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Spatial and temporal control of evolution through replication-transcription conflicts

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Abstract

Evolution could potentially be accelerated if an organism could selectively increase the mutation rate of specific genes that are actively under positive selection. Recently, a mechanism that cells can use to target rapid evolution to specific genes was discovered. This mechanism is driven by gene orientation-dependent encounters between DNA replication and transcription machineries. These encounters increase mutagenesis in lagging strand genes, where replication-transcription conflicts are severe. Due to the orientation and transcription-dependent nature of this process, conflict-driven mutagenesis can be used by cells to spatially (gene-specifically) and temporally (only upon transcription induction) regulate the rate of gene evolution. Here, I summarize recent findings on this topic, and discuss the implications of increasing mutagenesis rates and accelerating evolution through active mechanisms.

Keywords

Replication-transcription conflicts; lagging strand genes; mutagenesis; evolution; genome organization; TC-NER; evolution of evolvability

Mechanisms that increase mutation rates for adaptive purposes

Global mechanisms that increase mutation rates genome-wide have been identified [1-4]. These mechanisms include stress-induced adaptive mutagenesis, which is activated during stationary phase of growth and/or the DNA damage induced SOS response [1, 3-5]. Increasing the mutation rate of genes globally during stress will improve the chances of acquiring adaptive mutations, allowing the cell to survive environmental changes that require improved traits. However, this strategy also increases the chances of acquiring lethal mutations.

It seems that cells would benefit from mechanisms that could target mutagenesis to only genes under positive selection, and only when increased mutation rates could be beneficial.

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Yet, such a mechanism had not been discovered until 2013. Based on evolutionary analyses of *Bacillus subtilis* genomes and laboratory experiments, we proposed that gene orientation can be used to increase or decrease mutation rates in a gene-specific manner [6]. This mechanism is driven by the inevitable encounters between DNA replication and transcription machineries, a.k.a. replication-transcription conflicts, which have different consequences for genes encoded on the leading versus the lagging strand of the replication fork. Recent advances on this topic are discussed below.

Replication-transcription conflicts promote mutagenesis

The anti-parallel nature of DNA allows genes to be encoded on either strand. Yet, the expression of genes from one versus the other strand has differential consequences for the DNA replication machinery: When a gene is encoded on the leading strand of the replication fork, the transcription machinery moves in the same direction as the replication machinery (co-directionally). On the other hand, when the gene is encoded on the lagging strand, the transcription machinery moves opposite to the replication machinery (head-on). These generate two different types of conflicts (Figure 1) which have significantly different outcomes. Although co-directional conflicts do occur (due to the slower progression of RNA polymerase relative to the replication fork), head-on conflicts are much more detrimental as they lead to severe stalling and disassembly of the replication machinery, breaks in the DNA, deletions and insertions, and mutagenesis. [6-15]. This article is primarily focused on conflict-driven mutagenesis and thus the other consequences of conflicts will only be minimally discussed (see recent reviews on the topic [16, 17]).

We found that when transcription occurs head-on to replication, it increases mutagenesis within the open reading frame of a given gene (~2.5 times relative to an otherwise identical co-directional gene) [6, 8]. We observed similar results for three different reporter genes, and at two different chromosomal locations, indicating that the differences in rates of mutagenesis are neither gene sequence nor genomic context-dependent [8]. The sequence independence of this mechanism was also later confirmed through comparative evolutionary studies [18]. Because gene orientation-dependent differences in spontaneous mutation is transcription-dependent, we proposed that replication-transcription conflicts, rather than the inherently elevated rate of lagging strand mutagenesis, is responsible for the increased mutations rates in head-on genes [19].

Wang and colleagues also found that head-on gene orientation increases mutagenesis and genome instability in *B. subtilis* [15]. They found that reporters in the two orientations are subject to equivalent rates of mutagenesis in the absence of transcription. However, transcriptional de-repression led to an asymmetric pattern of mutagenesis where the head-on oriented gene experiences an elevated mutation rate. One novel aspect of the Sankar et al. study was the identification of a transcription-dependent increase in base substitution rates in the promoter (~3-fold), suggesting that conflict-derived mutations can alter gene expression patterns. Another important observation was that indels are most pronounced at the 5' end of co-directional and the 3' end of head-on genes; the locations where RNA polymerase is most likely to initially contact the replisome (Sankar et al., Figure 2).

