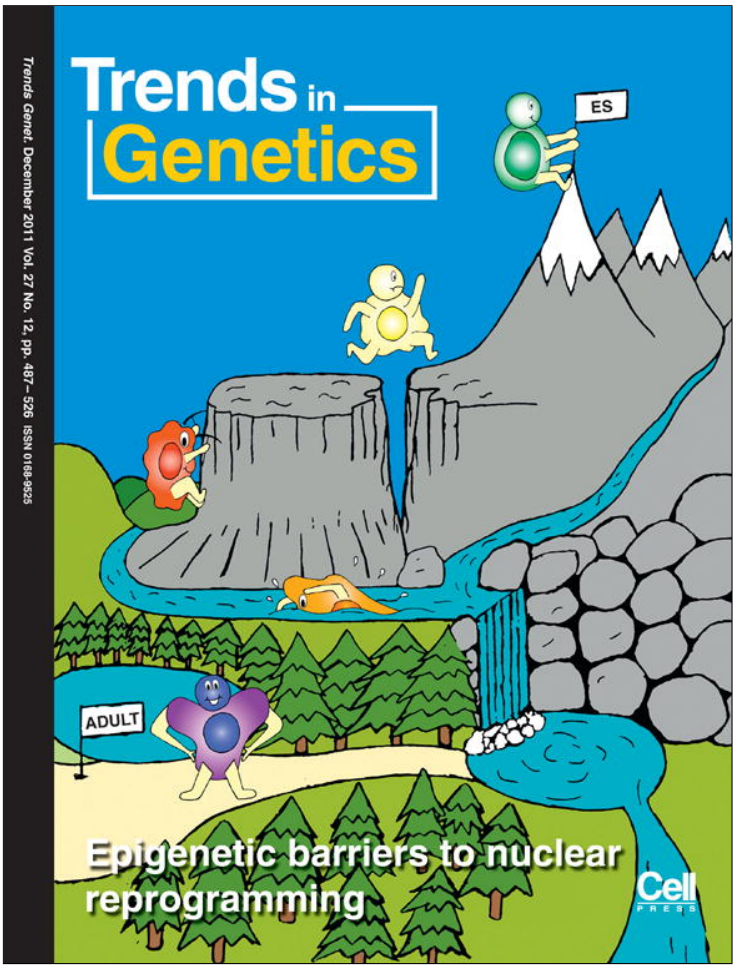

Opinion

Cell PRESS

Sticking a fork in cohesin – it's not done yet!

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To identify the products of chromosome replication (termed sister chromatids) from S-phase through M-phase of the cell cycle, each sister pair becomes tethered together by specialized protein complexes termed cohesins. To participate in sister tethering reactions, chromatin-bound cohesins become modified by establishment factors that function during S-phase and bind to DNA replication-fork components. Early models posited that establishment factors might move with replication forks, but that fork progression takes place independently of cohesion pathways. Recent studies now suggest that progression of the replication fork and/or S-phase are slowed in cohesion-deficient cells. These findings have led to speculations that cohesin ring-like structures normally hinder fork progression but coordinate origin firing during replication. Neither model, however, fully explains the diverse effects of cohesion mutation on replication kinetics. I discuss these challenges and then offer alternative views that include cohesin-independent mechanisms for replication-fork destabilization and transcription-based effects on S-phase progression.

Sister chromatid tethering reactions require cohesins, deposition complexes and establishment factors

The viability of cell progeny requires that each chromosome is faithfully replicated and that the resulting sister chromatids are segregated with high fidelity, ensuring that each newly forming daughter cell receives a genetic complement identical to that of the parent cell. The temporal separation of the DNA synthesis phase (S-phase) from the sister chromatid segregation phase (M-phase or mitosis) requires cells to identify chromatids as sisters throughout this interval. Identity is achieved and maintained through the combined activities of several complexes: cohesins, deposition complexes and establishment factors [1]. Cohesins tether together sister chromatids from early S-phase until anaphase onset and thus maintain identity over time. Deposition complexes load cohesins onto chromatin, but deposition is not sufficient to tether sisters together. Establishment factors must first convert chromatin-associated cohesins into a tethering-competent state.

