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Biochemistry

Reconstitution of functional human single-stranded DNA-binding protein from individual subunits expressed by recombinant baculoviruses

(DNA replication/DNA polymerase/simian virus 40)

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ABSTRACT **Human single-stranded DNA-binding protein (HSSB), also known as replication protein A, is composed of a 70-kDa single-stranded DNA-binding subunit (p70) and 34-kDa and 11-kDa (p34 and p11, respectively) subunits of unknown functions. We have examined interactions among the HSSB subunits *in vivo* by coinfecting insect cells with different combinations of recombinant baculoviruses encoding p70, p34, or p11. *In vivo*, coexpressed p34 and p11 subunits formed stable complexes, whereas neither p34 nor p11 formed stable complexes with p70. In cells coinfected with viruses expressing all three subunits, the stable heterotrimer formed, which, when purified, replaced HSSB isolated from HeLa cells in various assays, including simian virus 40 DNA replication *in vitro*. These data suggest that, in the assembly of functionally active HSSB, formation of the p34–p11 complex precedes p70 addition to the complex.**

Human single-stranded DNA-binding protein (HSSB; also known as replication protein A) was originally identified as a HeLa cell protein required for the *in vitro* replication of simian virus 40 (SV40) origin-containing DNA (ori$^+$DNA) (1–3). HSSB is also required for the nucleotide-excision repair of UV-damaged DNA *in vitro* (4) and may also be involved in genetic recombination (5, 6).

The HSSB heterotrimer consists of a tight complex of 70-, 34-, and 11-kDa subunits (p70, p34, and p11, respectively) (1). This subunit structure is common to yeast, mice, *Drosophila*, and humans, suggesting that its functional activity is conserved among eukaryotes (7–10). Nevertheless, yeast single-stranded DNA-binding protein (SSB) is a poor substitute for HSSB in the *in vitro* replication of SV40 DNA (7), indicating that specific interactions between HSSB and human replication proteins are important for biological activity.

HSSB functions in the initiation and elongation stages of SV40 DNA replication. In the initiation stage, it is involved in the SV40 large tumor antigen (T antigen)-dependent unwinding of ori$^+$DNA (11–13). The unwinding reaction is supported by other SSBs, such as those isolated from *Escherichia coli*, adenovirus, or yeast (7, 14); however, initiation of DNA synthesis, measured by synthesis of small RNA primers by the DNA polymerase α–DNA primase (pol α–primase) complex, specifically requires HSSB (15). Functional and direct interactions between HSSB, T antigen, and pol α–primase have been shown (9, 16–18).

During the elongation reaction, HSSB is the only DNA-binding protein that stimulates the DNA polymerases that participate in the synthesis of both leading and lagging strands. HSSB stimulates pol α activity on a primed template DNA and also DNA polymerase δ and ε activities in the presence of proliferating cell nuclear antigen and activator 1 (also known as replication factor C) (14, 19, 20).

The p34 subunit of HSSB is phosphorylated in a cell-cycle-dependent process. The phosphorylation occurs at the G_1/S transition of the cell cycle, and dephosphorylation occurs during mitosis (21). Dutta and Stillman (22) have shown that the cdc2–cyclin B complex phosphorylates the p34 subunit of HSSB complex *in vitro* at the sites necessary for its phosphorylation *in vivo* and also showed that the addition of the cdc2 kinase stimulated SV40 DNA replication *in vitro* with G_1-phase HeLa cell extracts. Furthermore, Fotedar and Roberts (23) observed that the phosphorylation of p34 *in vitro* only occurred after it associated with single-stranded DNA, possibly at the site where the replication initiation complex forms. Several reports also indicated that the cdc2 and/or its related kinase are involved in DNA replication (24–26), although no direct evidence has been presented that activation of DNA replication by cdc2 kinase is from the phosphorylation of p34 of HSSB.

We have examined the mechanism of HSSB complex formation by using coinfection of recombinant baculoviruses encoding the individual subunits. Purified baculovirus-derived HSSB efficiently replaced HSSB from HeLa cells in supporting SV40 DNA replication *in vitro*, as well as other reactions requiring HSSB.

MATERIALS AND METHODS

Cell Extracts, Proteins, and Antibodies. Cytosolic extracts, SV40 T antigen, HSSB, topoisomerase I, and the pol α–primase complex were isolated by published procedures (refs. 2, 19, 27, and 28, respectively). Purified cdc2–cyclin B was provided by Z.-Q. Pan (Sloan–Kettering Cancer Center). Antisera against HSSB p70 and p11 were prepared by immunizing rabbits and rats, respectively, with subunits isolated from SDS/PAGE. Antibodies against p70 and p34 were purified from hybridoma supernatants by protein A-Sepharose columns (29, 30).

