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Different Cellular Substrates of Abelson Leukemia Virus Transforming Protein Kinase in Murine Fibroblasts and Lymphocytes

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A. Introduction

Abelson murine leukemia virus (A-MuLV) is a replication-defective retrovirus capable of rapidly inducing leukemia in mice as well as of transforming in vitro mouse bone marrow cells and fibroblasts [1, 2, 14]; the A-MuLV was derived by passage in vivo of the replication-competent Moloney leukemia virus (M-MuLV) and its genome codes for a single polypeptide which is a hybrid molecule containing a portion of the parental M-MuLV genome (*gag* gene) and a portion of the cellular gene termed *abl* [18]. This protein, which varies in size from 160 to 90 kilodaltons, depending on the specific A-MuLV strain, is associated mainly with the detergent-insoluble cell fraction and possesses a tyrosine kinase activity [4, 15, 19]. In this regard, the A-MuLV protein resembles the pp60*src* encoded by the Rous sarcoma virus (RSV) [10] and the protein kinases encoded by other retroviruses such as the feline sarcoma virus (FeSV) [3, 17] and the Fujinami sarcoma virus (FuSV) [9]. The fact that the RSV pp60*src* is linked with specialized cellular areas such as cell–cell junctions and adhesion plaques has suggested that the alteration of these structures is involved in the origin of the transformed phenotype [12, 13] (Marchisio et al., Exp. Cell Res., in press). However, it has also been demonstrated that, in the same cell type, different cellular substrates are phosphorylated at tyrosine residues by diverse protein kinases (Di Renzo et al., submitted). Accordingly, we found it of interest to study the cellular substrates of the A-MuLV protein kinase in cells having different cytoskeletal architecture and adhesion properties such as fibroblasts and lymphocytes.

B. Materials and Methods

I. Cell Lines

TA-3 and TA-4, non-B lymphoma cell lines were established from two independent thymic lymphomas induced by inoculating intrathymically (i.t.) newborn BALB/c mice with the complex A-MuLV(M-MuLV). MZ-5, a pre-B lymphoma cell line, was established from a splenic lymphoma induced by inoculating the complex A-MuLV(M-MuLV) subcutaneously (s.c.) in newborn BALB/c mice. ABC-1, a A-MuLV-transformed line of pre-B cells, was kindly provided by Dr. Natalie Teich [16]. ANN-1 is an A-MuLV-transformed line of fibroblasts [14]. As control, a T cell lymphoma line (TB-5) induced by M-MuLV in BALB/c mice was also included in this study.

Lymphoma cell lines were cultured in complete medium consisting of Dulbecco-MEM (Gibco Europe, Glasgow, Scotland) supplemented with L-glutamine, HEPES, 2-mercaptoethanol, antibiotics, and 10%

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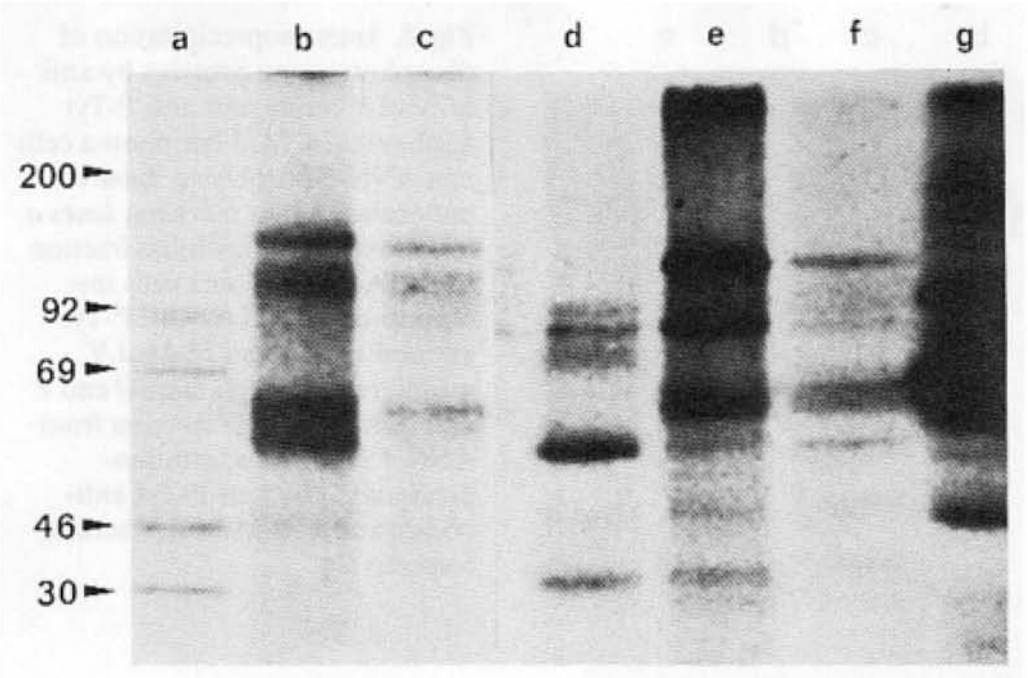

