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A Set of Stage-Dependent Embryonic Antigens Expressed in Cell Cultures of BALB/c Mouse Embryos and in Transformed Cell Lines

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A rabbit antiserum (A2) directed against the detergent-solubilized fraction of the simian virus 40-transformed mouse embryo fibroblast cell line VLM detects common antigens in primary cell cultures from BALB/c mouse embryos and in transformed cell lines from various species. Positively reacting cell cultures show a set of polypeptides with molecular weight species p86, p74, p68, p46, p42, p40, and p35. As tested by Western blotting procedures, all immunoprecipitated proteins carry immunologically reactive determinants. By analysis with two-dimensional gel electrophoresis, all precipitated polypeptides show charge heterogeneities. Concerning the two major members of the protein set, p40 consists of at least four subspecies with isoelectric points in the range of pH 6.2–6.8, whereas p35 is composed of two subspecies focusing between pH 6.4 and pH 7.2. By comparison of the two-dimensional patterns of p35 of various transformed cell lines, a basic (pH 6.6–7.2) and an acidic (6.4–6.6) charge type of p35 could be observed. Comparative analyses of primary cell cultures from 12–16-day mouse embryos show the immunoprecipitated set of polypeptides only in the 16-day embryo cell cultures. After six further propagations, these cells express the immunoreactive proteins as strongly as the primary cell cultures. In embryonic cell cultures of day 14 of gestation the expression of this set of antigens is induced only when cells are propagated at least six times. Under identical conditions these proteins could not be induced in cell cultures of 18-day-old mouse embryos. None of the polypeptides could be immunoprecipitated from primary mouse kidney cell cultures of 12-day-old mice even when the cultures were propagated at least 15 times. This set of polypeptides is also present in simian virus 40-transformed cells of hamster, rat, monkey, and human origin. These findings suggest that in simian virus 40-transformed mouse cells, in addition to p53, the synthesis of other embryonic antigens is reactivated. The presence of the described set of polypeptides in polyoma virus-transformed cells of rat and mouse origin and in cell lines

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derived from malignant human tumors might indicate common functions in metabolic patterns of transformed cells.

Key words: stage-dependent embryonic antigens, transformed cell lines, tumor cell lines, two-dimensional gel electrophoresis, immune precipitation, Western blotting

Common aspects of embryogenesis and cancerogenesis are well known [1]. Indeed, activated cellular oncogenes have been found during mouse development [2], and, conversely, fetal antigens have been detected in transformed cells [3–6]. The expression of genes that are silent in mature cells may be a predominant feature of cancer cells [7]. Therefore, it seems reasonable to regard neoplastic development as a capactity of eukaryotic cells in the multicellular organism to express genes encoding for cellular characters that were important in a different context of ontogenesis, namely embryogenesis and fetal development. Simian virus 40 (SV40)-transformed cell lines are widely used systems. The activity of fetal antigens in SV40-transformed cells and their impact for tumorigenicity have been discussed for years [6,8–10]. Recently, the suggestion has been put forward that there might be families of similar antigens activated in different transformed cells [7,11]. Scott et al [7] described four sets of transcriptionally active genes in SV40-transformed mouse cells that were not detected in their nontransformed counterparts. The set 1 transcription unit was associated with transformation and required a functional SV40-tumor antigen (T-ag) but was also found to be active in cells transformed by other agents, including retroviruses and chemical carcinogens, and also in embryonal carcinoma cells and embryonic tissues. A stage-specific appearance was demonstrated for p53 [12], a protein synthesized in elevated levels in many transformed cell lines compared to their nontransformed counterparts [13,14]. In SV40-transformed cells, p53 was found to be complexed with SV40-T-ag [15]. Mora et al [12] reported that p53 was expressed specifically in primary cell cultures of 12–14-day-old mouse embryos, whereas on day 16 of gestation, no p53 could be immunoprecipitated from embryo cells. Kurth and Bauer [16], who investigated the same gestational period, found an antigen expressed on the surface of transformed mouse and chicken fibroblasts and on mouse embryo cells exclusively of day 16 of gestation as tested by cytotoxic antibodies.

We have investigated this in primary cell cultures of 12–18-day-old BALB/c mouse embryos, in SV40-transformed embryonal and postnatal BALB/c mouse fibroblasts, in polyoma virus- and SV40-transformed cell lines of various species, and in cell lines derived from spontaneously developed human neoplasias. In SV40-transformed cells, analyses concerning transformation-associated antigens have been carried out mainly with T-ag-specific antisera and monoclonal antibodies or with sera from tumor-bearing hamsters (TBH). In an attempt to characterize antigens that might be relevant both in embryonic and transformed cells, we have raised a xenogeneic rabbit antiserum against detergent-solubilized proteins of VLM cells. Using this antiserum, we have analyzed transformed and nontransformed cell lines by immunoprecipitation, two-dimensional gel electrophoresis (2D-PAGE), and Western blotting procedures. In the present report, we describe developmental antigens detected in primary cell cultures obtained from BALB/c mouse embryos at day 16 of gestation. These antigens were also found in polyoma virus- and SV40-transformed cells of different species and in cell lines derived from spontaneously developed human tumors but not in nontransformed counterparts. The molecular weights (M_r) and

isoelectric points (IEP) of these proteins were determined, and their 2D-PAGE characteristics are presented.

