Preventing replication stress to maintain genome stability: resolving conflicts between replication and transcription

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Abstract:

DNA and RNA polymerases clash along the genome as they compete for the same DNA template. Cells have evolved specialized strategies to prevent and resolve replication and transcription interference. Here we review the topology and architecture at sites of replication fork clashes with transcription bubbles as well as the regulatory circuits that control replication fork passage across transcribed genes. In the case of RNA Polymerase II transcribed genes, co-transcriptional processes such as mRNA maturation, splicing and export influence the integrity of replication forks and transcribed loci. Fork passage likely contributes to reset the epigenetic landscape, influencing gene expression and transcriptional memory. When any of these processes are not properly coordinated, aberrant outcomes such as fork reversal and R-loop formation arise and trigger unscheduled recombinogenic events and genome rearrangements. The evolutionary implications of such conflicts on genome dynamics, and their potential impact on oncogenic stress are discussed.

Introduction

Replication and transcription are the two major processes that track along the chromosomes, using the DNA duplex as a template for the synthesis of nucleotide chains, essential for the transmission and expression of all genetic information. In S-phase, as replication forks progress throughout the genome, they inevitably compete for the DNA template with active RNA polymerases (RNAPs). Both replication and transcription must be highly coordinated in order to prevent potential clashes that can be detrimental for genome integrity. Both DNA replication and transcription unwind the DNA helix generating topological perturbations that are solved by specialized enzymes, the DNA topoisomerases, which prevent abnormal DNA transitions. Furthermore, co-transcriptional processes (such as RNA maturation and export) impose a series of architectural constraints that potentially counteract fork progression. Specialized regulatory circuits dismantle these architectural domains to allow replication fork passage.

Recent findings have shed light on the molecular events associated with S phase transcription mediated by the different RNAPs. Here we review the topological and architectural events coordinating fork progression with RNA synthesis and co-transcriptional processes, as well as the pathological events resulting from uncoordinated clashes between replication and transcription.

DNA topology at the replication fork and the transcription domain.

The double helical structure of DNA implies that all DNA transactions generate torsional energy. The spatial organization and the topological state of the DNA fiber are influenced by the crossing of DNA segments, by nucleosome organization and by DNA loop formation. DNA can be negatively or positively

supercoiled when the double helix underwinds or overwinds, respectively (Wang, 2002). The torsional stress created during biological processes cannot simply diffuse by twisting chromosomes extremities; chromosomes are large and complex structures and the DNA fiber is often anchored to fixed structures, such as the nuclear envelope or the chromosome scaffold, that impose topological barriers and impede the rotation of the DNA segments.

The topological transitions are mediated by DNA topoisomerases, either to relieve excessive torsional stress or to generate and maintain adequate levels of torsion. DNA topoisomerases catalyze the transient breakage of DNA to allow the passage of single or double DNA strands through one another (Champoux, 2001; Wang, 2002). Type I topoisomerases make transient single-strand DNA breaks without the requirement for ATP, whereas type II topoisomerases introduce transient double-strand breaks through ATP hydrolysis. The two types of topoisomerases can be divided into type IA, type IB, type IIA and type IIB. Type IA topoisomerases un-pair short stretches of double-stranded DNA, introduce a break in the single-stranded region, hold the broken ends and, by bridging the gap, allow the passage of a second DNA strand. They function preferentially on under-wound or negatively supercoiled DNA. Type IB topoisomerases interact with DNA double helix, cleave one of the DNA strands and while one DNA segment of the nick is tightly held by the enzyme, the other is free to rotate. Type IB enzymes relax both positively and negatively supercoiled DNA. Differently from type IA and type IB, type IIA and type IIB DNA topoisomerases are dimeric enzymes. The type IIA enzymes produce a double-strand break in DNA, causing a conformation change that pulls the two ends of the cleaved duplex DNA apart to create an open DNA gate. A second duplex DNA from either the same molecule or

different molecule is then passed through the DNA gate before resealing of the break. This mechanism allows several topological transformations, including the relaxation of positively or negatively supercoiled DNA, as well as catenation and decatenation of different DNA molecules. Type IIB topoisomerases have distinct structural differences compared to the type IIA topoisomerases but share common mechanistic features (Champoux, 2001; Wang, 2002). In eukaryotes, the DNA torsional state is mainly regulated by the actions of type IB (topo I) and type IIA (topo II) topoisomerases. Although topo I and topo II are implicated in supporting replication and transcription, their relaxation functions are redundant in many instances.

Both DNA polymerases (DNAPs) and RNAPs can rotate around the double helix while moving along the DNA (Doksani et al., 2009; Gamper and Hearst, 1982; Harada et al., 2001; Liu and Wang, 1987; Reyes-Lamothe et al., 2008) (Figure 1). The topological context as well as the type of topoisomerase that will deal with the torsional constrains arising during replication and transcription strongly depends on whether DNAPs and RNAPs rotate freely or whether their mobility is constrained. For example, at the replication fork, swiveling at the fork branching point redistributes the torsional stress to the replicated regions, forming precatenanes through the intertwining of the two replicated duplexes (Postow et al., 2001a; Schvartzman and Stasiak, 2004; Wang, 2002) (Figure 1B). Analogously, swiveling of the moving RNAP is expected to entangle the newly synthesized RNA molecule with DNA behind the transcription bubble (Figure 1D) (Koster et al., 2010; Liu and Wang, 1987). When DNAP is prevented from rotating around the helix, the unreplicated DNA is forced to rotate and accommodates the torsional stress by generating positive supercoiling ahead of

the fork (Figure 1A). In the case of transcription, any impediment preventing RNAP rotation likely generates positive supercoiling ahead of the RNAP and negative supercoiling behind the transcription machinery, thus forming the so-called twin-supercoiled domains (Koster et al., 2010; Liu and Wang, 1987; Wu et al., 1988) (Figure 1C).

Accumulation of supercoiling ahead of the replication forks or in front and behind transcription bubbles can be solved by type IB or type II topoisomerases, while precatenane resolution is mediated by type II topoisomerases (Champoux, 2001; Wang, 2002). In the yeast *Saccharomyces cerevisiae*, either Top1 (type IB enzyme) or Top2 (type IIA enzyme) support progression of the replication machinery (Bermejo et al., 2007; Brill et al., 1987; Kim and Wang, 1989). Both Top1 and Top2 travel with replication forks and likely cooperate in positive supercoiling and precatenane relaxation (Bermejo et al., 2007). While the simultaneous inactivation of Top1 and Top2 prevents DNA and ribosomal RNA synthesis, poly(A)+ RNA and transfer RNA synthesis can still occur (Brill et al., 1987). A genome-wide study has shown that Top2 binds promoters of transcribed genes specifically in S phase (Bermejo et al., 2009).

