Cohesin codes – interpreting chromatin architecture and the many facets of cohesin function

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Journal of Cell Science 126, 31–41

doi: 10.1242/jcs.116566

Summary

Sister chromatid tethering is maintained by cohesin complexes that minimally contain Smc1, Smc3, Mcd1 and Scc3. During S-phase, chromatin-associated cohesins are modified by the Eco1/Ctf7 family of acetyltransferases. Eco1 proteins function during S phase in the context of replicated sister chromatids to convert chromatin-bound cohesins to a tethering-competent state, but also during G2 and M phases in response to double-stranded breaks to promote error-free DNA repair. Cohesins regulate transcription and are essential for ribosome biogenesis and complete chromosome condensation. Little is known, however, regarding the mechanisms through which cohesin functions are directed. Recent findings reveal that Eco1-mediated acetylation of different lysine residues in Smc3 during S phase promote either cohesion or condensation. Phosphorylation and SUMOylation additionally impact cohesin functions. Here, we posit the existence of a cohesin code, analogous to the histone code introduced over a decade ago, and speculate that there is a symphony of post-translational modifications that direct cohesins to function across a myriad of cellular processes. We also discuss evidence that outdate the notion that cohesion defects are singularly responsible for cohesion-mutant-cell inviability. We conclude by proposing that cohesion establishment is linked to chromatin formation.

Key words: DNA replication, Eco1/Ctf7, SMC3, Sister chromatid cohesion, Acetylation, Condensation

Introduction

One goal of cell division is to ensure that sister chromatids, produced during S phase, become properly segregated into the newly forming daughter cells during mitosis. To accomplish this, cohesin complexes, consisting of Smc1, Smc3, Mcd1 (Rad21 in mammals) and Scc3 (Irr1 in budding yeast SA1 and SA2 in metazoans, STAG in mammals), tether sister chromatids together (Skibbens, 2008; Onn et al., 2008). This mechanical coupling identifies chromatids as sisters from the time of DNA replication until the onset of anaphase (Fig. 1). The structural basis through which cohesins maintain sister chromatid pairing remain controversial, but crystal structure analyses of highly conserved SMC complexes provide new insights into this important topic (Box 1). There is also clear evidence that cohesin subsets exhibit different and distinct chromatin-associated dwell times, supporting the notion that cohesin structures cycle between soluble pools and weakly or tightly bound chromatin complexes (Gause et al., 2010; Gerlich et al., 2006; McNairn and Gerton, 2009; Onn and Koshland, 2011). Ascertaining the extent to which altered cohesin conformations impact cohesion–DNA architecture may provide key insights into cohesin function in a wide variety of processes (Fig. 2). Scc2 (Mis4 in fission yeast, NIPBL in mammals,) and Scc4 (also known as Ssl3 in fission yeast, Mau-2 in *C. elegans*, and MAU2 in mammals) are required for cohesin to associate with DNA (Kogut et al., 2009; Bernard et al., 2006; Ciosk et al., 2000; Furuya et al., 1998; Rollins et al., 2004; Seitan et al., 2006). Cohesins first associate with DNA during late G1 in budding yeast or in telophase of the preceding cell division in vertebrate cells, but the majority of studies conclude that Scc2 and Scc4 are dispensable from G2 onward, such that cohesin deposition is essential only during S phase (Fig. 1) (Watrin et al., 2006; Bernard et al., 2006; Lengronne et al., 2006; Furuya et al., 1998; Ciosk et al., 2000; Bermudez et al., 2012; Gerlich et al., 2006; Skibbens, 2008). Regardless of the uncertainties concerning cohesin deposition and structure, it is clear that cohesin loading is not sufficient to lead to the tethering of sister chromatids. A separate activity, termed ‘establishment’, is provided by the Eco1 family of proteins and converts chromatin-bound cohesins to a tethering-competent state (Skibbens et al., 1999; Tóth et al., 1999; Tanaka et al., 2000; Milutinovich et al., 2007).

The discovery and characterization of the evolutionary conserved family of Eco1 acetyltransferases (Ctf71 in budding yeast; EFO1 or ESCO1 and EFO2 or ESCO2 in humans; DECO in *Drosophila melanogaster*; ESO1 in fission yeast; hereafter generally referred to as Eco1) significantly advanced the understanding of sister chromatid tethering reactions (Bellows et al., 2003; Hou and Zou, 2005; Ivanov et al., 2002; Skibbens et al., 1999; Tóth et al., 1999; Vega et al., 2005; Williams et al., 2003). Eco1 family members perform their essential function specifically during S phase. In yeast, Eco1 interacts with a host of DNA replication components that include the clamp-like DNA proliferating cell nuclear antigen (PCNA), replication factor C (RFC) complexes that load clamps onto DNA, the DNA helicase Chl1 and the Okazaki fragment maturation flap endonuclease 1 (Fen1/Rad27 in yeast) (Kenna and Skibbens, 2003; Mayer et al., 2004; Moldovan et al., 2006; Petronczki et al., 2004; Rudra and Skibbens, 2012; Skibbens, 2004; Skibbens et al., 1999). Yeast contains four different RFC complexes, in which the large Rfc1 subunit (which forms a complex with Rfc2–Rfc5) is replaced

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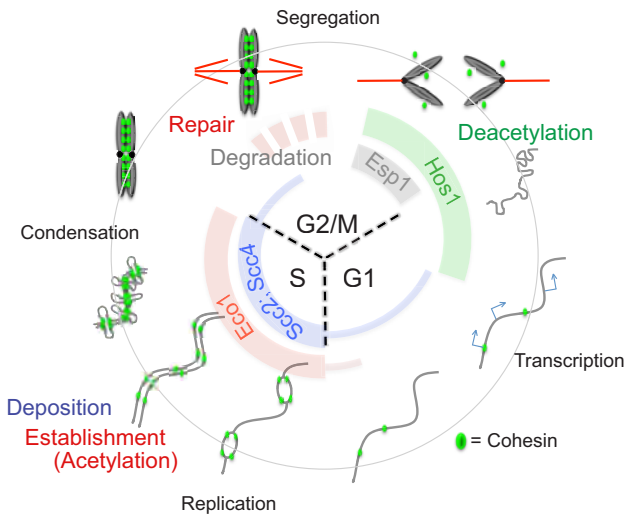