MATERIALS AND METHODS

Cell Lines and Cultures

Primary embryonic fibroblasts were prepared from 12–18-day-old mouse embryos (MEZ 12–18). Female BALB/c mice were inspected for vaginal plugs after mating overnight (day 1 of gestation), and 12–18 days later primary embryo cell cultures were prepared according to Dulbecco and Vogt [17]. The cells were seeded into plastic flasks (Nunc, 175 cm^2, 10^7 cells per flask). BALB/c kidney cells (MNZ) derived from 12-day-old mice were prepared as described for the embryos. The following cell lines were used: embryonic SV40-transformed [VLM, SV3T3, Fr(tsA 58)F2b], embryonic polyoma virus-transformed (PyA31), embryonic nontransformed (3T3) BALB/c mouse cells, and SV40-transformed BALB/c mouse kidney cells (mKSA). SV40-transformed cell lines originated from various species: SV80, human fibroblasts; AGMK-EVa, monkey kidney; and H65/90B, hamster tumor. Polyoma virus-transformed embryonic rat cells were TClA and B_4Cl_3. Tumor cell lines were HeLa (cervix carcinoma), Tu-22 and LyMet (renal cell carcinoma and lymph node metastasis thereof, both established by M. Ruprecht and F. Falkenberg of our university), and RD (rhabdomyosarcoma). Nontransformed cell lines were FH 99 (human fetal lung fibroblasts, established by Lia Diagnostika, Munich), BSC-1, CV-1, Vero (monkey kidney), and BHK (baby hamster kidney).

All cells were propagated at 37°C in Dulbecco-Vogt modified Eagle medium (DMEM; Gibco Europe, Paiseley, Scotland) buffered with 15 mM N,N-bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), 15 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), 15 mM N-tris-(hydroxymethyl)-methyl-2-aminoethanesulfonic acid (TES), and 15 mM sodium bicarbonate (all from Sigma Chemie GmbH, Munich, Federal Republic of Germany). DMEM with 5,000 IE/liter penicillin G and 50 mg/liter dihydrostreptomycin (Sigma Chemie GmbH) was supplemented with 10% fetal bovine serum (FCS; Biochrom KG, Berlin, Federal Republic of Germany) for propagation of primary embryonic mouse cells. MNZ and the transformed cells were propagated in DMEM supplemented with 10% and 5% newborn calf serum (NCS; Gibco Europe), respectively.

Labeling of Cells

Subconfluent cell cultures were washed three times with Hanks' balanced salt solution (HBSS) and incubated for 3 hr at 37°C in 4 ml methionine-free DMEM containing 250 μCi per 10^7 cells of [^{35}S]methionine (900 Ci/mmol; NEN Chemicals GmbH, Dreieich, Federal Republic of Germany) and 5% NCS or FCS. After 3 hr, the radioactive supernatants were removed, and the cells were rinsed three times with HBSS; thereafter, cell fractionation followed immediately.

Cell Fractionation

The cell monolayers were incubated for 5 min on ice with solution A (0.25 M sucrose, 10 mM Tris HCL, pH 7.4, 3 mM $MgCl_2$, 5 mM KCl; 1,000 U aprotinin/ml; aprotinin was a generous gift from Novo Industrie GmbH, Pharmaceutika, Mainz, Federal Republic of Germany). Cells were lysed by incubation for 10–15 min on ice

in solution A containing detergents (0.5% Triton X-100, Sigma Chemie GmbH; 0.25% sodium deoxycholate, Merck, Darmstadt, Federal Republic of Germany). The lysates were centrifuged (800g, 10 min, 4°C) and the supernatants designated as detergent-solubilized fractions. Sediments were used for further investigations of chromosomal proteins.

Serum

To induce antibodies against SV40-transformed embryonic mouse fibroblasts in rabbits, detergent-solubilized proteins of VLM cells were used. Preimmune sera were taken before the first immunization. Primary and booster injections were given at monthly intervals followed by bleeding of the animals 14 days later (A2-sera).

Immunoprecipitation

Formaldehyde-fixed *Staphylococcus aureus* Cowan I immunoadsorbent (SAC) was prepared and used essentially as described by Kessler [18]. Briefly, 100 μg [^{35}S]methionine-labeled cytoplasmic proteins were preadsorbed for 30 min with 10 μl preimmune serum or control serum and for 2 hr with 100 μl SAC. Samples were centrifuged (12,000g, 2 min) and the supernatants immunoprecipitated with 10 μl of TBH or A2-serum by incubation for 1 hr at 4°C. SAC was added as before, and the mixture was incubated for another 2 hr or over night at 4°C. Thereafter, SAC was sedimented (12,000g, 2 min) and washed as described by McCormick et al [19]. Adsorbed immune complexes were dissolved by boiling the SAC pellet for 10 min in 50 μl electrophoresis sample buffer (62.5 mM Tris HCl, pH 6.8; 3% sodium dodecyl sulfate [SDS]; 5% 2-mercaptoethanol; 10% glycerol; 0.005% bromophenol blue) for separation by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) or lysis buffer (2% SDS, 5 mM EDTA, 5% 2-mercaptoethanol) for separation by two-dimensional gel electrophoreses.

