

The Conflict of Transcription-Replication Coordination: Understanding in the Perspective of Genome Integrity

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Abstract

In every organisms, chromosomes are involved in two critical metabolic process, gene transcription and DNA replication, which seems to conflict with one another. In S phase the replication forks progress throughout the genome; inevitably compete for the DNA template with active RNA polymerases (RNAPs). The question how replication forks compete with transcription complexes during the process of replication is been noticeable in biology. Replication fork arrest due to the collision with transcription complex will lead to DNA damage response, mutagenesis and chromosomal deletions. This indicates that replication fork may occasionally collapse after collision with a RNA polymerase (RNAP). Several recent reports have highlighted the problems that occur when bacterial replication forks encounter transcription complexes, linking conflicts between genome duplication and gene expression to replication fork breakdown and Genetic instability. In this review we will look towards the reasons for collisions, and how cell counteracts these collisions?

Introduction

With each cycle of cell growth and division, several DNA base pairs are uncoiled and replicated to produce either the RNA transcripts or the new DNA strands [1]. In every organisms, chromosomes are involved in two critical metabolic process, gene transcription and DNA replication, which seems to conflict with one another. DNA replication copies the genetic information in preparation for cell division and is initiated at sites called origins [2]. Each origin consists of two replication complex (replisomes) which are having a replicative DNA helicase, leading and lagging strand DNA polymerases and many other accessory factors. The two replisomes move away from the origin in opposite directions, copying both strands of the duplex as the replisome move. In eukaryotic cells several hundreds to thousands of origins are involved in every S phase but in prokaryotes only a single origin is involved. Similar to DNA replication, transcription performs copying the information present in the genome, here by an RNA polymerase that transcribes one DNA strand into RNA. When a replication and transcription complex collides, the post collision effects are potentially catastrophic [2].

In S phase the replication forks progress throughout the genome; inevitably compete for the DNA template with active RNA polymerases (RNAPs). The question how replication forks compete with transcription complexes during the process of replication is been noticeable in biology since 25 years [3]. Replication fork arrest due to the collision with transcription complex will lead to DNA damage response, mutagenesis and chromosomal deletions [4,5]. This indicates that replication fork may occasionally collapse after collision with a RNA polymerase (RNAP) [6].

Initial attempts to understand the post effect of the interaction of replication forks with stationary and/or transcribing RNA-polymerase molecules were made in 1983 by Bruce Albert's and colleagues [3]. Since then, many other examples of transcription-replication collisions in bacteria, yeast and mammalian cells have been understood. In prokaryotes, both co directional and head-on encounters seems to be unavoidable, as replication and transcription move simultaneously on the same template DNA, but replisomes proceed approximately 12-fold faster (~600-730 nucleotides s^{-1}) [7,8] than do RNA polymerases (~50 nt s^{-1}) [9-11]. The initial evidence in vivo for the occurrence of transcription-replication collisions came from EM visualization of ColE1.

Since then, many studies in bacteria have described transcription complexes as natural impediments to replication [12]. Moreover, in contrast to those of prokaryotes, eukaryotic replication and transcription machineries progress with comparable speeds of 17-33 nt s^{-1} and 17-72 nt s^{-1} , respectively.

Several recent reports have highlighted the problems that occur when bacterial replication forks encounter transcription complexes, linking conflicts between genome duplication and gene expression to replication fork breakdown and Genetic instability. In a recent paper, Duch et al., [2] reports an elegant new mechanism that prevents collisions between replication and transcription during a heightened transcriptional response. Both replication and transcription must be highly coordinated in order to prevent potential conflict that can be catastrophic for genome integrity.

In this brief review we are going to understand what these conflicts, reasons for these conflicts are and how the cell counteracts these conflicts.

The conflicts of collision in cell

Since DNA replication complexes moves faster than transcription complexes, the two must inevitably collide given that they occupy the same template. It is not only actively transcribing RNAPs that could pose a problem for replisomes. In many genes RNAP pauses at regulatory sequences and RNAP can also pause or stall at sites of DNA damage [13-16].