Numerous studies reveal that the establishment of cohesion (Glossary) may be intimately coupled to DNA replication in unperturbed cells. Establishment factors function during S-phase and physically interact with numerous DNA replication factor components [2]. Despite the preponderance of findings documenting that establishment may be intimately linked to replication, little if any evidence over the last decade suggests that cohesion plays a role in replication-fork progression. Recent human cell studies, however, suggest that defects in cohesion pathways may indeed slow S-phase progression [3,4]. One model proposed to explain the S-phase progression defect was that cohesins coordinate origin firing: fewer cohesins lead to fewer forks and thus a longer S-phase. A second model focused on reduced fork migration in establishment-depleted cells. The model advanced stipulated that cohesins normally exist as barriers that physically impede the replication fork. Only during establishment would cohesins undergo a structural change to allow for fork progression. Both hypotheses require additional validation, and several lines of evidence appear to be inconsistent with the cohesin barrier model in particular, including observations that normal replication takes place in cohesion mutants in other model cell systems (see below).

In this article I briefly review replication-coupled cohesion establishment and recent models of cohesins as fork barriers and coordinators of origin firing. Evidence that challenges these models is then examined, from which I offer alternative hypotheses including that mutation of

Glossary

Aneuploidy: incorrect DNA content of a cell – often the consequence of chromosome mis-segregation during mitosis.
Cohesin: the complex of Mcd1/Scc1, Smc1, Smc3 and Irr1/Scc3 that tethers sister chromatids together from S-phase until anaphase onset. Cohesins also play a role in transcription regulation.
Cohesion: the process by which sister chromatids are identified and tethered together.
Establishment: the process by which chromatin-associated cohesins are converted to a tether-competent state. Ctf7 is an acetyltransferase that modifies the cohesin subunit Smc3 to establish cohesion. In the absence of Ctf7, sister chromatids are fully decorated with bound cohesins but remain unpaired.
FLIP (fluorescence loss in photobleaching); FRAP (fluorescence recovery after photobleaching: microscopy-based techniques that permit the detection of moving fluorescently labeled proteins, lipids or macromolecules.
PCNA (proliferating cell nuclear antigen): a ring structure (homotrimeric complex in eukaryotes). By encircling DNA and binding to DNA polymerase, PCNA facilitates processive DNA replication and is often referred to as a sliding clamp.
RFC (replication factor C) complex: the complex that loads sliding clamps such as PCNA onto DNA to facilitate DNA replication and repair. There are currently four different RFC complexes in yeast which differ primarily by the identity of the unique large subunit (Rfc1, Ctf18, Rad24 or Elg1) that associates with Rfc2–Rfc5.
Single fiber analysis: sequential but temporally distinct incorporation of nucleotide analogs that allows for measurement of replicated DNA regions over time.

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human establishment factors may directly destabilize the replication forks to which they bind.

Yeast cell studies reveal that cohesion establishment appears coupled to the DNA replication fork in unperturbed cells

Cohesin complexes (composed of Mcd1/Scc1, Smc1, Smc3 and Scc3/Irr1) tether sister chromatids together from early S-phase until anaphase onset [1]. The establishment factor Ctf7/Eco1 (herein termed Ctf7; additional gene nomenclature is given in Table 1) is an acetyltransferase that modifies the cohesin subunit Smc3 to convert chromatin-bound cohesins to a sister chromatid-tethering state [5–8]. Cohesion establishment appears to be intimately coupled to DNA replication during S-phase: Ctf7 associates with DNA replication factors such as PCNA, RFC complexes (Glossary and below) and Chl1 DNA helicase-like protein [9–11]. Mutation in any one of these DNA replication factors (and a host of fork-related factors) is sufficient to produce cohesion defects that result in random segregation of sister chromatids [1,2,9–22]. Although many DNA replication factors promote cohesion, others appear to antagonize Ctf7-dependent sister chromatid tethering [23–26]. For instance, Elg1 and Ctf18 bind to Rfc2–Rfc5 in a mutually exclusive fashion to form alternative RFC complexes – but deletion of *ELG1* rescues, whereas deletion of *CTF18* exacerbates, the conditional growth and cohesion defects of *ctf7* mutated cells [23,24,27]. That alternative RFC complexes promote (establishment) or antagonize (anti-establishment) sister chromatid tethering reactions strengthens the notion that Ctf7 activity is coupled to the replication fork [1]. Ctf7 activity is limited to S-phase by degradation during G_2/M [28], but Ctf7 becomes active outside of S-phase in response to DNA damage, raising an important issue that I will return to later [29–34]. Despite the wealth of evidence that establishment is coupled to replication, little if any evidence suggested that S-phase progression in turn depends on cohesion. New studies, however, hint at just such a reciprocal relationship. Mutation of cohesion pathways produced S-phase progression defects – leading to models that cohesins might either act as barriers to fork progression or coordinate origin firing [3,4].

