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Mini review

DNA polymerases and repair synthesis in NER in human cells

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ABSTRACT

The late steps of nucleotide excision repair, following incisions to remove the damaged section of DNA, comprise repair synthesis and ligation. In vitro and in vivo studies have shown the size of the repaired patch to be about 30 nucleotides. In vitro studies implicated the replicative polymerases in repair synthesis, but recent in vivo data have shown that several DNA polymerases and ligases are involved in these steps in human cells.

Contents

1. Introduction

Research described in the preceding articles has given us a rather deep insight into the proteins that are involved in the pre-incision and incision steps of NER, although much still needs to be learned about their regulation. Incision breaks are difficult to detect during NER. This led to the idea that pre-incision/incision was rate-limiting, and that, following incision, rapid and simple repair synthesis and ligation reactions ensued. Recent work has suggested a much more complicated scenario, with different polymerases and ligases as well as accessory proteins being involved.

Historically, research on repair synthesis can be divided into three phases. (1) Discovery and analysis of repair patches in cultured cells. (2) Use of cell free systems to analyse NER. (3) Use of genetics, siRNA and fluorescence microscopy to study mechanisms, dynamics and control in whole cells.

2. Repair replication in cultured human cells

The classical technique for measuring repair synthesis uses isopycnic centrifugation of density-labelled DNA in CsCl gradients, the technique of repair replication, which unequivocally distinguishes between replicative and repair synthesis [1]. In mammalian systems, repair replication was found to correlate well with unscheduled DNA synthesis (UDS), measured as the incorporation of ^{3}H-thymidine into DNA in cells outside of the S phase of the cell cycle [2]. UDS is usually examined either by autoradiography or by liquid scintillation counting. It is relatively easy to measure and was used in the 1960's for the discovery of the NER defect in xeroderma pigmentosum (XP) [3] and for the assignment of XP patients into different genetic complementation groups [4]. As is well known and described elsewhere in this volume, the defects in XP are in all cases (except for the variant class) in pre-incision or incision steps, rather than in repair synthesis. However the beauty of using repair synthesis for screening for NER deficiency in cells from patients is that it is dependent on all the earlier steps being functional and will therefore detect defects in any of the XP groups (except variants). It has therefore been widely used in screening for repair deficiencies. In a recently developed variation of the technique, the thymidine analogue 5-ethynyluridine (EdU) is used instead of

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^{3}H thymidine. Incorporation of EdU can be quantitated by fluorescence microscopy and image analysis, thereby obviating the need for radioactivity [5,6].

Work in the Smith/Hanawalt and Mullenders/van Zeeland labs in the mid 1970's to mid 1980's used repair replication to characterize the size of the repair patches and the effects of inhibitors on repair synthesis in human cells. Extensive studies by the former group led them to estimate a patch size of about 30 nucleotides in mammalian cells following UV-irradiation in a wide variety of conditions [7,8]. Three inhibitors, hydroxyurea (HU), 1-β-D-arabinofuranosylcytosine (araC) and aphidicolin (APC) are all powerful inhibitors of semi-conservative DNA replication, albeit by very different mechanisms. HU inhibits ribonucleotide reductase, thereby depleting the pool of deoxyribonucleotides. Repair synthesis is in general refractory to HU and indeed HU is often included in measurements of repair synthesis to repress replicative synthesis. The effects of araC and APC are dependent on dose and state of the cells. They have relatively minor effects at low doses in dividing cells, but inhibit repair synthesis significantly at higher doses or in confluent cells [9,10]. The repair synthesis that does take place in the presence of araC or APC results in patches that are not ligated [10–12]. XP-C cells are defective in global genome repair but proficient in transcription-coupled repair. The repair patch size was found to be similar in normal and XP-C fibroblasts, indicating that repair synthesis was similar for global and transcription-coupled repair [8].

3. Repair synthesis in cell-free systems

The development by Wood et al. of a cell free system for studying NER [13] and its reconstitution using recombinant proteins [14] enabled this group and others [15] to determine many parameters of the NER process. This work showed that dual incision by the XPG and XPF-ERCC1 proteins resulted in a gap of about 30 nucleotides, similar to the size calculated from the earlier in vivo studies. They also investigated which polymerase could fill this gap. 13 DNA polymerases are now known to exist in mammalian cells, including the high fidelity processive replicative polymerases, polδ and polε, although at the time that most of the in vitro NER studies were carried out, only six of these polymerases had been recognised. Initial studies showed that polε could carry out the repair synthesis step, which also required the sliding clamp accessory protein, PCNA, and its loader, replication factor C (RFC) [16]. Subsequent work showed that polδ could also carry out repair synthesis, but in this case DNaseIV was also required [16,17]. Using the reconstituted system, repair synthesis could be achieved by polε, PCNA, RFC and DNA ligase I [14]. In a more defined system with a plasmid containing a single lesion, either polδ or polε could be used with RPA, RFC and PCNA [18]. Antibodies to polδ strongly inhibited repair synthesis using HeLa cell extracts, implicating polδ in the in vitro system [19]. Taken together, these in vitro studies suggested that repair synthesis could be achieved using either or both of the replicative DNA polymerases together with their accessory proteins. This conclusion is consistent with genetic studies in yeast that suggested that either polδ or polε was necessary for repair synthesis but that there was some redundancy between them [20].