Construction of Recombinant Baculoviruses. The cDNAs encoding p70, p34, and p11 were independently cloned from HeLa cell cDNA libraries (Stratagene) by using oligonucleotides deduced from peptide sequences of the proteins (S.-H.L., F.B.D., and J.H., unpublished results); the DNA sequences of our clones were the same as those published by others (31–33), except that our cDNA for p11 (0.89 kb) contained 5′- and 3′-untranslated regions longer than the reported one (0.69 kb). A *Nco* I–*Kpn* I fragment containing the entire coding region of p70 was isolated from a pBlue-

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Abbreviations: SV40, simian virus 40; SSB, single-stranded DNA-binding protein; HSSB, human SSB; ori$^+$DNA, origin-containing DNA; pol α, DNA polymerase α; mAb, monoclonal antibody; T antigen, large tumor antigen; primase, DNA primase.

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script vector and cloned into baculovirus transfer vector pVL941-SW (34). A *Bst*BI–*Bam*HI fragment was excised from a plasmid containing the full-length p34 cDNA in pBluescript vector and ligated to a synthetic linker (5′-GATCCATGTGGAACAGTGGATT-3′):(5′-CGAATC-CACTGTTCCACATG-3′) to create a *Bam*HI site at the 5′-end. The product was isolated by gel electrophoresis and cloned into the *Bam*HI site of pVL941 (35). For p11, PCR was done by using a Perkin–Elmer/Cetus DNA thermal cycler and a plasmid containing the full-length cDNA and two primers (5′-CCGCCATGGTGGACATGATG-3′ and 5′-CGCCATGGTCAATCATGTTGCA-3′), representing N- and C-terminal sequences with *Nco* I restriction sites. PCR (30 cycles) was done with 1-min denaturation at 94°C, 1 min hybridizing at 42°C, and 2-min extension of primers at 72°C. The PCR product was isolated from the gel, restricted with *Nco* I, and cloned into the *Nco* I site of pVL941-SW. Both strands of the insert were sequenced to confirm the sequence.

Insect Cell Culture and Baculovirus Infection. Insect cells, *Spodoptera frugiperda* (Sf9), were grown in Grace's medium (GIBCO)/10% fetal calf serum/0.33% (wt/vol) yeastolate/0.33% (wt/vol) lactalbumin hydrolysate/fungizone at 10 μg/ml/Gentamycin at 50 μg/ml) in spinner bottles. Baculovirus transfer vectors, each containing an HSSB subunit, were cotransfected with linearized baculovirus DNA (PharMingen) into Sf9 cells, and the recombinant viruses were purified using dot-blot hybridization with labeled oligonucleotide probes and plaque assays as described (35).

Coinfection, Metabolic Labeling, and Immunoprecipitation. Sf9 cells (2.0×10^6) were seeded onto a 60-mm dish and infected with different combinations of recombinant viruses, each at a multiplicity of infection of 30. After 40 hr at 27°C, the cells were labeled for 4 hr with Tran^{35}S-labeled methionine at 200 μCi/ml (1000 Ci/mmol; 1 Ci = 37 GBq) in 1.5 ml of methionine-free medium/5% dialyzed fetal calf serum. Cells were then washed and lysed as described (36). For *in vitro* experiments, the lysates were prepared in modified EBC buffer (36) with 0.2% Nonidet P-40 and without NaF, β-glycerophosphate, and sodium orthovanadate. Lysates (50 μl), clarified by centrifugation, were mixed with 0.15 ml of EBC buffer/bovine serum albumin (200 μg/ml) and then incubated with either purified monoclonal antibody (mAb) or antiserum for 3 hr at 4°C with rocking. Protein A (or protein G)-Sepharose was added, the mixture was incubated 1 hr at 4°C, and immunoprecipitates were collected by centrifugation, washed five times in EBC buffer, and separated by SDS/12% PAGE. Gels were fixed with 10% acetic acid and treated with 1 M sodium salicylate (pH 6.0) for 30 min and visualized by autoradiography.

