suggests that a tenfold increase in expression resulting from a mutation such as the one described here could play a decisive role in tumorigenesis.

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Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product

Peter Whyte*†, Karen J. Buchkovich*, Jonathan M. Horowitz‡, Stephen H. Friend‡§, Margaret Raybuck*†, Robert A. Weinberg‡ & Ed Harlow*‖

* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA
‡ Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142, USA and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
§ Division of Hematology-Oncology, The Children's Hospital, Dana-Farber Cancer Institute, Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115, USA

One of the cellular targets implicated in the process of transformation by the adenovirus E1A proteins is a 105K cellular protein. Previously, this protein had been shown to form stable protein/protein complexes with the E1A polypeptides but its identity was unknown. Here, we demonstrate that it is the product of the retinoblastoma gene. The interaction between E1A and the retinoblastoma gene product is the first demonstration of a physical link between an oncogene and an anti-oncogene.

REGULATION of cellular proliferation is a complex process that involves both positively and negatively acting signals. Tumourigenesis results from alterations in genes whose protein products are involved in these signalling pathways. The DNA tumour viruses encode a set of proteins that are capable of overriding and reprogramming normal regulation of cellular growth; consequently, they have been widely used as model systems for studying cellular transformation. The oncogenes—tumour-inducing genes—from polyomavirus, simian virus 40 (SV40) and adenovirus are able to induce a number of distinct changes in cell phenotype, including immortalization, secretion of growth factors, loss of contact inhibition, anchorage-independent growth and morphological transformation. Unlike the transforming retroviruses, these DNA viruses contain oncogenes that do not appear to have cellular homologues. Although functional similarities have been shown between cellular oncogenes

† Present addresses: Fred Hutchinson Cancer Center, 1124 Columbia Street, Seattle, Washington 98104, USA (P.W.) and Amersham International plc., Forest Farm Industrial Estate, Whitchurch, Cardiff, UK (M.R.).
‖ To whom correspondence should be addressed.

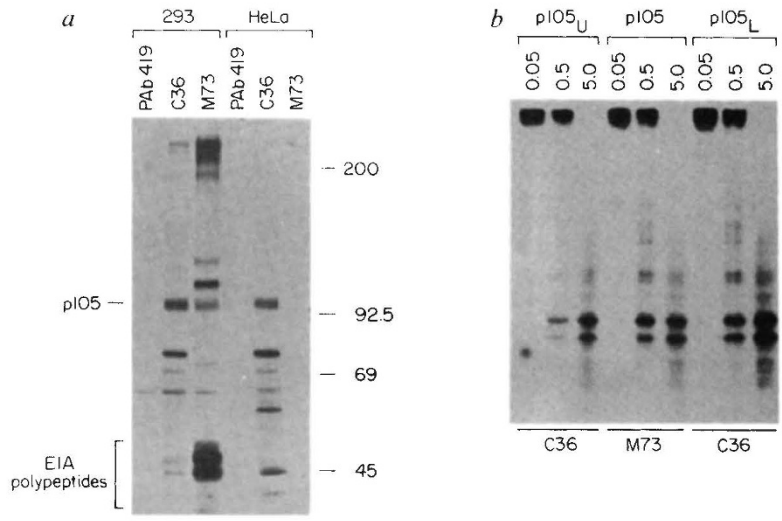

Fig. 1 Characterization of the monoclonal antibody C36. *a*, Extracts from [^{35}S]methionine-labelled cultures of human 293 or HeLa cells were immunoprecipitated with M73, a monoclonal antibody specific for the E1A proteins[30], C36, a monoclonal antibody specific for the 105K protein, or PA6419, a control monoclonal antibody specific for SV40 large T antigen[32]. Proteins were resolved on a 6% polyacrylamide gel and detected by fluorography[47]. *b*, Partial proteolytic maps of the 105K proteins immunoprecipitated by either C36 or M73 antibodies. Proteins from 293 cells were labelled with [^{35}S]methionine and immunoprecipitated using either C36 or M73. After SDS-PAGE, the autoradiogram of the gel was used as a template to excise the 105K bands. The bands that form the 105K doublet immunoprecipitated by C36 were excised and analysed separately. The upper band is labelled $p105_U$ and the lower $p105_L$. Only the lower band was excised from the M73 immunoprecipitates. The proteins were subjected to partial digestion with increasing amounts of *Staphylococcus aureus* V8 protease, given in micrograms per reaction[48].

