

A B-cell-specific DNA Recombination Complex*

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We have purified and biochemically characterized a multiprotein complex designated SWAP. In a DNA transfer assay, SWAP preferentially recombines (“swaps”) sequences derived from Ig heavy chain switch regions. We identified four of the proteins in the SWAP complex: B23 (nucleophosmin), C23 (nucleolin), poly(ADP-ribose) polymerase (PARP), and SWAP-70. The first three are proteins known to be present in most cells. B23 promotes single-strand DNA reannealing and the formation of joint molecules in a D-loop assay between homologous, but also between S_{μ} and S_{γ} sequences. SWAP-70 is a novel protein of 70 kDa. Its cDNA was cloned and sequenced, and the protein was overexpressed in *Escherichia coli*. SWAP-70 protein expression was found only in B lymphocytes that had been induced to switch to various Ig isotypes and in switching B-cell lines. SWAP-70 is a nuclear protein, has a weak affinity for DNA, binds ATP, and forms specific, high affinity complexes with B23, C23, and poly(ADP-ribose) polymerase. These findings are consistent with SWAP being the long elusive “switch recombinase” and with SWAP-70 being the specific recruiting element that assembles the switch recombinase from universal components.

The constant (C)¹ region of its heavy (H) chain determines the class of an Ig molecule. Mouse H chains are designated μ , δ , $\gamma 3$, $\gamma 1$, $\gamma 2b$, $\gamma 2a$, ϵ , and α . Upon stimulation by antigen, expression of the early IgM class usually changes to that of another class (IgG3, IgG1, IgG2b, IgG2a, IgE, or IgA). At the DNA level, the process is mediated by recombination that generally involves DNA transfer between a switch (S) region within the intron 5' to C_{μ} and another S region within the intron 5' to the particular C-gene segment that is to be expressed (1–5).

S regions, several kilobase pairs in length, contain short, G-rich repetitive sequence elements, often arranged in tandem arrays. They may adopt secondary structures, such as stem loops, and many break points are located at such structures (6). In marked contrast to those observed in V(D)J rearrangement (7–11), the switch recombination break-and-rejoining points

are imprecise. They lie scattered all over the S regions or, less often, even outside the S regions. Thus, class switch recombination is region-specific rather than site-specific. Rearrangements combining three S regions are occasionally detected (12), and, although switch recombination is usually intrachromosomal (13), intermolecular rearrangements have also been observed (14).

Following transcriptional activation (3, 15), switch rearrangements generally result in deletion of the DNA sequences between the two breakpoints. The mechanism for most class switching events can be described by a loop-excision model (16). The exact structure of the loop intermediate is unknown; it may resemble a crossed (α) or a stem loop (Ω) conformation. In that model, after loop formation, four DNA ends are generated by endonucleolytic cleavage of the two S regions to be recombined; these ends are possibly protected or even fixed by proteins. Either (i) the four ends can be rejoined in their original configuration, (ii) the four ends can be inverted, or (iii) the intervening sequence can be circularized and removed while the remaining chromosomal DNA ends are joined together to yield the switched gene. Both inversions (16) and excised circular DNAs, the so-called switch circles of various sizes (17, 18), were observed *in vivo*, but mechanistic details of the reaction remain unknown.

To date, no isolation and biochemical characterization of an S-region-specific recombination activity has been reported (19), although several S-region-binding proteins have been described, primarily in gel migration shift experiments (20–23). To our knowledge, no recombination-specific function or recombination-related enzyme activity has yet been described for these proteins. We have screened extracts from switching B-cells for activities that preferentially recombine S-regions. Our assay measures stable DNA transfer, a central step of DNA recombination, from a plasmid containing S_{μ} to a plasmid containing another S region. A similar DNA transfer assay was instrumental in isolating a mammalian protein complex that repairs DNA gaps and deletions through recombination of homologous DNA substrates (24–26).

Here we report the isolation of a protein complex—we call it SWAP—with a recombination activity that is specific for switching B-cells and prefers S-region substrates. We also report the identity of four of the proteins in the SWAP complex: B23 (nucleophosmin), C23 (nucleolin), poly(ADP-ribose) polymerase (PARP), and SWAP-70. B23 promotes single-strand DNA reannealing and the formation of joint molecules in a D-loop assay between homologous sequences but also between S_{μ} and S_{γ} sequences. SWAP-70 is a novel protein of 70 kDa. Its cDNA was cloned and sequenced, and the protein was overexpressed in *Escherichia coli*. SWAP-70 protein expression was found only in B lymphocytes that had been induced to switch and in switching B-cell lines. SWAP-70 is a nuclear protein, has a weak affinity for DNA, binds ATP, and forms specific, high affinity complexes with B23, C23, and PARP.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF053974.

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¹ The abbreviations used are: C, constant; H, heavy; bp, base pair(s); DTA, DNA transfer assay; ds, double-stranded; ss, single-stranded; nt, nucleotide(s); EPPS, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid; LPS, lipopolysaccharide; DTT, dithiothreitol; S, switch region; ConA, concanavalin A.

EXPERIMENTAL PROCEDURES

DNA Transfer Assay (DTA)—The DTA was done as described (24, 25). Input ^3H radioactivity was between 150,000 and 350,000 cpm and the same for each experimental series. The S_{μ} substrate consisted of an M13 double-stranded (ds) DNA carrying a 1.3-kilobase pair *Hind*III S_{μ} fragment (27), and was labeled with digoxigenin (24). The S_{γ} plasmid consisted of pSP72 containing a 3.7-kilobase *Eco*RI-*Hind*III $S_{\gamma 2b}$ fragment (28) and was internally labeled with [^3H]thymidine. For the standard DNA transfer assay, 0.18 μg of the ^3H -labeled DNA substrate (e.g. pSP- S_{γ}) was incubated together with 0.02 μg of the digoxigenin-labeled substrate (e.g. M13- S_{μ}) and varying amounts of protein in 50 μl containing 3 mM MgCl_2 , 30 mM EPPS, pH 7.4, 1 mM DTT, less than 50 mM ammonium sulfate, and 1 mM ATP. After 6 min, the reaction was terminated by the addition of SDS to 0.05% and EDTA to 50 mM. Further processing was as described (24) and included a phenol-chloroform extraction step of the DNA prior to binding the products to anti-digoxigenin beads. The activity is expressed as a percentage of input ^3H cpm, stably transferred to the recipient and bound to the beads.

Purification of the SWAP—The protein purification procedures were carried out at 4 °C, and are summarized in Table I. Nuclear extracts were prepared as described (25) from 1×10^8 to 8×10^8 lipopolysaccharide (LPS) (50 $\mu\text{g}/\text{ml}$) blasts (0.7 mg of nuclear protein/ 10^8 cells). Fraction I (2 mg of protein) was loaded onto a Superdex 200 FPLC gel filtration column (Amersham Pharmacia Biotech) and fractionated at a flow rate of 1 ml/min in Buffer E (5 mM KCl, 5 mM MgCl_2 , 2 mM DTT, 0.2 mM EDTA, 15 mM Tris-HCl, pH 7.5 at 4 °C, and 1 mM PMSF, 10 mM $\text{Na}_2\text{S}_2\text{O}_5$, 1 $\mu\text{g}/\text{ml}$ aprotinin, 0.5 $\mu\text{g}/\text{ml}$ TLCK, 0.7 $\mu\text{g}/\text{ml}$ pepstatin A) containing 80 mM ammonium sulfate. 1.4-ml fractions were collected, active fractions were pooled (5.6 ml, 0.6 mg of protein, Fraction II), diluted 1:4 with Buffer E, and loaded at 1 ml/min onto a 1-ml Macro S cation exchange FPLC column (Bio-Rad). After washing the column with 20 column volumes of Buffer E-20 (E plus 20 mM ammonium sulfate), the proteins were eluted at a 1 ml/min flow rate with a gradient from 20 to 600 mM ammonium sulfate in Buffer E in 1.2-ml fractions. The switch-specific activity (2.4 ml, 0.011 mg of protein, Fraction III) eluted in two fractions at around 280 mM ammonium sulfate. For further purification, Fraction III was diluted 1:2 with Buffer E and loaded at 0.3 ml/min onto a 1 ml blue Sepharose (HiTrap, Amersham Pharmacia Biotech) FPLC column (pre-equilibrated in E-140). Elution was with a linear gradient from 0 to 1000 mM ammonium sulfate in Buffer E, and the activity eluted between 740 and 810 mM (0.6 ml, 6 ng of protein, Fraction IV). On ice or at -70 °C, the active fractions were stable for a short period only; frozen in liquid nitrogen, samples remained active for at least several weeks.

