All living organisms must go through cycles of replicating their genetic information and then dividing the copies between two new cells. This cyclical process, in cells from bacteria and human alike, requires a protein complex known as cohesin. Cohesin is a structural maintenance of chromosomes (SMC) complex. While bacteria have one form of this complex, yeast have several SMC complexes, and humans have at least a dozen cohesin complexes alone. Therefore the ancient structure and function of SMC complexes has been both conserved and specialized over the course of evolution. These complexes play roles in replication, repair, organization, and segregation of the genome. Mutations in the genes that encode cohesin and its regulatory factors are associated with developmental disorders such as Roberts syndrome, Cornelia de Lange syndrome, and cancer. In this review, we focus on how acetylation of cohesin contributes to its function. In Roberts syndrome, the lack of cohesin acetylation contributes to nucleolar defects and translational inhibition. An understanding of basic SMC complex function will be essential to unraveling the molecular etiology of human diseases associated with defective SMC function.

The Cohesin Ring

In order to understand the function of cohesin, it is important to consider its motifs and structure. Cohesin forms a ring, consisting of two long coiled-coil proteins known as SMC proteins, a third component known as the kleisin subunit Rad21/Mcd1/Scc1 (hereafter referred to as Rad21), which closes the complex, and a fourth subunit Scc3/STAG of unclear function (Fig. 1). Smc1 and Smc3 are ATP binding proteins that belong to the structural maintenance of chromosomes (SMC) family, which also includes condensin subunits (Smc2 and Smc4) and Smc5/6 complex components (Smc5 and Smc6). All SMC proteins help modulate chromosome structure and topology. SMC proteins are characterized by ~50 nanometer anti-parallel coiled coils flanked by a globular hinge domain and N and C-terminal domains. The N and C-terminal domains of SMC proteins contain conserved Walker A and Walker B motifs that come together to make an ATPase referred to as the head domain. ATPase activity is important for association of the cohesin complex with the chromosome and relocation of the cohesin ring along DNA.2,3

Smc1 and Smc3 interact via their hinge domains. The head domains are held together by a kleisin subunit. Kleisins are a superfamily of bacterial and eukaryotic SMC protein partners.4 The N terminus of Rad21 binds to the head domain of Smc3 while the C terminus binds to the head domain of Smc1,5 which links the head of the SMC proteins. Electron microscopy reveals that the Smc1-Smc3 heterodimer forms a V-shaped structure.5,6

Because Rad21 bridges the heads of Smc1 and Smc3, the three proteins are thought to form a circular complex.5,7,8 The cohesin complex appears to be able to open and close via either the Smc1-Smc3

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Chromosomes derived from a cohesion subunit are arranged to form a ring. The long coiled-coil Smc1 and 3 subunits are shown in blue and red, respectively, interacting at their hinge domain. The head domains of each subunit form an ATPase. The head domains are held together with the Scc1/Rad21/Mcd1 kleisin subunit (green). The Scc3/STAG subunit (yellow) associates with the kleisin. Adapted from reference 1.

Cohesin Acetylation

The opening and closing of the cohesin ring must be regulated in order to control its stability and association with chromatin throughout the cell cycle. Cohesin can interact with DNA in at least two modes, one of which is extremely stable and one of which is more transient. Both acetylation and sumoylation promote stable cohesin-DNA association in budding yeast. Phosphorylation of cohesin subunits can promote or destroy cohesion, depending on the context. Both the acetylation mechanism for promoting stable cohesin-DNA interactions and the acetyltransferase are evolutionarily conserved from yeast to human cells. Eco1 (establishment of cohesion) was first discovered in budding yeast as an acetyltransferase required for the establishment of cohesion in S phase. Deacetylation of Smc3 by Hos1 (known as HDAC8 in human) is also important for the establishment of cohesion in S phase. In addition to cohesion establishment, acetylation plays an important role in DNA damage repair. Instead of Smc3, Eco1 acetylates the kleisin subunit Rad21 in response to a DNA double-strand break to promote cohesion both at the break site and genome-wide.

Budding yeast only contain a single enzyme, Eco1, to acetylate cohesin. Mammalian cells have two enzymes, ESCO1 and ESCO2, both implicated in sister chromatid cohesion. This begs the question as to which are the unique and which are the overlapping actions of these two enzymes. Although both are acetyltransferases, knockdown of ESCO1 reduces the acetylation of lysines 105 and 106 on Smc3 more than knockdown of ESCO2, suggesting ESCO1 is responsible for the bulk of Smc3 acetylation on these lysines. ESCO1 acetylation of Smc3 is important for cell survival in response to ionizing radiation-induced DNA damage, in association with genome-wide reinforcement of cohesin binding in the G2/M cell cycle phase. ESCO2 mutant cells are also sensitive to DNA damage, suggesting ESCO2 also functions as part of the DSB repair response. While our current understanding of the functional overlap of these two enzymes is poor, insight into their function will help us understand regulation of cohesin association throughout the genome.

