Opinion

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Buck the establishment: reinventing sister chromatid cohesion

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The time between chromosome replication and segregation can be from hours to decades. Cohesion is thus crucial for identifying replication products as sister chromatids from S-phase until mitosis. Early models posited active sister chromatid tethering reactions in which cohesins deposited onto each sister chromatid are converted to a paired state by replication-fork-associated establishment factors. Subsequent, but now largely marginalized, models suggested instead that establishment occurs passively – requiring only cohesin preloading and passage of the replication fork through huge cohesin rings. More recent models return to active establishment reactions but remain predicated on preloaded ring structures. Here, new models are presented in which replication-coupled cohesin deposition is followed by conversion to a pairing-competent C-clamp structure that does not require DNA entrapment.

Introduction

The cell cycle can be viewed in terms of chromosome processing. Each chromosome must be faithfully replicated during S-phase to produce two identical sister chromatids. Later, during mitosis, sisters segregate away from each other and into the newly forming daughter cells. To identify chromatids as sisters over the extended periods of time that can exist between S-phase and mitosis, replication products are tethered together (Figure 1). During mitosis, maintaining sister identification is especially difficult given the tremendous poleward-directed forces produced by kinetochores associated with spindle microtubules. Cohesion resists opposing forces produced by sister kinetochores, resulting in visibly stretched centromeric chromatin. In generating tension across sister chromatids, cohesion fulfills a second duty by providing a physical cue to the cell that sister chromatids are properly bi-oriented [1]. In the absence of tension, mitotic checkpoints delay the metaphase-to-anaphase transition – a checkpoint dependency recapitulated in cohesion mutants [2–4]. Finally, by keeping sister chromatids in close proximity, cohesion provides the template required for double-strand-break repair; indeed, cohesion establishment reactions can be induced by DNA damage [5–11].

Elucidating the molecular underpinnings of cohesion is clinically relevant and of immediate importance. Given the roles of cohesion it is not surprising that cohesion mutant cells exhibit an array of phenotypes (aneuploidy, genome instability, defects in DNA repair, hyper-recombination and chromosomal translocations) that are all hallmarks of cancer cells. Mutations in cohesion factors are now correlated to specific cancer subsets that include breast, melanoma and testicular cancers [12–15]. However, cohesion defects are not only implicated in cancer. A significant body of evidence now links cohesion factor (cohesin) mutations to developmental abnormalities such as Cornelia de Lange syndrome, Roberts syndrome/SC phocomelia, and Warsaw breakage syndrome [16–20]. These maladies manifest a wide spectrum of phenotypes that include mental deficiencies, growth retardation, small and/or flipper-like appendages, heart defects and much more. New studies are beginning to uncover potential underlying mechanisms of these cohesinopathies, in part by pursuing early evidence that cohesion factors play crucial roles in transcription regulation [20–24]. Regardless of their role in sister chromatid pairing and transcription, little is known of cohesin architecture *in vivo*. Although one popularized view maintains that cohesin forms a huge triangular ring-like structure capable of entrapping sister chromatids, new studies confirm instead that cohesion requires a large aggregate of proteins that all participate equally in sister chromatid pairing. This paper highlights evidence from budding yeast that cohesins form a much more complicated and compact structure than a simple ring that simply entraps DNA. I further discuss the role of antiestablishment factors in regulating this process.

Cohesin basics

In *Saccharomyces cerevisiae*, the cohesin subunits that maintain sister chromatid pairing are clearly defined. Cohesin complexes contain at least Smc1, Smc3, Mcd1/Scc1 and Irr1/Scc3 [1]. Pds5 is also essential for maintaining cohesion but performs additional regulatory roles (see below). Vertebrate cell cohesins require an additional factor termed Sororin to maintain sister chromatid pairing [25–27]. There is also a consensus regarding basic aspects of cohesin subunit interactions (Box 1). The complexities arise in considering how these associations result in structure (Figure 2). Although several lines of evidence support some version of embrace in which a huge cohesin ring-like structure entraps DNA within a central lumen, many others confound such a structure (see section 'Revisiting cohesin structure'). Certainly, that pairing maintenance requires many factors strongly indicates the so far unappreciated complexity of cohesin structure *in vivo*. Equally plausible models of cohesin structure now include bracelets and snaps that either enwrap or associate laterally with chromatin (Box 1). Whereas the cohesin structure that

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Box 1. Cohesin subunit interactions

Both Smc1 and Smc3 contain globular N- and C-termini connected by a long α-helical domain. The long α-helical domain is interrupted by a centrally-positioned hinge that allows each SMC protein to fold in half. One consequence of hinge folding is that the α-helical domains entwine tightly together into a single and relatively rigid antiparallel coiled-coil structure approximately 40 nm long. Hinge folding also brings the globular N and C termini together to form an ATP binding cassette-type head domain. Both hinges and head domains provide for strong heterotypic binding: hinges from Smc1 and Smc3 bind together and head domains from Smc1 and Smc3 bind together to form an Smc1/3 heterodimer. In some fashion, Mcd1 sits atop the Smc1/3 head domains and recruits Irr1/Scc3 – but this is insufficient to complete assembly. Pds5 and in vertebrate cells, Sororin (not shown), are required to maintain pairing. Various findings position Pds5 at both the head and hinge domains of Smc1/3.