Cloning and Overexpression of the SWAP-70 cDNA—The gene encoding SWAP-70 was cloned using a polymerase chain reaction strategy based on degenerate oligonucleotides derived from the N-terminal 26 amino acid residues and subsequent screening of a λ -ZAP mouse spleen cDNA library (Stratagene Inc.) with a nondegenerate oligonucleotide. The cDNA contained in the plasmid vector pBluescriptSK was excised from the phages, and the sequence of the SWAP-70 cDNA was determined. The cDNA was subcloned into the pQE-30 vector (Qiagen, Inc.) for expression as a His-tagged protein in *E. coli*. Purification of the protein was according to standard methods, including lysozyme-lysis of the cells, chromatography of the clear cell extract on a nickel-Sepharose column and elution at 80 mM imidazole, followed by gel filtration on a Superdex75 FPLC column (Amersham Pharmacia Biotech).

DNA and ATP Binding—Gel retardation were performed in 10- μl reaction mixtures containing 0.2–1 ng (3000–6000 cpm) of ^{32}P -end-labeled DNA (230-bp *Eco*RI-KasI M13mp18 DNA fragment, or 49-nt ss oligonucleotide) in 20 mM HEPES (pH 7.5), 1 mM DTT, 100 $\mu\text{g}/\text{ml}$ bovine serum albumin, and protein as indicated. After 20 min of incubation at room temperature, DNA-protein complexes were resolved by electrophoresis at 4 °C in nondenaturing polyacrylamide gels in 20 mM HEPES (pH 7.5), 0.1 mM EDTA. All gels were fixed (60 min in 10% acetic acid, 10% ethanol), dried, and exposed for autoradiography.

For azido-ATP labeling (29), reactions were carried out in 200 μl of 20 mM Tris-HCl, pH 7.5, 10 mM MgCl_2 , 0.1 mM DTT, 5 μM 8- N_3 -[α - ^{32}P]ATP (9–20 Ci/mmol; ICN), and proteins (200 ng). Reaction mixtures were incubated on ice for 60 min and then irradiated for 50 s at a distance of 10 cm from a XL-1500 UV cross-linker (Spectronics Co.). After irradiation, proteins were precipitated with trichloroacetic acid and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography.

DNA Renaturation and D-loop Assays—The DNA single-strand reannealing assay (3' ^{32}P -labeled, 422-nt DNA substrates) was performed as described (26).

For the standard D-loop assay, 100 ng (24 fmol) of predominantly supercoiled plasmid pUC- S_{γ} DNA or, for controls, DNA linearized outside the region of homology (*Asp*700 restriction enzyme digest) and 0.8 ng (100 fmol) of 5'- ^{32}P -labeled, 49-nt ss S_{γ} consensus sequence oligonucleotide (5'-GGGACCAGTCCTAGCAGCTGTGGGGGAGCTGGGGGAA-GGTGGGAGTGTGA; G/C content, 65%) (21) were incubated together with protein for 30 min at 37 °C in the standard DNA transfer reaction buffer (20 mM EPPS, pH 7.4, 5 mM MgCl_2 , 1 mM DTT). Reactions were stopped by addition of SDS to 0.1% and 4 μg of proteinase K and further incubation at 37 °C for 45 min. Routinely, products were analyzed in 0.7% agarose gels containing 3 mM magnesium acetate in the TAE buffer system. Gels were run at 2 V/cm for 16–24 h, stained with ethidium bromide, photographed to determine positions of the duplex DNA bands, dried, and exposed for autoradiography for 2–24 h. In later experiments, the magnesium acetate in the agarose gels was found not to be necessary, and the gels could be run at 4 V/cm without reducing the yield of joint molecules. For quantification of products, the ethidium bromide-stained bands were excised, the agarose was melted, and the ^{32}P radioactivity was directly measured in a scintillation counter. The percentage of input ^{32}P -oligonucleotide paired with the duplex DNA in the joint molecules was calculated. Many variations of the assay were performed and are described under "Results." Some used an oligonucleotide, CS-3, containing the first 25 nt of the 49-nt S_{γ} sequence (G/C content, 68%) or the 22-nt T7 oligonucleotide (5'-GTAATACGACTCAC-TATAGGGC) (G/C content, 45%), which is fully homologous to the plasmid pBluescriptSK. The pH variations were based on compounds of the polysulfonate buffer family.

RESULTS

A DNA Transfer Activity with Preference for S Regions—To assay for a DNA transfer activity that prefers S-region substrates, we labeled a plasmid (pSP72) containing $S_{\gamma 2b}$ sequences with [^3H]thymidine and ds M13 containing S_{μ} sequences with a small number of digoxigenin ligands. Recombinant DNA molecules were quantified by counting ^3H in plasmid DNA that had been immunoprecipitated by an anti-digoxigenin antibody (Fig. 1A); in this way, a direct and quantitative measurement of the catalytic activity leading to DNA transfer was obtained (19, 24). To an equimolar mixture of these two constructs, we added nuclear extracts from untreated spleen cells or from spleen cells cultivated for 2–3 days in the presence or absence of LPS (Fig. 1B). Because these cultures contain non-B lymphocytes and other nonswitching cells, we separated the (switching) blasts from the nondividing (nonswitching) cells according to size by cell elutriation (30) and tested the nuclear extracts prepared from both cell pools for DNA transfer activity on S substrates *versus* non-S substrates (Fig. 1B).

Despite the expected presence of general DNA transfer activities in such an extract (24, 25), the crude nuclear extracts from LPS blasts recombined S region substrates 2–3-fold better than non-S region substrates (Fig. 2A); *i.e.* a newly induced, specific activity is added on top of the general DNA swapping. This activity, called SWAP, is not present in extracts from nonswitching cells (Fig. 2A) or in extracts from thymus cells (not shown).

We purified SWAP by eluting it from a sizing column at a position corresponding to a molecular mass of globular proteins of 200–300 kDa (Fraction II). This fraction showed a 4-fold preference for S region substrates and was inactive when isolated from nonswitching cells (Fig. 2A); no other previously observed DNA transfer assay activity, *e.g.* from HeLa cells or calf thymus (24, 25), eluted at that position. The SWAP was further purified by cation exchange and blue Sepharose chromatography yielding Fraction IV with a preference for S region substrates of 10-fold (Figs. 2B and 3). With these purification steps, the specific activity increased more than 1000-fold (Table I). When an unrelated DNA, *e.g.* from M13, SV40, or $\Phi\text{X}174$, was paired with an S region, SWAP activity was reduced, on the average, by 6-fold (Fig. 2B). Fully homologous DNA substrates (5.7 kilobase pairs) recombined with less than half the

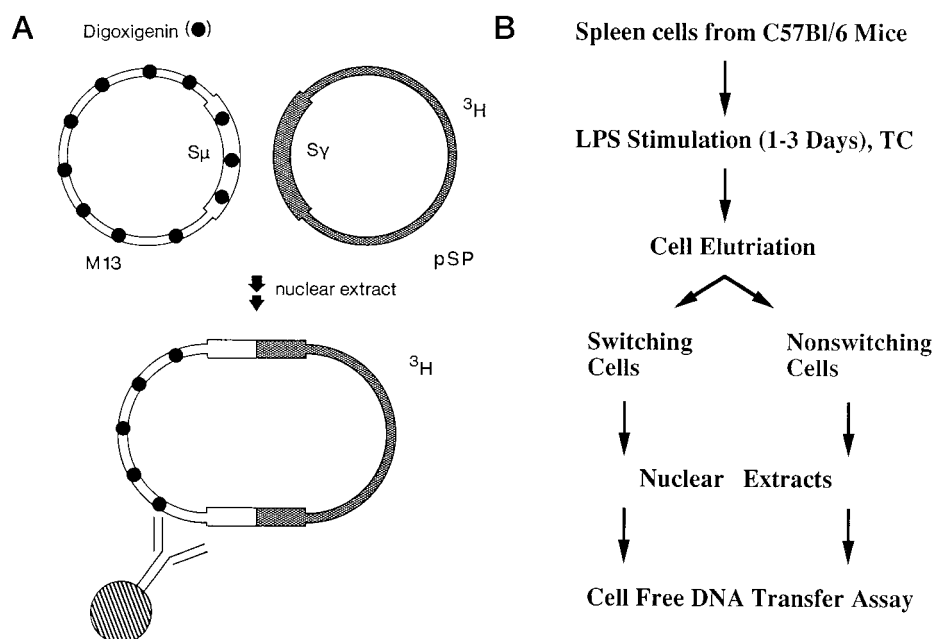


FIG. 1. **Switch region DNA transfer assay.** A, schematic representation. The ds M13 DNA contains S_{μ} and is digoxigenin-labeled; the pSP72 (or pUC19) plasmid contains S_{γ} and is ^3H -labeled. The two substrates were mixed together and incubated with a nuclear extract, e.g. from switching B-cells. DNA transfer from the S_{γ} plasmid to the S_{μ} plasmid results in plasmids containing both ^3H and digoxigenin label. The recombined plasmid is precipitated by a bead-bound antibody to digoxigenin and its radioactive content is measured. B, experimental design.

efficiency of two S region substrates (not shown).

