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Molecular Model of the A Subunit of Protein Phosphatase 2A: Interaction with Other Subunits and Tumor Antigens

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Protein phosphatase 2A consists of three subunits, the catalytic subunit (C) and two regulatory subunits (A and B). The A subunit has a rod-like shape and consists of 15 nonidentical repeats. It binds the catalytic subunit through repeats 11 to 15 at the C terminus and the tumor antigens encoded by small DNA tumor viruses through overlapping but distinct regions at N-terminal repeats 2 to 8. A model of the A subunit was developed on the basis of the fact that uncharged or hydrophobic amino acids are conserved at eight defined positions within each repeat. Helical wheel projections suggested that each repeat can be arranged as two interacting amphipathic helixes connected by a short loop. Mutational analysis of the A subunit revealed that the proposed loops are important for binding of tumor antigens, the B subunit, and the C subunit. Native gel analysis of mutant A subunits synthesized in vitro demonstrated that the binding region for the B subunit, previously thought to include repeats 2 to 8, covers repeats 1 to 10 and that the B and C subunits cooperate in binding to the A subunit.

The small DNA tumor viruses simian virus 40 (SV40), polyomavirus, and BK virus cause cell transformation via complex formation between the virus-encoded T antigens and cellular proteins involved in growth control (see reference 17 for a review). One such cellular protein is protein phosphatase 2A (PP2A), a serine/threonine-specific protein phosphatase that associates with the small T antigens of SV40, polyomavirus, and BK virus as well as with polyomavirus medium T (3, 9, 10, 12, 13, 18, 20). PP2A consists of three subunits, the catalytic C subunit (37 kDa), the regulatory A subunit (65 kDa), and one of several regulatory B subunits (approximately 55 kDa) (see references 2 and 8 for reviews). The A subunit consists of 15 nonidentical repeats, 38 to 43 amino acids in length, that are arranged in a linear fashion to form a rod-shaped molecule (1, 4, 16). Previously we demonstrated that the T antigens bind to overlapping regions in the N terminus of the A subunit; SV40 small T binds to repeats 3 to 6, while polyomavirus small T and medium T bind to repeats 2 to 8. The C subunit was shown to bind to repeats 11 to 15 in the C terminus of the A subunit. T antigens and the C subunit cooperate in binding. This effect is most pronounced for medium T, which does not detectably bind to the A subunit in the absence of the C subunit. Since T antigens form complexes only with the AC form and not with the ABC form of PP2A, it was concluded that the B subunit competes with T antigens for binding to the N terminus of the A subunit (11).

The biological role and molecular mechanism of the T antigen-PP2A interaction in transformation has yet to be elucidated. In vitro, PP2A dephosphorylates a wide variety of substrates, including proteins involved in growth control (see references 2, 8, and 17 for reviews). Small T inhibits dephosphorylation of myelin basic protein, myosin light chains, SV40 large T, and p53 by the AC form (14, 19). Inhibition of PP2A might explain the biological function of small T as a cofactor in transformation.

In this paper, the basis for a structural model of the A subunit is presented, in which this protein is proposed to consist mainly of alpha helixes joined by connecting loops. By site-directed mutagenesis we have shown that the proposed loops play an important role in protein-protein interactions. We also provide direct evidence that the B subunit binds to the N terminus and that it cooperates with the C subunit in binding to the A subunit.

MATERIALS AND METHODS

Mutants of PP2A-Aα. Site-directed mutagenesis was performed according to the method of Kunkel (7) as described previously (11). The intrarepeat loop mutants were synthesized by using mutagenic oligonucleotides that encode intrarepeat loop 9 (ANQHVKS) to replace the sequences DWFTSRT (loop 4), DTPMVRR (loop 5), EQDSVRL (loop 6), PNYLHRM (loop 13), PVANVRF (loop 14), or QDVDVKY (loop 15). The interrepeat loop mutants were synthesized by using mutagenic oligonucleotides that encode interrepeat loop 9/10 (GKDNTIEH) to replace the sequences SSAVKAE (loop 4/5), ELDNVKSE (loop 5/6), GQDITTKH (loop 13/14), or DNSTLQSE (loop 14/15) (Fig. 1). The 5′ and 3′ annealing sequences were 15 to 25 nucleotides long and ended with at least one G or C. Mutants were identified by changes in restriction patterns as compared with the wild type, as follows. (i) Substitutions with intrarepeat loop 9 conferred this loop's natural *Afl*3 restriction site to the site of substitution, and substitutions with interrepeat loop 9/10 conferred this loop's natural *Taq*1 site to the site of substitution; in two mutants, SUB and 5, the substitution eliminated a natural *Nco*I restriction site. (ii) All mutant plasmids were digested with different restriction enzymes and yielded fragments of expected sizes. The truncation and deletion mutants and mutant SUB were described previously (11).