Early electron micrographs depict cohesins in an open V-like configuration with separated SMC heads anchored by dimerized hinge domains in an arms-spread configuration. Whereas such images provide important first steps to appreciating what might be relaxed cohesin conformations, there is mounting evidence that such images do not represent cohesin structure *in vivo* (see section 'Revisiting cohesin structure'). Regardless, subsequent biochemical studies of differentially tagged Mcd1 ends suggested that the Mcd1 N-terminus binds to the head of Smc3 head whereas the Mcd1 C-terminus binds to the head of Smc1. One model emanating from these findings is that Mcd1/Scc1 might bridge separated Smc1/3 head domains to form a huge triangular ring or proteinaceous loop that could encircle DNA (often referred to as an 'embrace' model). However, these subunit interactions equally predict a variety of structures that include double rings and bracelet spirals that enwrap DNA and also snap configurations that associate laterally with DNA. In the latter case, snap or lateral association models are supported by findings that both SMC hinge domains and SMC head domains are separately sufficient to bind DNA in the absence of a complete ring. Ultimately, any model of cohesin structure must conform to studies suggesting that chemical cross-linking of a cohesin subset is sufficient to retain chromatin-association even in the presence of denaturing agents [59,60]

tethers sisters together *in vivo* remains controversial, new findings regarding both structure and initial sister chromatid pairing reactions provide an opportunity to revisit this issue.

Establishment: converting chromatin-associated cohesins to a paired state

The timing of sister chromatid pairing is typically limited specifically to S-phase – but we know little of the mechanisms that preclude rampant pairing (sister to sister to non-sister) from taking place throughout the cell cycle. Another major challenge for the cell is that genomes are replete with conserved gene families, motifs, repetitive DNA elements, sisters, non-sisters and homologous chromosomes. How do cells identify which chromatids to pair together without relying on DNA sequences? The answers to these questions revolve around the *establishment factor* Ctf7/Eco1 that converts chromatid-bound cohesins to a paired state and a host of *antiestablishment factors* that appear to regulate inappropriate sister chromatid pairing reactions.

Early studies revealed that Ctf7/Eco1 was functionally distinct both from the cohesins that maintain sister chromatid pairing and also from deposition factors that load cohesins onto chromatin [1]. Particularly telling were observations that *ctf7/eco1* mutant cells contain sister chromatids fully decorated with cohesions – but still remain unpaired. This *cohesin without cohesion* phenotype exemplifies establishment mutations and highlights the role of Ctf7/Eco1 in converting chromatid bound cohesins into a paired state (Figure 1). The answer to 'when' came early – cell-cycle mapping studies revealed that Ctf7/Eco1 is required during S-phase in normal cells. Over the past several years, three significant advances contributed to answering the question 'how'.

The first major finding coupled Ctf7/Eco1 function to DNA replication-fork components. Both genetic and biochemical analyses revealed interactions between Ctf7/Eco1 and several DNA replication-fork components including proliferating cell nuclear antigen PCNA (sliding clamp processivity factor), the Ctf18–replication factor C (RFC) complex (promotes PCNA loading/unloading) and DNA helicase. In turn, mutations in any one of these replication-fork factors produce cohesion defects – lending strong support for a *replication-coupled cohesion establishment* model [1]. The second major advance came from computer-assisted sequence alignments that led to characterization of Ctf7/Eco1 as an acetyltransferase [28]. The third advance culminated in identification of Ctf7/Eco1 substrates, but produced some unexpected results in that two separate establishment pathways (and targets) exist: one during S-phase and one during G2/M in response to DNA damage [29]. The combination of these findings suggest that replication-fork-associated Ctf7/Eco1 converts chromatid-bound cohesins into a pairing-competent state and that Ctf7/Eco1 targets change in a cell-cycle-dependent fashion.

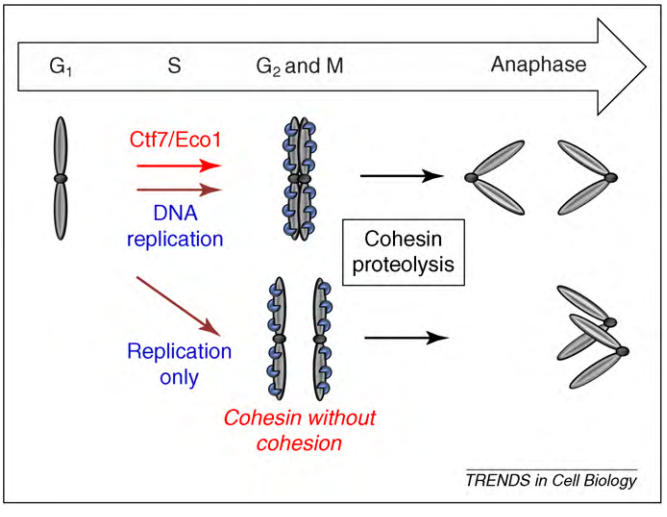

Figure 1. Cohesion during the cell cycle. Linear views of the cell cycle (arrow, above) and chromosome cycle (schematic, below). Left, unreplicated chromosome; middle, post-replicated sister chromatids are decorated with cohesins (blue clasps). Sister chromatids are paired if Ctf7/Eco1 was present during S-phase (upper panel) but remain unpaired if Ctf7/Eco1 was absent during S-phase – resulting in a *cohesin without cohesion* phenotype (lower panel). Right, during anaphase, paired sisters segregate normally (upper panel) whereas unpaired sisters segregate at random and can result in cell aneuploidy – shown are both sisters moving to right (lower panel). Chromosome structure not representative of cell-cycle changes.