Because the DNA is treated with SDS/EDTA and phenol extraction after the DNA transfer reaction, the linkage of the two substrates is considered stable and independent of the continuous presence of protein or Mg^{2+} . More than 80% of the transfer products are heat-stable (20 min at 85°C); the rest probably represents noncovalent reaction intermediates. Maximum product formation by Fraction IV (1 ng) occurred after 6 min of incubation at 37°C and 3 mM MgCl_2 . Omission of the four dNTPs did not affect the reaction significantly, but lack of ATP rendered the reaction 88% less efficient. Polymerase chain reaction analysis and subcloning of the bead-bound recombination products confirmed the presence of covalent S_{γ} - S_{μ} junctions among the DNA transfer products (not shown). Purified SWAP fractions had no detectable DNA polymerase, DNA helicase, general endonuclease, or general exonuclease activity (not shown).

Identification of Components of the SWAP Complex—On silver-stained SDS-polyacrylamide gels there were about 12 and 7 polypeptides left in Fractions III and IV, respectively (Fig. 4). When heavily stained, species were seen in Fraction IV with molecular masses of approximately 38, 50, 70, 75, 100, 115, and 160 kDa, of which the 70-kDa species is clearly the most prominent. We gel-eluted and partially sequenced the 38-, 70-, and 115-kDa proteins. The 38-kDa protein yielded tryptic peptides TVSLGAG and FINYVK, which are identical to residues 46–52 and 266–271 of mouse B23 (nucleophosmin) (31). The 115-kDa protein yielded tryptic peptides TLGDFLA EYAK and TTNFAGILSQG, which are identical to residues 108–118 and 864–874, respectively, of mouse PARP (32). These protein identifications were confirmed by Western blotting experiments (not shown).

The N terminus of the 70-kDa protein was MRGLKDELLKAIWHAF TALDLDRS, which was not present in the molecular biology data bases. The cDNA of SWAP-70, the novel 70-kDa protein, was cloned and sequenced (Fig. 5). SWAP-70 does not belong to a known protein family, and its 586-amino acid sequence contains nuclear localization signals at positions 222–225, 281–284, and 361–376. There is a possible coiled-coil re-

gion between amino acids 320 and 450, as well as a potential *O*-glycosylation site at amino acids 314–315, a continuous hydrophilic region near its C terminus, and several potential phosphorylation sites for several protein kinases. A fourth SWAP component is described in the next section.

High Affinity Binding of SWAP Components to the SWAP-70 Protein—The 200–300-kDa gel filtration elution position of SWAP suggests a protein complex, and we looked for high affinity interactions between B23, PARP, and SWAP-70. Histagged SWAP-70 was overexpressed from *E. coli* and purified to near homogeneity as described under “Experimental Procedures” (Fig. 6A). Purified SWAP-70 protein (Fraction II) was bound to Sepharose beads as an affinity tag for proteins contained in a nuclear extract from LPS-induced, switching B-cells. About 1 mg of extract was loaded at 25 mM ammonium sulfate. The bound material was stepwise eluted with 80, 120, 300, 600, and 1200 mM ammonium sulfate, and the fractions were analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting. The 300 and 600 mM fractions contained about 10 polypeptides, as judged from silver-stained gels (not shown). On immunoblots, PARP peaked in the 300 mM fraction (Fig. 6B), and B23 peaked in the 300 and 600 mM fractions (Fig. 6C), corresponding to 600 and 1200 mM ionic strength, respectively, of a monovalent salt; this result suggests high affinity binding. Preincubation of the SWAP-70 affinity column with polyclonal anti-SWAP-70 antibodies completely abolished its ability to retain any of the other components of the complex (not shown).

Using the affinity column to look for additional proteins forming a complex with SWAP-70, we identified the 100-kDa polypeptide observed in Fractions III and IV of the SWAP complex (Fig. 4). Because it associates with B23 (33) and has a similar molecular mass, C23 (nucleolin) was a good candidate for this protein. Using anti-C23 antibodies on Western blots, we detected two cross-reacting polypeptides of 95–100 kDa in the 300 mM ammonium sulfate fraction, likely representing two phosphorylation forms of this heavily modified protein. A third band of lower molecular mass may be a degradation product (34) (Fig. 6D). Thus, a fourth component of the SWAP complex

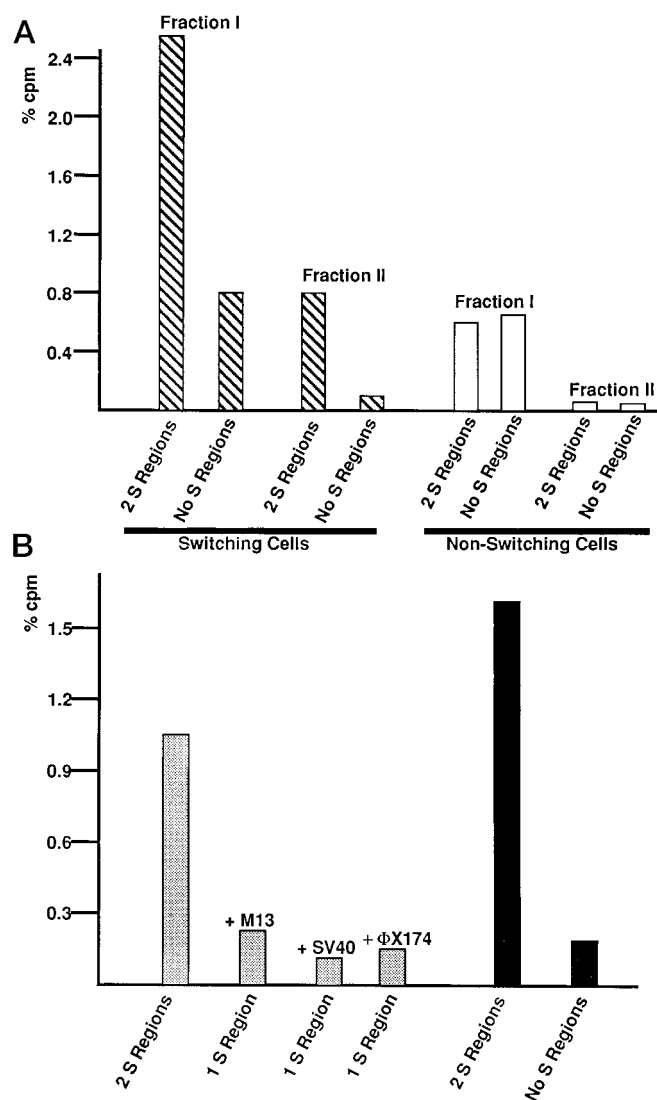


FIG. 2. SWAP in extracts and extract fractions from switching and nonswitching spleen cells. **A**, nuclear extract (Fraction I; 850 ng/reaction; striped) and SWAP Fraction II (80 ng/reaction; white) tested with DNA substrates with or without S-regions. **B**, SWAP Fraction IV (1 ng/reaction) tested in two independent experiments (gray and black) with DNA substrate combinations containing either both S regions, one S region and an unrelated DNA (M13 RF, SV40, or ΦX174 RF), or no S regions (pSP + M13 RF).

is C23, which also binds to SWAP-70 with high affinity. C23 has been described as a subunit of LR1, a B-cell-specific heterodimer that specifically binds switch region DNA (21, 35).

Expression Pattern of SWAP-70—Equal amounts of nuclear extracts of various tissues from 4-week-old C57BL/6 mice were analyzed by Western blotting with affinity purified polyclonal anti-SWAP-70 antibodies. Although there may be low level expression in other tissues, SWAP-70 is strongly expressed only in B-cells that have been induced to switch (Fig. 7A). Low expression in spleen and in lymph nodes presumably results from the few activated B-cells present in any 4-week-old mice. However, only spleen cells stimulated with LPS and concanavalin A (ConA) express SWAP-70 at high levels. Importantly, SWAP-70 is not expressed in thymocytes, nor in activated, proliferating T-cells. ConA stimulates T-cells, and these in turn stimulate B spleen cells, which then express SWAP-70 at high levels (Fig. 7, A and B); about half of the cells in ConA-stimulated spleen cell cultures are activated B-cells (not shown). In mice lacking B-cells (36), ConA did not induce the

