# COMMENTARY

# DNA polymerase δ/PCNA: actions and interactions

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## Introduction

Recent work has shown that the proliferating cell nuclear antigen (PCNA) is an essential component for the replication of the eucaryotic virus, SV40, in a human cell extract. This is due to its interaction with a hitherto overlooked DNA polymerase (pol δ), and this discovery has brought these two proteins under the scrutiny of those working in the cell cycle, replication and DNA polymerase fields. However, there has been some confusion in the literature surrounding the description of these two important proteins. Through the years PCNA has been independently described as three separate proteins, namely PCNA, cyclin and the DNA polymerase δ auxiliary protein (see below). To add to the confusion, a totally distinct protein, which was identified in fertilised sea-urchin and clam eggs (Evans *et al.* 1983), was also called 'cyclin'. This cyclin bears no relation to the protein described here. If this were not enough, as soon as opinion appeared to agree that pol δ was in fact a separate enzyme and not a derivative of pol α as had been suggested (one of the distinguishing criteria being that the mode of action of pol δ was dramatically affected by PCNA), then several groups began to find two types of pol δ. Both of these exhibited 'δ-like' properties but differed in their ability to respond to the auxiliary factor.

The aim of this article is to clarify this picture, first by describing pol δ and how this enzyme differs from pol α, and also by briefly reviewing the history of PCNA, especially in its relation to pol δ. In conclusion I would like to suggest how the two different types of pol δ, one PCNA-dependent and the other PCNA-independent, may have different functions in eucaryotic cells.

## DNA polymerase δ

Polymerase δ was initially described by Byrnes *et al.* in 1976 as a novel polymerase, distinguished from the previously described DNA polymerases (α, β and γ) by its tightly associated 3′–5′ exonuclease activity (for review, see Bambara *et al.* 1989). This enzyme was originally partially purified from rabbit bone marrow, and later purified to homogeneity from calf thymus (Lee *et al.* 1980). Subsequently, other polymerase preparations that also contain a 3′–5′ exonuclease activity have been isolated from other tissues, for example from calf thymus (Lee *et al.* 1984; Crute *et al.* 1986; Wahl *et al.* 1986), from rabbit bone marrow (Goscin and Byrnes, 1982) and from human placenta (Lee and Toomey, 1987). Other groups have also reported the purification of 'α-like' polymerases with exonuclease activity, but on closer examination these are probably types of pol δ (Chen *et al.* 1979), while one of the two enzymes (δI and δII) described by Crute *et al.* (1986) is probably an α-type enzyme.

Until recently, pol α has been generally accepted as the main replicative polymerase of eucaryotic cells and had been the subject of intense study by many groups (for review, see Lehman and Kaguni, 1989). Owing to difficulties of low abundance and proteolysis during often tortuous purification schemes, the subunit composition and activities associated with pol α were subject to considerable debate. Recently developed, rapid purification methods have permitted the isolation of largely intact enzymes, which has enabled detailed biochemistry to be carried out and the properties of this enzyme to be characterised. The uncertainty surrounding pol α has, however, aided the initial response, which was to discount pol δ as just another variant of pol α. The discovery of a cryptic exonuclease in the isolated $182\times10^3 M_r$ polymerase/primase subunit of the main replicative polymerase of *Drosophila melanogaster* (Cotterill *et al.* 1987), which was not present in the intact enzyme, added much credence to this theory, although this appears to be unique to *Drosophila*.

However, there is growing evidence to suggest that pol α and pol δ are indeed separate enzymes. Physically, the two activities can be separated either by conventional chromatographic resins such as hydroxyapatite or on immunoaffinity matrices, as the two enzymes are immunologically distinct. Activities associated with each enzyme also differ. It is now generally accepted that only preparations of pol δ contain a 3′–5′ exonuclease activity, whilst pol α preparations have a tightly bound primase subunit that is lacking in pol δ. pol δ can also function in strand displacement reactions, while pol α is inactive in this assay. The two enzymes show different template specificities, with pol α being most active on activated (nicked) calf thymus DNA, while pol δ shows maximal synthesis on synthetic poly(dA)/oligo(dT) templates.

