J. Biochem. 125, 904-909 (1999)

Nucleolar Protein B23.1 Binds to Retinoblastoma Protein and Synergistically Stimulates DNA Polymerase α Activity[1]

Masaharu Takemura,* Keiji Sato,† Masahiro Nishio,†,2 Tetsu Akiyama,‡ Hayato Umekawa,† and Shonen Yoshida*,3

**Laboratory of Cancer Cell Biology, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Nagoya, Aichi 466-8550; †Department of Agricultural Chemistry, Faculty of Bioresources, Mie University, Tsu, Mie 514-8507; and ‡Department of Molecular and Genetic Information, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo 113-0032*

Received December 17, 1998; accepted February 4, 1999

Phosphorylated retinoblastoma protein and nucleolar protein B23 are putative stimulatory factors for DNA polymerase α. We showed that these two factors interacted with each other and stimulated the activity of DNA polymerase α synergistically. B23 exists in two isoforms designated as B23.1 and B23.2. While B23.1 bound to a retinoblastoma protein-conjugated column, B23.2 did not. These results indicate that B23.1 can directly bind to retinoblastoma protein. It was also shown that B23 was co-immunoprecipitated with both retinoblastoma protein and DNA polymerase α from a HeLa cell extract by monoclonal antibodies raised against these components. These results suggest that these three proteins exist as a complex in cells, at least in part. The simultaneous addition of both B23.1 and retinoblastoma protein caused stimulation of DNA polymerase α activity that is much higher than the sum of the stimulation by retinoblastoma protein and B23.1 alone. The maximal stimulation was attained at the molar ratio of DNA polymerase α/retinoblastoma protein/B23.1=1:1:12. Since B23 exists as a hexamer in solution, it may act as a stimulator of DNA polymerase α in a form of double-hexamer, in concert with the phosphorylated retinoblastoma protein.

Key words: B23, DNA polymerase α, DNA replication, oligomerization, retinoblastoma protein.

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

The common initiation step of DNA elongation of either the leading or lagging strand is the synthesis of short RNA primers, followed by the synthesis of short DNA chains, and these reactions are catalyzed by DNA polymerase α (pol α)-primase complex (*1, 2*). Although pol α has been shown to exist in the cell nucleus throughout the cell cycle (*3*), it may act only in the S phase. Therefore, pol α may be regulated posttranslationally by some nuclear factors. A number of stimulatory/accessory proteins have been reported, *e.g.*, C-factor (*4-6*), poly(ADP-ribose) polymerase (*7*), replication protein A (RP-A) (*8*), replication factor C (RF-C) (*9*), proliferating cell nuclear antigen (PCNA) (*10*), *etc.* Some stimulators for DNA polymerases and for a cell-free DNA replication system are now recognized as essential factors for DNA replication, *e.g.*, RP-A as an essential single-stranded DNA binding protein (*11*), RF-C as a clamp-loader for DNA polymerases δ and ε (*12-14*), and PCNA as a sliding clamp for DNA polymerases δ and ε (*11, 13, 14*). In eukaryotic cells, DNA replication occurred through the concerted action of plural DNA polymerases, α, δ, and ε, with other enzymes such as DNA helicase (*15*), DNA topoisomerases (*16*), and DNA ligases (*17*). However, we still do not completely understand the regulation of the pol α reaction that is involved in the initiation step of DNA replication.

We found that two nuclear proteins stimulate pol α activity *in vitro* (*18, 19*). One is a nucleolar protein, B23, which is a major nucleolar protein and a putative ribosome assembly factor (*20*), and the other is a phosphorylated retinoblastoma (Rb) protein, which has been thought to be one of the negative regulators of cell cycle progression (*21*). B23/nucleophosmin/numatrin/NO38 is a 37 kDa nucleolar protein which is more abundant in tumor cells and normal growing cells (*22-26*). Feuerstein *et al.* reported that B23 is copurified with pol α on conventional purification (*27*). B23 exists in two isoforms, which are produced through alternative splicing, designated as B23.1 and B23.2. We have shown that purified B23.1 stimulates the activity of purified pol α (*18*) but that B23.2 does not. These results suggest that B23.1 might be involved in DNA replication, besides the ribosome assembly process. On the other hand, Rb protein is a 110 kDa nuclear protein which sequestrates

[1] This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

[2] Present address: Department of Pharmacology, Nagoya University School of Medicine, Nagoya, Aichi 466-8550.

[3] To whom correspondence should be addressed. Tel: +81-52-744-2453; Fax: +81-52-744-2457; E-mail: syoshida@tsuru.med.nagoya-u.ac.jp

Abbreviations: pol α, DNA polymerase α; Rb, retinoblastoma; PCNA, proliferating cell nuclear antigen; RP-A, replication protein-A; RF-C, replication factor-C; OVA, chicken egg albumin; GA, glutaraldehyde; SV40 Tag, simian virus 40 T antigen; MCM, minichromosome maintenance.