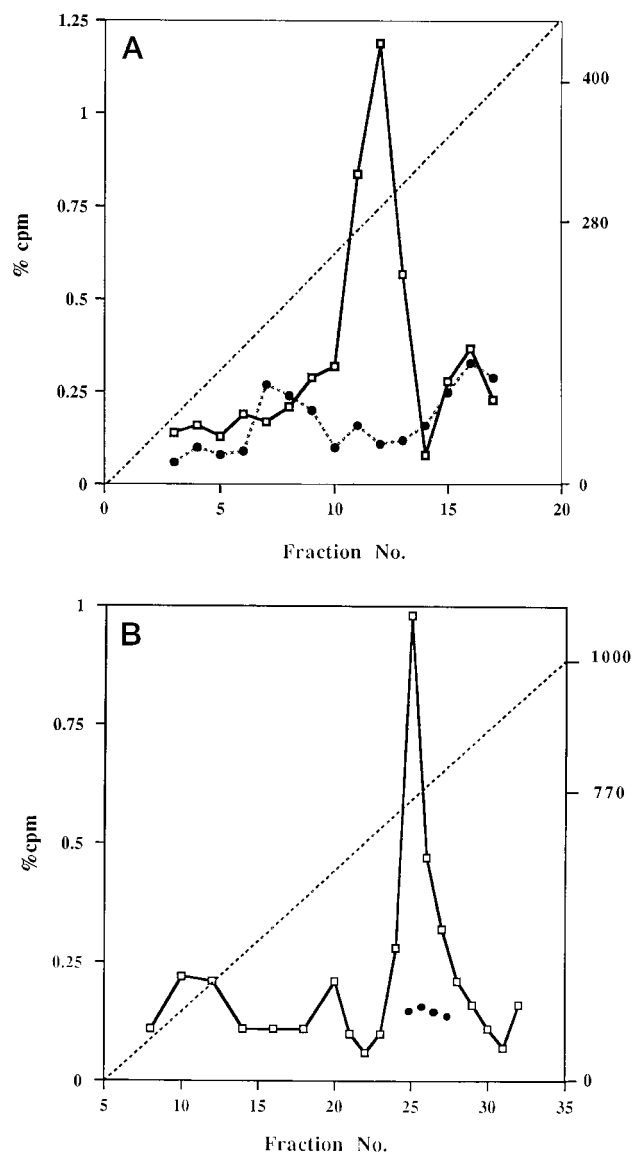


FIG. 3. Elution profiles, SWAP Fractions III and IV. **A**, macro S anion exchange chromatography elution profile. Open squares, DTA with 2 S region substrates; filled circles, DTA with substrates lacking S regions. Dotted line, salt gradient; %cpm, DTA activity. **B**, blue Sepharose chromatography elution profile; labeled as in A.

TABLE I
Purification of SWAP

Protein fraction	Total protein	Activity	Protein per reaction	Specific activity	Total units ^a
	μg	% cpm	ng	units/mg	
I	2000	2.7	3000	0.9	18000 ^b
II	600	1.1	400	2.8	1680
III	11	1.2	15	80	880
IV	0.6	0.96	0.9	1067	640

^a 1 unit = 0.1% cpm substrate DNA radioactivity transferred.

^b Contains large amounts of several different transfer activities.

expression of SWAP-70 (Fig. 7B). However, LPS-stimulated cells from mice lacking T-cells (34) contain high levels of SWAP-70 (Fig. 7B). In a culture of proliferating pre-B-cells (37), SWAP-70 is highly expressed only when the cells are stimulated with anti-CD40 antibodies and IL-4 to induce switching primarily to the IgE isotype (38) (Fig. 7C). Similarly, high expression of SWAP-70 was seen 3–5 days after induction of switching to IgA in the cell line I.29-D22 by treatment with LPS and transforming growth factor-β (39) (not shown).

SWAP-70 is also expressed at high levels expressed in the pre-B-cell line 18-81, which undergoes class switching (40, 41), but only at low levels in the pre-B-cell line 70Z/3, which does not switch (Fig. 7D). In these cell lines, an approximately 45-kDa polypeptide cross-reacts with the antibodies and might be a proteolytic degradation product of SWAP-70.

Molecular Properties of SWAP-70—To directly study DNA binding properties of SWAP-70, gel shift experiments were performed. As shown in Fig. 8A, SWAP-70 bound to DNA. This binding does not require Mg^{2+} and is even somewhat reduced

by its presence. Increasing amounts of SWAP-70 (20 and 40 ng) bound more DNA, but only about 5% of the input DNA was bound, even under optimal conditions. The oligonucleotide used for the experiment shown in Fig. 8A contains the 49-nt S_{γ} consensus signal (28). The same binding efficiency, however, was observed with other, non-S oligonucleotide substrates. SWAP-70 binds ds and ss DNA with about the same efficiency (not shown). Addition of ATP after preincubation of the protein with DNA abolished the signal, as did incubation with the nonhydrolyzable analog ATP- γ -S. Binding of SWAP-70 to ^{32}P -azido-ATP was not inhibited by DNA (Fig. 8B). Thus, either ATP has a higher affinity than DNA to the same binding site of SWAP-70, or ATP induces a conformational change such that DNA cannot bind anymore. At any rate, ATP is a potential modulator of DNA binding of SWAP-70.

Promotion of DNA Reannealing by B23—It is unknown whether the short stretches of homology found between different S regions play a role in switch recombination, but DNA transfer of switch regions probably requires juxtaposition and possibly pairing of the substrate DNAs (19). Pairing of two complementary DNA single strands is promoted by most known recombination proteins (42), and we tested individual

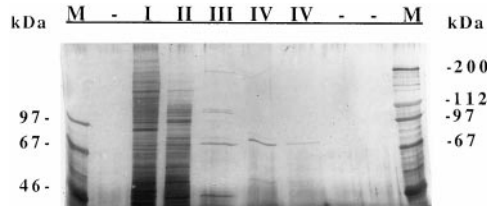


FIG. 4. SDS-polyacrylamide gel electrophoresis of SWAP, Fractions I-IV. The fractions were loaded on a 7.5% SDS-polyacrylamide gel, and the gel was stained after electrophoresis with silver according to standard procedures. M, marker.

FIG. 5. Nucleotide and amino acid sequence of SWAP-70. Boxed regions indicate nuclear localization signals. The sequence has been submitted to GenBank™ (accession number AF053974).

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1  ATGAGGGGGTTGAAAGACGAAGTCTCAAGCCATTGGCACGCCTTCACCGCGCTCGAC   20
   M R G L K D E L L K A I W H A F T A L D
61  CTGGACCGCAGCGGCAAGGTCTCAAGTCGCAAGGTCTTCCCAATACCTGTGC   40
   L D R S G K V S K S Q L K V L S H N L C
121 ACGGTGTGAAGTTCCACATGACCCGGTTGCCCTTGAGGAGCACTTTAGGGATGACGAT   60
   T V L K V P H D P V A L E E H F R D D D
181 GAGGGGCTGTCTCAATCAGGGCTACATGCCATATTTAAACAAGTTCATTTTGGAAAAG   80
   E G P V S N Q G Y M P Y L N K F I L E K
241 GTCCAAGACAACCTTTGACAAGATTGAATCAATAGAATGTGTGGACACTTTGTGTCAAG   100
   V Q D N F D K I E F N R M C W T L C V K
301 AAAAACCTCACAAGAGTCTCTACTCATTACAGAAGATGATGCATTTAAAGTGTGGGTC   120
   K N L T K S P L L I T E D D A F K V W V
361 ATTTTCAACTTTTGTGAGGACAAAGTATCCACTAATTATTGTGCCAGAAGAGATTGAA   140
   I F N F L S E D K Y P L I I V P E E I E
421 TACCTGCTTAAGAAGCTTACAGAAGCTATGGGAGGAGGTGTGGCAACAAGAATTTGAA   160
   Y L L K K L T E A M G G G W Q Q E Q F E
481 CATTACAAATAAAGTTGATGACAATAAAGATGGCTTTCTGCATGGGAACCTTATTGAG   180
   H Y K I N F D D N K D G L S A W E L I E
541 CTAATTGGGAATGGACAGTTTAGCAAGGGCTGACCGCTGACAGCGTATCTATGGCCATT   200
   L I G N G Q F S K G M D R Q T V S M A I
601 AACGAAGTCTTCAATGAGCTTATTTAGATGTATTGAAGCAGGGTTACATGATGAAGAAA   220
   N E V F N E L I L D V L K Q G Y M M K K
661 GGTCAACAAACGGAACCTGGAGCTGAGCGCTGGTTGTATTAAACCAACATAATTTCC   240
   G H K R K N W T E R W F V L K P N I I S
721 TACTATGTGAGCGAGGATCTGAAAGATAAAGAGGAGACATCTCTGGATGAAACCTGC   260
   Y Y V S E D L K D K K G D I L D E N C
781 TGTGTGGAGTCTCTGCTGACAAAGATGGAAGAAATGCTTTTCTAATAAAATGCTTT   280
   C V E S L P D K D G K K C L F L I K C F
841 GATAAGACCTTTGAAATCAGTGCCTCAGATAAGAAGAAAGAAACAGATGATGATCAGGCC   300
   D K T F E I S A S D K K K K Q E W I Q A
901 ATTTACTCCACCATCTGTTGAAGCTGGCAGCCPCCACCAACAGGAAGCCCGC   320
   I Y S T I H L L K L G S P P P H K E A R
961 CAGCGTCGGAAGAGCTCCGAAGGAAGCTGTAGCCGAGCAGGAGAGCTGGAGCGGAG   340
   Q R R K E L R R K L L A E Q E L L E R Q
1021 ATGAAGGAACCTCAAGCCCAATGAAACAAGCAACAGGAGCTGGAAGCGTGAGGAAG   360
   M K E L Q A A N E N K Q Q E L E S V R K
1081 AAATGGAGGAAGCAGCTCTCGTGGCAGAGAGGAAAGAAACGCTTGACAGATCAG   380
   K L E E A A S R A A D E E K K R L Q T Q
1141 GTGGAGCTACAGACAGGTTTCAGCAGGAGCTGGAGCGGAGAAGCTGATCAGACAGCAG   400
   V E L Q T R F S T E L E R E K L I R Q Q
1201 ATGGAGGAGCAGGTTGCCAGAGTCTCCGAAGTGGAGCAGTATCTGACGAGGAGTTCCG   420
   M E E Q V A Q K S S E L E Q Y L R V R
1261 GAGCTGGAAGACATGTACCTAAAGTGCAGGAGGCTCTTGAGGACGAGAGGAGGCGCCG   440
   E L E D M Y L K L Q E A L E D E R Q A R
1321 CAGGATGAAGAGACTGTGCGCAAGCTTCAGGCCAGGTTGCTGGAGGAAGAGTCTTCTAAG   460
   Q D E E T V R K L Q A R L L E E E S S K
1381 AGGCGAGAGCTGGAAGTGGACCTGGAGCAGCAGCAGGCCATTGACAGCAACAGAGCG   480
   R A E L E K W H L E Q Q Q A I Q T T E A
1441 GAGAAGCAGGAGCTGGAACAGCAGCGTGTCTGATGAAGGAGCAGGATTCAGGAGGCCAT   500
   E K Q E L E Q Q R V M K E Q A L Q E A M
1501 GCACAGCTGGAACAGTTGGAGTTGGAGCGGAAGCAGGCCCTGGAGCAGTATGAGGGAGTT   520
   A Q L E Q L E L E R K Q A L E Q Y E G V
1561 AAAAAGAAGCTAGAGATGGCAACACATATGACCAAGAGCTGGAAGGACAAAGTGGCCAT   540
   K K K L E M A T H M T K S W K D K V A H
1621 CATGAGGGATTAATCAGATTGATAGAACCAGGTTTCAAGAACCCTCATCTGATCAACAC   560
   H E G L I R L I E P G S K N P H L I T N
1681 TGGGACCCGAGCGTTACCCAGGAGAGCTCGAGGAGAGAGAGAAGAGCTGGAAGAG   580
   W G P A A F T Q A E L E E R E K S W K E
1741 AAGAAGACCACAGAGTGA 1758
   K K T T E * 586

