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Interaction of poly(ADP-ribose)polymerase with DNA polymerase α

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Abstract

Homogeneously purified poly(ADP-ribose) polymerase (PARP) specifically stimulated the activity of immunoaffinity-purified calf or human DNA polymerase α by about 6 to 60-fold. Apparently, poly(ADP-ribosyl)ation of DNA polymerase α was not necessary for the stimulation. The effects of PARP on DNA polymerase α were biphasic: at very low concentrations of DNA, it rather inhibited its activity, whereas, at higher DNA concentrations, PARP greatly stimulated it. The autopoly(ADP-ribosyl)ation of PARP suppressed both its stimulatory and inhibitory effects. By immunoprecipitation with an anti-DNA polymerase α antibody, it was clearly shown that PARP may be physically associated with DNA polymerase α. Stimulation of DNA polymerase α may be attributed to the physical association between the two, rather than to the DNA-binding capacity of PARP, since the PARP fragment containing only the DNA binding domain showed little stimulatory activity. The existence of PARP-DNA polymerase α complexes were also detected in crude extracts of calf thymus. (Mol Cell Biochem **138**: 39–44, 1994)

Key words: DNA polymerase α, poly(ADP-ribose)polymerase, immunoprecipitation, autopoly(ADP-ribosyl)ation

Introduction

Accumulating evidence suggests that DNA synthesis may closely be correlated with an increased level of PARP in several tissues [1–6]. Newly replicated chromatin [7] as well as DNA fragments rich in replication forks [4] were co-purified with PARP. On the other hand, addition of a PARP inhibitor arrested Hela cell proliferation [8]. These effects have been correlated with poly(ADP-ribosyl)ation of nuclear proteins, such as histones [9], endonucleases [10], reverse transcriptase [11], DNA polymerase β [12], topoisomerase 1 [13, 14], DNA ligase II [15], and SV40 T-antigen [16, 17]. In contrast, Eki and Hurwitz recently demonstrated that PARP strongly suppressed the *in vitro* replication of SV40 DNA, without poly(ADP-ribosyl)ation of proteins [18]. The role(s) of PARP in DNA replication, therefore, has yet to be elucidated.

Increasing evidence also suggests that PARP participates in DNA repair by binding to DNA strand breaks, constituting a signal that switches off DNA synthesis temporarily to ensure that lesions are not replicated before repair [19]. As a signal for repair, PARP might also interact with repair enzymes, such as DNA polymerases, but this possibility has not yet been examined. In the present study, we demonstrate for the first time that PARP can bind physically to DNA polymerase α and directly affect its activity [20].

Results and discussion

PARP Specifically Stimulates DNA Polymerase α

PARP was purified from calf thymus by affinity chromatography on a 3-aminobenzamide Affi-gel column, followed by hydroxylapatite and Sephadex G-200 column chromatographies [21], with the final preparation showing a single polypeptide of 116 kDa after SDS-PAGE. The DNA polymer-

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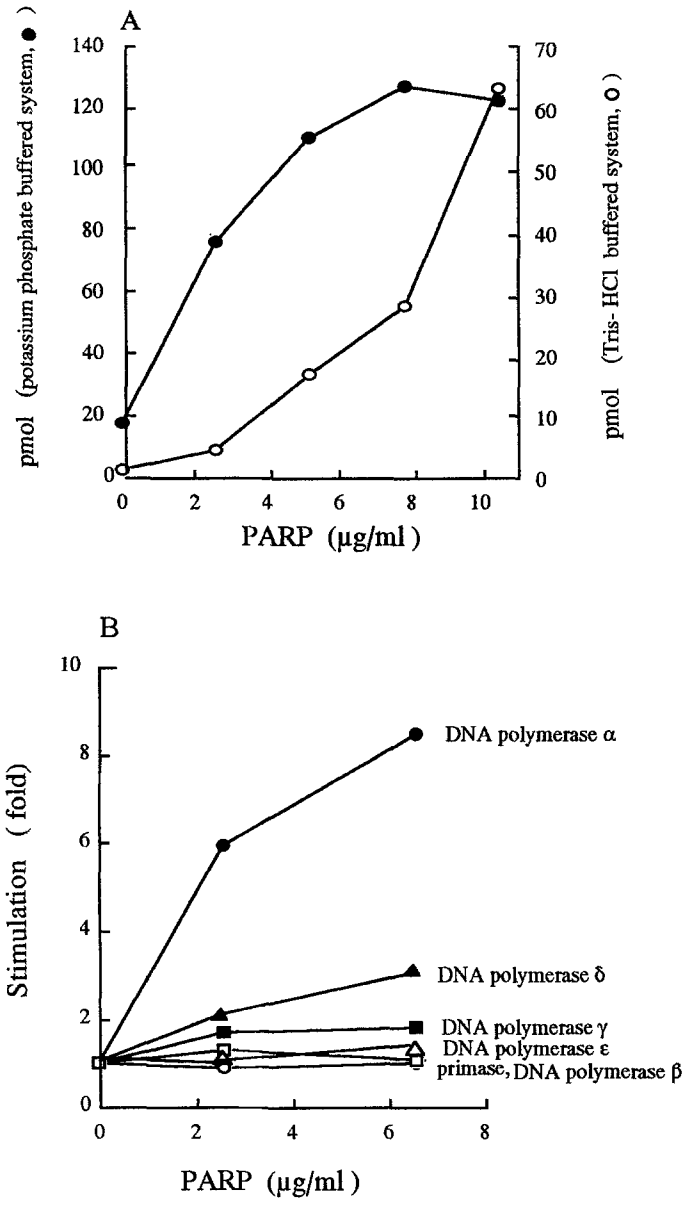