Isolation of Recombinant HSSB Complex. One liter of Sf9 cells (10^6 cells per ml) was coinfected with recombinant viruses encoding p70, p34, and p11, each at a multiplicity of infection of 30. After 48 hr, cells were harvested, lysed with EBC buffer/0.25 M NaCl, and the mixture was centrifuged (35,000 rpm in a Beckman Ti45 rotor; 4°C; 30 min). The supernatant contained ≈20% of the expressed protein subunits (the remainder was in the pellet) present in an equal ratio as judged by immunoblot analysis (data not shown). The supernatant (325 mg of protein) was adjusted to 0.5 M NaCl and applied to a single-stranded DNA cellulose column (20 mg of protein per ml of bed volume), which was then washed successively with buffers containing 0.5 M and 0.8 M NaCl. HSSB was eluted with buffer/1.5 M NaCl/40% (vol/vol) ethylene glycol. The eluate (1.2 mg of protein) containing HSSB, confirmed by immunoblot analysis, was dialyzed against 2 liters of buffer A [25 mM Tris·HCl, pH 7.5/1 mM dithiothreitol/10% (vol/vol) glycerol/0.5 mM EDTA/0.1 mM phenylmethylsulfonyl fluoride/0.02% Nonidet P-40] containing 0.15 M NaCl. The dialyzed sample was adjusted to 0.05 M NaCl with buffer A and applied to a Mono Q column (Pharmacia). The protein was eluted with a linear NaCl gradient (0.05–0.4 M), and the fractions were screened for HSSB activities.

SV40 DNA Replication *in Vitro*. Reaction mixtures (40 μl) were the same as described by Wobbe *et al.* (2).

DNA Unwinding Assay. The unwinding of SV40 ori$^+$DNA (pSV01ΔEP) in the presence of the recombinant baculovirus HSSB was done as described (12). Reactions (30 μl) were as described for the replication assay with 300 units of topoisomerase I, 0.6 μg of SV40 T antigen, 4 mM of ATP, and the indicated source and amount of HSSB. The reaction was stopped by adding 10 μl of 0.1 M EDTA, proteinase K (1 mg/ml), 2% SDS, and glycogen (1 mg/ml); then this mixture was incubated at 37°C for 15 min. Samples were ethanol-precipitated and electrophoresed at 110 V for 3.5 hr in a vertical agarose gel (1.5%).

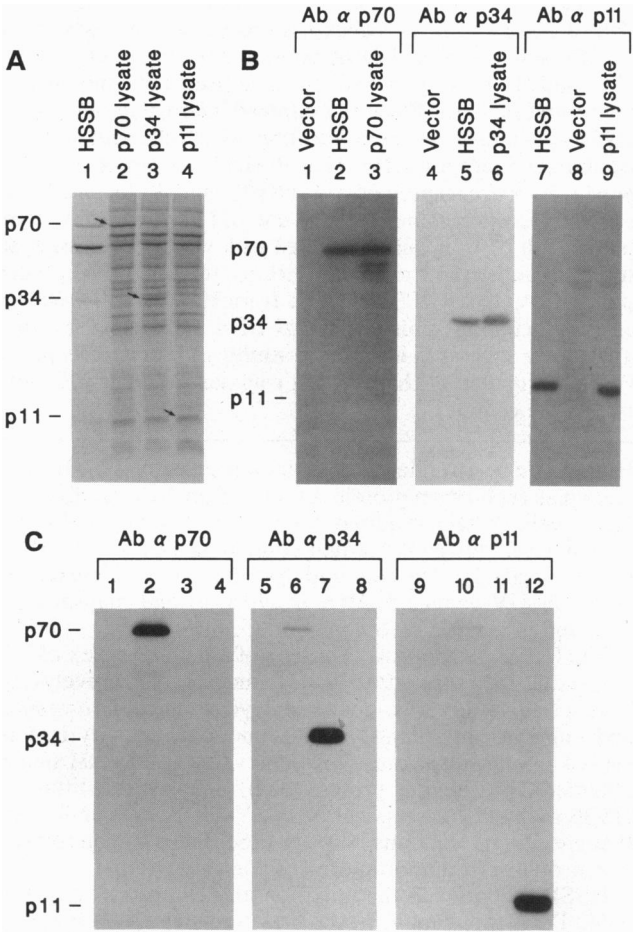

FIG. 1. Expression of HSSB subunits in Sf9 cells infected with recombinant baculovirus. (*A*) Insect cells infected with p70, p34, and p11 were lysed with SDS/PAGE sample buffer, analyzed by SDS/14% PAGE, and stained with Coomassie blue. Position of each subunit is indicated at left, and arrows denote each expressed subunit. (*B*) Lysates were prepared and electrophoresed as described above. For immunoblotting, proteins were transferred to a nitrocellulose filter, incubated with either a 1:2000 dilution of anti-p70 (lanes 1–3), 20 μg of purified mAb against p34 (lanes 4–6), or a 1:500 dilution of anti-p11 (lanes 7–9) followed by detection of antibodies with ^{125}I-labeled protein A (Amersham). The Vector lane indicates lysate prepared from cells infected with wild-type baculovirus. (*C*) [^{35}S]Methionine-labeled lysates from cells infected with vector alone (lanes 1, 5, and 9), p70 (lanes 2, 6, and 10), p34 (lanes 3, 7, and 11), and p11 (lanes 4, 8, and 12) were prepared as described. The lysates were incubated with antibodies against p70 (lanes 1–4), p34 (lanes 5–8), or p11 (lanes 9–12) as described in *B*, and the samples were precipitated with either protein A-Sepharose (lanes 1–4) or protein G-agarose (lanes 5–12) and analyzed by SDS/14% PAGE.

