishikawa et al., 2003). Instead, we found that the Mps3 N-terminal acidic domain functions to anchor chromosome arms at the nuclear envelope through the Sir4 pathway of telomere tethering. Thus, telomere positioning at the nuclear membrane is a conserved function that Mps3 plays in both mitotic and meiotic cells. The fact that mutants in the Mps3 N terminus also display defects in proper localization of Sir4 to the nuclear periphery and in telomeric silencing indicates that Mps3 likely functions as a nuclear membrane receptor for the SIR complex, which includes Sir4.

Results
Mps3 N-terminal mutants
To examine the role of Mps3 in telomere tethering during mitotic growth, we constructed a series of deletion mutants in the
nucleoplasmic Mps3 N terminus (Fig. 1 A) and transformed them into the LEU2 locus of a strain containing a complete deletion of the chromosomal copy of MPS3. These cells were kept alive by MPS3 on a centromeric URA3-marked plasmid. By plating cells to 5-fluoroorotic acid (5-FOA), we could select for loss of the URA3 plasmid containing MPS3 and analyze the growth phenotype of the N-terminal deletion mutants. We found that the Mps3 N terminus is not required for viability (Fig. 1 B), although its complete elimination (amino acids 2–150) did result in reduced fitness, SPB duplication defects, and a temperature-sensitive growth phenotype (not depicted).

Deletion of the acidic region in the Mps3 N terminus (amino acids 75–150) did not result in any apparent growth abnormalities nor did cells display defects in SPB duplication (Fig. 1, B–D). It also did not affect localization to the SPB, although levels of mps3Δ75–150 fused to GFP (mps3Δ75–150–GFP) were reduced compared with Mps3–GFP (Fig. 1, E and F). Analysis of the nuclear envelope staining pattern of Mps3–GFP by single photon imaging methods revealed that Mps3–GFP was present in multiple punctuate foci on the nuclear envelope in 96% of cells imaged (n = 93; Fig. 1 F). mps3Δ75–150–GFP, however, showed significantly reduced nuclear envelope staining and a decrease in foci formation; only 8% of cells showed any signal in the peripheral nuclear membrane besides the SPB signal (n = 71; Fig. 1 F). Loss of mps3Δ75–150–GFP localization to the nuclear envelope was rescued by coexpression of a wild-type, untagged copy of MPS3 (n = 68; Fig. 1 G), indicating that our inability to detect mps3Δ75–150–GFP at the nuclear membrane is not the result of detection methods but rather reflects a requirement for the N-terminal acidic domain in localization of Mps3 to the nuclear periphery.

The Mps3 acidic domain is required for telomere tethering
Using the new mps3Δ75–150 allele, we could test the requirement of the Mps3 N terminus in telomere tethering at the nuclear periphery. Tandem repeats of the lactose operator (LacO₉) were integrated into individual telomeres, and expression of a GFP fusion to the DNA-binding region of the lactose repressor (GFP-LacI) allowed visualization of the telomeric locus in living cells. The position of each spot relative to the nuclear periphery, as well as nuclear diameter, was monitored by coexpression of the nucleoporin Nup49 fused to GFP (NUP49-GFP, Fig. 2 A). By dividing the locus-to-periphery distance by the nuclear diameter, we assigned each spot a position into one of three concentric zones of equal volume that approximates its position within the nucleus (Hediger et al., 2002). Loci in the most peripheral zone (zone 1) are anchored to the nuclear envelope, whereas loci in the intermediate zone (zone 2) region may have partial contacts with the nuclear envelope. The innermost zone (zone 3) contains loci that are not associated with the nuclear periphery.

Examination of LacO₉ integrated at telomere VI-R, VIII-L, or XIV-L in wild-type cells revealed the preferential association of telomeres with the nuclear periphery that others have previously observed (Fig. 2 B; Hediger et al., 2002). In mps3Δ75–150 mutants, the nuclear envelope appeared intact and was similar to wild-type cells based on Nup49-GFP epifluorescence as well as immunofluorescence analysis with other nucleoporin antibodies (unpublished data). However, association of telomeres with the nuclear envelope was lost and chromosome ends assumed a random distribution within the nucleus (Fig. 2 B). The fact that we observed loss of anchoring of all three telomeres, which are known to have different molecular requirements for nuclear envelope association (Hediger et al., 2002), suggests that the Mps3 N terminus plays a fundamental role in telomere tethering at the nuclear periphery.