Fig. 1. Chromosomes and cohesin throughout the cell cycle. During S phase, each chromosome (gray line) is replicated to produce two sister chromatids. Each sister also becomes chromatinized (histone assembly and/or modification) and competent to condense. Cohesins (green) are deposited onto each chromatid by Scc2 and Scc4 during most of the cell cycle, but deposition (blue arc) is essential specifically during S phase (thick blue arc). The Smc3 subunit of chromatid-bound cohesin is acetylated by Eco1 and this modification is essential during S phase to convert cohesins to a tethering-competent state termed cohesion establishment (red arc). Eco1-dependent acetylation can occur prior to origin firing during S phase but does not participate in sister chromatid cohesion establishment. Cohesins remain chromatin associated through G2 phase and into mitosis (spindle microtubules are shown in red, condensed chromosomes are shown as gray ovals) to maintain sister chromatid identity over time. In response to DNA damage during G2 and M phase, Eco1 is reactivated (broken red arc). At anaphase onset, the Mcd1 component of the cohesin complex is degraded by Esp1 (gray arc). During this time, the Smc3 component of the cohesin complex is deacetylated (green arc) by Hos1. Although Hos1 activity appears to be crucial in late mitosis and into G1, some deacetylation can precede anaphase (not shown). During G1, a soluble pool of cohesins continues to interact with DNA, consistent with the known roles of cohesin in both promoting and repressing transcription (blue arrows).

with Elg1, Ctf18 or Rad24. Although most DNA replication components promote cohesion establishment, the RFC complex that contains Elg1 antagonizes Eco1 function, revealing a complex network of cohesion regulation (Maradeo and Skibbens, 2009; Maradeo and Skibbens, 2010; Parnas et al., 2009; Rowland et al., 2009; Sutani et al., 2009). These findings support the model that cohesion is established as nascent sister chromatids emerge from behind the DNA replication fork (Skibbens, 2000).

Defining ‘establishment’ (the process through which sister chromatids first become paired) at the molecular level depends on the lens through which one views the physical basis of sister chromatids cohesion. For instance, a previous model referred to as ‘replication through a ring’ posits that establishment occurs passively when the DNA replication fork passes through huge cohesin rings that are deposited onto chromatin during the G1 phase of the cell cycle (Gruber et al., 2003; Haering et al., 2002; Skibbens, 2009). The resulting notion that cohesin deposition onto DNA during G1, and subsequent DNA replication, result in the entrapment of both sisters within single rings appears refuted by numerous reports. First, yeast *eco1* mutant cells contain sister chromatids that are fully decorated with cohesins and subsequently undergo DNA replication, yet sister chromatids remain untethered (Lengronne et al., 2006; Milutinovich et al., 2007; Skibbens et al., 1999; Tóth et al., 1999). In fact, less than 50% of chromatid-bound cohesins participate in cohesion (Farcas et al., 2011; Haering et al., 2008). The simplest interpretation for such a ‘cohesin without cohesion’ phenotype is that each sister is bound by individual cohesins that subsequently become tethered together (Fig. 1). Second, there are secondary sister-chromatid-tethering complexes that include a role for cohesins but are inconsistent with the single-ring model (Chang et al., 2005; Gartenberg, 2009). For instance, transcriptionally repressed loci on sister chromatids are tethered together in a reciprocating manner by interactions between cohesins and silencing heterocomplexes – each of which is assembled onto sister chromatids. This way, silencing defects lead to loss of cohesion despite the retention of chromatid-bound cohesins (Chang et al., 2005). This clear demonstration that cohesins bind each chromatid oddly remains relegated to the exception instead of serving as an exemplar of genome-wide cohesion. The third challenge to the ‘replication through a ring’ model involves Eco1 stabilization in response to DNA damage that occurs during the G and M phases of the cell cycle [Eco1 is targeted for degradation by mitotic cyclin-dependent kinase or CDK (Lyons and Morgan, 2011)]. Importantly, this DNA-damage-induced establishment promotes the tethering of newly deposited cohesins throughout the entire genome – not just a sites of DNA damage (Heidinger-Pauli et al., 2008; Heidinger-Pauli et al., 2009); Lyons and Morgan, 2011; Ström et al., 2007; Unal et al., 2007). In fact, simply overexpressing Eco1 (presumably to overcome CDK-dependent targeting of Eco1 for degradation) in the absence of DNA damage is sufficient to engender genome-wide cohesion during mitosis (Heidinger-Pauli et al., 2008). Thus, establishment can occur independent of DNA replication forks or repair enzymes. Although it may be premature to completely discount models of huge cohesin rings that encompass two sister chromatids, far more likely are those models in which individual cohesins that are bound to each sister chromatid become modified in order to stabilize inter-cohesin assemblies and, thereby, achieve sister chromatid tethering (Box 1).

The discovery that Eco1 is an acetyltransferase that, at least *in vitro*, can modify several cohesin subunits, culminated in reports that acetylation of a highly conserved lysine residue within the cohesin Smc3 might be at the core of sister-chromatid-tethering reactions during S phase (Ivanov et al., 2002; Rolef Ben-Shahar et al., 2008; Unal et al., 2008; Zhang et al., 2008a). However, the situation is complicated owing to recent findings that acetylation of Smc3 lysine residues functions beyond sister-chromatid-tethering reactions (Guacci and Koshland, 2012). Thus, early studies that causally relate inviability to cohesion defects are premature (if not incorrect). Many cohesin subunits are subjected to several post-translational modifications (including SUMOylation, phosphorylation and acetylation), raising the possibility that specific modifications direct cohesins to participate in one cell process over another (Table 1). For instance, Mcd1 is phosphorylated in response to DNA damage but apparently not during cohesion establishment during S phase. Conversely, Smc3 is acetylated during S phase but not in response to DNA damage during mitosis (Table 1). Furthermore, new evidence suggests that acetylation of different Smc3 lysine residues differentially direct cohesins to function in cohesion or condensation (Guacci and Koshland, 2012).

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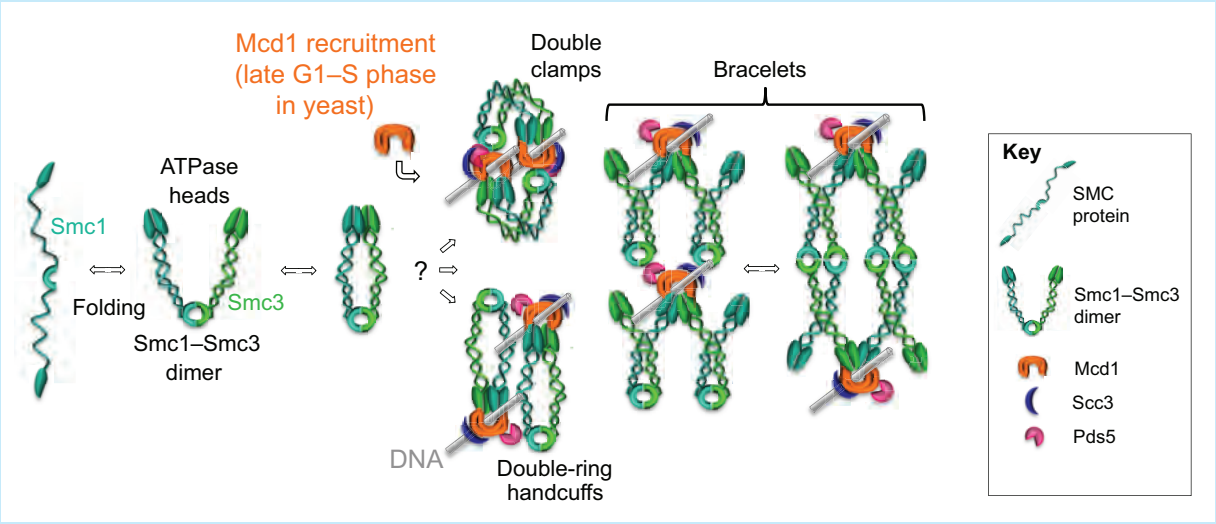
Box 1. Cohesins viewed through conserved structures