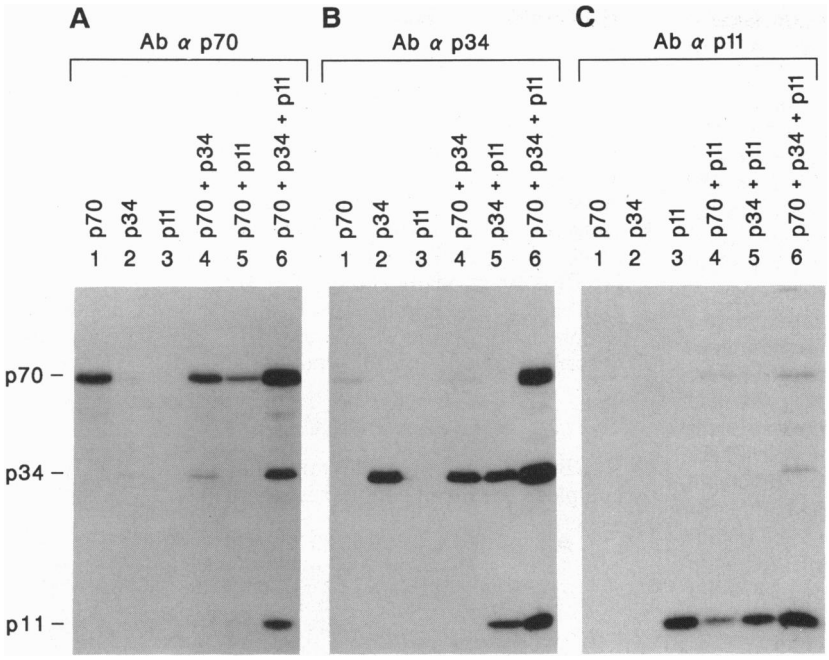

FIG. 2. Complex formation of various HSSB subunits *in vivo*. Lysates, singly or coinfected with different combinations of recombinant viruses, are indicated at top of *A–C*. Lysates were incubated with anti-p70 from rabbit (*A*), p34 mAb (Ab) (*B*), or anti-p11 from rat (*C*) as described. The immune complexes were precipitated with either protein A-Sepharose (*A*) or protein G-agarose (*B* and *C*) and analyzed by SDS/14% PAGE.

RESULTS

Recombinant Baculovirus Expression of HSSB Subunits. Insect cells (Sf9) infected with recombinant viruses encoding individual subunits expressed intact p70, p34, and p11, as shown by SDS/PAGE and immunological analysis of whole-cell lysates (Fig. 1). Our antibodies to the individual subunits of HSSB did not cross-react with insect SSB either by immunoblotting or immunoprecipitation techniques (Fig. 1 *B* and *C*, respectively). This result was not surprising because antibodies against HSSB also do not cross-react with yeast (21) or mouse SSBs (22). The human p34 subunit expressed in insect cells separated into doublet bands on SDS/PAGE (Fig. 1*B*, lane 6), probably representing phosphorylated (slower migrating) and unphosphorylated (faster migrating) forms reported in HeLa cells (21).

p11 Forms a Stable Complex with p34 *in Vivo* and Is Required for p70 Interaction. When cells were coinfected with p70 and p34, <10% of the expressed p34 immunoprecipitated with anti-p34 (Fig. 2*B*, lane 4), judging from densitometric scanning normalized to the number of methionine residues in each subunit (14, 9, and 6 methionine residues were present in p70, p34, and p11, respectively). This result indicates a weak or unstable interaction between p34 and p70 *in vivo*. Likewise, p70 and p11 showed little interaction (Fig. 2 *A*, lane 5, and *C*, lane 4). When cells were coinfected with recombinant viruses encoding p34 and p11 subunits, however, a stoichiometric amount of p34–p11 complex (p11/p34, 1:1.2) immunoprecipitated with p34 antibody (Fig. 2*B*, lane 5), indicating a strong interaction between these subunits. When cells were coinfected with the three different recombinant viruses, all three subunits coimmunoprecipitated with antibodies to p70 or p34 (Fig. 2 *A* and *B*, lanes 6, respectively). Rat polyclonal antibodies against p11 immunoprecipitated free p11 predominantly and only small amounts of the other subunits (Fig. 2*C*, lanes 4–6). As addressed below, this result is probably because our p11 antibody does not recognize p11 complexed to other subunits.

