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Biological role of *Escherichia coli* translesion synthesis DNA polymerase IV

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Abstract

Damage tolerance is a measure of last resort to rescue cells from DNA damage, without which cells would become highly sensitive to killing by DNA-damaging agents. DNA lesion can be tolerated via *different pathways, of which two best studied are homologous recombination and replicative lesion bypass. Replicative lesion bypass requires specialized DNA polymerases, most of which belong to the Y-family of DNA polymerases. These enzymes exhibit high error rates and low processivity when copying normal DNA but are able to synthesize DNA opposite damaged templates hence allowing completion of genome replication in the presence of the replication-blocking DNA damage. The most ubiquitous branch of the Y-family of DNA polymerases, a DinB branch, is typified by* Escherichia coli *Pol IV. Such remarkable conservation throughout evolution strongly suggests that the Y-family DNA polymerases from the DinB branch are extremely important for cell survival and fitness. We found that* E. coli *Pol IV is capable to counteract cytotoxic effects of DNA alkylation in error-free fashion. This activity is of major biological relevance because alkylating agents are continuously produced endogenously in all living cells and are also present in the environment.*

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INTRODUCTION

Despite proficiency of DNA repair, some DNA lesions persist. At least three factors may contribute to the persistence of DNA damage, i.e., high levels of damage, poorly repaired lesions and lesions located in inefficiently repaired genomic regions. Because persistent lesions can block the replication apparatus, natural selection has favored the emergence of damage tolerance systems that allow complete replication in the presence of DNA damage. Damage tolerance is a measure of last resort to rescue cells from DNA damage, without which cells would become highly sensitive to killing by DNA-damaging agents. DNA lesion can be tolerated *via* different pathways, of which two best studied are homologous recombination and replicative lesion bypass. The process of replicative DNA lesion bypass can be divided into two steps: (i) nucleotide incorporation opposite the lesion i.e., translesion synthesis, followed by (ii) extension of DNA synthesis. After a short stretch of extension, normal DNA synthesis by the replication apparatus can then resume.

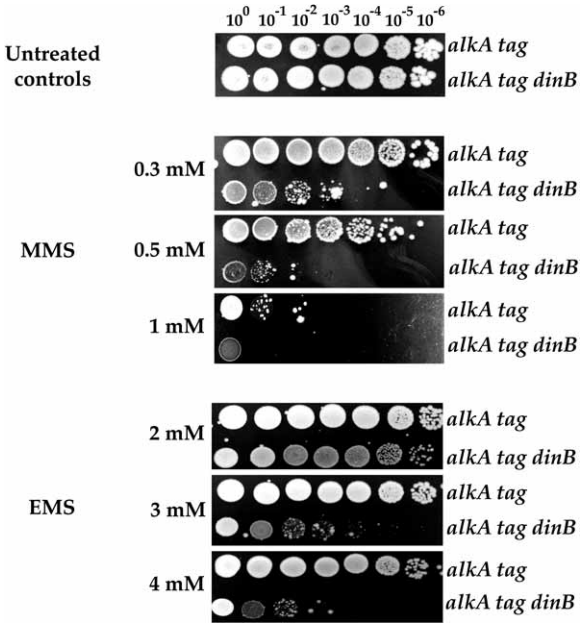

Figure 1.

Replicative lesion bypass requires specialized DNA polymerases *(1),* most of which belong to the Y-family of DNA polymerases that are found in prokaryotes, eukaryotes, and archaea *(2)*. The activity of these enzymes eluded researchers for long time, as they share no sequence homology with other known DNA polymerases. Their main characteristics are the lack of the 3'→5' exonuclease activity and a more open catalytic site compared to the replicative DNA polymerases *(3)*. These features enable the Y-family DNA polymerase to successfully bypass lesions, but also compromise the accuracy of replication of a nondamaged template. Lesion bypass can be either error-free or error-prone, when the correct or incorrect nucleotide is incorporated opposite the damage, respectively. Different translesion synthesis DNA polymerases have different substrate specificity. Therefore, bypass of a given lesion is expected to be error-free or error-prone depending on which DNA polymerase is involved, i.e., bypass of a cognate lesion is expected to be predominantly error-free and that of non-cognate lesion predominantly error-prone *(4)*. Conversely, for a given translesion synthesis DNA polymerase, nucleotide incorporation can be error-free or error-prone, depending on the specific lesion.