Cohesin as a barrier to replication-fork progression

Ctf18–RFC (and the RFC cofactor Dcc1) promotes sister chromatid tethering reactions presumably by facilitating Ctf7 acetylation of Smc3 [1]. Recent human cell studies confirmed that *DCC1* deletion produces robust cohesion defects. *DCC1* depletion also diminished Ctf18 protein levels and reduced Smc3 acetylation [3]. Single fiber combing analyses (where nucleotide analogs are used to detect replicated domains along straightened DNA) further revealed that cells depleted of Ctf18–RFC–Dcc1 exhibit significantly decreased replication-fork velocities and an increase in the incidence of stalled forks compared to control cells [3]. That cells diminished for replication components exhibit fork progression defects is hardly surprising, but that Ctf18–RFC–Dcc1 promotes Ctf7 activity raised the possibility that decreased fork velocities might result from establishment defects. Indeed, single fiber combing analyses revealed that human cells knocked down for either ESCO1 or ESCO2 exhibit significantly reduced replication-fork velocities – as did elevated expression of non-acetylatable Smc3 [3].

These results provide an exciting twist to previous models of replication-coupled cohesion in that fork progression now appears sensitive to completion of sister chromatid tethering reactions. How might this work? The possibility put forward was that chromatin-associated cohesins impede replication-fork progression [3] (Figure 1a). Based on a popular notion that cohesin forms a giant ring that encircles DNA upon deposition [35,36], acetylation of Smc3 was suggested to convert cohesin ring barriers into an opened ring that would allow for replication-fork progression, followed by ring closing around both sister chromatids to establish cohesion [3]. Whereas the complications of this model include a scenario in which cohesins remain bound to single-stranded DNA even during template-based synthesis of the complementary strand, evidence of cohesin interactions with fork stability factors provide at least in concept one mechanism for the retention of open cohesin rings [21]. In addition to findings that overexpression of non-acetylatable Smc3 reduced fork velocities in human cells, the cohesin barrier model was spurred by findings that co-depletion of Pds5

Table 1. Nomenclature of cohesion factors discussed in this review

Budding yeast	Fission yeast	Human	Function
Ctf7/Eco1	Eso1	EFO1/ESCO1 EFO2/ESCO2	Establishment; acetylation of Smc3
Rad61/Wap1	Wapl	WAPL	Anti-establishment; bind to Pds5
Pds5	Pds5	PDS5A PDS5B	Establishment, anti-establishment, and maintenance
Mcd1/Scc1	Rad21	RAD21	Maintenance; cohesin subunit
Smc1	Psm1	SMC1	Maintenance; cohesin subunit
Smc3	Psm3	SMC3	Maintenance; cohesin subunit
Irr1/Scc3	Psc3	SA1, SA2 STAG1,2	Maintenance; cohesin subunit
Scc2	Mis4	NIPBL	Deposition
Scc4	Ssl3	Scc4	Deposition
Elg1	Elg1	ELG1	Processivity clamp loader Bind to Rfc2–Rfc5, interact with Ctf7, oppose establishment
Ctf18	Ctf18	Ctf18	Processivity clamp loader Bind to Rfc2–Rfc5, interact with Ctf7, promote establishment
Pol30/PCNA	Pcn1	PCNA	Processivity clamp for DNA polymerase; promote establishment

