Molecular Microbiology (2004) **54**(3), 598–603 doi:10.1111/j.1365-2958.2004.04289.x

MicroReview

Why is transcription coupled to translation in bacteria?

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Summary

Active mechanisms exist to prevent transcription that is uncoupled from translation in the protein-coding genes of bacteria, as exemplified by the phenomenon of nonsense polarity. Bacterial transcription–translation coupling may be viewed as one among several co-transcriptional processes, including those for mRNA processing and export in the eukaryotes, that operate in the various life forms to render the nascent transcript unavailable for formation of otherwise deleterious R-loops in the genome.

The lack of a membrane-enclosed nucleus, a classical feature that distinguishes a prokaryote from a eukaryote, explains *how* transcription may be coupled to translation in the former but does not explain *why* this should happen. That such coupling is not an incidental consequence of the absence of a spatial barrier is underscored by the phenomenon of nonsense polarity in bacteria, first identified in the *lac* and *trp* operons of *Escherichia coli* nearly 40 years ago (Newton *et al*., 1965; Yanofsky and Ito, 1965). Nonsense polarity refers to the abolition of expression of intact promoter-distal genes in an operon that bears a nonsense mutation which stops translation in a promoter-proximal gene, and is mediated by premature termination of transcripts in the region immediately downstream of the nonsense mutation (Adhya and Gottesman, 1978; Nudler and Gottesman, 2002).

The common thinking is that transcription–translation coupling is a means for the cell to prevent accumulation of non-functional transcripts in the cytoplasm (Richardson, 1991; 2002), and hence that it is functionally analogous to nonsense-mediated mRNA decay that occurs in eukaryotic cells (Hilleren and Parker, 1999; Wilusz *et al*., 2001; Maquat, 2004). That the stability of a bacterial mRNA species is influenced by the efficiency with which its translation is coupled to transcription has also been demonstrated earlier (Iost and Dreyfus, 1994; 1995). As discussed below, however, recent evidence may be interpreted in support of a second (not necessarily mutually exclusive) model that the purpose of bacterial transcription–translation coupling is to preclude the occurrence of otherwise lethal R-loops on the bacterial chromosome. In this sense, translation may play the same role in the prokaryotes as do other co-transcriptional events such as mRNA processing and export in eukaryotic cells.

Co-transcriptional R-loops from untranslated RNA in bacteria

The premature termination of untranslated transcripts in *E. coli* is mediated by the Rho protein acting together with its associated factors such as NusG (Adhya and Gottesman, 1978; Nudler and Gottesman, 2002; Richardson, 2002). In the absence of a translating ribosome, Rho binds a suitable exposed site on nascent mRNA and, in a process that is kinetically rather than thermodynamically controlled, signals the elongating RNA polymerase to terminate transcription. Rho and NusG are essential for viability in many species of bacteria (Richardson, 2002).

The R-loop is a structure in which RNA is heteroduplexed with one strand of double-stranded DNA. Negative supercoiling of DNA will be expected to favour R-loop formation (Masse and Drolet, 1999a), and the twin-supercoiling domain model states that DNA is negatively supercoiled behind a moving RNA polymerase (Liu and Wang, 1987; Wang, 2002); together, these two features may explain why it is that the two known biochemically characterized examples of R-loop formation in bacterial cells have both involved nascent RNA transcripts in ternary elongation complexes (rather than free RNA molecules in the cytoplasm). The first example is the R-loop formed by (plasmid-encoded) RNA-II in the process of replication of ColE1-like plasmids (Itoh and Tomizawa, 1980; Selzer

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and Tomizawa, 1982), and the second is transcription-associated R-loop formation in *topA* mutants (whose DNA is hypernegatively supercoiled because of topoisomerase I deficiency) (Drolet *et al*., 1995; Masse *et al*., 1997; Masse and Drolet, 1999a; Broccoli *et al*., 2004). In *E. coli*, RNase H1 and RecG are the only enzymes known to disrupt R-loops (by hydrolysis and unwinding, respectively), and the finding that cells with a combined deficiency of both enzymes are inviable suggests that R-loops do occur in wild-type cells and can be lethal (Kogoma, 1997).

Apparently therefore both R-loop formation and Rho-mediated premature transcription termination are kinetic phenomena associated with the nascent untranslated and unstructured transcript as it emerges from the RNA polymerase in the ternary elongation complex, raising the possibility that the latter (Rho-mediated termination) has been selected in evolution to prevent the occurrence of the former (R-loop formation). Several genetic observations have provided indirect evidence that mutants deficient in either Rho or NusG indeed suffer increased R-loops on the chromosome (Harinarayanan and Gowrishankar, 2003). They include (i) the synthetic lethality of cells with combined deficiencies of Rho and RecG, or of NusG and RNase H1, (ii) uncontrolled replication of the R-loop-dependent ColE1-like plasmids in the *rho* or *nusG* mutants (see below) and (iii) rescue of some of the *rho*- or *nusG*-associated phenotypes by RecG or RNase H1 overexpression.