We also conducted SNP analyses on several *B. subtilis* isolates and determined the rate of synonymous (dS) and non-synonymous (dN) point mutation in genes of both orientation. These studies showed that head-on genes have a significantly elevated dN (a difference of 50%) and convergent mutations relative to co-directional genes [6]. Convergent mutations are indicators of both positive selection and adaptive evolution, suggesting that an increased rate of spontaneous mutation in head-on genes drives their accelerated rate of evolution.

Genome organization and gene orientation bias

The majority of genes, especially highly transcribed and/or essential genes, are encoded on the leading strand in bacteria (Figure 2). The bias for essential and core genes is stronger than for all genes, varying from 75% to 95% depending on the organism [6, 20-23], with 100% of rRNA genes being co-oriented with replication in all bacterial genomes [21, 24-27]. This co-orientation bias largely (but not completely) avoids the potentially detrimental head-on replication-transcription encounters [6, 7, 9, 10, 13]. Interestingly, there are some species, such as *Escherichia coli*, where the orientation bias is low, except for highly transcribed and essential genes [20, 21, 27]. Whether this is due to their ability to tolerate head-on conflicts (different conflict resolution factors [11, 28, 29]), and/or a lack of selection for co-orientation of genes, is unclear. Nevertheless, the high conservation of co-orientation bias across bacteria suggests that head-on conflicts are universally problematic.

Explaining the presence of head-on genes

Given the detrimental effects of head-on conflicts, and the elaborate essential mechanisms required to resolve them, it is unclear why any gene would remain in the head-on orientation [11, 28]. Head-on genes are transcribed to some degree during normal growth and are highly induced during stress exposure [28, 30, 31]. Because the environment can change at any time, cells will at least occasionally be exposed to stress during active replication, causing severe conflicts. One example is the exposure of pathogenic bacteria to host defenses (reactive oxygen species and nitric oxide (NO)) during infections, where bacteria are actively replicating [32, 33].

Genomic rearrangements can be used to change the orientation of a given gene: in general, DNA rearrangements, such as inversions, are relatively common events that can be facilitated through recombination-dependent mechanisms, even when there is minimal homology between DNA fragments [34-40]. Therefore, cells have the ability to evolve genomes composed of only co-directional genes. Since this has not occurred, other pressures may be driving the production or maintenance of head-on genes. One such driver could be horizontal gene transfer, which can lead to the integration of newly acquired genes (which are frequently transcriptionally silenced) in either orientation. Another is the lack of transcription during replication [41].

In the absence of any selection pressure, head-on transcription is more likely to destabilize the region given that conflicts with replication can lead to DNA breaks and genomic rearrangements [9, 42, 43], and thus, there should be a higher inversion rate from the head-on to co-directional orientation. However, selection to preserve the head-on orientation or a

lack of selection to co-orient the remaining head-on genes may minimize co-orientation biases despite this higher inversion rate. Because second-order selection is generally weak, it is unlikely that selection directly promotes the head-on orientation of genes. Rather, I propose that cells with an inverted (to head-on) gene could fix in the population because of its higher mutation rates. In other words, cells with the inverted allele could gain a selective advantage before their counterparts harboring the co-directional allele. In this hypothetical circumstance, head-on orientation is not directly selected for. Instead, it is subject to the “hitchhiker effect”: an increase in the frequency of an allele in a population, not because it is directly under selection, but because it is *associated* with a DNA sequence that is under positive selection [44].