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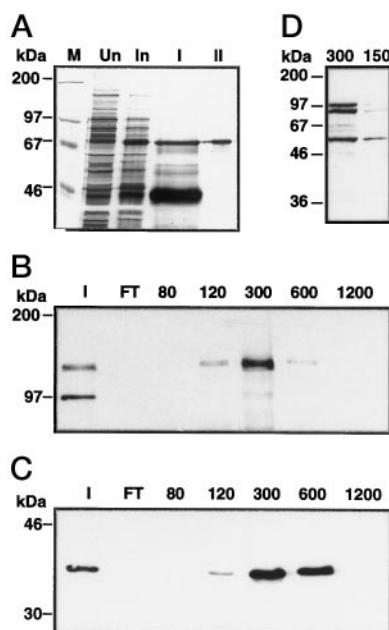


FIG. 6. SWAP-70 affinity chromatography. A, purification of SWAP-70 from *E. coli*. Proteins were stained with silver. Un, uninduced; In, induced; I and II, fractions I and II. M, marker. B, immunoblot of affinity column-eluted fractions, probed with anti-PARP antibodies. C, as in B, but probed with anti-B23 antibodies. D, as in B, but probed with anti-C23 antibodies. I, nuclear extract, load. FT, flow-through, 80–1200 mM ammonium sulfate.

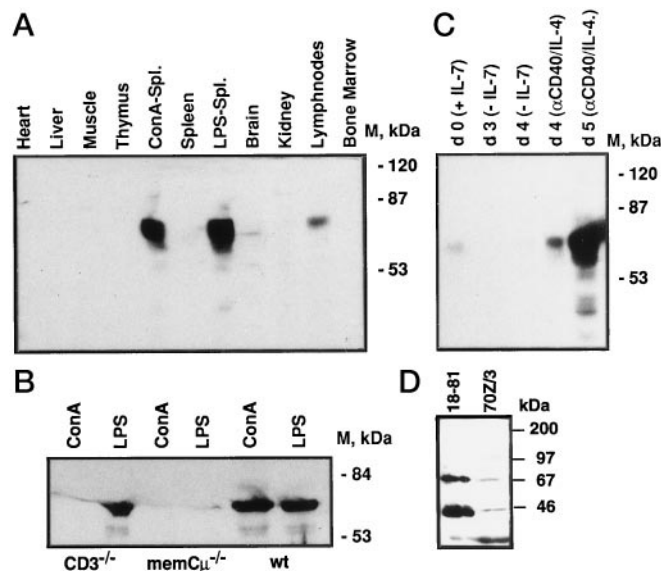


FIG. 7. Expression pattern of SWAP-70 on Western blots of nuclear extracts (10 μ g of protein each) using affinity-purified rabbit antibody against SWAP-70. A, various tissues; B, Con A- and LPS-stimulated spleen cells from CD3^{-/-} (i.e. T-cell-negative) mice; membrane (mem) C μ ^{-/-} (i.e. B-cell-negative) mice; and wild-type (wt) mice; extracts prepared at day 3 after induction. C, pre-B-cells induced to switch to IgE by anti-CD40/IL-4 treatment (38); d, day after induction; the cells proliferated undifferentiated in the presence of IL-7. D, mouse B-cell lines 18–81 (switching, 5 μ g of protein) and 70Z/3 (non-switching, 20 μ g of protein).

components of the SWAP complex for this activity. A 422-bp, linear, 3'-labeled ds DNA fragment was heat-denatured and reannealing to the duplex form visualized by gel electrophoresis (26). Although SWAP-70 was not active in this pairing assay (not shown), purified B23 was at least as efficient as the *E. coli* RecA protein (Fig. 9). A titration of B23 into the reaction showed that 15 ng of B23 (390 fmol) yielded as much ds DNA as

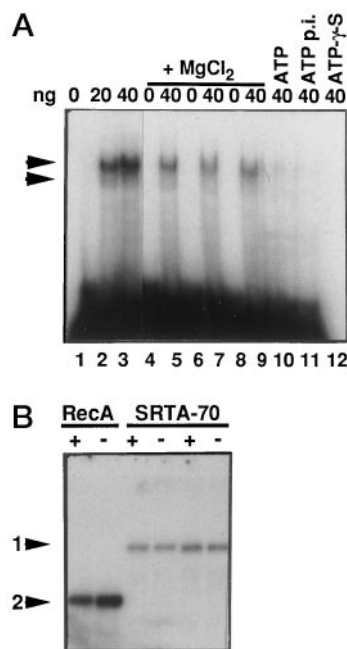


FIG. 8. DNA and ATP binding by SWAP-70. A, 0, 20, or 40 ng of SWAP-70 was incubated for 20 min with a ³²P-labeled 49-nt oligonucleotide either without MgCl₂ (lanes 1–3 and 10–12) or with MgCl₂ (lanes 4 and 5, 1 mM; lanes 6 and 7, 5 mM; lanes 8 and 9, 10 mM); with 1 mM ATP (lane 10) or 1 mM ATP- γ -S (lane 12); or with 1 mM ATP added for the last 5 min of incubation (lane 11). One major binding product (top arrow) and one minor binding product (bottom arrow) are labeled. B, ³²P-azido-ATP was incubated with either *E. coli* RecA (2; 38 kDa, 0.3 μ g), or SWAP-70 (1; 70 kDa, 1 μ g, 2 μ g) proteins. DNA was added to (+) or omitted from (–) the reaction.

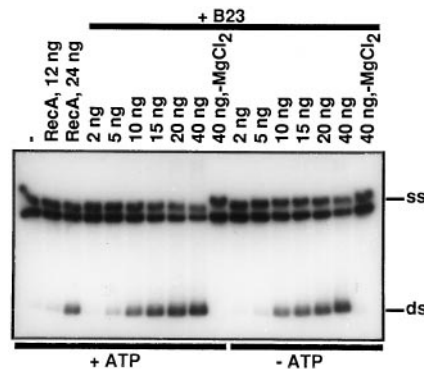
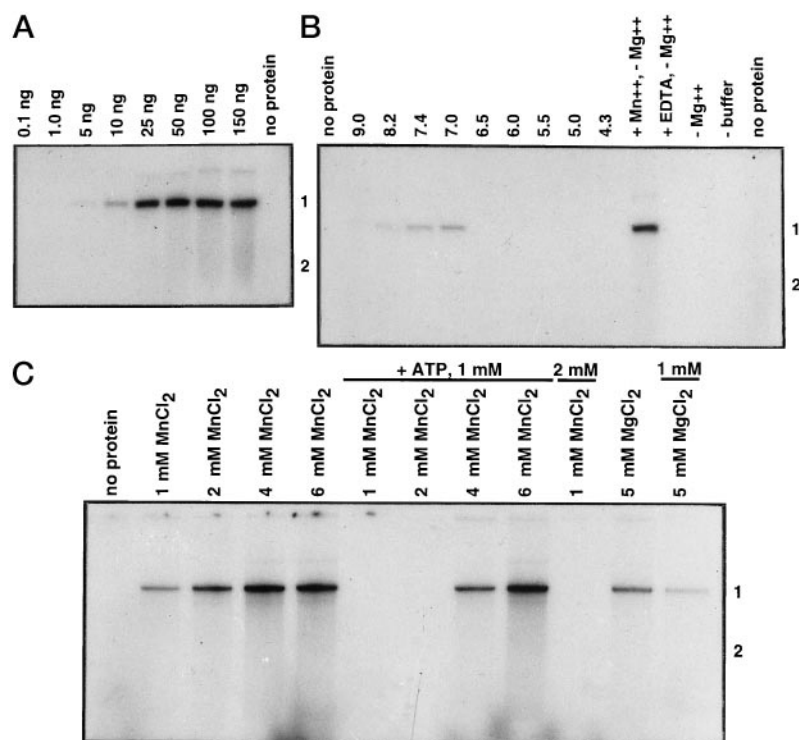


