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PROMINENT INCREASE IN THE AMOUNT OF A CYTOSOL PROTEIN IN TRANSFORMED FIBROBLASTS AND ASCITES HEPATOMA CELLS

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Summary

Analysis of cytosol proteins by sodium dodecyl sulfate polyacrylamide slab gel electrophoresis revealed a prominent increase in the amount of a cytosol protein with molecular weight of 88 000 in transformed human adult, human embryo, mouse adult, and hamster embryo fibroblasts as compared with normal fibroblasts. The cytosol protein with M_r 88 000 is also increased in the cytosol of four kinds of rat ascites hepatoma cell as compared to normal and regenerating liver. The protein with M_r 88 000 exists as one of the major cytosol proteins in transformed fibroblasts, hepatoma cells and HeLa cells, constituting 7—10% of total cytosol proteins. The data suggest that the cytosol protein with M_r 88 000 is associated with certain growth characteristics of cells.

Analysis of HeLa cell cytoplasm by two-dimensional polyacrylamide gel electrophoresis revealed the presence of 532 cytoplasmic protein components [1]. Most of these proteins, however, are not characterized as to their physiological role and pattern of intracellular distribution. Finding a correlation between a cytoplasmic protein and a specific cellular phenomenon may provide a clue for further characterization of the protein. In the present paper we report that a cytosol protein is increased, and is present in large amounts in various kinds of tumor cell.

Normal and transformed fibroblasts and HeLa cells were grown in Dulbecco's modified Eagle's medium supplemented with 15% fetal calf serum (Gibco), 100 units/ml penicillin and 100 μg/ml streptomycin at 37° C in an

atmosphere of 5% of CO_2 in air. Cells were inoculated into 100-mm Falcon plastic dishes at a density of 10 000 cells cm^2. They were fed every other day. When the cells had grown to near confluence, they were washed twice with phosphate-buffered saline and scraped from the plate with a rubber policeman. In some experiments, normal human adult fibroblasts were harvested during logarithmic growth. Four human skin fibroblasts (PL65, PL67, PL69 and PL78) and a human embryo fibroblast (IMR90) were kindly provided by Dr. M.S. Sasaki, Kyoto University, and by Dr. T. Matsumura, The University of Tokyo, respectively. The human adult fibroblasts were used at passage no. 5 or 6 and the human embryo fibroblast was used at passage no. 26. Simian virus (SV)40-tansformed human adult and embryo fibroblasts (W98-Va-C and WI26-Va-4) were kind gifts from Dr. H. Koprowski, The Wister Institute. A hamster embryo fibroblast (HEC) and SV 40-transformed HEC (SV-HE-Cl 2) were established as previously described [2]. A mouse adult fibroblast was prepared by incubating back skin fragments from a 5-week-old *ddy* mouse and used at passage no. 3. A mouse adult fibrosarcoma cell line (FRUKTO), derived from a *dd* mouse [3], was kindly provided by Dr. A. Niwa, Dokkyo University.

Four kinds of fast-growing rat ascites hepatoma cell, AH 109A, AH130, AH7974 and Yoshida sarcoma were obtained by drainage of the ascites fluid 5 days after implantation into male Donryu rats of 175—200 g.

