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Some Lymphoid Cell Lines Transformed by Abelson Murine Leukemia Virus Lack a Major 36,000-Dalton Tyrosine Protein Kinase Substrate

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Fibroblasts transformed by Abelson murine leukemia virus differ from normal fibroblasts in that they contain several cellular proteins, including one of 29 and one of 36 kilodaltons, which are phosphorylated at tyrosine residues. Since it has been shown before that these proteins also become phosphorylated at tyrosine after transformation of fibroblasts by a number of other retroviruses, their phosphorylation may play an important role in the transformation of these cells. In contrast, the 36-kilodalton phosphoprotein was not detectable in three of the four lines of Abelson virus-transformed B lymphoma cell lines studied here. These three cell lines, RAW307.1.1, 18-48, and 18-81, and a B lymphoma induced by mineral oil, WEHI 279, were all found to lack both the phosphorylated and unphosphorylated forms of the 36-kilodalton protein. It thus appears that expression of this major cell protein is not essential for the survival of B lymphoma cells in culture and that the phosphorylation of the 36-kilodalton protein at tyrosine is not essential for transformation of pre-B lymphocytes by Abelson virus.

Abelson murine leukemia virus induces a rapid and characteristic lymphosarcoma in mice, with tumors consisting predominantly of pre-B cells (22, 35). The virus also induces the transformation of a broad spectrum of mouse cells in culture. Whereas infection of cells from late-gestation mouse embryos or from adult marrow yields colonies of transformed pre-B cells (26, 37), infection of cells from early and mid-gestation mouse embryos induces rapid proliferation of erythroid cells (37). In addition, the virus causes frank transformation of cells from established fibroblastic lines (29).

Abelson virus arose in the laboratory during passage of Moloney murine leukemia virus in a mouse (1). More than three quarters of the coding capacity of Moloney virus is deleted in the genome of Abelson virus and has been replaced with a 3.5-kilobase segment of cellular genetic information, termed *abl* (12, 34). Genetic evidence indicates that expression of the *abl* sequences plays an essential role in both tumor formation in mice and cellular transformation in culture (25, 27).

In cells transformed by Abelson virus, the *abl* sequences are expressed in the form of a hybrid protein (24, 41). The amino-terminal domain of this transforming protein is encoded by a remnant of the *gag* gene of Moloney virus, and the carboxy-terminal domain is encoded by *abl* (24, 41). The Abelson virus protein has an associated protein kinase activity which almost certainly is an inherent property of the viral polypeptide (39). This activity has a strict specificity for tyrosine residues in its protein substrates. In this respect, Abelson virus resembles a number of other RNA tumor viruses which also encode transforming proteins with associated tyrosine protein kinase activities (14). Genetic and biochemical analysis of nontransforming and weakly oncogenic mutants of Abelson virus showed that the tyrosine protein kinase activity of the transforming protein is necessary for tumor formation in mice and transformation of both fibroblasts and lymphoid cells in culture (25, 27).

The most compelling evidence that the Abelson virus protein functions as a tyrosine protein kinase in vivo is that cells transformed by the virus contain a number of cellular proteins which are phosphorylated on tyrosine to an increased extent (6, 31, 33). We have identified three such cellular polypeptides in Abelson virus-transformed mouse fibroblasts. One is the cytoskeletal protein vinculin (31). The other two are proteins which are apparent when total cellular phosphoproteins are analyzed by two-dimensional gel electrophoresis (6). One of these is a 39-kilodalton (39K) protein which is the murine homolog of a phosphoprotein first described by Radke and Martin in chicken fibroblasts transformed by Rous sarcoma virus (20, 21). This polypeptide is a highly conserved (6), abundant

(6, 8), cytoplasmic protein (7) which is also phosphorylated on tyrosine in murine and avian fibroblasts which have been transformed by other tumor viruses encoding transforming proteins with associated tyrosine protein kinase activities (6, 8, 19). In addition, the homolog of this protein in human A-431 cells is phosphorylated on tyrosine when these cells are treated with epidermal growth factor, a polypeptide which activates a tyrosine protein kinase associated with its receptor (10, 13). Because estimates of the molecular weight of this polypeptide have ranged from 34K to 39K (6, 9, 21), we will refer to it here by its original designation, the 36K protein (21).

The second protein detected by this procedure is a cytoplasmic protein of 29K (6). This protein is also phosphorylated on tyrosine in mouse fibroblasts transformed by two otherwise unrelated retroviruses, Rous sarcoma virus (6) and Snyder-Theilen feline sarcoma virus (unpublished data), whose transforming proteins also have an associated tyrosine protein kinase activity. A 28K protein is phosphorylated on tyrosine in chicken cells transformed by a number of different retroviruses (5, 6). Peptide mapping shows that this 28K protein in chicken cells is closely related to the 29K protein in mouse cells (unpublished data). We will refer to it as the 29K protein. Because neither the 36K nor the 29K protein is phosphorylated on tyrosine in cells transformed by a number of other viruses or by other means (3, 6), it is likely that the 36K and 29K proteins are primary substrates of these several viral transforming proteins rather than of a cellular protein kinase which is activated by transformation of any sort.