The structure of SMC complexes is highly conserved. All SMC proteins (Smc1, Smc2, Smc3, Smc4, Smc5, Smc6 and Rad50) are folded in half (see far left of Figure for unfolded Smc1) at a central hinge to produce a long coiled-coil and an ATPase 'head' that is formed through the association of N- and C-termini. SMC proteins associate through hinge-to-hinge and head-to-head binding (progressive Smc1 and Smc3 associations, see centre of the left part of the Figure). In turn, recruited factors, which may exhibit enzymatic activities, reside on top the closely apposed SMC heads. For cohesion, Mcd1 recruits Scc3, Pds5, Rad61 (not shown) and sororin (not shown). Pds5 and Rad61 are expendable in some model systems, whereas sororin is present only in vertebrate cells (Rankin et al., 2005; Díaz-Martínez et al., 2007; Shintomi and Hirano, 2009; Nishiyama et al., 2010). Thus, Pds5, Rad61 and sororin are unlikely to be structural components but regulators of potential therapeutic value. The highly conserved nature of SMC complexes provides crucial insight into the structure of cohesion but, thus far, remain largely underutilized. For instance, analyses of interactions of cohesin subunits and their release upon linearization of circular DNA (Haering et al., 2002; Gruber et al., 2003; Ivanov and Nasmyth, 2005; Haering et al., 2008; Farcas et al., 2011) led to a model in which huge cohesin rings encircle DNA (for alternative models, see McNairn and Gerton, 2008; Skibbens, 2008; Onn et al., 2008; Díaz-Martínez et al., 2008; Nasmyth and Haering, 2009). The presumption that DNA is embraced by SMC arms, however, is speculative, lacks support from DNA–protein mapping studies and is confounded by findings that Smc1 and Smc3 heads remain closely apposed during anaphase (Mc Intyre et al., 2007). Here, we rely on analyses of the crystal structure of the Rad50-containing MRN complex, which instead suggest that DNA resides between Mre11 dimers (analogous to Mcd1) and closely apposed SMC-like Rad50 heads (Hopfner and Tainer, 2003; Williams et al., 2008; Zhang et al., 2008b; Williams et al., 2009; Rupnik et al., 2010; Schiller et al., 2012; Möckel et al., 2012). Additional evidence suggests that SMC heads reside near SMC hinges (Sakai et al., 2003; Mc Intyre et al., 2007). Several models (see right side of Figure) highlight DNA positioning to the Mcd1–Smc1–Smc3 interface and allow for cohesin dimerization (oligomerization) to promote sister chromatid tethering.



Here, we discuss findings that are relevant to the establishment of cohesion during S phase, and the requirement for cohesion in yeast cell viability, before turning to the roles of acetylation in regulating cohesion establishment and chromosome condensation. We conclude with a review of cohesin modifications and speculate on the existence of a cohesin code, analogous to the histone code posited ~15 years ago, through which cohesins are directed to different processes such as chromosome segregation, DNA repair, transcription regulation, ribosome biogenesis, recombination and chromosome condensation.

Smc3 acetylation – a prelude to the cohesin code

Three independent studies identified Smc3 as the acetylation target of Eco1 and mapped two contiguous lysine residues (K112 and K113 in budding yeast and K105 and K106 in vertebrate cells) as crucial sites of modification (Rolef Ben-Shahar et al., 2008; Unal et al., 2008; Zhang et al., 2008a). The essential role of acetylation is undisputed: dual lysine-to-arginine (K→R) replacements (K112R and K113R), which result in non-acetylatable Smc3, render cells inviable (Rolef Ben-Shahar et al., 2008; Unal et al., 2008; Zhang et al., 2008a). Identical results are obtained when these lysine residues are replaced with alanine (K112A and K113A) (Zhang et al., 2008a). Two studies also addressed whether acetylation of both lysine residues is required for cells to remain viable and showed that either of the single non-acetylatable mutations K112R or K112A support cell viability (Unal et al., 2008; Zhang et al., 2008a). Thus, acetylation of K112 does not appear to be essential; but this result does not exclude the possibility that it regulates additional chromatin functions (discussed below). K113 thus appears to be the key acetylation event and, in fact, either non-acetylatable single mutation K113R or K113A renders cells inviable (Rolef Ben-Shahar et al., 2008; Zhang et al., 2008a). Intriguingly, the non-acetylatable K113T allele bypasses eco1 mutant cell temperature sensitivity (Rowland et al., 2009). Taken together, these studies suggest that acetylation of Smc3 K113 is essential