One- and Two-Dimensional PAGE

SDS-PAGE was performed as described by Laemmli [20] and 2D-PAGE essentially as described by O'Farrell [21] with minor modifications according to Peters and Comings [22]. Acrylamide, bisacrylamide, ampholytes, and reference proteins were purchased from Serva Feinbiochemica GmbH (Heidelberg, Federal Republic of Germany). The isoelectric points and M_r values of the separated polypeptides were estimated using the following reference proteins: bovine serum albumin (pI 6.2; M_r 68,000), carbonic anhydrase (bovine erythrocyte, pI 6.7; M_r 29,000), conalbumin (chicken egg, pI 7.2, M_r 86,000), and ovalbumin (chicken egg, pI 5.6, M_r 45,000). Gels with radioactive material were subjected to fluorography [23] and exposed to X-Omat AR5 X-ray films (Kodak AG) at −70°C.

Electrophoretic Transfer to Nitrocellulose and Immunochemical Detection of Proteins

SDS-PAGE-separated proteins were electrotransferred to nitrocellulose as described by Brunette [24]. The nitrocellulose membrane was quenched according to Batteigner et al [25]. Blots were incubated with antisera diluted (1:100) in quenching solution containing 1% bovine serum albumin (BSA). Washings were performed in quenching solutions. The immunoenzyme reaction was carried out as described by

Blake et al [26]. For molecular weight determination, the lane with the reference proteins was cut off from the blot and stained with india ink [27].

Protein Determination

TCA-precipitable proteins contents of the samples were determined using the method of Lowry et al [28] with BSA as standard in an autoanalyzer (Skalar, Breda, NL).

RESULTS

Serum A2 Detects a Set of Seven Polypeptides in SV40-Transformed Embryonal BALB/c Mouse Fibroblasts

Using subcellular fractions of the SV40-transformed embryonal mouse cell line VLM for immunization, we tried to detect common antigenic patterns of embryonic and transformed cells. The serum described here (A2) was raised against detergent-solubilized VLM proteins. The reliability of the test system has been proven by a T-ag immunoprecipitation with TBH sera. Antiserum A2 recognized a set of polypeptides consisting of seven discrete M_r species (p35, p40, p42, p46, p68, p74, and p86; Fig. 1, lane 1). Polypeptides p35 and p40 showed the most [^{35}S]methionine level. With 2D-PAGE analysis, p35 segregates into at least two subspecies localized between pH 6.4 and 6.6. p40 consists of at least four subspecies, their isoelectic points ranging from pH 6.3 to 6.7. The other precipitated proteins (p86, p74, and p68) segregated into at least two subspecies with IEPs of pH 6.2, 6.7, and 5.4, respectively.

To determine which of the precipitated molecular weight species were actually immunoreactive, ie, to exclude unspecific coprecipitation, immunoprecipitations were

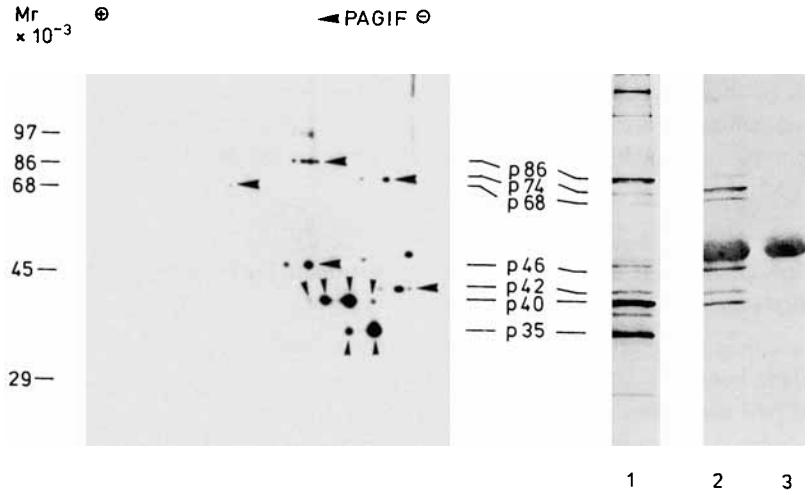


Fig. 1. Fluorograms and immune blots of one- and two-dimensional polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled VLM cytoplasmic proteins immunoprecipitated by antiserum A2. At left the two-dimensional polypeptide pattern is shown. Arrows indicate the subspecies of the precipitated proteins. The immunoprecipitated polypeptides separated by SDS-PAGE (lane 1) were analyzed by immunoblotting with A2 serum (lane 2). VLM proteins precipitated by preimmune serum were separated by SDS-PAGE and analyzed by immunoblotting (lane 3).

carried out with nonimmune and A2 serum and analyzed by SDS-PAGE. After electroblotting onto nitrocellulose membranes, adsorbed proteins were incubated with A2 serum and developed by an antirabbit IgG-alkaline phosphatase detection system. The complete set of polypeptides was reactive with A2 serum (Fig. 1, lane 2), whereas in immunoprecipitates with a control system only the IgG band had reacted (Fig. 1, lane 3). Therefore, we conclude that all M_r species of the set carried antigenic determinants that are individually recognized by A2 antibodies.