The Mps3 N terminus is sufficient to restore telomere tethering to mps3Δ75–150 mutants
We expressed Mps3 N-terminal fragments from the MPS3 promoter in mps3Δ75–150 mutants to determine the minimal region of Mps3 necessary for recruitment of telomeres to the nuclear periphery (Fig. 3 A). Although none of these N-terminal constructs was able to complement growth of mps3Δ cells (not depicted), the soluble Mps3 N-terminal domain consisting of amino acids 1–150, as well as a version of Mps3 containing the entire N terminus

Figure 2. mps3Δ75–150 mutants have defects in telomere tethering. (A) Yeast cells expressing GFP-LacI and Nup49-GFP fusions were tagged with ~256 copies of the LacO₉ at telomere VI-R, VIII-L, or XIV-L. The subnuclear position of the telomere was scored with respect to the distance from the nuclear envelope in a single plane image and assigned a position into one of three zones of equal volume (see text for details). Bar, 5 μm. (B) The distribution of telomere ends in zone 1 (black), zone 2 (white), and zone 3 (gray) was determined in asynchronously growing wild-type and mps3Δ75–150 cells at 30°C for each telomere. The dotted line at 33% corresponds to a random distribution. Confidence values (P) for the χ² test were calculated for each dataset between random and test distributions. The number of cells examined in each dataset is indicated (n).
and the transmembrane domain (mps3 1–181), was able to restore tethering of both telomeres VI-R and XIV-L to the nuclear membrane in mps3 Δ75–150 mutants (Fig. 3 B). A version of the Mps3 N terminus lacking the acidic domain (mps3 1–75) failed to efficiently anchor telomeres (Fig. 3 B), suggesting that the acidic domain may be involved in an important binding interaction that tethers the chromosome ends at the nuclear periphery. Alternatively, mps3 1–75 may simply not be targeted correctly to the nucleus, although it is small enough to freely diffuse through nuclear pore complexes.

**mps3Δ75–150 mutants are defective in S phase telomere tethering**

In G1 phase of the cell cycle, yKu70–yKu80–dependent tethering is the dominant mechanism of nuclear envelope localization of telomeres, whereas binding to the Sir4–Esc1 complex is thought to keep telomeres at the nuclear periphery during S phase (Hediger et al., 2002; Taddei et al., 2004a). We examined recruitment of telomeres VI-R and XIV-L to the nuclear envelope in G1 and S phase cells, which were scored in an asynchronous population of cells based on bud morphology, to determine if the yKu70–yKu80 or Sir4–Esc1 pathway is predominately affected in mps3 Δ75–150 mutants. Over 50 and 44% of G1 mps3 Δ75–150 cells contained telomeres VI-R and XIV-L, respectively, in the outermost zone of the nucleus compared with 34 and 29% of S phase cells (Fig. 4). This suggests that telomere tethering is most affected in S phase cells in mps3 Δ75–150 mutants and that Mps3 is likely involved in the Sir4–Esc1 pathway of telomere recruitment to the nuclear membrane.

**The Mps3 acidic domain interacts with Sir4**

To test if Mps3 physically interacts with Sir4, Esc1, or yKu70, we immunoprecipitated Mps3 fused at its C terminus to three copies of the HA epitope (Mps3-HA3) from yeast cells that had been lysed after liquid nitrogen grinding and Western blotted with anti-FLAG (to detect yKu70-FLAG3), anti-MYC (to detect Esc1-MYC13), or Sir4 antibodies. Mps3-HA3 bound to Sir4 (Fig. 5 A), but it did not reproducibly bind to Esc1 or yKu70 (not depicted). Binding was dependent on the Mps3 acidic domain, as mps3 Δ75–150–HA3 showed significantly reduced interaction with Sir4 (Fig. 5 A). We were unable to coimmunoprecipitate...
Mps3-HA3 with Sir4 from sir2Δ or Δ3 cells (Fig. 5 B), indicating that stable Sir4 binding to Mps3 is dependent on other members of the SIR complex. However, Sir4 binding to Mps3 did not depend on yKu70, Esc1, or the myosin-like proteins Mlp1 and 2, which play a role in nuclear membrane organization, telomere length regulation, and SPB assembly (Strambio-de-Castillia et al., 1999; Hediger et al., 2002; Niepel, et al., 2005). The ability of Mps3 to bind to Sir4 in the absence of Esc1 suggests that Mps3 could be a novel nuclear receptor for silencing complexes at the nuclear periphery during S phase.

We observed a two-hybrid interaction between the Mps3 N terminus and a Sir4 fragment containing amino acids 839–934 but not with a fragment containing amino acids 934–1358 (Fig. 5 C), indicating that amino acids 839–934 of Sir4 are required for Mps3 binding. This is adjacent to the predicted binding site of Esc1, which is located at amino acids 950–1262 (Andrulis et al., 2002), and overlaps the Sir2 binding site, which is located at amino acids 731–1358 (Cockell et al., 2000). We were unable to coimmunoprecipitate a bacterially expressed and purified Mps3 N terminus and Sir4 C terminus (unpublished data), suggesting that additional proteins such as Sir2 and 3, posttranslational modifications, and/or DNA itself are important for Mps3 binding to Sir4 in vitro.

