A PRECURSOR OF CYTOPLASMIC ACTIN IN CULTURED *DROSOPHILA* CELLS

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ABSTRACT

In continuous lines of cultured *Drosophila* cells two forms of cytoplasmic actin, designated II and III, are detected after a 25- to 120-min pulse label with [^{35}S]methionine. However, only one of these, actin II, accumulates in the cell.

With the use of a pulse-chase protocol and two-dimensional gel electrophoresis, it has been found that actin III is synthesized as a precursor of the more stable cytoplasmic actin II. The half-life of actin III was estimated to be ~50 min.

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KEY WORDS actin precursor · *Drosophila* · electrophoresis

In a recent study of myogenesis in primary cell cultures from *Drosophila melanogaster* embryos, Storti et al. (6) reported that three electrophoretically distinguishable forms of actin are synthesized. Two of the three forms, designated actins II and III, were also found to be synthesized in a continuous nonmuscle cell line originally established from *Drosophila* embryos by Schneider (5). These two actins were subsequently considered to be cytoplasmic actins. The third form, actin I, was produced only in myogenic and muscle cells. The criteria used for identifying these three proteins as actins included: a mol wt of 44,000 daltons, isoelectric points similar to that of actin purified from adult chick muscle, and a high affinity for DNase I immobilized on agarose. The isoelectric points of chicken actin and *Drosophila* actins I, II, and III were 5.72, 5.72, 5.77, and 5.84, respectively.

It was also observed (6) that when the Schneider line cells were allowed to incorporate [^{35}S]methionine into protein for 1 h, and the cell proteins were separated by two-dimensional gel electrophoresis, both actins II and III appeared as radioactive protein spots on autoradiographs. However, when these gels were stained for protein with Coomassie Blue, only actin II could be visualized, suggesting that actin III is a short-lived form which does not accumulate. This same result was obtained in studies on myogenic *Drosophila* cells (6), and similar observations were cited from studies on rat (2) and chick (6) cells. The authors conclude that the role of these unstable actins in the cell is unknown. In this paper, we show that *Drosophila* actin III is a kinetic precursor of the stable cytoplasmic actin II.

MATERIALS AND METHODS

Echalier's Kc *Drosophila* cell line (1) was grown at 25° ± 1°C in D-20 medium containing 15% fetal calf serum, at concentrations up to 10^6 cells/ml. For labeling studies, ~10^7 cells were collected by low-speed centrifugation and resuspended in 3 ml of D-20 medium lacking methionine, yeastolate, lactalbumin hydrolysate, and

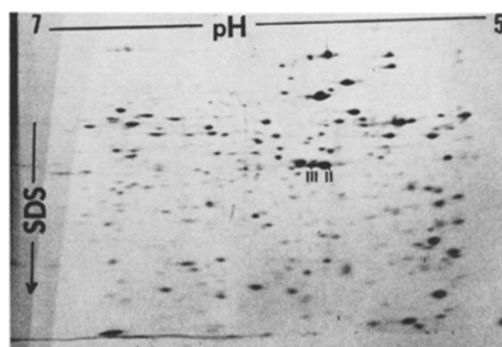

FIGURE 1 An autoradiograph of labeled *Drosophila* cell proteins separated by two-dimensional gel electrophoresis. Migration in the first isoelectric focusing dimension was from left to right. Migration in the second-dimension gel, containing SDS, was from top to bottom. Spots designated *III* and *II* represent the two cytoplasmic actins.

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serum. To each ml of suspension, 100 μCi of [^{35}S]methionine (New England Nuclear, Boston, Mass.) was added and incorporation was allowed to continue for 25 min at 25°C. At the end of the labeling period the cells were pelleted, washed twice with complete medium containing L-methionine, and finally resuspended in 10 ml of the same medium. At various intervals, aliquots of 10^6 cells were removed and prepared for two-dimensional polyacrylamide gel electrophoresis according to the method of O'Farrell (4), or for one-dimensional gel electrophoresis in 12.5% polyacrylamide slab gels containing 0.1% SDS (7).

