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Translesion synthesis in mammalian cells

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Translesion synthesis in mammalian cells

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Abstract

DNA damage blocks the progression of the replication fork. In order to circumvent the damaged bases, cells employ specialised low stringency DNA polymerases, which are able to carry out translesion synthesis (TLS) past different types of damage. The five polymerases used in TLS in human cells have different substrate specificities, enabling them to deal with many different types of damaged bases. PCNA plays a central role in recruiting the TLS polymerases and effecting the polymerase switch from replicative to TLS polymerase. When the fork is blocked PCNA gets ubiquitinated. This increases its affinity for the TLS polymerases, which all have novel ubiquitin-binding motifs, thereby facilitating their engagement at the stalled fork to effect TLS.

Introduction

The ability of all organisms to replicate their genomes is a pre-requisite for life. In order to accomplish this with maximum efficiency and fidelity, organisms have evolved superbly tailored replication machines. Central to these machines are replicative DNA polymerases, which are able to replicate DNA at high speed, with high processivity and with a very low error-rate. High fidelity is achieved by the active sites of these polymerases having stringent requirements, matching the incoming nucleotide to the template base by the appropriate Watson-Crick base-pairing. In addition the 3'-5' exonucleases associated with replicative polymerases remove any base that might, on rare occasions, be mis-inserted. DNA is however subject to continual damage from both endogenous and exogenous sources, and although most types of damage are removed by the cellular repair machinery, these processes are often slow and incomplete. Damage often remains in the DNA during replication and the price paid for the efficiency and accuracy of replicative polymerases is that many types of damage block their progress. An important mechanism for overcoming these blocks, particularly in mammalian cells, entails the use of specialised DNA polymerases to carry out translesion synthesis (TLS) past the damaged sites. Most of these polymerases belong to the Y-family [1], and in contrast to the replicative polymerases, they operate at low speed, low processivity and with low fidelity. However because their active sites adopt a much more open structure than replicative polymerases, they are less stringent and can accommodate altered bases in their active sites (eg see [2]). There are two Y-family polymerases in *Escherichia coli* (Polymerases IV and V), two in *Saccharomyces cerevisiae* (Pol η and Rev1) and four in mammalian cells (Pols η , ι , κ and Rev1). In addition, the B-family DNA polymerase ζ also plays an important role in TLS in eukaryotes. The conserved

active site structure of the Y-family polymerases is usually located in the N-terminal two-thirds of the protein. The C-terminal third is not conserved between the different Y-family polymerases and is involved with localisation, recruitment and protein-protein interactions (see below).

TLS polymerases

Pol η was discovered in mammalian cells as the protein deficient in the variant form of the skin cancer-prone genetic disorder xeroderma pigmentosum (XP) [3, 4]. Most XP patients are deficient in the ability to remove UV photoproducts from their DNA by nucleotide excision repair (NER), but about 20 % are normal in this respect and have problems in replicating their DNA after UV-irradiation [5]. The gene defective in these XP variants encodes pol η . In vitro pol η is able to replicate past a cyclobutane dimer (CPD), the major UV photoproduct, as efficiently as past undamaged bases, and in the majority of cases the “correct” bases are inserted [6, 7]. Because of its poor processivity, pol η is likely to dissociate relatively soon after it has bypassed the damage [7]– an important requirement because of its low fidelity on undamaged DNA. XP variant cells have an elevated UV-induced mutation frequency [8], indicating that in normal cells pol η plays an important role in maintaining mutations at a low level on exposure to UV light. In its absence, it is likely that TLS is carried out by one of the other TLS polymerases, or more than one acting in combination. They are less effective than pol η in carrying out this task, resulting in an elevated mutation frequency in pol η -defective XP variants. The nature of this back-up process has been the subject of speculation, based on the in vitro properties of the polymerases (eg pol ι and pol ζ acting in concert), but convincing evidence is lacking at present. Pol η is likely to have evolved to carry out TLS past CPD photoproducts

generated by exposure to sunlight. It can also carry out TLS past some other lesions in vitro (eg see [6]) with reduced efficiency, but whether it also does so in vivo is uncertain (eg see [9])

The other major UV lesion, the pyrimidine (6-4) pyrimidone photoproduct, generates a much greater distortion in DNA than the CPD and cannot be bypassed by pol η .

Studies in yeast and human cells have indicated that pol ζ and Rev1 are required for TLS past this lesion [10, 11], but the mechanism is not yet understood.

Our understanding of the roles of the other TLS polymerases in vivo is much less advanced. Many in vitro studies have been carried out using different damaged DNA substrates, and it has been concluded that some of the polymerases are more effective at inserting nucleotides across from damaged bases but are unable to extend from the inserted nucleotide (eg pol ι), whereas others are less efficient at this insertion step but can extend from a nucleotide inserted by another polymerase opposite a damaged base. Both in vivo and in vitro studies have shown that pol κ can carry out TLS past DNA containing benzo[a]pyrene –guanine adducts [12, 13].

Pol ζ is a heterodimer containing the Rev3 catalytic subunit and the Rev7 regulatory subunit [14]. Rev1, Rev3 and Rev7 were originally identified in *S. cerevisiae* as being required for mutations induced by most DNA damaging agents [15]. A similar requirement has also been found in human cells [16, 17]. This implies that they are involved in TLS, often inserting the “wrong” bases.

Rev1 is an enigmatic protein. It is not a polymerase, but a dCMP transferase, inserting a dCMP residue in a template-directed manner [18]. In the crystal structure, the incoming dCTP pairs with an arginine in the active site [19]. However there is convincing evidence that this catalytic activity is not required for UV mutagenesis [20], although it is required for bypass past other lesions. The properties of *rev1*, *rev3* and *rev7* mutants are in most cases identical, suggesting that pol ζ and Rev1 act in concert.