Because translesion synthesis DNA polymerases exhibit high error rates when copying normal DNA, and because their activity can be mutagenic or even toxic when they are acting on non-cognate lesions, the activity of translesion synthesis DNA polymerases must be subject to tight regulation. However, in spite of existence of such regulation (not yet fully understood), the translesion synthesis DNA polymerases are one of the major sources for the generation of spontaneous random mutations – this is clearly the case in bacteria and in yeast. Therefore, the comprehension of the regulation of activity and of the substrate specificity of different translesion synthesis DNA polymerases is essential for better understanding the control of mutation rates in prokaryotes and in eukaryotes.

Escherichia coli DNA polymerase IV

The most ubiquitous branch of the Y-family of DNA polymerases, a DinB branch, is typified by *Escherichia coli* Pol IV, human Pol k, and the archaeal Dbh/Dpo4 enzymes *(2)*. Such remarkable conservation throughout evolution strongly suggests that the Y-family DNA polymerases from the DinB branch are extremely important for cell survival and fitness. Besides Pol IV, encoded by the *dinB* gene, *E. coli* possesses two more DNA polymerases capable of bypassing lesions: Pol V, encoded by the *umuDC* genes, belonging to the Y-family and Pol II, encoded by the *polB* gene, belonging to the B-family of DNA polymerases *(5)*. Genes coding for these three DNA polymerases are regulated by the SOS system *(6, 7)*. *E. coli* SOS system is composed of, at least, 40 genes of which many code for DNA repair functions, e.g., nucleotide excision repair and homologous recombination. SOS is induced by a wide variety of genotoxic stresses that all have one common characteristic; they increase intracellular concentration of single-strand DNA. The persistent contact with single-strand DNA activates co-protease activity of the RecA protein, which promotes the self-cleavage of the LexA protein, the SOS repressor, thus inducing the SOS response. When DNA lesions are repaired and replication restored, SOS functions are again repressed.

In the unstressed, growing cell, there are 30-50 molecules of the DNA polymerase Pol II and 250 of Pol IV, whereas Pol V is undetectable. For comparison, under such conditions there are approximately 30 molecules per cell of replicative DNA polymerase Pol III. Such high spontaneous expression level of *dinB* gene indicates that Pol IV could play an important metabolic function. Therefore, it is intriguing that inactivation of the *dinB* gene has no strong phenotype in unstressed cells *(8-10)*. Upon SOS induction, the number of Pol II and Pol IV rapidly increases to 250 and 2500 molecules per cell, respectively, while Pol V reaches about 60 molecules per cell one hour after SOS induction *(5)*. In addition to the SOS system, the transcription of the *dinB* gene is controlled by RpoS, a sigma subunit of RNA polymerase, which regulates a general stress response *(11)*. Pol IV is also regulated by the heat shock chaperone GroE *(12)*. Therefore, Pol IV is a component of several important cellular stress responses.

The overexpression of the *dinB* gene substantially increases spontaneous mutagenesis in the unstressed, growing cells *(13),* probably by competing with Pol III for binding to the β-clamp *(14)*. In stressed cells, Pol IV was also shown to considerably contribute to mutagenesis. For example, Pol IV is responsible for the untargeted mutagenesis of non-irradiated λ phage in UV irradiated cells *(15),* and for the increased generation of mutations under carbon source starvation and stationary phase *(9, 16, 17)*. Pol IV was also shown to be required for long-term survival in stationary phase *(18)*.

Many studies have been performed in order to determine what is cognate lesion(s) for *E. coli* Pol IV. *In vitro,* Pol IV can perform DNA synthesis across a variety of base modifications, but *in vivo* it is involved in the bypass of only a subset of these base modifications, i.e., those induced by benzo[*a*]pyrene, 4-nitroquinolone *N*-oxyde, nitrofurazone and reactive oxygen species *(19, 20)*. For example, Pol IV bypasses abasic sites *in vitro* but not *in vivo (21)*. Such discrepancies indicate that the access to the DNA damage, and the activity of Pol IV and other bypass DNA polymerases is regulated *in vivo*. Most of these studies were performed using man-made chemical DNA damaging agents. Consequently, it is not clear what is what is natural cognate lesion for *E. coli* Pol IV DNA polymerase.