Collision between replication and transcription.

The coordinated action of type I and type II topoisomerases allows cells to deal with the topological problems arising when two forks converge during replication termination or when one fork clashes with a transcription bubble in a head-on collision mode (Azvolinsky et al., 2009; Brewer and Fangman, 1988; Deshpande and Newlon, 1996; Olavarrieta et al., 2002). The unreplicated DNA becomes very short, rendering it difficult for DNA topoisomerases to bind and

remove the last few intertwines. These residual and unsolved intertwines may represent a topological barrier for replication and transcription, unless they diffuse into precatenanes behind the forks (Wang, 2002). In *E. coli,* as well as *S. cerevisiae* and *Schizosaccharomyces pombe,* type II topoisomerases are required for timely replication termination and to prevent chromosomal breaks during chromosome segregation (Champoux, 2001; Fachinetti et al., 2010; Wang, 2002), likely by mediating precatenane resolution (Wang, 2002; Wang et al., 2008). Prokaryotes which have only one origin of replication have partially solved the problem of the collision between replication and transcription by evolutionarily selecting co-directionality between forks and transcription bubbles, thus avoiding a head on clash (Brewer, 1988; Rocha, 2004). In contrast, eukaryotic chromosome replication that is mediated by multiple replicons has to frequently deal with head on collisions between forks and transcribed genes. The S phase architecture of RNAPII transcribed genes is rather complex and, in *S. cerevisiae* is mediated by Top2 and the high mobility group protein Hmo1 (Bermejo et al., 2009). It has been proposed that RNAPII transcribed units are organized in loops (Ansari and Hampsey, 2005) and that the integrity of these loops as well as their topological complexity in S phase depends on the concerted action of Top2 and Hmo1 (Bermejo et al., 2009). DNA looping at transcribed genes has intriguing implications: It facilitates RNAP recycling and fuels concomitant rounds of transcription events within the same gene (Ansari and Hampsey, 2005). It also topologically insulates transcription from other chromosomal processes and influences the capability of mRNA genes to memorize the previous transcriptional status through a process known as transcription memory (Tan-Wong et al., 2009). Furthermore, it generates a barrier for incoming forks

independently of the directionality of transcription (Azvolinsky et al., 2009). Top2 seems to bind at the base of the loops specifically in S phase (Bermejo et al., 2009) and this observation raises the question as to whether the loops form only in S phase or, rather, Top2 resolves the complexity of the loops particularly when transcription clashes with replication.

In head-on collisions the replisome (a large molecular complex comprising DNAPs and a number of accessory factors) on the lagging strand directly faces the front edge of RNAPs, while in co-directional collisions, the replisome on the leading strand encounters the rear edge of RNAPs (Figure 2). Both types of collisions slow down the replication fork progression (Azvolinsky et al., 2009), although this observation might reflect the peculiar DNA looping organization of RNAPII transcribed regions (Ansari and Hampsey, 2005; Bermejo et al., 2009; Perkins et al., 2008; Tan-Wong et al., 2009). At least in theory, in a codirectional collision between a fork and a transcribed unit not organized in a loop, the negative supercoiling generated behind the RNAP might adsorb the positive supercoiling generated by the incoming fork, thus facilitating fork progression (Wang, 2002). In the case of tRNA transcription it is clear that the head on collision with a fork causes replication pausing (Deshpande and Newlon, 1996) and fork progression under these circumstances is facilitated by a specialized replisome associated DNA helicase, Rrm3, that likely dislodges the RNAPIII machinery by moving with a 5' to 3' directionality (Ivessa et al., 2003).

At the ribosomal DNA repeats, specialized mechanisms have evolved to avoid the interference between replication and the RNAPI machinery. A Replication Fork Barrier (RFB), constituted by a *CIS*-chromosomal motif together with effector proteins, arrests any forks that are progressing head-on towards the heavily

transcribed 35S rRNA gene. The 35S rDNA gene is then replicated by those forks advancing co-directionally and emanating from origins flanking the rDNA repeats (Brewer and Fangman, 1988). Yeast Top1 and Top2 are required for rDNA transcription (Brill et al., 1987) and to suppress hyper-recombination at rDNA repeats (Christman et al., 1988). It has been proposed that Top1 and Top2 play differential roles in assisting RNAPI progression (French et al., 2011): while Top2 is required to relax positive supercoiling in front of the transcription bubble to facilitate its progression, Top1 seems to relax negative supercoiling behind it.

Head-on collisions between replication and transcription generates fork pausing (Liu and Alberts, 1995; Olavarrieta et al., 2002; Wang, 2002). Fork restart can take place through the displacement of the RNAP machinery from the DNA template (Pomerantz and O'Donnell, 2008). *In vitro*, the codirectional collision between replication and transcription barely affects fork progression (Liu and Alberts, 1995; Pomerantz and O'Donnell, 2008; Srivatsan et al., 2010; Wang et al., 2007) unless the RNAP is stalled (Dutta et al., 2011; Elias-Arnanz and Salas, 1997). It has been suggested that, occasionally, the replication machinery can use mRNAs to re-prime DNA synthesis after colliding codirectionally with the RNAP (Kogoma, 1997; Pomerantz and O'Donnell, 2008).

Coordinating transcription coupled events with replication

Transcription and transcription-coupled processes have the ability to shape chromatin and to affect chromosome architecture and integrity. In addition to having a profound impact on nucleosome density and positioning, transcription determines the establishment of higher order structures. A classical example of

such a structure is the interaction between transcription enhancers and gene promoters that can take place both in *CIS* (leading to the formation of chromatin loops) and in *TRANS* (determining the interaction between different chromosomes). Moreover, DNA looping at transcribed genes seems to be mediated by the interaction between promoter and terminator regions (Ansari and Hampsey, 2005).