Coupled transcription and translation in vitro. Fifty nanograms of wild-type or mutant plasmid was transcribed with T7 RNA polymerase and translated in the presence of L-[^{35}S]cys-

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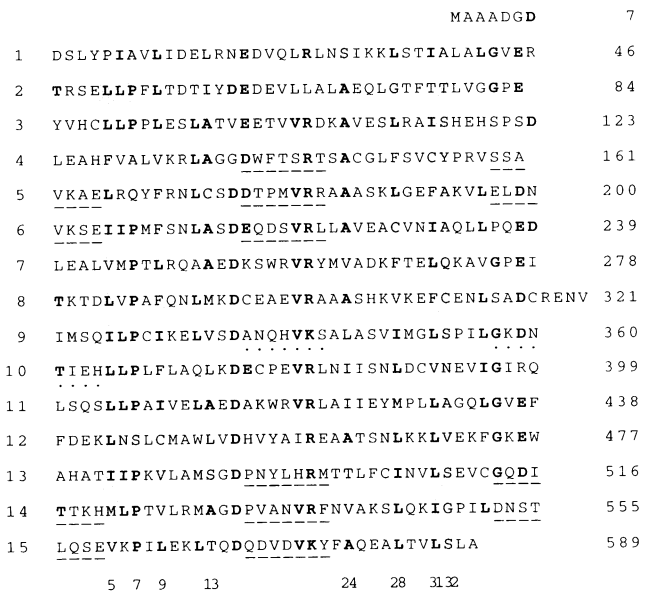

FIG. 1. Amino acid sequence and location of mutations in PP2A-Aα. Conserved amino acids are shown in boldface. Conserved uncharged or hydrophobic amino acids are indicated by shading and are numbered at bottom according to their position in the repeats. Mutated amino acids are underlined by dashes; amino acids used for replacement are underlined by dots. Numbers on the left indicate repeats, and numbers on the right indicate amino acids.

teine (>22 TBq/mmol) (NEN) or stabilized L-[^{35}S]methionine (>37 TBq/mmol) (Amersham) with Promega's TNT T7 Coupled Reticulocyte Lysate System (10 μl) at 30°C for 1.5 to 2.5 h.

Complex formation with tumor antigens and PP2A-C. The sources of tumor antigens and the conditions for complex formation with in vitro-synthesized A subunits were described previously (11). Briefly, 10-μl translation mixtures were incubated on ice with 200 ng of purified SV40 small T, provided by Kathy Rundell; with approximately 200 ng of polyomavirus small T in Sf9 cell lysate, provided by Anders Bergqvist and Goeran Magnusson; with approximately 500 ng of polyomavirus medium T in lysates of 293 cells infected with hybrid adenovirus type 5 containing the cDNA for polyomavirus medium T under control of the late adenovirus promoter (13); or with endogenous C subunit present in the reticulocyte lysate at an estimated concentration of 50 ng/10 μl. As controls, corresponding solutions or extracts without tumor antigens were used. SV40 small T was precipitated with 5 μl of hamster antitumor serum, polyomavirus small T was precipitated with 1 μl of rat antipolyomavirus tumor ascites, polyomavirus medium T was precipitated with 1 μl of monoclonal antibody (ascites) against the medium-T peptide GluGlu, and the C subunit was precipitated with 0.5 μl of anti-KL serum (11). KL designates the peptide KVTRRTPDYFL derived from the C terminus of PP2A-Cα. As a control for C subunit binding, the immunoprecipitation with anti-KL serum was inhibited by 6.6 μg of peptide KL. The immunoprecipitates of SV40 or polyomavirus small T were washed with RIPA buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1% deoxycholate, 1% Triton X-100), and the immunoprecipitates of polyomavirus medium T or of the C subunit were washed with TBST buffer (10 mM Tris-HCl [pH 7.5], 150 mM NaCl, 0.2% Tween 20, 0.02% sodium azide) at 0 to 4°C.

Complex formation with PP2A-B. Translation mixtures of 10 μl were diluted with 30 μl of buffer containing 60 mM Tris-HCl (pH 6.8), 10% glycerol, and 0.001% bromophenol blue. Aliquots of 4 μl were loaded on nondenaturing 4 to 12% polyacrylamide gradient gels (Novex). The gels were electrophoresed in the mini-V 8 · 10 chamber (Bethesda Research Laboratories) with 25 mM Tris–192 mM glycine (pH ≈ 8.3) as the running buffer. The gels were treated with acetic acid and 2,5-diphenyloxazole for fluorography, dried, and exposed to preflashed Hyperfilm MP (Amersham). It should be pointed out that no exogenous B or C subunits were added to the translation mixtures, since complex formation of [^{35}S]methionine-labeled A subunits took place with the endogenous PP2A. Purified A subunit (>90% pure), C subunit (80% pure), AC complex (90% pure), AB′C complex (85% pure), and ABαC complex (40% pure) were analyzed under the same gel conditions and stained with Coomassie brilliant blue.