We tried to identify cognate lesion for Pol IV DNA polymerase by investigating the consequence of Pol IV-mediated bypass of different types of spontaneous DNA damage *in vivo (22)*. In order to increase the amount of one specific lesion in the genome, and to prevent other DNA repair systems to remove the lesion before Pol IV has an opportunity to perform the bypass, an exhaustive set of mutants affected in their DNA repair ability was constructed. DNA repair function involved in the repair of alkylation and oxidation damage, as well as those involved in the repair of abasic sites and in the nucleotide excision repair have been inactivated. Because several DNA repair enzymes can act on the same lesions, in some cases we inactivated two enzymes that exhibit overlapping functions. The advantage of this approach is that it increases only DNA damage and not the damage of other cell constituents, as is frequently the case when cells are treated with chemical agents. The DNA lesions present at high concentration in these genetic backgrounds are presumably the most relevant for the evolutionary conservation of DNA repair proteins. We were looking for a genetic background in which inactivation of *dinB* will significantly increase mutation frequency. All mutants used were also mismatch-repair deficient (*mutS* mutants) because mismatch-repair has been shown to correct Pol IV generated errors *(23)*. Finally, many DNA lesions that can be bypassed by Pol IV can also be bypassed by two other translesion synthesis DNA polymerases: Pol II and Pol V *(19)*. Therefore, we also inactivated genes coding for tehse two DNA polymerase. The goal was to identify DNA damage that is bypassed specifically and accurately by Pol IV, which, by doing this, prevents other DNA polymerases from performing (error-prone) lesion bypass.

Bypass of the cytotoxic alkylation DNA lesions

We found that *E. coli* Pol IV DNA polymerase participates in the error-free processing of DNA damage that accumulate in the genome of the *alkA tag* double mutant strain (22). *tag* is a constitutively expressed gene, while the expression of the gene coding for AlkA is controlled by an adaptive response, an inducible alkylation-specific DNA repair response *(24, 25)*. The adaptive response is

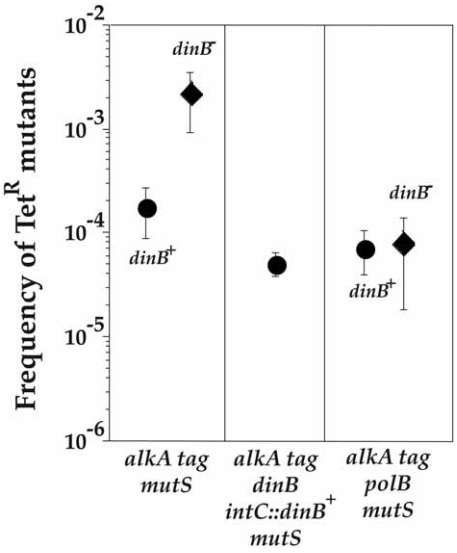

Figure 2.

under the positive control of the Ada protein, which removes alkyl groups from DNA and stimulates the expression of the *ada, alkB*, *alkA* and *aidB* genes. Tag glycolysase excises for 3-methyladenine (3-meA), and to a much lesser extent, 3-methylguanine (3-meG) from DNA. AlkA has a much broader range of substrates, but it also excises 3-metA and 3-meG from DNA *(26, 27)*. 3-meA and 3-meG are cytotoxic lesions that block both replication and transcription due to the aberrant alkyl group protruding into the minor groove of DNA. Therefore, these two DNA lesions are the most likely candidates to be substrate for Pol IV. We also showed that Pol IV also contributes to the tolerance of cytotoxic alkylating DNA lesions induced by methylating and ethylating agents, which indicates that its activity is not limited to methyl adducts *(22)*.

3-meG and 3-meA are mutagenic probably because they block DNA replication, induce the SOS response and consequently induce the expression of genes coding for SOS DNA polymerases *(28)*. In addition, SOS induction is enhanced in bacteria deficient for the repair of alkylation cytotoxic lesions. We showed that in the absence of Pol IV, mutations are generated by the activity of Pol II and Pol V *(22)*. It was previously known that mutations induced by alkylating agents depend on Pol V activity *(29),* but this is the first report concerning the involvement of Pol IV in the error-free processing of 3-meA and 3-meG. By doing this, Pol IV prevents access of Pol II and Pol V to these lesions.

Pol IV is also contributing resistance to the killing effect of high doses of the methylating agent methylmethane-sulfonate (MMS), in the *alkA tag* proficient background *(22)*. This may be one of the reasons why the *dinB* gene is expressed at high level in unstressed cells, i.e., when cells are suddenly exposed to high doses of alkylating agents, constitutive level of 3-methyladenine DNA glycosylases is not sufficient to ensure survival. In

order to resist high doses of alkylating agents, bacteria must induce adaptive response *(24)*. This response best protects cells when they are first exposed to low doses of alkylating agents, which, by inducing an adaptive response allow cells to become resistant to the lethal and mutagenic effects of subsequent high-level challenge from alkylating agents. So, Pol IV may be important for survival of cells exposed to high doses of alkylating agents prior to induction of an adaptive response.