Transcription can be coupled with RNA splicing and RNA maturation and these processes also have an impact on chromosome architecture and genome integrity, particularly in the S phase topological context. When factors involved in RNA maturation and processing engage the RNA molecule while it is being transcribed, they might hinder the rotatory movement of the RNAP, thus facilitating the formation of the twin-supercoiling domains (Koster et al., 2010; Wu et al., 1988). The coupling of transcription with mRNA export through the nuclear pore complex (NPC) implies that the ejection of the nascent RNA prevents its entanglement behind the transcription bubble while RNAPII keeps rotating. This mechanism, in theory, could counteract the formation of the twin topological domains (Figure 2).

The association of RNAPII transcribed genes to fixed structures, such as nuclear pore complexes or large macromolecular structures creates physical connections between transcribed chromatin and the fixed matrix, giving rise to topological barriers. In linear eukaryotic chromosomes topological barriers confine the distortions accumulated in response to topological stress, contribute to their timely resolution, insulate entire chromatin segments generating topological domains, and also impede replication fork progression (Bermejo et al., 2011; Postow et al., 2001a; Roca, 2011).

Gene gating couples mRNA transcription and export by bringing chromatin into contact with NPCs and is mediated by the THO/TREX and TREX-2 complexes, as well as by key nucleoporins (Cabal et al., 2006; Drubin et al., 2006; Rougemaille et al., 2008; Tan-Wong et al., 2009). Recent evidence in yeast suggests that every RNAPII transcribed gene associates with gating factors (Casolari et al., 2004; Gomez-Gonzalez et al., 2011). Thus, in theory, it is possible that gene gating actively contributes to DNA looping at transcribed genes (Figure 2 A-C). The RNAP at the beginning of mRNA synthesis can rotate while synthesizing RNA and therefore entangle the nascent RNA molecule with DNA behind the bubble. The gene gating machinery could generate a coil in the transcribed DNA segment by engaging the entangled mRNA molecule and dragging it to the NPC, giving rise to a loop. The loop could then facilitate the interaction between the promoter and the terminator and be stabilized by HMG-like proteins that have strong affinity for cruciform nucleosome free regions, such as the ones that form at the base of the loop (Bermejo et al., 2009). At this stage, productive and efficient transcription can take place. In S phase the loop could also recruit Top2 to deal with the replication-induced topological stress. Recent observations in yeast suggest that transcribed genes are unleashed from the nuclear envelope to allow fork progression across them (Bermejo et al., 2011) (Figure 2 E1, E2). This process is mediated by the Mec1-Rad53 checkpoint that phosphorylates Mlp1, a key protein located in the inner basket of the nuclear pore. One possibility is that fork passage across transcribed genes, besides counteracting gene gating, might also dismantle the DNA loop. Once the loop is unfolded, the fork may occasionally experience a head-on collision with the residual transcription machinery (Figure 2 D2-F2). This scenario has several implications: forks

replicating across transcribed units will reset the architecture of transcription, which should be then re-established following fork passage, thus implying that the assembly and disassembly of the transcription domains is cell cycle dependent. This is consistent with observations indicating that gene gating is regulated by Cyclin Dependent Kinases (CDKs) through phosphorylation of the Nup1 nucleoporin (Brickner and Brickner, 2010). Moreover, since transcription memory has been linked to the chromosomal architecture of transcribed genes (Light et al., 2010; Tan-Wong et al., 2009), it is expected that fork passage across RNA genes would dismantle the gene loops and therefore influence the capability of genes to memorize their transcriptional status. Intriguingly, the checkpoint target Mlp1 and the chromatin protein Htz1 (that inversely correlates with the presence of Hmo1 (Bermejo et al., 2009; Tan-Wong et al., 2009) besides mediating gene gating and assisting fork progression at transcribed genes, also impact transcription memory (Light et al., 2010; Tan-Wong et al., 2009).

The architecture of the rDNA locus is also peculiar (Figure 3). The function of the RFB relays on the Fork Blocking protein 1 (Fob1), which is essential to prevent DNA breaks and unscheduled recombination events at rDNA repeats (Kobayashi and Horiuchi, 1996). Fob1 interacts with a network of proteins mediating the anchoring of the rDNA repeats to the nuclear periphery (Mekhail et al., 2008) (Figure 3B). Fob1 recruits Tof2 and the Cohibin (Csm1/Lrs4) complex, and the latter interacts with the inner nuclear matrix associated CLIP complex, formed by Heh1/Src1 and Nur1 (Huang et al., 2006). Cohibin/CLIP association mediates the perinuclear attachment of rDNA repeats. Releasing rDNA repeats from the nuclear envelope through CLIP disruption destabilizes the repeats (Mekhail et al., 2008). It was recently proposed that Csm1/Lrs4 forms a molecular clamp

crosslinking rDNA repeats and therefore forming chromatin loops spanning two RFBs, which could be attached to the inner nuclear membrane through CLIP (Corbett et al., 2010) (Figure 3A). Attachment of the rDNA loops to the nuclear envelope might impose a series of topological constraints affecting both fork pausing and RNAPI bubble progression. Arrest of the replication fork approaching the RFB in a head-on orientation with RNAPI, might determine, or be in part mediated by, the accumulation of positive supercoils. Intriguingly, Tof2 and cohibin interact with Top1 (Chan et al., 2011; Park and Sternglanz, 1999), raising the possibility that these factors coordinate the architectural organization of rDNA repeats with topological simplification. Importantly, Tof2 is required for rDNA condensation, segregation and cohesin recruitment to the RFB (Corbett et al., 2010). Furthermore, *TOP1* and *TOP2* double mutants excise rDNA repeats as extrachromosomal circles (Kim and Wang, 1989), perhaps due to improper recombination in the absence of topological simplification at the base of the cohibin-established loops.

