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Centromere Glue Provides Spindle Cue

During cell division, accurate distribution of the genome by the mitotic spindle requires that sister chromatids remain tethered until the right moment. Studies of an uncharacterized vertebrate protein, Sgo (Salic et al., 2004 [this issue of *Cell*]), reveal dual roles as a chromosome cohesion factor and a regulator of spindle microtubule dynamics.

Faithful transmission of the genome to daughter cells requires accurate segregation of duplicated chromosomes during mitosis. To achieve this, chromatids remain linked to their sisters following DNA replication then condense and attach to a complex cytoskeletal machine, the mitotic spindle. At metaphase, the sister chromatids align at the center of the spindle and face opposite spindle poles, so that when they detach in anaphase, they can be separated into two sets before the cell pinches into two. Kinetochores, specialized structures assembled at the centromere of each chromatid, mediate dynamic connections between chromosomes and the microtubule polymers of the spindle. The kinetochores also serve as sensors for the spindle assembly checkpoint, transmitting a signal that prevents anaphase onset until all the chromosomes are properly attached and aligned. In this issue of *Cell*, Salic et al. (2004) investigate the function of a kinetochore protein, a vertebrate homolog of *Drosophila* MEI-S332 and yeast Sgo, and find that it is not only essential for sister cohesion in mitosis but also for proper kinetochore microtubule dynamics and mitotic progression.

Previous studies of chromosome cohesion have focused primarily on a highly conserved, multisubunit protein complex called cohesin (reviewed in Haering and Nasmyth [2003]). Cohesin loads onto chromatin during DNA replication, forming a proteinaceous glue linking sisters together along their arms and especially at their centromeres. In budding yeast, the glue is precipitously dissolved at the metaphase/anaphase transition as cleavage of a cohesin subunit, Scc1/Rad21, by the cysteine protease separase is induced by the anaphase promoting complex (APC). However, in higher eukaryotes the bulk of cohesin dissociates from chromosome arms during prophase by a cleavage-independent mechanism, while residual cohesin persists between sister centromeres until separase activation at anaphase. A wealth of data in multiple organisms supported a vital role for cohesin as the major mediator of chromosome cohesion (Haering and Nasmyth, 2003).

However, studies of meiosis in *Drosophila* and fungi have revealed the existence of other essential cohesion factors. Meiosis presents a unique chromosome cohesion problem because it involves two successive chromosome divisions, during which cohesion is lost in multiple discrete steps. In the first (meiosis I) anaphase, paired homologous chromosomes linked along their arms separate, while sister chromatids remain tethered at their centromeres. Cohesin cleavage is required for homolog separation, yet some cohesin must persist to enable sister chromatids to remain physically linked until they separate during the second division (meiosis II). The *Drosophila* MEI-S332 protein was a strong candidate regulatory factor, as it localizes to centromeres until anaphase of meiosis II, and its loss of function results in premature sister chromatid separation during anaphase I (Kerrebrock et al., 1995; Moore et al., 1998; Tang et al., 1998). Recently it became apparent that MEI-S332 is conserved, as three different screens in fission and budding yeasts identified related proteins, named "shugoshin" (which means "guardian spirit" in Japanese), that protect Rec8, the meiotic version of Scc1, from cleavage during meiosis I (Katis et al., 2004; Kitajima et al., 2004; Rabitsch et al., 2004; Marston et al. 2004). Several observations indicate that the function of Sgo proteins is not restricted to meiosis. MEI-S332- and Sgo-related proteins localize to centromeres in mitotic cells and are required for accurate chromosome segregation (Katis et al., 2004; Kitajima et al., 2004; Marston et al., 2004; LeBlanc et al., 1999; Moore et al., 1998). However, the precise function of mitotic Sgo proteins, their role in chromosome cohesion, and the extent of their conservation has been unclear.

Notably, Salic et al. did not identify vertebrate Sgo via its role in cohesion but rather in an assay for microtubule stabilizing proteins in *Xenopus* egg extracts. An N-terminal fragment of Sgo can bind along the length of purified microtubules and stabilize them, suggesting a direct function in regulating microtubule dynamics. However, endogenous Sgo is not found on microtubules but localizes to kinetochores during prophase and disappears during anaphase due to proteolysis induced by the APC. Furthermore, depletion of Sgo from HeLa cells by RNAi causes dramatic defects in sister chromatid cohesion, in line with the meiotic roles of MEI-S332 and Sgo1. What is the significance of the microtubule binding domain? Careful long-term live imaging also revealed a second mitotic function for Sgo. Early after RNAi treatment, prior to chromatid separation, Sgo inhibition induced a mitotic arrest indicative of faulty kinetochore-spindle interactions. Although spindles formed in the absence of Sgo and microtubules attached to kinetochores, the distance between sister centromeres was decreased, reflecting a loss of tension that activates the spindle assembly checkpoint. A careful analysis of kinetochore fiber microtubule dynamics using photoactivatable GFP-tubulin revealed that kinetochore microtubules are less stable in cells depleted of Sgo compared to controls, while microinjection of Sgo antibodies dramatically dampened kinetochore microtubule dynamics, presumably due to stabilization of Sgo.