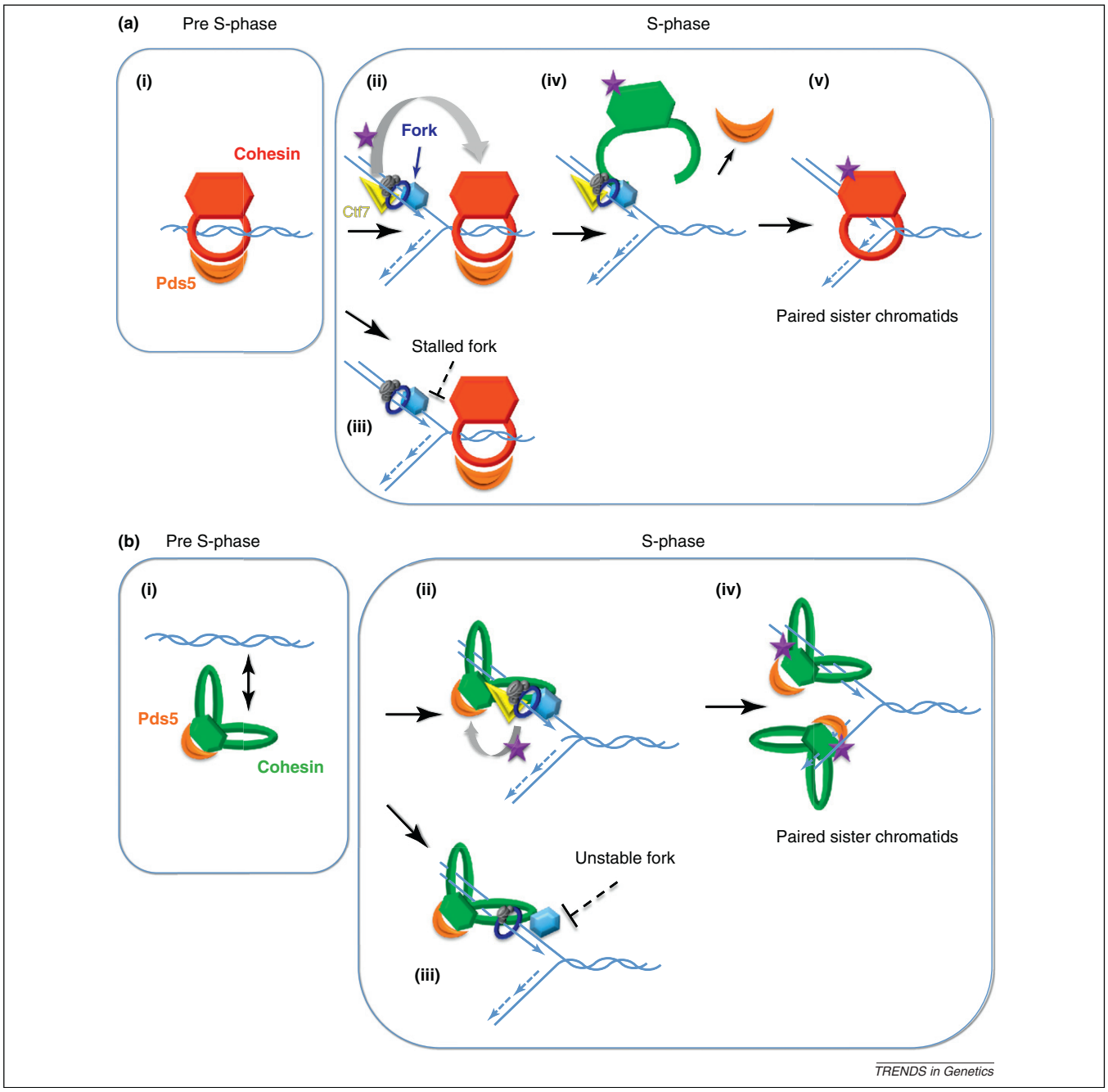

Figure 1. Alternative views of replication-fork progression defects in cohesion-deficient cells. **(a)** Cohesin as a barrier to DNA replication-fork movement. **(i)** As proposed, cohesins form rings (ring model in red) that are loaded onto DNA during G_1 or earlier. **(ii)** Upon entering S-phase, replication forks (DNA polymerase, blue hexagon; PCNA, dark blue ring; RFC complexes, five-ball grey cluster) assemble and move along the DNA. Only leading-strand synthesis is shown for simplicity. **(iii)** In cells lacking Ctf7, cohesin barriers block fork progression (dashed black line). **(iv)** In cells that retain functional Ctf7 (yellow triangle), fork-associated Ctf7 reaches forward to acetylate (purple star) cohesin. (iv) This results in Pds5 (orange crescent) displacement and cohesin ring opening (in green). Cohesin remains bound during template-based synthesis of the complementary strand. **(v)** After fork passage, cohesin ring re-closes around both sisters (fork components not shown). **(b)** Human Ctf7 effect on replication-fork progression. **(i)** Cohesins (clamp model in green) can associate with DNA before S-phase, but are highly dynamic and are therefore unlikely to load as rings or form effective barriers. **(ii)** Functional cohesin loading takes place during DNA replication and may be coordinated with fork progression. Conceptually, this model allows for the deposition of cohesins on both sister chromatids (deposition shown only for the leading strand). Fork-associated Ctf7 acetylates chromatin-bound cohesin for conversion to a state competent for sister pairing. **(iii)** In human cells diminished in Ctf7 function, DNA polymerase may transiently release from PCNA, resulting in stalled forks (black dashed line) and short gaps in synthesis. **(iv)** In cells that retain Ctf7, sister chromatid tethers may occur by any one of many conformations including a single ring around both sisters, paired rings – one around each sister, and clamps or bracelets. Cohesin clamps attached to one another and associated with each sister chromatid are shown here.