Neither the function of the 36K protein in normal cellular metabolism nor the role that it plays in viral transformation is known. It has been observed, however, that some avian lymphoid cells do not contain the 36K protein (R. L. Erikson, personal communication). This raised the possibility that murine pre-B cells also might lack the 36K protein. If this were the case, it would mean that the transformation of these cells by Abelson virus does not require the phosphorylation of this protein. Because this would greatly delimit the possible physiological roles which the 36K protein might play in malignant cellular metabolism, we examined whether the 36K polypeptide was present in a number of normal and Abelson virus-transformed murine lymphoid cells.

MATERIALS AND METHODS

Cells. The ANN-1 line of Abelson virus-transformed mouse NIH 3T3 fibroblasts (29), the 18-48 (35), 18-81 (35), RAW307.1.1 (18), and RAW309.1.1 (23) lines of Abelson virus-transformed mouse B lymphoid cells, the BW 5147 line of spontaneously transformed mouse T lymphoma cells (15), and the WEHI 279 line of mouse B lymphoma cells, whose transformation was induced by mineral oil (38), were grown in Dulbecco-Vogt modified Eagle medium (DMEM) supplemented with 10% fetal calf serum and 5×10^{-5} M 2-mercaptoethanol.

Biosynthetic labeling. For two-dimensional gel analysis, cells were labeled at a concentration of 10^6 cells per ml in phosphate-free DMEM which was supplemented with 5% complete fetal calf serum and 5% dialyzed (against phosphate-free saline) fetal calf serum and contained $^{32}P_i$ (carrier-free; ICN) at a concentration of 0.6 mCi/ml. Labeling was done for 16 h at 36°C. Cells were labeled with [^{35}S]methionine (>1,000 Ci/mmol, Amersham/Searle) in two ways: by incubation at a concentration of 10^6 cells per ml in methionine-free DMEM which was supplemented with 10% fetal calf serum for 18 h at 36°C, and by incubation at a concentration of 2×10^6 cells per ml in methionine-free DMEM which was supplemented with 10% dialyzed fetal calf serum for 2 h at 36°C. In both cases, the labeling medium contained [^{35}S]methionine at a concentration of 100 μCi/ml. All labeling was done in plastic 35-mm dishes in a CO_2 incubator. The poorly adherent ANN-1 cells were handled in exactly the same manner as the nonadherent lymphoid cells. At the end of the labeling period, the cells were pelleted by sedimentation at $100 \times g$, suspended in cold Tris-buffered saline, and pelleted again. The cell pellets were then dissolved in either phosphate-buffered RIPA buffer (30) or two-dimensional gel lysis buffer (11).

Antisera. Anti-36K protein serum was prepared by a procedure to be described elsewhere (J. Cooper and T. Hunter, J. Biol. Chem., in press). Basically, the 36K protein was partially purified by a procedure similar to the analytical procedure described previously (6). Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis showed the 36K protein to be the major protein in the preparation. An estimated 150 μg of 36K protein was emulsified with complete Freund adjuvant and injected intradermally at multiple sites into the back of an adult rabbit. Four weeks later immunization was repeated with 75 μg of the 36K protein, and serum was collected 12 days later.

Immunoprecipitation. All lysis of cells and washing of immunoprecipitates was done with phosphate-buffered RIPA buffer. When lysates of ^{32}P-labeled cells were prepared, 2 mM EDTA was included in the RIPA buffer. The procedures for immunoprecipitation, with fixed *Staphylococcus aureus* bacteria (Calbiochem) used to collect the immune complexes, have been described in detail previously (30).

SDS-polyacrylamide gel electrophoresis. Immunoprecipitates were dissolved in 2% SDS–5 mM sodium phosphate (pH 7.0)–0.1 M dithiothreitol–5% 2-mercaptoethanol–10% glycerol by heating the mixture to 100°C for 60 s and then analyzed by electrophoresis on 13.5-cm-long by 1-mm-thick gels which consisted of 15% acrylamide and 0.09% bisacrylamide. All other procedures have been described previously (30).

Analysis by partial proteolysis. The 36K protein, labeled biosynthetically with [^{35}S]methionine, was isolated by immunoprecipitation and purified by SDS-polyacrylamide gel electrophoresis as described above. The protein was detected by autoradiography. Gel slices containing the 36K protein were then insert-

ed into the wells of a 20% polyacrylamide gel, 5 or 50 ng of *S. aureus* protease was loaded on top of the slices, and digestion and analysis of the digestion products was performed as described previously (4), except that the buffers contained 1 mM 2-mercaptoethanol. Detection of the proteolytic fragments was accelerated by fluorography.

Two-dimensional gel electrophoresis. ^{32}P-labeled cells were lysed and analyzed by two-dimensional gel electrophoresis as previously described (5, 11). Extract from approximately 2×10^5 cells was loaded on each isoelectric focusing gel, which contained pH 6 to 8 ampholytes, and was focused for 14,000 V h. Second-dimension gels (15% polyacrylamide) were fixed, dried, incubated in 1 M KOH at 55°C for 2 h, fixed, and dried as previously described (5).