p11 Forms a Stable Complex with p34 *in Vitro*. Because coinfection showed that the three HSSB subunits assembled into a complex *in vivo*, we asked whether the same subunits could form a complex *in vitro*. Lysates of singly infected cells were mixed, incubated on ice, and then immunoprecipitated (Fig. 3). Similar to what was observed *in vivo*, only background amounts of p34 or p11 were complexed with p70 when p70–p34 or p70–p11 mixtures were precipitated with anti-p70 (Fig. 3*A*, lanes 4 and 5). Anti-p34 precipitated p34 complexed

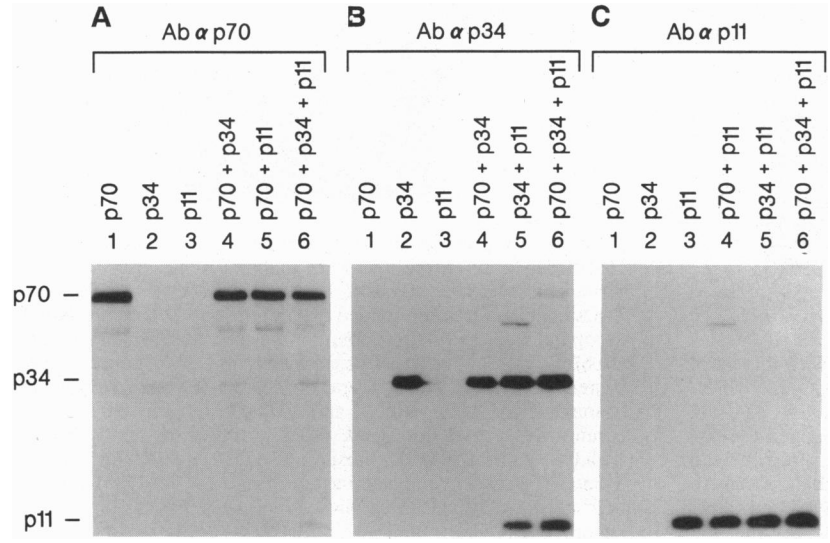

FIG. 3. Complex formation between HSSB subunits *in vitro*. Lysates, singly infected with p70, p34, and p11, were mixed as indicated and incubated on ice for 1 hr. Complexes formed *in vitro* were precipitated with the antibodies (Abs) against p70 (*A*), p34 (*B*), and p11 (*C*), and the immune complex was analyzed as described for Fig. 2.

with p11, but not p34 complexed with p70 (Fig. 3*B*, lanes 4 and 5). As seen *in vivo*, only small amounts of p34 were precipitated by anti-p11 (Fig. 3*C*, lane 5).

One difference noted between complex formation *in vivo* and *in vitro* was that *in vitro* the addition of p70 to the p34–p11 complex was less efficient than seen *in vivo* (compare Fig. 3 *A* and *B*, lanes 6, with Fig. 2 *A* and *B*, lanes 6). Because p70 binds single-stranded DNA, it was possible that DNA in the crude lysates inhibited addition of p70 to the p34–p11 complex. However, pretreatment of p70 lysate with DNase I had no effect on complex formation (data not shown).

p11 Is in the Pocket of p34 of the Complex. Even though p11 formed a stable complex with p34 *in vitro* that was immunoprecipitated by anti-p34, p34 was not detected when an extract of cells was precipitated with anti-p11 (compare Fig. 2 *C* and *B*, lanes 5). This was also the case in the *in vitro* experiment (compare Fig. 3 *C* and *B*, lanes 5). One explanation for these observations is that the p11 epitope(s) for the polyclonal antibody is in contact with p34 and thus unavailable for interaction with anti-p11. Thus, only free p11 could be immunoprecipitated with anti-p11 from extracts coinfected with viruses expressing p34 and p11. Similarly, p70 and p34 would be poorly precipitated with anti-p11 (Fig. 2*C*, lane 6) from cells coinfected with viruses expressing all three subunits.

If the epitopes of p11 recognized by anti-p11 were in contact with p34, incubation of p11 with anti-p11 before p34 addition should prevent p34–p11 complex formation. Preincubation of p11 lysate with increased amounts of anti-p11 reduced the amount of p11 coprecipitated with anti-p34 plus anti-mouse IgG-agarose (Fig. 4, lanes 5–8). The anti-mouse IgG interacted specifically with the p34 mAb because no p11 was detected in reactions containing only p11 lysate (Fig. 4, lane 9). Neither the mAb against p34 (lanes 1–4) nor the rat preimmune serum (lane 10) had any effect on p34–p11 complex formation *in vitro*.