Pathological outcomes of the topological interference between replication and transcription

Replication forks accumulate torsional energy when paused at termination zones or when they approach transcribed genes. The torsional stress, unless promptly resolved, can generate aberrant DNA transitions leading to genome instability (Branzei and Foiani; Helmrich et al., 2011) (Figure 4). *In vitro* studies have shown that positive supercoiling in front of a fork can be accommodated by fork reversal through the extrusion and re-annealing of nascent DNA strands (Postow et al., 2001b). Reversed forks can also derive from the run off of hemicatenane

structures that likely represent precatenane derivatives (Bermejo et al., 2008; Cotta-Ramusino et al., 2005). Reversed forks are cruciform DNA structures resembling Holliday junctions and, being highly recombinogenic, they can lead to genome rearrangements (Lopes et al., 2001; Sogo et al., 2002). *In vivo*, fork reversal is likely prevented by a stable replisome-fork association (Lucca et al., 2004). In yeast mutants altered in the Mec1/ATR pathway, replication forks that undergo pausing rapidly collapse into a reversed fork conformation (Lopes et al., 2001; Sogo et al., 2002). Mec1/ATR-mediated Mlp1 phosphorylation assists replication across gated genes whose architecture hinders fork progression. In checkpoint mutants the transcribed chromatin remains perinuclear and accumulates torsional stress in the proximity of forks encountering gated genes, thus generating the context for fork reversal (Bermejo et al., 2011).

The advancing RNAP machinery also generates torsional energy that can lead to the formation of long stretches of DNA:RNA hybrids – the so called R-loops (Thomas et al., 1976). These structures form while RNAP is transcribing, as the negative supercoiling of the twin topological domain can accommodate the tail of the nascent RNA molecule (Drolet, 2006; Phoenix et al., 1997) (Figure 4D)**.** In the R-loop structure the RNA anneals with the template leaving the non-transcribed strand unpaired. The R-loop is also highly genotoxic as the unpaired strand can occasionally form G quartets and plectonemic structures, or, as in the case of B cell immunoglobulin class switching, be targeted by AID-mediated DNA cytosine deamination (Gomez-Gonzalez and Aguilera, 2007; Helmrich et al., 2011; Roy and Lieber, 2009; Wahba et al., 2011). Moreover, the DNA:RNA hybrid in the R-loop is highly recombinogenic and can generate a block for incoming forks or even provide unscheduled RNA primers for DNA polymerases (Gomez-Gonzalez

et al., 2011; Pomerantz and O'Donnell, 2008). It is unclear whether R-loops form physiologically and specialized enzymatic activities dismantle them or, rather, they represent the pathological consequences of genetic abnormalities in certain transcription-coupled processes. In yeast, R-loops accumulate in mutants in the THO/TREX and TREX-2 complexes (Huertas and Aguilera, 2003). Intriguingly, the Hpr1 subunit of the THO/TREX complex has a DNA topoisomerase domain (Wang et al., 1990) and perhaps the complex itself may play a role in organizing the topology of the transcribed DNA template to prevent the annealing of the nascent RNA. THO/TREX mutants fail to gate genes to the NPCs and likely build up the topological context that results into R-loop formation. THO/TREX mutants do not accumulate reversed forks but, rather, they rescue fork reversal in checkpoint defective cells (Bermejo et al., 2011). We speculate that the topological stress of THO/TREX mutants can be only accommodated by R-loop formation. We propose the following scenario that might explain why accumulation of topological energy in gene gating and checkpoint mutants results in two different outcomes, R-loops and reversed forks. As discussed above, an efficient gene gating should allow RNAPII rotation thus preventing the establishment of the twin domain context. The twin topological domain is a prerequisite for R-loop formation (Drolet, 2006), which depends on the accumulation of negative supercoiling (Figure 4 C, D). Accordingly, the THO/TREX complex prevents R-loop formation. We speculate that the twin loop domains form soon after gene gating is switched off by the checkpoint when forks approach mRNA genes as, despite the dismantlement of the loop, residual RNAPIIs could still transcribe (Figure 2 D-F). However, this would be a transient event since soon after fork passage the loop would reform (Brickner and

Brickner, 2010). It is possible that at certain genomic locations, where highly transcribed genes collide head on with replication forks, the transient formation of twin topological domains might generate the ideal context for R-loops even under physiological conditions. A specialized Rrm3-like DNA helicase could efficiently prevent their formation. This hypothesis also implies that the ideal context for R-loop formation is when cells experience S phase. Consistently, genomic instability associated with DNA:RNA hybrids occurs preferentially in S-phase (Gomez-Gonzalez et al., 2011; Helmrich et al., 2011). Gene gating defective mutants would inevitably transcribe mRNAs using the twin topological domain mode and, therefore, would be more prone to form R-loops to accommodate the topological stress accumulated at those regions where forks and transcribed units clash head on. Conversely, checkpoint defective cells, which maintain genes gated at the nuclear envelope, even when forks approach, would not be able to form R-loops, and would accommodate the topological stress through fork reversal (Figure 4 A,B). In this scenario the topological energy accumulated when forks encounter transcription units would be the main source of either R-loop or fork reversal formation. Both situations are highly recombinogenic and lead to genome rearrangements.

Similar topological constraints might arise when forks hit chromosomal regions that are physically tethered to large cellular structures, as in the case of ribosomes in prokaryotes or the spliceosome and PML (promyelocytic leukemia) bodies in higher eukaryotes (Lallemand-Breitenbach and de The, 2010). For instance, the recruitment of the splicing machinery behind RNAPs might hinder negative supercoiling accommodation and would need to be highly coordinated with topological stress resolution ahead of replication forks to prevent R-loop

formation. Moreover, the splicing machinery might actively interfere with the binding of the nascent RNA to the complementary DNA. In human cells R-loop-based instability of transcribed regions is suppresses by the type IB Topo 1 that coordinates topological simplification with splicing by regulating the ASF/SF2 splicing factor (Tuduri et al., 2009). Noteworthy, splicing proteins were highly represented in screens performed in mammalian cells designed to score for factors preventing spontaneous DNA breaks (Paulsen et al., 2009).

Mechanisms influencing sister chromatin cohesion and chromatin condensation.

The topological context at sites where replication forks approach transcribed units might influence sister chromatid cohesion. If the replisomes are allowed to rotate, the accumulation of positive supercoiling as forks approach NPC-gated loops might fuel precatenane formation behind the fork. Precatenanes would locally contribute to sister chromatid cohesion by tethering sister chromatids together until they are solved by type II topoisomerases. It has also been proposed that precatenanes might also give rise to hemicatenane joints (Bermejo et al., 2008) that would be refractory to type II topoisomerases and rely on type IA enzymes for resolution. Hemicatenanes might also contribute to sister chromatid cohesion (Lopes et al., 2003).