Analysis of gel-fractionated proteins by electrophoretic transfer to nitrocellulose. Unlabeled ANN-1, 18-48, and 18-81 cells were rinsed in Tris-buffered saline and solubilized by boiling in 2% SDS–20% 2-mercaptoethanol–50 mM Tris-hydrochloride (pH 6.8)–10% glycerol. All samples were diluted in this buffer to a protein concentration of 200 μg/ml, and 25-μl samples were separated on a 15% SDS-polyacrylamide gel. Proteins from part of the gel were electrophoretically transferred to nitrocellulose (2, 36). Duplicate tracks were stained, and it was confirmed that similar amounts of cell protein had been analyzed. The filter was pretreated and incubated with antibody and ^{125}I-labeled *S. aureus* protein A as previously described (2), except that the bovine serum albumin in the buffers was replaced by 1% ovalbumin (Sigma) and incubation was done at 42°C. The antiserum was used at a concentration of 2%, and the ^{125}I-labeled protein A (New England Nuclear) was used at a concentration of 0.25 μCi/ml.

RESULTS

Analysis by two-dimensional gel electrophoresis. Initially we analyzed cellular phosphoproteins which had been labeled biosynthetically with $^{32}P_i$ by two-dimensional gel electrophoresis. In these experiments the gels were treated with alkali before fluorography. This treatment greatly facilitates the detection of many proteins which contain phosphotyrosine, because this phosphoamino acid is much more stable to alkali than either phosphoserine or RNA (3, 5).

The phosphorylated 29K protein was present in the two lines of Abelson virus-transformed lymphoid cells examined, 18-48 and RAW 309.1.1, as well as in the ANN-1 line of Abelson virus-transformed NIH 3T3 cells (Fig. 1). It was not detectable in either the BW 5147 line of spontaneously transformed murine T lymphoma cells or the WEHI 279 murine B lymphoma cells, which are derived from a tumor induced by mineral oil (Fig. 1). The extent of phosphorylation of the 29K protein in these cell lines was difficult to estimate because other proteins in this part of the gel were phosphorylated to variable extents. Careful examination of autoradiographs exposed for various lengths of time suggested however that the 29K protein in the two Abelson virus-transformed lines contained more than five times as much alkali-stable phosphate as did the 29K protein in the control cell lines. We have not analyzed the phosphoamino acids of the 29K protein from the 18-48 and RAW309.1.1 cells. The 29K protein in ANN-1 fibroblasts was found to contain phosphotyrosine and a trace of phosphoserine after being isolated from an alkali-treated gel (6).

This set of lymphoid cells therefore resembled the fibroblasts we examined before in that the 29K protein was phosphorylated in the two cell lines transformed by a virus which encodes a transforming protein with an associated tyrosine protein kinase activity, but was not phosphorylated in two cell lines transformed by means other than viral infection.

Such was not the case for the 36K protein. Although a trace of the phosphorylated 36K protein was present in the Abelson-virus transformed RAW 309.1.1 cells, no phosphorylated 36K protein was detectable in the 18-48 line of Abelson virus-transformed B lymphoid cells (Fig. 1). The phosphorylated 36K protein could not be detected in the two lines of nonvirally transformed lymphoid cells (Fig. 1).

The absence of the phosphorylated 36K protein in the Abelson virus-transformed cell line 18-48 could have resulted either from failure of the viral transforming protein to effect the phosphorylation of the 36K protein or from the simple absence of the protein from these cells. This question was difficult to answer by two-dimensional gel electrophoresis because the isoelectric point of the unphosphorylated form of the 36K protein is too high to allow identification of the polypeptide by our customary method for such analysis. To distinguish between these possibilities, we therefore employed immunoprecipitation.

Analysis by immunoprecipitation. We prepared an antiserum which recognizes the murine 36K protein by immunizing a rabbit with a preparation of the chicken 36K protein. Although the serum was not monospecific, analysis of immunoprecipitates prepared with it by SDS-polyacrylamide gel electrophoresis allowed unambiguous identification of the 36K protein. Six murine lymphoid cell lines were labeled with [^{35}S]methionine, and the 36K protein was isolated by immunoprecipitation. The 36K protein was undetectable in the 18-48, 18-81, and RAW307.1.1 lines of Abelson virus-transformed B lymphoid cells (Fig. 2) and in the WEHI 279 line of B lymphoma cells (Fig. 2). It was present, however, in the BW 5147 line of spontaneously transformed T cells (Fig. 2) and, as expected from the two-dimensional gel analyses, in the

