

Dna tranlession polymerase in prokaryotic cells



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DNA Tranlesion Polymerase in prokaryotic cells: History, structures and function

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DNA is one of the most important part of the cell that gives cell integrity and character. This part of the cell can be exposed to different kinds of damages that may put the cell's integrity in jeopardy. The only part of the cell that has this ability to be repaired is DNA. Basically repairing should be done due to a reasonable reason. Repairing the other macromolecules are not profitable. For example, if a defective protein forms, the protein can be simply be replaced by another one. But defects in DNA can cause problem in the whole cell organisms and the character of cell [1]. Usually the whole repairing process is happening fast, although there are defects that persist against this process. The repairing process is done by special polymerases and the whole process of DNA repair is called "translesion DNA synthesis" (TLS) [2].

DNA can be damaged due to different reasons, such as base modification, elimination or addition of nucleotides, crosslinking of DNA strands and breakage of phosphodiester backbone [1]. These reasons can be due to some environmental conditions such as radiation or insertion of certain chemicals in to the body or due to malfunction of polymerases and enzymes in cellular process, such as putting wrong nucleotide in the DNA strand chain [1]. Up to now, it is known that there are three translesion DNA polymerases (TLS polymerases) in E. coli and about fifteen polymerases in eukaryotes that can run this process [2].

History

For the first time it was in the early 1940s, that it was found agents causing mutational changes such as ionizing and radiation of UV, interact with cells and can damage their genome [3]. Also it was found that these cells can survive and recover from theses damages [4] and the term DNA repair was found. “ DNA repair is a biochemical term that defines biological processes during which alterations in the chemistry of DNA (DNA damage) are removed and the integrity of the genome is restored” [3].

The first DNA repair mechanism to be discovered was enzymatic photo reactivation (EPR) [3]. This process is referred to the elimination of cyclobutane pyrimidine, which are generated by UV radiation and can block both DNA replication and transcription, from the genome [4]. This reaction can be catalyzed by photoreactivating enzyme in a reaction that needs a visible range light. The second mechanism found was excision repair [3]. This mechanism is referred to DNA damages cut out from genome that leaves some gaps in DNA duplex. These gaps are repaired by a non-semiconservative mode of DNA synthesis called repair synthesis [5].

By the end of the 1970s, it was known that cells are using various mechanisms for DNA repair process that focus around two basic principles: the excision of base damage or its direct reversal such as EPR [3]. In the mid1970s Miroslav Radman proposed a new hypothesis called SOS hypothesis [5, 6]. The SOS hypothesis proposes an overall response to DNA damage in which the cell cycle is stopped and DNA repair is induced. Genetics experiments demonstrated that main players involved in damage-induced mutagenesis are *lexA*, *recA* along with *umuD* and *umuC* [2]. *LexA* cleavage from *recA** and also *umuD* cleavage that form *umuD'* use the same

mechanism and is an absolute requirement for SOS mutagenesis. For showing that, *E. coli* because of its simple structure was used as a model for translesion DNA synthesis and mutagenesis.

Later Harrison Echols proposed another model and suggested that in order to help the replication process against the lesions it is possible to reduce the fidelity of proteins so when DNA replication process is stopped at a location of unrepaired DNA damage, certain SOS-regulated genes can encode proteins that interact with the hindered replication process in a manner that reduces their fidelity [3]. In the late 1980s and early 1990s, it was demonstrated that Echols genes are in fact specialized low-fidelity DNA polymerases that enhance low-fidelity replication across the lesion, the so-called translesion DNA synthesis (TLS) [3]. Their highly reduced fidelity allows the replicative bypass of sites of DNA damage, but with a high chance of combining incorrect nucleotides [5].

Early TLS models and PolV

Bridges and Woodgate were the first ones who defined the function of Umu proteins during UV-induced TLS in 1985 [7]. According to them, TLS happened in two steps. In the first step Pol III add a nucleotide opposite the first (3') T of a T-T cytidine diphosphate diacylglycerol (CPD). Bounding a RecA protein to the template proximal to the lesion is a requirement for this step. In the second step, Pol III interacts with UmuDC proteins to incorporate another nucleotide at the second (5') T of the cytidine diphosphate diacylglycerol (CPD). At least one of these two steps are non-WC, causing a

mutation targeted at the site of the CPD [2]. Figure 1 shows the process schematically.

Another model was proposed by Echols and Goodman in 1990 [3]. In this model they proposed that when Pol III encounters a template lesion, its holoenzymes (Pol III core, beta sliding clamp, gamma-clamp-loading complex) are completely blocked. This process follows by the assembly of a damage localized nucleoprotein complex involving RecA, UmuC, UmuD', SSB, and Pol III holoenzyme, a mutasome, to copy past a template lesion [2]. The fact that RecA* simplifies the cleavage of UmuD to UmuD' was used in this model [8]. Later, it was demonstrated that it was actually a dimeric UmuD₂ that is cleaved to UmuD'₂ and that next interacts with UmuC to form a stable complex of UmuD'₂C [9]. This complex was named as Pol V in 1999 by Tang et al. [10]. It can be said that genome replication done on undamaged DNA by Pol III is rapid and error-free [11], the TLS process carried out by mutasome is slow and error-prone [2].

“ A key feature of the mutasome model is the assembly of RecA* on ssDNA proximal to the lesion (Fig. 1). When a replication fork encounters a lesion, an uncoupling of leading-and lagging-strand synthesis may ensue. Then, one of the TLS Pols can replace Pol III on the β - clamp and copy the damaged DNA” [2]. For both leading and lagging strands it be easily seen that RecA* can be assembled on the form of template strand, proximal to lesion. If the lesion occurred in the leading strand, RecA filaments can be formed on a region of ssDNA that is created by DNA unwinding by DnaB helicase downstream from the lesion but if lesions exist in lagging strand ssDNA is present as a result of Okazaki fragment synthesis [2].