4. Polymerases, ligases and accessory proteins

The in vitro systems enabled huge strides to be made in our understanding of the mechanism of NER. However the substrate used in these systems was unchromatinised simple plasmid DNA and the amount of repair synthesis achieved was relatively low. This therefore left open the possibility that repair synthesis in chromosomal DNA might be more complex. More recent studies using immunofluorescence, localised irradiation through micropore filters, chromatin immunoprecipitation and siRNA technology, have indeed revealed that this is the case.

PCNA is required for DNA synthesis by most of the DNA polymerases. In most circumstances it is loaded onto the DNA by replication factor C (RFC). Green and Almouzni used micropore irradiation to show that PCNA was recruited to the sites of damage and that this recruitment was dependent on the presence of pre-incision and incision XP factors [21]. This result, together with similar findings by Essers et al. [22], implicates PCNA in post-incision repair synthesis in vivo and is consistent with the data obtained using cell-free systems. Recent in vitro studies using immobilized DNA containing a single cisplatin-DNA intrastrand crosslink showed that, once incision at the damage had taken place, XPG and RPA were needed to recruit PCNA, whereas the other XP proteins were no longer required [23]. In vivo micropore irradiation studies showed that although PCNA recruitment is dependent on the presence of XPG, its catalytic activity is not required. In contrast, XPF must be not only present but also catalytically active [24]. This supports a model in which, at least under some conditions, incision by XPF 5′ to the damage occurs prior to 3′ incision, as described in detail in the article by Orlando Schärer in this volume. Repair synthesis, which requires PCNA, can proceed without cleaving by XPG 3′ to the damage. This was confirmed by showing partial rescue of UDS in cells expressing the catalytically dead XPG, whereas there was no rescue in the catalytically dead XPF [24]. Interestingly, recruitment of PCNA to sites of local damage did not require RFC [25], although it is likely that RFC is necessary to load the PCNA onto the DNA.

In contrast to the replicative DNA polymerases, polymerases of the Y family have low fidelity and relatively poor processivity. Their principal function is thought to be carrying out translesion synthesis past different DNA lesions, though other roles have also been identified [26]. Ogi and Lehmann showed that, contrary to expectation, embryonic fibroblasts from mice defective in Y-family DNA polymerase κ had substantially reduced levels of repair synthesis, especially in the presence of HU. These findings implicated polκ in this process, especially under conditions of low deoxynucleotide concentrations [27]. In a further study, Ogi et al. showed that polκ was recruited to sites of local damage in UV-irradiated quiescent human primary fibroblasts, and was recruited to NER repair complexes in a UV- and NER incision-dependent manner. In the absence of polκ, polδ or both, UDS was reduced by 50%, suggesting that polκ and δ were involved in the same repair synthesis pathway. In contrast, depletion of polδ and/or polκ as well as polε reduced UDS to background levels, suggesting that polε was involved in a different repair synthesis pathway. Interestingly, the three polymerases implicated in repair synthesis by these studies appeared to be recruited to damage sites by different mechanisms. Recruitment of polκ required ubiquitinated PCNA and XRCC1, whereas polδ needed conventional RFC and PCNA, and polε needed a variant form of RFC. The authors proposed that there were two modes of repair synthesis, the first requiring polδ and κ, the other employing polε [28]. Models to account for this suggested that in the former pathway the repair site might be in a less accessible conformation, whereas in the latter case the DNA was more accessible.

Recently the nuclease Exo1 was shown to compete with repair synthesis for incised NER intermediates in *Saccharomyces cerevisiae*. In most instances, repair synthesis is more efficient and relatively short 30 nt NER patches result. Occasionally however, ExoI is able to compete and it extends the gap, resulting in occasional long repair patches, especially under conditions where repair synthesis was obstructed [29] (See also article by Novarina et al. in this issue). Since repair synthesis is measured as the total incorporated nucleotides, somewhat paradoxically, more repair synthesis due to