RESULTS

Model of the A subunit. The most striking feature of the A subunit is its repeat structure. Conserved amino acids, including glutamic and aspartic acids, arginine, lysine, proline, glycine, and several hydrophobic amino acids, are distributed over the entire length of each repeat (4, 16) (Fig. 1). Another interesting feature, although less obvious, is that eight positions within each repeat, namely, positions 5, 7, 9, 13, 24, 28, 31, and 32, are occupied exclusively by uncharged or hydrophobic amino acids. In contrast, none of the charged amino acid positions are highly conserved, indicating that the uncharged positions have been retained during evolution. The structural basis of this conservation becomes apparent if one assumes that each repeat is composed of two alpha helixes. Figure 2 shows helical wheel projections of amino acids 5 to 13 (helix A) and 24 to 32 (helix B) derived from each half of all repeats. All the helixes are amphipathic and have the potential to interact through their hydrophobic sides. We propose that each A helix binds to the B helix from the same repeat. Amino acids 14 to 23 between the two helixes could form hydrophilic loops (designated intrarepeat loops). The sequences from amino acid 33, at the end of each repeat, to amino acid 4 of the next repeat could also form loops (designated interrepeat loops). Whereas all intrarepeat loops have the same length, the interrepeat loops vary in size, depending on the lengths of individual repeats that range from 38 to 43 amino acids. It has been reported that the A subunit has a rod-like shape (1). To accommodate this shape, we assume that the A-B helix pair from repeat one makes close contact with the helix pair from repeat two, which contacts the helix pair from repeat three, etc. The 15 A-B helix pairs would stack next to each other to form a rod.

From this model of the A subunit one can make several testable predictions. (i) The binding sites for T antigens and other subunits are arranged linearly on the rod-shaped molecule. This prediction was shown to be correct in an earlier study (11). (ii) Exchange of a conserved uncharged amino acid with a charged amino acid would weaken the amphipathic nature of the alpha helix and reduce the strength of its hydrophobic interaction with the adjacent helix. (iii) Addition or deletion of amino acids within the amphipathic helix would have a similar destabilizing effect. (iv) Exchange, addition, or deletion of amino acids within loops would have little effect on protein stability. Instead, such changes within loops might affect interactions of the A subunit with other proteins. We tested the last prediction and found that the intrarepeat loops play an important role in protein-protein interactions.

Effect of loop mutations on protein interaction. We reported previously that the replacement of seven amino acids (Asp-16 to

FIG. 2. Helical wheel projections of amino acids 5 to 13 (helix A) and 24 to 32 (helix B) of all repeats. These two regions contain the conserved uncharged or hydrophobic amino acids shaded in Fig. 1. Circle 1 represents amino acids of repeat 1, circle 2 indicates amino acids of repeat 2, etc. Positions 5, 9, and 13, which are uncharged in all A helixes, face the same direction. In all B helixes the uncharged positions 24, 28, 31, and 32 face the same direction. Charged amino acids are shaded and boldface.

Arg-22) from the intrarepeat loop 5 with seven amino acids from the corresponding loop of repeat 12 completely destroyed the ability of the A subunit to bind T antigens. This provided an initial indication that loops might be important for protein-protein interaction (11). To test this possibility further, other loop substitution mutants, schematically shown in Fig. 3, were constructed. In mutants 4, 5, 6, 13, 14, and 15, seven amino acids (no. 16 to 22) from intrarepeat loops in repeats 4, 5, 6, 13, 14, and 15, respectively, were substituted with seven corresponding amino acids from repeat 9. In addition, the interrepeat loops between repeats 4/5, 5/6, 13/14, and 14/15 were replaced by a sequence from interrepeat loop 9/10. To measure the ability of the mutant proteins to bind T antigens and the C subunit, they were synthesized in vitro in the presence of [^{35}S]cysteine, mixed with unlabeled T antigen, and immunoprecipitated with specific antisera against T antigen or the C subunit (see Materials and Methods). The immune complexes were analyzed on sodium dodecyl sulfate-polyacrylamide gels. As shown in Fig. 4, substitution of N-terminal intrarepeat loop 4, 5, or 6 strongly reduced or eliminated binding of all T antigens to the A subunit (Fig. 4a, b, and c; mutants 4, 5, and 6) but had no effect on C subunit binding (Fig. 4d, mutants 4, 5, and 6). Note that in panels a, b, and c, + indicates addition of T antigen, whereas in panel d, + indicates addition of competing peptide KL against which the antiserum to the C subunit was raised. Weak binding of SV40 small T to mutant 4 was observed in some experiments. Replacement of C-terminal intrarepeat loop 13, 14, or 15 eliminated binding of the C subunit (Fig. 4d, mutants 13, 14, and 15) and of medium T (Fig. 4c, mutants 13, 14, and 15). Binding of polyomavirus small T was strongly reduced (Fig. 4b, mutants 13, 14, and 15), whereas that of SV40 small T was not affected (Fig. 4a, mutants 13, 14, and 15). These data are summarized in Fig. 3. The results show that the intrarepeat loops are essential for protein-protein interaction. They also confirm previous data demonstrating that T antigens bind to the N terminus and that the C subunit binds to the C terminus of subunit A. The finding that polyomavirus medium T antigen was unable to associate with C-terminal intrarepeat loop mutants is consistent with the earlier observation that it also does not bind to mutants with deletions of C-terminal repeats (11). We assume that polyomavirus medium T depends on cooperation with the C subunit for binding to the A subunit. Thus, since C-terminal intrarepeat loop and repeat deletion mutants do not bind the C subunit, they also do not bind medium T. The weak binding of polyomavirus small T to mutants 13, 14, and 15 indicates that it also strongly depends on cooperation with the C subunit. SV40 small T, on the other hand, showed no cooperation with the C subunit, although under more stringent conditions, a weak effect has been observed, as shown previously (11).

