Multiple domains of chromosomes are associated with the nuclear envelope (NE) in interphase. The association between chromosomes and the NE is involved in a variety of chromosomal reactions, such as gene expression and DNA repair. However, efficient chromosome movements are required for the fidelity of chromosome segregation in mitosis. Most higher eukaryotes perform open mitosis, in which the NE is broken down, enabling chromosomes to be released from the NE as well as spindle microtubules to access to kinetochores. By contrast, lower eukaryotes, such as Schizosaccharomyces pombe, perform closed mitosis, during which NE breakdown does not occur. In S. pombe, telomeres are tethered to the NE in interphase. Phosphorylation of the telomere-binding protein Rap1 at M phase promotes transient dissociation of telomeres from the NE, facilitating the faithful chromosome segregation. These findings imply a common mechanism for genome stability via the dissociation of chromosomes from the NE in eukaryotic mitosis.

**S. pombe Telomeres are Dissociated from the NE at M Phase**

Telomeres are localized at the ends of linear chromosomes and play critical roles in the genome stability. In S. pombe, a fission yeast, the Taz1 protein (a homolog of mammalian TRF1 and TRF2) directly binds to the double-stranded telomere DNA. Taz1 directly interacts with the Rap1 protein (a homolog of mammalian Rap1) and recruits it to telomeres.\(^3\) Rap1 in turn interacts with multiple partner proteins to regulate various telomere functions, such as maintenance of telomere DNA length, protection of chromosomal ends and meiotic telomere clustering (Fig. 1A).\(^4\)

In S. pombe, telomeres moderately cluster themselves and continuously move inside the nucleus in interphase, keeping their positions near the NE (Fig. 1B).\(^7\) Telomeres are tethered to the NE via the interaction between Rap1 and the inner nuclear membrane protein Bqt4 (Fig. 1A).\(^6\) Microscopic analyses have revealed the M phase-specific movement of telomeres. Immediately after entry into mitosis, telomere clustering begins to break down, and telomeres are dissociated from the NE. At the end of mitosis, telomeres return to the NE and cluster again (Fig. 1B).\(^9\)

**Transient Telomere Dissociation from the NE is Required for Faithful Chromosome Segregation**

To elucidate the physiological importance of the transient telomere dissociation from the NE, telomeres were forced to be tethered to the NE at M phase by expressing the fusion protein of Taz1 with the C-terminal region of Bqt4 (Bqt4\(\Delta N\)), which associates with the NE. The Taz1-Bqt4\(\Delta N\)-expressing cells displayed a higher frequency of abnormal chromosome segregation than did wild-type cells. Moreover, the Taz1-Bqt4\(\Delta N\)-expressing cells exhibited chromosome entanglement at anaphase, suggesting that telomere dissociation from the NE is required for the efficient movement of chromosomes during...

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residues at the five phosphorylation sites with alanine decreases the distances between the telomeres and the NE. By contrast, substitution with phospho-mimic amino acid residues (aspartic acid or glutamic acid) increases the distance, as in the case of the rap1+ or bqt4+ deletion. The phosphorylation of Rap1 specifically influences the interaction between Rap1 and Bqt4, but not the interactions between Rap1 and Taz1, Poz1 and Bqt1-Bqt2. Consistently, alanine- or phospho-mimic mutations of rap1+ at the phosphorylation sites do not exhibit any defect in telomere DNA length or the progression of meiosis (Fig. 1A). Moreover, among the five phosphorylation sites of Rap1, the phosphorylation at Ser513 is most influential on the Rap1-Bqt4 interaction and, consequently, the distance between the telomeres and the NE.9 These findings indicate that the activation of Cdc2 kinase at early M phase is the major trigger for the release of telomeres from the NE via Rap1 phosphorylation, which reduces the association between Rap1 and Bqt4 (Fig. 3).