Methods. Immunoprecipitations were described previously[30]. To prepare C36, E1A/host-protein complexes were purified from lysates of 293 cells on immunoaffinity columns prepared by cyanogen bromide coupling of M73 antibody to Sepharose 4B beads and then eluted with 1 M acetic acid. The acetic acid was removed by lyophilization and the proteins were resuspended in phosphate buffered saline (PBS). Purified proteins from $\sim 2 \times 10^8$ cells were used to immunize mice and monoclonal antibodies were prepared by fusing splenocytes to NS-1 myeloma cells[49] three days after the final boost. Positive tissue culture supernatants were identified by immunoprecipitation of [^{35}S]methionine-labelled extracts of 293 cells.

and those carried by DNA tumour viruses, it is not known whether these functional similarities extend to the biochemical level.

The proteins encoded by several DNA tumour-virus oncogenes form stable complexes with host-cell proto-oncogene proteins. Examples are the polyoma middle T/pp60 *c-src* complex[1], the SV40 large T/p53 complex[2-4] and the adenovirus E1B/p53 complex[5]. In these cases, the formation of the complex has a profound effect either on the catalytic activity of the cellular protein, as in the case of the kinase activity of pp60 *c-src*[6,7], or on protein stability, as with p53[8,9]. These changes are thought to potentiate the transforming functions of these proteins.

The mechanism of action of the adenovirus oncogene E1A is less well resolved. Acting on its own E1A can immortalize primary cells[10-12]; it can also cooperate with the adenovirus E1B gene or an activated *ras* gene to transform cells in culture, and these cells will induce tumours in animals[11,13]. In addition the E1A-encoded proteins are potent regulators of gene expression, being able to modulate transcription of both viral and cellular genes (reviewed in ref. 14). The E1A proteins activate transcription of the other adenovirus early genes and certain cellular genes. They also repress transcription of genes linked to certain viral or cellular enhancers[54,55].

We and others have previously shown that the E1A oncoproteins associate with host-cell polypeptides known only by their relative molecular masses (M_r) of ~28,000 (28K), 40K, 50K, 60K, 80K, 90K, 105K, 107K, 130K and 300K[15,16]. These complexes appear to mediate some of the physiological alterations induced by E1A, as any mutation that destroys binding of an E1A protein to the most predominant of the bound host proteins 300K, 107K or 105K, also inactivates the ability of the E1A oncogene to cooperate with a *ras* oncogene in transforming primary baby-rat kidney cells (ref. 17 and P.W., N. Williamson and E.H., submitted). These correlations suggest that the transforming properties of the E1A proteins depend on their binding to these host proteins.

Another class of genes involved in tumourigenesis, but apparently unrelated to either E1A or the other DNA tumour-virus oncogenes, is that of the 'tumour-suppressor' genes or 'anti-oncogenes' (reviewed in refs 18, 19). The inactivation of these genes has been implicated as a causal event in the generation of a number of different human tumours. It appears that when both copies of an anti-oncogene are inactivated, cells initiate uncontrolled growth. Hence, the normal function of genes of this class seems to lie in blocking cell proliferation, although the molecular mechanisms are obscure.

The best studied anti-oncogene is the retinoblastoma gene (*RB* gene) inactivation of which favours the appearance of retinoblastomas and certain soft-tissue sarcomas (reviewed in refs 20-24; the abbreviation *RB* will be used for the gene and RB for the encoded protein, while retinoblastoma will be used to reference to the tumour). The *RB* gene exhibits large deletions in as many as 30% of the retinoblastoma tumour DNAs examined[25,26]. Recently, we and others have isolated a full length *RB* complementary DNA[25-27]. The cDNA was sequenced and found to encode a protein of 928 amino acids[27,28]. The identification of this protein as a nuclear phosphoprotein of ~110K[29] led us to compare it with the E1A-associated 105K and 107K proteins, which are also nuclear phosphoproteins.

We report here that the 105K protein is the product of the *RB* gene, thereby demonstrating a physical and presumably functional association between the products of the E1A oncogene and the *RB* anti-oncogene. This association has unexpected implications for the mechanisms of action of these two types of gene products.

