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Mini review

Tolerance of lesions in *E. coli*: Chronological competition between Translesion Synthesis and Damage Avoidance

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ABSTRACT

Lesion tolerance pathways allow cells to proceed with replication despite the presence of replication-blocking lesions in their genome. Following transient fork stalling, replication resumes downstream leaving daughter strand gaps opposite replication-blocking lesions. The existence and repair of these gaps have been know for decades and are commonly referred to as postreplicative repair [39,38] (Rupp, 2013; Rupp and Howard-Flanders, 1968). This paper analyzes the interaction of the pathways involved in the repair of these gaps. A key repair intermediated is formed when RecA protein binds to these gaps forming ssDNA.RecA filaments establishing the so-called SOS signal. The gaps are either "repaired" by Translesion Synthesis (TLS), a process that involves the transient recruitment of a specialized DNA polymerase that copies the lesion with an intrinsic risk of fixing a mutation opposite the lesion site, or by Damage Avoidance, an error-free pathway that involves homologous recombination with the sister chromatid (Homology Directed Gap Repair: HDGR). We have developed an assay that allows one to study the partition between TLS and HDGR in the context of a single replication-blocking lesion present in the *E. coli* chromosome. The level of expression of the TLS polymerases controls the extent of TLS. Our data show that TLS is implemented first with great parsimony, followed by abundant recombination-based tolerance events. Indeed, the substrate for TLS, i.e., the ssDNA.RecA filament, persists for *only* a limited amount of time before it engages in an early recombination intermediates (D-loop) with the sister chromatid. Time-based competition between TLS and HDGR is set by mere sequestration of the TLS substrates into early recombination intermediates. Most gaps are subsequently repaired by Homology Directed Gap Repair (HDGR), a pathway that involves RecA. Surprisingly, however, in the absence of RecA, some cells manage to divide and form colonies at the expense of losing the damage-containing chromatid.

Contents

Abbreviations: ssDNA.RecA, single-stranded DNA.RecA filament; TLS, translesion synthesis; DA, damage avoidance; HDGR, homology directed gap repair; DDT, DNA damage tolerance; PRR, post-replication repair; NER, nucleotide excision repair; MMR, mismatch repair; SSB, single strand binding protein; G-AF, *N*-(2′-deoxyguanosin-8-yl)-2-aminofluorene; G:AAF, *N*-(2′-deoxyguanosin-8-yl)-2-acetylaminofluorene; CPD, cyclobutane pyrimidine dimers; TT(6-4), thymine–thymine pyrimidine (6-4) pyrimidone.

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1. Introduction

This paper analyzes the fate of lesions that escape DNA repair and are thus present on the DNA template during replication. The mechanisms by which cells manage to replicate their genome despite the presence of non-coding lesions in the DNA are referred to as DNA damage tolerance (DDT) pathways (Fig. 1); DDT pathways are sometimes considered as repair mechanisms although they are not strictly speaking repair pathways since the lesion is not being removed during the process but merely tolerated. There are two distinct DDT strategies, Translesion Synthesis (TLS) and Damage Avoidance (DA). Failure to properly execute one of these lesion tolerance pathways can lead to replication fork collapse that in turn may result in genetic rearrangements or cell death.

Since the discovery of the specialized DNA polymerase family about 15 years ago [32], TLS pathways have received a huge interest namely in view of their prime role in generating point mutations. Point mutations can be beneficial for evolution but are also highly detrimental in somatic cells where they trigger cancer or in pathogens where they can elicit antibiotic resistance. As pointed out in this paper, TLS represents only a minor fraction of tolerance events since most lesions are tolerated by error-free homologous recombination-based pathways [9]. As DA pathways are "genetically silent" they are technically more challenging to study by lack of a phenotype compared to mutagenic TLS pathways. A major task for the future will be to unravel the cross-talk and genetic regulation of TLS and DA pathways. In principle, if it were possible to control the respective usage of TLS versus DA pathways, one could modulate the mutation frequency in a living cell. The present paper describes our recent efforts to address this challenge.

2. Structure and recovery of the damaged *E. coli* genome at early times following UV

Early work on replication of the UV damaged *E. coli* genome in a NER deficient (*uvrA*) strain has shown that following UV irradiation (at 1–2 J/m^2, leading to 50–100 lesions per genome), *E. coli* cells synthesize the same amount of DNA as un-irradiated control cells with a 15–20 min delay, the size of the nascent DNA fragments is short and corresponds approximately to inter-lesion distance [8,39]. The average delay inflicted on fork progression has thus been estimated