(an auxiliary cohesin-associated protein [37–41]) rescued the fork progression defect produced by diminished ESCO 1 or ESCO2 [3]. If this model is correct, then Pds5 must contribute to a cohesin conformation that blocks fork movement.

Challenging the barrier – will the blockade hold?

The underpinnings of the cohesin barrier model are that (i) cohesins deposited before DNA replication are stable and block subsequent fork progression, (ii) diminished Ctf7 function results in slowed replication forks, and (iii) Pds5

Box 1. Cohesins may be in the form of rings, bracelets or clamps

Electron microscopy and biochemical analyses provide strong evidence that Smc1 and Smc3 (Smc1/3) are elongated molecules in which associations between distal tips and splaying apart of the kinked central domains produce a closed structure with a central lumen [1,75,76]. Despite the popularity of a 'ring' model in which both sister chromatids are thought to somehow fit inside a single cohesin ring, this model fails to explain how non-SMC components such as Mcd1, Scc3 and Pds5, which are clearly required to maintain sister identity but are not part of the contiguous 'ring', participate in tethering. Opposite ends of the Smc1/3 structure appear to interact with one another in a head-to-tail fashion, which suggests that cohesins may bind other cohesins through one or more non-SMC components. These findings are consistent with a model that cohesins decorate each sister and that establishment results from the pairing of rings [1,75,76]. However, experimental evidence in support of higher-order cohesin complexes is limited. A second possibility that explains interactions between opposite ends of Smc1 and Smc3 is that non-SMC subunits cause Smc1/3 to fold over – a conformation that would eliminate a cohesin ring lumen. This folded-over conformation is supported by both atomic force microscopy and FRET (fluorescent resonance energy transfer – a microscopy-based technique that allows for the detection of closely apposed molecules labeled with electrically coupled fluorophores) [62,77]. Thus, rings may simply represent an assembly mid-point for a more compact and functional cohesin structure such as a C-clamp that could grasp one or both chromatids. Another model equally consistent with the elongated Smc1/3 heterodimer structure is that cohesins enwrap chromatids as a bracelet instead of a ring. Elucidating the cohesin structure (single ring, double ring, C-clamp or bracelet; Figure I) that tethers sister chromatids and also affects chromosome condensation, DNA repair and transcription regulation remains a high priority in the field [1,73–76].

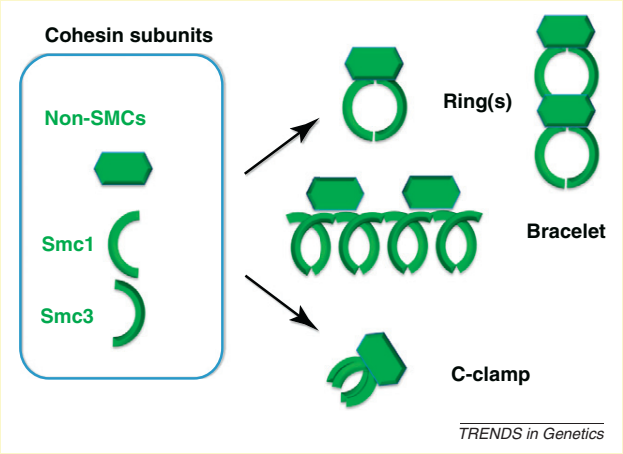

Figure I. Models of cohesin conformation.

displacement is required for establishment. An additional consideration is the cohesin ring structure posited to impede fork progression. Despite documented roles of cohesins in sister chromatid tethering, chromosome condensation and transcription regulation, it might be surprising to learn that we still do not have any clear view of how cohesin structures look *in vivo* nor whether cohesins adopt altered conformations within these different contexts [1]. This crucial facet of the barrier model and specifically the notion that giant cohesin rings encircle DNA are discussed in Box 1.

The first tenet of the cohesin barrier model is that cohesins deposited during G_1 block subsequent replication-fork progression. This implies a level of stability regarding the association of cohesin with chromatin before replication. By contrast, detailed FRAP and/or FLIP analyses performed in flies, mammalian cell systems and yeast all clearly document that cohesins are highly dynamic and quite transiently chromatin-associated before S-phase. In fact, stable cohesin association requires DNA replication-fork passage [42–46]. These results raise the following 'chicken and egg' conundrum: stable cohesin barriers block replication-fork progression but replication-fork progression stabilizes otherwise highly dynamic cohesins. It is equally likely that cohesins 'deposited' onto chromatin before replication are not in the form of rings that entrap DNA nor relevant to sister chromatid tethering reactions [42,47]. This view is consistent with findings that cohesins can be deposited as early as late G_1 (yeast) or during mitotic exit (vertebrate cells), but that deposition is essential only during S-phase and when cohesion is established [48–51].