Depending on the orientation of a particular gene, the replication fork can face RNA polymerases in either a head-on or a co directional

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manner. In the head-on conflicts, a given gene encoded on the lagging strand is transcribed in the opposite direction from leading-strand replication. In contrast, conflicts are co directional when genes are coded on the leading strand and transcription occurs in the same direction as leading-strand replication [17]. Consequently, the mode of transcription-replication encounters depends on the direction of transcription. DNA damage can be caused by head-on or co directional encounters or even upon collisions in both directions. There are five major cause which will be leading to collision of Replisome and RNAP complex, which are: 1.co-directional collision, 2. head-on collision between the replisome and transcribing RNAP, 3. stable backtracked RNAP complexes form at specific sequences, 4. RNAP is unable to progress through roadblocks and 5. A single blocked RNAP leads to the formation of RNAP arrays.

Co-directional collision

This kind of collision does occur mainly due to differences in rate of replication by replisome and transcription by RNAP complex. Co-directional collisions are detrimental when transcription elongation is compromised by extensive backtracking.

In the co directional case, by contrast, the front edge of the leading strand DNA polymerase collides with the rear edge of RNA polymerase. Co directional collisions are detrimental when transcription elongation is compromised by extensive backtracking, which is the reversible sliding of the RNA-polymerase elongation complex backward along DNA and RNA [18]. Bacteria have several mechanisms to suppress backtracking or to eliminate backtracked complexes (Figure 1).

Head-on collisions

In the case of head-on collisions, the front edge of RNA polymerase meets the hexameric DNA helicase DnaB that moves along the lagging strand template [17]. In bacteria, the majority of genes are encoded on the leading strand in the direction of DNA replication, which argues for a selection toward co-orientation of replication and transcription. When chromosomal fragments are inverted to a head-on orientation, replication rates are reduced, levels of the recombination protein RecA are increased, and the SOS DNA-damage response is activated [4,19]. One critical explanation for this is that transcription leads positive super coiling in front of the transcription bubble, potentially providing a topological rather than a direct physical block to continued fork movement [20,21] However, mapping of head-on collisions between bacterial forks and transcription complexes suggests that blockage occurs by direct contact between replisomes and RNAPs rather than indirectly via positive super coiling [22] (Figure 2).

Stable backtracked RNAP complexes form at specific sequences

Stable backtracked RNAP complexes forms at specific sequences, at sites containing roadblocks or under conditions of nucleotide starvation. At these sites when replisome complex meets RNAP complex it collides and which results in halting of replisome [23,24].

RNAP is unable to progress through roadblocks

RNAP is unable to progress through roadblocks such as DNA damage or tightly bound proteins. So at these points where the RNAP complex has been halted the replisome comes and collide to RNAP complex during the process of replication of the DNA [23]. A single blocked RNAP leads to the formation of RNAP arrays

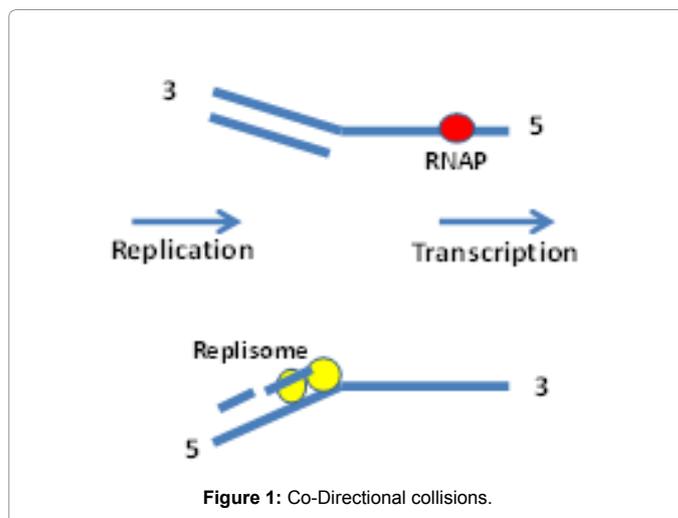


Figure 1: Co-Directional collisions.