The cells were centrifuged at 600 × *g* and the cell pellet was washed three times with phosphate-buffered saline. Normal, 24-h regenerating, and 72-h regenerating livers from normal male Donryu rats were perfused with phosphate-buffered saline. Cultured cells, ascites hepatoma cells, and normal and regenerating livers were homogenized with a Potter-Elvehjem homogenizer in homogenization buffer (0.25 M sucrose/3 mM Tris-HCl, pH 7.4/0.1 mM EDTA/1 mM phenylmethylsulfonyl fluoride) at 0°C. The homogenate was centrifuged at 140 000 × *g* for 60 min at 4°C and the supernatant was stored at −80°C.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was carried out on 2 mm thick, 135 mm long and 160 mm wide slabs in buffer system, as described by Laemmli [4]. Samples containing 30 μg of cytosol proteins were prepared for electrophoresis by dissolving them in sample buffer (10 mM Tris-HCl, pH 7.4/1% SDS/0.5% 2-mercaptoethanol/0.001% bromphenol blue/5% sucrose) and by incubation at 80°C for 3 min. Samples were applied to 7.5% gels with 4.5% stacking gels and electrophoresed for 3 h at 40 mA, until the bromphenol blue dye marker reached the front of the gel. After electrophoresis, the gel was stained with Coomassie brilliant blue according to Fairbanks et al. [5]. Differences in Coomassie brilliant blue staining bands were determined by absorbance at 550 nm with a Gelman model ACD-15 densitometer (Gelman Instrument Co., Ann Arbor) and expressed as percent of total protein staining. Molecular weight standards included chicken skeletal muscle actin (M_r 42 000), bovine serum albumin (M_r 68 000) and phosphorylase a (M_r 94 000). The purified actin was kindly provided by Dr. M. Kuroda, University of Tsukuba. Protein concentrations were determined by the method of Lowry et al. [6]. Fig. 1A shows the protein composition of the cytosol of normal and transformed human fibroblasts.

TABLE I

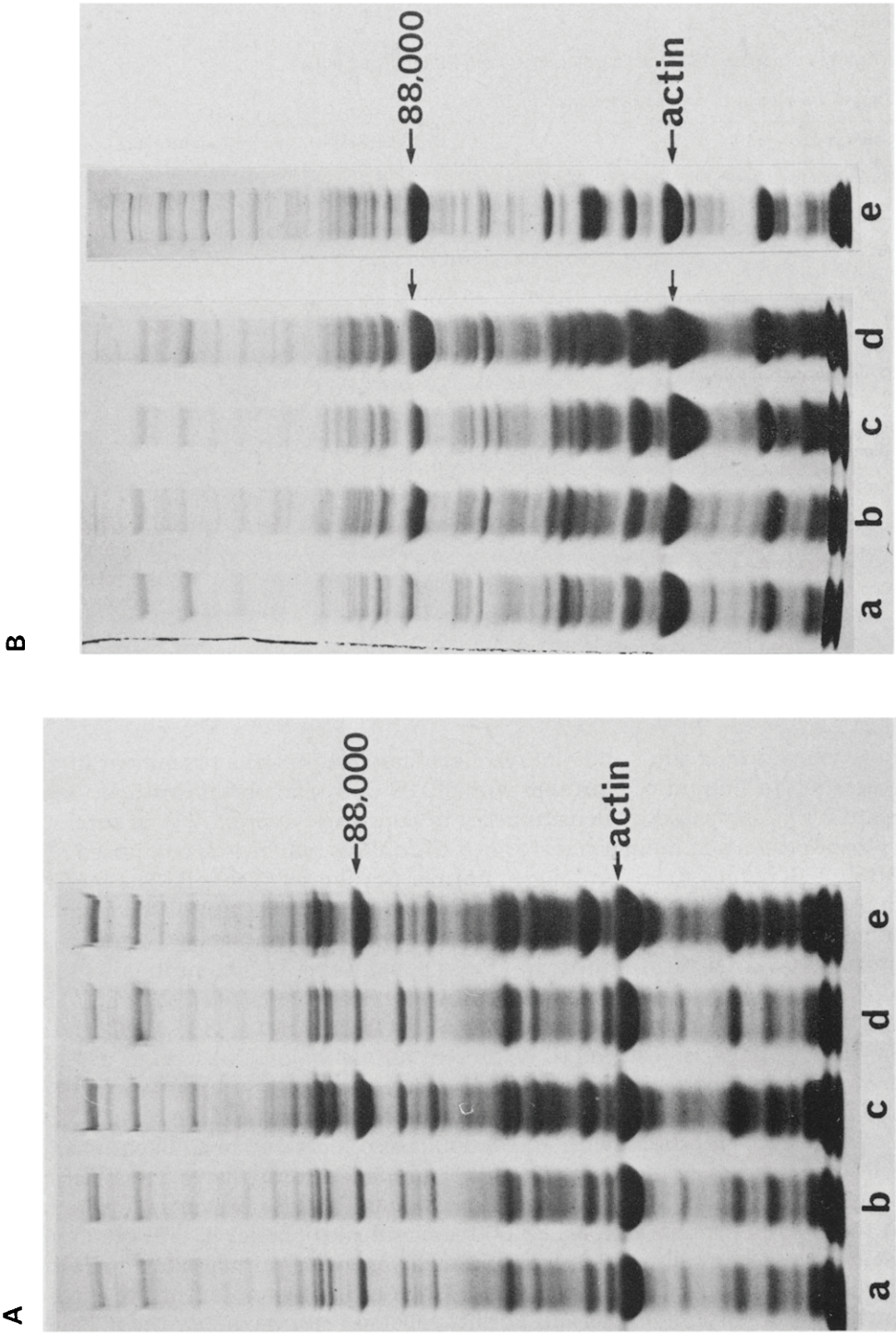
RELATIVE AMOUNTS OF CYTOSOL PROTEIN WITH M_r 88 000