The mutagenic nature of TC-NER: the driver of head-on gene evolution

We found that transcription-coupled nucleotide excision repair (TC-NER) is required for the increased mutagenesis of head-on genes [8]. Without the TC-NER proteins Mfd and UvrA, head-on genes do not accumulate mutations more than co-directional genes [8]. In the context of replication-transcription conflicts, we attributed the mutagenicity of NER to the function of an error prone polymerase, PolY1 in *B. subtilis* [8]. Traditionally, after a lesion is excised by the Uvr proteins, the remaining gap in the DNA is thought to be filled by DNA polymerase I. However, one study suggested that in *B. subtilis* PolII and PolY1 interact, potentially providing a platform for multiple polymerases collaborating to complete NER [45]. (Notably, another study did not observe orientation-based effects on UV damage in *E. coli*, but this was performed in *uvrA* mutants which should be incapable of producing the asymmetry [5, 46]). Interestingly, our studies were not performed under exogenously added DNA damage, consistent with the model that error prone polymerases can function not only during DNA damage, but also under regular growth conditions in DNA repair. However, the mutation rates were measured in cells facing starvation stress, which could induce some DNA repair genes, including Mfd [47].

Is this mechanism adaptive?

The conflict-mediated increase in the mutation rate of head-on genes may or may not provide an adaptive advantage. First, not all genetic changes induced by conflicts (for instance, the deletions and insertions identified by Sankar et al. [15]) are expected to be adaptive. Base substitutions in particular are more likely to produce conservative changes and thus should be the major type of genetic changes that provide an adaptive advantage, potentially by improving protein function.

Second, though it is possible that head-on genes are simply more tolerant of diversity and therefore can accumulate mutations over time more rapidly without much consequence, evidence suggests that this is not the case. First, certain functions are overrepresented in lagging strand genes, suggesting that for many genes, positive selection promotes the maintenance of the head-on orientation. Second, the elevated rate of convergent mutations in head-on genes [6] suggest that they frequently come under positive selection.

One study argues that lagging strand genes do not undergo accelerated evolution [48]. This opinion is based largely on an overly restrictive and scholastic definition of convergent mutation. In essence, convergent evolution is the development of similar traits in separate lineages. Therefore, same-site mutations can be considered convergent if they produce the same functional effect, regardless of whether they are identical (parallel) or different (coincidental) [49-52]. In our study, we defined convergent mutation accordingly. Chen and Zhang however dismiss the adaptive significance of coincidental mutations, requiring that residues in the same position to be initially different. This greatly underestimates the frequency of convergent events. However, even when Chen and Zhang analyze only parallel mutations, they still observe a 2.7-fold higher rate of parallel (i.e. adaptive) mutations in head-on genes (although the P value for the difference between genes of the two orientations was not significant due to the low sample number). This trend is actually in keeping with our original proposal. Therefore, the model that orientation is an adaptive mechanism for accelerating evolution remains quite attractive.

Future studies of gene orientation and transcription-driven mutagenesis

The underlying factors driving transcription-dependent mutagenesis, especially in head-on genes are of particular interest. Although we previously established that some repair factors, including TC-NER proteins, are critical for this process, how this repair system is activated in an orientation-dependent manner remains unknown. Furthermore, we do not necessarily have a comprehensive list of all of the factors that play a role in this process. A list of open questions can be found in the Outstanding Questions.

Genetic and biochemical studies in combination with evolutionary analyses of wild strains could provide further insight into the mechanisms of transcription-dependent mutagenesis. One tool that is used to study this problem is laboratory-based mutation accumulation (MA) line experiments. These experiments are a powerful means of identifying many global mutation patterns with minimal bias. However, MA line experiments have not detected orientation-specific difference in mutation rates [53, 54], potentially because: (i) the endogenous head-on and co-directional genes are by definition different in sequence, mutability, length, and transcription levels - key factors that influence conflict severity and mutation rates [55, 56], and (ii) MA line experiments minimize stress, which should also minimize transcription of head-on genes and their mutagenesis [30, 31]. In the wild, however, where stress must occur at least occasionally, head-on genes will be periodically induced, increasing conflict-driven mutation rates. Therefore, the most accurate approach for studying gene orientation-dependent mutagenesis should include analyses of SNPs from natural strains found in the wild combined with experimental methods that specifically isolate the impact of gene orientation on mutagenesis.