FIG. 9. DNA reannealing activity of B23 protein. A heat-denatured 422-bp ³²P-end-labeled DNA fragment was incubated with various amounts of B23 protein, with *E. coli* RecA, or without protein (–), and with or without ATP or MgCl₂, as indicated (described in Ref. 26). ss, single-stranded substrate; ds, double-stranded reannealing product.

24 ng of RecA (600 fmol). The B23 mediated reaction is ATP-independent. There is, however, a strict dependence on Mg²⁺ (Fig. 9).

Formation of D Loops and Joint Molecules by B23—For a more complex three-strand pairing reaction (probably closer to the switch recombination reaction) we investigated formation of joint molecules generated by invasion of ss oligonucleotide into a ds DNA target, the so-called D-loop formation (42–50). For most reactions, a 5'-³²P-labeled ss oligonucleotide (49 nt; S_Y consensus sequence (28)) was incubated with a ds supercoiled pUC plasmid carrying a 3.7-kilobase pair fragment of the S_Y region (27) and various amounts of B23 protein. In some assays, a known unspecific pairing product (the so-called “Birnbom band”) appears, resulting from annealing of the oligonucleotide to partially and irreversibly denatured plasmid DNA,

FIG. 10. Formation of joint molecules: analysis of reaction characteristics. Formation of joint molecules (*band 1*) with the 49-nt S_{γ} oligonucleotide and the superhelical pUC- S_{γ} duplex target. *A*, titration of B23 into the reaction; amounts (in ng) of B23 are indicated. *B*, varying reaction conditions. The pH variations range from 4.3 to 9.0 (indicated at the top). *C*, titration of $MnCl_2$ or $MgCl_2$ in the presence or absence of 1 mM ATP into the reaction, which lacked any metal cofactor.



which is generated in alkaline lysis plasmid preparation methods (51) (*band 2* in Fig. 10, *A* and *B*, and see Fig. 13; *band 3* in Figs. 11 and 13 indicates the free oligonucleotide). This band migrates at the position of the supercoiled plasmid and does not represent a true D-loop formation product, which migrates slower. The side product, however, is not present if the supercoiled plasmid was purified under nondenaturing conditions (Figs. 10 and 11, *C–E*).

B23 promotes the formation of D-loops under a variety of conditions. Titration of B23 into the reaction is shown in Fig. 10*A*. With 48 fmol of ss and 22 fmol of ds substrate DNAs, product formation required at least about 5 ng (130 fmol) of B23 protein, and saturation was reached at about 100 ng (2.6 pmol) of B23. The optimum pH of the reaction buffer was about 7.0–7.4, with very little product visible at pH 8.2 (Fig. 10*B*). A metal cofactor is required. $MgCl_2$ can be replaced by $MnCl_2$ (Fig. 10*B*), but $CaCl_2$ does not support the reaction, and addition of Zn^{2+} strongly inhibits it (not shown). Preincubation of B23 with the ss oligonucleotide was unnecessary.

Initially, an inhibitory effect of ATP added to a reaction was observed (Fig. 10*C*, *right two lanes*). Titration of the metal cofactor in the presence or absence of 1 mM ATP, however, showed that the inhibitory effect is most likely due to the well-known metal ion chelating effect of ATP. Increasing the concentration of $MnCl_2$ reversed the inhibitory effect of ATP (Fig. 10*C*). Roughly the same amounts of joint molecules were formed in the presence of 5 mM $MgCl_2$ and 1–2 mM $MnCl_2$. ATP inhibition was also reversed when PARP enzyme was added to the reaction, independent of whether NAD was added or not (not shown). The nature of this reversion remains to be determined.

In Fig. 11*A*, joint molecules were formed in a standard reaction with 50 ng (1.3 pmol) of B23 in a buffer containing 5 mM $MgCl_2$. SWAP-70 is not active in the assay, and titration of increasing amounts of SWAP-70 into the B23 mediated D-loop reaction had no effect (Fig. 11*A*). The complete SWAP fraction, which contains B23, was also active in the assay (Fig. 11*B*). For both the complete SWAP complex and purified B23 protein, no joint molecules were formed with the linear duplex DNA sub-

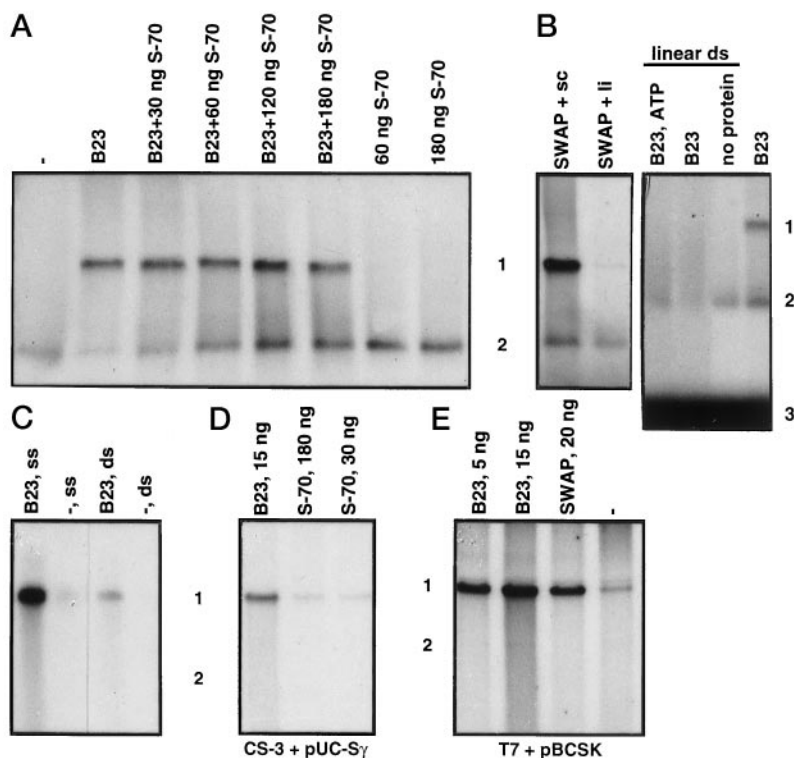
strates, but they were easily detectable with the supercoiled substrate (Fig. 11*B*). Linear duplex DNA is also not an efficient substrate for D-loop formation mediated by *E. coli* RecA (42). If the ss oligonucleotide was replaced by a ds oligonucleotide of the same sequence, the reaction efficiency was markedly reduced, and the remaining minor signal was probably caused by unannealed oligonucleotides (Fig. 11*C*). B23 also mediated D-loop formation with a shorter 25-nt oligonucleotide of the S_{γ} sequence (68% G/C; Fig. 11*D*) as well as with a 22-nt oligonucleotide (T7, 45% G/C) fully homologous to a different duplex DNA substrate (pBluescriptSK) (Fig. 11*E*). SWAP-70 was inactive in this reaction.

Joint Molecule Formation: Heat Stability, Reaction Kinetics, and Protein Titrations—Once they are formed by B23, the joint molecule products do not require the presence of protein. The quantitative results in Fig. 12 were obtained by excising gel bands of joint molecules and direct scintillation counting. Measurement of the free ^{32}P oligonucleotides excised from the same lane of the gel, permitted calculation of percentage of ss substrate converted into product. Variations among experiments were less than $\pm 15\%$.

Under reaction conditions with 22 fmol of duplex and 48 fmol of ss substrates, about 3.2% of the S_{γ} oligonucleotide (1.5 fmol) was converted by 100 ng (2.64 pmol) of B23 protein into joint molecules (Fig. 12*A*). The reaction appears to be linear over more than 1 h, and some product formation could be detected after 1 min incubation (Fig. 12*B*). In this set-up, with 50 ng of B23, 100 fmol of oligonucleotide, and 44 fmol of duplex DNA, after 20 min 3.0% and at the 90-min time point, 5.7% of the ss oligonucleotide was converted into joint molecules. Similar to the negative controls shown in Figs. 10 and 11, little oligonucleotide was incorporated into the duplex target upon omission of protein, even in the 90-min reaction (1.1%).