Fig. 1. DNA polymerase α is stimulated specifically and dose-dependently by PARP. A. Dose-dependent stimulation of DNA polymerase α activity by PARP, in potassium phosphate-buffered system (●) and in Tris-HCl-buffered system (o). Incubation was carried out at 37°C for 60 minutes. The reaction mixture (50 μl) contained 40 mM potassium phosphate (pH 7.2) or Tris-HCl (pH 7.5), 4 mM 2-mercaptoethanol, 40 μM each of dATP, dGTP, dCTP, 20 μM [^{3}H]-dTTP (500 cpm/nmol), 4 mM $MgCl_2$, 5 'μg BSA, 5 μg activated calf thymus DNA, and 0.05 unit of calf thymus DNA polymerase α-primase complex. (from [20]) B. Enzyme specificity of inhibition. DNA polymerase β (o), DNA polymerase γ (■) [26] and DNA primase (□) [27], as well as calf thymus DNA polymerase α (●), δ(▲), and ε (Δ) were assayed, in the presence of indicated amounts of PARP (from [20] and unpublished results).

ase α-primase complex was also purified from calf thymus on an immunoaffinity Sepharose 4B column conjugated with monoclonal antibody (MT17) directed against the DNA polymerase α catalytic subunit [22]. The final preparation showed 4 subunits of 150, 70, 53, and 45 kDa [23]. Addition of homogeneously purified PARP (5–10 μg/ml) to the reaction with immunoaffinity-purified DNA polymerase α dose-dependently stimulated DNA polymerase α activity in either potassium phosphate-buffered assays or Tris-HCl-buffered assays. Stimulation was 6 to 8-fold in potassium phosphate and up to 60 fold in Tris-HCl (Fig. 1A), since the immunoaffinity-purified DNA polymerase α enzyme showed little activity in Tris-HCl [23]. This stimulation was evidently specific for DNA polymerase α because DNA polymerases β and γ, DNA primase, as well as DNA polymerase ε were not stimulated at all, while DNA polymerase δ was also dose-dependently stimulated, but to a much lesser extent (Fig. 1B).

An anti-PARP antibody, which recognizes the catalytic domain [24], conjugated to CNBr Sepharose, immunoprecipitated PARP, resulting in a concomitant decrease in stimulatory activity. Therefore, it was concluded that the DNA polymerase α stimulation is indeed due to PARP itself.

Mode of Action

As shown in Fig. 2, the curve of the activity versus DNA concentration became sigmoidal in the presence of PARP. Maximum stimulation was observed at concentrations higher than the saturation level (100 μg/ml, the reaction was rather inhibited by the addition of PARP. At DNA doses less than 10 μg/ml, PARP apparently inhibited the reaction in competition with the DNA template-primer (Fig. 2B). This is consistent with results of Eki and Hurwitz [18], wherein the binding of PARP to DNA termini also inhibited the elongation of Okazaki fragments by DNA polymerase α in the SV40 DNA replication system. In this system, they used 0.3 μg/ml of supercoiled [18], which is approximately 16-fold less than the concentrations of activated DNA used in our usual assay system. At low DNA concentrations, the inhibitory effect of PARP caused by DNA binding might become predominant. On the other hand, at high DNA concentrations, a 4-fold increase in V_{max} was observed, suggesting an entirely different mechanism for stimulation. Interestingly, although essentially the same results were observed with another DNA primed-template, primed M13 DNA, when an RNA-primed DNA template, poly(dT)-oligo(rA), was used as template, there was no inhibition by PARP at low DNA concentrations, and stimulation was observed at all DNA concentrations. This suggests that PARP does not compete for binding to RNA primer ends, thus the stimulatory effect of PARP was predominant at all DNA concentrations.