After electrophoresis the gels were stained, destained, dried, and an autoradiograph was prepared. Exposure times were 4-6 d. In some experiments the autoradiograph was analyzed quantitatively by densitometric scanning in a Joyce-Loebl microdensitometer (Joyce Loebl, Gateshead-on-Tyne, England). Alternatively, quantitation of radioactivity in proteins was carried out by excising the gel fragment containing the protein spot of interest, using the autoradiograph as a map, and then dissolving the gel fragments overnight in 0.5 ml of 30% hydrogen peroxide at 50°C, and counting in Aquasol (New England Nuclear).

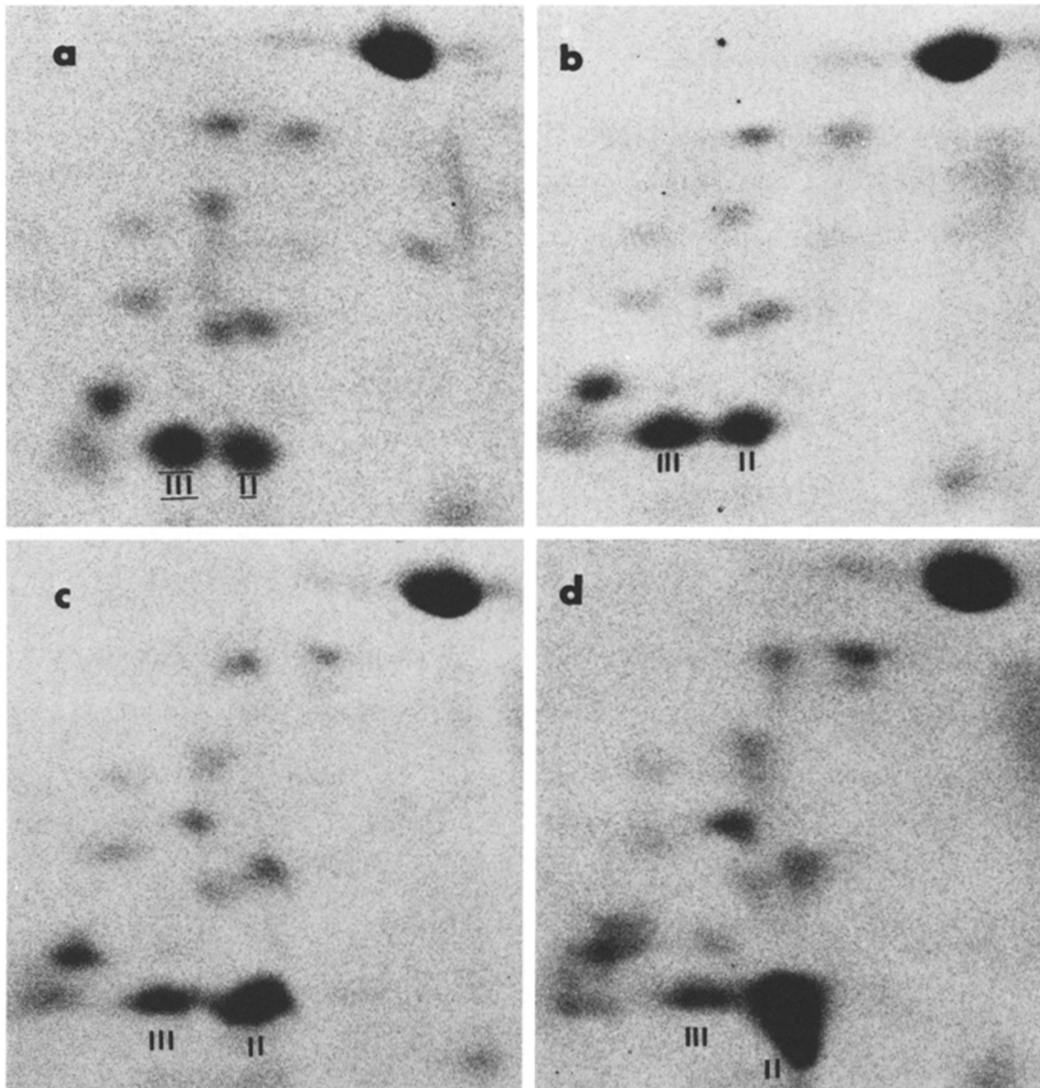

FIGURE 2 Autoradiograph of labeled proteins in the actin region during the chase period. Samples represent chases of (*a*) 0 min, (*b*) 15 min, (*c*) 40 min, and (*d*) 120 min. Actins are designated *III* and *II*.

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TABLE I
Specific Radioactivity of Drosophila Cell Proteins during a Chase Period

min of Chase	TCA-precipitable cpm/10^6 cells
0	21,200
15	22,590
40	22,047
120	20,165

TABLE II
Radioactive Labeling in Actins II and III Spots Excised from Two-Dimensional Gels

Chase time	Actin Form	cpm	Ratio of cpm in II/III
min			
0	III	1211	0.68
	II	823	
	II & III	2034	
15	III	882	0.84
	II	739	
	II & III	1621	
40	III	710	1.78
	II	1264	
	II & III	1974	
120	III	221	8.37
	II	1849	
	II & III	2070	

Time zero of the chase begins after a 25-min pulse label and several washes

RESULTS AND DISCUSSION

Fig. 1 is an autoradiograph of *Drosophila* total cell proteins labeled for 2 h with [^{35}S]methionine and subjected to two-dimensional electrophoresis. The labeled protein spots numbered *II* and *III* correspond to the cytoplasmic actins described by Storti et al. (6). Both actin forms bind specifically to DNase I immobilized on agarose, and actin II comigrates with the cytoplasmic actin isolated from chick brain (6). With the use of the pulse-chase protocol described in Materials and Methods, the metabolic relationship between actins II and III was investigated. Fig. 2 shows the autoradiographic pattern of labeled protein spots in the actin region of the gels during the chase period. The relative labeling intensity of actin III decreases with time, while the relative labeling intensity of actin II increases. We interpret this to represent a precursor-product