The joint molecules were tested for heat stability (Fig. 12*C*). After treatment with proteinase K and SDS, the reaction mixture was heated for 20 min to various temperatures. Although none could be detected at 95 °C, the amount of joint molecules was not reduced at 55 °C. Considering the 92% homology between the S_{γ} consensus sequence and the pUC- S_{γ} duplex, the

FIG. 11. Formation of joint molecules in various reactions. 1, joint molecules; 2, unspecific annealing product (Birnboim band). Equal amounts of ss (80 fmol; 49-nt S_{γ} or noted otherwise) and ds (22 fmol; pUC- S_{γ} or noted otherwise) substrates were incubated for 30 min at 37 °C with protein. **A**, formation of joint molecules by B23, SWAP-70 (S70), and B23 plus SWAP-70. **B**, SWAP, Fraction III (left panel) or B23 (right panel) incubated with linear or supercoiled pUC- S_{γ} target substrates. **C**, B23 incubated with either the ss (left) or the ds (right) 49-nt/bp S_{γ} oligonucleotide; **D**, B23 or SWAP-70 (S70) incubated with a 25-nt S_{γ} oligonucleotide substrate and pUC- S_{γ} target duplex DNA; **E**, B23 or SWAP, Fraction III, incubated with a 22-nt oligonucleotide (T7, 100% homology) and supercoiled pBlue-script target DNA.



joint molecules possess the stability expected for true base pairing (Fig. 12C). Using the formula $T_m = 81.5 + 16.6 (\log[\text{Me}^{2+}] + 0.41 \times P - 600/N)$, where P = fraction G/C; N = number of nt, with $[\text{Me}^{2+}] = 2 \text{ mM MnCl}_2$, and deducting 2 °C for each percent of nonhomology, we calculate the approximate T_m to be 58 °C. At 58 °C, the number of joint molecules is reduced by $\frac{1}{3}$ (Fig. 12C). The slight increase in joint molecules after heating to 45–55 °C is probably due to protein-independent annealing of the oligonucleotide to duplex DNA, which began to denature at these temperatures. The joint molecules do not require the continuous presence of Mg^{2+} or Mn^{2+} ions, because addition of 50 mM EDTA during the proteinase K/SDS treatment did not reduce the joint molecule signal (not shown).

Varying the amount of the ds DNA substrate also had an effect on the reaction. Most efficient product formation was found using 1.1 μg (245 fmol) of duplex DNA in a reaction containing 50 ng (1.3 pmol) of B23 protein and 100 fmol of ss oligonucleotide (5.1% ss substrate converted into joint molecules after 20 min of incubation). Based on the binding site size of B23 on ss DNA of 11 nt (52), the 49-nt ss substrate can theoretically be bound by 4.5 molecules of B23; the binding site size on ds DNA is not known, but if it is similar, the 6800-bp duplex substrate may bind at least 618 molecules of B23. The affinity of B23 for ds DNA, however, is much lower than that for ss DNA (52), so that the ss substrate would be covered first. About 450 ($4.5 \times 100 \text{ fmol}$) monomeric B23 molecules may be bound by the ss substrate, leaving roughly 850 (of 1.3 pmol) molecules of B23 available for the duplex molecule. This results in three or four B23 molecules per duplex DNA molecule. Thus, the ds DNA does not need to be coated with the protein. An alternative calculation of the reaction efficiency based on the amount of duplex substrate, which is limiting, converted into joint molecules yielded a 4-fold higher efficiency (up to 20%).

Joint Molecules Formed with Different S Regions—The homology between the 49-nt S_{γ} consensus sequence oligonucleotide and the pUC- S_{γ} target is 92%, and four mismatches were generated upon pairing. Because there exist patches of homologies between different S regions and switch recombination

occurs between two different S regions, we tested joint molecule formation using the S_{γ} oligonucleotide and a pUC- S_{μ} or M13- S_{μ} ds target DNA (Fig. 13A). The maximal homology here was 75% over the entire 49-bp sequence or 90% in a 11-bp stretch. Although not as efficient as the S_{γ} - S_{γ} pairing (Fig. 13A, right two lanes), B23 was able to produce joint molecules with the two different S regions. No joint molecules were obtained with the pUC plasmid DNA or the M13 ds DNA as target; in these cases, the homologies were lower (46% for pUC19, 51% for M13; maximum of 70% in stretches of 15–20 bp). Comparable results were obtained using substrates with an S_{γ} , an S_{μ} , or no S region cloned into a different plasmid vector (Fig. 13B). Thus, small patches of homologies in the S regions may support pairing of different S regions, but possibly other sequence or structural elements or a minimal length of multiple homology patches (like that present in S regions) is necessary to form stable products.

DISCUSSION

The SWAP Complex—We have used a modified version of a DNA transfer assay (19, 24–26) to identify activities that preferentially recombine Ig H-chain S-region sequences. Such an activity, the SWAP complex, was isolated from nuclear extracts of switching B-cells. So far, four components of SWAP have been identified. Three of these, B23, C23, and PARP, are previously sequenced proteins that are present in many sorts of cells. SWAP-70 is a novel protein that, like the SWAP complex, is only present in the nucleus of switching B-cells.

Although SWAP works best with two S regions, it does work at a lower level in reactions in which only one of the substrates contains an S region. In this case, SWAP might use the S region as a preferred substrate and recombine it with whatever partner is available, a process similar to the myc translocations in plasmacytomas and Burkitt's lymphoma (53), which are thought to be mediated by the switch recombinase, as are other illegitimate translocations into S regions (54). The preference of purified SWAP for S regions in the cell-free system is about 10-fold. Although this might be sufficient for the switch recom-

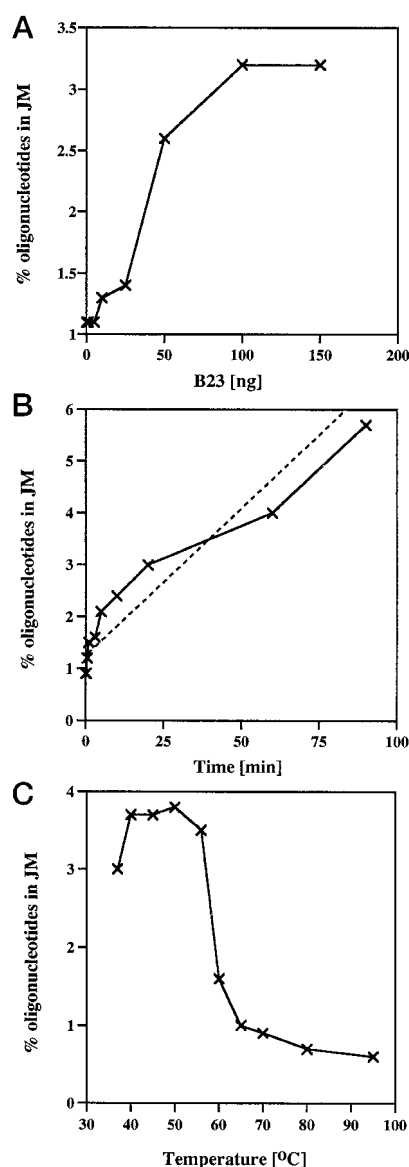


FIG. 12. **Quantitation of joint molecules.** Joint molecules (JM) were formed with the 49-nt S_{γ} oligonucleotide and pUC- S_{γ} duplex DNA substrates. Radioactive joint molecule and free oligonucleotide bands were excised from the agarose gel, and the gel was melted and directly counted in the scintillation counter. **A**, titration of B23 and % of oligonucleotide converted into joint molecules (30-min incubation, 22 fmol of ds, 48 fmol of ss substrates). **B**, kinetics of joint molecule formation using 1.3 pmol of B23, 100 fmol of ss substrate, and 44 fmol of ds substrate. Input ss substrate radioactivity was 85,000 cpm. *Dotted line*, idealized linear curve. **C**, heat stability of the joint molecules. Input radioactivity was 88,000 cpm.

binase, additional specificity for a particular S region is probably contributed by transcriptional activation of the locus and/or the germ line switch transcripts (3–5, 15). It is also a matter of debate whether or not limited homologies contribute to the *in vivo* switch rearrangement. But there is no *a priori* reason why a switch activity has to be inactive on homologous substrates. The short patches of homology that exist between different switch regions may well support the switch recombination reaction *in vivo*, and, as we show, they seem to be used by B23 in the formation of joint molecules.