A2 Serum Recognizes a Set of Developmental Antigens in Primary Mouse Embryo Cells on Day 16 of Gestation

We then addressed the question of the reactivity of A2 serum with nontransformed embryonal mouse cells. The midgestational period was chosen with regard to experiments of other investigators [12,16] and to its high transformation susceptibility. This is also the period when high levels of p53 are synthesized in the cells [12]. Primary cultures of BALB/c mouse embryos of days 12–16 of gestation were labeled for 3 hr with [^{35}S]methionine and separated into a detergent-solubilized and a chromosomal protein fraction. Samples were absorbed with preimmune serum to reduce nonspecific binding. To exclude disappearance of proteins of interest, we also analyzed preabsorption sediments with staphylococci and with staphylococci plus preimmune serum (Fig. 2A, lane 1 and 2). On day 16 of gestation, polypeptides that closely resembled the 2D-PAGE characteristics of the above-described VLM polypeptides were detected in the immunoprecipitates (Fig. 2A, lane 7, and Fig. 2B). To exclude differences in protein labeling induced by variations in methionine metabolism, we tested the A2 precipitates of each day of gestation by Western blotting procedures (Fig. 2A, lanes 3′–7′). Only the 16-day embryo cell cultures contained significant amounts of antigenic polypeptides. On day 18 of gestation (Fig. 2A, lane 8′), the polypeptides could no longer be detected. On day 13 of gestation, however, minimal levels could be detected using Western blotting techniques. The data indicate that, excluding for day 16 of gestation, cells from all other days did not contain significant amounts of the polypeptides. We conclude, therefore, that the genes of the above-described antigens are regulated in a stage-dependent manner during embryonic development and reach their maximum of synthesis on day 16 of gestation, at least for the period tested.

Induction of the Set of Developmental Antigens by Propagation of 14-Day-Old Embryos

To confirm the stage-specific appearance of the set of embryonic antigens, we tested their behavior after several propagations of cultures. The rate of synthesis of p35 and p40 and concomitantly precipitated proteins remained constant when 16-day mouse embryo cell cultures were propagated six times. To investigate whether these embryonic antigens could be induced in cells of an earlier (14) or later (18) day of gestation, cells were kept in culture over several passages. In primary cell cultures, none of the stage-specific proteins was precipitated by A2 serum on day 14 of gestation (Fig. 3A, lane 1). These precipitates contained predominantly a polypeptide comigrating with actin. After two passages, a similar result was obtained (Fig. 3A, lane 2). After six propagations, however, the complete set of proteins could be