Schlacher and Goodman [12] showed RecA* act in trans form on a non-template ssDNA strand and this transactivation of PolV by RecA* to perform TLS happens in-vitro. And this lead to the PolV mutasome model of TLS (Fig. 1). Jiang et al. [13] demonstrated this new PolV form as PolV Mut = UmuD2'C-RecA-ATP. PolV Mut has this ability to copy both damaged and undamaged DNA (e. g. performs TLS) when RecA* is not present [2]. So, the straight role of RecA* in SOS-mediated TLS is to transfer a RecA molecule from the 3'-filament tip with a molecule of ATP to convert into Pol V Mut, that can cross a different number of DNA lesions on its own. (Fig. 1) [2, 14]. PolV Mut can have two conformations. One is activated form that can copy DNA, the other one is deactivated form that is unable to copy the DNA. The activation of PolV Mut is depended on the location of RecA-ATP bond to the polymerase subunits UmuD2' and UmuC [13]. By representing the RecA* again, the deactivated form of PolV Mut can be activated. In this case, the old RecA-ATP is substituted by a new RecA-ATP from the 3'-filament tip [13]. This type of switching on and off is unique to this kind of polymerase and has not been seen in other types of polymerases. This method can be useful specially preventing the undamaged DNA to go under mutation in E. Coli, and give the cell this power to activate Pol V Mut whenever replication fork have stuck at DNA template damage site [2].

PolIII and PolIV in E. Coli TLS

PolIII discovered in 1970[15]. At first, it was thought that mutation is non-informative in PolIII [16]. Pol II has an activity isolated from UV-irradiated cells that has this ability to replicate past abasic template lesions [2, 17]. This polymerase has some responses to UV radiation and this activity derives <https://assignbuster.com/dna-tranlesion-polymerase-in-prokaryotic-cells/>

from that [2]. By purification it was proved that the induced lesion-copying protein was Pol II [17]. In 1980, Kenyon and Walker [18] discovered a DNA damage-inducible gene called *dinA* that can encode PolIII. Also, one of the features of PolIII is bypassing N2-deoxyguanosine-acetyl aminofluorene (AAF) adducts, this behavior can be error-free and produces 2-frame shift mutations [19]. "AAF adducts are of family of aromatic amides that induce frame shift mutations within GpC sequences, such as the NarI sequences" [19]. These adducts are able to increase the GC dinucleotide loss in NarI sequence (CGC GCC) by 107 times when they are bound to the G in middle of sequence [20]. PolIII and PolIV can complement each other, but it does not mean that their activities are functionally unneeded [21]. As Pham et al. [21] mentioned PolIV job is to copy UV-damaged DNA in an error-prone manner in TLS. But Pol II is able to copy chromosomal DNA in an error-free replication process.

Kenyon and walker also introduced another gene called *dinB* gene that can be induced by cellular SOS response to DNA damage [18]. For many years, the function of this gene was unknown. After some year Ohmori et al. [22] found other gene, *dinP* gene, in the same section that *dinB* gene was found and Wagner et al. showed that they are able to encode Y-family DNA PolIV [23]. This kind of polymerases like other polymerases used in TLS are not crucial for life. Their role is to bypass certain N2-dG adducts (such as N2-furfuryl-dG) in an error-free manner [2]. Kumari et al. demonstrated they can copy past N2- N2-guanine interstrand cross-links in a high fidelity manner [24].

Regulation of TLS polymerases

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Different polymerases have this ability to traverse an extensive range of DNA lesions but this ability may cause in reducing the fidelity during replicating the undamaged DNA. Usually cells have several mechanisms to check and control the TLS polymerases because except PolIII, all of them has this potential to delete errors made when duplicating an undamaged DNA [2].

Usually no regulation is needed for PolIII. Because it has high fidelity derived by high intrinsic 3'-5' exonucleolytic proofreading. On the other hand, the Y-family polymerases such as PolIV and PoIV are exo-nuclease deficient and needed to be controlled [2].

PoIV activity can be regulated by many proteins and many ways. First as said before the UmuD' should be activated by UmuD. All UmuD, UmuC and UmuD' proteins are all exposed to degradation by Lon and ClpXP protease. RecA* that forms PoIV Mut can interact with UmuD2'C complexes and active them. The PoIV Mut itself activity can be enhanced by binding to the β -clamp [2].

As Wagner et al. showed the PolIV activity can be stimulated by protein interaction with RecA, UmuD and β -clamp [25].

Although the main mechanisms of DNA repair by various polymerases are known now, more studies can be conducted on E. Coli cells to find more details about the regulation and side reactions happening in this process. E. Coli cells as simple cells are an appropriate model to analyze these functions. Jarosz et al. proposed well questions about the future studies on Y-family DNA polymerases [26]:

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“(1)How do Y-family polymerases gain access to an appropriate primer terminus and how is their action coordinated with that of replicative polymerases?

(2)How do protein–protein interactions regulate the activity of Y-family polymerases?

(3)Are there families of cognate lesions for each different Y-family polymerase?

(4) Can mutations introduced by Y-family polymerases be corrected by exonucleolytic proofreading in trans?”

Different polymerases act in different paces after the damaged. For example PolII is induced immediately after DNA damage but PolIV is induced about 50 min after the damage [21]. An area of interest could be study on how they can be regulated to be induced in shorter time.

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