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Transcription fidelity and its roles in the cell

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Accuracy of transcription is essential for productive gene expression, and the past decade has brought new understanding of the mechanisms ensuring transcription fidelity. The discovery of a new catalytic domain, the Trigger Loop, revealed that RNA polymerase can actively choose the correct substrates. Also, the intrinsic proofreading activity was found to proceed via a ribozyme-like mechanism, whereby the erroneous nucleoside triphosphate (NTP) helps its own excision. Factor-assisted proofreading was shown to proceed through an exchange of active centres, a unique phenomenon among proteinaceous enzymes. Furthermore, most recent *in vivo* studies have revised the roles of transcription accuracy and proofreading factors, as not only required for production of errorless RNAs, but also for prevention of frequent misincorporation-induced pausing that may cause conflicts with fellow RNA polymerases and the replication machinery.

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Introduction

Gene expression relies on the accurate copy of genetic information. The fidelity of RNA synthesis results from the accuracy of correct NTP selection (versus non-complementary NTPs and complementary 2′-deoxy NTPs), the proofreading of misincorporation events, and the efficiency of extension of the misincorporated nucleotide. In this review, we summarize the structural and biochemical determinants of transcription fidelity that have been uncovered in the last decade, and we describe very recent insights on the consequences that stalled misincorporated complexes may have on cellular functions and gene expression.

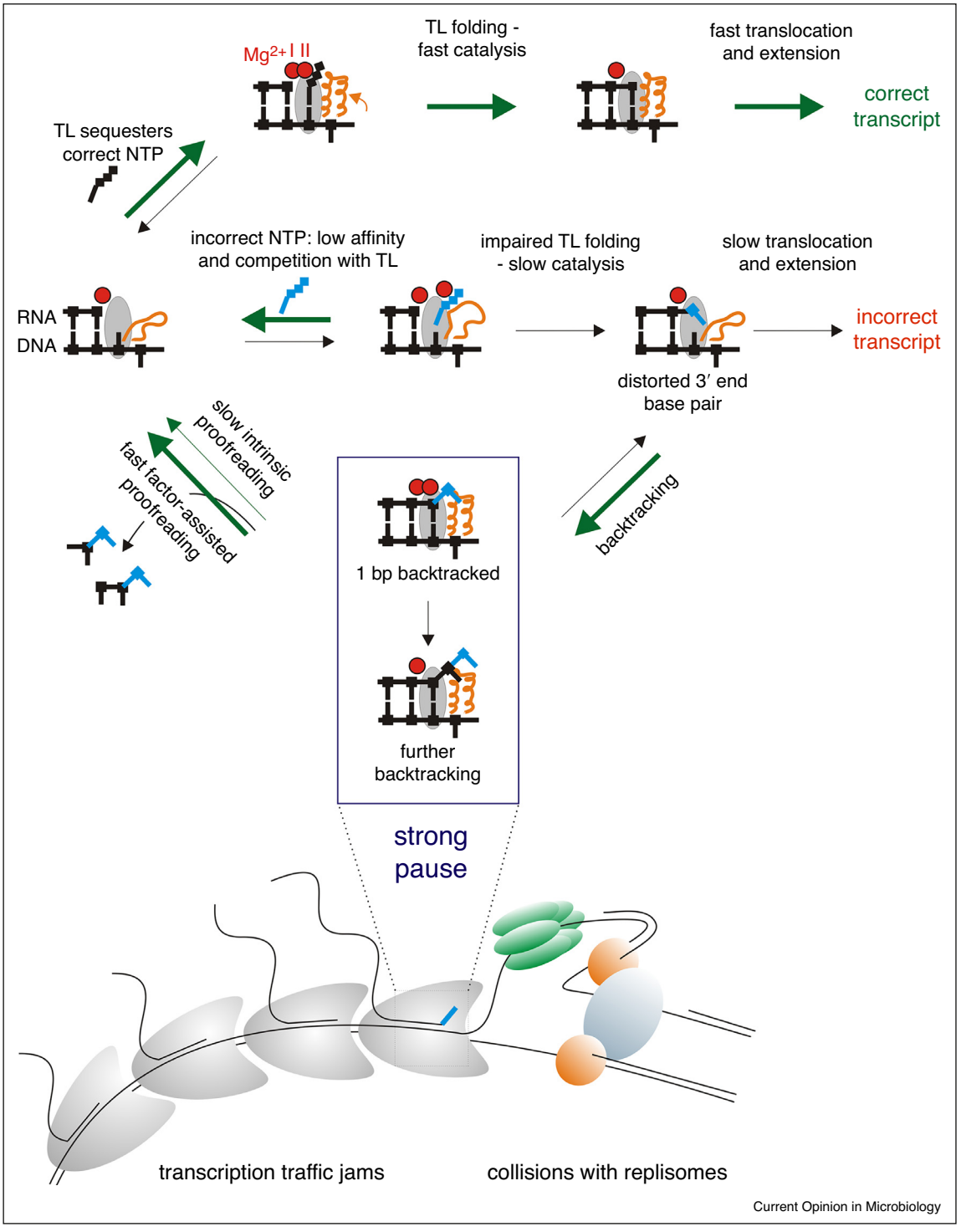
Determinants of the accuracy of NTP choice

