equation), and ϕ is the instantaneous angle between neighboring base pairs for the particular conformation. The first two terms are the energy required to change the equilibrium twist angle from ϕ_0 to ϕ_0', and the last two terms represent the energy arising from fluctuations about the new equilibrium angle. The twisting potential energy as a function of twist angle between base pairs is shown in Fig. 4 for $F = 0.33$.

The magnitude of the anharmonicity for the relaxed molecule can be estimated by determining the size of the cubic term relative to the quadratic term in the potential energy expression when $(\phi - \phi_0') \approx 5°$, the root-mean-square deviation due to thermal energy. For $F = 0.33$, the correction term represents a change of ~15% in the height of the potential energy surface for the relaxed topoisomer at $U = 1/2\ k_BT$. This value is sufficiently small that the perturbation-type expansion we have used is expected to be valid. Moreover, the Barkley-Zimm model, a harmonic approximation, is reasonable to use in analyzing each narrow topoisomer distribution, as we have done.

Our results have consequences for the formation of protein complexes on supercoiled DNA. The araC protein forms looped structures on negatively supercoiled DNA, but not on relaxed minicircle DNA (*23*). Similarly, the *lac* repressor can form stable loops on negatively but not positively supercoiled minicircle DNA when a twist is required to bring protein binding sites onto the same side of the helix (*24*). Our results imply that the preference for negatively supercoiled DNA is not due to increased twisting flexibility of the negatively supercoiled DNA. Rather, we speculate that these DNA-looping proteins may instead be sensitive to the spatial orientation of juxtaposed DNA strands, which changes for supercoils of opposite sign.

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Interaction of p107 with Cyclin A Independent of Complex Formation with Viral Oncoproteins

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The p107 protein and the retinoblastoma protein (RB) both bind specifically to two viral oncoproteins, the SV40 T antigen (T) and adenoviral protein E1A (E1A). Like RB, p107 contains a segment (the pocket) that, alone, can bind specifically to T, E1A, and multiple cellular proteins. Cyclin A bound to the p107 pocket, but not the RB pocket. Although both pockets contain two, related collinear subsegments (A and B), the unique sequence in the p107 pocket that occupies the space between A and B is required for the interaction with cyclin A.

THE CELLULAR PROTEIN, p107, shares a number of properties with the retinoblastoma product (RB), a known tumor suppressor. Both proteins form complexes with SV40 large T antigen (T) and the adenoviral protein E1A (*1–3*), and RB interacts with the human papilloma virus E7 protein (*4*). Complex formation may, in part, underlie the transforming functions of these viral proteins (*2–6*). RB also interacts specifically with several cellular proteins in vitro (*13–15*). These interactions may account for the proposed regulation of the cell cycle by RB (*7–12*). RB and cyclin A, another protein that functions to regulate the cell cycle, both bind specifically to the transcription factor, E2F (*16–21*). These interactions may also contribute to cell cycle control.

The p107 protein has clear sequence similarity to RB. The RB sequence contains a stretch of ~400 amino acids (the pocket) that can alone bind T, E1A, and the aforementioned cellular proteins (*13, 22–24*). The pocket is composed of two subsegments (A and B) that are separated by a spacer of ~75 residues (Fig. 1). Foreign sequences can be substituted for the spacer without disturbing binding of T and E1A (*22, 23*). Similarly, a segment of p107 containing ~600 residues (residues 252 to 816) can also bind T and E1A. The segment composed of two subsegments similar to A and B of RB. Parts A and B of p107 are separated by a spacer of ~200 amino acids (Fig. 1) (*1*). Substituting the RB spacer for the spacer in p107 did not affect binding of T or E1A (*1*), suggesting that the