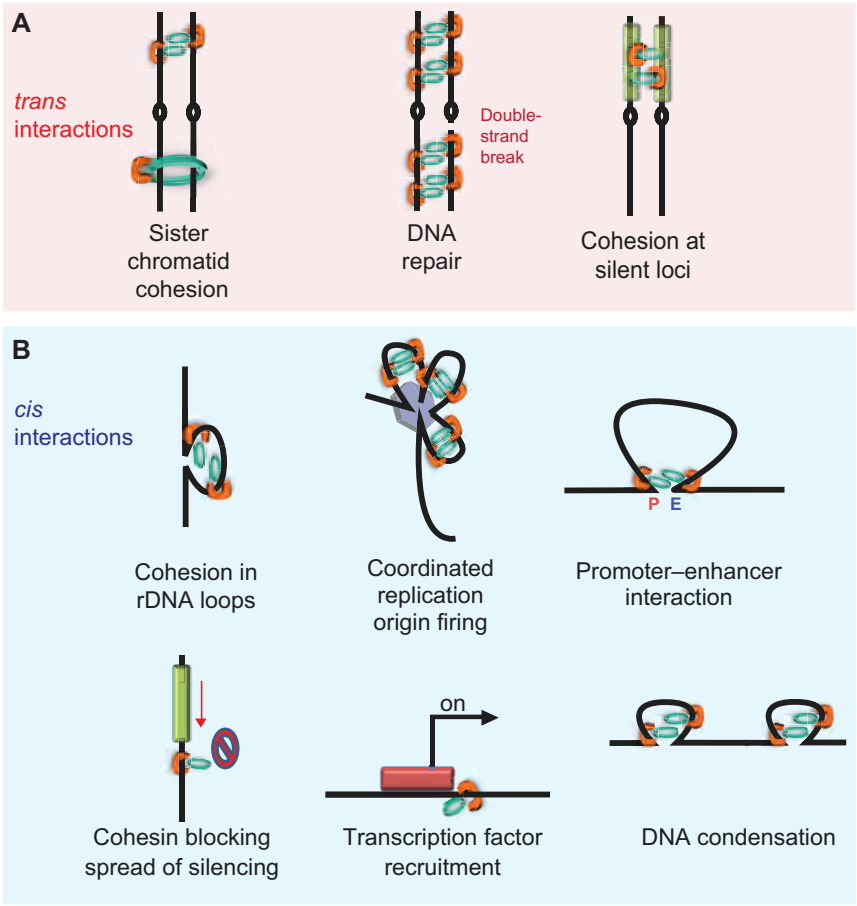


Fig. 2. Putative *cis* and *trans* configurations of cohesin functions. Cohesins perform essential roles in a number of cellular processes and in the context of either *cis* (intramolecular) or *trans* (intermolecular) DNA orientations. (**A**) *Trans*-based mechanisms of cohesin function include: sister chromatid tethering established during S phase that occurs along the length of the chromosome (left), DNA repair in which double-strand breaks induce new rounds of Eco1-dependent sister chromatid tethering reactions during G2 and M phases to provide a template for error-free DNA repair (middle), and cohesion at silenced loci by cohesins bound to one chromatid interacting with Sir2 complexes assembled on silenced loci of the sister chromatid (right). Evidence that cohesins topologically embrace DNA suggest models in which each sister is either bound by cohesins that become tethered together (top part of sister chromatid cohesion – left) or two sisters may be bound by a huge single cohesin ring (lower portion of sister chromatid cohesion – left). See Box 1 for further discussion of cohesin structure. (**B**) *Cis*-based mechanisms of cohesin function are more numerous. Top: Cohesins stabilize rDNA loops to promote efficient ribosome assembly, coordinate replication-origin firing by clustering origins within a zone of initiating activity (purple) and promote registration of promoter-enhancer DNA elements required for transcription. Bottom: Cohesins also block the spread of repressor complexes or preclude promoter enhancer-registration (not shown) to suppress transcription, recruit or stabilize transcription factors to promote transcription and promote chromosome condensation.

but that rectifying mutations exist in specific yeast genetic backgrounds that support the growth of cells with non-acetylatable lysine residues at this position (Guacci and Koshland, 2012). Differences in *RAD61* (termed WAPL in vertebrate cells) may provide the required rectification. Deletion of *RAD61* in yeast bypasses (at least conditionally) the requirement for both Smc3 acetylation and Eco1 activity (Maradeo and Skibbens, 2010; Rolef Ben-Shahar et al., 2008; Rowland et al., 2009; Sutani et al., 2009). Thus, reduced Rad61 function in otherwise wild-type cells could account for tolerance to some *smc3* mutations in yeast cells (Guacci and Koshland, 2012).

As Smc3 acetylation of K113, but not K112, appears crucial for cell viability, one would predict that replacement of K113 with either of two structurally related acetyl-mimic residues, such as glutamine or asparagine, should support cell viability. By contrast, similar replacements of K112 should have little adverse effect on cell growth. Indeed, the first prediction was confirmed because either acetyl-mimic *smc3* mutants (K113N or K113Q) result in a dramatic suppression of *eco1*-deletion-mediated conditional lethality (Rolef Ben-Shahar et al., 2008; Unal et al., 2008). However, the second prediction proved to be more intriguing. Although acetyl-mimic K112 *smc3* mutants (K112N or K112Q) had little effect on cell growth, the combination of both acetyl-mimic mutations (K112Q, K113Q) failed to rescue *eco1*-deletion-mediated conditional growth defects (Guacci and Koshland, 2012; Unal et al., 2008). It is noteworthy that analogous double K→Q mutations in *Smc3* in vertebrate cells did suppress the cohesion defects in cells otherwise knocked down for ESCO1 function, but in cells that retained wild-type *Smc3*, which is likely to mask any adverse effects (Guacci and Koshland, 2012; Unal et al., 2008; Zhang et al., 2008a) Therefore, acetylation of K112 appears to be a crucial regulator of cohesion but is, on its own, insufficient to engender establishment.

Anti-establishment and the dangers of death

What is the role of Smc3 acetylation? Answers exist both at the molecular and phenomenological levels. In general, the role of acetylation at the molecular level has been well-studied (reviewed by Polevoda and Sherman, 2002; Izzo and Schneider, 2010; Yang and Seto, 2008; Miller et al., 2010; Xiong and Guan, 2012). On the one hand, histone acetylation sequesters the positive charge of lysine and, thus, disrupts contacts between the N-terminal tail of histone and DNA. Acetylation also can disrupt protein–protein interactions (for instance, in the case of hormone receptors and co-activators) and inhibit higher-order chromatin complex formations (such as 30 nm solenoid fibers). On the other hand, lysine acetylation near an enzymatic active site can inhibit that enzyme – a situation reported numerous times for SOD2 (reviewed by Miller et al., 2010). Such a relationship was similarly posited for Smc3, i.e. that acetylation would inactivate Smc3 – here, to create a 'locked' cohesin structure (Chan et al., 2012). This simple model,

Table 1. Cohesin modifications

	Acetylation	Phosphorylation	SUMOylation
Cohesion	Smc3 (K112, K113) Mcd1 (K84, K210) Smc1 Scc3 Pds5 Eco1	Mcd1 (S83) Scc3 Eco1	Smc3 Mcd1 Smc1 Scc3 Pds5 Eco1
DNA repair	Mcd1 (K84, K210) Eco1	Mcd1 (S83)	Mcd1 (K165, K290, K460) Smc1
Condensation	Smc3 (K113) Mcd1		
Unknown	Smc3 (K309, K316, K699, K700, K931, K940) Scc3 (K13, K36, K78, K106, K224, K1071, K1086)		Mcd1 (K252, K345, K391, K392, K394, K500, K509, K521)
References	(Ivanov et al., 2002; Rolef Ben-Shahar et al., 2008; Unal et al., 2008; Unal et al., 2007; Heidinger-Pauli et al., 2008; Heidinger-Pauli et al., 2009; Zhang et al., 2008)	(Brands and Skibbens, 2005; Heidinger-Pauli et al., 2008; Heidinger-Pauli et al., 2009; Lyons and Morgan, 2011)	(Almedawar et al., 2012; McAleenan et al., 2012; Williams et al., 2003)