Anti-105K protein antibodies

In an initial study of the E1A proteins, a series of E1A-specific monoclonal antibodies[30] were prepared and used to analyse the E1A proteins in both adenovirus-infected and adenovirus-transformed cells. Immunoprecipitations of lysates from these cells yielded not only the E1A-encoded proteins, but also a series of other proteins of differing M_rs (ref. 16 and see above) that were physically associated with the E1A polypeptides. To examine these cellular proteins directly, we sought to prepare antibodies that react specifically with them. To this end, we purified the E1A/cellular protein complexes from 293 cells, a line of human embryonic cells that has been transformed by a fragment of the adenovirus 5 genome[31], using the M73 anti-E1A monoclonal antibody, and used the complexes as immunogens for the production of further monoclonal antibodies. Among the various resulting antibodies was one, designated C36, that reacted specifically with the E1A-associated 105K host-cell protein.

The initial characterization of the C36 antibody is shown in Fig. 1*a*. In this experiment, lysates of [^{35}S]methionine-labelled

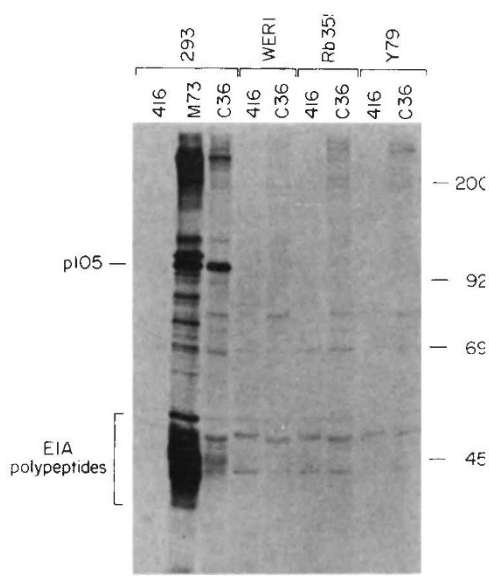

Fig. 2 Immunoprecipitations using the C36 monoclonal antibody from lysates of retinoblastoma cells. Cultures of 293, WERI-1, Y79, or RB355 cells were radiolabelled with [^{35}S]methionine, and lysates were precipitated with either C36, M73 or PAb416 monoclonal antibodies. PAb416 is a monoclonal antibody specific for SV40 large T antigen[32]. Immune complexes were collected on protein A-Sepharose beads and analysed on an 8% SDS-polyacrylamide gel by fluorography.