© 1999 by The Japanese Biochemical Society.

several transcription factors such as E2F (*28, 29*) and UBF (*30*), resulting in cell cycle arrest. Rb protein becomes an "inactive" on phosphorylation by several protein kinases without degradation throughout the cell cycle (*21*). Recently, we suggested that the phosphorylated form of Rb protein play positive roles in cell cycle progression, such as a stimulator of pol α (*19*). We demonstrated in the present study that two pol α stimulators, B23.1 and Rb protein, could interact each other, as well as with pol α. Furthermore, B23.1, presumably in its double-hexameric form, and Rb protein stimulated pol α synergistically.

MATERIALS AND METHODS

Antibodies—Anti-B23 monoclonal antibodies (NB23) were produced in a BALB/c mouse that had been immunized with 100 μg of purified rat recombinant B23.1 in Freund's complete adjuvant. Cell culture and the fusion were performed as previously described (*31*). At 10 to 12 days after hybridoma formation, antibody-producing cells were selected by enzyme-linked immunosorbent assaying against B23.1. Western blotting analysis revealed that NB23 recognized both B23.1 and B23.2. The anti-Rb protein monoclonal antibodies (3H9) were a kind gift from Dr. Katsuyuki Tamai of MBL, Ina.

Purification of B23.1 and B23.2—Two rat B23 isoforms designated as B23.1 and B23.2 have been expressed in *Escherichia coli* using the pKK223-3 vector (*32*), and were purified by means of ammonium sulfate fractionation, and column chromatographies on DEAE-cellulose, heparin-Sepharose, and Bio-Rad Q, as described previously (*32*). The electrophoretic profiles of the purified proteins are shown in Fig. 1.

Immunoaffinity Purification of Rb Protein—Human Rb protein was purified using a recombinant baculovirus system as described previously (*33-35*). The electrophoretic profile of the purified protein is shown in Fig. 1.

Immunoaffinity Purification of Pol α—Pol α-primase complex was purified from a calf thymus extract on a column of Sepharose conjugated with monoclonal antibodies (MT-17) directed against calf pol α as described previously (*7, 36*). The purified pol α showed specific activity of 15,000-20,000 units/mg. One unit corresponds to 1 nmol of deoxyribonucleotide incorporated in 1 h under the conditions described below except that potassium phosphate buffer (pH 7.2) was used instead of Tris-HCl buffer (pH 7.5). The electrophoretic profile of the purified protein is shown in Fig. 1.

Affinity Column Chromatography on Rb Protein-Conjugated Sepharose—The purified Rb protein (30 μg) was conjugated with 300 μl of CNBr-activated Sepharose 4B, and then packed into a small column (100 μl), which was equilibrated with buffer A (50 mM Tris-HCl, pH 7.5, 2 mM 2-mercaptoethanol, and 0.1 M NaCl). The purified B23.1 or B23.2 (15 μg each, in 100 μl) containing 1 mg/ml of bovine serum albumin (BSA) was applied to the column. After washing with an excess amount of buffer A, the adsorbed protein was eluted (200 μl/fraction) with buffer A containing 0.4 and 1.0 M NaCl, and then analysed by 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (*37*) followed by silver staining.

Immunoprecipitation of Rb-B23 or Pol α-Rb-B23 Complex from a HeLa Cell Extract—HeLa S3 cells were grown in RPMI medium containing penicillin (100 U/ml) and kanamycin (60 μg/ml), and supplemented with 5% calf serum, and then harvested at the logarithmic phase. The cells were washed three times with PBS and then lysed in 20 mM Hepes-OH, pH 7.5, containing 5 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM PMSF, 1 mM DTT, and 2 μg/ml leupeptin, followed by centrifugation at 7,000 $\times g$ for 10 min, the resulting supernatant being collected. In this extract, there is mainly cytoplasmic protein, but some nuclear proteins such as Rb protein and pol α also exist (data not shown). Sepharose-beads conjugated with anti-Rb protein (3H9), anti-B23 (NB23), and anti-pol α (MT-17) monoclonal antibodies, respectively (100 μl each), were added to 100 μl of extract, followed by incubation at 4°C for 2 h. The sepharose beads were collected by centrifugation at 2,000 $\times g$ for 5 min, and then washed three times with 50 mM Tris-HCl, pH 7.5, containing 0.1 M NaCl. The adsorbed protein was analysed by 10% SDS-PAGE followed by immuno-detection using anti-Rb protein or anti-B23 monoclonal antibodies.

DNA Polymerase Assay—Pol α was assayed (*38*) in a 25 μl reaction mixture comprising 80 mM Tris-HCl, pH 7.5, 8 mM 2-mercaptoethanol, 200 μg/ml of activated calf thymus DNA, 80 μM each of dATP, dGTP, and dCTP, 40 μM [^{3}H]dTTP (18.5 kBq), 8 mM $MgCl_2$, and 2.5 μg of BSA. After incubation at 37°C for appropriate times, acid-insoluble radioactivity was measured as described previously (*38*). The stimulatory activities of components were measured as described previously (*19*).