One may therefore envisage that all bacterial transcription is R-loop prone (Fig. 1A), and that the R-loops are avoided by one of the following (Fig. 1B–D): RNA secondary structure formation as in rRNA and tRNA; coupling of translation with transcription; or Rho- and NusG-mediated premature termination of transcription in situations where the mRNA fails to be translated. Indeed, Drolet and co-workers have shown that transcription–translation coupling does serve to prevent the occurrence of R-loops in *topA* strains (Masse and Drolet, 1999a; Broccoli *et al*., 2004). Furthermore, in the *topA* mutants, even rRNA transcription is abnormally associated with R-loop formation (Masse *et al*., 1997; Hraiky *et al*., 2000), but only so when the *boxA* sequence in the rRNA leader region is intact and functional (Drolet *et al*., 2003; Broccoli *et al*., 2004). *boxA* is a motif that mediates an active mechanism of anti-termination during rRNA transcription (Condon *et al*., 1995), and the fact that R-loops do not occur in its absence suggests once again that Rho-mediated transcription termination is important for R-loop avoidance.

With reference to Fig. 1D, a nonsense mutation (in the DNA) is not the only means by which untranslated mRNA may be generated during transcription of a bacterial protein-coding gene. The transcript region, even from a wild-type gene, may fail to be translated in any of the following instances: (i) generation of a nonsense codon in the mRNA by transcriptional error (as illustrated in Fig. 1D) (Libby *et al*., 1989; Taddei *et al*., 1997; Bridges, 1999; Bregeon *et al*., 2003), (ii) stochastic failure of ribosome binding to mRNA, (iii) ribosomal frameshifting on mRNA (Bregeon *et al*., 2001), leading to the premature termination of translation or (iv) endonucleolytic mRNA cleavage, resulting in the absence of translation on the 3′ side of the cleavage site. Untranslated mRNA regions may also occur in wild-type cells as a consequence of inefficient Rho-independent transcription termination at the ends of genes or operons (Abe *et al*., 1999).

The notion that factor-dependent transcription termination may have evolved primarily to prevent R-loops from occasional untranslated RNAs (rather than as a regular means to terminate transcription at the ends of genes or operons) obtains indirect support from the fact that there are very few examples of Rho-dependent termination sites *outside* the coding regions of genes (Platt, 1986; Pichoff *et al*., 1998). On the other hand, *intragenic* Rho-dependent terminators occur in abundance (Richardson, 1991; 2003; Nudler and Gottesman, 2002); for example, not less than four sites have been mapped within the first 500 bp of the *lacZ* coding region (Ruteshouser and Richardson, 1989).

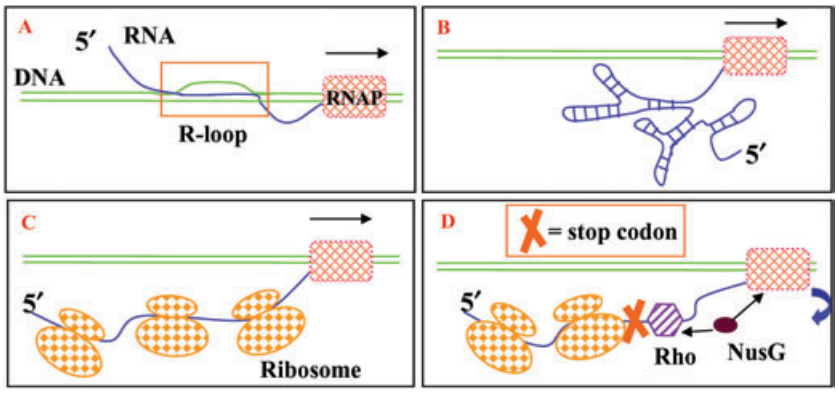

Fig. 1. Schematic depiction of R-loop formation by re-annealing of the nascent unstructured transcript to the template DNA strand upstream of the transcription elongation complex (A), and of its avoidance by either RNA secondary structure formation (B), coupling of translation with transcription (C), or Rho- and NusG-mediated termination of transcripts with premature stop codons (D). RNAP, RNA polymerase.

Additional instances of bacterial transcription–translation uncoupling

Transcription–translation uncoupling in the Rho- or NusG-deficient strains is associated with greatly increased content of several ColE1-family plasmids whose replication is R-loop dependent. The uncontrolled or runaway replication of the plasmids is explained as a consequence of titration, by the chromosomal R-loops in the mutant strains, of host factors such as RNase H1 or RecG that may otherwise act to destabilize the R-loops at the plasmid replication origin (Harinarayanan and Gowrishankar, 2003).