Figures are average of two experiments.

Cell Type		Percent of total cytosol proteins
Human normal adult fibroblasts:	PL65	2.3
	PL67	3.0
	PL69	2.5
	PL78	3.1
	PL67 (log growth)	3.5
	PL78 (log growth)	3.5
Human normal embryo fibroblast:	IMR90	2.5
Human transformed adult fibroblast:	W98-Va-C	7.1
Human transformed embryo fibroblast:	WI-26-Va-4	7.0
Mouse normal fibroblast		3.3
Mouse transformed fibroblast:	FRUKTO	7.9
Hamster normal fibroblast:	HEC	4.3
Hamster transformed fibroblast:	SV-HE-Cl 2	8.0
HeLa cells		8.0
Normal liver		1.9
24-h regenerating liver		2.3
72-h regenerating liver		2.6
Ascites hepatoma cells:	Yoshida sarcoma	9.7
	AH7974	10.5
	AH130	9.2
	AH109A	9.7

Both transformed adult and embryo fibroblasts exhibited a prominent increase in the amount of a protein with M_r 88 000. The protein with M_r 88 000 was estimated by densitometry to constitute approx. 7 % of total cytosol proteins in human transformed fibroblasts, whereas it comprised 2–3.5% of total cytosol proteins in normal fibroblasts (Table I). The increase in the amount of the cytosol protein with M_r 88 000 was also observed in transformed mouse adult and hamster embryo fibroblasts, constituting approx. 8% of total cytosol proteins (Fig. 1B and Table I). The protein with M_r 88 000 exists as one of major cytosol proteins in Hela cells, too (Fig. 1B and Table I). The cytosol protein with M_r 88 000 of HeLa, W98-Va-C, and SV-HE-Cl 2 cells, is observed to be present as one major spot with p*I* of 5.9 in two-dimensional polyacrylamide gel electrophoresis by the method of O'Farrell [7] (Hamaguchi, H. et al., unpublished results).