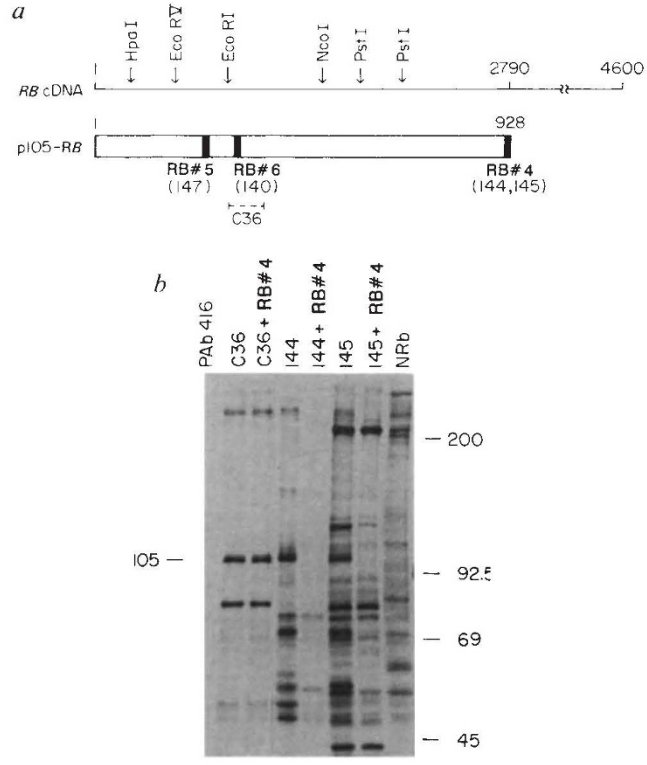

Fig. 3 Comparison of proteins immunoprecipitated with the C36 monoclonal antibody and rabbit antisera raised against peptide RB#4. *a*, Location of peptides used to produce rabbit polyclonal anti-RB antisera. Peptide RB#4 corresponds to the carboxy-terminal 15 residues of the deduced RB protein sequence (Q-K-M-N-D-S-M-D-T-S-N-K-E-E-K). Two rabbits were injected with peptide Rb#4 and the sera are designated 144 and 145. The sequence of peptide RB#5 is G-S-P-R-T-P-R-R-G-Q-N-R-S-A-R and yielded serum 147. Peptide RB#6 is R-Y-E-E-I-Y-L-K-N-K-D-L-D-A-R and yielded serum 140. The predicted location of the C36 epitope is also indicated. *b*, Extracts from [^{35}S]methionine-labelled 293 cells were immunoprecipitated with the 144 or 145 anti-RB#4-peptide sera, the C36 monoclonal antibody specific for the E1A-associated 105K protein, or control antibodies. Immunoprecipitations were done either in the absence or presence of a saturating amount of peptide RB#4. Immunoprecipitated proteins were separated on 6% polyacrylamide gels and located by fluorography.
Methods. Peptides were synthesized on an Applied Biosystems 430A protein synthesizer, purified by HPLC, and subjected to amino-acid analysis and mass spectrometry. In addition to the indicated residues of each peptide, an amino-terminal cysteine was included in the RB#4 peptide and carboxy-terminal cysteines were included in peptides RB#5 and RB#6 to allow convenient coupling to KLH. Coupling of the peptides to KLH was performed using N-succinimidyl bromoacetate according to Liu *et al.*[50]. The peptide/KLH conjugates were injected at multiple subcutaneous sites (100 μg peptide per injection) into male New Zealand rabbits every 2 weeks for 8 weeks.

293 cells were incubated with antibodies C36, M73 or PAb419, a control monoclonal antibody specific for the SV40 large T antigen[32]. As expected, the M73 antibody precipitated the E1A polypeptides together with a group of the host polypeptides, among them the 105K protein. (The heterogeneity of the E1A proteins is due to differential splicing of the E1A primary transcript and to post-translational modifications[32–42].) The C36 antibody precipitated the 105K antigen along with a portion of the E1A proteins. The identity of the co-precipitated E1A proteins was confirmed both by immunoblotting with a panel of monoclonal antibodies that recognize several distinct epitopes on E1A, and by high-resolution two-dimensional gel electrophoresis in which the E1A proteins yield a characteristic pattern (data not shown). As shown in Fig. 1*a* and in other experiments below, the C36 antibody also immunoprecipitated an 85K protein. This polypeptide was recognized directly by the C36 antibodies, as it can be precipitated from lysates and partially purified preparations that do not contain the 105K protein (Fig. 2 and data not shown). As shown in Fig. 1*a*, several minor bands were also detected by C36 immunoprecipitation, and the reasons for the precipitation of these bands are unknown at present. Figure 1*a* also shows the immunoprecipitation of the 105K proteins from HeLa cells which do not contain the E1A proteins. In addition, we have found that a 105K polypeptide is specifically precipitated by the C36 antibodies (data not shown) from about 20 different common laboratory cell lines.

The 105K proteins immunoprecipitated by C36 separated into at least two bands on low percentage polyacrylamide gels. To investigate the origin of these two bands and to demonstrate that the proteins immunoprecipitated by C36 were identical to the E1A-associated 105K protein, we compared the two sets of proteins by partial proteolysis with *Staphylococcus aureus* V8 protease. As shown in Fig. 1*b*, both bands of the 105K doublet precipitated by C36 were similar to the 105K polypeptides precipitated in association with E1A by M73, thus demonstrating that the C36 antibodies were specific for the 105K E1A-associated protein and that both bands of the doublet were closely related.

The report from Lee *et al.*[29] describing the product of the *RB* gene as a 110K nuclear phosphoprotein prompted us to assay retinoblastoma cells for the 105K protein. Lysates of three cell lines containing large deletions of the *RB* gene were immunoprecipitated with C36 antibodies. The 105K proteins were not detected (Fig. 2). The similar physical properties of the RB and 105K proteins, together with the apparent absence of the 105K polypeptides from these retinoblastoma cells, suggested that the two proteins might be related.