Recombinant Baculovirus HSSB Is Functionally Active. HSSB was isolated from insect cells coinfected with recombinant viruses encoding all three HSSB subunits. The chromatographic behavior of recombinant HSSB was the same as HSSB from HeLa cells. In addition, both HSSBs sedimented at 5.0S on glycerol-gradient centrifugation (data not shown). The purified recombinant HSSB (250 μg from 1-liter culture of Sf9 cells) was at least 95% pure as judged by SDS/PAGE analysis followed by silver staining. Mono Q fractions (linear NaCl gradient) were examined for HSSB activities in SV40 DNA replication and DNA unwinding and were also analyzed by SDS/PAGE (Fig. 5). Three protein bands of sizes 70, 34, and 11 kDa were coeluted from the Mono Q column (Fig. 5*A*), and these were the subunits of HSSB, as confirmed by immunoblotting analysis (data not shown). When the Mono Q fractions were assayed for HSSB activity in SV40 DNA replication *in vitro*, the peak fractions (fractions 36 and 38) containing three subunits of HSSB supported the replication activity (Fig. 5*B*). In addition, these fractions, when incubated with SV40 T antigen, topoisomerase I and ATP, yielded highly unwound superhelical DNA, form U, from SV40 ori$^+$DNA. These results indicate that the recombinant HSSB is functionally active. The Mono Q pool of recombinant HSSB also stimulated the elongation of primed DNA templates by purified pol α and DNA polymerase δ under described conditions (14) (data not shown).

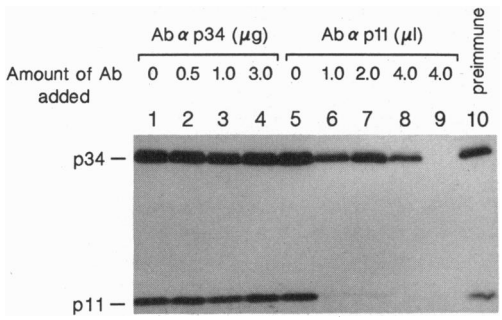

FIG. 4. Effects of antibodies (Abs) on formation of p34–p11. The [^{35}S]methionine-labeled p34 (lanes 1–4) and p11 (lanes 5–8) lysates (20 μl per reaction) were pretreated for 1 hr on ice with increased amounts of p34 mAb (lanes 1–4) and anti-p11 (lanes 5–9), respectively, followed by addition of the other lysate to allow p34–p11 complex formation. Lane 9 was same as lane 8 but lacked the p34 lysate, and lane 10 was pretreated with rat preimmune serum instead of anti-p11. All samples were immunoprecipitated with the p34 mAb and antimouse IgG-agarose (HyClone) and were analyzed on SDS/12% PAGE.

DISCUSSION

HSSB contains three subunits that form a highly stable complex. The intact complex is essential for DNA replication