Glutaraldehyde Treatment of B23.1—A glutaraldehyde (GA) solution was mixed with a B23.1 solution to give a final concentration of 0.1% GA. After incubation at 37°C for

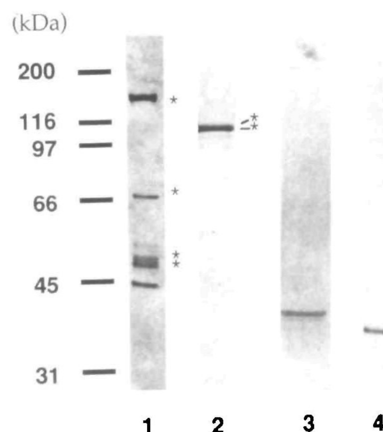

Fig. 1. **Electrophoretic profiles of the purified proteins.** Purified pol α, Rb protein, B23.1, and B23.2 were analysed by 10% SDS-PAGE. The proteins were visualized by silver staining. Purified pol α (lane 1) is composed of four subunits (asterisks), which migrate to positions corresponding to 140, 73, 50, and 47 kDa upon SDS-PAGE, respectively (*36*). A contaminating protein band around the 45 kDa position may be a contaminant because its amount varies with the preparation. Purified Rb protein (lane 2) gave two bands (asterisks) around the 110 kDa positions, which are its partially phosphorylated and underphosphorylated forms, respectively (*19*). Purified B23.1 (lane 3) and B23.2 (lane 4) migrated to the 37 and 35 kDa positions, respectively. The molecular mass standards (Bio-Rad) used were myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase B (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), and carbonic anhydrase (31 kDa).

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

5 min, the treated sample was subjected to 10% SDS-PAGE, followed by immuno-detection using anti-B23 monoclonal antibodies (NB23).

Quantitation of pol α, B23, and Rb Protein—The amounts of purified pol α, B23.1, B23.2, and Rb protein were estimated from the intensities of SDS-PAGE bands stained with Coomassie Brilliant Blue, taking various amounts of BSA as standards.

RESULTS

B23.1, but Not B23.2, Binds to Rb Protein—The direct protein-protein interaction of Rb protein with B23 was examined by affinity column chromatography on Rb protein-conjugated Sepharose. As shown in Fig. 2, B23.1 was adsorbed to the Rb protein-Sepharose column. All of the B23.1 applied was trapped on the Rb protein-Sepharose column and was eluted with 0.4 M NaCl. In contrast, B23.1 was not adsorbed to a chicken egg albumin (OVA)-conjugated control column. On the other hand, B23.2 was not adsorbed to the Rb protein-Sepharose column under the same conditions as for B23.1. These results indicate that B23.1, but not B23.2, can directly bind to Rb protein *in vitro*.

Association of B23 with Rb Protein and Pol α in Cells—The possible interaction of B23 and Rb protein in cells was examined with a HeLa cell crude extract by means of immunoprecipitation assays involving antibody-conjugated Sepharose beads. Western blotting of the resulting immunocomplex clearly showed that anti-Rb protein monoclonal antibodies (3H9) co-precipitated B23 in association with Rb protein (Fig. 3). Anti-B23 monoclonal antibodies (NB23) also co-precipitated Rb protein in association with B23 (Fig. 3). The control IgG-conjugated Sepharose beads could bind neither Rb protein nor B23 (Fig. 3). These results indicate that B23 interacts with Rb protein and exists as a complex in cells. Although the anti-B23 antibodies (NB23) can recognize both B23.1 and B23.2 (data not shown), the B23 protein band detected was identified as B23.1 from its mobility on SDS-PAGE (Fig. 3).

Since both Rb protein and B23.1 physically associate with pol α (*19*, Umekawa, H., *et al.*, unpublished observations), these interactions in cells were also examined in the same way using anti-pol α monoclonal antibodies (MT-17). As shown in Fig. 3, both B23 and Rb protein were co-immunoprecipitated with pol α, but not with contol IgG. Taken together, the data obtained on immunoprecipitation of a HeLa cell extract suggest that some parts of B23.1, Rb