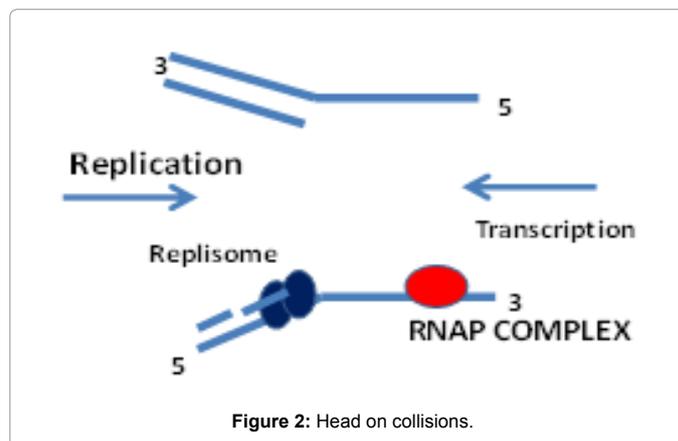


Figure 2: Head on collisions.

A single blocked RNAP leads to the formation of RNAP arrays

A single blocked RNAP leads to the formation of RNAP arrays behind the stalled complex in highly transcribed genes. These kinds of halts are more dangerous as the replisome will not be able to dissociate the RNAP arrays [23].

With this information of how these collisions occur in the cell, we shall look forward to know the catastrophic effect of these collisions in the cells.

Replication-transcription collisions cause genomic instability

The post collision effects between DNA-dependent DNA polymerases(replisome complex) and DNA-dependent RNA polymerases complex present a high risk for cells, as they may give rise to chromosomal recombination events, tumour formation, DNA damage or cell death [22,25,26]. EM, two-dimensional gel electrophoresis, replication-fork stalling is observed due to transcription-replication encounters that is often accompanied by DNA damage or recombination in both prokaryotic and eukaryotic cells [5,27-35]. The activities of the replicating polymerases as well as the function of replicative helicases and topoisomerases, such as Rrm3, Top1 and Top2, protect the genome from deleterious replication-transcription encounters [28,32,35].

In prokaryotes and eukaryotes, the generation of DSBs may result from two distinct and apparently independent consequences of transcription-replication interference: R loops and Topological stresses.

R Loops

These are the RNA-DNA hybrid structures are produced at the sites of RNA-polymerase pausing caused by DNA secondary structures at G-rich sequences [36,37] or extended trinucleotide repeats [38,39] or by collisions with the replication fork [40]. These are efficiently removed by the hydrolytic function of the RNA-DNA hybrid-targeting enzyme RNase H (in prokaryotes) or RNase H1 (in eukaryotes).

According to the 'thread-back' model, the newly synthesized RNA transcript can invade the DNA duplex to form a three-stranded nucleic acid molecule containing an RNA-DNA duplex and a single-stranded DNA: the R loop. R loops are natural intermediates of several transcription- or replication-linked processes. Although R loops occur at low frequency in normal cells [17,41] the number and/or the length of the R loops may increase when transcription or RNA processing is disturbed [35,41-44]. However, perturbing replication, either by hindering DNA-polymerase progression or by depleting replicative topoisomerases, also favours the creation or stabilization of R loops, which leads to increased DNA damage [17,29,35]. To prevent the disastrous result of R loops, the activity of RNase H1 needs to be tightly controlled.