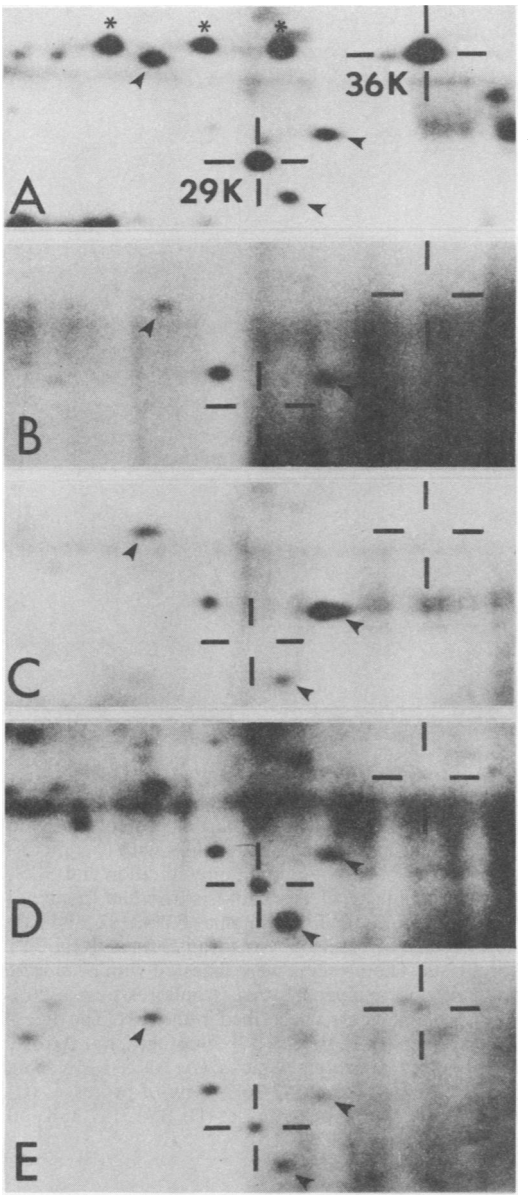

FIG. 1. Analysis of alkali-stable cellular phosphoproteins in Abelson virus-transformed lymphocytes by two-dimensional gel electrophoresis. Five cell lines were labeled biosynthetically with $^{32}P_i$ for 16 h at 36°C. The cells were then prepared for and subjected to two-dimensional gel electrophoresis as described in the text. All of the gels were treated with alkali. Autoradiography with an intensifying screen and prefogged film was done for 20 to 60 h. Isoelectric focusing was in the horizontal dimension, and SDS-polyacrylamide gel electrophoresis was from top to bottom. The acidic end of the pH gradient was to the left. Only the portion of the autoradiograms containing the 29K and 36K proteins is shown here. The arrowheads point to phosphoproteins present in all five samples. They were used to help align and identify the 36K and 29K phosphoproteins. The asterisks mark three phosphoproteins present at higher levels in ANN-1 cells than in uninfected 3T3 cells. They appear not to contain phosphotyrosine (unpublished data). (A) ANN-1 cells (Abelson virus-transformed fibroblasts); (B) WEHI 279 cells (nonvirally transformed B cells); (C) BW 5147 cells (spontaneously transformed T cells); (D) 18-48 cells (Abelson virus-transformed B cells); (E) RAW309.1.1 cells (Abelson virus-transformed B cells).

RAW309.1.1 line of Abelson virus-transformed B lymphoid cells (Fig. 2). The cells which did not contain detectable amounts of the 36K protein must contain very little of the polypeptide. Analysis of autoradiograms of these immunoprecipitates by densitometry revealed that we would have been able to detect the protein in these cells if it had been present at one fiftieth the level found in the BW 5147 and RAW309.1.1 cells.

The 36K protein appeared to be less phosphorylated in the RAW309.1.1 lymphoma cells than in the ANN-1 fibroblasts when analyzed by either two-dimensional gel electrophoresis (Fig. 1) or immunoprecipitation (data not shown). Comparison by partial proteolysis of the 36K proteins from the spontaneously transformed BW 5147 and the Abelson virus-transformed RAW309.1.1 cells with that from Abelson virus-transformed mouse fibroblasts revealed that the proteins from the lymphoid and fibroblastic cells were indistinguishable by this criterion (Fig. 3).

Analysis by transfer of gel-fractionated proteins to nitrocellulose. It was conceivable that the 36K protein was in fact present in the Abelson virus-transformed lymphoid cells but was not detectable by the procedures we had used either because it was synthesized extremely slowly or because its antigenic determinants were masked by other proteins. To test these remote possibilities, we looked for the protein in these cells by another technique. Unlabeled 18-48 and 18-81 Abelson virus-transformed B lymphoid cells were dissolved directly in SDS gel sample buffer and subjected to SDS-polyacrylamide gel electrophoresis. The proteins in the gel were then transferred electrophoretically to a sheet of nitrocellulose, and the filter was incubated with the anti-36K protein serum. Specifically bound antibody was detected with ^{125}I-labeled staphylococcal protein A and autoradiography (Fig. 4). The 36K protein was readily detected by this procedure in a sample of Abelson virus-transformed fibroblasts. It was not detectable in the samples from either the 18-48 or the 18-81 lymphoid cell lines. Densitometric measurements of autoradiograms exposed for different lengths of time suggested that these two lymphoid cell lines contained less than 2% of the 36K protein present in the fibroblastic ANN-1 cell line.

DISCUSSION

Fibroblasts transformed by Abelson virus, Rous sarcoma virus, Fujinami sarcoma virus, PRCII virus, and Y73 virus contain a number of cellular proteins which are newly phosphorylated on tyrosine (3, 5, 6, 8, 9, 19, 20, 31–33). The phosphorylation of these proteins on tyrosine is unquestionably induced, and in all probability catalyzed directly, by the transforming proteins of these viruses. It is likely that the phosphorylation of some or all of these proteins plays a role in the phenotypic changes which characterize a fibroblast transformed by these viruses.