Next, we wanted to examine the effect that the loss of Mps3 binding had on Sir4 localization in vivo. In 42% of wild-type cells enriched in S phase with hydroxyurea, Sir4 was present at multiple foci at the nuclear envelope (n = 100; Fig. 5 D). However, only 9% of mps3Δ 75–150 cells maintained this staining pattern (n = 100). Most mps3Δ 75–150 mutant cells contained an increased amount of Sir4 protein present in the diffuse nucleolar region that we frequently observed in the mutant (Fig. 5 D; unpublished data). Collectively, these data indicate that Mps3 interacts with Sir4 and functions to tether it throughout the nuclear periphery.

**Chromosome recruitment using LexA-Sir4<sup>839–934</sup>**

To further demonstrate the importance of binding between the Mps3 acidic domain and amino acids 839–934 of Sir4, we took advantage of a chromatin localization assay to test if Sir4<sup>839–934</sup> could impart a specific localization to an otherwise randomly positioned chromosomal segment in an Mps3-dependent manner (Taddei et al., 2004a). We constructed a fusion between the coding sequence for amino acids 839–934 of Sir4, the LexA DNA-binding domain, and the nuclear localization sequence from SV40 large T antigen (LexA-Sir4<sup>839–934</sup>) and expressed it...
from the galactose-inducible GAL1 promoter in wild-type, mps3AΔ75–150, and esc1Δ cells. These cells also contained four LexA operators (lexAop) linked to tandem copies of the LacO₅ linked to one of two loci on chromosome VI: the transcriptionally active ARS607 locus, which is located on the arm of chromosome VI >50 kb from either the centromere or the telomere (Fig. 6 A), or the subtelomeric ARS609 locus, 23 kb from telomere VI-R, which has been truncated by replacement of normal subtelomeric elements with an ADE2 reporter gene linked to terminal TG₁₃ telomeric repeats (Fig. 6 C; Hediger et al., 2002).

Analysis of chromosome position revealed that LexA-Sir4839–934 was able to efficiently tether chromosome arm sequences to the nuclear periphery in wild-type and esc1Δ cells but not in mps3Δ75–150 mutants (Fig. 6 B). In addition, expression of LexA-sir4839–934 was also able to overcome the partial telomere tethering defect observed in esc1Δ mutants (Fig. 6 D; Taddei et al., 2004a), but it had no effect on mps3Δ75–150 cells, which also have a partial tethering defect of the truncated telomere VI-R (Fig. 6 D). This strongly suggests that amino acids 839–934 of Sir4 interact with the Mps3 acidic domain at the nuclear periphery and that the effects of this domain on chromosome recruitment to the membrane are independent of Esc1 binding.

Figure 6. LexA-Sir4839–934 can recruit chromosomes to the nuclear periphery in the presence of the Mps3 acidic domain. (A) Schematic of ~256 LacO₅ and four lexAop binding sites integrated at ARS607, which is located on the arm of chromosome VI ~50 kb from the centromere and 70 kb from the telomere. (B) Wild-type, mps3Δ75–150, and esc1Δ cells that contained this version of chromosome VI as well as a galactose-inducible LexA-sir4839–934-NLS (SU2651, SU2652, and SU2653, respectively) were grown overnight in YEP/2% raffinose at 30°C to mid-log phase. Cultures were divided and 2% dextrose was added to repress expression (~) and 2% galactose was added to induce expression of LexA-sir4839–934-NLS for 2 h at 30°C. Localization of arm VI to zone 1 (black), 2 (white), and 3 (gray) was then analyzed. The dotted line at 33% corresponds to a random distribution. Confidence values (P) for the chi² test were calculated for each dataset between random and test distributions. The number of cells examined in each dataset is indicated (n). (C) Schematic of ~256 LacO₅ and four lexAop binding sites integrated at ARS609, near telomere VI-R. The subtelomeric sequence of telomere VI-R has also been truncated by the insertion of an ADE2 reporter linked to copies of the TG₁₃ telomeric repeats (Hediger et al., 2002). (D) Localization of truncated telomere VI-R in wild-type, mps3Δ75–150, and esc1Δ cells that contained a galactose-inducible LexA-sir4839–934-NLS (SU2647, SU2648, and SU2649, respectively), determined as described in B. (E) Expression of the telomeric ADE2 gene in strains from D was monitored by streaking cells to SD plates containing 10 μg/ml adenine and 2% dextrose or 2% galactose/2% raffinose to repress (~) or induce the expression of LexA-sir4839–934-NLS, respectively. After growth for 3 d at 30°C, plates were incubated for 1 wk at 4°C to allow the red pigment to develop. Expression of ADE2 results in white colored cells and blocks the accumulation of the red pigment in this strain background; this occurs in cells that have lost telomeric silencing. Bar, 1 cm.