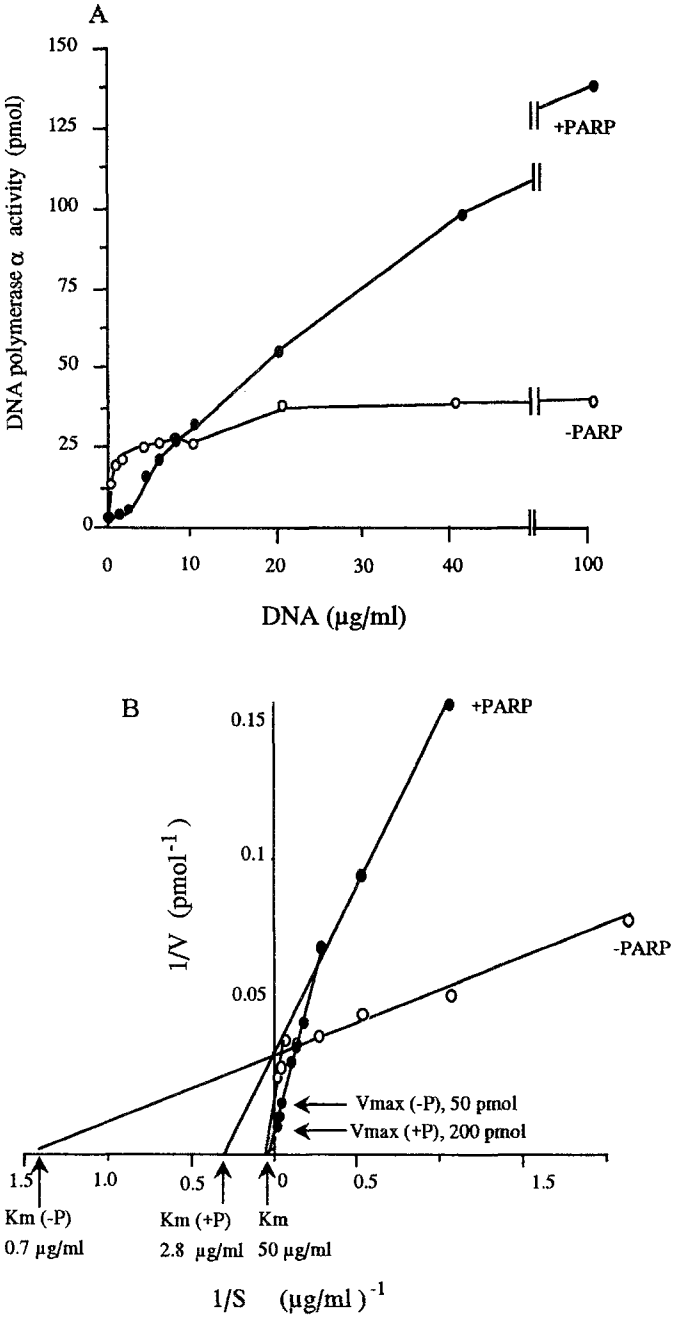

Fig. 2. Mode of inhibition: effects on Km and Vmax for the template-primer. A. The activity of DNA polymerase α was assayed using indicated concentrations of activated DNA as template-primer, in the presence of 5.2 μg/ml PARP (●) or without PARP (o). All experimental points are means of 3 independent experiments, with the standard deviation routinely less than 10% of the mean. B. Lineaweaver-Burk plots were made based on the data from A. Symbols used in A were the same as those in B (from [20]).

Effects of Autopoly(ADP-Ribosyl)ation and PARP Fragments

As shown in Fig. 3A, the stimulatory effect of PARP on DNA polymerase α activity was cancelled by its autopoly(ADP-ribosyl)ation. Kinetic analysis further showed that automodified PARP did not affect the kinetic parameters at either high or low DNA concentrations (see [20]).

Purified PARP (116 kDa) was cleaved with papain into 46 kDa DNA-binding and 74 kDa catalytic domain fragments ([25]; Fig. 3C) and then tested for any stimulatory activity on DNA polymerase α (Fig. 3B). Both fragments showed only slight stimulation, suggesting that an intact molecule of PARP is required for full stimulation.