The 36K phosphoprotein first described by Radke and Martin (21) in chicken fibroblasts transformed by Rous sarcoma virus has attracted considerable attention because it is the most easily detectable phosphotyrosine-containing protein in fibroblasts and myoblasts transformed by a number of different viruses (6, 9, 16, 19, 20). Martinez et al. (17) have estimated that as much

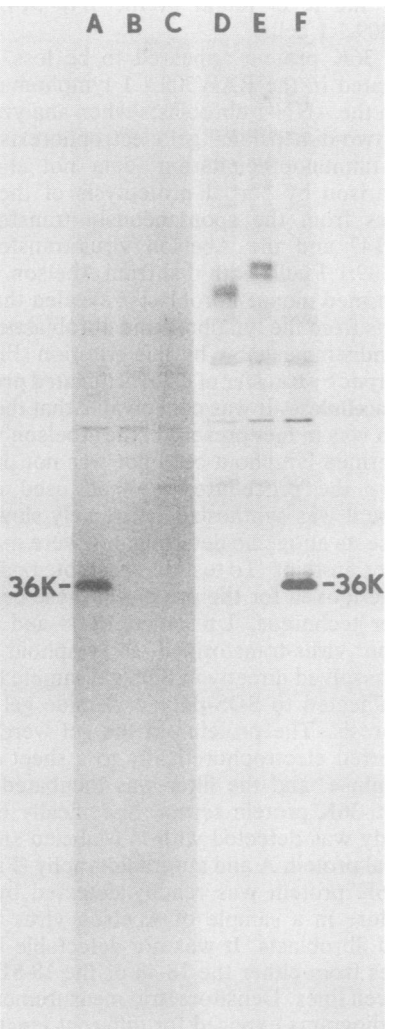

FIG. 2. Analysis of the 36K protein by immunoprecipitation. Cell lines 18-48, 18-81, RAW307.1.1, and RAW309.1.1 (Abelson virus-transformed B lymphoid cells) and BW 5147 and WEHI 279 (nonvirally transformed lymphoid cells) were labeled biosynthetically with [^{35}S]methionine for 2 h and immunoprecipitates were prepared with anti-36K serum as described in the text. The immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis. The precipitated proteins were detected by fluorography. Tracks A, B, and C were from one experiment, and tracks D, E, and F were from another. Tracks: (A) BW 5147; (B) 18-48; (C) 18-81; (D) WEHI 279; (E) RAW307.1.1; (F) RAW309.1.1 cells.

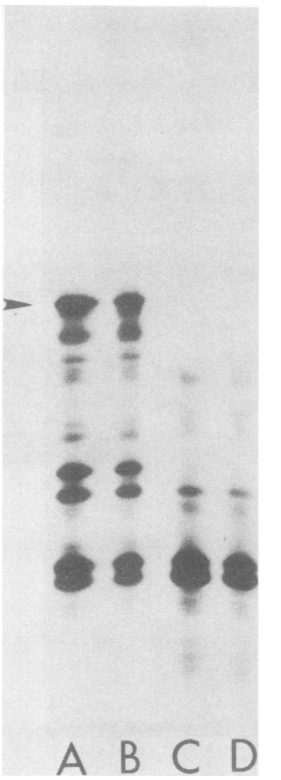

FIG. 3. Comparison of the 36K protein from lymphocytes and fibroblasts by partial proteolysis. The 36K protein, labeled biosynthetically with [^{35}S]methionine, was isolated by immunoprecipitation and SDS-polyacrylamide gel electrophoresis from the spontaneously transformed T lymphoma BW 5147 and the ANN-1 line of Abelson virus-transformed NIH 3T3 fibroblasts. The proteins were digested with *S. aureus* protease and analyzed by electrophoresis on a 20% polyacrylamide gel as described in the text. The digestion products were detected by fluorography. Arrow, Undigested 36K protein. Tracks: (A) ANN-1 36K, 5 ng of protease; (B) BW 5147 36K, 5 ng of protease; (C) ANN-1 36K, 50 ng of protease; (D) BW 5147 36K, 50 ng of protease.

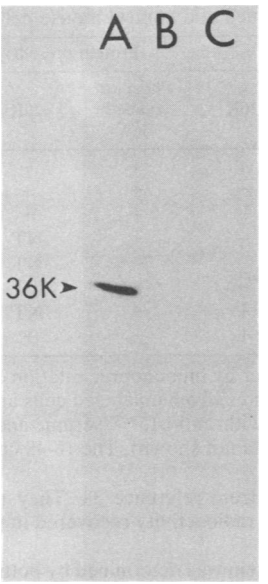

FIG. 4. Detection of the 36K potein after electrophoretic transfer to nitrocellulose. ANN-1 Abelson virus-transformed fibroblasts and 18-48 and 18-81 Abelson virus-transformed B lymphoid cells were dissolved directly in SDS gel sample buffer and subjected to SDS-polyacrylamide gel electrophoresis as described in the text. The proteins in the gel were transferred electrophoretically to a sheet of nitrocellulose and detected with rabbit anti-36K serum, ^{125}I-labeled protein A, and autoradiography as described in the text. Tracks: (A) ANN-1 fibroblasts; (B) 18-48 B lymphoid cells; (C) 18-81 B lymphoid cells.