Telomere silencing defects in mps3Δ75–150 mutants

These same strains contain an ADE2 marker that allowed us to determine if mps3Δ75–150 mutants have defects in transcriptional regulation of telomeric genes as a result of the loss of telomere tethering. Wild-type cells appeared as sectored red and white colonies after growth on plates containing limiting amounts of adenine caused by the stochastic expression of ADE2 (Fig. 6 E). ESC1 has previously been shown to be partially required for telomeric silencing (Andrulis et al., 2002; Taddei et al., 2004a), and, consistent with these reports, colonies harboring esc1Δ appeared light pink to white in color because of increased expression of ADE2 at the truncated telomere (Fig. 6 E). mps3Δ75–150 mutants also did not show sectoring, and colonies were light pink to white in color (Fig. 6 E), indicating that telomere silencing is decreased in cells lacking the Mps3 acidic domain. Furthermore, induction of LexA-sir4839–934 was able to rescue the silencing defect of esc1Δ but not mps3Δ75–150 mutants (Fig. 6 E). Collectively, these results demonstrate that defects in telomere tethering in mps3Δ75–150 mutants are directly correlated with effects on telomeric gene expression; the fact that neither can be rescued by LexA-sir4839–934 strongly suggests...
TELOMERE ANCHORING BY MPS3 • BUPP ET AL.

Discussion

In this paper, we have shown that the SUN protein Mps3 plays a role in the telomere position effect and telomere tethering at the nuclear periphery through the interaction of its N-terminal acidic domain with Sir4. As the first integral membrane protein in budding yeast known to play a role in chromosome positioning during mitotic growth, Mps3 could function to regulate chromatin structure or simply act as a DNA tether at the nuclear periphery. Based on the ability of LexA-sir4_{139-931} to recruit both telomeric as well as actively transcribed chromosome arm DNA to the nuclear membrane in an Mps3-dependent manner, we propose that the primary function of Mps3 is to be a DNA/chromatin anchor. Additional support for this hypothesis comes from recent findings that mps3-3 mutants fail to cluster telomeres at the nuclear periphery (Antoniacci et al., 2007), of the role of Mps3 in formation of the meiotic bouquet (Conrad et al., 2007), and of the fact that the N terminus lacks any motifs of functional significance that would suggest a role in chromatin assembly (Jaspersen et al., 2006).

that the interaction between the Mps3 N terminus and Sir4 is important for telomere tethering and the telomere position effect.

In addition to telomeres, the mating-type loci and the ribosomal DNA (rDNA) locus are also silenced from transcription by RNA polymerase II. We analyzed transcriptional regulation at these loci in mps3Δ75–150 mutants using a strain containing reporter constructs to allow simultaneous detection of expression at telomere V-R, the silent mating-type locus HMR, and the rDNA (Fig. 7 A; Ray et al., 2003). Consistent with our telomere VI-R findings, we observed derepression of URA3 integrated at telomere V-R in mps3Δ75–150 mutants, resulting in decreased growth on plates containing the suicide substrate 5-FOA (Fig. 7 B). Interestingly, we also observed a decrease in silencing at the rDNA locus in mps3Δ75–150 cells, whereas repression at HMR was increased ~10-fold (Fig. 7 B). The silencing defects observed in mps3Δ75–150 mutants are not as great as in sir2Δ cells, but the fact that mps3Δ75–150 sir2Δ double mutants do not have additive effects on silencing is consistent with the possibility that Mps3 and Sir2 are in the same pathway of transcriptional repression (Fig. 7 B).

Expression of the Mps3 N-terminal fragments mps3 1–150 and mps3 1–181 was able to restore telomere tethering at the nuclear periphery in mps3Δ75–150 mutants. We also found that when we expressed either mps3 1–150 or mps3 1–181 from the MPS3 promoter on a 2-μm plasmid in mps3Δ75–150 cells, it was sufficient to restore silencing at HMR, rDNA, and telomere V-R to similar levels observed in the wild type (Fig. 8). Therefore, the Mps3 N terminus is necessary and sufficient for both telomere tethering and the telomere position effect.
Based on our chromosome recruitment studies with LexA-
sir4Δ39–93A, we currently favor a model in which Mps3 does not
directly bind DNA but rather associates with chromosomes via
interaction of the N terminus with other proteins, including
Sir4. Binding to chromatin-associated proteins such as Sir4
would allow Mps3 to recognize particular chromosome do-
 mains for selective tethering at the nuclear periphery. Given that
we were unable to coimmunoprecipitate recombinant Mps3
and Sir4, it seems likely that the interaction between the Mps3
N terminus and Sir4 is facilitated by additional proteins or chro-
matin. Obvious candidates include Sir2 and 3 because binding
between Mps3 and Sir4 in vivo was dependent on both of these
proteins. Other candidates include the telomere elongation fac-
tor Est1, the establishment of cohesion protein Eco1/Ctfl, the
protein kinase Cdc5, and the histone 2A variant Htz1, all of
which are chromosome-interacting proteins that bind to Mps3
in the two-hybrid system (Uetz et al., 2000; Antoniacci et al.,
2004; Antoniacci et al., 2007).

