PERSPECTIVES: CELL CYCLE

Replication Meets Cohesion

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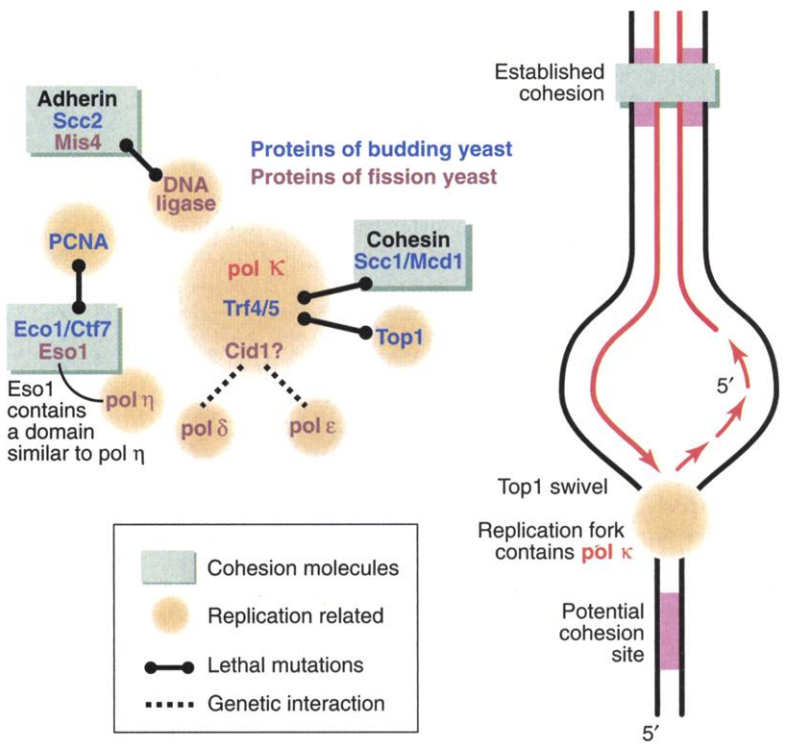
As the cell's DNA is replicated during S phase of the cell cycle, the original and duplicate chromosome of each sister chromatid pair become tightly bound to each other. Sister chromatids stay connected during G_2 and M phase of the cell cycle until anaphase, when the mitotic spindle pulls apart and the sister chromatids move to opposite poles of the cell. A tight connection between sister chromatids (called cohesion) must be established during DNA replication, otherwise the chromosomes separate prematurely (*1*).

Genetic screening in yeast has identified cohesion molecules, such as proteins of the cohesin complex, that hold the sister chromatids together; homologs of cohesion molecules have also been found in vertebrates (see the figure). In budding yeast, the cohesin complex is composed of at least four protein subunits (Scc1p/Mcd1p, Scc3p, Smc1p, and Smc3p), although other cohesion proteins, such as Scc2p and Eco1p/Ctf7p, are not part of this complex. All of these cohesion molecules are necessary for normal progression through S phase, but, so far, none of them have been implicated in DNA replication per se. Therefore, the Wang *et al.* report on page 774 of this issue comes as something of a surprise. These investigators identify in budding yeast a new DNA polymerase (encoded by the *TRF4* gene) called polymerase κ (pol κ). They show that pol κ is involved not only in DNA replication but also in the maintenance of sister chromatid cohesion (*2*). Pol κ is the fourth essential nuclear DNA polymerase identified in budding yeast (the other three are pol α, δ, and ε).

In previous work, Wang and colleagues identified two gene products in budding yeast, Trf4p and Trf5p, that had overlapping functions with each other and with DNA topoisomerase I (an enzyme that relieves torsional strain in chromosomal DNA during replication, transcription, and recombination). Trf4p and Trf5p are evolutionarily conserved and are required for proper segregation of sister chromatids during mitosis. Sequence analysis indicates that these two proteins have limited homology to the DNA pol β superfamily. Members of this superfamily have nucleotidyltransferase activity and have been implicated in DNA replication to repair damaged DNA and in the addition of poly A tails to mRNAs (*3*). Wang *et al.* demonstrate that Trf4p has pol β–like activity in vitro and can extend an oligo dT primer in a distributive fashion (that is, it extends the primer by several nucleotides before dissociating from it). Trf4p also extends a normal primer containing the four deoxynucleotides in a processive manner (that is, it extends the primer by up to 75 nucleotides). It is possible that Trf4p is located at the replication fork, which would be required for a DNA polymerase involved in replication.