The second tenet of the cohesin barrier model is based on evidence that knockdown of human ESCO1 or ESCO2 resulted in fork velocities reduced by half [3], doubling the duration of S-phase. Given the highly conserved nature of Ctf7-dependent Smc3 acetylation [6–8], diminished Ctf7 function should recapitulate S-phase defects in any model system. This does not appear to be the case. Budding-yeast cells deficient in Ctf7 (or Pds5) contain chromosomes fully decorated by cohesins ('barriers' in place) but exhibit relatively normal S-phase progression [27,37,38,51–54]. Identical results were obtained in fission yeast cells harboring mutations in Eso1: S-phase progression and duration appeared to be identical to those of wild-type cells [55]. Thus, one must consider that the replication-fork progression defect reported specifically for human cells is based on something other than unacetylated Smc3 or cohesin barriers.

The third tenet of the barrier model is predicated on findings that Ctf7-dependent Smc3 acetylation displaces Pds5 from cohesin. On the one hand, Pds5 both binds to cohesins and is essential to maintain sister chromatid pairing through M-phase [36–40] – findings inconsistent with Pds5 displacement during establishment. On the other hand, Pds5 appears to exert both pro- and anti-establishment functions, suggesting that biasing Pds5 activity or partners (Ctf7, Rad61 and cohesins) could tip the 'establishment' balance in one direction or the other during S-phase [25,26,37–45,55–61]. Admittedly, the characterization of Pds5 remains in its infancy, and resolving these issues will require further experiments regarding (i) Pds5 regulation of Ctf7 acetyltransferase activity during establishment, (ii) Smc3 acetylation effect on Pds5 positioning relative to the cohesin complex, and (iii) Pds5 influence on cohesin dynamics throughout the cell cycle.

Alternative views to a cohesin barrier model

Are there explanations other than a cohesin barrier model that could account for the fork progression defects observed in cells diminished for Ctf7? I propose here alternative scenarios based in part on the finding that human cells

depleted for either Ctf18–RFC–Dcc1 or Ctf7 homologs accumulate DNA damage [3]. Mutations in DNA replication factors such as PCNA or in one of many RFC subunits produce DNA damage and/or adversely affect S-phase progression via accumulation of nicks/gaps that stall fork progression. Moreover, all RFCs (including Ctf18) appear to play key and redundant roles in DNA repair [61–64]. In this light, it is not surprising that human cells accumulate DNA damage when depleted of Ctf18–RFC–Dcc1 [3]. Notably, Ctf7 associates with both PCNA and all RFC complexes and functions specifically during S-phase in unperturbed cells [9,11,27,28,52]. Thus, a plausible scenario is that diminished levels of Ctf7 adversely impact the assembly or stability of the replication fork to which it binds – the net result being stalled forks that resolve into DNA damage (Figure 1b). Prior findings that expression of non-acetylatable Smc3 alters Ctf7 activity could then account for the fork progression defect in those cells [3,8]. Analysis of Roberts syndrome (RBS), a developmental disease that arises from mutations in ESCO2 is germane to this model. RBS patient cells exhibit DNA damage foci and reduced fork velocities but contain predominantly paired sister chromatids. Thus, ESCO2 function in establishing cohesion appears to be separable from that of fork instability and DNA damage [65].

A second explanation for S-phase progression defects might reside in the many faces of Ctf7 throughout evolution. Budding yeast Ctf7 contains 281 residues, the vast majority of which form the acetyltransferase domain [5,27,52]. Fission yeast Eso1 contains 872 residues: the C-terminal domain is akin to budding yeast Ctf7 whereas the extended N-terminal domain contains a Rad30-like DNA repair polymerase (Pol eta/η). Both domains are functional in their own right and each domain can function independently of mutations in the other [55,58,66]. Importantly, mutation of either budding or fission yeast Ctf7 fails to produce DNA damage [27,55,58]. Why are human Ctf7 homologs different? Human ESCO1 (the first homolog identified) contains 840 residues: the C-terminus again containing a Ctf7-like acetyltransferase domain, but the extensive N-terminal contains a linker-histone-type domain [67]. It is tempting to speculate that this linker histone may be crucial for fork progression. If true, future experiments will be required to test whether mutation of the linker-histone domain destabilizes replication forks directly or through an as yet unrecognized chromatin remodeling error. Human ESCO2 contains 601 residues, but this N-terminal extension is different from any other homolog identified to date [65]. If each Ctf7 homolog is unique, then each is likely to manifest unique phenotypes when mutated – not all of which can be ascribed to cohesion defects. In support of this assertion, mutation of cohesion factors produce a myriad of disease states, many of which appear to be founded on different roles in non-overlapping cellular and developmental pathways (Box 2).