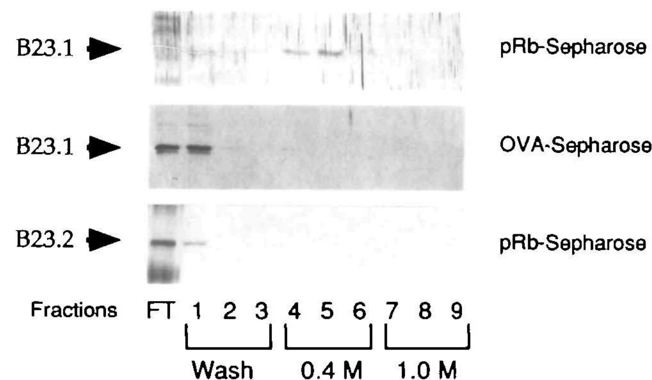

Fig. 2. **Association of B23 with Rb protein.** Purified Rb protein was immobilized on CNBr-activated Sepharose 4B (Pharmacia). Purified B23.1 or B23.2 contained an excess amount of BSA (1 mg/ml) to minimize the nonspecific adsorption. Upper panel, elution profile of B23.1 from an Rb protein-immobilized column; middle panel, elution profile of B23.1 from an OVA-immobilized column; and lower panel, elution profile of B23.2 from an Rb protein-immobilized column. The adsorbed proteins were eluted with 0.4 and 1.0 M NaCl. Each fraction was subjected to 10% SDS-PAGE followed by silver staining. FT, flow through; lanes 1, 2, and 3, washed fractions; lanes 4, 5, and 6, fractions eluted with 0.4 M NaCl; and lanes 7, 8, and 9, fractions eluted with 1.0 M NaCl.

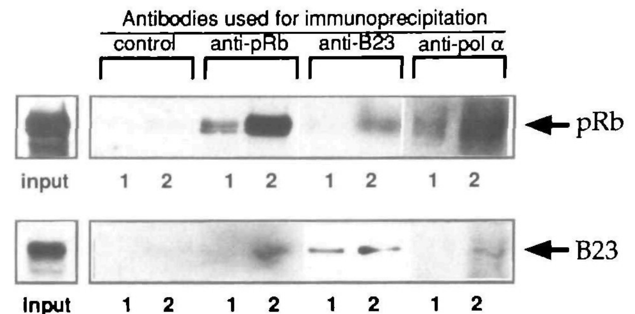

Fig. 3. **Immunoprecipitation assays.** Immunoprecipitation of Rb protein and B23 from a HeLa cell extract using anti-Rb protein monoclonal antibodies (3H9), anti-B23 monoclonal antibodies (NB23), and anti-pol α monoclonal antibodies (MT-17). Antibody-conjugated or control IgG [anti-plant amylase inhibitor monoclonal antibody (*49*)]-conjugated Sepharose was added to a HeLa cell extract, followed by incubation at 4°C for 2 h. After centrifugation, the immunoprecipitate was subjected to SDS-PAGE followed by immuno-detection using 3H9 (upper panel) or NB23 (lower panel). Input indicates all the proteins in the applied HeLa cell extract; 5 μl (lane 1) and 15 μl (lane 2) aliquots of the immunoprecipitate were subjected to SDS-PAGE followed by immuno-detection. Arrows indicate the positions of Rb protein (upper panel) and B23 (lower panel).

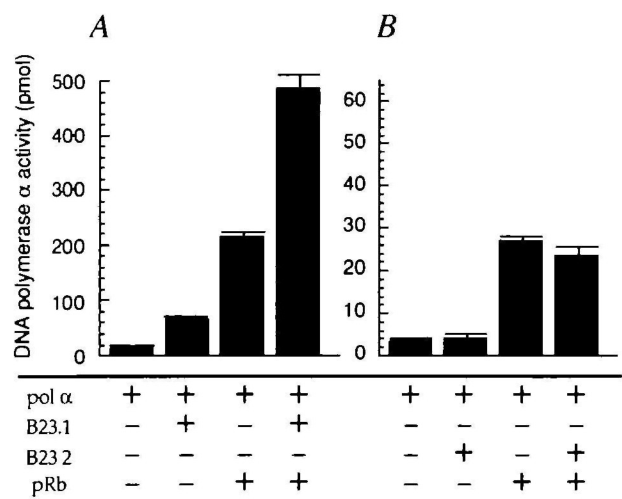

Fig. 4. **Synergistic stimulation of pol α by B23.1 and Rb protein.** Purified pol α (0.76 units) activity was assayed as described under "MATERIALS AND METHODS," after the addition of purified B23.1 (3 μg/ml), B23.2 (3 μg/ml) or purified Rb protein (1.4 μg/ml), as indicated. Incubation was carried out at 37°C for 60 min. The data represent the means of three determinations ± SD, and of three independent experiments.