The silencing factors Sir2, 3, and 4 are present in limiting
concentrations in the yeast nucleus except at the telomeres
(Buck and Shore, 1995; Maillet et al., 1996; Marcard et al.,
1996). Localization of silencing proteins as well as telomeres to
the nuclear periphery is thought to create a subcompartment of
the nucleus that favors silencing and protects the rest of the
genome from aberrant transcriptional repression by the SIRs (for
review see Akhtar and Gasser, 2007). Binding of Sir4 to Mps3
at the nuclear membrane could be an early step in the formation
of silent heterochromatin at the telomeres, although Mps3 also
may associate with telomere binding proteins to directly mediate
recruitment of chromosome ends to the periphery (Antoniacci
and Skibbens, 2006). Consistent with a role in recruitment of Sir4
and/or telomeres at the nuclear periphery, we observed a loss of
telomeric silencing in mps3Δ75–150 mutants. However, the

fact that we also did not observe a decrease in silencing at HMR
indicates that the Mps3 N terminus does not play an integral
role in silencing per se by altering chromatin structure but rather
is additional evidence for our hypothesis that Mps3 is a nuclear
envelope anchor. Increased silencing at the mating type loci in
mps3Δ75–150 mutants is likely caused by dissociation of SIR
proteins from telomeres followed by their redistribution to in-
ternal chromosomal sites such as HML and HMR, as has been
observed previously for other mutants in telomere and SIR teth-
ering (Martin et al., 1999; Hediger et al., 2002; Taddei et al.,
2004a). Our analysis of Sir4 staining demonstrates that at least
one component of the SIR complex is relocalized in mps3Δ75–150
mutants. However, the effects that this change in localization
has on DNA binding of Sir4 and other components of the SIR
complex at a molecular level are currently unknown.

Interaction between Mps3 and chromatin-associated proteins
including Sir4 is likely important for proper localization of
Mps3 to the inner nuclear membrane because Mps3 lacks any
nuclear targeting information (Lusk et al., 2007). Loss of bind-
ing to nuclear proteins would result in a lack of Mps3 retention
in the peripheral nuclear envelope; this is the phenotype we ob-
served with mps3Δ75–150-GFP. The fact that Mps3 N-terminal
fragments are able to restore both telomere positioning and
silencing suggests that they contain information sufficient for
nuclear localization, either through binding to mps3Δ75–150
protein still present in the cell or nuclear localization  information
present within the N-terminal fragments themselves. Localization
of other SUN proteins to the inner nuclear envelope requires
determinants in both the N- and C-terminal regions and in many
cases involves interaction between the SUN protein and nuclear
proteins, such as the lamins (Padmakumar et al., 2005; Starr
and Fischer, 2005; Crisp et al., 2006; Haque et al., 2006; Hasan et al.,
2006; Wang et al., 2006; Liu et al., 2007).
Isolation and characterization of mps3Δ75–150 mutants indicates that the role of Mps3 in telomere positioning and gene regulation is separable from its function at the SPB. In addition, the fact that different regions of the Mps3 N terminus and distinct Mps3 binding partners appear to be required for mitotic versus meiotic telomere tethering (Conrad et al., 2007) strongly suggests that distinct mechanisms of chromosome positioning likely exist during the different growth phases. The involvement of Mps3 in telomere tethering during both vegetative and meiotic growth raises the interesting possibility that other SUN proteins will also play a role in chromosome positioning during mitotic growth. Given the known connection between SUN proteins and the cytoskeleton, an additional interaction with chromatin could represent a novel mechanism of communication between the cytoplasm and the nucleus that does not require transport through the nuclear pore complex.

Materials and methods

Yeast strains and plasmids

All strains are derivatives of W303 (ade2-1, his3-11,15, leu2-3,12, trp1-1, ura3-1). All strains are derivatives of W303 (ade2-1, his3-11,15, leu2-3,12, trp1-1, ura3-1) and are listed in Table S1 (available at http://www.jcb.org/cgi/content/full/jcb.200706040/DC1). Standard techniques were used for DNA and yeast manipulations.

Deletions in the Mps3 N terminus were constructed in ps1148 (pRS305-MPS3) by introducing in-frame Nhel restriction sites to remove to the corresponding coding sequence after digestion and religation. Wild-type and mutant forms were digested with BstElF to direct integration into the LEU2 locus of SU2039 and single copy integration was verified by Southern blotting.

N-terminal truncation mutants in MPS3 were created in pRS306 and pRS423 by PCR amplification of the indicated region of the MPS3 open reading frame along with ~800 bp of promoter as XhoI–BamHI fragments. Plasmids were digested with XcmI to target integration to URA3 or were directly transformed into yeast.

To create pRS306–MPS3–GFP and pRS306–mps3Δ75–150–GFP, the coding sequence for GFP was first amplified by PCR and inserted into the XbaI–SacI sites of pRS306. MPS3 and mps3Δ75–150, including ~800 bp of promoter sequence, were then amplified by PCR and ligated in frame to the GFP at the KpnI–BamHI sites. Both plasmids were digested with EcoRV to direct integration into the URA3 locus of a diploid strain containing mps3Δ4–NATMX. Haploid strains were recovered by sporulation.