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pol $\alpha$ and pol $\delta$ can also be distinguished by their sensitivities to the inhibitors aphidicolin and butylphenyl dGTP, with pol $\alpha$ being approximately 100-fold more sensitive to the nucleotide analogue than pol $\delta$. pol $\alpha$ is also unaffected by PCNA, while the processivity (i.e. the length of time that the enzyme remains in contact with the template thus determining the length of product produced) of pol $\delta$ is dramatically increased in the presence of this factor. In spite of this, no direct evidence that the two enzymes are separate entities exists. However, the discovery of a pol $\delta$-like enzyme in the yeast *S. cerevisiae* (pol III), which is also dependent on the yeast equivalent of PCNA and is genetically separable from the main '$\alpha$-like' replicative polymerase (pol I), adds additional weight to the argument that these enzymes may be distinct in higher eucaryotes (Sitney *et al.* 1989; Boulet *et al.* 1989).

## The proliferating cell nuclear antigen (PCNA)

As with the confusion over the identity and role of polymerase $\delta$, PCNA has also suffered a similar fate. This protein was initially identified as a human auto antigen using sera from patients with systemic lupus erythematosis. Since it was found in the nucleus of cells and tissues that were actively dividing, the descriptive name of the proliferating cell nuclear antigen was used (Miyachi *et al.* 1978). At a similar time, a protein was identified using two-dimensional electrophoresis, that was synthesised only in S-phase. Therefore this protein is only present in proliferating cells (Bravo and Celis, 1980; Bravo *et al.* 1987), and was named cyclin. The similarity between PCNA and this protein in terms of size ($36\times10^3 M_r$) and characteristic cell cycle profile were noted relatively quickly and the formal proof that these two proteins were one and the same was provided by a comparison of the peptides generated by V8 proteolysis (Mathews *et al.* 1984).

The most intriguing twist in this complicated tale was the identification of a partially purified factor derived from a human 293 cell extract, which was essential in a reconstituted system, for the replication of the eucaryotic virus, SV40. This work was being carried out in the laboratory of Bruce Stillman at Cold Spring Harbor, which, by serendipity, was in close proximity to that of Mike Mathews, who had identified the initial relationship between cyclin and PCNA. The similarity in the chromatographic behaviour of PCNA and the replication factor and also the presence of a $36\times10^3 M_r$ band in the partially purified human extract made the simple substitution of pure PCNA for this factor in the replication reaction too hard to resist. Much to everyone's surprise, PCNA did indeed fully substitute for the replication factor (Prelich *et al.* 1987*a*). Once the link between PCNA, which had always been suspected of being involved in replication, because of its intimate relationship with the cell cycle, and its close association with DNA replication sites (Bravo and Macdonald-Bravo, 1985, 1987), was established, it was perhaps slightly less of a surprise to find that this protein also resembled a previously identified protein that regulates the activity of calf thymus DNA polymerase $\delta$ (Tan *et al.* 1986; Prelich *et al.* 1987*b*; Bravo *et al.* 1987). Again substitution experiments between the groups of Stillman and Downey quickly established that these proteins were also functionally interchangeable (Prelich *et al.* 1987*b*).

This was the first evidence implicating pol $\delta$ in chromosomal DNA replication. This has now been confirmed by yeast genetics, which show that two polymerases pol I ($\alpha$-like) and pol III ($\delta$-like) are required for complete synthesis in *S. cerevisiae* (for review, see Blow, 1989). In addition, recent immunological studies by Hutchinson and Kill (1989), also show that pol $\alpha$ and PCNA co-localize, thus implicating the presence of both pol $\alpha$ and pol $\delta$ at sites of replication.