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

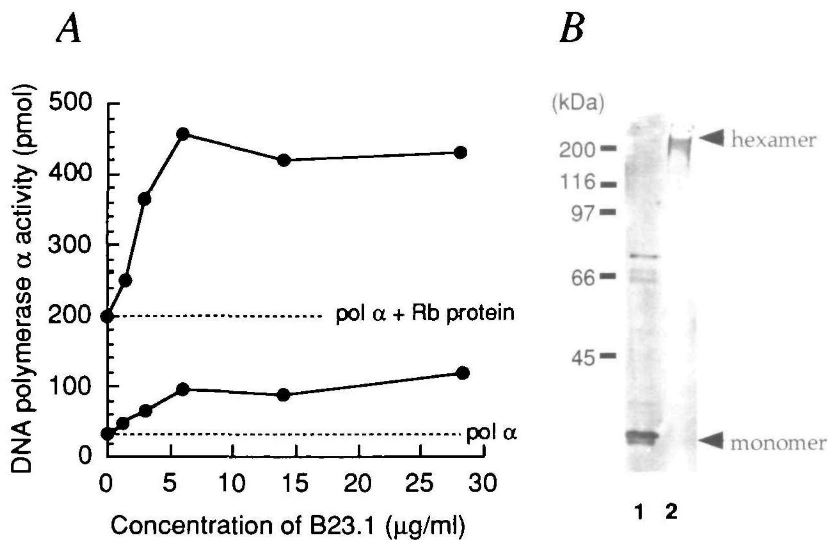

Fig. 5. **Hexamer formation of B23.1.** A, pol α activity was assayed in the presence of 1.4 μg/ml or absence of Rb protein and various amounts of B23.1. Incubation was carried out at 37°C for 60 min. The data represent the means of three determinations, and of two independent experiments. B, purified B23.1 (10 μg/ml) was subjected to SDS-PAGE with or without GA treatment. GA treatment was carried out as described under "MATERIALS AND METHODS." Then GA-treated (lane 2) and non-treated (lane 1) B23.1 were subjected to 10% SDS-PAGE followed by immuno-detection with anti-B23 monoclonal antibodies (NB23). The protein bands cross-reacting with NB23 around the 70 kDa position may be the B23-dimer. The molecular mass standards (Bio-Rad) used were myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase B (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), and carbonic anhydrase (31 kDa).

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

protein, and pol α in HeLa cells exist in a complex form. However, the amount of B23.1 immunoprecipitated with anti-pol α antibodies was much less than that with anti-Rb protein antibodies, suggesting that not all of the B23.1 formed the complex with both of pol α and Rb protein (Fig. 3).

B23.1 and Rb Protein Synergistically Stimulate Pol α Activity—Immunopurified pol α was mixed with purified Rb protein and B23.1 or B23.2, and then the DNA synthesis reaction was carried out (Fig. 4). In order to rule out the possibility that Rb protein and B23 might stabilize pol α in a nonspecific manner by raising the protein concentration, an excess amount of BSA (100 μg/ml) was added to the reaction system. As shown in Fig. 4A, each of B23.1 and Rb protein stimulated the pol α activity, about 5- and 16-fold, respectively. About 37-fold synergistic stimulation of pol α activity was observed when both B23.1 and Rb protein were added. On the other hand, B23.2 did not stimulate the pol α activity as previously described (*18*), in either the presence or absence of Rb protein (Fig. 4B).

Stoichiometry of the Stimulation by Rb Protein and B23.1—The stoichiometry of the protein-protein interaction was estimated by measuring the stimulation. Increasing amounts of B23.1 were added to a reaction mixture that contained both pol α and the saturation level of Rb protein (1.4 μg/ml, see Ref. *19*), resulting in a monophasic saturation curve with a plateau level at about 6 μg/ml of B23.1 (Fig. 5A). The molar ratio of pol α/Rb protein was 1:1, as quantitated as described under "MATERIALS AND METHODS." The maximal stimulation was observed at 6 μg/ml of B23.1 and 1.4 μg/ml of Rb protein. It was calculated from these data that the maximal stimulation can be attained with Rb protein and B23.1 at the molar ratio of pol α:Rb protein:B23.1 = 1:1:12. A similar saturation curve was obtained by adding B23.1 to pol α in the absence of Rb protein (Fig. 5A).

Previous studies indicated that B23.1 forms a hexamer in solution (*32, 39*), and it mainly exists as an oligomer in HeLa cells (*40*). The hexamer formation was confirmed with our B23.1 preparation, by crosslinking the proteins in an oligomer with GA. As shown in Fig. 5B, almost all the B23.1 was in the hexameric form. These results suggest that B23.1 may form a double-hexamer when it interacts with one molecule of Rb protein, and that this form stimulates pol α in collaboration with Rb protein.

DISCUSSION

B23 is one of the major nucleolar proteins that is thought to be involved in ribosome assembly (*20*) and also in the transport of ribosomes from the nucleolus to the cytoplasm (*41*). B23 is expressed in two isoforms, B23.1 and B23.2, presumably due to alternative splicing (*42*). B23.2 is shorter than B23.1 by 35 amino acid residues at its C-terminus. Wang *et al.* have reported that the C-terminal region of B23.1 is essential for DNA binding activity (*43*). We have reported that B23.2 exhibits no stimulatory activity against pol α, suggesting that the C-terminal region of B23.1 is essential for the stimulation (*18*). In the present study, we showed that B23.1, but not B23.2, could bind to another stimulator of pol α, Rb protein, *in vitro* (Fig. 2).

Yung *et al.* have shown that B23 is capable of being translocated from the nucleolus to the nucleoplasm (*44*). Furthermore, Feuerstein and Mond reported that B23.1, which they called "numatrin," tightly bound to the nuclear matrix (*45*). These previous reports and our present data suggest that B23.1 plays a role in nucleoplasmic DNA replication in collaboration with phosphorylated Rb protein. On the other hand, Cavanaugh *et al.* (*30*) reported that Rb protein is also localized to the nucleolus of U937 cells that are committed to differentiation by treatment with phorbor ester. Since B23.1 is mainly localized in the nucleolus, it is also possible that B23.1 participates in ribosomal DNA replication together with the nucleolus-localized Rb protein.