Constructs for two-hybrid analysis were made by PCR amplification of the indicated regions of MPS3 and SIR4 with primers containing NcoI and XhoI ends. After digestion, the products were cloned into the Ncol–SalI sites of pOAD1 or pOBD2 (Izet et al., 2000). pOAD1 plasmids were transformed into the prey strain SU1644, and pOBD2 plasmids were transformed into the bait strain SU1645.

The LexA DNA-binding domain was amplified by PCR as a Xhol–NcoI fragment and ligated into ps1100, a plasmid derived from pRS303 containing the GAL1/10 promoter at KpnI–XhoI, LacI at BamHI, and SV40 NLS at BamHI–Xbal. Next, the region of SIR4 encoding amino acids 839–934 was amplified as a Ncol–BglII fragment and cloned into the Ncol–BamHI sites of the previous vector. The entire GAL-LexA-sir4 (839-934)-NLS insert was then moved into pRS306 using KpnI–SacI to create pRS306-GALLexA-sir4 (839-934)-NLS, which was digested with Stul to target integration into the URA3 locus.

Cytological techniques

Analyses of DNA content by flow cytometry and protein localization by indirect immunofluorescence and epifluorescence microscopy were performed as described previously (Jaspersen et al., 2002, 2006). The following primary antibody dilutions were used: 1:500 rat anti-tubulin YOL1/34 (Accurate Chemical and Scientific Corporation), 1:500 affinity purified rabbit anti-Tub4 (Jaspersen et al., 2002), and 1:1,000 goat anti Sir4 (Santa Cruz Biotechnology, Inc.) antibodies. Secondary antibodies included 1:10,000 Cy3-conjugated goat anti–rabbit IgG (Millipore), 1:200 fluorescein-conjugated goat anti–rat IgG (Millipore), and 1:10,000 Alexa 555–conjugated donkey anti–goat IgG (Invitrogen). DNA was visualized by staining with 1 μg/ml DAPI for 5 min immediately before mounting with Citifluor (Ted Pella, Inc.). Cells were examined at room temperature with an imaging system (AxioImager; Carl Zeiss, Inc), using a 100× α-Plan Fluor objective (NA 1.45; Carl Zeiss, Inc.), and images were captured with a digital camera (Orca ER; Hamamatsu) and processed using Axiovision 4.6.3 (Carl Zeiss, Inc.). Constrained iterative deconvolution was used to generate the images presented in Fig. 5 D whereas images in Fig. 1 D are single z section slices.

To analyze nuclear envelope staining of Mps3–GFP, cells were grown to mid-log phase in synthetic complete media supplemented with 5× adenine and placed onto 25% gelatin pads as described previously (Maddock et al., 2000). Imaging was performed on a laser scanning microscope (LSM 510 META; Carl Zeiss, Inc.) equipped with a ConfoCor3 module with avalanche photodiode detectors (Carl Zeiss, Inc.) using a 100× α-Plan Fluor lens (NA 1.45) at room temperature. Excitation of Mps3–GFP was performed with a 488-nm Argon laser line and the appropriate filter sets. Data was acquired using AIM software (Carl Zeiss, Inc.). High resolution volume renderings of the acquired three-dimensional images were generated using software (Velocity 4.0; Improvision) and projected as two-dimensional images in Fig. 1 F and G.

The position of GFP spots was determined as described previously (Hediger et al., 2002; Todde et al., 2004a). In brief, an imaging system (AxioImager) with a 100× α-Plan Fluor objective (NA 1.45) and a digital camera (Orca ER) were used to capture 19-image stacks of 170-nm step size through nuclei of log-phase cells at room temperature. The spot-to-periphery distance and nuclear diameter were determined in a single z stack image where the spot was most concentrated using Axiovision 4.6.3, except in cases when the spot fell into one of the top or bottom three focal planes. By dividing the spot-to-periphery distance by the diameter, each spot fell into one of three zones of equal surface (Fig. 2 A). Zone 1 has a width of 0.184 × the nuclear radius (r), zone 2 has a width of 0.422 × r, and zone 3 has a width of 0.422 × r. Confidence values (P) for the χ2 test were calculated for each dataset between random and test distributions.

Immunoprecipitation and Western blotting

Liquid nitrogen ground lysates were prepared from 500 OD600 of mid-log phase cells as described previously (Jaspersen et al., 2006). 50 μl of anti-HA resin (Roche) was added to lysates to immunoprecipitate Mps3–HA3. After 2 h of incubation at 4°C, beads were washed five times and 1/10 of the bound protein was analyzed by SDS-PAGE followed by Western blotting. The following primary antibody dilutions were used: 1:1,000 anti-HA 16812 (Covance), 1:1,000 anti-GFP B34 (Covance), 1:2,000 anti-glucose–6-phosphate dehydrogenase (Sigma–Alich), and 1:1,000 anti-Sir4 (Santa Cruz Biotechnology, Inc.). Alkaline phosphatase–conjugated secondary antibodies were used at 1:10,000 (Promega).