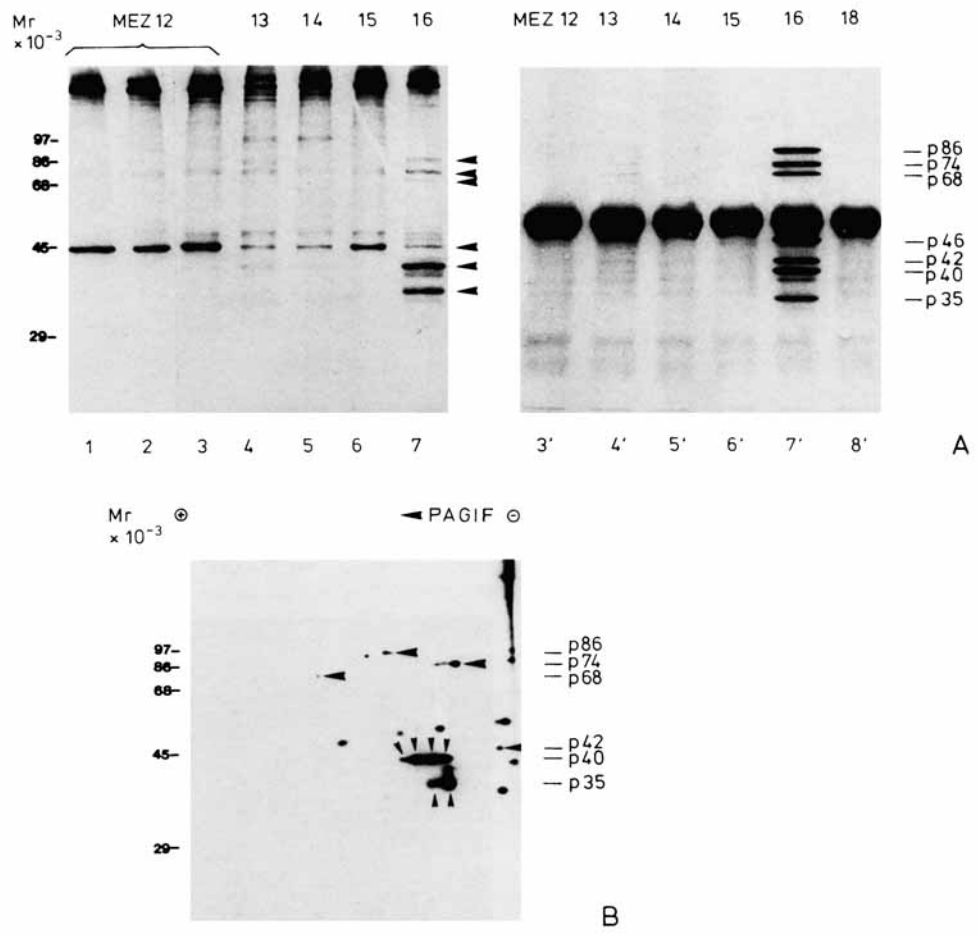


Fig. 2. Fluorograms and immune blots of one- and two-dimensional separated [^{35}S]methionine-labeled proteins immunoprecipitated from primary embryonic mouse cells (MEZ) with A2 serum. A) SDS-PAGE, MEZ proteins of day 12–16 of gestation (lanes 3–7) immunoprecipitated with serum A2. Lanes 3′–7′ show the same samples analyzed by immunoblotting with A2 serum. Lane 8′ shows the enzyme immune reaction of MEZ polypeptides on day 18 of gestation. Patterns of control precipitates obtained from detergent-solubilized proteins of MEZ 12 by staphylococci with (lane 1) and without preimmune serum (lane 2) are presented. B) two-dimensional pattern of the immune precipitate shown in A, lane 7. Arrows indicate subspecies of the precipitated proteins.

detected by immunoprecipitation (Fig. 3A, lane 4). The conformity of the antigen set with the VLM pattern was further confirmed by two-dimensional protein analysis (Fig. 3B and C). The corresponding experiment with 18-day embryo cell cultures showed none of the relevant proteins up to the sixth passage (data not shown). This was also the case for postnatal (day 12) BALB/c mouse kidney cell cultures (MNZ; Fig. 4, lane 6; and see below), which did not contain any of the developmental polypeptides in primary cell cultures or in the cell line up to the fifteenth passage.