PARP is Physically Associated with DNA Polymerase α

After preincubation of PARP with DNA polymerase α for 60 min at 0 °C, immunoprecipitation was performed with a monoclonal antibody, MT17 [22], which is directed against the 150 kDa polypeptide of DNA polymerase α, conjugated to CNBr-Sepharose 4B. The immunocomplex was then subjected to SDS-polyacrylamide gel electrophoresis. It was clearly shown that PAPR was coimmunoprecipitated with DNA polymerase α (Fig. 4). Consistently, the stimulatory activity by PARP in the supernatant was also depleted by anti-DNA polymerase α antibody but not with control IgG. These results demonstrate that PARP can bind directly to DNA polymerase a, thereby stimulating its activity. To determine whether any DNA polymerase α-PARP complexes exist in cell extracts, gel filtration chromatography was performed using crude fractions. Repeated gel filtrations revealed large complexes of 400 kDa and 700 kDa containing both PARP and DNA polymerase α (Fig. 5), indicating the existence of DNA polymerase α-PARP complexes *in vivo*.

Conclusion

The remarkable stimulation of DNA polymerase α by PARP, especially in the Tris-HCl-buffered system (about 60-fold) may suggest that PARP positively regulates DNA replication, in agreement with observations that PARP is induced by proliferation stimuli. On the other hand, the physical association of PARP with DNA polymerase α may be implicated in DNA repair. Accumulating evidence suggest that PARP participates in DNA repair, as a sensor for DNA damage, by binding to DNA strand breaks and locating the DNA repair enzyme complex to the damaged sites [19]. The physical binding between PARP and DNA polymerase α described here might play a role in this process. A model illustrating