Dissociation of Other Chromosomal Domains from the NE at M Phase

Because telomeres are tethered to the NE by the interactions between the Rap1 and Bqt4 proteins in interphase, it has been speculated that the function of Rap1 changes in M phase to release telomeres from the NE. In fact, extensive band shifts of the Rap1 protein were observed at M phase in western analyses, whereas no band shift was observed for Taz1 (Fig. 2A). The band shifts of Rap1 were primarily due to its phosphorylation at Ser213, Thr378, Ser422, Ser456 and Ser513; the timing of each phosphorylation during the cell cycle varies from site to site (Fig. 2B).9

Among these phosphorylations, the phosphorylations at Thr378, Ser422 and Ser513 were observed during early to middle stages of mitosis, when Cdc2, a major mitotic kinase in S. pombe, is highly active. By contrast, these phosphorylations were barely detectable when Cdc2 was inactivated. Moreover, these phosphorylations were completely abolished when the proline residues following the three phosphorylation residues were mutated to alanine, eliminating the consensus target sequence (Ser/Thr-Pro) of Cdc2. Furthermore, p13nuc1 bead-associated kinases (among which the major kinase is Cdc2) efficiently phosphorylated these three residues in vitro. Taken together, these results strongly suggest that Cdc2 phosphorylates these three residues.9 However, the identity of the kinase(s) that phosphorylate Ser213 and Ser456 of Rap1 is unknown (Fig. 2B).

Phosphorylation of Rap1 Promotes Telomere Dissociation from the NE

Phosphorylation of Rap1 inhibits the interaction between Rap1 and Bqt4. Furthermore, substitution of amino acid residues at the five phosphorylation sites with alanine decreases the distances between the telomeres and the NE. By contrast, substitution with phospho-mimic amino acid residues (aspartic acid or glutamic acid) increases the distance, as in the case of the rap1+ or bqt4+ deletion. The phosphorylation of Rap1 specifically influences the interaction between Rap1 and Bqt4, but not the interactions between Rap1 and Taz1, Poz1 and Bqt1-Bqt2. Consistently, alanine- or phospho-mimic mutations of rap1+ at the phosphorylation sites do not exhibit any defect in telomere DNA length or the progression of meiosis (Fig. 1A). Moreover, among the five phosphorylation sites of Rap1, the phosphorylation at Ser513 is most influential on the Rap1-Bqt4 interaction and, consequently, the distance between the telomeres and the NE.9 These findings indicate that the activation of Cdc2 kinase at early M phase is the major trigger for the release of telomeres from the NE via Rap1 phosphorylation, which reduces the association between Rap1 and Bqt4 (Fig. 3).

Figure 1. S. pombe telomeres are dissociated from the NE at M phase. (A) Telomere-binding proteins and their functions. (B) Telomeres are dissociated from the NE in interphase. After entry into mitosis, telomeres are dissociated from the NE, and telomere clustering breaks down. For simplicity, only two chromosomes are shown.
fact, *S. pombe* centromeres are clustered toward the spindle pole body (SPB) at the nuclear periphery in interphase. Upon entry into mitosis, centromeres are dissociated from the SPB and from the NE and are relocated at the center of the nucleus. However, how these other chromosomal regions at the nuclear periphery become released from the NE in mitosis remains unclear.

**Telomere-NE Dissociation and Telomere Declustering for the Progression of Mitosis**

In *S. pombe*, telomeres continuously move inside the nucleus in interphase. Therefore, it is highly possible that the long chromatin fibers are entangled with each other because a large number of chromosomal domains are associated with the NE. For the normal activation of spindle assembly checkpoint, which detects the tension between the sister kinetochores, each chromosome must be aligned at the center of the nucleus at metaphase without any entanglement or tension between the kinetochores and the NE. Thus, dynamism of telomeres in mitosis, i.e., detachment from the NE and declustering, may facilitate the resolution of chromatin fibers before metaphase. In fact, entanglement of chromosomes has been observed at anaphase when telomeres are artificially tethered to the NE in mitosis. Although Rap1 is crucial for the “meiotic” telomere clustering toward SPB, telomeres are normally clustered in interphase in *rap1* or *bqt4* deletion strains (Fig. 4A), indicating that the other proteins are involved in telomere clustering in the mitotic cell cycle; however, how telomere clustering and declustering are regulated is largely unknown.