The stimulation of pol α by B23.1 and Rb protein alone differs in several points. First, the most remarkable difference is the level of stimulation. Rb protein causes much stronger stimulation than B23.1 when activated calf thymus DNA is used as a template-primer (Fig. 4; *18, 19*). However, the stimulation by B23.1 is higher than that by Rb protein with poly(dA)-$(dT)_{12-18}$ (*19*, Umekawa, H., *et al.*, unpublished observations). Second, for the stimulation

of pol α, phosphorylation is essential for Rb protein (*19*), but is not required for B23.1, because unphosphorylated B23.1 expressed in *E. coli* can stimulate pol α to the same level as native B23.1 isolated from Novikoff hepatoma cells (*18*). Actually, B23.1 could stimulate the fully-stimulated pol α in the presence of a saturating amount of Rb protein (Fig. 5). Strikingly, the stimulation by these two factors was not additive but synergistic (Fig. 4). The protein-protein interactions among B23.1, Rb protein, and pol α may produce the optimum conformation of pol α.

It has been reported that B23 can form oligomers, especially the hexamer (*32, 39, 40, 46*), but the biological significance of this oligomerization remains obscure. Among the enzymes and protein factors involved in DNA metabolism, hexamer or double-hexamer structures are sometimes observed. For instance, the simian virus 40 large T-antigen (SV40 Tag) forms a double-hexamer when it acts either in replication origin recognition or as DNA helicase (*47*). PCNA, a sliding clamp for pol δ and ε (*11, 13, 14*), has two functional domains and forms a trimer resulting in hexameric domain structures. Minichromosome maintenance (MCM) protein complex form a hetero-hexamer composed of MCM4, MCM6, and MCM7, which exhibits DNA helicase activity (*48*). In this context, B23, especially B23.1, also forms a hexamer, and stimulates pol α presumably by forming a double-hexamer in concert with Rb protein, as shown in the present study. It might also participate in DNA replication in its oligomeric form through the regulation of pol α activity.

We wish to thank Dr. Mark O.J. Olson of the University of Mississippi Medical Center for the kind gift of the pKK223-3 expression vectors, Drs. Katsuyuki Tamai and Susumu Suzuki of MBL Co., Ltd., for the kind gift of anti-Rb protein monoclonal antibodies (3H9), Dr. Yukio Furuichi of the Faculty of Bioresources, Mie University, for the kind gift of anti-amylase inhibitor monoclonal antibodies, Dr. Yoshinori Watanabe of the Institute for Molecular and Cellular Biology, Osaka University, and Drs. Keiko Tamiya-Koizumi, Motoshi Suzuki, Masanori Ogawa, Katsura Nozawa, Aki Tosaka, and Yukiko Mizutani of our laboratory for the helpful discussions, and Ms. Miwa Takahashi and Ms. Tazuko Tomita of our laboratory for their excellent technical assistance.