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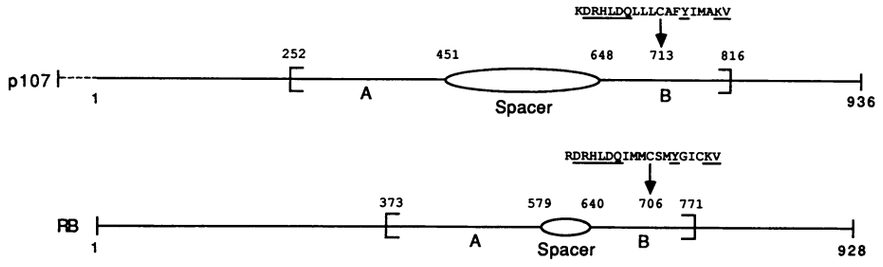

Fig. 1. Sequence landmarks of p107 and RB. The major elements of similarity in the p107 and RB sequences lie within subsegments of their respective T and E1A binding regions (pockets) (*1*). These subsegments are indicated by A and B. Subsegment A spans residues 252 to 451 in p107 and residues 373 to 579 in RB. Subsegment B spans residues 648 to 816 in p107 and residues 640 to 771 in RB (*1*). The spacer regions situated between the A and B subsegments are indicated. The sequences of the RB and p107 spacers are different (*1*). Cys^{706} is the site of a naturally occurring RB mutation (*26*). Cys^{706}, the corresponding residue in p107, Cys^{713}, and their surrounding sequences are indicated. Identical residues in these two segments are underlined. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

sequence composition of the spacer is not essential for this function.

Given the similarities between the p107 and RB pockets, we tested whether these domains bound the same set of cellular proteins. Various RB- and p107–glutathione S-transferase (GST) fusion proteins were used as affinity reagents in search of cellular proteins that can bind specifically to them. A set of comigrating cellular proteins from a human retinoblastoma line (WERI-Rb27) bound to both the GST-RB and GST-p107 pockets. If either fusion protein was first incubated with peptide replicas of the RB binding domains of either T or E7, the comigrating proteins were not bound (Fig. 2) (*25*). Two polypeptides (X and Y) bound only to the GST-p107 pocket.

A point mutation (Cys^{706}→Phe) in the B subdomain of RB prevents binding of the protein to T, EA, and cellular proteins (*13, 26*). Cys^{713} of p107 is also in subdomain B, and its neighboring sequences are similar to those surrounding Cys^{706} in RB (Fig. 1). A GST-p107 fusion protein in which Cys^{713} was changed to Phe did not bind cellular proteins, again implying that there is some similarity in the mechanisms by which these two pockets operate.

Protein Y, which bound only to the p107 pocket (Fig. 2), migrated during SDS–gel electrophoresis similarly to cyclin A, a protein known to associate with p107 (*27*). To test its relationship to cyclin A, various GST-p107 pocket derivatives were mixed with in vitro translated human cyclin A (Fig. 3). Cyclin A bound the intact pocket [GST-p107 (252 to 816)] with and without the Cys^{713}→Phe mutation, and T peptide did not compete for binding. The RB pocket did not bind cyclin A.

The most obvious structural difference between the RB and p107 pockets is the presence of dissimilar spacer elements (*1*). Thus, the p107 spacer sequence might contribute specific binding of cyclin A to p107.