Little is known on how gene gating is re-established after fork passage in S-phase. The gene gating apparatus colocalizes genome wide with RNAPII genes (Gomez-Gonzalez et al., 2011) and, in theory, gating twin genes (the pair of homologous genes located on sister chromatids) to the same NPCs would keep

them in close proximity, thus contributing to sister chromatid cohesion (Figure 5A). Perhaps precatenanes might then indirectly contribute to twin gene gating.

Co-transcriptional gene gating in S phase might also impact on replicon dynamics. As an example, origin firing is influenced by the nuclear architecture and the topological state of the chromatin (Courbet et al., 2008; Lemaitre et al., 2005). Consequently, chromatin tethering to the nuclear envelope might have an impact on replicon activation. In budding yeast there are around 350 replication origins but only a fraction of them are efficiently fired. Moreover, efficient origins are fired throughout S phase, meaning that, at any given time, only few origins are active. For instance, in yeast cells treated for 1 hour with hydroxyurea, approximately 150 origins are active (Raghuraman et al., 2001). Intriguingly, the number of active origins and NPCs (Winey et al., 1997) lie within the same order of magnitude. Considering the relatively small number of NPCs and the fact that all transcribed units associate with gene gating factors (Casolari et al., 2004), it is likely that more genes are gated to the same NPC (Figure 5B). This complex nuclear architecture mediated by gene gating has therefore the potential to globally shape chromosome dynamics in S phase. Moreover, the number of transcriptional units gated together and the distribution of genes gated to the same NPCs along chromosomes likely influences chromosome condensation mechanisms. For instance, if nearby transcribed genes associate with a given NPC (Figure 5B), a large chromatin loop would form in which several genes could be encompassed within a single domain. Such architectural organization would be reset following fork passage across transcribed units and may contribute to prime chromatin condensation at the end of S-phase.

Concluding remarks

Clashes between replication and transcription could have relevant implications for cancer, particularly following oncogene activation. Oncogene over-expression deregulates transcription and generates DNA damage associated with replication stress (Di Micco et al., 2006; Dominguez-Sola et al., 2007; Srivatsan et al., 2010). Based on the scenarios described above, it is possible that the unscheduled hyper-activation of transcriptional units without coordinated epigenetic and co-transcriptional programs might determine a higher propensity for topological stress that would inevitably result in pathological outcomes such as fork reversal and R-loop accumulation. Consistent with this hypothesis, genes involved in RNA processing and splicing rank as the most abundant classes in screens for factors targeted by the DNA damage checkpoint kinases (Matsuoka et al., 2007) and those required to suppress spontaneous DNA breaks in unchallenged human cells (Paulsen et al., 2009). Intriguingly, recent findings directly link the presence of DNA:RNA hybrids at fragile sites in mammalian cells (Helmrich et al., 2011).

The idea that replication fork progression could contribute to re-set transcriptional memory mechanisms by dismantling the high-order organization of transcribed genes might have implications for those cells in which the reprogramming of transcription is coupled to proliferation, such as stem cells or hematopoietic precursors (Semerad et al., 2009). It is tantalizing to think that by entering the cell cycle, and S phase in particular, these cells could trigger certain plasticity in their transcriptional regulation.

It is worth noting that the embryonic cell cycle, in which chromosome replication is very fast and transcription does not occur, lacks a checkpoint response (Tan-

Wong et al., 2009). This only becomes functional later on when the somatic cell cycle begins and transcription, and co-transcriptional processes, are established, raising the possibility that the checkpoint response has evolved as a mechanism for controlling the clashes between replication and transcription machineries.

It will be a challenge in the future not only to integrate the knowledge coming from studies in the replication, transcription, recombination, RNA processing, nuclear organization and DNA topology fields, but also to understand how local mechanical forces impact on chromosome integrity.

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Figure Legends

Figure 1. Topological consequences of DNAP and RNAP rotation. The DNA topological context is influenced by the ability of the replication fork to rotate around its axis. (A) If the rotation of the DNAP machinery is prevented, the DNA

helix overwinds generating positive supercoiling (positive and negative supercoiling are indicated by + and – signs, respectively). (B) If the DNAP is able to freely rotate, torsional stress can be transmitted backwards generating intertwinings of the replicated chromatids known as precatenanes. (C) Similarly, a non rotating RNAP machinery generates a transient increase and decrease of the linking number ahead and behind the transcription bubble, respectively, which is accommodated as positive or negative supercoiling. (D) If the RNAP can rotate around the DNA axis as it transcribes, the linking number will not change, though the nascent RNA entangles around the duplex. Relevant DNA topoisomerase types acting on the different topological substrates are shown in bold type. Yellow arrows indicate the rotation of polymerases around the DNA double helix axis.

Figure 2. Gene gating and replication forks. Gene loops are established by the association of promoter and terminator regions. As RNAP initiates transcription at the promoter generates a short nascent RNA chain and keep rotating around the DNA template. (A) As it becomes increasingly longer, the nascent RNA could intertwine with the template DNA. Engagement of the nascent RNA by the NPC gating machinery (B), could lead to the formation of gated DNA loops (C). As the replication forks approach gated loops (D1-2), these are dismantled through a process dependent on local checkpoint activation (E1-2). Disengagement from the NPC might facilitate the transient formation of twin supercoiled domains (F1-2). Light blue and red arrows indicate the direction of RNAP and DNAP movement respectively.

Figure 3. Architectural organization of ribosomal DNA repeats imposed by cohibin-mediated RFB association to the nuclear envelope. (A) Hypothetical architecture of rDNA repeats. Chromatin loops are stabilized by cohibin-mediated association of RFB elements and bound to the nuclear matrix via association with the CLIP complex. Cohibin is shown in green and RFB in red. (B) The functionality of the replication fork barrier (RFB) at rDNA repeats depends on Fob1, which associates to the Csm1/Lrs4 cohibin complex through Tof2. Cohibin, in turn, associates with the Src1/Nur1 CLIP complex, thus tethering the repeats to the nuclear envelope. Cohibin forms a molecular clamp crosslinking rDNA repeats through its association to Tof2 and Fob1 at the RFB.