The 105K protein is encoded by *RB*

To produce antisera that react with the RB protein, six peptides representing predicted hydrophilic regions of the RB protein

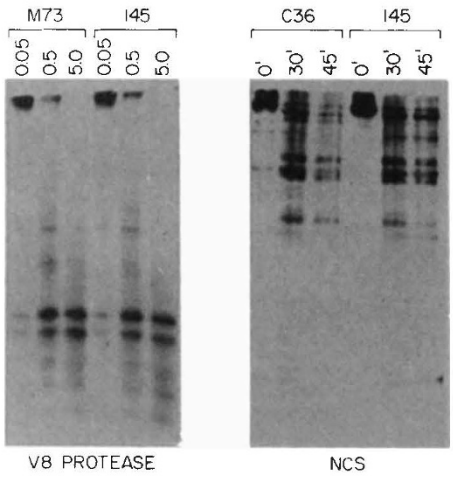

Fig. 4 Partial proteolysis of the 105K polypeptides immunoprecipitated by the 145 anti-RB#4 peptide sera and by the C36 or M73 monoclonal antibodies using *S. aureus* V8 protease[48] or N-chlorosuccinimide (NCS)[51]. Proteins were prepared as described in Fig. 1 and located by autoradiography. The respective bands were excised using the autoradiogram as a template. The proteins were either digested with increasing amounts of V8 protease (given in micrograms per reaction) or for increasing time with NCS, and the partially degraded products were separated on 15% SDS-polyacrylamide gels. The location of the partial proteolytic products was determined by fluorography.

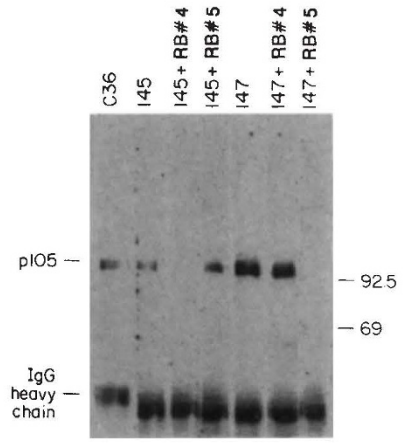

Fig. 5 Immunoblots of 105K E1A-associated protein with anti-RB antibodies. The E1A-associated 105K protein was immunoprecipitated from 293 cells using the M73 anti-E1A monoclonal antibody. The immunoprecipitated polypeptides were resolved by SDS-PAGE and then transferred to nitrocellulose membranes using standard immunoblotting techniques[52]. Strips were cut and reacted with C36, 145 (anti-RB#4), or 147 (anti-RB#5) antibodies. The binding of the anti-RB-peptide antibodies was performed with and without a saturating amount of peptide RB#4 or RB#5 and the addition of the appropriate peptide blocked the binding of these anti-peptide antibodies to 105K. After washing, the C36-reacted strips were probed with 10^6 c.p.m. of [^{125}I]labelled rabbit anti-mouse immunoglobulin (New England Nuclear) and the anti-RB-peptide-reacted strips were probed with 10^6 c.p.m. of [^{125}I]labelled goat anti-rabbit immunoglobulin antibodies (New England Nuclear). The location of the [^{125}I]labelled reagents was determined by autoradiography.

were synthesized and conjugated to keyhole limpet haemocyanin (KLH). Three of these peptides, one carboxy-terminal (RB#4) and two internal (RB#5 and RB#6), induced antibodies against a protein with the properties of the *RB* gene product (see Fig. 3*a* for the location of peptides). Antisera against RB#4 were chosen for more detailed studies.

To compare the 105K E1A-associated protein and the *RB* gene product directly, we first measured their relative molecular masses. As shown in Fig. 3*b*, the 105K protein immunoprecipitated with C36 migrated similarly to RB precipitated with anti-RB#4 sera and this was true, regardless of the polyacrylamide concentration (data not shown). Immunoprecipitation by anti-RB#4 sera of the band of M_r 105K was inhibited by the addition of saturating amounts of peptide RB#4, but the addition of the RB#4 peptide to reactions using the C36 antibody did not block the precipitation of the 105K E1A-associated antigen. We have also compared the relative molecular masses of the 105K proteins immunoprecipitated by C36 and the RB protein recognized by an anti-trp E/RB-fusion protein antibody[29] (prepared and kindly supplied by Jin-Yuh Shew and Wen-Hwa Lee). Again the RB protein comigrated with the E1A-associated 105K protein (data not shown).

We next compared the polypeptides by partial proteolysis (Fig. 4). The 105K proteins were prepared either by immunoprecipitation using the M73 anti-E1A monoclonal antibody, the C36 anti-105K monoclonal antibody, or the anti-Rb#4 peptide antiserum. Partial proteolysis products were prepared either by digestion with *S. aureus* V8 protease or by chemical cleavage with N-chlorosuccinimide (NCS). V8 protease cleaves after acidic residues (aspartic or glutamic acid) while NCS cleaves after tryptophan residues. Both cleavage reagents yielded identical products from all 105K proteins, showing that the positions of accessible aspartic acid, glutamic acid, and tryptophan residues were identical in these 105K polypeptides. These data prove that the 105K proteins immunoprecipitated by the C36 and anti-RB#4 antibodies were identical or closely related.