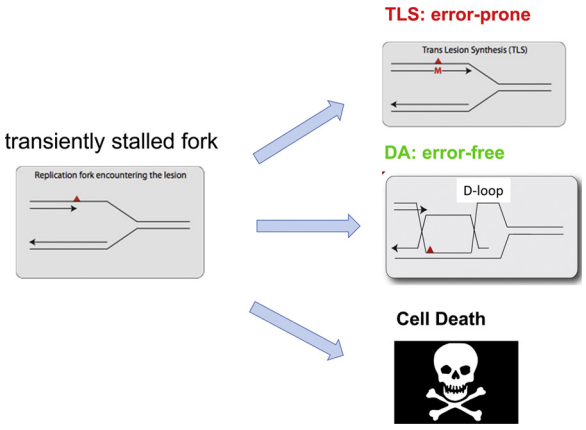

Fig. 1. DNA Damage Tolerance (DDT) strategies: a non-coding lesion leads to a transiently stalled replication fork. Cells implement different strategies to overcome the stall: 1. Translesion Synthesis (TLS) involves the recruitment of a specialized DNA polymerase; the process is error-prone as the TLS polymerase may introduce a mutation opposite the damaged base. 2. Damage Avoidance (DA) a strategy uses the information present in the sister chromatid; this process is related to homologous recombination and is error-free. 3. If TLS and DA fail, the fork my collapse and lead to cell death. TLS and DA are referred to as DNA Damage Tolerance strategies, they allow cells to cope with lesion in DNA during replication.

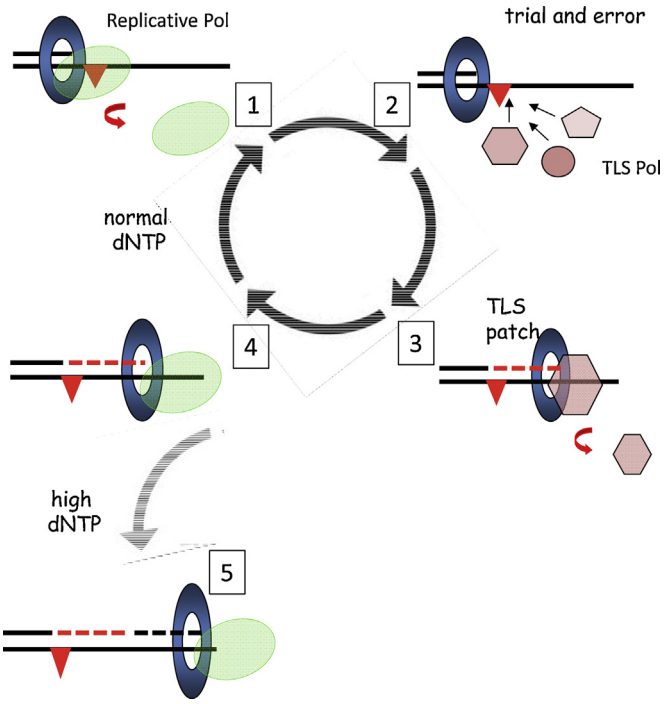

Fig. 2. An integrated view of TLS pathways: Step 1: the replicative DNA polymerase dissociates from the primer template upon encounter with a noncoding template base. Step 2: the vacant primer template becomes the substrate for binding by specialized DNA polymerases; to the best of our present knowledge, there is no active selection process for the binding of a specific polymerase; binding is stochastic and obeys classical mass-action law. Step 3: a successful specialized polymerase is one that is able to synthesize, in a single binding event, a patch long enough to resist proofreading (TLS patch in dotted red). The interaction of the TLS polymerase with the β-clamp left behind on the template upon dissociation of the replicative DNA polymerase is essential to confer limited processivity to the TLS polymerase that are otherwise highly distributive. For all three SOS polymerases (Pol II, Pol IV, and Pol V), mutations that inactivate the β-clamp binding motif abrogate their TLS activity *in vivo* [3]. Step 4: upon dissociation of the TLS polymerase, the "TLS patch" is extended upon reloading of the replicative polymerase leading to complete TLS (Step 5). If the TLS patch is too short, the proofreading activity of the replicative DNA polymerase may abort the TLS pathway back to step 1. We have recently shown that the balance between exonucleolytic degradation and polymerization by the replicative DNA polymerase is modulated by the dNTP pool size [16]. Increased dNTP pools that arise as a consequence of genotoxic stress favor elongation over proofreading.

to be in the range of 10–20 s/lesion in two independent studies [39,37]. Upon further incubation for 45′–60′, these short DNA fragments are converted into larger molecules of the size observed in the un-irradiated control [38,39]. These data are compatible with a model in which the replication fork initially skips over lesions via downstream re-priming leaving gaps opposite lesions. While re-priming is a natural property in lagging strand replication it was shown, *in vitro*, to be possible in the leading strand as well [44]. We suggest that these gaps are repaired *simultaneously*, rather than *sequentially* behind the advancing fork, either by TLS [33] or via recombination [40,25], during a process classically referred to as post-replication repair (PRR) [8]. In *S. cerevisiae*, it was also found that UV-irradiated cells uncouple leading and lagging strand replication. EM pictures revealed that small ssDNA gaps accumulate along replicated duplexes, likely resulting from re-priming events downstream of the lesions on both leading and lagging strands [26]. Translesion synthesis and homologous recombination counteract gap accumulation, without affecting fork progression. Using a genetic tool, it was confirmed that repair of UV-induced gaps is a process that is separable in time and space from genome replication [10]. Compared to the fork skipping reaction, repair of a single gap is a slow process estimated to take ≈ 30–40 min per lesion [33]. It is thus important that these gap-repair events can be dealt with