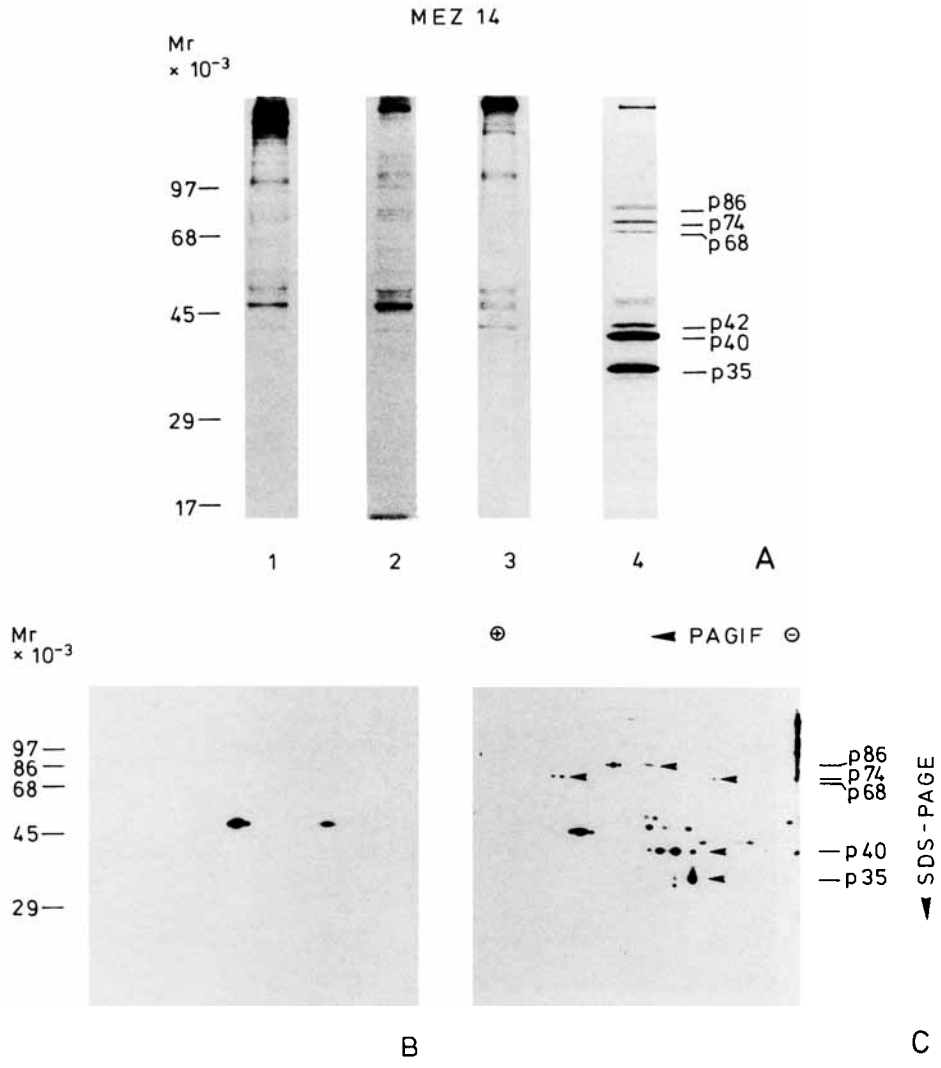


Fig. 3. Expression of stage-specific antigens in serially propagated cell cultures derived from mouse embryos at day 14 of gestation (MEZ 14). Fluorograms of electrophoretically separated immunoprecipitates obtained with A2 serum from [^{35}S]methionine-labeled detergent-solubilized proteins from primary embryonic mouse cells (A, lane 1) and from cell cultures after two, four, and six propagations (A, lanes 2–4). The two-dimensional patterns of immunoprecipitated polypeptides from primary MEZ 14 cultures corresponding to lane 1 in A are presented in (B) and of immunoprecipitated polypeptides after six propagations corresponding to lane 4 in A are presented in (C).

Stage-Dependent Embryonic Antigens (SDEA) Recognized Immunochemically by A2 Serum in SV40- and Polyoma Virus-Transformed BALB/c Mouse Fibroblasts

To study whether the appearance of SDEA is a characteristic of the transformed embryonic mouse cells, immunoprecipitates with A2 serum from detergent-solubilized fractions of SV3T3, VLM, and PyA31 were analyzed (Fig. 4). All the cells

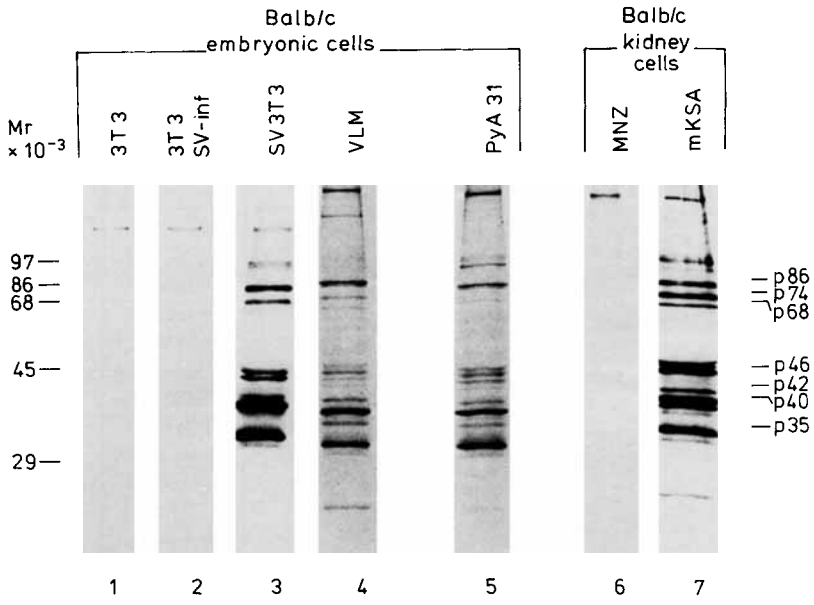
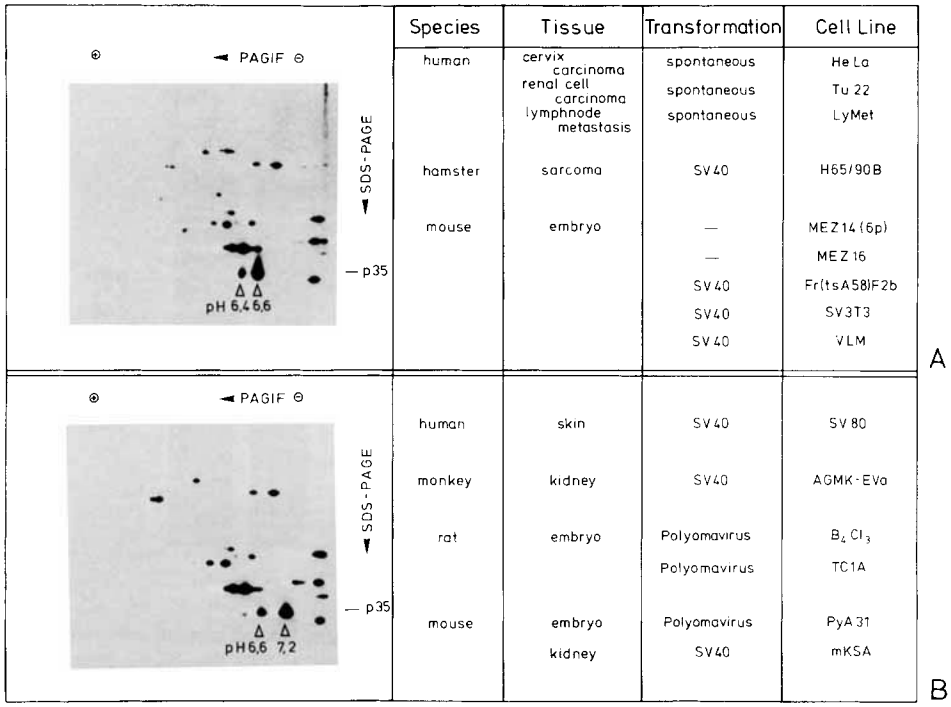