Remarkably, increased plasmid replication is also observed in at least two other instances in which transcription is uncoupled from translation. First, the classical method for amplification of plasmid content in cultures has been by the addition of chloramphenicol, which is an inhibitor of translation (Clewell and Helinski, 1969; Clewell, 1972). Second, cells of ppGpp-deficient (*relA* mutant) strains subjected to amino acid limitation also exhibit increased content of ColE1-like plasmids (Hecker *et al*., 1988; Riethdorf *et al*., 1989). Both perturbations are expected to lead to global translational arrest in the absence of any limitation of the transcription potential; possibly therefore it is the increased occurrence of chromosomal R-loops, rather than the selective inhibition of chromosomal DNA replication (Clewell, 1972), that is the basis for increase in ColE1 plasmid copy number in these situations.

Increased R-loops associated with the uncoupling of transcription from translation during cold shock

Translational arrest is a critical component of cold-induced stress in the bacteria, which is suggested to be the consequence of both secondary structure formation in mRNA and the inactivation of ribosomes at low temperature (Thieringer *et al*., 1998; Xia *et al*., 2003). Consistent with this model are the findings that inhibitors of translation such as chloramphenicol or the ribosome-inactivating toxin colicin E3 induce the cold shock response (Thieringer *et al*., 1998; Walker *et al*., 2004). Null mutations in genes such as *dbpA* or *srmB* that interfere with ribosome assembly also confer a cold-sensitive phenotype (Charollais *et al*., 2003; Perutka *et al*., 2004). As has been suggested by Masse and Drolet (1999b), one may therefore expect an increased propensity for transcription to be uncoupled from translation in bacteria that are subjected to cold stress.

Data from two sets of studies suggest that the increased tendency for uncoupling of transcription from translation during cold shock is also associated with an increased occurrence of R-loops on the chromosome. Thus, the frequency of occurrence of R-loops in *topA* mutants is markedly elevated during low-temperature growth (Masse and Drolet, 1999b; Broccoli *et al*., 2000). Likewise, there is evidence also for increased R-loops in the Rho- or NusG-deficient cells during cold stress (Harinarayanan and Gowrishankar, 2003).

Topological constraints in co-transcriptional R-loop formation

As mentioned above, the domain of negative DNA supercoiling that is created behind the moving RNA polymerase is probably essential for R-loop formation during transcription (Masse and Drolet, 1999a), but additional topological constraints on the process by which R-loops occur also need to be considered. In the scheme shown in Fig. 1D, the absence of Rho or NusG is expected to lead to increased R-loops from the region of untranslated mRNA downstream of its nonsense codon; however, this region is not free to twist around the template DNA strand for the reasons that (i) on its 3′ side, it is tethered to the transcription elongation complex and (ii) on the 5′ side, the translating ribosomes are loaded on it (upstream of the nonsense codon). One could speculate on several alternative mechanisms by which the RNA·DNA hybrids are formed in this situation, the first of which is that the R-loop is generated by the action of a novel topoisomerase activity within the cells. A second possibility is that the untranslated RNA segment is cleaved by an endonuclease such as RNase E (Iost and Dreyfus, 1995; Lopez *et al*., 1998), with the region on the 3′ side of the cut now free to twist around the DNA strand. A third possibility is that the R-loop represents an RNA·DNA hybrid with a zero linking number, which may in turn be (i) a duplex with equal numbers of left- and right-handed helical turns, perhaps similar to that described previously in form V supercoiled DNA (Brahmachari *et al*., 1987) or (ii) one with base-pairing contacts that entail no twist between the DNA and RNA strands.

In this context, it is also worth noting that our model (for R-loop formation during transcription elongation, and for its avoidance by coupled translation or by Rho binding) assumes, and in fact requires, that the R-loops are generated by re-annealing, to the upstream region of the template DNA strand, of the nascent transcript *after* it has emerged from the exit channel of the RNA polymerase (see Fig. 1A). An alternative mechanism for R-loop formation during transcription, and one that is not expected to be affected by the presence or absence of simultaneous translation of the nascent transcript, will invoke the continued extension of the (otherwise transient) RNA·DNA hybrid within the transcription bubble, that is, without the transcript ever dissociating from the template DNA strand; indeed, the model for R-loop formation in the *ori* region of

ColE1-like plasmids (Selzer and Tomizawa, 1982; Eguchi *et al*., 1991), admittedly proposed before the determination of RNA polymerase structure, was based on the latter mechanism. The distinctions between these two mechanisms have been discussed in a recent review which concluded that, although both mechanisms are likely to operate for R-loop generation, it is difficult to discern their *inter se* importance, given that the point of initiation of the R-loop behind a moving RNA polymerase has so far not been directly visualized (Drolet *et al*., 2003).

Is transcription in the eukaryotes also R-loop prone?