The cytosol protein with M_r 88 000 is also increased in all hepatoma cells examined as compared with normal and regenerating livers, constituting 9–10.5% of total cytosol proteins (Fig. 1C and Table I). Because the amount of the cytosol protein with M_r 88 000 is significantly larger in W98-Va-C and ascites hepatoma cells than in logarithmically growing normal human adult fibroblasts and regenerating liver, the difference in the amount of the cytosol protein between tumor cells and normal cells may not be due to the difference in the phase of cell cycle. Recently, we observed that the cytosol protein with M_r 88 000 is increased in both rat and human colonic cancers as compared with normal colonic mucosa, (unpublished results).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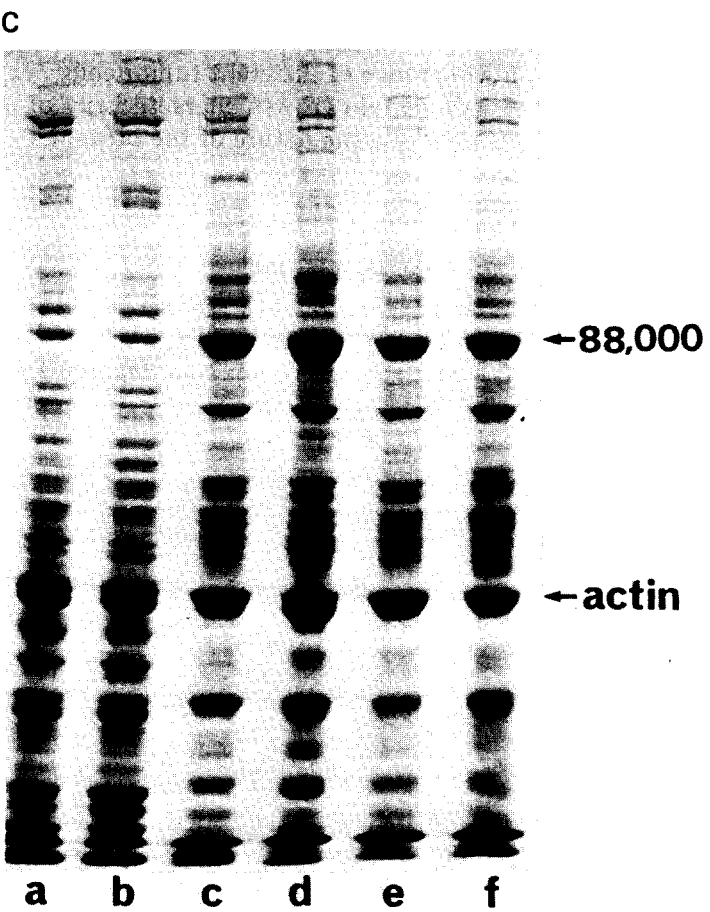

Fig. 1. SDS-polyacrylamide slab gel electrophoresis patterns of cytosol proteins. 30 μg of protein was applied to each lane. An apparent molecular weight of a prominently increased protein in tumor cells and the position of actin are indicated. (A) a and b, normal human adult fibroblasts (PL 67 and 69); c, transformed human adult fibroblast (W98-Va-C); d, normal human embryo fibroblast (IMR90); e, transformed human embryo fibroblast (WI26-Va-4). (B) a, normal mouse fibroblast; b, transformed mouse fibroblast (FRUKTO); c, normal hamster fibroblast (HEC); d, transformed hamster fibroblast (SV-HE-Cl 2); e, HeLa cells. (C) a, 24-h regenerating liver; b, normal liver; c—f, ascites hepatoma cells: Yoshida sarcoma (c); AH7974 (d); AH 130 (e); AH 109A (f).

The nature of cytosol protein with M_r 88 000 remains to be elucidated. In the case of HeLa and W98-Va-C cells, about one fourth of total cell proteins was recovered in the cytosol fraction. Therefore, the cytosol protein with M_r 88 000 consits at least 1.5—2% of total cell proteins in these cells. The cytosol protein with M_r 88 000 cannot be detected in the cultured medium before the use for cell culture by SDS-polyacrylamide gel electrophoresis. The protein with M_r 88 000 seems to be cell-derived rather than serum-derived, although the experiment with amino acid label is required for the final conclusion as to the origin of the protein. The cytosol protein migrates faster than the cytosol phosphoprotein (M_r 96 000) associated with cell growth described by Kletzien et al. [8] (Hamaguchi, H. et al., unpublished results). Because the cytosol protein is present in normal cells, it also

seems to be different from a tumor-associated cytosol antigen with M_r 85 000 reported by Leung and Feldman [9]. Presence of the cytosol protein with M_r 88 000 in large amounts in a wide range of different tumor cells suggests that the protein is associated with certain growth characteristics of cells. Current efforts are directed at establishing the nature of the protein.

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