REFERENCES

1. Wang, T.S.-F. (1991) Eukaryotic DNA polymerases. *Annu. Rev. Biochem.* **60**, 513-552
2. Waga, S. and Stillman, B. (1994) Anatomy of a DNA replication fork revealed by reconstitution of SV40 DNA replication *in vitro*. *Nature* **369**, 207-212
3. Nakamura, H., Morita, T., Masaki, S., and Yoshida, S. (1984) Intracellular localization and metabolism of DNA polymerase α in human cells visualized with monoclonal antibody. *Exp. Cell Res.* **151**, 123-133
4. Lamothe, P., Baril, B., Chi, A., Lee, L., and Baril, E. (1981) Accessory proteins for DNA polymerase α activity with single-strand DNA templates. *Proc. Natl. Acad. Sci. USA* **78**, 4723-4727
5. Pritchard, C.G. and DePamphilis, M.L. (1983) Preparation of DNA polymerase α, C_1C_2 by reconstituting DNA polymerase α with its specific stimulatory cofactors, C_1C_2. *J. Biol. Chem.* **258**, 9801-9809
6. Pritchard, C.G., Weaver, D.T., Baril, E.F., and DePamphilis, M.L. (1983) DNA polymerase α cofactors C_1C_2 function as primer recognition proteins. *J. Biol. Chem.* **258**, 9810-9819
7. Simbulan, C.M.G., Suzuki, M., Izuta, S., Sakurai, T., Savoysky, E., Kojima, K., Miyahara, K., Shizuta, Y., and Yoshida, S. (1993) Poly(ADP-ribose) polymerase stimulates DNA polymerase α by physical association. *J. Biol. Chem.* **268**, 93-99
8. Wobbe, C.R., Weissbach, L., Borowiec, J.A., Dean, F.B., Murakami, Y., Bullock, P., and Hurwitz, J. (1987) Replication of simian virus 40 origin-containing DNA *in vitro* with purified proteins. *Proc. Natl. Acad. Sci. USA* **84**, 1834-1838
9. Tsurimoto, T. and Stillman, B. (1989) Purification of a cellular replication factor, RF-C, that is required for coordinated synthesis of leading and lagging strands during simian virus 40 DNA replication *in vitro*. *Mol. Cell. Biol.* **9**, 609-619
10. Hübscher, U. and Spadari, S. (1994) DNA replication and chemotherapy. *Physiol. Rev.* **74**, 259-304
11. Hübscher, U., Maga, G., and Podust, V.N. (1996) DNA replication accessory proteins in *DNA Replication in Eukaryotic Cells* (DePamphilis, M.L., ed.) pp. 525-543, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
12. Stillman, B. (1994) Smart machine at the DNA replication fork. *Cell* **78**, 725-728
13. Yoder, B.L. and Burgers, P.M.J. (1991) *Saccharomyces cerevisiae* replication factor C: I. Purification and characterization of its ATPase activity. *J. Biol. Chem.* **266**, 22689-22697
14. Burgers, P.M.J. (1991) *Saccharomyces cerevisiae* replication factor C: II. Formation and activity of complexes with the proliferating cell nuclear antigen and with DNA polymerases δ and ε. *J. Biol. Chem.* **266**, 22698-22706
15. Borowiec, J.A. (1996) DNA helicases in *DNA Replication in Eukaryotic Cells* (DePamphilis, M.L., ed.) pp. 545-574, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
16. Hangaard Andersen, A., Bendixen, C., and Westergaard, O. (1996) DNA topoisomerases in *DNA Replication in Eukaryotic Cells* (DePamphilis, M.L., ed.) pp. 587-617, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
17. Nash, R. and Lindahl, T. (1996) DNA ligases in *DNA Replication in Eukaryotic Cells* (DePamphilis, M.L., ed.) pp. 575-586, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
18. Takemura, M., Ohta, N., Furuichi, Y., Takahashi, T., Yoshida, S., Olson, M.O.J., and Umekawa, H. (1994) Stimulation of calf thymus DNA polymerase α activity by nucleolar protein B23. *Biochem. Biophys. Res. Commun.* **199**, 46-51
19. Takemura, M., Kitagawa, T., Izuta, S., Wasa, J., Takai, A., Akiyama, T., and Yoshida, S. (1997) Phosphorylated retinoblastoma protein stimulates DNA polymerase α. *Oncogene* **15**, 2483-2492
20. Olson, M.O.J. (1991) The role of protein in nucleolar structure and function in *The Eukaryotic Nucleus: Molecular Biochemistry and Macromolecular Assemblies* (Strauss, P.R. and Wilson, S.H., eds.) Vol. 2, pp. 541-546, Telford Press, Caldwell, NJ
21. Weinberg, R.A. (1995) The retinoblastoma protein and cell cycle control. *Cell* **81**, 323-330
22. Orrick, L.R., Olson, M.O.J., and Busch, H. (1973) Comparison of nucleolar proteins of normal rat liver and Novikoff hepatoma ascites cells by two-dimensional polyacrylamide gel electrophoresis. *Proc. Natl. Acad. Sci. USA* **70**, 1316-1320
23. Prestayko, A.W., Klomp, G.R., Schmoll, D.J., and Busch, H. (1974) Comparison of proteins of ribosomal subunits and nucleolar preribosomal particles from Novikoff hepatoma ascites cells by two-dimensional polyacrylamide gel electrophoresis. *Biochemistry* **13**, 1945-1951
24. Olson, M.O.J., Orrick, L.R., Jones, C., and Busch, H. (1974) Phosphorylation of acid-soluble nucleolar proteins of Novikoff hepatoma ascites cells *in vivo*. *J. Biol. Chem.* **249**, 2823-2827
25. Schmidt-Zachmann, M.S., Hügle-Dörr, B., and Franke, W.W. (1987) A constitutive nucleolar protein identified as a member of the nucleoplasmin family. *EMBO J.* **6**, 1881-1890
26. Chan, W.Y., Liu, Q.R., Borjigin, J., Busch, H., Rennert, O.M., Tease, L.A., and Chan, P.K. (1989) Characterization of the cDNA encoding human nucleophosmin and studies of its role in normal and abnormal growth. *Biochemistry* **28**, 1033-1039
27. Feuerstein, N., Mond, J.J., Kinchington, P.R., Hickey, R., Lindsberg, M.L.K., Hay, I., and Ruyechan, W.T. (1990) Evidence for DNA binding activity of numatrin (B23), a cell cycle-regulated nuclear matrix protein. *Biochim. Biophys. Acta* **1087**, 127-136