Figure 4. Pathological events caused by replication and transcription interference. (A) NPC-associated gene loops can behave as barriers for the diffusion of the torsional stress generated by incoming replication forks. (B) In checkpoint defective cells, persistence of topological barriers at gated genes locally increases positive supercoiling accumulation that, in the context of an unstable replisome, could result in fork reversal (C). In normal cells twin supercoiled domains might form transiently following the checkpoint mediated dismantling of gated DNA loops. (D). In gene gating defective mutants twin supercoiled domains would persist longer and the underwound DNA helix behind the transcription bubble might favor the annealing of the nascent RNA molecules thus leading to the formation of R-loops. Dashed boxes mark pathological events challenging genome integrity.

Figure 5. Hypothetical impact of gene gating on sister chromatid cohesion and chromosome condensation. (A) Association to the same NPC of twin gene copies located on newly replicated chromatids, following transcription reactivation after replication, would restrict their movement within the nucleus. By keeping sister chromatids in close proximity this mechanism could contribute to cohesion establishment and/or maintenance. (B) Gene gating might have a profound impact on chromosome architecture and condensation. The association of neighboring genes to the same NPC would generate large chromosomal loops restricted to certain nuclear territories that could serve as a framework for chromatin loops condensation prior to mitosis.

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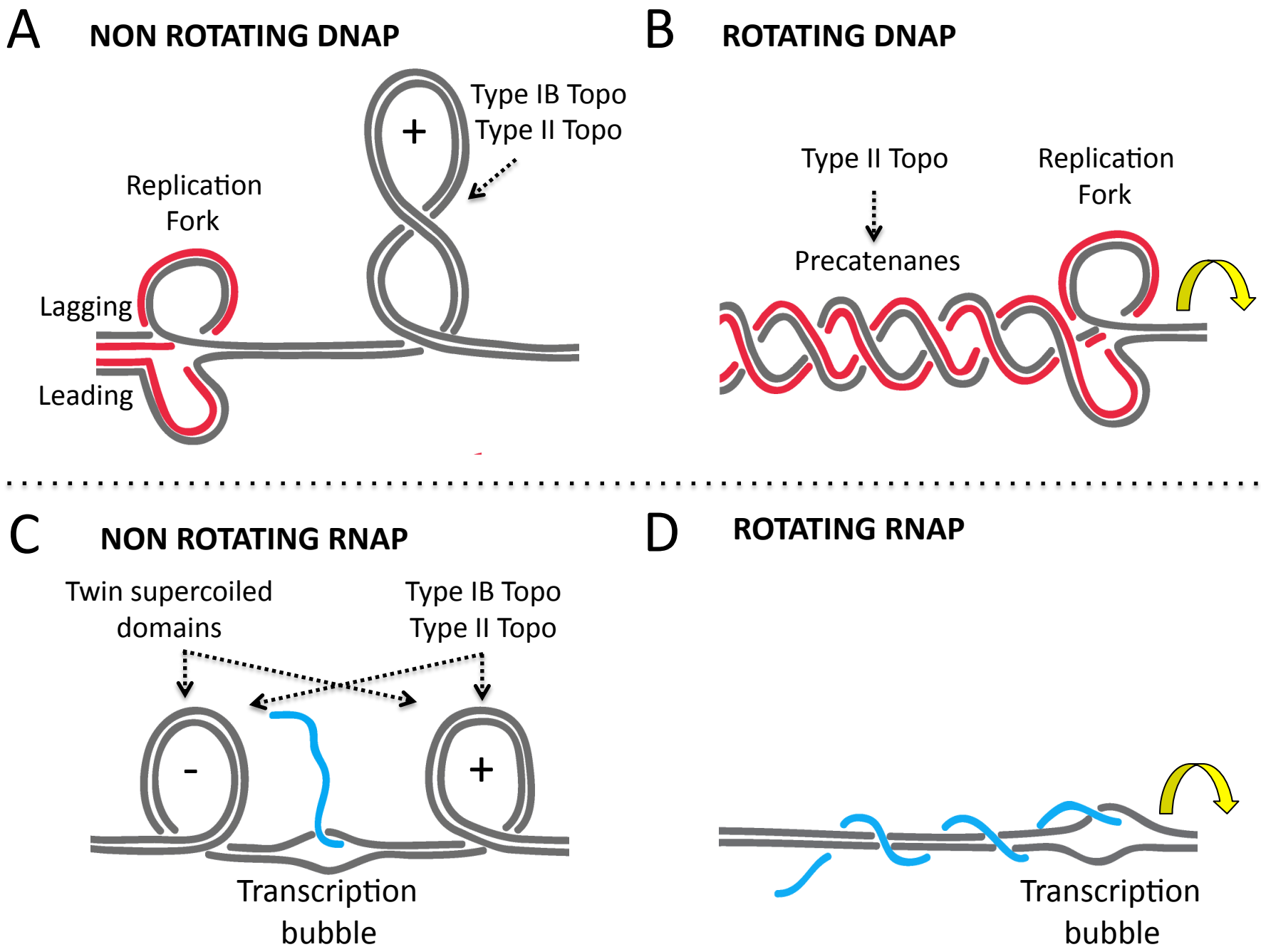
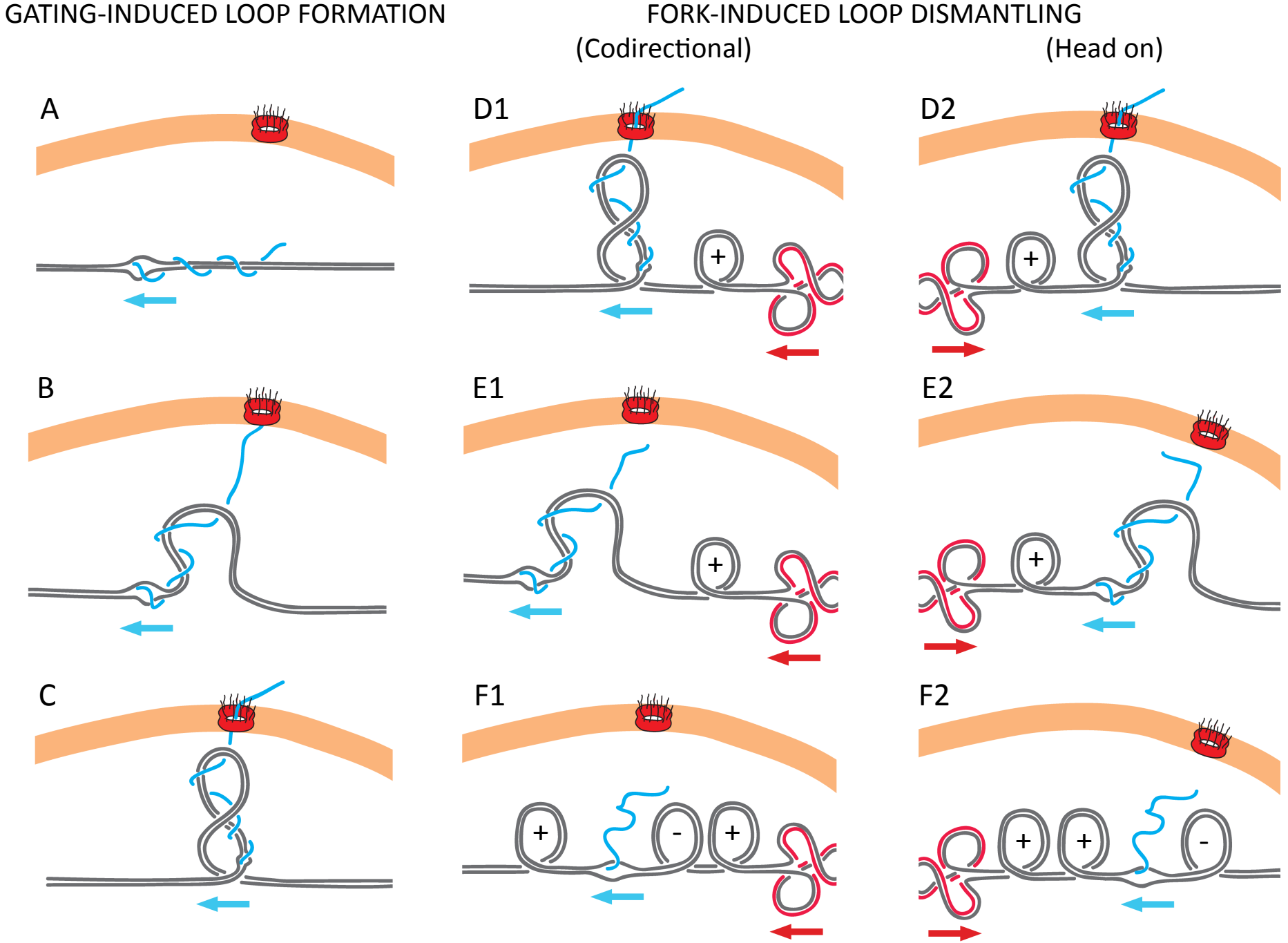
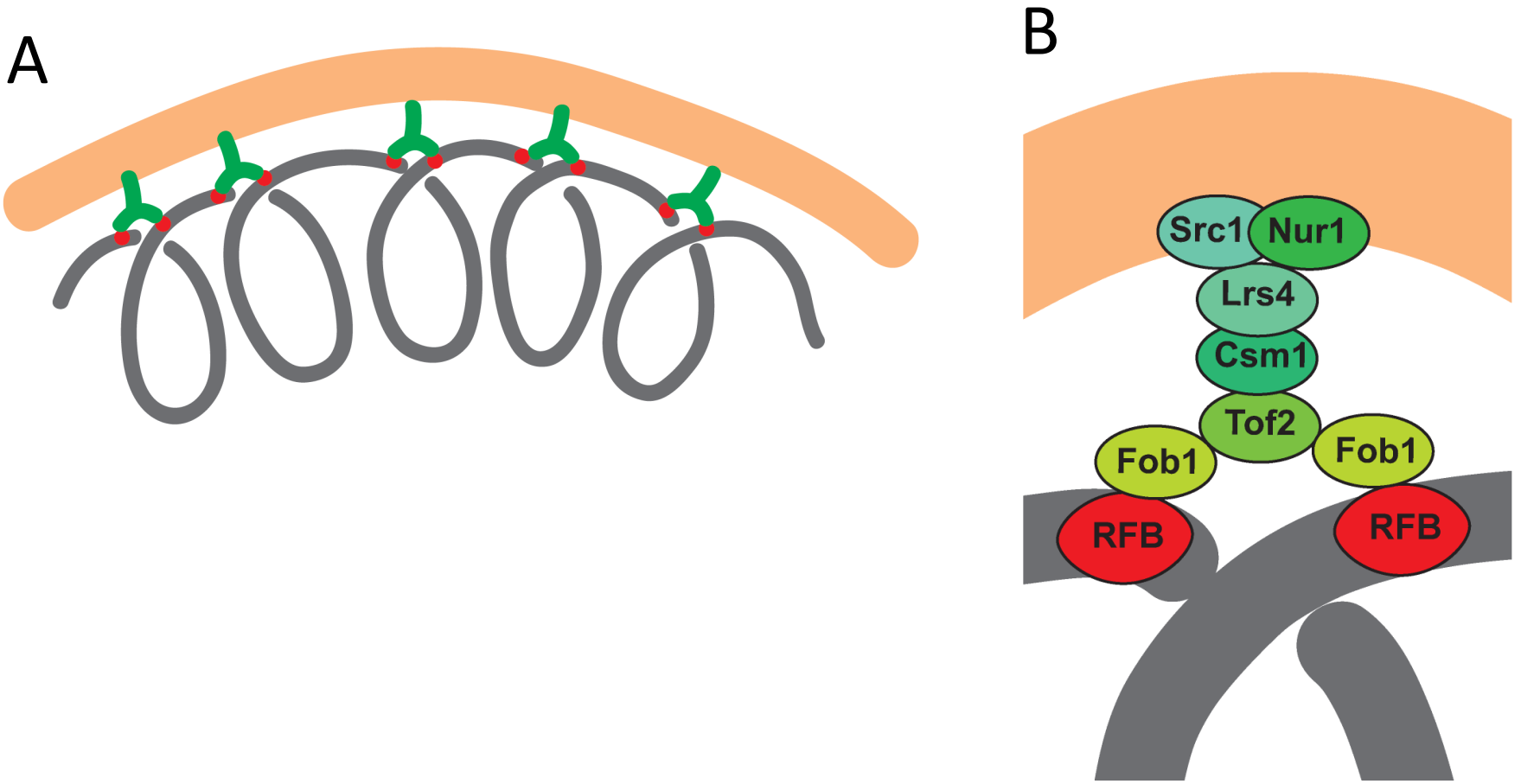