A partial compilation of post-translational modifications reported for cohesins (Mcd1, Smc1, Smc3 and Scc3) cohesin accessory factors (Pds5, Rad61) and the establishment factor Eco1. Where known, post-translation modification of amino acids are listed across from the implicated function. Code 'writers' include Eco1, Mms21, Nfi1, Nse2, Cdc28, and Skp1–cullin-F-box-containing complex SCF^{Cdc4}. 'Erasers' such as Hos1 or Smt4 (SUMO isopeptidase) are not shown. Apart from cohesion-related factors, Eco1 also acetylates Mps that is crucial for duplication of the spindle pole body, telomere motility and the association of the chromosome with the nuclear envelope 3, a nuclear envelope yeast protein (Antoniacci et al., 2007; Antoniacci et al., 2004; Antoniacci and Skibbens, 2006; Ghosh et al., 2012; Haas et al., 2012).

however, fails to account for the fact that Smc3 is acetylated prior to DNA replication (Song et al., 2012). *A priori*, pre-replicative acetylation that produces locked cohesin rings might be expected to form a barrier to the progression of the DNA replication fork. However, the opposite is true: ESCO2 knockdown adversely affects the progression of the DNA fork (Terret et al., 2009). Acetylation as a 'cohesin ring lock mechanism' is furthermore confounded by evidence that pre-replication cohesin acetylation is inconsequential to the tethering of sister chromatids (Song et al., 2012). Thus, it appears that Smc3 acetylation per se is critical, but that acetylation must occur in context (during maturation of sister chromatids) and extend to accessory factors that otherwise preclude the conversion of chromatin-bound cohesins to a tethering-competent state. The fact that histone acetylation can serve as a docking site for bromodomain transcription activators (reviewed by Sanchez and Zhou, 2009) supports the notion that Smc3 acetylation may recruit additional factors that either bridge cohesin complexes bound to each sister chromatid (Box 1) or alter the position of anti-establishment factors that otherwise preclude the tethering of sister chromatids.

At the phenomenological level, the predominant view is that Eco1-dependent Smc3 acetylation at K113 inactivates the cohesin-bound anti-establishment activities that are provided by Rad61 and Pds5, i.e. that Smc3 acetylation may displace or inactivate cohesin-associated factors that would otherwise drive cohesin dissociation from DNA (Chan et al., 2012; Rowland et al., 2009; Sherwood et al., 2010; Terret et al., 2009). In part, this hypothesis is based on observations that either *RAD61* deletion or specific alleles of *PDS5* bypass the requirement in cell viability for Eco1 and that the association of cohesin to chromatin is sensitive to Rad61 protein levels (Chan et al., 2012; Gandhi et al., 2006; Hartman et al., 2000; Kueng et al., 2006; Panizza et al., 2000; Rowland et al., 2009; Sutani et al., 2009; Tanaka et al., 2001). One of many possible models is that anti-establishment factors maintain cohesins in a highly dynamic state. Surprisingly, mutation (or depletion) of *RAD61* family members can have different effects on cohesin dynamics (i.e. hyperstabilizing or destabilizing cohesin binding), depending on the cell type or the RAD61 allele tested within a cell type (Gandhi et al., 2006; Gause et al., 2010; Kueng et al., 2006; Bernard et al., 2008; Sutani et al., 2009; Rowland et al., 2009). Contrary to prediction, overexpression of Rad61 has no effect on cohesin binding to DNA in yeast, unless Eco1 function is perturbed in parallel (Chan et al., 2012). Thus, the anti-establishment role of Rad61 in yeast occurs primarily on inactive cohesins because of severely limited or absent Eco1. In contrast to Rad61 (and Pds5), which are thought to act through cohesins, other anti-establishment factors – such as the RFC component Elg1 – instead appear to act through Eco1 (Maradeo and Skibbens, 2009; Maradeo and Skibbens, 2010; Parnas et al., 2009). Regardless of the underlying mechanisms, the fact that deletion of *RAD61* allows *eco1*-null cells to remain viable is a remarkable rescue of an otherwise lethal scenario (Feytout et al., 2011; Rowland et al., 2009; Skibbens et al., 1999; Sutani et al., 2009; Tanaka et al., 2001; Tóth et al., 1999). The recent study by Guacci and Koshland, however, demonstrates that cell death is rather an unfortunate measure for assessing loss of cohesion, and reveals that defects in other processes such as chromosome condensation are equally linked to inviability of yeast cohesin mutants (Guacci and Koshland, 2012).

The prediction is simple: if Rad61 is, indeed, an anti-establishment factor that becomes inactivated by Eco1, then *eco1 rad61* double null mutant cells should not only be viable, but also exhibit normal sister chromatid pairings. By contrast,

these double mutant cells exhibit both conditional lethality and, even at permissive temperatures, extensive cohesion defects that are identical to those observed in cohesin mutant cells (Guacci and Koshland, 2012; Rowland et al., 2009; Sutani et al., 2009; Feytout et al., 2011). To put it simply, the rescue of viability of *eco1*-null cells is not due to the rescue of cohesin-based sister chromatid tethering, as previously posited in yeast studies (Rowland et al., 2009; Sutani et al., 2009). What then is the molecular mechanism underlying this rescue? Guacci and colleagues realized the importance of previous evidence that mutations in either *PDS5*, *ECO1* or *MCD1* all produce chromosome condensation defects as well as cohesion defects in yeast chromatid pairing in budding and fission yeast (Guacci et al., 1994; Guacci et al., 1997; Hartman et al., 2000; Lavoie et al., 2004; Skibbens et al., 1999). Could the *RAD61* deletion bypass of *eco1*-null cells occur through rescue of condensation defects as opposed to cohesion defects? Their detailed analysis supports exactly this model in that the condensation defect – but not the cohesion defect – in *eco1*-null cells is rescued by the deletion of *RAD61* (Guacci and Koshland, 2012). Therefore, Eco1-dependent Smc3 acetylation is essential for inactivating the anti-condensation activity that is mediated by Rad61.