as 10% of the phosphotyrosine in Rous sarcoma virus-transformed chicken fibroblasts is present in the 36K protein. The protein is an abundant cytoplasmic protein, constituting between 0.25 and 0.9% of total cellular protein (8, 20). Some of it is found in association with membranes after cell fractionation, and it remains in the detergent-insoluble matrix after treatment of a monolayer culture of fibroblasts with a low concentration of a nonionic detergent (S. Courtneidge, personal communication; K. Radke, P. Moss, and G. S. Martin, personal communication) (3, 7). The role that this protein plays in cellular transformation and normal metabolism is however still unclear. It has been suggested that the protein may by cytosolic malate dehydrogenase (28).

One approach to understanding the function of this and other substrates of the viral transforming proteins is to examine the tissues in which they are expressed. Abelson virus is unique among the viruses identified to date which encode transforming proteins with associated tyrosine protein kinase activities in that it transforms both fibroblasts and lymphoid cells. We therefore compared here the cellular proteins newly phosphorylated on tyrosine in Abelson virus-transformed fibroblasts with those present in Abelson virus-transformed B lymphoid cells.

We focused on two cellular proteins, the 36K and 29K proteins. Like Abelson virus-transformed fibroblasts, both of the Abelson virus-transformed B lymphoid cell lines we examined contained the phosphorylated 29K protein. We do not know, however, whether every Abelson virus-transformed lymphoid cell line contains this phosphorylated protein. In contrast, one Abelson virus-transformed lymphoid cell line examined here, 18-81, contained no detectable phosphorylated 36K protein, and another, RAW309.1.1, contained only a trace (Table 1). The explanation for the absence of the phosphorylated 36K protein in the 18-81 cell line is simple; these cells do not contain detectable amounts of the 36K polypeptide. Additionally, the RAW307.1.1 and 18-48 lines of Abelson virus-transformed B lymphoid cells were also found not to contain this polypeptide.

These three transformed lymphoid cell lines are not unique. The WEHI 279 line of murine B lymphoma cells, a tumor line induced by mineral oil, also did not contain detectable amounts of the 36K protein. The simple fact that the 36K protein is not present in three lines of Abelson virus-transformed lymphocytes or one line of nonvirally transformed cells reveals much about the protein. It shows that the phosphorylation of this protein is not essential for the transformation of lymphocytes by Abelson virus. (ii) It demonstrates that even though the protein constitutes 0.25 to 0.9% of total cellular protein in fibroblasts, it is not necessary for either the survival or the growth of some lymphoid cells. The 36K protein would appear to be a polypeptide which is not required at high levels for any of the metabolic or physiological activities shared by lymphoid cells and fibroblasts. These would seem to include macromolecular synthesis, mitosis, glycolysis, respiration, and most aspects of intermediary metabolism.

The most obvious difference between the lymphoid cells studied here and the cultured cells in which the 36K protein is found—fibroblasts, myoblasts, epidermoid cells (10, 13), and glial cells (unpublished data)—is that they are nonadherent. It seems possible therefore that the 36K protein plays some role in cell adhesion or migration on a substratum.

In general, lymphoid cells contain less total phosphotyrosine in protein than do fibroblasts (33). This is true both of spontaneously transformed lymphoid cells, which we assume have an abundance of phosphotyrosine which is similar to that of their normal progenitors, and

TABLE 1. Protein phosphorylation in Abelson virus-transformed and control mouse cell lines

Cell line	Lineage	Abelson virus transforming protein[a]	Phosphotyrosine (% of total cell phosphoamino acids)[b]	Protein present[c]			
				36K	Phosphorylated 36K	29K	Phosphorylated 29K
ANN-1	Fibroblast	P120	0.391	+	+	+	+
RAW309.1.1	B	P160	0.158	+	+	+	+
18-48	B	P90 and P120	0.170	−	−	+	+
RAW307.1.1	B	P160	NT	−	−	NT	NT
18-81	B	P120	0.109	−	−	NT	NT
WEHI 279	Pre-B		0.047	−	−	NT	−
BW 5147	T		0.015	+	−	NT	−
3T3	Fibroblast		0.033	+	−	+	−

[a] The size of Abelson virus transforming protein was determined either by immunoprecipitation of the protein from [^{35}S]methionine-labeled cells or by immunoprecipitation of the protein from unlabeled cells and incubation of the precipitate with [γ-^{32}P]ATP. In both cases precipitation was with anti-p15^{gag} serum, and the labeled proteins were analyzed by SDS-polyacrylamide gel electrophoresis (data not shown). The 18-48 cells used here contained both P90 and P120. P90 was much more abundant.

[b] The values for phosphotyrosine in whole cell protein are taken from reference 33. They represent the radioactivity recovered as phosphotyrosine as a percentage of the total radioactivity recovered in phosphotyrosine, phosphothreonine, and phosphoserine combined.