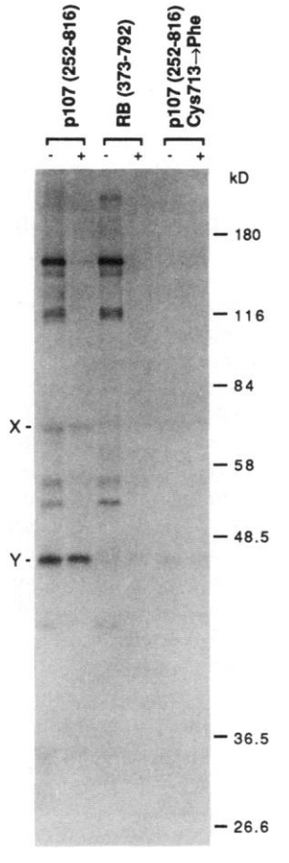

Fig. 2. Detection of cellular proteins specifically bound by p107. Various segments of RB and p107 and the Cys^{713}→Phe mutant of p107 were cloned into a variant of pGEX-2T (Pharmacia) called pGEX-2TK (*31*). The RB and p107 sequences encoded by each resulting fusion protein are indicated. The Cys^{713}→Phe mutant was created by site-directed mutagenesis (*32*) with a mutant primer, 5′-CAGCTCCTCCTTTTTGCCTTTTATATC-3′ [nucleotides 2125 to 2151 in the p107 cDNA (*1*)]. The GST fusion proteins (~500 ng) bound to glutathione-Sepharose (Pharmacia) were generated as described (*13*). Where indicated (+), a peptide (2 μg) spanning amino acid residues 16 to 32 (QPETTDLYCYEQLNDSS) of HPV-16 E7 was added to the reaction mixture. Similar peptides inhibit formation of complexes between RB and E7 or cellular proteins (*15, 33*). A centrifuged, [^{35}S]methionine-labeled extract of ~6 × 10^6 WERI-RB27 cells was then added. After incubation, the beads were washed (*13*). Bound proteins were eluted, resolved by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (10% gel), and detected by fluorography. The migration positions of prestained molecular size markers (Sigma) are indicated.

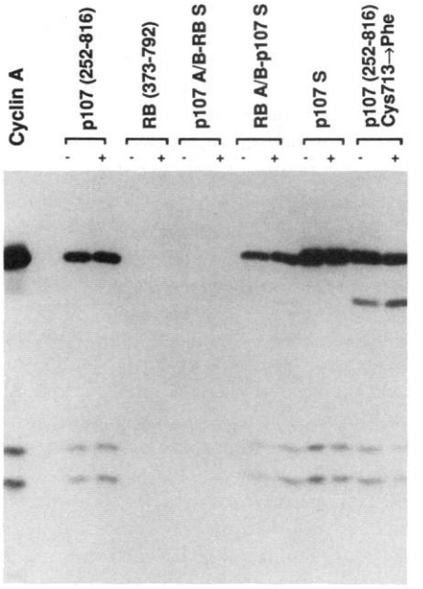

Fig. 3. Binding of cyclin A to the p107 spacer element. Identical amounts of various GST fusion proteins synthesized in *Escherichia coli* and bound to glutathione-Sepharose were generated as described (*13*) (Fig. 2). Where indicated (+), T peptide (50 μg) spanning amino acids 102 to 115 in SV40 T antigen (*7*), was added to the bound GST fusion proteins (~500 ng). In each case 700 μl of buffer containing 50 mM tris-HCl (pH 8.0), 120 mM NaCl, Nonidet P-40 (0.5%), and aprotinin (Sigma), leupeptin (BMB), and phenylmethyl sulfoxide (Sigma) (all at 5 μg/ml) was added. In vitro translated ^{35}S-labeled cyclin A (20 μl) was added and the mixture was incubated for 1 hour at 4°C. The beads were washed as described (Fig. 2) (*13*). The proteins were resolved by SDS-PAGE (10% gel), and labeled proteins were detected by fluorography. The p107 pocket (subsegments A and B) containing an RB spacer element in place of its native RB spacer (indicated by p107 A/B-RB S) was constructed as described (*1*). A chimeric RB pocket composed of A and B subdomains of RB separated by a p107 spacer in place of the native RB spacer (defined as RB A/B-p107 S) was constructed in an analogous manner. In this chimera, the A subdomain of RB spans residues 373 to 571, the B subdomain spans amino acids 646 to 792, and the p107 spacer extends from p107 residue 443 to 654. The p107 spacer alone (residues 443 to 654) is indicated by p107 S. The cyclin A DNA template used for in vitro transcription and translation contains the cyclin A sequences described previously (*30*).

A series of chimeric pockets and pocket subsegments were tested for binding to cyclin A. The p107 pocket containing an RB spacer in place of its own spacer segment failed to bind cyclin A. In contrast, the RB pocket containing a p107 spacer did bind cyclin A, and T peptide did not inhibit binding. Both chimeric pockets did bind to T and E1A (*1, 25*), indicating that switching the spacer elements did not affect viral protein binding to the pocket. Thus, the p107 pocket and, in particular, its spacer can bind directly or indirectly to cyclin A. The following results (Fig. 3) further indicate that cyclin A binding is independent of cellular or viral protein binding: (i) GST-p107 (Cys^{713}→Phe), which does not bind T or E1A still bound cyclin A. (ii) Peptides from T and E7 did not compete with cyclin A for pocket binding. (iii) The spacer alone conferred cyclin A binding to RB.