To account for the finding that binding of T antigens and the B subunit (see below) to the A subunit is affected by both N- and C-terminal mutations, one could also consider the possibility that these proteins possess two sites that bind simultaneously to N- and C-terminal domains of the A subunit. As discussed previously (11), it seems unlikely that the small T antigens extend over the entire length of the A subunit. Furthermore, all T antigens, as well as the B and C subunits, require the same intrarepeat loops at the C terminus of the A subunit. Nevertheless, the T antigens and the B subunit do not compete with the C subunit for binding to the A subunit, as indicated by the fact that only ABC and T-antigen–AC complexes have been described, not BA or T-antigen–A complexes. Further support for the cooperative model comes from cross-linking experiments demonstrating that the B and C subunits contact each other when bound to the A subunit (6). One could also postulate that the A subunit can bind T antigens or the B subunit at its N and/or C terminus. In this case one would expect to find the following complexes: A-T antigen-T antigen, ABB, and AB-T antigen. However, these complexes have not been described.

Mutation of the interrepeat loops between repeats 5/6, 13/14, or 14/15 had no detectable effect on binding of T antigens and

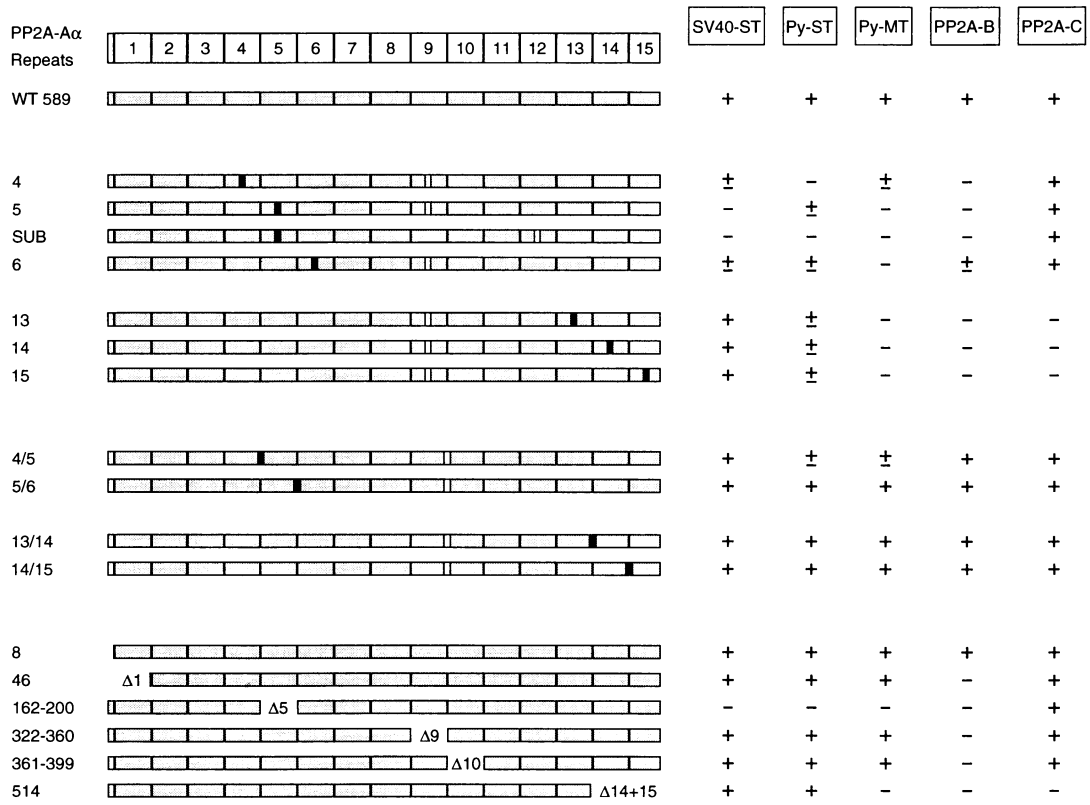

FIG. 3. Schematic representation of PP2A-Aα mutant proteins; summary of complex formation with tumor antigens and B and C subunits. The top bar represents the A subunit in a linear arrangement of its 15 repeats. The numbers on the left are designations for the mutants. Black bars indicate the sites of substitutions; white bars indicate the sequences used for substitutions. Δ indicates deletion of repeats. The deletion mutants were described previously (11). Mutants 8, 46, 162–200, 322–360, 361–399, and 514 have deletions of amino acids 2 to 7, 2 to 45, 162 to 200, 322 to 360, 361 to 399, and 515 to 589, respectively. WT 589 is the wild-type A subunit. The results of complex formation are summarized on the right. SV40-ST, SV40 small T; Py-ST, polyomavirus small T; Py-MT, polyomavirus medium T; PP2A-B, regulatory subunit B of PP2A; PP2A-C, catalytic subunit of PP2A. Scoring: −, no detectable binding; ±, barely detectable binding; +, strong binding. The data for binding of the three tumor antigens to mutants SUB, 8, Δ1, Δ5, Δ9, Δ10, and Δ14+15 are from reference 11.