[c] The presence of the phosphorylated and unphosphorylated 36K protein was determined by both immunoprecipitation and two-dimensional gel electrophoresis. The 29K protein was analyzed only by two-dimensional gel electrophoresis. Autoradiographs of two-dimensional gels of ^{32}P-labeled normal 3T3 cells were presented in reference 6. The presence of the 29K protein in uninfected 3T3 cells has been established by analysis of [^{35}S]methionine-labeled cells by two-dimensional gel electrophoresis (unpublished data). We have concluded that RAW307.1.1 and 18-81 cells do not contain the phosphorylated 36K protein because they do not contain detectable amounts of the polypeptide. NT, Not tested.

Abelson virus-transformed lymphocytes, which have a level of phosphotyrosine which is manyfold higher than most lymphoid cells but noticeably less than that in Abelson virus-transformed fibroblasts. The absence of the 36K protein is almost certainly one of the factors contributing to the relatively low level of phosphotyrosine in Abelson virus-transformed lymphoid cells.

At present it is difficult to generalize about the tissue distribution of the 36K protein. Although it was absent from three lines of Abelson virus-transformed B lymphoid cells, it was present and phosphorylated to a very limited extent in one such line. Additionally, of the two nonvirally transformed lymphoid cell lines which we studied, one B lymphoma contained no detectable 36K protein, whereas a T lymphoma contained considerable amounts of it. It is possible that the presence of the 36K protein will prove to be indicative of the particular lineage or stage of differentiation of murine lymphoid cells. Analysis of additional cell lines and fractionated hematopoietic cell populations should reveal whether this is the case. These experiments are under way.

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LITERATURE CITED

1. **Abelson, H. T., and L. S. Rabstein.** 1970. Lymphosarcoma: virus-induced thymic independent disease in mice. Cancer Res. **30**:2213–2222.
2. **Burnette, W. N.** 1981. "Western Blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112**:195–302.
3. **Cheng, Y.-S. E., and L. B. Chen.** 1981. Detection of phosphotyrosine-containing 36,000-dalton protein in the framework of cells transformed with Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **78**:2388–2392.
4. **Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli.** 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. **252**:1102–1106.
5. **Cooper, J., and T. Hunter.** 1981. Changes in protein phosphorylation in Rous sarcoma virus-transformed chicken embryo cells. Mol. Cell Biol. **1**:165–178.
6. **Cooper, J., and T. Hunter.** 1981. Four different classes of retroviruses induce phosphorylation of tyrosine present in similar cellular proteins. Mol. Cell. Biol. **1**:394–407.
7. **Cooper, J. A., and T. Hunter.** 1982. Discrete primary locations of a tyrosine protein kinase and of three proteins that contain phosphotyrosine in virally transformed chick fibroblasts. J. Cell Biol. **94**:287–296.
8. **Erikson, E., R. Cook, G. J. Miller, and R. L. Erikson.** 1981. The same normal cell protein is phosphorylated after transformation by avian sarcoma viruses with unrelated transforming genes. Mol. Cell. Biol. **1**:43–50.
9. **Erikson, E., and R. L. Erikson.** 1980. Identification of a cellular protein substrate phosphorylated by the avian