Wang and co-workers now show that budding yeast with mutations in both Trf4p and Trf5p have delayed DNA replication and fail to complete S phase, suggesting that these two proteins are involved in DNA replication in vivo. The delay in S phase could be caused by activation of a cell cycle checkpoint (a surveillance pathway that ensures that DNA replication or repair of damaged DNA is complete before the cells exit S phase). Budding yeast with mutations in both Top1p and Trf4p die, probably as a result of the combined loss of the primary replication swivel protein (Top1p) and a replication factor (Trf4p). A possible homolog of Trf4 in fission yeast, Cid1, interacts with pol δ and ε and is required for activity of the cell cycle checkpoint that regulates entry of S phase cells into M phase (*4*).

Budding yeast with a mutation in Trf4p but not in Trf5p are viable (*2*). Surprisingly, these single mutants are defective in sister chromatid cohesion, although they are able to complete DNA replication in S phase (probably because normal Trf5p is able to substitute for defective Trf4p). Wang and co-workers provide convincing evidence for the loss of sister chromatid cohesion in the Trf4p mutant with two independent assays, fluorescence in situ hybridization and a green fluorescent protein–marker chromosome assay. Cohesion was lost in 41% of the mutant yeast cells but in only 13% of wild-type control cells. Consistent with the notion that Trf4p is required for cohesion, double mutant yeast (with mutations in both Trf4p and Mcd1p) die. Trf4p may be required to maintain as well as to es-



The ties that bind. Coordinated DNA replication and sister chromatid cohesion. Yeast Trf4p is both a DNA polymerase (pol κ) involved in DNA replication and a cohesion molecule, which binds sister chromatids together during S phase of the cell cycle until their separation at anaphase. **(Left)** Trf4p (pol κ) and other cohesion molecules genetically interact with replication-related gene products. The cohesion proteins of budding and fission yeast are depicted in blue and brown, respectively. **(Right)** Trf4p (pol κ) located at the replication fork may specifically replicate DNA at a sister chromatid cohesion site.

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tablish cohesion. Double mutant yeast with mutations in both Trf4p and Trf5p (arrested in early M phase with nocodazole) show a marked increase in loss of cohesion if they are shifted to 36°C (the restrictive temperature) for 3 hours. Wang *et al.* go on to show that a site-directed substitution mutation in the Trf4p domain, which is essential for polymerase activity, resulted in a defect in sister chromatid cohesion, suggesting that proper cohesion seems to require polymerase activity of Trf4p.

It is still not clear how Trf4p is mechanistically involved in cohesion. It may establish sister chromatid cohesion during DNA replication as it sits at the replication fork. All known cohesion molecules are essential for S phase and some, such as Ctf7p, Mis4p, and Eso1p, interact with enzymes implicated in DNA replication. Mutations in Ctf7p—together with mutations in Pol30p (proliferating cell nuclear antigen) or Ctf18p (replication factor C–like protein)—are lethal for budding yeast (*5*). In fission yeast, mutation of Mis4p combined with a DNA ligase mutation is also lethal (*6*). Another fission yeast cohesion molecule, Eso1p, consists of two domains: one related to budding yeast Eco1p/Ctf7p and the other related to DNA pol η (*7*).

The finding that Trf4p (pol κ) is essential for cohesion adds another function for DNA polymerases to a list that already includes genomic DNA replication (pol α, δ, and ε), mitochondrial DNA replication (pol γ), and repair replication (pol α, β, δ, ε, ζ, η, θ, and ι) (*8*). Trf4p is the first cohesion molecule found to have enzymic activity as well. Wang *et al.* propose that cohesion may be established in such a way that when the replication fork encounters a cohesion site, there is a switch from DNA polymerase α, δ, or ε to Trf4p and Trf5p (pol κ).