Origin firing: back to the basics of cohesin tethering functions

Studies of DNA helicases provide a further and different link between cohesion and S-phase progression pathways. The MCM complex (Mcm2–Mcm7) is the major helicase that unwinds DNA in preparation for replication. Biochemical studies suggest that human Mcm4 binds to other MCM complex components as well as to cohesins [4]. In pursuing a physiological link between cohesins and DNA helicase, cells knocked-down for cohesin were tested for altered S-phase progression. S-phase progression was indeed delayed, but single fiber combing and immunodetection methods failed to uncover diminished replication-fork velocities or DNA damage [4]. What mechanism prolonged S-phase? Extended single-fiber analysis revealed far fewer DNA replication forks compared to control cells [4]. Moreover, cohesin knockdown resulted in increased Halo diameters (a technique used to measure the spread of relaxed chromatin emanating from a nucleoplasmic scaffold [68]). These findings raised an intriguing possibility that cohesins cluster replication origins into foci to coordinate firing [4]. In the absence of cohesins, it was argued that fewer origins became clustered which resulted in longer loops (larger halos), greater interfork distances and a longer S-phase (Figure 2a).

Presently, the notion that cohesins coordinate origin firing is not supported in lower eukaryotic model cell systems. For instance, analysis of *mcd1/scc1, pds5* or *scc3/irr1* mutant homologs (Table 1) in budding and/or fission yeast cells failed to uncover any significant S-phase progression defect [52,69–72]. At the very least, little evidence supports the notion that origin clustering is conserved through evolution. Are there other models to consider? Cohesion mutations also impact upon transcription (Figure 2b), an effect that could reduce expression of replication initiation proteins [73,74]. Cohesin roles in transcription and chromosome condensation (most notably in yeast [27,37,69]) could further complicate interpretation of Halo-based assays performed in cohesion mutants (Figure 2b).

Box 2. Cohesion pathways play key roles in cell and developmental pathways

It is well-documented that cohesion helps ensure that each daughter cell receives a full genetic complement upon cell division and that errors in chromosome segregation can have devastating consequences. For instance, defects in cohesin, deposition, or establishment pathways result in massive chromosome mis-segregation and cell aneuploidy – a hallmark of cancer. Cohesion gene mutation or upregulation is tightly correlated with aggressive melanoma, breast, astrocytic and colorectal cancers with additional links on the horizon [78–81]. As chromatin-binding proteins, cohesins also impact upon chromosome metabolism. For instance, yeast cell studies reveal that cohesion defects prevent chromosome condensation [27,37,69] that potentially could lead to chromosome amputation by the cytokinetic cleavage furrow. Results from multiple model systems also demonstrate that cohesins play diverse roles in transcriptional regulation of embryonic development [73,74]. In humans, mapping studies linked cohesion mutations to severe developmental abnormalities including Cornelia de Lange syndrome, Roberts syndrome/SC Phocomelia, Rothmund–Thompson syndrome and Warsaw breakage syndrome [65,82–87]. Some of these maladies are reminiscent of the severe birth defects that result from thalidomide exposure as witnessed in the late 1950s. In light of these links to tumorigenesis and developmental abnormalities, it is clear why cohesion pathways have received such intense attention over recent years.