**Chromosome Release from the NE: The Universal Principle of Eukaryotic Mitosis Involving Phosphorylation Events**

A budding yeast, *Saccharomyces cerevisiae*, also performs closed mitosis. In this organism, telomeres are attached to NE through multiple pathways that involve telomere-binding proteins, Sir4 and yKu80, and inner nuclear membrane proteins, Esc1 and Mps3. The redundant mechanisms of telomere tethering to NE imply that telomere localization is important for cellular activities. In fact, recombination between sub-telomeres is repressed, and DNA repair in sub-telomeric region is regulated by telomere
In mouse meiosis, telomeres are clustered to a limited area at the nuclear periphery at the zygote stage as in *S. pombe* meiosis, and dissociation of the NE occurs to facilitate the faithful chromosome segregation in mitosis.

A mechanism similar to that in *S. pombe* mitosis exists in *S. cerevisiae* to facilitate the faithful chromosome segregation in mitosis.

**Figure 4.** Conservation of chromosome release from the NE in eukaryotic cell division. (A) Mitotic telomere clustering in *rap1Δ* and *bqt4Δ* cells. Strains JK81 (wild-type, *taz1-mCherry*), JP836 (*rap1Δ*) and JP842 (*bqt4Δ*) were grown in YES medium at 30°C, and the numbers of *Taz1-mCherry* signals per cell were counted. More than 100 cells in G1 phase were analyzed for each strain. Note that *S. pombe* has three chromosomes and six telomeres. (B) Modification of the Rap1 protein in meiosis. Strain JP791 (*h+/h pat1–114/pat1–114 mat-Pc*) was grown in YE medium to mid-log phase at 25°C and then transferred to EMM-N medium (supplemented with 1% glucose) at 25°C. After 6.5 h of incubation (G1 arrest), the temperature was shifted to 34°C to inactivate Pat1. Samples were taken every 10–15 min after 120 min of incubation at 34°C. The percentage of cells with 1, 2 or 3–4 nuclei was determined by counting >200 cells stained with DAPI (4',6-diamidino-2-phenylindole). The whole-cell extracts were analyzed by immunoblotting with anti-Rap1 antibodies, anti-Cdc13 (Cyclin-B) antibodies and anti-PSTAIRE antibodies for Cdc2 (loading control). (C) Models for the regulation of closed and open mitoses. The breakdown of telomere tethering to the NE is triggered primarily by Cdc2 in closed mitosis in *S. pombe*. Dissociation of the other chromosomal regions from the NE is also regulated by Cdc2. By contrast, breakdown of the NE triggered by CDK1 and other mitotic kinases occurs in open mitosis. Both induce the release of chromosomes from the NE, which likely leads to accurate chromosome segregation.
telomeres from the NE occurs in later stages. Thus, the regulatory mechanism of telomere dissociation from the NE is most likely not specific to closed mitosis, that is, open and closed meioses may also follow a mechanism similar to that of closed mitosis to release telomeres from the NE. Consistent with this suggestion, S. pombe Rap1 is highly modified during meiosis I and II (Fig. 4B).

Telomere dissociation from the NE in S. pombe mitosis is promoted by Rap1 phosphorylation, which is primarily dependent on Cdc2. In open mitosis, phosphorylation of lamin, some components of the nuclear pore complex (NPC) and several inner nuclear membrane proteins by CDK1 and other mitotic kinases is important for disassembly of the NE and NPC. In addition, the mitotic kinase of the VRK (vaccinia-related kinase) family phosphorylates the chromatin-binding protein BAF (barrier-to-autointegration factor), which links chromosomes with the NE, to reduce the affinity of BAF for chromosomes. Therefore, phosphorylation of proteins at the nuclear periphery appears to be a key and universal mechanism to release chromosomes from the NE in both open and closed mitoses. As described above, a large number of chromosomal regions in addition to telomeres are located near the NE in S. pombe interphase. It will be of interest to determine whether mitotic dissociation of the other chromosomal regions from the NE is also regulated by phosphorylation of the chromatin-associated proteins. Taken together, in closed mitosis, cells show breakdown of tethering of the telomere (and possibly other chromosomal regions) to the NE, while chromosomes become free in open mitosis by breakdown of the NE. In both cases, chromosomes are transiently released from the NE, which may contribute to efficient chromosome movements and, consequently, fidelity of chromosome segregation (Fig. 4C).

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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