Writing the cohesin code for cohesion and condensation

The observation that Eco1 has essential roles in condensation and cohesion raises the obvious question of whether the acetylation of different residues supports separable activities. This question is crucial, as the answer might determine whether condensation proceeds as a consequence of cohesion establishment or whether these separable activities both depend on Smc3 acetylation – but at different lysine residues. Considering the results discussed above in the context of acetylation becomes instructive. The K113Q mutation only partially suppresses *eco1* mutant cell conditional growth, and neither K113Q single nor K112Q–K113Q double mutations support cell viability on their own (Unal et al., 2008; Zhang et al., 2008a). By contrast, K112R–K113Q supports robust cell growth as the sole source of Smc3 function. This inclusion of K112R (providing both a non-acetylatable and non-acetylmimic residue) in the double-mutant cells, however, rescues only the chromosome condensation defects of *eco1*-mutant cells – K112R K113Q cells continue to exhibit massive cohesion defects (Guacci and Koshland, 2012). These results support the notion of a cohesin code that is in part written through acetylation. By extension, note that arginine methylation (at least in histones) recruits HEAT-repeat proteins (Jenuwein, 2006; Jenuwein and Allis, 2001). It would be interesting to test whether the *smc3* double mutant K112R–K113Q rescues condensation through K112R methylation and subsequent recruitment of HEAT proteins, such as Pds5 and Scc2, which then could either stabilize cohesins or drive additional rounds of either cohesin or condensin recruitment. Toward this end, we speculate that histones and their modifications provide a powerful tool by analogy through which cohesin functions can be comprehensively dissected.

Chromatin research greatly benefited from the description of a histone code in which temporal and context-specific histone modifications act as crucial regulators of replication, epigenic maintenance and transcription (Gardner et al., 2011; Jenuwein and Allis, 2001; Strahl and Allis, 2000; Turner, 2000). A similar epigenetic readout has been posited for PCNA (Shibahara and Stillman, 1999; Zhang et al., 2000; Hoege et al., 2002; Gazy and Kupiec, 2012). Is there an analogous cohesin code? For Smc3, K113 acetylation is essential for cell viability. Both K112 and K113 are acetylated *in vivo*, but deacetylation of K112 appears to be crucial for condensation (Unal et al., 2008; Zhang et al., 2008a; Guacci and Koshland, 2012). Peptide-mapping studies of an Smc3 fragment centered on K112–K113 further suggest sequential modifications, as both mono- (K113) and diacetylated peptides were recovered (Unal et al., 2008). Currently, it is unknown whether K112 can be acetylated in the absence of K113 acetylation. Conceptually, there are three possible paths with regard to Smc3 acetylation that include a di-acetylated intermediate: (1) K112 acetylation precedes that of K113; (2) K113 acetylation precedes that of K112 or (3) K112 and K113

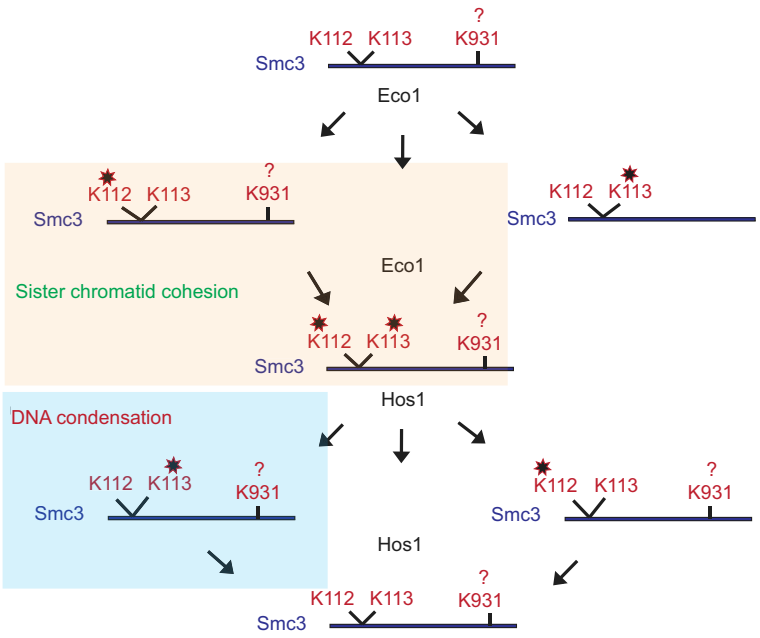


Fig. 3. Cohesin code for cohesion and condensation. Lysine residues K112 and K113 of Smc3 are both acetylated *in vivo* by Eco1. Acetylation of K113 appears essential for cohesion establishment during S phase. Even though K112 is similarly acetylated during S phase, it is the unacetylated form of K112 that supports chromosome condensation. Analysis of Smc3 peptides suggests a step-wise acetylation pathway on the basis of the recovery of both diacetylated (K112, K113) and mono-acetylated (K113) fragments. At present, monoacetylated K112 cannot be excluded as a path to diacetylation. Thus, unacetylated Smc3 could become bi-acetylated and then monoacetylated via numerous paths. On the basis of the differential roles of acetylation in cohesion and condensation, coupled with the peptide mapping studies, we hypothesize a cohesin code algorithm that mediates these processes in a stepwise fashion. Lysine residue K931 is also highly conserved through evolution and, thus, may have a regulatory role. Acetylation reactions that appear to promote cohesion are shown in red, those that seem to promote condensation are shown in blue.

are simultaneously acetylated (Fig. 3). Given that K112 acetylation appears to be problematic for condensation (Guacci and Koshland, 2012), a subsequent deacetylation step might be required in each of the above scenarios.

In addition to the putative sequential deacetylation steps that may balance cohesion and condensation reactions, several studies reveal a role for deacetylases in recycling cohesins back to an unmodified state upon which new codes can be written in the next cell cycle (Beckouët et al., 2010; Borges et al., 2010; Deardorff et al., 2012a; Xiong et al., 2010). Cohesin inactivation by Mcd1 proteolysis defines a main step in anaphase onset and sister chromatid separation (Uhlmann et al., 1999). It is at this cell cycle transition that Smc3 becomes deacetylated. Intriguingly, the yeast histone de-acetylase Hos1 (HDAC8 in vertebrate cells) that erases the acetylation code from Smc3 is not essential (Beckouët et al., 2010; Borges et al., 2010; Xiong et al., 2010; Deardorff et al., 2012a), suggesting that Smc3 deacetylation is not crucial. However, Hos1 overexpression in cells that are diminished for Eco1 function exhibit severe growth defects. Similarly, cells genetically engineered to retain high Eco1 acetyltransferase activity in the absence of Hos1 deacetylase activity exhibit elevated levels of sister chromatid cohesion defects. Both studies argue that unmodified Smc3 must be present during S phase so that establishment can occur in the context of replicated sister chromatids – but not before (Beckouët et al., 2010; Xiong et al., 2010).