the C subunit (Fig. 4a, b, c, and d; mutants 5/6, 13/14, and 14/15), whereas mutation of the interrepeat loop between repeats 4/5 reduced binding of SV40 small T (Fig. 4a, mutant 4/5) and strongly reduced or eliminated binding of polyomavirus small and medium T (Fig. 4b and c, mutant 4/5) but had no effect on the C subunit (Fig. 4d, mutant 4/5). These results emphasize the important role of the intrarepeat loops in complex formation and are consistent with the model in which the A subunit is a rod composed of helix pairs from which intrarepeat loops protrude on one side and interrepeat loops protrude on the other. The intrarepeat loop-containing surface provides a matrix for protein binding and interaction (see Fig. 6).

Binding of the B subunit. A determination of the binding region on the A subunit for the B subunit was hampered by the lack of B-specific antibodies for precipitating AB complexes. To circumvent this problem an alternative approach was used. Wild-type or mutant A subunit was synthesized in a reticulocyte lysate in the presence of [^{35}S]cysteine. Following synthesis, the entire lysate was analyzed by polyacrylamide gel electrophoresis under nondenaturing conditions, and radioactive bands were visualized by fluorography. As shown in Fig. 5b, wild-type radioactive A subunit was present in monomeric form and in AC dimers and ABC trimers, indicating that it equilibrated with unlabeled AC and ABC present in the reticulocyte lysate. If a mutant A subunit associates with the C but not the B subunit, then radioactive A subunit will appear as free A subunit and in the AC form but not as ABC. On the other hand, if a mutant A subunit is unable to complex with the C subunit, then radioactive A subunit appears only at the position of free A subunit. The migrations of A, AC, and ABC on native gels were verified by the analysis of the purified components, as demonstrated in Fig. 5a. Hemoglobin from reticulocyte lysate was used as a standard, migrating slightly faster than ABC. On radioactive gels it appeared as a white band because of its ability to quench fluorescence. All loop substitution mutants and several previously described deletion mutants were analyzed with this method. Figure 5b, lane 2, shows that deletion of amino acids 2 to 7 from the N terminus of the A subunit (mutant 8) had no effect on binding of B or C. However, replacement of intrarepeat loop 4, 5, or 6 (Fig. 5b, lanes 3, 5, and 7) resulted in the formation of very little if any ABC, indicating that these loops are essential for binding of the B subunit. By contrast, substitution of N-terminal interrepeat loop 4/5 or 5/6 (Fig. 5b, lanes 4 and 6) had no effect on ABC formation. ABC was frequently detected as multiple bands, which were not further analyzed. They might represent different modified forms of the enzyme, or they might differ in subunit composition. A band migrating between hemoglobin