To ensure that the 105K proteins were recognized directly by the anti-peptide and C36 antibodies, and were not coprecipitated via another antigen, we performed two further experiments. First, as shown in Fig. 5, anti-peptide antibodies raised against two different regions of the RB protein were able to bind directly to the E1A-associated 105K protein in immunoblotting experiments. These results also confirm the specificity of the C36 antibody by demonstrating that these antibodies can also bind directly to 105K.

A complementary second set of experiments was used to test whether the C36 antibodies would specifically bind to the protein product of the *RB* gene. We synthesized RNA directly from the *RB* cDNA using an *in vitro* transcription reaction[43]. The resulting cRNA was used as a template for translation in rabbit reticulocyte lysates. In repeated experiments, we have been unable to synthesize full-length RB protein *in vitro* using the cRNA from the human *RB* cDNA. Instead, several short products were synthesized which appear to result from initiation of translation at internal methionine residues, as their sizes (Fig. 6) agree with the predicted size of carboxy-terminal proteins beginning at internal methionines. As seen in Fig. 6, the anti-RB#4 antiserum precipitated the translation products synthesized *in vitro*, confirming its reactivity with the *RB* gene products. The anti-RB#6 and anti-RB#5 antisera, raised against internal peptides, precipitated only the polypeptides that initiate upstream of the position of their respective peptides. As expected, the C36 antibody immunoprecipitated a portion of the products of the *in vitro* translations, confirming that this antibody can bind directly to the product of the *RB* gene. These results also suggest that the epitope recognized by the C36 monoclonal antibody lies in the amino-terminal half of the protein between amino acids 300 and 380.

These last two experiments confirm that antibodies raised against either the E1A-associated 105K protein or the RB protein not only specifically recognized their respective immunogens, but also bound directly to the other protein. These data, coupled

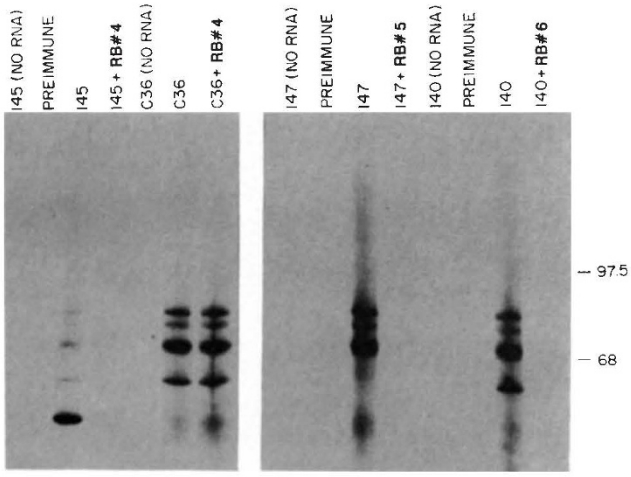

Fig. 6 Immunoprecipitation of polypeptides synthesized from the *in vitro* transcription/translation of *RB* cDNA. RB-related polypeptides synthesized *in vitro* were immunoprecipitated with C36, 145 (anti-RB#4), 147 (anti-RB#5) or 140 (anti-RB-#6) antibodies in the presence or absence of saturating amounts of peptide RB#4, RB#5, or RB#6. Preimmune rabbit sera were used in parallel immunoprecipitations as were rabbit reticulocyte lysates without addition of *RB* cRNA.

Methods. The *RB* cDNA was cloned downstream of the T7 promoter in Bluescript pBSK$^+$ plasmid (Stratagene). The plasmid was linearized by cleavage within the polylinker sequences immediately downstream of the inserted cDNA sequences and cRNAs were synthesized from the linearized templates using T7 RNA polymerase, and then translated in rabbit reticulocyte lysates (3 μg per 35 μl of reticulocyte lysate)[43,53]. Immunoprecipitated proteins were resolved on 8% polyacrylamide gels and detected by fluorography.

with the biochemical data showing that the two polypeptide chains share very similar structures, lead us to conclude that these two proteins are indeed identical and are the products of the same gene. We propose to follow the nomenclature of other oncoproteins in designating this protein p105-RB.