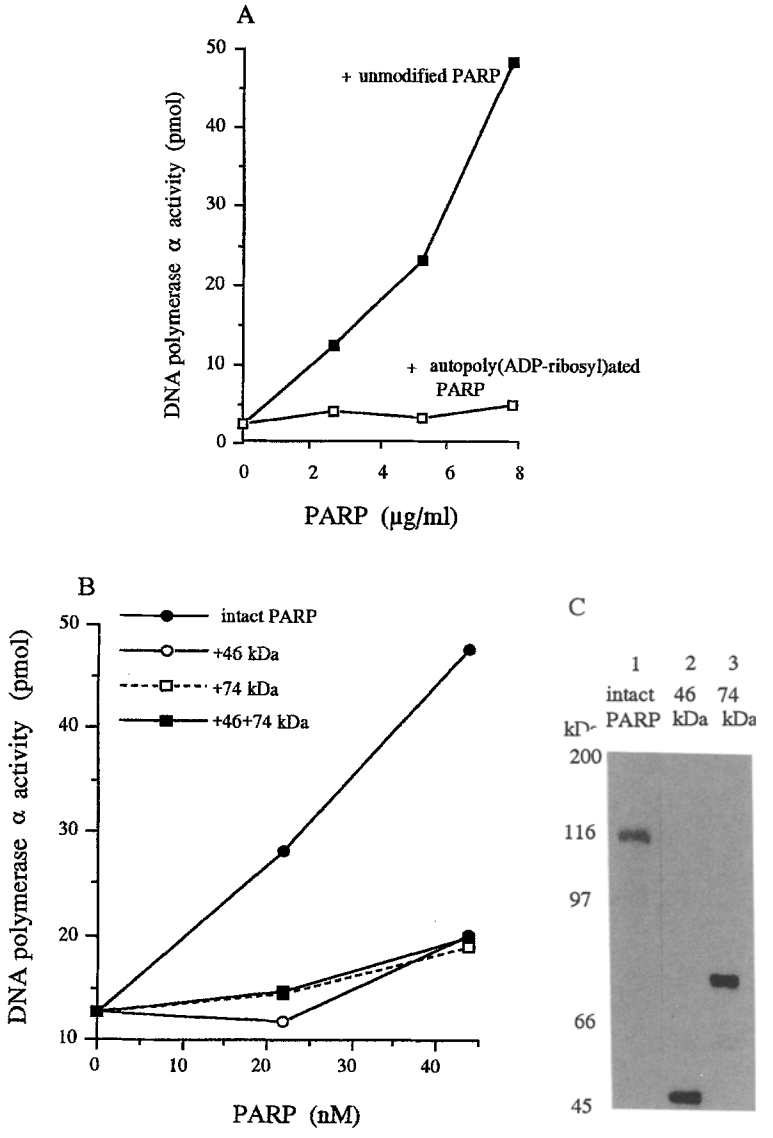

Fig. 3. Effects of autopoly(ADP-ribosyl)ation and PARP fragments. A. Autopoly(ADP-ribosyl)ation of PARP was carried out by incubating purified PARP (1.32 μg) at 25 °C for 20 min in 10 μl of 100 mM Tris HCl (pH 8.0), containing 2 mM dithiothreitol, 10 mM $MgCl_2$, 100 μM NAD, 10% glycerol, and 100 μg/ml activated DNA (25), resulting in an average chain length of more than 10 ADP-ribose units. Indicated amounts of autopoly(ADP-ribosyl)ated PARP (o,□), as well as untreated PARP (●,■), were then assayed for stimulation of DNA polymerase α activity in Tris-HCl buffered system (from Ref. 20). B. Effect of PARP fragments on DNA polymerase a activity, as compared to intact enzyme. Equimolar amounts of purified 46 kDa DNA binding fragments (o), the 74 kDa catalytic domain fragments (□), as well as the intact 116 kDa PARP enzyme (●), were assayed for stimulation of DNA polymerase α activity. Reconstitution was also tried with equimolar amounts of both fragments added to the reaction mixture (■) and then assayed for stimulatory activity. C. SDS Polyacrylamide gel electrophoresis of intact 116 kDa PARP, the 46 kDa DNA binding and the 74 kDa catalytic domain fragments. C. (from [20]).

the interaction of PARP with DNA polymerase α-primase (Fig. 6) proposes that both enzymes, being localized near DNA rich in replication forks or damaged DNA, could physically associate and bind to each other, resulting in a conformational change at the catalytic site of DNA polymerase α, which stimulates its activity after binding to DNA ends (if not in limiting amounts) or to RNA primer ends. In the same way that automodification of PARP reduces its affinity for DNA, it may also lose its ability to bind to DNA polymerase α, thereby releasing it from the complex, to be later regenerated into unmodified PARP by the action of specific glycohydrolases. When DNA termini are limiting, however, PARP and DNA polymerase α compete for binding to the DNA, and PARP temporarily blocks access of the α enzyme to these DNA ends; at this time, association between the two enzymes may localize the α enzyme to this site, until automodification of PARP releases it from the DNA, allowing the α enzyme to bind and later elongate the DNA strand.

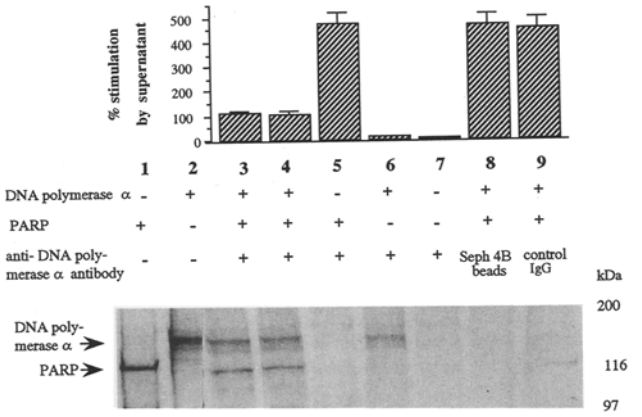

Fig. 4. Immunoprecipitation with anti-DNA polymerase α monoclonal antibody. Equimolar amounts of purified PARP and DNA polymerase α were pre-incubated for 60 minutes on ice, and immunoprecipitated with anti-DNA polymerase α antibody (MT17) conjugated to CNBr-activated Sepharose 4B (lanes 3 to 7), with control IgG (anti- Rb protein antibody, lane 9), or with Sepharose 4B beads alone (lane 8). The pellets were washed extensively, resuspended in electrophoresis buffer, and loaded on a 6.5% polyacrylamide gel. The immunocomplex bound to the beads was then subjected to SDS polyacrylamide gel electrophoresis and visualized by silver-staining (lower panel). Lane 1, input PARP; lane 2, input DNA polymerase α. Ten μl aliquots of the supernatants were also assayed for DNA polymerase α stimulation (upper panel).