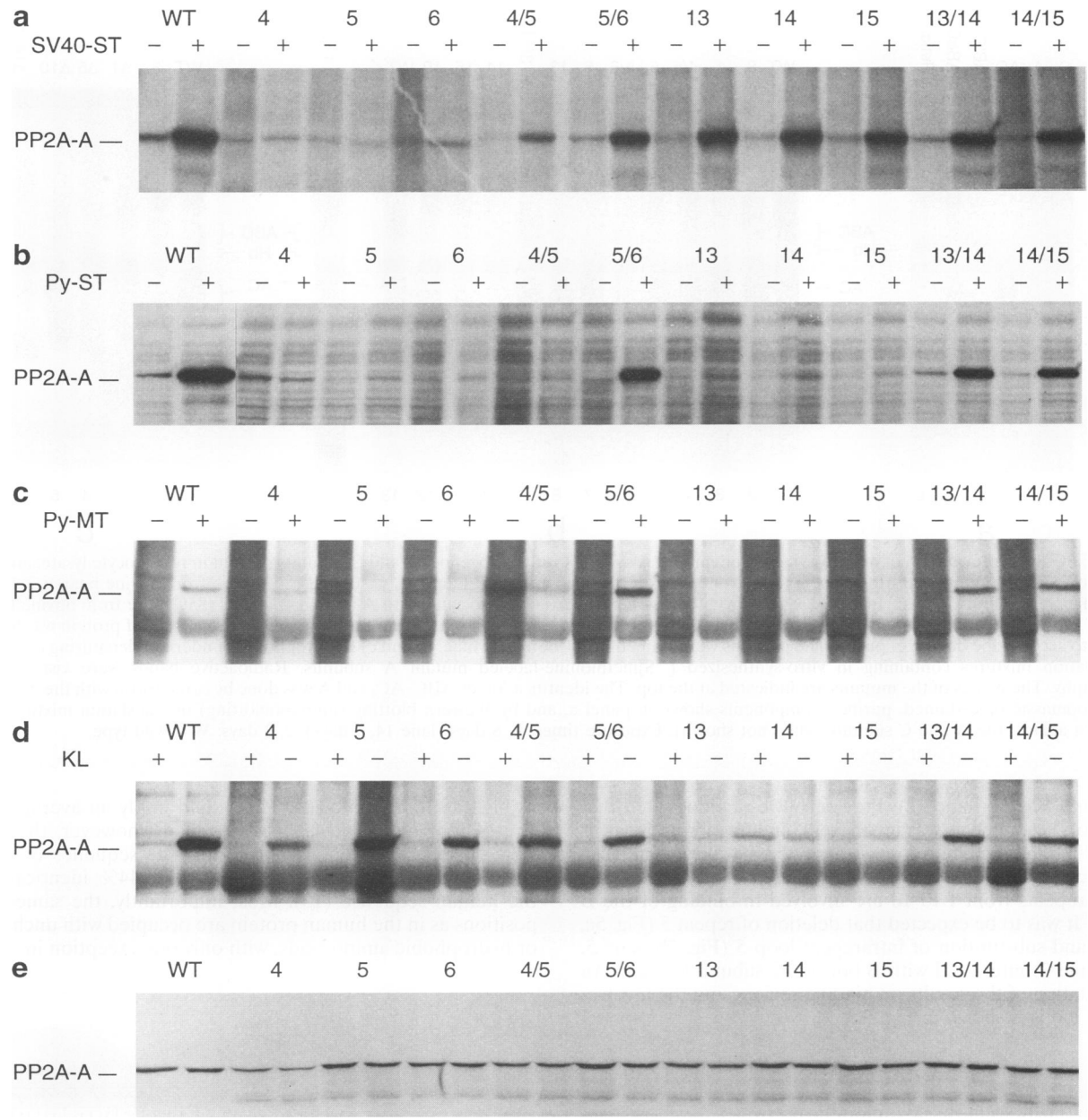

FIG. 4. Complex formation of mutant A subunits with T antigens and the C subunit of PP2A. Mutant A subunits, named at the top of each panel, were synthesized in vitro in the presence of [^{35}S]cysteine. Translation mixtures were incubated with SV40 small T (a), polyomavirus small T (b), polyomavirus medium T (c), or competing peptide KL corresponding to the C terminus of Cα (d) (+ lanes) or with solutions without T antigens (a to c) or without peptide KL (d) (− lanes). Complexes were immunoprecipitated with hamster antitumor serum (a), rat antipolyomavirus tumor ascites (b), anti-medium T peptide GluGlu (c), or anti-C subunit peptide KL (d). Aliquots of the translation mixtures used in panel d are shown in panel e. The fraction of immunoprecipitates loaded was 0.5 in panels a to d, and the fraction of translation mixture loaded was 0.05 in panel e. Exposure times (days): a, 7; b, 7; c, 10; d, 7; e, 0.7. For abbreviations, see the legend to Fig. 3.

and AC was not further analyzed. A striking result was obtained with the intrarepeat loop mutants 13, 14, and 15, which exhibited only the free A subunit (Fig. 5b, lanes 8, 10, and 11). As shown above, these mutants are defective in C subunit binding. Therefore, ABC was also not found. For the same reason the simultaneous deletion of repeats 14 and 15 did not lead to formation of AC and ABC (Fig. 5c, lane 6). Since we also did not find AB complexes, the B subunit in the reticulocyte lysate is most likely Bα, which does not bind to A subunit in the absence of C subunit (6a). The B subunit also does not bind to the C subunit in the absence of the A subunit (6). The finding that replacement of interrepeat loop 13/14 or 14/15 permitted formation of AC and ABC (Fig. 5b, lanes 9 and 14) confirms that these loops are not involved in binding of the C subunit (Fig. 4d). We were surprised to find that the deletion of repeat 1, 9, or 10 repressed ABC formation (Fig. 5c, lanes 3