A two-polymerase hypothesis of eucaryotic replication is not a new concept, as the properties needed for leading and lagging strand are significantly different. Polymerase $\alpha$, with its tightly associated primase activity and semi-processive mode of action, is ideally suited for synthesis of the lagging strand. This has previously been shown to occur by the joining of short Okazaki length fragments (Ogawa and Okazaki, 1980). Conversely, pol $\delta$ has no associated primase activity and, in the presence of PCNA, is highly processive and thus is capable of synthesising the long stretches of DNA required of a leading-strand polymerase. The strand displacement activity of pol $\delta$, which is also required by a leading-strand polymerase, again demonstrates that this enzyme is perfectly adapted for this function. This hypothesis was tested using the SV40 *in vitro* system, as in the absence of PCNA polymerase $\delta$ was virtually inactive. Analysis of the products of SV40 replication showed that the presence of PCNA in the reconstituted system enabled the normal products of SV40 replication to be synthesised, while in the absence of PCNA (i.e. removing the effect of pol $\delta$, while not affecting the activity of pol $\alpha$), only short Okazaki-like fragments were produced. Further elegant experiments involving the hybridisation of replication products to strand-specific clones that contained DNA representing either leading or lagging strand synthesis provided evidence that pol $\alpha$ was responsible for both initiation and synthesis of the lagging strand, while pol $\delta$, in the presence of PCNA, was responsible for leading strand synthesis in this system (Prelich and Stillman, 1988).

## Roles of polymerase $\delta$

On closer examination, pol $\delta$ can be divided into two types depending on its sensitivity to PCNA, with the independent form being fully processive even in the absence of this auxiliary protein. Although the function of PCNA-dependent pol $\delta$ now appears to be established, little is known about the PCNA-independent form (Focher *et al.* 1989; Syvaoja and Linn, 1989). Recent work by Linn's group, however, has implicated this enzyme in the process of DNA repair (Nishida *et al.* 1988). In cells permeablized by Brij-58, DNA repair synthesis following ultraviolet light (u.v.) irradiation was impaired by comparison with intact cells. This could be

fully restored by a fraction obtained from the supernatant of similarly treated HeLa cells. Purification of this factor revealed it to be pol δ and further characterisation showed it to be of the independent type (Syvaoja and Linn, 1989). Although this implicates the PCNA-independent form of pol δ in DNA repair, PCNA itself may also be involved in this process. Work by Celis and Madson (1986) describes an increase in the nuclear staining of non-S-phase cells when human amnion cells were irradiated with u.v. Under conditions that induce nucleotide excision repair synthesis, a redistribution of PCNA occurred in such a way that it appeared to migrate to the sites of DNA damage. Similar differences in the appearance of PCNA in human fibroblasts, before and after u.v. irradiation, have also been reported by Toschi and Bravo (1988). However, as differences in the immunoflurescent staining patterns of PCNA have been shown to be variable (for review, see Mathews, 1989), these observations must be viewed with caution. Also PCNA may not be interacting with pol δ directly but with other factors that are involved in DNA repair.

Although known data appear to suggest set roles for the two types of pol δ, i.e. PCNA-dependent for replication and PCNA-independent for repair, the two enzymes may be found to have common features and therefore it may be prudent to consider their involvement in a range of cellular processes. Intriguing experiments by Hurwitz's group demonstrate the apparent synthesis of SV40 *in vitro* in the absence of either PCNA or pol δ (Lee *et al.* 1988). It will be interesting to see how this relates to the roles of pol δ and PCNA found by other groups using this system. An interesting experiment would be the substitution of a PCNA-independent pol δ in a SV40 reconstituted system to see if this can function at a replication fork in a manner similar to the PCNA-dependent form. As groups studying DNA replication and DNA repair are realising the importance of both types of pol δ, it may also be instructive to see if other proteins that are involved in replication are also involved in DNA repair.

## Conclusion

In summary, DNA pol α, and pol δ in conjunction with PCNA, appear to be essential for the complete replication of chromosomal DNA in eucaryotes (for review see Blow, 1989). Evidence also shows that the PCNA-independent form of pol δ is involved in DNA repair. However, the observation that PCNA is relocated to sites of DNA damage suggests that it may also be involved in processes other than DNA replication.

It is clear that proteins, identified and named in one system, will be shown to be involved in another. This may sometimes relate otherwise divergent fields and reveal hitherto unknown links between cellular processes.

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