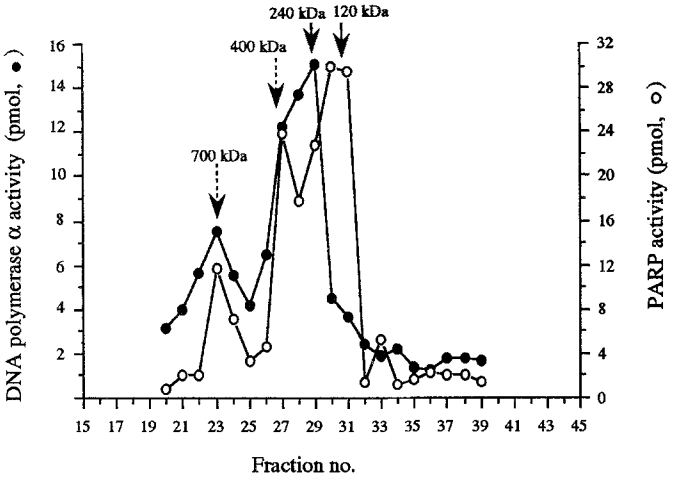

Fig. 5. Partial purification of DNA polymerase α-PARP complexes from calf thymus crude extracts by gel filtration chromatography. Calf thymus crude extract (10 ml, 22) was concentrated on a small phosphocellulose column and by overnight dialysis in solid sucrose. This concentrated fraction (0.2 ml) was applied to a Superose 6 gel filtration column (24 ml, FPLC), and fractions containing both PARP and DNA polymerase α activity, which eluted at 300–500 kDa, were rechromatographed on the same Superose 6 column. Both activities were measured in the same way. Recoveries of enzyme activities from the column were over 90 % (from [20]).

The fact that poly(ADP-ribosyl)ation function of PARP accounts for only a few percent of its molecular activity in

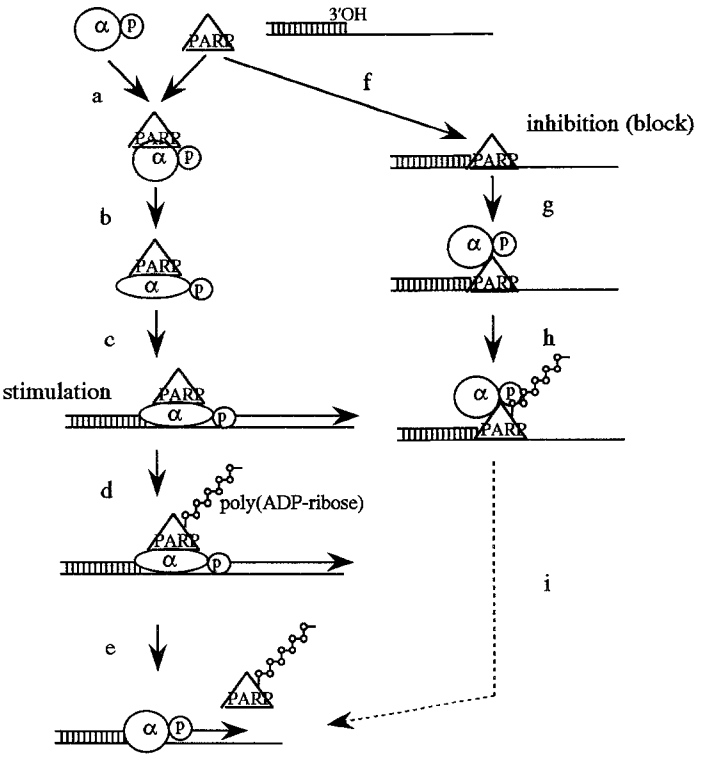

Fig. 6. Model for the interaction of PARP with DNA polymerase α-primase. Free DNA polymerase α-primase complex(α-p) and free PARP, both localized near DNA rich in replication forks, physically associate (a); PARP binds at an allosteric site, resulting in conformational changes at the catalytic site of DNA polymerase α (b), thereby stimulating its activity upon binding to the DNA template-primer (c); autopoly(ADP-ribosyl)ation of PARP (d) releases the modified PARP from the complex (e), and unmodified PARP is later regenerated. At low DNA concentrations, PARP and DNA polymerase α compete for binding to the limited 3′ OH ends of DNA; although binding of PARP blocks the binding of the α enzyme to the DNA (f), it specifically localizes DNA polymerase α to this site (g); automodification of PARP reduces its affinity to DNA (h), releasing the modified PARP (i), and allowing access to the 3′ OH end for DNA polymerase α-primase, which then proceeds to elongate the DNA strand.

intact cells [28] indicates that its DNA binding function, as well as association with other proteins, such as DNA polyase α, may also represent other potentially significant biological roles of PARP.

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