Topological constraints

Topological constraints arise when a replication fork meets a chromosomal region that cannot rotate freely, for example, as it may be attached to the nuclear pore [45]. If the release of DNA from the pores is prevented, positive super coils accumulate in front of the fork, leading to fork collapse, reversed forks and increased genomic instability. In eukaryotes, mRNA export occurs simultaneously with transcription, DNA instability can arise from topological tension that occurs when transcription and replication machineries meet at loci that are bound to the nuclear periphery [28]. When DNA is attached to the nuclear pore and is unable to freely rotate, the topological tension generated in front of the replisome may lead to replication-fork stalling. According to the topological model, the nuclear pore complex (NPC)-bound DNA region will be removed from the NPC by the activities of the Mec1 (ATR) replication-damage checkpoint proteins to permit progression of the replication fork [45].

The collisions lead to genomic instability as we briefly explained it above. This disastrous effect on the genome integrity is being counteracted by the cell or prevented in the following manner.

Strategies that a Cell Adopt to Minimise these Collision

Avoiding co-occupancy at the DNA template

Collisions can be avoided by spatially or temporally separating DNA replication and transcription. Spatial separation could be achieved by separating replication and transcription into different 'factories', allowing concurrent gene expression and genome duplication. A complete temporal separation of transcription and DNA replication is clearly difficult in rapidly dividing bacteria where there is no window during the cell cycle in which DNA replication is not occurring [23]. Furthermore, the need to produce proteins such as histones during genome duplication, as well as the existence of very large genes whose transcription takes longer than a cell cycle [46].

Genome organisation

Many bacterial and viral genomes have evolved in such way that the majority of highly transcribed genes are oriented co-directionally with replication fork movement [47]. In almost all bacteria, the highly transcribed rRNA and tRNA genes are co-oriented with replication, and the majority of (but not all) other genes also show a co directional bias. In *B. subtilis*, 75% of all genes are co oriented with replication, whereas in *E. coli*, there is little bias when considering the entire genome; only 55% of genes are co oriented with replication. However, 70% and 90% of the essential genes (rRNA, tRNA and other essential genes) in *E. coli* and *B. subtilis*, respectively, are transcribed co directionally with replication [26]. Mechanisms to avoid head-on collisions are present in eukaryotes. Each highly transcribed ribosomal DNA transcription unit in *Saccharomyces cerevisiae* contains a potential origin of replication, risking multiple head-on collisions between forks and RNAPs. However, these are prevented by a specific replication fork barrier downstream of the transcribed region [47].

Reducing the immobile RNAPs

Reducing the accumulation of stalled RNAPs, and the consequent risk of formation of RNAP arrays, can be achieved by reducing the probability of stalling occurring, reactivating stalled RNAPs to allow transcription to resume or dissociation of stalled RNAPs [23].

Coupling of translation and transcription

Ribosome translocating along the emerging transcript also suppress backtracking and associated genome instability in *E. coli*, implying that the coupling of translation and transcription facilitates RNAP movement [11,42].

RNA translocase Rho

The RNA translocase Rho also reduces the conflict between gene expression and genome duplication [48]. Rho catalyses programmed termination of transcription and abortion of transcripts that are not being utilized [49] but may also translocate 5'-3' along emerging transcripts to displace stalled RNAPs [48]. This Rho function may be particularly important when transcription is not coupled to translation.

DNA translocase Mfd

Anti-backtracking factors include a double stranded DNA translocase, Mfd that binds to stalled transcription complexes and the DNA upstream of the complex [50]. Translocation by Mfd along the DNA results in forward translocation of backtracked RNAP and resumption of transcription [15,50]. Mfd also acts to dissociate RNAP that is stalled by template damage, and in this situation facilitates lesion repair [12,50].

GreA and GreB protein

GreA and GreB proteins provide a solution to the problem of backtracking. These small proteins insert a long coiled-coil hairpin motif into the secondary channel of RNA polymerase, placing two acidic residues in the vicinity of the catalytic site. This leads to re-coordination of one of the two Mg²⁺ in the active centre, allowing endogenous hydrolysis of the transcript [51,52]. In the case of backtracked complexes, this positions the 3'-end at the catalytic site, thereby resetting the system [9,53]. Transcriptional elongation may then resume provided the way ahead is clear.