sarcoma virus transforming gene product. Cell **21**:829–836.
10. **Erikson, E., D. J. Shealy, and R. L. Erikson.** 1981. Evidence that viral transforming gene products and epidermal growth factor stimulate phosphorylation of the same cellular protein with similar specificity. J. Biol. Chem. **256**:11381–11384.
11. **Garrels, J. I.** 1979. Two-dimensional gel electrophoresis and computer analysis of proteins synthesized by clonal cell lines. J. Biol. Chem. **254**:7961–7977.
12. **Goff, S. P., E. Gilboa, O. N. Witte, and D. Baltimore.** 1980. Structure of the Abelson murine leukemia virus genome and the homologous cellular gene: studies with cloned viral DNA. Cell **22**:777–785.
13. **Hunter, T., and J. A. Cooper.** 1981. Epidermal growth factor induces rapid tyrosine phosphorylation of proteins in A431 human tumor cells. Cell **24**:741–752.
14. **Hunter, T., and B. M. Sefton.** 1982. Protein kinases and viral transformation, p. 337–370. *In* P. Cohen and S. van Heyningen (ed.), Molecular aspects of cellular regulation, vol. 2: The molecular actions of toxins, viruses and interferon. Elsevier/North-Holland, Amsterdam.
15. **Hyman, R., and V. Stallings.** 1974. Complementation patterns of THY-1 variants and evidence that antigen loss variants "pre-exist" in the parental population. J. Natl. Cancer Inst. **52**:429–436.
16. **Kobayashi, N., and A. Kaji.** 1980. Phosphoprotein associated with activation of the *src* gene product in myogenic cells. Biochem. Biophys. Res. Commun. **93**:278–284.
17. **Martinez, R., K. D. Nakamura, and M. J. Weber.** 1982. Identification of phosphotyrosine-containing proteins in untransformed and Rous sarcoma virus-transformed chicken embryo cells. Mol. Cell. Biol. **2**:653–665.
18. **North, J. R., and W. C. Raschke.** 1979. Differentiation antigens on normal and Abelson virus-transformed lymphocytes. J. Immunol. **122**:2571–2576.
19. **Pawson, T., J. Guyden, T.-H. Kung, K. Radke, T. Gilmore, and G. S. Martin.** 1980. A strain of Fujinami sarcoma virus which is temperature-sensitive in protein phosphorylation and cellular transformation. Cell **22**:767–776.
20. **Radke, K., T. Gilmore, and G. S. Martin.** 1980. Transformation by Rous sarcoma virus: a cellular substrate for transformation-specific protein phosphorylation contains phosphotyrosine. Cell **21**:821–828.
21. **Radke, K., and G. S. Martin.** 1979. Transformation by Rous sarcoma virus: effects of *src* gene expression on the synthesis and phosphorylation of cellular polypeptides. Proc. Natl. Acad. Sci. U.S.A. **76**:5212–5216.
22. **Raschke, W. C.** 1979. Transformation by Abelson murine leukemia virus: properties of the transformed cells. Cold Spring Harbor Symp. Quant. Biol. **44**:1187–1194.
23. **Raschke, W. C., S. Baird, P. Ralph, and I. Nakoinz.** 1978. Functional macrophage cell lines transformed by Abelson leukemia virus. Cell **15**:261–267.
24. **Reynolds, F. H., T. L. Sacks, D. N. Deobagkar, and J. Stephenson.** 1978. Cells nonproductively transformed by Abelson murine leukemia virus express a high molecular weight polyprotein containing structural and nonstructural components. Proc. Natl. Acad. Sci. U.S.A. **75**:3974–3978.
25. **Reynolds, F. H., Jr., W. J. M. van de Ven, and J. R. Stephenson.** 1980. Abelson murine leukemia virus transformation-defective mutants with impaired P120-associated protein kinase activity. J. Virol. **36**:374–386.
26. **Rosenberg, N., and D. Baltimore.** 1976. A quantitative assay for transformation of bone marrow cells by Abelson murine leukemia virus. J. Exp. Med. **143**:1453–1463.
27. **Rosenberg, N. E., D. R. Clark, and O. N. Witte.** 1981. Abelson murine leukemia virus mutants deficient in kinase activity and lymphoid cell transformation. J. Virol. **36**:766–774.
28. **Rübsamen, H., K. Saltenberger, R. R. Friis, and E. Eigenbrodt.** 1982. Cytosolic malic dehydrogenase activity is associated with a putative substrate for the transforming gene product of Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **79**:228–232.
29. **Scher, C. D., and R. Siegler.** 1975. Direct transformation of 3T3 cells by Abelson murine leukemia virus. Nature (London) **253**:729–731.
30. **Sefton, B. M., K. Beemon, and T. Hunter.** 1978. Comparison of the expression of the *src* gene of Rous sarcoma virus in vivo and in vitro. J. Virol. **28**:957–971.
31. **Sefton, B. M., T. Hunter, E. H. Ball, and S. J. Singer.** 1981. Vinculin: a cytoskeletal target of the transforming protein of Rous sarcoma virus. Cell **24**:165–174.
32. **Sefton, B. M., T. Hunter, K. Beemon, and W. Eckhart.** 1980. Evidence that the phosphorylation of tyrosine is essential for cellular transformation by Rous sarcoma virus. Cell **20**:807–816.
33. **Sefton, B. M., T. Hunter, and W. C. Raschke.** 1981. Evidence that the Abelson virus protein functions in vivo as a protein kinase which phosphorylates tyrosine. Proc. Natl. Acad. Sci. U.S.A. **78**:1552–1556.
34. **Sheilds, A., S. Goff, M. Paskind, G. Otto, and D. Baltimore.** 1979. Structure of the Abelson murine leukemia virus genome. Cell **18**:955–962.
35. **Siden, E. J., D. Baltimore, D. Clark, and N. Rosenberg.** 1979. Immunoglobulin synthesis by lymphoid cells transformed in vitro by Abelson murine leukemia virus. Cell **16**:389–396.
36. **Towbin, H., T. Staehlin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. **76**:4350–4354.
37. **Waneck, G. L., and N. Rosenberg.** 1981. Abelson leukemia virus induces lymphoid and erythroid colonies in infected fetal cell cultures. Cell **26**:79–89.
38. **Warner, N. L.** 1978. Neoplasms of immunoglobulin-producing cells in mice. Recent Results Cancer Res. **64**:316–324.
39. **Witte, O. N., A. Dasgupta, and D. Baltimore.** 1980. Abelson murine leukemia virus protein is phosphorylated in vitro to form phosphotyrosine. Nature (London) **283**:826–831.
40. **Witte, O. N., S. Goff, N. Rosenberg, and D. Baltimore.** 1980. A transformation-defective mutant of Abelson murine leukemia virus lacks protein kinase activity. Proc. Natl. Acad. Sci. U.S.A. **77**:4993–4997.
41. **Witte, O. N., N. Rosenberg, M. Paskind, A. Shields, and D. Baltimore.** 1978. Identification of an Abelson murine leukemia virus-encoded protein present in transformed fibroblasts and lymphoid cells. Proc. Natl. Acad. Sci. U.S.A. **75**:2488–2492.