Localisation

All the Y-family polymerases are localised in the nucleus, and during S phase, pol η , ι and Rev1 relocate to replication factories, visible as bright fluorescent foci if the polymerases are tagged with green fluorescent protein (eg [21]). Here they colocalise with the polymerase sliding clamp PCNA, and other proteins involved in or associated with DNA replication. Thus during replication, they are present in the environment where replication is taking place, presumably “on stand-by” in case they are required. It is often suggested that this poses a danger to the cell, which might recruit one of these low fidelity polymerases to replicate the DNA. However, under normal circumstances, because of its high processivity it is unlikely that the replicative polymerase will be displaced by one of the other polymerases [22].

Recruitment to the replication fork

We can then pose the opposite question, namely how are the TLS polymerases recruited to the replication fork when the replication machinery is blocked? This replacement of replicative with TLS polymerase is designated the “polymerase switch”. A seminal paper by Jentsch and co-workers identified the central role of

PCNA in the polymerase switch [23]. They showed that in *S. cerevisiae*, when the replication fork was blocked, in this case by damage inflicted by methyl methanesulfonate, PCNA became modified by ubiquitination on lysine-164. This ubiquitination was effected by the products of genes which had long been known to be involved in replication of damaged DNA, but whose role had up till then not been understood. The mono-ubiquitination of PCNA was carried out by the E2 ubiquitin-conjugating enzyme Rad6 and the E3 ubiquitin ligase Rad18. Further ubiquitin molecules were added in a lysine-63 linkage by the E2 heterodimer Mms2-Ubc13 and the E3 Rad5. It was proposed that mono-ubiquitination channelled the damage through an error-prone TLS pathway, whereas poly-ubiquitination channelled into an error-free pathway of damage avoidance [23, 24]. This latter pathway has been postulated to involve a copy choice type of recombination involving template switching, but it is poorly understood and will not be discussed further.

How might ubiquitination of PCNA channel events into a TLS pathway? In mammalian cells mono-ubiquitination of PCNA is easily detected after a variety of treatments which block the progression of the replication fork, and this is dependent on the orthologs of Rad6 and Rad18 [25, 26]. Poly-ubiquitination of PCNA has been very hard to detect. A further interesting feature of the regulation of PCNA ubiquitination following exposure to DNA damaging treatment is its association with the degradation of the de-ubiquitinating enzyme (DUB) USP1 [27], which is able to remove mono-ubiquitin from PCNA. Thus DNA damaging treatments result in both the activation of proteins that ubiquitinate PCNA (Rad6 and Rad18) and the degradation of the protein that de-ubiquitinates it (USP1).

Polymerases η , ι and κ all have classical PCNA-binding “PIP” motifs, and have been shown to bind PCNA in vitro (eg see [28, 29]), but not in vivo. This suggests that the interactions are weak. Mono-ubiquitination of PCNA increased its affinity for pol η , so that their interaction could be detected in cell extracts [25, 26]. It was shown subsequently that not only pol η , but also pol ι , Rev1 and pol κ have novel ubiquitin-binding domains (UBDs), and that at least in the case of pol η and pol ι (and likely for pol κ and Rev1 also), the polymerases were able to bind to ubiquitin [30]. Thus, the combination of binding to ubiquitinated PCNA via both the PIP motif and the UBDs strengthens the interactions between the polymerases and PCNA, facilitating their recruitment to the stalled fork and facilitating the polymerase switch.

In vitro replication assays have shown that ubiquitination of PCNA did not alter its properties as a processivity factor for the replicative pol δ or pol ϵ , or for pol η on an undamaged template. However when the template contained an abasic site, ubiquitination of PCNA substantially increased the efficiency of TLS by pol η and Rev1 [31].

Weak interactions have also been identified between the polymerases themselves.

Pols η and ι interact directly with each other, and this interaction facilitates the localisation of pol ι into replication factories [21]. Rev1 interacts with pol η , ι , κ and Rev7, in all cases via the same domain contained in its C-terminal 150 aa [32-34]. It should also be borne in mind that PCNA is a homotrimer, and the available evidence suggests that ubiquitination is an all or nothing process, ie that all three monomers become ubiquitinated in one trimer [25, 31]. Each monomer may therefore be able to interact with a different polymerase, providing a “toolbelt” of different polymerases

that can attempt to deal with the blocked fork [35] (Figure 1A). Thus a medley of weak interactions occurs at the stalled fork enabling the polymerases to bind and attempt to carry out TLS (summarised in Figure 1B). In the case of a blocking CPD, pol η will do the job. With other lesions other polymerases will be able to effect TLS. In the case of a fork stalled by hydroxyurea, which results in depletion of deoxynucleotides, PCNA is ubiquitinated, but none of the polymerases will be able to relieve the situation significantly because of the lack of their crucial substrate.

Concluding remarks

Mammalian cells have evolved a variety of specialised polymerases in order to carry out TLS, either singly or in combination, past different types of DNA damage. Their recruitment to stalled replication forks requires the modification of PCNA by ubiquitination and is regulated by a series of weak interactions between the each polymerase and ubiquitinated PCNA and between the polymerases themselves.

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Figure Legend

Figure 1 Interactions between polymerases and PCNA

A, A PCNA trimer at a fork stalled by a lesion (black rectangle). All three monomers are ubiquitinated and are shown interacting with different polymerases. B, Summary of interactions, indicated by double-headed arrows.