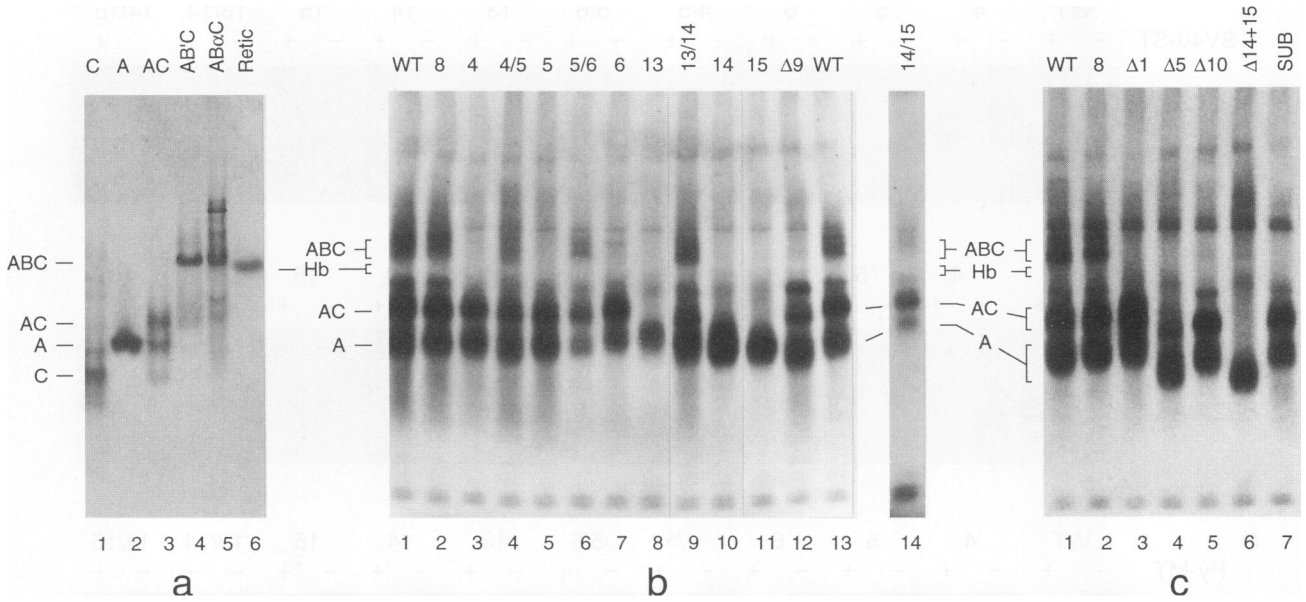

FIG. 5. Complex formation of in vitro-synthesized mutant A subunits with endogenous B and C subunits present in reticulocyte lysate, analyzed by electrophoresis in nondenaturing gels. (a) Electrophoresis of purified enzymes. Lanes: 1, C subunit (80% pure from bovine heart); 2, human Aα subunit (>95% pure); 3, AC form (90% pure from bovine heart, partially dissociated into A and C); 4, AB′C (85% pure from bovine heart); 5, ABαC (40% pure from bovine heart); 6, reticulocyte lysate, 0.01 μl (Hb, hemoglobin). For all enzyme preparations 4 μg of protein was loaded, taking into account the degree of purity. The proteins were stained with Coomassie blue. (b and c) Electrophoresis under nondenaturing conditions of translation mixtures containing in vitro-synthesized [^{35}S]methionine-labeled mutant A subunits. Radioactive bands were visualized by fluorography. The names of the mutants are indicated at the top. The identification of ABC, AC, and A was done by comparison with the migration of the Coomassie blue-stained, purified components shown in panel a, and by Western blotting (immunoblotting) of translation mixtures with antibodies against the A and C subunits (data not shown). Exposure times: b, 8 days (lane 14, 7 days); c, 3 days. WT, wild type.

and 5, and Fig. 5b, lane 12). Previously we demonstrated that neither of these repeats was involved in binding of T antigens or of the C subunit, and therefore we assumed that they would also not be required for B subunit binding. The present data suggest that all repeats from 1 to 10 are involved in binding of the B subunit. It was to be expected that deletion of repeat 5 (Fig. 5c, lane 4) and substitution of intrarepeat loop 5 (Fig. 5b, lane 5, and c, lane 7) interfered with B but not C subunit binding. An interpretation of the results on binding regions and the involvement of loops is shown in Fig. 6.