Implications

The association of the adenovirus E1A proteins and p105-RB has important implications for the possible functions of these proteins and the genes that they represent. Perhaps the most significant of these is the connection that is now forged between these two classes of genes and their associated regulatory pathways. Oncogenes like E1A, and analogously acting cellular counterparts, allow the establishment of cells in culture ('immortalization') and collaborate with other oncogenes such as *ras* to induce full malignant transformation. The contrasting 'tumour-suppressor' or 'anti-oncogenes', represented by *RB*, have also been implicated in tumourigenesis but clearly act in a different way. Tumour formation ensues when these anti-oncogenes are inactivated, suggesting that they normally act to limit cellular proliferation. The present results show that these two classes of genes, one acting positively on cell growth, the other negatively, may directly confront one another through the interaction of their encoded proteins. Oncogenes and anti-oncogenes thus appear as constituents of a common regulatory pathway. In the absence of these results, it was plausible that oncogenes and the tumour-suppressor genes like *RB* acted as elements of two distinct regulatory pathways, each concerned with a different aspect of growth or differentiation.

The known properties of the E1A and RB proteins suggest a provocative model for the function of the E1A/p105-RB complex. It is known that the E1A proteins can efficiently transform cells in cooperation with an activated *ras* gene. It is also known that the disruption of both copies of the *RB* gene often leads to the appearance of retinoblastomas or other genetically related tumours. Presumably any mechanism that interferes with the normal function of the RB protein would produce results similar to the loss of the *RB* gene. One possibility is that the E1A proteins inhibit the function of p105-RB by complex formation, thus achieving the same effect as the loss of the *RB* gene.

What might be the physiological role of a cellular tumour-suppressing gene like *RB*? We favour a model in which anti-oncogene proteins like p105-RB are elements of a signalling pathway that allows cells to respond to environmental signals. These signals might be differentiation inducers, cell cycle regulators, or other factors that carry an inhibitory signal to the cell. The loss of the *RB* gene and associated loss of the RB protein would remove an essential link in such a pathway and in this way disrupt signal transduction. As a consequence, the cell would lose its ability to respond normally to the inhibitory signal, while retaining its proliferative ability. Any resulting tumours would represent the progeny of this expanding cell population. Following this model, the E1A proteins act to block the passage of growth-inhibiting signals and in this way serve as indirect stimulators of cell proliferation.

What does the virus gain through the binding of E1A to p105-RB? For adenovirus itself, the modulation may be important for its replication. The major cell targets for adenovirus infection are epithelial cells lining the upper respiratory tract or the intestines, cells in which proliferation is inhibited or tightly regulated. One of the major goals of viral early protein expression must be to alter the physiological state of these cells and to drive them into S-phase (nuclear DNA replication). In this way, the viral oncogene creates an intracellular environment that is more permissive for viral DNA synthesis. The E1A proteins have been shown to stimulate host DNA synthesis[44-46] and the virus may achieve this by antagonizing the function of cell proteins like p105-RB that normally serve to constrain cell growth within a tissue.

A growing body of literature supports the existence of genes whose actions suppress cell proliferation[18]. Overcoming the actions of these genes appears to be a prerequisite for achieving the tumourigenic phenotype in many cancers. The findings presented in this study suggest that one of the functions of the adenovirus E1A proteins is to overcome the action of the *RB* suppressor gene. This lends weight to the notion that cellular transformation requires not only growth stimulation, but also other equally important functions that override the mechanisms holding cell growth in check.

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LETTERS TO NATURE

Theory of adhesion for the large-scale structure of the Universe

Lev A. Kofman* & Sergey F. Shandarin†

* Institute of Astrophysics and Atmospheric Physics, Estonian Academy of Sciences, Tartu, Toravere 202444, USSR
† Institute for Physical Problems, Academy of Sciences, Kosygina 2, Moscow 117334, USSR

At present we see the large-scale distribution of matter in the Universe primarily as clusters and superclusters of galaxies, with giant voids between them[1,2]. Understanding the origin and evolution of the large-scale structure (LSS) is one of the central problems in cosmology; it is of direct concern in understanding both the nature of the dominant dark matter in the Universe and physical processes in the very early Universe when primordial inhomogeneities were generated[3-5]. Here we use a new theoretical approach[6-8] to the formation of LSS by applying the Burgers' equation[9] that mimics the gravitational sticking of matter at the non-linear stage of gravitational instability. In this theory the non-linear evolution, including both the formation and clustering of clumps of matter separated from the Hubble expansion, is directly determined by the geometrical structure of the initial random field of linear newtonian gravitational potential fluctuations ϕ which may be gaussian or non-gaussian, depending on the model.