Fig. 4. Fluorograms of electrophoretically separated [^{35}S]methionine-labeled proteins immunoprecipitated with A2 serum from various BALB/c mouse cell lines. Lane 1) 3T3 fibroblasts, lane 2) 3T3 fibroblasts 24 hr after SV40 infection (50 pfu/cell), lanes 3 and 4) SV40-transformed cell lines SV3T3 and VLM, respectively, lane 5, polyoma virus-transformed cell line PyA31; lanes 6 and 7) mouse kidney cells (MNZ) and their SV40-transformed counterpart, mKSA, respectively. Arrows indicate the position of stage-dependent embryonic antigens (SDEA) in the particular precipitates.

synthesized SDEA. However, the [^{35}S]methionine labeling of the individual polypeptides varied between the different cell strains. The appearance of SDEA in polyoma virus-transformed cell line PyA31 indicates that the induction of synthesis of these polypeptides is not restricted to SV40-transformed cells (Fig. 4, lane 5). Moreover, SDEA are not only a characteristic of transformed embryonic mouse cells but were also found in an SV40-transformed cell line derived from differentiated kidney cells (postnatal day 12; Fig. 4, lane 7). Nontransformed kidney cells (MNZ) from 12-day-old mice did not synthesize SDEA in significant amounts (Fig. 4, lane 6). In 3T3 cells (Fig. 4, lane 1), SDEA were not synthesized in detectable amounts under our culture conditions nor were they synthesized 24 hr after SV40 infection (m o i 50; Fig. 4, lane 2).

SDEA in Polyoma Virus- and SV40-Transformed Cell Lines of Different Species and in Cell Lines Derived From Human Malignant Neoplasias

SDEA were detected by immunoprecipitation in polyoma virus-transformed rat cell lines, in polyoma virus- and SV40-transformed mouse cell lines, and in SV40-transformed cell lines of human, monkey, and hamster origin (see table in Fig. 5). In the human cell lines derived from a human cervical adenocarcinoma (HeLa), from a human cell carcinoma, from a lymph node metastasis thereof (Fig. 5), and from a rhabdomyosarcoma (data not shown), immunoprecipitable SDEA were present in the



Species	Tissue	Transformation	Cell Line
human	cervix carcinoma	spontaneous	He La
	renal cell carcinoma	spontaneous	Tu 22
	lymphnode metastasis	spontaneous	LyMet
hamster	sarcoma	SV40	H65/90B
mouse	embryo	—	MEZ14(6p)
		—	MEZ16
		SV40	Fr(tsA58)F2b
		SV40	SV3T3
		SV40	VLM
human	skin	SV40	SV80
monkey	kidney	SV40	AGMK-EVa
rat	embryo	Polyomavirus	B_4Cl_3
		Polyomavirus	TC1A
mouse	embryo	Polyomavirus	PyA31
	kidney	SV40	mKSA

Fig. 5. Two-dimensional analysis of immunoprecipitated p35 from various transformed and embryonic cell lines. [^{35}S]methionine-labeled detergent-solubilized proteins were immunoprecipitated with A2 serum and analyzed by two-dimensional PAGE. Patterns in A (HeLa) and B (AGMK-EVa) are examples for the two different separation types of p35 (A, IEP pH 6.4–6.6; B, IEP pH 6.6–7.2). Cell lines with different etiologies of transformation from various species are listed according to their separation type corresponding to the fluorogram patterns at right.

detergent-solubilized protein fractions. In *bona fide* nontransformed cell lines of human, monkey, and hamster origin, SDEA could not be detected (data not shown).

p35 2D-PAGE Pattern Segregates Transformed Cell Lines Into Two Types

For further characterization, all immunoprecipitates were analyzed by 2D-PAGE. Special attention was paid to the migrational behavior of p35 and p40. p40 segregates into at least four and p35 into at least two charge-dependent subspecies. Among different cell lines, variations in the amount of labeling could be observed for the individual subspecies. Whereas p40's position remained stable for all cell lines investigated, p35 exhibited a basic charge shift in certain transformed cells (Fig. 5B) compared to the pattern of VLM cells (Figs. 1 and 5A). In the p35 acidic type (IEP pH 6.4–6.6), the following cell characteristics were grouped: all cell lines derived from tumors, all SV40-transformed embryonal cells, and certain cell cultures derived from mouse embryos (Fig. 5A). The p35 basic type (IEP pH 6–7.2) comprised the polyoma virus-transformed cell lines (Fig. 5B). Because of the low number of cell lines tested in the individual groups, no conclusion concerning the nature of the p35 charge types could be drawn.