relationship. Since the specific radioactivity of labeled protein remained fairly constant during the chase period (Table I), and since the total number of TCA-precipitable counts applied to each gel was the same, it was possible to estimate the rate of conversion by cutting out the radioactive gel spots, dissolving them, and counting. In Table II it can be seen that while the total number of counts in actin II and III remained fairly constant throughout the chase period, the ratio of counts in actin II/actin III increased from 0.68 at the start of the chase to 8.4 after 2 h of chase. The time required to convert 50% of the actin III counts present at time zero to actin II was ~50 min. In a final experiment the same samples seen in Fig. 2 were electrophoresed in one-dimensional slab gels containing 0.1% SDS (7). Here, proteins are separated solely on the basis of their molecular weight. The gel was dried, an autoradiograph was prepared, and the negative was then scanned in a densitometer. When peak heights or areas were quantitated (data not shown), there was no difference in the relative abundance of radioactivity in the actin region as a function of chase time compared to other proteins. This again supports the notion that the disappearance of actin III is not due to its degradation but rather to its conversion to the more stable form. One technical point deserves attention. It is clear from Fig. 2*a* that at the start of the chase period there is already a significant amount of actin II present. This may represent conversion occurring during the pulse period and during the subsequent washing step. Finally, we have observed the conversion of actin III to actin II in three other *Drosophila* cell lines: Schneider's lines 1, 2, and 3 (5).

The conversion of actin III to actin II involves a shift of isoelectric point from 5.84 to 5.77, without any apparent change in molecular weight. We now have preliminary evidence that this conversion involves a post-translational acetylation reaction, as had been suggested earlier from studies on vertebrate cells (2).

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Note Added in Proof: Fyberg and Donady (1979, *Dev. Biol.* **68**:487–502) have shown that actin III is unstable in primary embryonic culture cells from *D. melanogaster*.

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