This study of vertebrate Sgo is exciting for a couple of reasons. First, it demonstrates that cohesin is not the whole story of sister chromatid cohesion in mitosis and begs the question of the relationship between these

two factors. Does vertebrate Sgo act as a protector of cohesin as in yeast meiosis? What is the molecular mechanism behind this protection? Or do Sgo and cohesin mediate separate cohesion pathways? Both are targets of the APC, but in what order? Second, this analysis illuminates an unexpected molecular connection between centromere cohesion and kinetochore microtubule dynamics. Given its potent microtubule stabilizing activity in vitro, and the fact that microtubules penetrate the kinetochore, Sgo could modulate microtubule behavior directly. In addition, Salic et al. observed a loss of outer kinetochore proteins CENP-E and CENP-F upon knockdown of Sgo. Downstream effects on these and other kinetochore proteins could also contribute to the observed defects in spindle microtubule dynamics.

Which Sgo functions are conserved? The limited homology among Sgo proteins is confined to a coiled-coil in the amino-terminal region and a carboxy-terminal domain, both of which are required for targeting MEI-S332 to centromeres in *Drosophila* cells (Lee et al., 2004). More precise delineation of the region required for microtubule stabilization will reveal whether it lies in the conserved N-terminal domain. Unlike in yeast and *Drosophila*, loss of vertebrate Sgo activates the spindle assembly checkpoint by inhibiting the APC, halting cell cycle progression (Salic et al., 2004). It is unclear whether this effect is direct, due to defects in microtubule-kinetochore interactions, or indirect due to loss cohesion between sister kinetochores, both of which would result in a loss of tension. Future experiments will illuminate how Sgo contributes to these functions.

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Epithelial Stem Cells: Stepping out of Their Niche

In this issue of *Cell*, Blanpain et al. (2004) have shown that two subpopulations of cells exist within the hair follicle stem cell niche. Despite being partially differentiated, clonal populations of suprabasal bulge region cells can regenerate skin and hair follicles as well as a new stem cell niche. The findings suggest that early lineage commitments of epithelial cells in the hair follicle may be reversible.

The "bulge" region of the human hair follicle was described morphologically over 100 years ago (Figure 1) (Stöhr, 1903) and is now known to be the home of the hair follicle stem cell niche (reviewed in Panteleyev et al. [2001]; Fuchs et al., [2004]). Despite its early recognition as a specialized structure, advances in hair follicle stem cell biology have been relatively slow. The breakthrough of in vivo marking of stem cells by the Fuchs lab (Tumbar et al., 2004) and the Morris and Cotsarelis team (Morris et al., 2004) earlier this year, followed by the elegant work of Blanpain et al. (2004) in this issue of *Cell*, have provided powerful new tools for stem cell lineage analysis. Some of the most fundamental questions in keratinocyte stem cell biology, such as self-renewal, multipotency, and the nature of the niche itself, are now being addressed.

Keratinocytes within the hair follicle stem cell niche are believed to be primed to respond to at least two sets of stimulatory signals to generate a bidirectional flow of stem cells. An upward flow of stem cells out of the bulge into the epidermis is generated in response to epidermal injury. A downward flow is induced by a periodic signal from a cluster of hair follicle dermal cells, known as the dermal papilla, at the beginning of each hair cycle. It is believed that these short-range morphogenic signals awaken a small number of epithelial stem cells to differentiate along the hair follicle lineage. Together, these early committed epithelial cells then descend with the papilla to generate the new hair shaft of the next cycle. The mechanisms involved in governing these exquisitely timed cellular dynamics, the delicate banter between epithelium and mesenchyme, and the precise signals mobilizing stem cells to step out of the niche are largely unknown.

Despite the lack of knowledge of the specific signals, through the use of a mouse model that retains GFP-labeled histone proteins in slowly cycling, long-lived cells, Blanpain et al. (2004) have made an unexpected discovery regarding hair follicle stem cell niche architecture. In mouse pelage follicles, they have uncovered *two* spatially distinct subpopulations of stem cells within the niche. One population, the newly identified asymmetrical suprabasal bulge cells, arises only following first contact of the bulge by the dermal papilla at the onset of the first postnatal hair cycle, at approximately 3 weeks of age. This developmental stage coincides with the formation of the club hair "companions," which represent the product of the first anagen hairs resulting from morphogenesis. While it is acknowledged that stem