A code for all processes

Is Smc3 acetylation the only modification of cohesin and is this code limited to functions in cohesion and condensation? The answer to both questions is likely to be a resounding "No". First, cells that harbor mutations in cohesin genes exhibit a wide spectrum of abrogated or aberrant processes that include ribosome biogenesis, transcription regulation, cohesion, condensation and DNA repair. Second, and most importantly, there are reports of separation-of-function alleles that adversely affect one cell process while leaving other processes unaffected (Bose et al., 2012; Brands and Skibbens, 2005; Brands and Skibbens, 2008; Dorsett, 2010; Skibbens, 2010; Ström et al., 2007; Unal et al., 2007). The list of cohesin modifications – including phosphorylation, SUMOylation, acetylation and ubiquitination – is impressive (Table 1). Even more impressive are the early indications that different modifications denote cohesin participation in different functions. For instance, a DNA damage response during mitosis includes acetylation of Mcd1 (by Eco1) and phosphorylation [dependent on Mec1 (ATR) and Chk1 kinase] – neither of which occur during cohesion establishment during S phase (Heidinger-Pauli et al., 2008; Rolef Ben-Shahar et al., 2008; Unal et al., 2008; Zhang et al., 2008a; Heidinger-Pauli et al., 2009). Mcd1, as well as most other cohesins, are also hyper-SUMOylated (by Mms21/Nse2 in yeast) in response to DNA damage – possibly to promote repair by stabilizing the association of a DNA template at the site of damage. Additional examples are provided in Table 1, but this list is not exhaustive. New cohesin modifications appear to be discovered almost monthly, such that we are only beginning to identify the writers (modifying enzymes), readers (complexes that respond to cohesin modifications) and erasers (enzymes that return cohesins to a 'ground state') of the cohesin code. Cohesin participation in DNA repair, condensation and cohesion appears to be dose-dependent (Heidinger-Pauli et al., 2010), further suggesting that specifically modified cohesin pools exist that are under-represented and, thus, difficult to uncover.

Clearly, we are only at the very beginning of code exploration. Although we appreciate the speculative nature of such a code, we predict that additional modifications [much like the bevy of histone tinkerings, which are deciphered both combinatorally and contextually (e.g. Strahl and Allis, 2000; Turner, 2000; Jenuwein and Allis, 2001; Gardner et al., 2011)] are likely to be discovered within this decade. It will then become important to test the scenarios of cohesin modification and phenotypic rescues in the context of different cellular processes. Identification of new code writers (kinases, acetyltransferases, SUMOylating and ubiquitylating proteins), targets and readers (cohesins and their regulators, such as Pds5, Rad61), and erasers (deacetylases, phosphatases and proteins that deSUMOylate) will be important to understand the impact of a code on cohesin function. For instance, the histone deacetylase Hos1 in budding yeast (HDAC8 in vertebrate cells) provides an important tool to study not only the recycling of acetylated Smc3 subunits for the next cohesion cycle, but also the step-wise removal of modifications that may direct cohesins to different cell processes (Beckouët et al., 2010; Borges et al., 2010; Deardorff et al., 2012a; Xiong et al., 2010). We fully expect that future investigation of these 'code erasers' will provide important new insights into the cellular processes and the regulation of the processes that are facilitated by cohesin modifications.

Deciphering such a code is likely to be of great clinical relevance. A number of cohesins and cohesion regulators (including Mcd1, Smc1 and Eco1) are important for ribosome biogenesis – a pathway that is directly linked to several developmental deformities that include Treacher Collins syndrome and Diamond–Blackfan anemia (Bose et al., 2012; Narla and Ebert, 2010; Skibbens et al., 2010). Cohesin and establishment mutations also result in developmental maladies, such as Roberts Syndrome/SC-phocomelia or Cornelia de Lange syndrome, that impart growth and mental retardation, severe limb reduction, and numerous facial abnormalities (Gordillo et al., 2008; Krantz et al., 2004; Musio et al., 2006; Tonkin et al., 2004; Vega et al., 2005; Deardorff et al., 2007; Deardorff et al., 2012b). A subset of cohesinopathies arise through deregulation of transcription programs (Gartenberg, 2009; Dorsett, 2011), based on the observation that cells from Cornelia de Lange patients can appear almost normal in terms of sister chromatid tethering (Castronovo et al., 2009; Revenkova et al., 2009). Cohesins similarly play a key role in DNA repair, and defective DNA repair in combination with transcription deregulation further impacts development (Dorsett and Ström, 2012). A link between these syndromes and the cohesin code is further supported by findings that mutations in the vertebrate cohesin de-acetylase HDAC8 are found in a subset of Cornelia de Lange syndrome phenotypes (Deardorff et al., 2012a; Deardorff et al., 2012b; Harakalova et al., 2012). Elucidating the extent to which cohesinopathies and ribosomopathies share underlying molecular mechanisms is likely to provide significant advances to both clinical and basic science research.

In the wake of the fork: cohesion establishment and chromatin formation

If a cohesin code, indeed, differentiates between cohesin functions in cohesion, condensation, transcription, ribosome biogenesis and DNA repair, when does such a code become

embedded? Various cohesin modifications take place in different phases of the cell cycle and in response to specific challenges (such as DNA damage), but a significant portion of the code appears to be embedded immediately behind leading and lagging DNA polymerases as each sister strand matures (Okazaki maturation, histone deposition, etc.) (Fig. 4). In support of this model, numerous studies show that Eco1 physically interacts with a number of DNA replication components, all of which reside behind DNA polymerase and promote maturation of replicated DNA strands (reviewed by Sherwood et al., 2010; Skibbens, 2010). Most recently, Eco1 was shown to physically (and genetically) interact with Fen1, which has a dedicated role in Okazaki fragment maturation. The most compelling evidence regarding the timing of Eco1-dependent acetylation of Smc3, however, comes from the *Xenopus* system. As in the human genome, the *Xenopus* genome encodes for two Eco1 homologs (XEco1 and XEco2). Only XEco2 is active in early frog embryos, therefore, egg extracts that are induced to enter the cell cycle but are depleted of XEco2 exhibit dramatic cohesion defects (Song et al., 2012). Addition of XEco2 prior to (but not after) DNA replication results in normal sister chromatid pairing, suggesting that XEco2 is required during S phase. Song and colleagues also tested whether XEco2 acetylates Smc3 prior to S phase. Surprisingly, extracts in which DNA replication origins cannot fire remain competent to acetylate Smc3. Careful cell cycle mapping revealed that, pre-S phase, Smc3 acetylation occurs also in cultures of human cells (Song et al., 2012), raising the crucial question whether pre-S phase acetylation contributes to cohesion? To address this question, Song and colleagues turned to an allele of XEco2 that is deficient in binding to PCNA ($Xeco2^{-PCNA}$). $Xeco2^{-PCNA}$ is fully competent to both bind chromatin and acetylate Smc3 in extracts that contain no other XEco2. However, $Xeco2^{-PCNA}$ completely fails in cohesion establishment (Song et al., 2012). These results mirror that of Scc2 and Scc4 deposition function: deposition can occur before DNA replication but is incapable of producing sister chromatid pairing [inviability of *scc2* and *scc4* mutant cells mirrors S-phase progression (Bernard et al., 2006; Ciosk et al., 2000; Furuya et al., 1998)]. Therefore, acetylation and deposition must occur in the presence of both sister chromatids.