Online supplemental material

Table S1 shows yeast strains used. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200706040/DC1.

We thank K. Ruge, D. Moazed, S. Gasser, O. Cohen-Fix, and D. Gotschlich for strains and plasmids. We are grateful to S. Gasser for valuable advice and to J.C. Workman, P. Baumann, S. Hawley, J. Getton, M. Winey, and S. Biggins for comments on the manuscript.

S.I. Jaspersen is supported by the Stowers Institute for Medical Research, a Special Fellow Award from the Leukemia and Lymphoma Society, and a Basil O’Connor Award from the March of Dimes.

Submitted: 7 June 2007
Accepted: 30 October 2007

References

Akhtar, A., and S.M. Gasser. 2007. The nuclear envelope and transcriptional control. Nat. Rev. Genet. 8:507–517.

Andrulis, E.D., A.M. Neiman, D.C. Zappulla, and R. Stern galanz. 1998. Peri nuclear localization of chromatin facilitates transcriptional silencing. Nature. 394:592–595.

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

Antonacci, L.M., and R.V. Skibbens. 2006. Sister chromatid telomere cohesion is nonredundant and resists both spindle forces and telomere motility. Curr. Biol. 16:902–906.

TELOMERE ANCHORING BY MPS3 • BUPP ET AL.
Antoniacci, L.M., M.A. Kenna, P. Uetz, S. Fields, and R.V. Skibbens. 2004. The spindle pole body assembly component Mps3p/Nep98p functions in sister chromatid cohesion. *J. Biol. Chem.* 279:49542–49550.

Antoniacci, L.M., M.A. Kenna, and R.V. Skibbens. 2007. The nuclear envelope and spindle pole body-associated Mps3 protein bind telomere regulators and function in telomere clustering. *Cell Cycle.* 6:75–79.

Buck, S.W., and D. Shore. 1995. Action of a Rap1p carboxy-terminal silencing domain reveals an underlying competition between HMR and telomeres in yeast. *Genes Dev.* 9:370–384.

Chikashige, Y., C. Tsutsumi, M. Yamane, K. Okamasa, T. Haraguchi, and Y. Hiraoka. 2006. Meiotic proteins bqt1 and bqt2 tether telomeres to form the bouquet arrangement of chromosomes. *Cell.* 125:59–69.

Cockell, M.M., S. Perrod, and S.M. Gasser. 2000. Analysis of Sir2p domains required for RNA and telomeric silencing in *Saccharomyces cerevisiae.* *Genetics.* 154:1069–1083.

Conrad, M.N., C.-Y. Lee, J.L. Wilkerson, and M.E. Dresser. 2007. MPS3 mediates meiotic bouquet formation in *Saccharomyces cerevisiae.* *Proc. Natl. Acad. Sci. USA.* 104:8063–8068.

Crisp, M., Q. Liu, K. Roux, J.B. Rattner, C. Shanahan, B. Burke, P.D. Stahl, and D. Hochstr. 2006. Coupling of the nucleus and cytoplasmic role of the LINC complex. *J. Cell Biol.* 172:41–53.

Ding, X., R. Xu, J. Yu, T. Xu, Y. Zhuang, and M. Han. 2007. SUN1 is required for telomere attachment to the nuclear envelope and gametogenesis in mice. *Dev. Cell.* 12:863–872.

Fraser, P., and W. Bickmore. 2007. Nuclear organization of the genome and the potential for gene regulation. *Nature.* 447:413–417.

Gartenberg, M.R., F.R. Neumann, T. Laroche, M. Blaszczyk, and S.M. Gasser. 2004. Sir-mediated repression can occur independently of chromosome subnuclear contexts. *Cell.* 119:955–967.

Gottschling, D.E., O.M. Aparicio, B.L. Billington, and V.A. Zakian. 1990. Evidence for silencing compartments within the yeast nucleus: a role for telomere proximity and Sir protein concentration in silencing factors at telomeres by Rap1 protein. *Annu. Rev. Genet.* 23:1301–1312.

Hasan, S., S. Guttinger, P. Mullhauser, F. Anderggen, S. Burgler, and U. Kutay. 2006. Nuclear envelope localization of human UNC84A does not require nuclear lamins. *EMBO J.* 25:403–413.

Hamada, K., T. Laroche, A. Formenton, L. Maillet, H. Schertahn, and S. Gasser. 2006. The clustering of telomeres and colocalization with Rap1, Sir3, and Sir4 proteins in wild-type *Saccharomyces cerevisiae.* *J. Cell Biol.* 134:1349–1363.