DISCUSSION

The discovery that the A subunit of PP2A is composed of 15 repeats suggested that its three-dimensional structure contains some element of regularity. Two findings were pivotal for developing a structural model. One is the observation by Chen et al. (1) that the A subunit has a rod-like shape with an axial ratio of 10.5 to 1. The other is the finding described in this paper that eight equivalent positions in every repeat are occupied by uncharged or hydrophobic amino acids, four of which are clustered together in the first and second half of every repeat. The use of helical wheel projections demonstrated that each repeat can give rise to two amphipathic helixes that could form a pair. To obtain a stiff rod, neighboring helix pairs have to interact with each other. The sum of all postulated helixes would result in a protein with approximately 50% alpha helix content. Preliminary circular dichroism measurements showed a helix content of 80% for the purified A subunit (6a), suggesting that the length of individual helices (helix A from position 5 to 13 and helix B from position 24 to 32) represents a minimum and that the average length could be as high as 16 amino acids. This would imply an average loop size of four amino acids. It seems likely, however, that loop sizes vary between different repeats. The sequence of the A subunit from *Saccharomyces cerevisiae* is 44% identical with the human sequence (15). Most importantly, the same eight positions as in the human protein are occupied with uncharged or hydrophobic amino acids, with only one exception in repeat

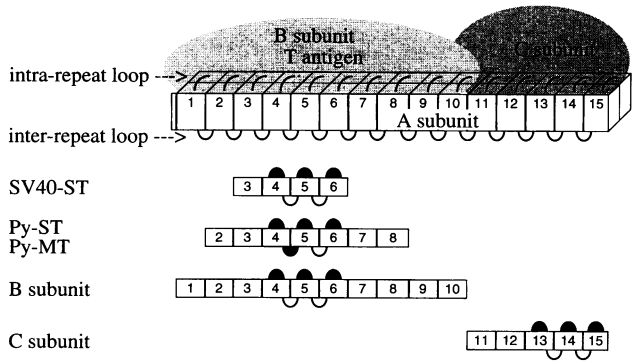

FIG. 6. Model of the A subunit of PP2A showing the roles of loops in binding of T antigens and the B and C subunits. The A subunit is drawn as a rod with 15 repeats. Each repeat is subdivided into two α helixes. Intrarepeat loops and interrepeat loops are indicated by arrows. Presumed binding regions for the T antigens, the B subunit, and the C subunit are shown as bars below the A subunit. Loops involved in binding are solid, loops not involved in binding are white. Loops not shown in the bars were not mutated. For abbreviations, see the legend to Fig. 3.

12, in which position 7 is occupied with aspartic acid (serine in the human sequence). This indicates that the yeast and human A subunits have very similar helical structures, which have been conserved for more than one billion years.

Although our model of the A subunit is speculative and final proof can come only from X-ray crystallography, it has provided a useful basis for the design of mutants. So far, the biological properties of the mutant proteins are consistent with the model. The first set of mutants established the binding regions for T antigens, the C subunit, and, indirectly, the B subunit. These regions extend linearly and without interruption over several repeats, as one might expect for a rod-shaped molecule. In the present study, the role of hypothetical loop structures in protein-protein interaction was investigated. We demonstrated that the loops between the helices within each repeat are most important for the binding of T antigens and the C and B subunits. The loops connecting alpha helices between successive repeats seem to be less important. Our results do not exclude the possibility that the binding regions include other parts of the A subunit, e.g., exposed helical regions. This question could be addressed by investigating additional mutants. It is remarkable that most A helices contain a conserved proline not located in the hydrophobic face. Being helix breakers, these prolines might introduce a bend into the A helices.

The binding region for the B subunit was determined by measuring the exchange between radioactive mutant A protein synthesized in vitro and wild-type A subunit present in multimeric complexes (ABC) in the reticulocyte lysate. If mutant A protein appeared in the ABC complex, then the binding region for the B subunit was intact. On the other hand, if it was found only in AC, then the binding region for the C but not for the B subunit was intact. The results confirm the previous notion that B subunit competes with T antigens and binds to the N terminus. The binding of the B subunit involves a large area on the A subunit (repeats 1 to 10). Since the C subunit binds to repeats 11 to 15, one side of the A subunit appears to be completely covered by the associated subunits.

An unexpected homology between the A subunit and a serine/threonine-specific protein kinase from *S. cerevisiae*, designated vps15 kinase, has recently been noticed (5). This kinase is required for the sorting of soluble vacuolar proteins. It contains a stretch of 117 amino acids in the middle of the polypeptide that consists of three nonidentical repeats of 39 amino acids and is homologous to repeats 9, 10, and 11 of the A subunit. This region partially overlaps with the binding sites for the C and B subunits. Surprisingly, the vps15 repeats are equally homologous to the human and *S. cerevisiae* A subunits. We recently reported (11) a short stretch of homology between the human A subunit and the yeast *cdc25* gene product, a tyrosine-specific protein phosphatase. It may be that the A subunit is composed of motifs that are used in various proteins for the interaction with other proteins.

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