A general propensity for R-loop formation by the nascent RNA transcript may be expected to exist also in eukaryotic cells, given the substantial similarities in structures of the transcription complexes in the eukaryotes and the prokaryotes (Darst, 2001; Cramer, 2002). For example, R-loops do occur as a special feature of immunoglobulin gene transcription (Reaban and Griffin, 1990; Yu *et al*., 2003), akin to R-loops at the bacterial plasmid *ori* region occurring as a special feature of RNA-II transcription. In general, however, mechanisms to prevent co-transcriptional R-loops appear to have been selected in eukaryotic evolution as they have in the prokaryotes.

For one, transcription–translation coupling has been reported both to occur within the nuclei of some mammalian cells and to participate in the mechanism of nonsense-mediated mRNA decay (Iborra *et al*., 2001; 2004), although it must be mentioned here that these claims have been questioned by some other groups (Dahlberg *et al*., 2003; Nathanson *et al*., 2003). Recent evidence also suggests that the events of RNA processing (capping, splicing and polyadenylation) as well as that of RNA export occur co-transcriptionally (Bentley, 2002; Maniatis and Reed, 2002; Neugebauer, 2002; Proudfoot *et al*., 2002; Reed, 2003; Stutz and Izaurralde, 2003), so that they are likely to render the RNA unavailable as a naked molecule for initiation of R-loop formation. Likewise, the facilitator of chromatin transcription (FACT) complex of proteins mediates the disassembly of nucleosomes downstream of the elongating RNA polymerase and their sequential reassembly upstream of it (Belotserkovskaya *et al*., 2003), thereby probably serving to sequester the DNA substrate necessary for R-loop formation during eukaryotic transcription.

Perhaps the most persuasive evidence, however, for the notion that eukaryotic transcription is generally R-loop prone has come from a recent study it was demonstrated that yeast *hpr1*Δ mutants exhibit phenotypes of hyperrecombination and impaired transcription elongation as a consequence of co-transcriptionally formed R-loops (Huertas and Aguilera, 2003). HPR1 is a subunit of the conserved THO/TREX complex of proteins implicated in the coupling of transcription with mRNA export (Strasser *et al*., 2002), and, interestingly, *hpr1* mutants are extremely sick in combination with mutations in the topoisomerase I gene (Aguilera and Klein, 1990; Sadoff *et al*., 1995). Remarkably, furthermore, RNase H1 overexpression was associated with suppression of the *hpr1*Δ-conferred phenotypes (Huertas and Aguilera, 2003), just as had been described previously for RNase H1 overexpression in the bacterial mutants deficient in either topoisomerase I or the transcription termination factors Rho or NusG (Drolet *et al*., 1995; Masse and Drolet, 1999a,b; Hraiky *et al*., 2000; Harinarayanan and Gowrishankar, 2003; Broccoli *et al*., 2004). The results therefore suggest that one of the functions of the THO/TREX protein complex is to prevent R-loop formation during eukaryotic transcription.

Why are R-loops on the chromosome toxic?

Although the reasons for R-loop toxicity are not fully understood, the following possible explanations have been advanced. In both *E. coli* (Hraiky *et al*., 2000; Drolet *et al*., 2003) and yeast (Huertas and Aguilera, 2003), chromosomal R-loops are associated with an impairment of transcription elongation, and it has been suggested that they act as roadblocks to the succeeding molecules of RNA polymerase. Whether the frequency with which a gene is transcribed will influence the propensity for R-loops occurring in it needs to be determined. It has also been speculated that the stalled transcription elongation complexes may interfere with replication fork progression and so lead to fork breakage or collapse (Drolet *et al*., 1995; 2003; Huertas and Aguilera, 2003). Such a possibility may, by our model, explain why some *rho* mutations in *E. coli* exhibit synthetic lethality with mutations in the *ssb* or *rep* genes involved in DNA replication (Fassler *et al*., 1985). R-loops may also mediate the aberrant initiation of chromosomal DNA replication (constitutive stable DNA replication, cSDR) in bacteria (Kogoma, 1997).

Concluding remarks

Although much of the supporting evidence is indirect, the view that emerges is that, during transcription, 'the nascent RNA transcript generally has an inherent capacity to cause trouble' (Svejstrup, 2003). The 'trouble' is in the form of R-loops generated upstream of the transcription elongation complex, a situation that is true in both the prokaryotes and the eukaryotes. Several co-transcriptional activities involving RNA as the substrate serve to prevent the occurrence of R-loops, and the coupling of transcription to translation in the bacteria may be

seen as a special instance of such a co-transcriptional activity.

Acknowledgements

J.G. is Honorary faculty member of the Jawaharlal Nehru Centre for Advanced Scientific Research. R.H. was a CSIR Research Fellow. Work in the authors' laboratory has been supported by a grant-in-aid from the Department of Biotechnology (Government of India) project BT/PR2430.

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