DISCUSSION

The present data indicate the presence of previously undetected embryonic antigens in primary cell cultures of BALB/c mouse embryos and in SV40-transformed cells of mouse, hamster, monkey, and human origin. These antigens were also detected in polyoma virus-transformed cell lines of mouse and rat origin and in four cell lines derived from human malignant neoplasms. The results are consistent with the hypothesis that transformed cells reactivate embryonic metabolic patterns to gain competence for adaption to an oncogenic environment. These antigens were recognized with an antiserum directed against the detergent-solubilized fraction SV40-transformed embryonic BALB/c mouse cell line VLM. For the chosen analytical conditions (M_r 10^4–10^5 and IEP pH 3–10), this antiserum immunprecipitates a set of polypeptides (p86, p74, p68, p46, p42, p40, and p35). These antigens may have the following, mutually exclusive, characteristics: 1) they may belong to molecules that are restricted to VLM cells only; 2) they may be strain- or species-specific; ie, they are detected only in BALB/c cells or in cells of any mouse strain and may be characteristic for a particular differentiated state, ie, embryonic and/or transformed and/or tissue type-specific; 3) they may be species-independent but characteristic for a particular differentiated state, ie, embryonic and/or transformed and/or tissue type-specific; and 4) they may be specific for the transforming agent. The tissue type specificity has not yet been analyzed in detail. We have, however, tested the other possibilities and detected with antiserum A2 a set of polypeptides that were not restricted to VLM or BALB/c cell lines but were also found in SV40- and polyoma virus-transformed cell lines of various species and in cell lines derived from spontaneous human neoplasms. These polypeptide antigens could not be detected in all *bona fide* nontransformed cell lines nor in primary cultures of differentiated mouse kidney tissues and cell lines thereof.

By analyzing primary cell cultures of BALB/c mouse embryos with A2 serum, we detected the same set of polypeptides, which correspond in their 2D-PAGE behavior to the antigens detected in VLM cytoplasm. These polypeptides seemed to be synthesized stage specifically in primary cell cultures of 16-day-old mouse embryos at least for the period studied (days 12–18). Primary embryonic cell cultures, however, change their protein pattern in a day-to-day manner depending on their gestational stage [unpublished data]. Although the appearance of these proteins may depend on in vitro culture conditions or be due to proliferation of cells belonging to a particular type of tissue, the phenomenon remains to be correlated to the gestational day of the embryo. Western blotting procedures reveal the presence of low-level concentrations of the polypeptides even in cell cultures of 13-day embryos. This finding and the ability to induce these proteins on day 14 but not on day 18 of gestation by propagating cells at least six times indicate activation of sequential genomic patterns during embryofetal development. Furthermore, varying relative and absolute intracellular levels of the individual subspecies in different cell lines and the observed charge variabilities especially of p35 may be important for the function of the whole set of proteins detected. Induction of the proteins beyond day 16 of gestation is not as easily performed as, eg, on day 14 of gestation. This is also demonstrated by the lack of the polypeptides in 3T3 cells derived from 17–19-day-old embryos [29] and propagated thereafter many times. Although polyoma virus- and SV40-transformed embryonic cell lines synthesize the whole set of polypeptides, these could not be

detected in 3T3 cells even after infection with SV40 (infection was tested by T-antigen immunofluorescence; data not shown). Analysis, however, was only performed 24 hr postinfection. Therefore, the possibility of a transiently induced synthesis of SDEA during the first 24 hr postinfection is not excluded. We never found any of the SDEA among the polypeptides investigated in the chromosomal protein fraction but precipitated some of the SDEA from culture fluid supernatants [unpublished results]. Especially p40 and p35 were always found in relatively high amounts in the culture fluid supernatants. A transformation-associated secretory protein with a molecular weight similar to p35 is described by Gottesmann [30] and Scher et al [31].

The reported data give evidence that SDEA are developmental antigens reactivated in transformed cells of various species independent of the transforming agent. Moreover, the polypeptides of interest were always precipitated as a set, a fact that indicates a functional relationship. Variations in charge and intracellular concentrations of the individual polypeptides may be modulated by species- or tissue-specific factors or the transformation-initiating proteins themselves. In the case of SV40 transformation, T-ag may be only the initial factor in maintaining transformation and establishing tumorigenicity. It is known that SV40-transformed diploid mouse embyro cells expressing T-age are not tumorigenic for many passages after their initial isolation [32]. Chen et al [33] propose that a "postcrisis factor" is required in combination with p53 to establish transformation. A similar mechanism may also be relevant for the initiation of transformation by other viral oncogenes: Characteristic, and therefore triggering, ratios of different oncogene products and other cellular factors may act upon cells and, therefore, lead to the transformed phenotype as discussed by Land et al [34]. The role of SDEA needs to be studied in this respect. Posttranslational modifications as indicated by charge shift of p35 and varying intracellular concentrations are of particular interest.

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