If pol κ activity per se is required for maintenance of sister chromatid cohesion, then currently held notions about cohesion may have to be substantially revised. A structural model has been proposed in which the cohesin complex binds the two sister chromatids together. The cohesin complex contains heterodimeric SMC (structural maintenance of chromosome) proteins that have head and tail domains connected by rods and a central hinge region. Because the SMC complex interacts with adenosine triphosphate (ATP), the structural link may well be altered during the cell cycle in an ATP-dependent manner. The Wang *et al.* study shows that formation and maintenance of sister chromatid cohesion may require the catalytic activity of a DNA polymerase. It will be interesting to determine how passage of the replication fork results in the establishing of effective cohesion by Trf4p and whether de novo DNA replication dependent on pol κ restores broken cohesion in cells after S phase.

References

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PERSPECTIVES: BIOMINERALIZATION

Naturally Aligned Nanocrystals

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By what sequence of events does a mineral form? In traditional models, individual atoms or molecules are added or subtracted as crystals grow. But a different view is emerging. In a series of laboratory experiments, Penn and Banfield have previously shown that inorganic nanocrystals, made up of hundreds or even thousands of atoms, can be the fundamental building blocks for the creation of highly ordered extended solids. On page 751 of this issue, Banfield *et al.* (*1*) provide strong evidence that some natural minerals also grow through such a mechanism of "oriented attachment" of nanocrystals. The results will be important not only for geochemistry but also for the creation of advanced artificial materials.

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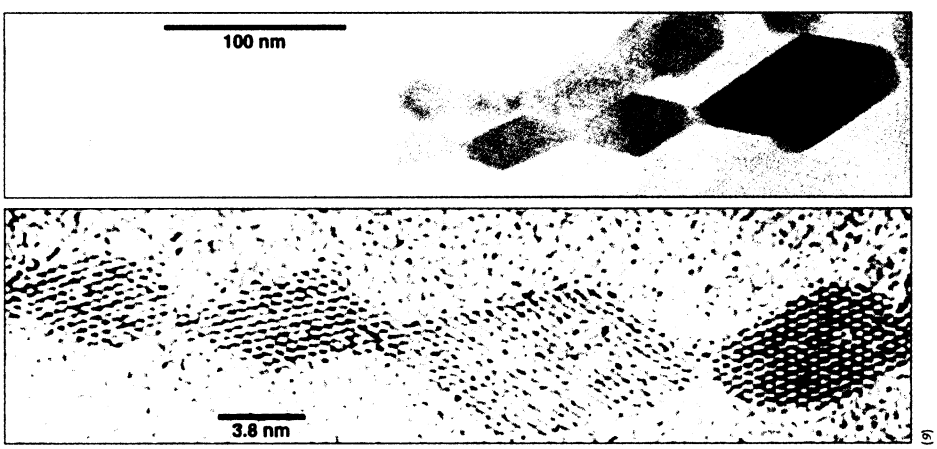
A nanocrystal typically has a diameter of between 1 and 10 nm and may contain as few as a hundred or as many as tens of thousands of atoms. Many fundamental properties of nanocrystals depend strongly on their size in smooth and predictable ways. Examples include the external field required to switch a magnetized particle [of great importance in magnetotactic bacteria (*2*) and in hard disk drives (*3*)] and the color of light emission from a semiconductor [used for the fluorescent labeling of cells (*4*) and in lasers (*5*)]. This facile tuning of properties by size variation is one reason why nanocrystals are widely viewed as promising components for new artificial optical and electrical materials.

But there is another reason why nanocrystals are particularly attractive as a building block for larger structures. Extended solids always contain a certain number of defects, which must be controlled to achieve desirable properties (*6*). If the number of atoms is large and the free energy of defect formation is finite, then a certain density of defects, such as vacancies, is inevitable even at equilibrium. However, when this same number of atoms is partitioned into nanometer-sized crystals, then each nanocrystal on average need not contain any interior defects. It is possible—even easy—to prepare nanocrystals that are highly perfect, because the time required to anneal a



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An example of oriented attachment. Transmission electron micrograph of TiO_2 nanocrystal aggregates (*9*). Primary particles align, dock, and fuse to form these oriented chains.

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