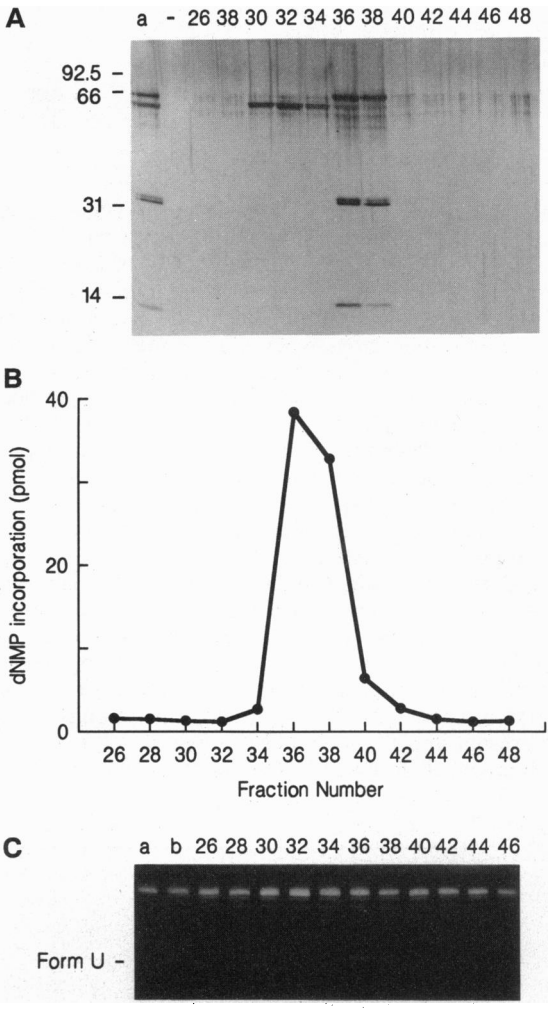

FIG. 5. Analysis of Mono Q fractions of recombinant baculovirus HSSB. (*A*) Mono Q fractions were analyzed by SDS/14% PAGE and visualized by silver staining. Lane a denotes the single-stranded DNA cellulose fraction loaded onto the Mono Q column. Numbers at top indicate the Mono Q column fraction. Size markers are at left. (*B*) Mono Q fractions (2 μl) were added to the SV40 monopolymerase system containing pol α–primase (0.3 and 0.6 unit, respectively), topoisomerase I (10^3 units), and SV40 T antigen (0.6 μg). Other conditions were as described. (*C*) The unwinding assay was done with 0.6 μg of T antigen, topoisomerase I (10^3 units), and 2 μl of each Mono Q fraction as indicated at top. Lanes: a, no HSSB; b, 0.8 μg of single-stranded DNA cellulose fraction. Form U, highly wound superhelical DNA.

because antibodies against p70, p34, and p11 inhibit SV40 replication and individual subunits (p70 or p34) cannot support DNA replication (29, 31–33). Our work suggests a molecular mechanism for formation of the HSSB complex. Initially, p34 complexes with p11 to which p70 is added. The *in vivo* formation of the HSSB complex from subunits expressed by recombinant baculovirus clones probably reflects the complex formation in human cells because the purified recombinant HSSB is the same as HSSB isolated from HeLa cells in its physical and functional properties. *In vitro*, efficient complex formation between p34 and p11 occurred, but p70 addition was inefficient. Treatment of p34 lysates with alkaline phosphatase or the cdc2–cyclin B kinase complex had no effect on p70 addition to the p34–p11 complex *in vitro* (data not shown). Incubation of lysate mixtures in the presence of single-stranded ϕX174 DNA or at 25°C or 37°C also was without effect (data not shown).

The role of each subunit in the activities associated with HSSB has not been clearly defined. The p70 directly binds single-stranded DNA, whereas the other subunits do not (3, 29, 32). Recombinant p70 substitutes for the multisubunit HSSB in stimulating pol α activity on a primed template DNA (32) but does not substitute for the HSSB complex in SV40 DNA replication *in vitro*. mAbs directed against p34 as well as antibodies against p11 block SV40 DNA replication, in keeping with important roles for these subunits in replication (29, 32, 33). From our study, p11 is clearly essential for formation of the HSSB complex. Interestingly, our polyclonal antibodies to p11 inhibited formation of the p34–p11 complex but failed to precipitate HSSB complexes or to neutralize the HSSB activity required for SV40 DNA replication. The epitopes of p11 may be buried due to the interaction of p11 with p34.

In yeast, all three genes appear essential for cell viability. Disruption of any one of them yields cells arrested in budded stages (8). This lethality, however, has not been shown to be due to their inability to replicate DNA. Based on our data, disruption of either the p34 or the p11 gene should render cells unable to form the three-subunit HSSB complex; this inability could block DNA replication.

Din *et al.* (21) suggested that the phosphorylation of p34 depends on its association with the other HSSB subunits. Fotedar and Roberts (23) showed that the phosphorylation of p34 occurred during replication and was catalyzed by a kinase(s) (not a cdc2 kinase) associated with the replication complex. However, free human p34 expressed from Sf9 cells infected with recombinant p34 baculovirus contained two distinct bands on SDS/PAGE, representing phosphorylated and unphosphorylated forms (Fig. 1*B*, lane 6). The *E. coli*-expressed free p34 also can be phosphorylated by cdc2–cyclin B complex *in vitro* (data not shown). These results suggest that free p34 can be phosphorylated directly in the absence of other subunits or DNA. Whether the sites phosphorylated on free p34 are the same as those phosphorylated in the HSSB complex is unknown. Both phosphorylated and unphosphorylated forms of p34 were coprecipitated with p70 and p11 when the cotransfected lysates were immunoprecipitated with p70 antibody (Fig. 2*A*, lane 6), indicating that the phosphorylation of p34 is not necessary to maintain the HSSB complex. This result is consistent with the fact that most of the HSSB complexes purified from HeLa cells contain unphosphorylated p34 (9). It will be of interest to determine whether a mutant p34 that lacks the phosphorylation sites (22) can still support complex formation of HSSB *in vivo*.

Even though the level of each HSSB subunit remains constant throughout the cell cycle (21), posttranslational regulation of HSSB activity via phosphorylation or restriction of HSSB complex formation at specific stages may operate to control its activity in a cell-cycle-dependent manner. The three-subunit structure specific for eukaryotic SSBs implies that they have functions not possessed by prokaryotic SSBs. Identification of the function of the individual subunits will be crucial to understanding their role in replication and possibly the regulation of cell-cycle-dependent replication.