Resolving Post Collision Effect

Displacement of RNAP by the replisome itself

If collisions do occur between forks and RNAPs, replication might be able to proceed to completion in many ways [23]. The *E. coli* replisome itself can displace an RNAP stalled by nucleotide deprivation in the co-directional orientation in vitro [6] although it remains unknown whether displacement of tandem stalled RNAPs with this orientation occurs efficiently.

Displacement of RNAP by accessory replicative motors

Helicases UvrD and Rep have long been implicated in replication and thought to promote replication through protein blocks such as transcription complexes [54]. However, until recently, convincing evidence indicating a role for UvrD and Rep in assisting replication through roadblocks has been lacking. UvrD and Rep are SF1 superfamily helicases that translocate along single-strand DNA (ssDNA) with 3'-5' polarity. Recent in vivo data provide strong evidence that auxiliary helicases such as UvrD and Rep promote replication through transcription complexes and repressors in *E. coli*. For example, UvrD and Rep along with the SF2 family helicases, DinG were shown to be required for cell growth when ribosomal genes were inverted [55]. Similarly in vitro for the Bacteriophage T4 Dda helicases, but Dda destabilizes transcription complexes independently of DNA replication. Thus, Dda may target transcription complexes rather than replication forks [3].

Rebooting genome duplication

Replisome does become blocked then replicative function is lost within a short time [25,56,57] although this instability has been doubted recently [6]. Loss of function of the replisome calls for reloading of the replication machinery via PriA or PriC in *E. coli* [54]. Cells lacking PriA are viable but extremely sick in rich media [58] a phenotype that can be suppressed by a mutation in RNAP that reduces backtracking [42,50]. Therefore fork breakdown due to transcription complexes creates a significant need for replisome reloading under rapid growth conditions [26] even in the presence of the many other mechanisms that reduce or resolve such conflicts. To directly bypass replicative blocks, recombination must occur with downstream homologous sequences, resulting in deletion of a part of the chromosome. Alternatively, blocked fork processing by recombination enzymes might simply facilitate replication reinitiating upstream of the block, presents a second chance for a replisome to move successfully through the block [59]. Recombination may also be critical in overcoming replication/transcription conflicts in [60] although mechanistic details are again lacking.

Upon environmental changes or extracellular signals, cells are subjected to marked changes in gene expression [2]. Dealing with high levels of transcription during replication is critical to prevent collisions between the transcription and replication pathways and avoid recombination events [6]. In response to osmotic stress, hundreds of stress-responsive genes are rapidly induced by the stress-activated protein kinase (SAPK) Hog1, even during S phase. In *Saccharomyces cerevisiae* a single signalling molecule, Hog1, coordinates both replication and transcription upon osmotic stress. Hog1 interacts with and phosphorylates Mrc1, a component of the replication complex. Phosphorylation occurs at different sites to those targeted by Mec1 upon DNA damage. Mrc1 phosphorylation by Hog1 delays early and late origin firing by preventing Cdc45 loading, as well as slowing down replication-complex progression [2].

Conclusion

It is crucial to ensure proper replication and gene transcription. The transcription-replication conflict is catastrophic for genomic integrity, multiple regulatory mechanisms have evolved to prevent and avoid such encounters. However, at many loci in bacteria and also within distinct regions of eukaryotic DNA, transcription-replication interference is unavoidable and may sometimes even be beneficial for the cell. Researchers are still just beginning to understand the complex mechanisms operating at the sites of collisions, and we therefore anticipate the elucidation of many more details of these mechanisms in the future. A better comprehension of these molecular events will clarify the view of how genomic stability is maintained. At present, very little is known about how epigenetic or chromatin-associated changes influence transcription-replication interference globally or at specific genomic loci. In addition, the way in which defined chromatin structures with specific epigenetic marks influence genomic stability awaits further investigation. Thus, we are expecting the discovery of an expanding array of functional links between epigenetic, chromatin architecture, transcriptional regulation, replication initiation and DNA repair.

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