Roberts Syndrome

The developmental disorder known as Roberts syndrome (RBS) is caused by mutations in ESCO2 that eliminate its acetyltransferase activity. RBS patients have growth and mental retardation, limb deformities, and craniofacial defects. Dephosphorylated cohesin represents the unstable form which is not fully capable of holding sisters together. Metaphase chromosome spreads from RBS patients show heterochromatic repulsion.
suggesting the possibility that ESCO2 is especially important for cohesion at these regions (Fig. 2). In mouse cells, ESCO2 associates with pericentric heterochromatin and depletion leads to reduced cohesin acetylation, lagging chromosomes, and increased apoptosis. In contrast to the case in humans, loss of ESCO2 in mice is lethal. ESCO1 has never been reported to be mutated in association with human disease, possibly because mutations in this gene would be lethal.

Because heterochromatic domains are difficult to replicate, it is possible that there is a cohesin acetyltransferase devoted to establishing cohesion in these regions in mammalian cells. The regions with heterochromatic repulsion in human RBS cells include pericentric domains and nucleolar organizing regions (NORs or rDNA). While the repulsion at pericentric domains might be predicted to cause chromosome missegregation and aneuploidy that could contribute to cell death, it remains to be determined if these contribute to the disease etiology.

Gene expression is clearly disrupted and contributes to the etiology of both RBS and related cohesinopathies, although the mechanisms behind the changes in gene expression are not clear. A budding yeast strain with a mutation in ECO1 that genocopies an RBS allele was found to have defects in nucleolar organization, transcription and cohesion of the rDNA, and translation. Cohesion within the rDNA appears to promote nucleolar structure and function in budding yeast. In budding yeast the rDNA is present in 100–200 copies at a single locus. At any given moment, about half of the repeats are highly transcribed by RNA polymerase I to produce the rRNAs that become structural components of ribosomes. These RNAs can be limiting for ribosome formation. The repeats form loops that are reduced in budding yeast bearing an RBS mutation, although RNA polymerase I occupancy remained normal (see Fig. 3 for a model of the role of cohesion at the rDNA). Furthermore, depletion or artificial destruction of cohesion in a single cell cycle was associated with loss of nucleolar integrity. Given these observations in budding yeast, we wondered whether nucleolar deficits would accompany ESCO2 mutation in human RBS cells. In human cells there are 5 chromosomes with rDNA repeats. RBS cells have profound defects in transcription of the rDNA and ribosome production. Furthermore, the nucleolus is highly fragmented (Fig. 4). This leads to global changes in gene expression and cell physiology (Xu and Gerton, unpublished).

mTORC1 is a protein kinase that senses cellular stress, such as amino acid deprivation, and responds by shutting down protein translation. Given the translation defects in RBS cells, we examined the mTOR pathway. Based on several key effector proteins, we found that the TOR pathway was inhibited and p53 was activated in RBS cells. A zebrafish model for RBS showed that depletion of ESCO2 was associated with disruption of the cell cycle and high levels of apoptosis. Using zebrafish RBS models, we found that the mTOR and p53 pathways were inhibited and activated, respectively, as observed in human RBS cells. Stimulation of the TOR pathway...
Cornelia de Lange Syndrome and Cancer

Cornelia de Lange syndrome (CdLS) is a genetically dominant disorder caused by deletion of FOB1 reveals that the differential expression of hundreds of genes is also corrected.30 This experiment suggests that a substantial portion of the differential gene expression associated with a mutation in ECO1 in budding yeast is due to defects at the rDNA locus and reduced translation.

Translational defects are associated with both human developmental disorders and cancer.64,65 A recent study found that 13% of acute myeloid leukemia (AML) cases were caused by mutations in cohesin genes, including RAD21, STAG2, SMCI, and SMC3.66 In general, AML is not associated with abnormal karyotypes, raising the possibility that the cohesin mutations are causing transcriptional or translational changes that lead to AML, and emphasizing the need to understand
the molecular origin of these changes. If we can understand the contribution of translational mechanisms and particular key regulatory pathways, such as p53 and mTOR, in Roberts syndrome and CdLS, we might also understand how cohesion mutations lead to meiodyne neoplasms.6,67

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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