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

28. Bagchi, S., Weinmann, R., and Raychaudhuri, P. (1991) The retinoblastoma protein copurifies with E2F-1, an E1A-regulated inhibitor of the transcription factor E2F. *Cell* **65**, 1063-1072
29. Chellappan, S.P., Hiebert, S., Mudryj, M., Horowitz, J.M., and Nevins, J.R. (1991) The E2F transcription factor is a cellular target for the RB protein. *Cell* **65**, 1053-1061
30. Cavanaugh, A.H., Hempel, W.M., Taylor, L.J., Rogalsky, V., Todorov, G., and Rothblum, L.I. (1995) Activity of RNA polymerase I transcription factor UBF blocked by Rb gene product. *Nature* **374**, 177-180
31. Harlow, E. and Lane, D. (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
32. Umekawa, H., Chang, J.H., Correia, J.J., Wang, D., Wingfield, P.T., and Olson, M.O.J. (1993) Nucleolar protein B23: bacterial expression, purification, oligomerization and secondary structures of two isoforms. *Cell. Mol. Biol. Res.* **39**, 635-645
33. Wang, N.P., Qian, Y., Chung, A.E., Lee, W.-H., and Lee, E.Y.-H.P. (1990) Expression of the human retinoblastoma gene product pp110RB in insect cells using the baculovirus system. *Cell Growth Differ.* **1**, 429-437
34. Akiyama, T., Ohuchi, T., Sumida, S., Matsumoto, K., and Toyoshima, K. (1992) Phosphorylation of the retinoblastoma protein by cdk2. *Proc. Natl. Acad. Sci. USA* **89**, 7900-7904
35. Savoysky, E., Suzuki, M., Tamai, K., Sakurai, T., Ohuchi, T., Akiyama, T., Kojima, K., and Yoshida, S. (1992) Efficient purification of a full length and biochemically active p110Rb, the retinoblastoma gene product. *Biochem. Biophys. Res. Commun.* **187**, 697-702
36. Tamai, K., Kojima, K., Hanaichi, T., Masaki, S., Suzuki, M., Umekawa, H., and Yoshida, S. (1988) Structural study of immunoaffinity-purified DNA polymerase α-DNA primase complex from calf thymus. *Biochim. Biophys. Acta* **950**, 263-273
37. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* **227**, 680-685
38. Yoshida, S., Kondo, T., and Ando, T. (1974) Multiple molecular species of cytoplasmic DNA polymerase from calf thymus. *Biochim. Biophys. Acta* **353**, 463-474
39. Yung, B.Y.M. and Chan, P.K. (1987) Identification and characterization of a hexameric form of nucleolar phosphoprotein B23. *Biochim. Biophys. Acta* **925**, 74-82
40. Chan, P.K. and Chan, F.Y. (1995) Nucleophosmin/B23 (NPM) oligomer is a major and stable entity in HeLa cells. *Biochim. Biophys. Acta* **1262**, 37-42
41. Borer, R.A., Lehner, C.F., Eppenberger, H.M., and Nigg, E.A. (1989) Major nucleolar proteins shuttle between nucleus and cytoplasm. *Cell* **56**, 379-390
42. Chang, J.H. and Olson, M.O.J. (1989) A single gene codes for two forms of rat nucleolar protein B23 mRNA. *J. Biol. Chem.* **264**, 11732-11737
43. Wang, D., Baumann, A., Szebeni, A., and Olson, M.O.J. (1994) The nucleic acid binding activity of nucleolar protein B23.1 resides in its carboxyl-terminal end. *J. Biol. Chem.* **269**, 30994-30998
44. Yung, B.Y.M., Busch, R.K., Busch, H., Mauger, A.B., and Chan, P.K. (1985) Effects of actinomycin D analogs on nucleolar phosphoprotein B23 (37,000 daltons/pI 5.1). *Biochem. Pharmacol.* **34**, 4059-4063
45. Feuerstein, N. and Mond, J.J. (1987) "Numatrin," a nuclear matrix protein associated with induction of proliferation in B lymphocytes. *J. Biol. Chem.* **262**, 11389-11397
46. Liu, Q.R. and Chan, P.K. (1991) Formation of nucleophosmin/B23 oligomers requires both the amino- and the carboxyl-terminal domains of the protein. *Eur. J. Biochem.* **200**, 715-721
47. Hassell, J.A. and Brinton, B.T. (1996) SV40 and polyomavirus DNA replication in *DNA Replication in Eukaryotic Cells* (DePamphilis, M.L., ed.) pp. 639-677, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
48. Ishimi, Y. (1997) A DNA helicase activity is associated with an MCM-4, -6, and -7 protein complex. *J. Biol. Chem.* **272**, 24508-24513
49. Furuichi, Y., Takemura, M., Uesaka, N., Kamemura, K., Shimada, S., Komada, H., Ohta, H., Itoh, Y., Umekawa, H., and Takahashi, T. (1993) Some characteristics of an alpha-amylase inhibitor from *Phaseolus vulgaris* (cultivar Great Northern) seeds. *Biosci. Biotech. Biochem.* **57**, 147-148

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012