Fig. 1. Immunoprecipitation by anti-phosphotyrosine (anti-P-Tyr) antibodies of ^{32}P-labeled detergent-insoluble proteins from A-MuLV-transformed lymphocytes and fibroblasts. *Lane a* molecular weight markers; *lanes b* and *f* pre-B lymphoma cells MZ-5 and ABC-1; *lanes c* and *e* non-B lymphoma cells TA-4 and TA-3; *lane d* TB-5, M-MuLV-transformed T-cells; *lane g* ANN-1 fibroblasts

heat-inactivated fetal calf serum (FCS Gibco). ANN-1 fibroblasts were cultured in Dulbecco-MEM plus 10% FCS.

II. Specific Antisera

Antibodies against phosphotyrosine residues (anti-P-Tyr antibodies) were produced as previously described [6]. Anti-M-MuLV serum (Lot No. 71S/161) was obtained from the Office of Program Resources and Logistics, NCI, Bethesda, Maryland.

III. Immunoprecipitation Assay

Detergent-insoluble fractions were labeled according to Burr [5] by incubating detergent-insoluble proteins with ^{32}P-labeled γ-ATP (specific activity 5000 mCi/m*M*, Amersham) in conditions allowing phosphorylation catalyzed by the kinase coded by A-MuLV [4, 5]. After phosphorylation, the proteins were immunoprecipitated either by anti-P-Tyr antibodies or by anti-M-MuLV serum, as previously described [6]. After elution from protein A-Sepharose with Laemmli buffer [11], proteins were separated by SDS-PAGE. Dried gels were exposed to Kodak X-Omat film and processed for autoradiography.

C. Results and Discussion

The natural targets for in vivo transformation by A-MuLV are pre-B lymphocytes [1, 2]; however, the A-MuLV, if inoculated i.t., is also able to induce thymic lymphomas [7]. Thus, in addition to ABC-1 cells we also studied some lines derived from A-MuLV-induced thymic lymphomas; these cells do not express either T cell or B cell markers, even after stimulation with Con-A or LPS, respectively, and accordingly were putatively defined as non-B cells.

In order to investigate whether a specific substrate for A-MuLV kinase could be detected in different A-MuLV transformed cell types, the detergent-insoluble cellular fraction was studied with the aid of monospecific antibodies directed against the phosphorylated form of protein tyrosine residues (anti-P-Tyr antibodies).

As shown in Fig. 1, two main proteins of 70 and 120 kilodaltons were precipitated from ANN-1 fibroblasts (Fig. 1, lane *g*),