SWAP-70 binds only weakly to DNA, but it interacts strongly enough with the other SWAP components to be used in affinity chromatography. The possibility that only one or a few components of the SWAP complex bind directly to SWAP-70, whereas others may bind indirectly via interactions with these compo-

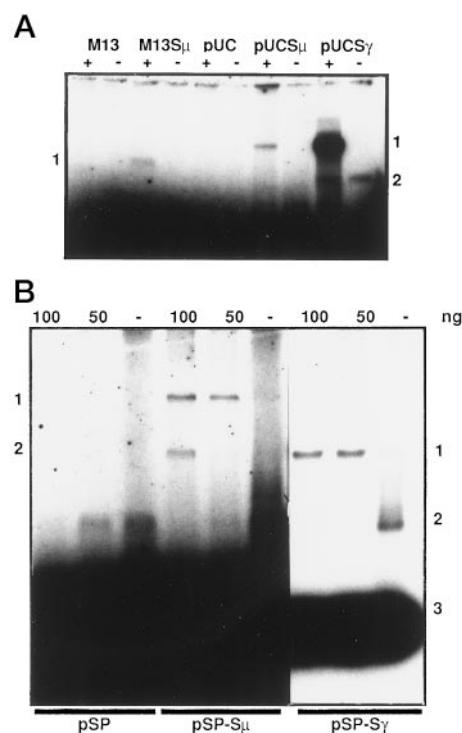


FIG. 13. **Formation of joint molecules with different S region DNA substrates.** The S_{γ} oligonucleotide (160 fmol) was incubated in **A** with (+) or without (–) 50 ng (1.3 pmol) of B23 protein and the plasmid DNAs with or without S regions as indicated; in **B** without (–) or with 50 or 100 ng of B23 protein and another set of plasmid DNAs (50 fmol each) containing either S_{γ} , S_{μ} , or no S region sequence. The *left* portion (pSP/pSP-S $_{\mu}$) of **B** was exposed twice as long (34 h). 1, joint molecule product; 2, unspecific band; 3, free oligonucleotide substrate.

nents, cannot be ruled out. In any case, all known components in the fraction elute from a SWAP-70 affinity column only at high salt concentrations; that is, the binding affinities are high.

The binding of ATP and the inhibition of DNA binding by ATP or a nonhydrolyzable analog suggest a regulative role of ATP binding on SWAP-70 activity. The DNA transfer reaction requires ATP, but the role of ATP in this reaction, catalyzed by the full SWAP fraction only, remains to be determined. Based on the ATP effect, direct interactions between SWAP-70 and DNA do not seem to be essential for the transfer reaction. The amino acid sequence of SWAP-70 (Fig. 5) does not contain a canonical Walker A NTP binding motif (55), but amino acid residues 183–190 resemble that motif to some extent.

Protein B23, also called nucleophosmin, numatrin, or NO38, has become known as a predominantly nucleolar phosphoprotein bound to the nuclear matrix (60). Although the precise function(s) of B23 remain unknown, it was hypothesized to act as a protein shuttle involved in ribosome assembly with migration between the nucleus and the cytoplasm (60). However, B23 seems to have other functions as well. The protein binds nucleic acids cooperatively with a preference for ss over ds DNA, but without preference for either RNA or ss DNA (52, 61). Similar to other ss binding proteins, B23 exhibits a helix-destabilizing activity, disrupting the base stacking, observed on synthetic ss nucleic acids such as poly(rA) and poly(rC) (52). A recent study showed increased phosphorylation and poly(ADP)ribosylation of B23 after x-ray treatment of mammalian cells (58). These observations and the higher affinity of B23 to ss DNA foster speculation about a possible involvement in DNA repair and recombination.

Since it promotes DNA single-strand reannealing, B23 contributes previously unknown recombination activities to the SWAP complex. Promotion of reannealing of ss DNA is a fea-

ture common to most established recombination proteins (42). However, other proteins that are very unlikely to be involved in DNA recombination, *e.g.* fatty acid synthase, also support the reaction. Furthermore, certain nonprotein agents, such as polyethylene glycol, also stimulate DNA reannealing (42). Thus, although the presence of a reannealing activity in a protein does not necessarily mean it is a recombination factor, reannealing activity might well be expected of a recombination protein. This expectation is fulfilled by B23, which promotes the reaction in an ATP-independent but Mg^{2+} -dependent manner with an efficiency even higher than that of *E. coli* RecA protein.

The formation of joint molecules constitutes a more complex three-strand reaction, because it entails the invasion of a single-strand DNA into a DNA duplex. This reaction reflects an early and specific step in DNA recombination, and only a few known recombination proteins promote this reaction (43–49). Our data show that protein B23 mediates D-loop formation in a protein concentration-dependent manner. B23 can use ss “invader” substrates varying in length from 22 to 49 nt, in G/C content from 45 to 65%, and in homology with the ds target DNA from about 75 to 100%. Various linear duplex DNA substrates did not support the reaction and serve as negative controls for unspecific, *e.g.* helicase or nuclease-driven pairing. The reaction requires Mg^{2+} or Mn^{2+} but not ATP. In theory, formation of joint molecules with superhelical acceptor DNA does not necessarily need an external source of energy, and strand exchange in the absence of ATP or in the presence of nonhydrolyzable ATP analogs has been demonstrated for *E. coli* RecA protein (42). The reaction may be driven by the gain in entropy as a consequence of loss of superhelicity (42, 50). Considering the degree of homology between the standard DNA substrates, the joint molecules are remarkably stable; heating to 55 °C, close to the T_m , leaves the joint molecules intact (Fig. 12C). This argues for full base pairing between the ss and ds substrates. Conversion of about 6% of the oligonucleotide substrate into joint molecules after a 90-min incubation of a standard reaction appears to be less efficient than what has been reported for D-loop formation by *E. coli* RecA protein or the meiotic human homolog HsDmc1 (49, 59). The kinetics are also different; RecA-mediated formation of joint molecules decreased significantly after the initial 2-min incubation (59), whereas no such decrease was seen in the B23 mediated reaction.

Is SWAP the Switch Recombinase?—The expression pattern of SWAP and of the SWAP-70 protein is consistent with it being involved in the process of class switching. The nuclear localization signals in SWAP-70 suggest its presence in the nuclei, and in immunoblotting experiments, it has not been seen at any comparable level in cytoplasmic extracts. It is strongly expressed only in switching B-cells. Importantly, the expression pattern of SWAP-70 could not be explained by activation of cell proliferation, which accompanies switching in spleen cell cultures. Indeed, the 70Z/3 and 18–81 cell lines proliferate at the same rate, yet SWAP-70 is expressed in the switching line 18–81 only; several of the tissues (*e.g.* thymus) tested in Western blotting experiments contain proliferating cells but are SWAP-70-negative, as are ConA-stimulated, proliferating spleen cells from mice lacking B-cells. Moreover, pre-B-cells induced to switch by anti-CD40 and IL-4 treatment actively proliferate before induction and cease to do so afterward. However, SWAP-70 is highly expressed several days after switch induction, at a time when switching is most prominent (35). There is no detectable expression of SWAP-70 during V(D)J recombination, which occurs after removal of IL-7 from these pre-B-cell cultures (35). It should be noted that SWAP-70 has

been observed to be expressed in B-cells switching to several different Ig isotypes: IgG2b (after LPS treatment), IgE (after treatment with anti-CD40 and IL-4), and IgA (after treatment with transforming growth factor- β and LPS).

Although their precise functions remain to be determined, all four components of the SWAP complex thus far identified seem to fit into roles one may expect from a switch recombinase complex. (i) The role of SWAP-70 is likely to reside in protein-protein interactions. Thus, SWAP-70 might act as a specific class switch recombinase assembly factor. (ii) C23 is identical to a component of the heterodimeric LR1, which has been reported earlier in independent studies to be B-cell-specific and to bind specifically to switch region DNA (21, 35). Thus, C23 might act as a switch region targeting factor in the SWAP complex. (iii) B23 might provide the activities necessary to perform the first steps in a recombination reaction: pairing of the DNA substrates and strand invasion with formation of stable joint molecules. (iv) PARP may regulate components of the complex (39). Based on switch induction end point studies, PARP-negative mice seem to have no serious deficiency in class switching (56).² Although PARP is probably not essential for class switching, it is difficult to assess the impact of selection for isotypes other than IgM. A decreased efficiency in switched isotype production may be counterbalanced by greater expansion of cells that are switched. PARP may have a regulatory role in switching, because treatment of switching I.29 B-cells with PARP inhibitors results in an increase in switch frequency (39). The possible existence of a second enzyme similar to PARP has not entirely been ruled out and would obscure the phenotype of the knockout mouse. PARP has also been shown to bind not only to DNA ends but also to bind DNA secondary structures such as stem loops (57). Such structures have been suggested to be substrates for the switch recombinase (6). It is notable that B23 protein has been found to be modified by PARP (58).

In terms of class switch recombination, the utilization by B23 of two different S region DNA sequences, S_μ and S_γ , as ds and ss DNA substrates is most remarkable. It was observed with S regions cloned in several vector DNAs. No other mammalian protein has been described to mediate such a reaction, which may resemble formation of an early recombination intermediate in the class switch reaction (7, 10).

The lines of evidence discussed in this section strongly suggest that the SWAP complex is involved in at least one of the genetic events that take place at the Ig loci during the later stages of the differentiation of B-cells, most likely the Ig H-chain class switch. A cogent conclusion will have to await the outcome of genetic knockout experiments.

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