Biological role of *Escherichia coli* DNA polymerase IV

In the light of our results, it is interesting that the expression of the *dinB* gene is elevated under carbon source starvation and stationary phase *(11)*. The induction of *dinB* gene transcription during stationary phase is controlled by RpoS. RpoS also upregulates the expression of *ada* and downregulates the expression *alkA* in stationary phase *(30, 31)*. Importantly, treatment with MMS does not induce expression of *alkA* in stationary phase cells, while in *rpoS* mutant cells *alkA* expression is significantly increased *(30)*. Such dual regulation of *alkA* gene expression, by RpoS and Ada, may result from the fact that the activity of AlkA may be deleterious in stationary phase. The overproduction of AlkA, unlike overproduction of Tag, was shown to sensitize growing *E. coli* cells to alkylating agents *(32)* probably because AlkA generates more abasic sites and strand breaks as base-excision repair intermediates than can be efficiently repaired. Because the repair of abasic sites may be difficult in starving stationary phase cells, RpoS represses the *alkA* gene (AlkA produces abasic sites) but induces expression of the *dinB* gene. Intriguingly, Pol IV cannot bypass abasic sites *in vivo (21)*, but it can bypass 3-meA and 3-meG *(22)*. Furthermore, unlike replicative DNA polymerase Pol III, Pol IV and Pol V have the potential to operate efficiently at low dNTP concentrations *(33),* a condition encountered during stationary phase *(34)*. Interestingly, it was recently proposed, based on *in vitro* data, that the Pol IV human homologue, Pol k,? might also be utilized in repair replication under conditions of low nucleotide concentrations, for example in non-dividing cells *(35)*.

In stationary phase *E. coli* cells, spontaneous generation of an endogenous DNA alkylating agent increases considerably, as suggested by the enhanced generation of mutations in stationary phase *E. coli ada ogt* (these two genes code for O^6-methylguanine-DNA methyltransferases) mutant cells *(31, 36, 37)*. This may be true also for eukaryotes, because transcriptional profiles of *Saccharomyces cerevisiae* show that a large number of genes that were regulated in response to MMS are also regulated in response to being held at stationary phase *(38)*. Consequently, a high amount of Pol IV might help cells to survive cytotoxic alkylation DNA damage during stationary phase. This is particularly important in stationary phase when the synthesis of translation apparatus is inhibited, and the number of ribosomes and rRNA gene expression decreases resulting in a reduction in the rate of global protein synthesis *(39, 40)*. If *dinB* were only under regulation of the SOS system, the induction of which requires new protein synthesis, it would be difficult to synthesize enough Pol IV to survive exposure to alkylating agents during stationary phase. This may explain why Pol IV is required for long-term survival in stationary phase.

Concluding remarks

What would the biological relevance of our observation be? All examined organisms posses DNA repair mechanisms that can specifically counteract the deleterious effects of DNA alkylation, which indicates that they are continuously exposed to alkylating agents and that this was also the case during their evolution. Alkylating agents are produced endogenously in cells and present in the environment. For *E. coli*, there are many possible sources of endogenous alkylating agents. *S*-adenosylmethionine, a methyl donor in many biochemical reactions, is a weak methylating agent *(26, 41)*. Endogeneous nitrosation of amides, amines, amino acids and related compounds can also generate alkylating agents, particularly during stationary phase *(41)*. *E. coli* is exposed to exogenous alkylating agents in its primary habitat: the gastrointestinal tract of warm-blooded animals. Nitrosation of bile acids and food compounds that generate alkylating agents is mediated by bacterial flora, but also by a spontaneous chemical reaction in stomach, where low pH facilitates this process *(42-44)*. It is therefore intriguing that mouse Pol IV homologue Pol k, similarly to its *E. coli* homologue *(22),* is involved in translesion DNA synthesis across cytotoxic alkylation DNA damage *(45, 46)* is present in epithelial cells lining the stomach *(47)*. For humans, the involvement of Pol k in tolerance of alkylating DNA damage is, in addition to the above-mentioned examples, also relevant for cancer therapy because alkylating agents are used as cytostatic drugs. It can therefore be proposed that the capacity of the Y-family DNA polymerases from the DinB branch to bypass cytotoxic alkylating lesions in an error-free fashion is of major biological relevance.

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