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1. Fairman, M. P. & Stillman, B. (1988) *EMBO J.* **7**, 1211–1218.
2. Wobbe, C. R., Weissbach, L., Borowiec, J. A., Dean, F. B., Murakami, Y., Bullock, P. & Hurwitz, J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1834–1838.
3. Wold, M. S. & Kelly, T. J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2523–2527.
4. Coverly, D., Kenny, M. K., Munn, M., Rupp, W. D., Lane, D. P. & Wood, R. D. (1991) *Nature (London)* **349**, 538–541.
5. Alani, E., Thresher, R., Griffith, J. D. & Kolodner, R. D. (1992) *J. Mol. Biol.* **227**, 54–71.
6. Heyer, W.-D., Rao, M. R. S., Erdile, L. F., Kelly, T. J. & Kolodner, R. D. (1990) *EMBO J.* **9**, 2321–2329.
7. Brill, S. J. & Stillman, B. (1989) *Nature (London)* **342**, 92–95.
8. Brill, S. J. & Stillman, B. (1991) *Genes Dev.* **5**, 1589–1600.
9. Melendy, T. & Stillman, B. (1993) *J. Biol. Chem.* **268**, 3389–3395.
10. Mitsis, P. G., Kowalczykowski, S. C. & Lehman, I. R. (1993) *Biochemistry* **32**, 5257–5266.
11. Borowiec, J. A., Dean, F. B., Bullock, P. A. & Hurwitz, J. (1990) *Cell* **60**, 181–184.
12. Dean, F. B., Bullock, P., Murakami, Y., Wobbe, C. R., Weissbach, L. & Hurwitz, J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 16–20.
13. Wold, M. S., Li, J. J. & Kelly, T. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3643–3647.
14. Kenny, M. K., Lee, S.-H. & Hurwitz, J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9757–9761.
15. Matsumoto, T., Eki, T. & Hurwitz, J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9712–9716.
16. Collins, K. L. & Kelly, T. J. (1991) *Mol. Cell. Biol.* **11**, 2108–2115.
17. Murakami, Y. & Hurwitz, J. (1993) *J. Biol. Chem.* **268**, 11008–11017.
18. Dornreiter, I., Erdile, L. F., Gilbert, I. U., von Winkler, D., Kelly, T. J. & Fanning, E. (1992) *EMBO J.* **11**, 769–776.
19. Lee, S.-H., Pan, Z. Q., Kwong, A. D., Burgers, P. M. J. & Hurwitz, J. (1991) *J. Biol. Chem.* **266**, 22707–22717.
20. Tsurimoto, T. & Stillman, B. (1989) *EMBO J.* **8**, 3883–3889.
21. Din, S.-U., Brill, S. J., Fairman, M. P. & Stillman, B. (1990) *Genes Dev.* **4**, 968–977.
22. Dutta, A. & Stillman, B. (1992) *EMBO J.* **11**, 2189–2199.
23. Fotedar, R. & Roberts, J. M. (1992) *EMBO J.* **11**, 2177–2187.
24. Blow, J. J. & Nurse, P. (1990) *Cell* **62**, 855–862.
25. D'Urso, G., Marraccino, R. L., Marshak, D. R. & Roberts, J. M. (1990) *Science* **250**, 786–791.
26. Fang, F. & Newport, J. W. (1991) *Cell* **66**, 731–742.
27. Ishimi, Y., Claude, A., Bullock, P. & Hurwitz, J. (1988) *J. Biol. Chem.* **263**, 197231–9733.
28. Mastrangelo, I. A., Hough, P. V. C., Wall, J. S., Dodson, M., Dean, F. B. & Hurwitz, J. (1989) *Nature (London)* **338**, 658–662.
29. Kenny, M. K., Schlegel, U., Furneaux, H. & Hurwitz, J. (1990) *J. Biol. Chem.* **265**, 7693–7700.
30. Harlow, E. & Lane, D. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
31. Erdile, L. F., Wold, M. S. & Kelly, T. J. (1990) *J. Biol. Chem.* **265**, 3177–3182.
32. Erdile, L. F., Heyer, W.-D., Kolodner, R. & Kelly, T. J. (1991) *J. Biol. Chem.* **266**, 12090–12098.
33. Umbright, C. B., Erdile, E. F., Jabs, E. W. & Kelly, T. J. (1993) *J. Biol. Chem.* **268**, 6131–6138.
34. Frappier, L. & O'Donnell, M. (1991) *J. Biol. Chem.* **266**, 7819–7826.
35. Summer, M. & Smith, G. (1988) *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures* (Texas Agricultural Experiment Station, College Station), Bull. 1555.
36. Kato, J.-Y., Matsushime, H., Hiebert, S. W., Ewen, M. E. & Sherr, C. J. (1993) *Genes Dev.* **7**, 331–342.