The potential perturbations ϕ link the present non-linear picture to the earliest stages of the Universe in a unique way, and form the basic object of our investigation, in contrast to most previous works where the distribution of the matter density ρ is studied.

Voids form around high peaks of the field ϕ, so their statistics are determined by the statistics of the peaks of ϕ. The observed sizes of voids are determined by the mean amplitude of primordial inhomogeneities $\langle\phi^2\rangle^{1/2}$ which leads to a new method for scaling amplitude ϕ (L.A.K., preprint). Also, the falling of the nearest superclusters towards a great attractor may be interpreted as a shallow valley between high peaks of gravitational potential for corresponding space region.

The mean density of matter in the Universe is comparatively low at present, and close to the critical value $\rho_{cr} = 5\times10^{-30}h^2$ g cm^{-2} predicted by cosmological inflation (h is the Hubble constant in units of 50 km s^{-1} Mpc^{-1}). On small scales, <100 Mpc, the Universe is very inhomogeneous. The largest structures, superclusters and voids, have typical sizes $\sim$30-100 h^{-1} Mpc, but there is also evidence of structures up to 300 Mpc[10] in extent. We assume LSS originates by gravitational instability of primordial inhomogeneities. Both adiabatic (inflaton) or isocurvature (isoinflaton) gaussian perturbations are inevitably generated by vacuum fluctuations during the inflationary era; non-gaussian inhomogeneities (such as cosmic strings and bubbles) may be generated by nonperturbative mechanisms, and can be also progenitors of various gravitational instability models.

In the present Universe most mass is in the form of dark, non-luminous matter, generally accepted to consist of weakly interacting particles (perhaps experimentally unknown), which are apparently of relic origin. Possible candidates are neutrinos (comprising so-called hot dark matter, HDM), or axions, gravitinos and so on (cold dark matter, CDM). At the epoch of LSS formation, dark matter is well described as collisionless, pressureless, dust-like matter. It is governed by gravitation only; the newtonian approximation suits our purposes very well[4,5].

The motion of dust-like matter in the expanding Universe obeys a well-known basic nonlinear system comprising a continuity equation, Euler's equation and Poisson's equation, for density $\rho(t, \mathbf{r})$, peculiar velocity $\mathbf{v}(t, \mathbf{r})$ and gravitational potential $\phi(t, \mathbf{r})$ in an expanding uniform background described by a scale factor $a(t)$ and a mean density $\bar{\rho}(t)$ (refs 4, 5). The peculiar velocity is the difference between physical velocity $\mathbf{w}$ of an object and its Hubble velocity $H\mathbf{r}$; $\mathbf{v} = \mathbf{w} - H\mathbf{r}$. The initial conditions are defined at the linear stage when all values $\mathbf{v}, \phi$ and $(\rho - \bar{\rho})/\bar{\rho}$ are small. The usual tool to study the nonlinear evolution of the structure is N-body numerical simulation, but this approach is limited by the capabilities of present-day computers[11,12]. Fortunately, there is the very useful Zel'dovich approximation[13-14] (we assume $\bar{\rho} = \rho_{cr}$)

$$\mathbf{r}(t, \mathbf{q}) = a(t)(\mathbf{q} + a(t)\nabla\Phi_0(\mathbf{q})) \qquad (1)$$

describing the growing mode of gravitational instability in terms of particle displacements from unperturbed positions specified by lagrangian coordinates q. The initial perturbation is given by the potential vector field $\nabla\Phi_0$, which is proportional to the peculiar velocity field in the linear stage. However, approximation (1) breaks down soon after the appearance of the first nonlinear objects, 'pancakes', of supercluster size in HDM models and of cosmologically negligible sizes in CDM models.

Several essential features of 'pancake' formation and, more important, their subsequent evolution, can be simulated in an adhesion[6-8,15]. In this model the collisionless particles move according to equation (1) until they fall into pancakes. Once there, the particles stick together, conserving momentum and moving along the pancake to form filaments, and along the filaments to form clumps. This is obviously a considerable oversimplification of the real processes in pancakes, as well as of their inner structure, but it adequately describes the global evolution of LSS beginning from some minimal scale determined