For a long time, the catalysis of phosphodiester bond formation by RNA polymerase (RNAP) was thought to be performed solely via a two metal ion (Mg^{2+}) mechanism within a relatively rigid active centre. However, at saturating NTPs concentrations (close to cellular levels), such a 'motionless' active site would provide as low as ~10-fold kinetic discrimination against some non-complementary NTPs (though 10^3 for certain misincorporations), and would not discriminate at all against complementary 2′-deoxy NTPs [1••]. The discovery of a flexible domain of the active site, the Trigger Loop (TL) [2••], revealed that the active centre of RNAP actively participates in choosing NTPs via an induced fit mechanism [1••,3]. TL is essential for the catalysis of phosphodiester bond formation, and it acts by stabilising the transition state of the reaction [1••,4]. The key property of the TL for the accuracy of transcription is its ability to accommodate catalytically active (folded) and inactive (open) structural states. The correct NTP binding in the $i+1$ site (grey in Figure 1) induces folding of the TL (orange in Figure 1), which, in turn, participates in the catalysis of nucleoside monophosphate (NMP) incorporation into the transcript. Binding of a non-cognate NTP in the $i+1$ site cannot induce productive folding of the TL because of the wrong geometry of base pairing with the template (in case of non-complementary NTPs) or the lack of critical contacts of the NTP's sugar moiety with the TL (in case of complementary deoxy NTPs) [1••]. Such an induced fit mechanism of selection provides 1–3 extra orders of magnitude of kinetic discrimination against non-complementary NTPs, and 3 orders of magnitude against complementary dNTPs [1••].

The affinity discrimination against non-complementary NTPs takes place due to their weaker base pairing with the template, and may increase discrimination by more than an order of magnitude. Furthermore, the TL competes with non-cognate NTPs in the $i+1$ site [1••], while sequestering the correct NTPs bound there [5]. Such 'active' expulsion of only wrong substrates adds another order of magnitude to the discrimination against non-complementary NTPs. Notably, TL-mediated expulsion is the only 'affinity' component for discrimination against dNTPs because the affinity of their binding in the active site is the same as for ribonucleotides [1••].

It must be noted that, while the above-mentioned mechanisms are general and conserved, their efficiencies may vary greatly depending on the identity of incoming NTP,

Figure 1



Multistep processes ensuring transcription fidelity. A schematic representation of the active centre of RNAP is given for different transcription intermediates, and shows template DNA and RNA (black lines), metal ions (red circles), the $i + 1$ site (grey oval) and the Trigger Loop (orange ribbon). Correct and incorrect incoming NTPs are coloured in black and blue, respectively. Green arrows show the direction of reactions leading to a correct transcript. The different thickness of the arrows serves only as a qualitative indication of the rates of reactions or conformational changes. At the bottom of the figure, a cartoon depicts a stalled misincorporated elongation complex, which may potentially cause transcription traffic jams with trailing RNAPs (left), and conflicts with replication forks (right).