Jaspersen, S.L., T.H. Giddings Jr., and M. Winey. 2002. Mps3p is a novel component of the yeast spindle pole body assembly component Mps3p/Nep98p functions in sister chromatid cohesion. *Genetics.* 144:839–855.

Jiang, Y., R. Benavente, D. Hodicz, C. Hoog, C.L. Stewart, and M. Altschuler. 2007. Transmembrane protein Sun2 is involved in tethering mammalian meiotic telomeres to the nuclear envelope. *Proc. Natl. Acad. Sci. USA.* 104:7426–7431.

Niepel, M., C. Strambio-de-Castillia, J. Fasolo, B.T. Chait, and M.P. Rout. 2005. The nuclear pore complex–associated protein, Mlp2p, binds to the yeast spindle pole body and promotes its efficient assembly. *J. Cell Biol.* 170:225–235.

Nishikawa, S., Y. Terazawa, T. Nakayama, A. Hirata, T. Makio, and T. Endo. 2003. Nrp99p is a core component of the nuclear pore complex in yeast. *J. Cell Sci.* 118:3419–3430.

Niepel, M., C. Strambio-de-Castillia, J. Fasolo, B.T. Chait, and M.P. Rout. 2005. The nuclear pore complex–associated protein, Mlp2p, binds to the yeast spindle pole body and promotes its efficient assembly. *J. Cell Biol.* 170:225–235.

O. H 2000. The polarity and dynamics of membrane protein Sun1 mediates the anchorage of Nesprin-2 to the nuclear envelope. *J. Cell Sci.* 113:3419–3430.

Pentker, A., L. Tang, M. Novatchkova, M. Ladurner, A. Fridkin, Y. Gruenbaum, D. Schweizer, J. Lodv, and V. Jantsch. 2007. The nuclear envelope protein Matefin/SUN-1 is required for homologous pairing in *C. elegans* meiosis. *Dev. Cell.* 12:873–885.

Ray, A., R.E. Hector, N. Roy, J.-H. Song, K.L. Berkner, and K.W. Runde. 2003. Sir3p phosphorylation by the Sir2p pathway effects redistribution of silencing function and shortened lifespan. *Nat. Genet.* 33:522–526.

Rusche, I.N., A.L. Kirchmaier, and J. Rine. 2003. The establishment, inheritance, and function of silenced chromatin in *Saccharomyces cerevisiae.* *Annu. Rev. Biochem.* 72:481–516.

Schmidt, J., R. Benavente, D. Hodicz, C. Hoog, C.L. Stewart, and M. Altschuler. 2007. Transmembrane protein Sun2 is involved in tethering mammalian meiotic telomeres to the nuclear envelope. *Proc. Natl. Acad. Sci. USA.* 104:7426–7431.

Starr, D.A., and J.A. Fischer. 2005. KASH ‘n Kary: the KASH domain family of cargo-specific cytoskeletal adaptor proteins. *Bioessays.* 27:1136–1146.

Strambio-de-Castillia, C., G. Blobel, and M.P. Rout. 1999. Proteins connecting the nuclear envelope localization of human UNC84A does not require the nuclear lamina and the cytoskeleton. *J. Cell Biol.* 144:839–855.

Taddei, A., F. Hediger, F.R. Neumann, C. Bauer, and S.M. Gasser. 2004a. Separation of silencing from perinuclear anchoring functions in yeast Ku80, Sir4 and Es1 proteins. *EMBO J.* 23:1301–1312.

Taddei, A., F. Hediger, F.R. Neumann, and S.M. Gasser. 2004b. The function of nuclear architecture: a genetic approach. *Annu. Rev. Genet.* 38:305–345.

Tang, X., Y. Jin, and W.Z. Cande. 2006. Bqt2p is essential for initiating telomere clustering upon pheromone sensing in fission yeast. *J. Cell Biol.* 173:845–851.

Tzur, Y.B., K.L. Wilson, and Y. Gruenbaum. 2006. SUN-domain proteins: ‘Velcro’ that links the nucleoskeleton to the cytoskeleton. *Nat. Rev. Mol. Cell Biol.* 7:782–788.

Uetz, P., L. Giot, G. Cagney, T.A. Mansfield, R.S. Judson, J.R. Knight, D. Lockshon, V. Narayan, M. Srinivasan, P. Pochart, et al. 2000. A comprehensive analysis of protein–protein interactions in *Saccharomyces cerevisiae.* *Nature.* 403:623–627.

Vakoc, B.R., and D. Nature. 2006. Characterization of the structures involved in localization of the SUN proteins to the nuclear envelope and the centrosome. *DNA Cell Biol.* 25:554–562.

Vakoc, B.R., and D. Nature. 2006. Characterization of the structures involved in localization of the SUN proteins to the nuclear envelope and the centrosome. *DNA Cell Biol.* 25:554–562.

Wormian, H.J., and G.G. Gundersen. 2006. Here come the SUNs: a nucleocytoplasmic molecular missing link. *Trends Cell Biol.* 16:61–69.