Concluding remarks

Gene orientation can, in essence, serve as an architectural switch, toggling between the two possible types of conflicts, and serving as a mechanism to increase or decrease the rate of genetic changes in a gene orientation-specific manner. These conflicts are also controlled temporally: they only occur when transcription *and* replication are active. Therefore, cells

possess a mechanism that could specifically increase mutation rates in a subset of genes, only when they are transcribed (presumably when their functions are most needed). As suggested by our prior findings, genes that come under positive selection frequently may tend to be in the head-on orientation through the hitchhiker effect: a gene's orientation may fix within a population by gaining adaptive mutations faster. Interestingly, this leaves the same genes in a position to mutate quickly again. As such, the head-on orientation could represent a sort of evolutionary memory, previously observed for other systems [57-59].

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Outstanding Questions

- Are transcription-dependent mutations in both orientations due to replication-transcription conflicts? Or is transcription causing mutations without the influence of replication?
- Is the increased mutagenesis of head-on genes through TC-NER occurring during a conflict or post-replicatively?
- Are DNA lesions the source of conflict-driven mutations?
- Is the conflict-dependent mutagenesis mechanism adaptive? Or are head-on genes mutating faster because they are simply more tolerant of diversity?
- How are error-prone polymerases recruited to gaps generated during NER?

Trends

- Head-on genes mutate at a faster rate.
- Replication-transcription conflicts can target mutations to head-on genes.
- Conflict-driven accelerated evolution is likely adaptive.
- Mfd-dependent TC-NER can preferentially act on head-on genes.
- NER and error polymerases function in the same pathway.

Figure 1. Cartoon depiction of the two types of replication-transcription conflicts

The replisome is represented by the purple sphere and RNA polymerase is represented by the blue-green sphere. Newly synthesized strands of the DNA are black. The mRNA is dark gray. Cartoon represents conflicts occurring at a gene that is encoded on the lagging strand (head-on) or leading strand (co-directional).

Figure 2. Gene orientation bias across various bacterial species

The fraction of genes encoded on the leading strand are shaded in lavender, and the fraction encoded on the lagging strand are shaded in darker purple. Abbreviations (Percent of genes encoded on the leading strand): Top row: *Mg*, *Mycoplasma genitalium* G37 (80.8%); *Sp*, *Streptococcus pneumoniae* TIGR4 (80.3%); *Sa*, *Staphylococcus aureus* NCTC 8325 (76.8%); *Ss*, *Streptococcus sanguinis* SK36 (75.3%); *Sa*, *Staphylococcus aureus* N315 (74.8%); *Bs*, *Bacillus subtilis* 168 (73.8%); *Ab*, *Acinetobacter* ADP1 (60.7%). Middle row: *Bt*, *Burkholderia thailandensis* E264 (59.3%); *Mt*, *Mycobacterium tuberculosis* H37Rv (59.0%); *St*, *Salmonella enterica* serovar Typhimurium LT2 (58.6%); *Sty*, *Salmonella enterica* serovar Typhi Ty2 (58.1%); *Btm*, *Bacteroides thetaiotaomicron* VPI-5482 (58.0%); *Hp*, *Helicobacter pylori* 26695 (57.8%); *St*, *Salmonella enterica* serovar Typhimurium 14028S (57.3%). Bottom row: *Pa*, *Pseudomonas aeruginosa* PA01 (55.9%); *So*, *Shewanella oneidensis* MR-1 (55.7%); *Hi*, *Haemophilus influenzae* Rd KW20 (55.0%); *Ec*, *Escherichia coli* K-12 MG1655 (54.9%); *Cc*, *Caulobacter crescentus* NA1000 (54.7%); *Pa*, *Pseudomonas aeruginosa* UCBPP-PA14 (54.1%); *Pg*, *Porphyromonas gingivalis* ATCC 33277 (51.4%). Data were extracted and compiled from analyses presented in [60].