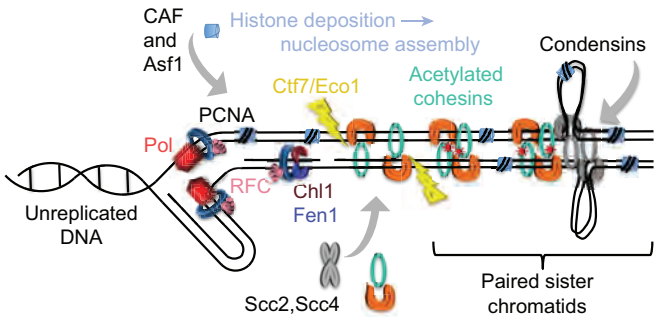


Fig. 4. Cohesion establishment probably occurs immediately behind the DNA replication fork. The core enzymes of the replisome (leading and lagging polymerases, i.e. DNA polymerases coupled to PCNA) move to the left (lagging strand at the bottom). Histone deposition and chaperone factors (CAF and Asf1) interact with PCNA (Corpet and Almouzni, 2009; Franco et al., 2005) to promote nucleosome assembly (blue barrels) on nascent DNA strands, providing a context in which cohesion establishment is likely to occur. In the context of continued maturation (RFC, PCNA, Chl1 and Fen1) of the nascent sister chromatids, Eco1 (yellow) acetylates (red star) chromatin-bound Smc3. Cohesins Mcd1 (orange), Smc1 and Smc3 (green) and Eco1 also promote condensation (illustrated by the looping DNA structures at the far right). Histone chaperones or deposition factors interact with PCNA and RFC, which suggests that cohesion establishment and chromosome condensation are coordinated with chromatinization (Corpet and Almouzni, 2009; Franco et al., 2005; Rolef Ben-Shahar et al., 2009). Here, cohesin that participates in cohesion is deposited by Scc2 and Scc4 in the context of replicated sister chromatids (behind DNA polymerase).

The above studies reveal that a unique context is required for cohesion establishment that goes beyond the simple presence of two sister chromatids. Insight regarding this context comes from analysis of the DNA helicase Chl1, which is crucial for cohesion and binds Eco1. Previous reports link the mutation of *CHL1* or decreased levels of the human CHL1 homolog DDX11 (also known as ChlR1), to decreased cohesin binding to chromatin (Parish et al., 2006; Inoue et al., 2007; Laha et al., 2011). This decreased cohesin binding seems to depend on altered heterochromatin that occurs in proximity to Fen1-dependent maturation of Okazaki fragments (Farina et al., 2008; Inoue et al., 2011). We have recently reported that Chl1 exhibits both genetic and physical interactions with Fen1 (Rudra and Skibbens, 2012). On the basis of these studies, Chl1 probably acts behind DNA polymerase during maturation of sister chromatids in order to facilitate cohesin loading by resolving aberrant DNA structures that might arise during Okazaki fragment maturation (Rudra and Skibbens, 2012; Wu et al., 2012). If correct, then establishment (and possibly deposition) occurs during histone deposition onto the newly replicated DNA – raising the possibility that cohesion, condensation and chromatinization are spatially and functionally coordinated (Fig. 4).

Several studies implicate specialized chromatin structures and nucleosome remodeling in cohesin deposition (Dorsett, 2011; Gartenberg, 2009; Skibbens, 2008). For instance, the ATP-dependent nucleosome remodeling complex RSC promotes efficient cohesin loading – especially in the context of double-strand breaks (Huang et al., 2004; Liang et al., 2007; Oum et al., 2011). Fission yeast Swi6 is a histone-binding factor required for cohesin association with heterochromatin (Pidoux and Allshire, 2005; Bernard et al., 2001; Nonaka et al., 2002). Intriguingly, the cohesin deposition factor NIPBL (Scc2 in yeast) binds to histone deacetylases in vertebrate cells, potentially linking cohesin deposition to histone modification (Jahnke et al., 2008). The histone deacetylase Sir2 is required for the binding of cohesin to repressed heterochromatin (repressed mating-type loci and rDNA in yeast), although the underlying mechanism appears to involve physical recruitment rather than enzymatic function (Chang et al., 2005; Kobayashi et al., 2004; Wu et al., 2011). The Arp-dependent ATPase chromatin remodeling complex INO80 does not appear to impact cohesin binding (Ogiwara et al., 2007), but its dual role in nucleosome positioning and cohesion is consistent with a chromatin-based model of cohesion establishment and cohesin deposition (Fig. 4). Finally, a recent study from Chen and colleagues used a genome-wide deletion collection to identify suppressors of a conditional eso1 mutant fission yeast strain (Chen et al., 2012). Their results are stunning for two reasons. First, over 200 gene deletions were identified that adversely impact the conditional growth of *eso1* mutant strains. Clearly, Eco1-type acetylation extends well beyond what has been appreciated thus far. Second, deletion of *spt2*, *not3* or *rox3* – all of which regulate transcription or effect chromatin architecture, can rescue *eso1* conditional growth defects (Chen et al., 2012). These links support a model, in which cohesion establishment occurs during chromatin formation (Fig. 4).

Concluding remarks

The past few years of cohesion research has provided important insights into a complex regulatory network that might be deciphered using a cohesin code. The mechanisms of cohesion establishment are only now coming to light, and earlier studies should be carefully reviewed in the context of chromatinization and DNA condensation. The general tendency of linking synthetic lethality that is observed for cohesion-associated genes to functions in sister-chromatid cohesion also needs careful reviewing. Future experiments that directly address the function of cohesin modifications in driving cohesion and condensation, their effects on DNA repair, transcription regulation and ribosome assembly or maturation, are crucial to tease out the mechanisms behind these intriguing cellular processes. In addition to these fundamental insights, elucidating the extent to which cohesinopathies (including Roberts syndrome, Cornelia de Lange syndrome and Warsaw breakage syndrome) and ribosomopathies (e.g. Treacher Collins syndrome and Diamond–Blackfan anemia) share their underlying molecular mechanisms is likely to provide significant advances to basic research and its translation into therapeutic strategies in the clinic.

Acknowledgements

The authors thank the anonymous reviewers for valuable suggestions Skibbens lab members Kevin Tong and Tina Sie Godfried, and the Cassbens group for helpful comments during the preparation of this Commentary.

Funding

The Skibbens lab is funded by the National Institute of General Medical Sciences [grant number 2R15GM083269-02] and a Faculty Innovation Grant from Lehigh University. Any opinions, findings, and conclusions or recommendations expressed in this study are those of the authors and do not necessarily reflect the views of the National Institutes of General Medical Sciences or Lehigh University. Deposited in PMC for release after 12 months.

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