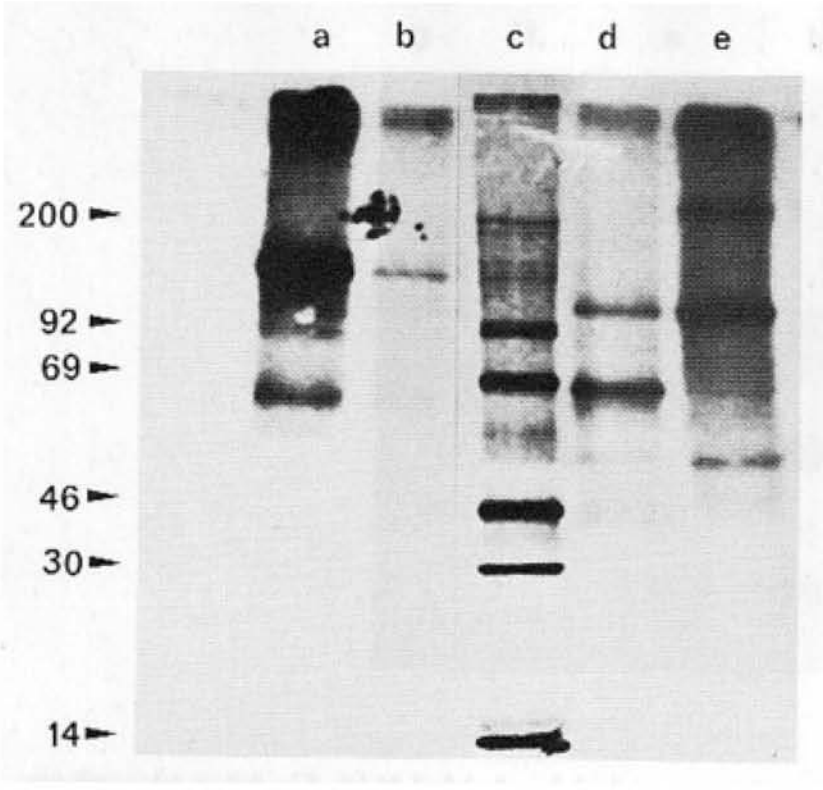

Fig. 2. Immunoprecipitation of phosphotyrosine proteins by anti M-MuLV serum and anti P-Tyr antibodies in TA-3 lymphoma cells and ANN-1 fibroblasts. *Lane c* molecular weight markers; *lanes a* and *b* detergent-insoluble fraction from TA-3 lymphoma cells immunoprecipitated by anti P-Tyr antibodies and anti M-MuLV serum, respectively; *lanes d* and *e* detergent-insoluble fraction from ANN-1 fibroblasts immunoprecipitated by anti-P-Tyr antibodies and anti-M-MuLV serum, respectively

whereas three different tyrosine phosphorylated proteins of 150, 100, and 65 kilodaltons were immunoprecipitated from pre-B and non-B lymphoma cells (Fig. 1, lanes *b, c, e, f*). No phosphorylated proteins were detected in nontransformed fibroblasts and normal thymus cells (data not shown in Fig. 1). On the contrary, in the TB-5 M-MuLV lymphoma cells, two proteins of 55 and 30 kilodaltons and an additional one of 100 kilodaltons, comigrating with the corresponding protein of A-MuLV lymphoma cells, were detected (Fig. 1, lane *d*). Moreover, in a preliminary experiment, using the anti-M-MuLV serum, which is able to recognize the *gag*-coded portion of the kinase protein, we observed only one immunoprecipitate band of 120 kilodaltons in ANN-1 fibroblasts (Fig. 2, lane *e*) and of 150 kilodaltons in lymphoma cells (Fig. 2, lane *b*). The anti-M-MuLV serum was not able to precipitate either the 70 kilodaltons phosphoprotein in fibroblasts (Fig. 2, lane *d*) or the 100 and 65 kilodaltons phosphoproteins in lymphoma cells (Fig. 2, lane *b*), suggesting that these latter are probably cellular substrates and not degradation products of the A-MuLV tyrosine itself.

These data seem to indicate that A-MuLV protein kinase, under identical experimental conditions, is able to phosphorylate at tyrosine residues different substrates in cells possessing diverse cytoskeletal architecture. It is known that A-MuLV-coded protein kinase, like other transforming protein kinases, does not discriminate for substrate phosphorylation [8, 18]; consequently, the different pattern of phosphorylation induced by A-MuLV protein kinase in fibroblasts and lymphocytes is probably imputable to a different association of the tyrosine kinase with the cytoskeletal macromolecules.

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