Bermejo et al. Fig.1



Bermejo et al. Fig. 2



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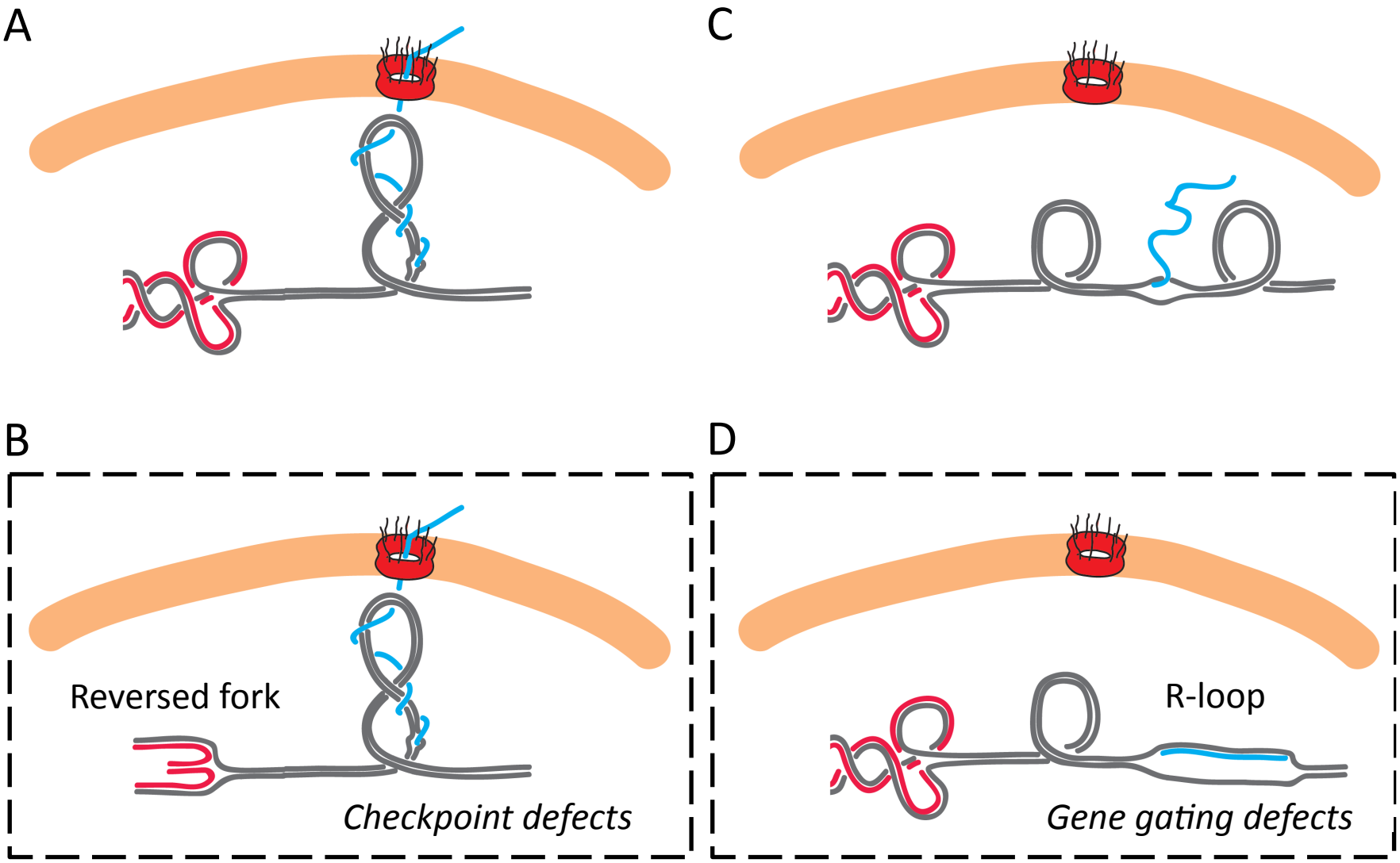
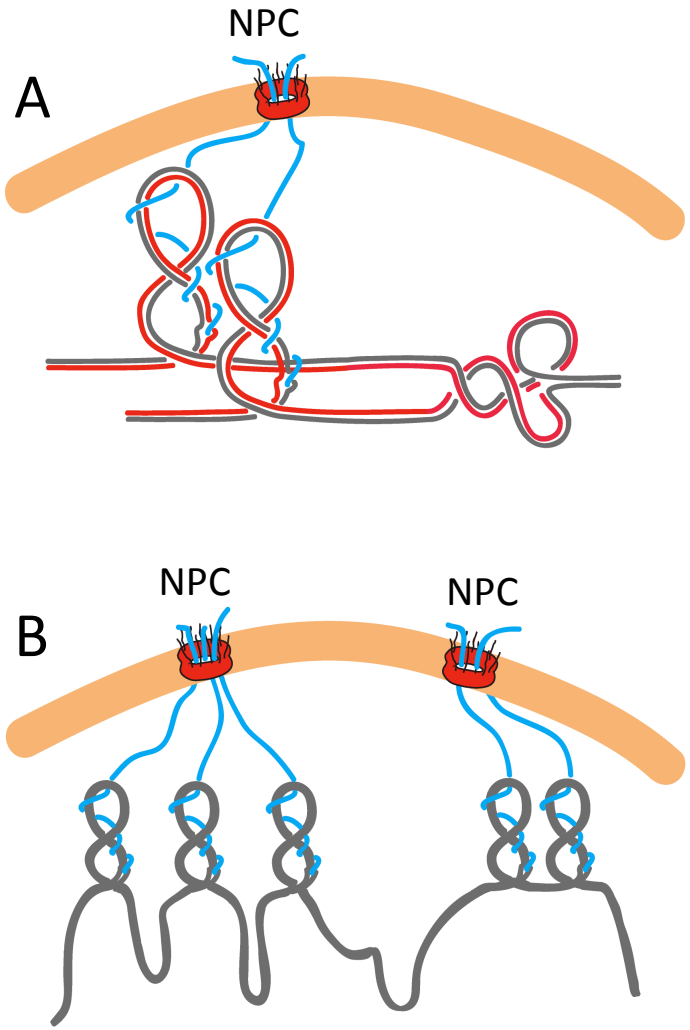


Bermejo et al Fig.4



Bermejo et al. Fig.5