The transcription factor E2F (DRTF1) can form a complex with RB and cyclin A (*16–21*). As defined by peptide competition and genetics, the pocket-binding proteins of p107 and RB appear to be the same (Fig. 2). E2F has been shown to be a pocket-binding protein (*19*), and would be predicted to form a complex with p107. Because cyclin A specifically binds to the p107 spacer, while other known pocket binding proteins likely interact with A and B domain sequences, E2F and cyclin A might be able to interact simultaneously with the p107 pocket (*28*).

If cyclin A operates by modulating the function of a bound cdc2 or cdk2 (cyclin-dependent kinase 2) kinase, at least three possibilities might be envisioned to explain how cyclin A complex formation could be linked to p107 function. (i) The target of a kinase–cyclin A complex might be p107, which, by analogy with RB (*7–12*), might be functionally altered by specific phosphorylation. (ii) The target of the bound cyclin A–kinase complex could be one or more p107 pocket-binding proteins such as E2F, which can be activated by phosphorylation (*29*) and binds cyclin A at a specific time in the cell cycle (*21*). (iii) The p107 protein might act as a transducer, sending a signal from a protein specifically bound in its pocket to the cyclin-kinase complex also bound on the pocket surface. Such a signal might regulate the kinase such that one or more proteins are or are not phosphorylated at a particular time in the cycle. These three models are not mutually exclusive. Finally, p107-bound cyclin A might, in theory, have functions other than regulating kinase activity of cdc2 or cdk2.

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Interaction Between Human Cyclin A and Adenovirus E1A-Associated p107 Protein

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The products of the adenovirus early region 1A (E1A) gene are potent oncoproteins when tested in standard transformation and immortalization assays. Many of the changes induced by E1A may be due to its interaction with cellular proteins. Four of these cellular proteins are the retinoblastoma protein (pRB), p107, cyclin A, and $p33^{cdk2}$. The pRB and p107 proteins are structurally related and have several characteristics in common, including that they both bind to the SV40 large T oncoprotein as well as to E1A. Cyclin A and $p33^{cdk2}$ are thought to function in the control of the cell cycle. They bind to one another, forming a kinase that closely resembles the cell cycle–regulating complexes containing $p34^{cdc2}$. Cyclin A is now shown to bind to p107 in the absence of E1A. The association of p107 with cyclin A suggests a direct link between cell cycle control and the function of p107.

CYCLIN A PARTICIPATES IN THE REGulation of the cell cycle (*1–5*), apparently by acting as a regulatory protein that associates with either the $p34^{cdc2}$ or $p33^{cdk2}$ kinase (*1, 6–8*). Cyclin A is also present in a protein complex that contains transcription factors such as E2F and DRTF1 (*9–11*). The biochemical significance of the interaction of cyclin A with both kinases and transcription factors is not clear at present.

We identified a 60-kD polypeptide, which was later shown to be cyclin A (*1*), as an E1A-associated protein. We have immunoprecipitated cyclin A and its associated proteins with two cyclin A–specific monoclonal antibodies, BF683 and C160 (*6, 8*). Proteins in lysates of [^{35}S]methionine-labeled 293 cells, which constitutively express E1A, or ML-1 myeloid leukemia cells were immunoprecipitated with these or control antibodies. Proteins from the 293 cell lysates were also precipitated with the E1A-specific antibody M73 (*12*). Both BF683 and C160 efficiently recognized cyclin A from ML-1 and 293 cells (Fig. 1, A and B). Each antibody precipitated several proteins, but our primary interest was in proteins that were detected with both antibodies to cyclin A.

Two groups of differently sized proteins were immunoprecipitated with either BF683 or C160. A set of proteins that migrate with molecular sizes of approxi-

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