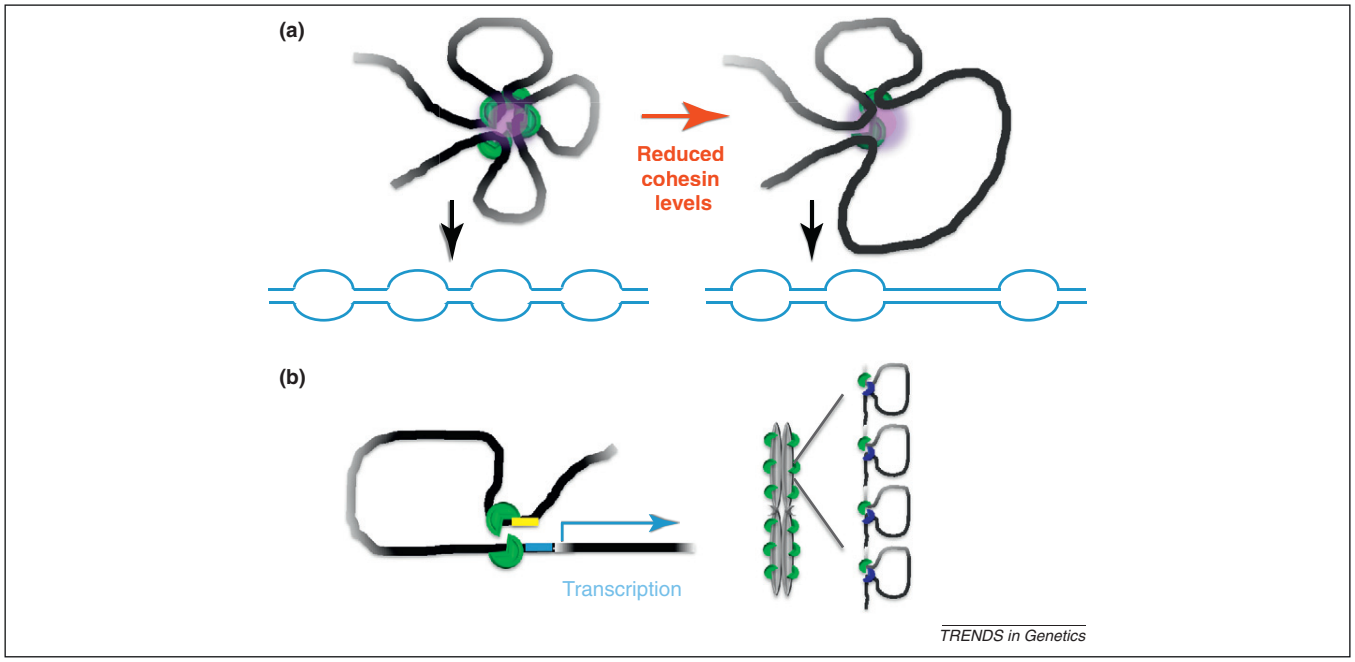

Figure 2. Cohesin in origin of replication clustering and other chromatin-gathering roles. **(a)** Left panel: cohesins (green clamps) cluster several origins of replication together to form numerous chromatin loops that emanate out from the cohesin anchor. The area of active origin firing is shown as a purple haze. Below, origin firing initiates DNA replication and assembly of bidirectional forks. Right panel: cells diminished for cohesins cluster together fewer origins – resulting in longer loops. Below, fewer origins that fire result in fewer bidirectional forks and thus require a longer S-phase to complete full duplication of the genome. **(b)** Additional roles for cohesins that could alter S-phase progression and/or chromatin topography. Left panel: diminished cohesin could reduce expression of S-phase initiation factors (origin proteins, cell cycle regulators). Left and right panels: diminished cohesin could reduce intramolecular chromatin fiber interactions (fewer but longer loops). Shown left is a cohesin-dependent chromatin loop that may form when an enhancer DNA element is brought into close proximity to a promoter. Shown right are cohesins (in green) that may stabilize loops in association with highly related condensing complexes (in blue) that emanate from an axial chromatin scaffold on condensed chromosomes. A condensed chromosome and an expanded view of chromatin loops are shown.

Concluding remarks

That cohesion mutations result in stalled and/or fewer forks are important observations [3,4]. In this article I offer alternative explanations regarding the molecular basis for these S-phase progression effects – including direct effects on fork stability or the transcription of initiation factors. The point of course is that researchers collectively interpret data to promote new research. At odds to this process is the premature transition of model into dogma. For instance, the notions of 'ring-shaped cohesins' or that cohesins encircle both sister chromatids may one day prove to be correct, but declarative statements to this effect are premature. Further efforts will be required to permit differentiation between cohesin as single rings, bracelets, clamps or hand-cuffs in the various contexts (cohesion, condensation, transcription, replication/repair, origin clusters) in which they function [1,73–76]. As another example, several lines of evidence argue that cohesion establishment is coupled to replication, but it is also true that Ctf7 becomes active during G_2/M in response to DNA damage. In this context, establishment occurs in the absence of replication factors and apparently without new rounds of Smc3 acetylation (Mcd1/Scc1 appears to be the target) [29–34]. Therefore, future efforts will reveal whether replication-coupling is a convenience, or a necessity, for establishment reactions during S-phase.

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