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ECDYSTERONE INDUCTION OF ACTIN SYNTHESIS AND POLYMERIZATION IN A *DROSOPHILA MELANOGASTER* CULTURED CELL LINE

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Actin pools have been evaluated in *Drosophila melanogaster* Kc 0 % cells, through an actin assay based on differential inhibition of DNase I by globular (G) and filamentous (F) actin. Total actin represents about 4 % of total proteins and 54 % is G-actin. In ecdysterone treated cells (0.1 μM), the total actin content increases up to 9 % of total proteins after 3 days of treatment. Ecdysterone induces increase of G-actin as well as F-actin. Increase of both actins, detectable after only 24 hrs of treatment, is roughly parallel during the first two days of treatment. For longer hormonal treatment, actin polymerization is more important than accumulation of G-actin. Indirect immunofluorescence microscopy with antibodies to exogeneous DNase I suggests that actin is widely distributed in the whole cytoplasm before and after ecdysterone treatment. These results suggest that ecdysterone induces actin synthesis and polymerization in *Drosophila melanogaster* cells.

Drosophila melanogaster cultured cell lines are extensively used as a model to study the action of ecdysteroid hormones. Many transformations of Kc 0 % cells under ecdysterone treatment have been previously reported (1-3). Essentially Kc 0 % cells undergo dramatic morphological modifications, including pseudopod formation and aggregation of the cells (3). Two enzymatic activities are greatly enhanced after hormonal treatment : eserine-sensitive acetyl cholinesterase (E.C.3.1.1.7.) and β galactosidase (E.C.3.2.1.2.3.). The protein pattern, as analysed by two-dimension electrophoresis (4) is specifically modified, less than three per cent of the detected spots are modified : three polypeptides are newly synthetized, two are repressed and six are increased. Two spots identified as cytoplasmic actins II and III (5) are more intensively labeled in treated cells suggesting the induction of actin synthesis.

Actins are a class of proteins involved in many cellular functions such as cytoskeletal support, cytokinesis, pseudopod formation and cell motility. Indeed, there have been numerous reports on changes in the synthesis and state of actin which correlate with modifications in cell shape and function (6-10). There is evidence that actin is in two forms in non-muscle cells : filamentous actin (F) and globular actin (G) (11). Moreover, organisation of microfilaments in non-muscle cells may be based on a controlled polymerization and depolymerization of actin. The method based upon differential inhibition of DNase I by actin (11)

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allows G-actin and F-actin pool evaluation. This technique was used to measure actin pools in extracts of Kc 0 % cells and to assess ecdysterone action on *Drosophila* cell actin.

The present work demonstrates that the previously described increase of (^{35}S) methionine incorporation into actin after ecdysterone treatment (4) parallels accumulation of actin in the cells. Moreover, this accumulation is concomittant with an increase of actin polymerization. Indirect immunofluorescence used to detect actin in whole fixed cells, shows that actin is diffusely distributed in the whole cytoplasm of untreated and treated Kc 0 % cells, including the pseudopod-like formations of ecdysterone treated cells.

MATERIALS AND METHODS

DNase I (Bovine pancreas type III), double stranded DNA (type I, from calf thymus) and pure chicken skeletal muscle G-actin were purchased from Sigma. All other reagents were obtained from Merck.

Cell cultures

The cells used for the present work were derived from the diploid cell line Kc established from embryos of *Drosophila melanogaster* by Echalier and Ohanessian (12) and were adapted to grow in medium without calf serum (0 %). The culture conditions have been described previously (12). Ecdysterone (SIMES-Milan) was added to the culture at a final concentration of 0.1 μM.

Cell extracts and DNase inhibitor activity measurement

Cell extracts were prepared by scraping the cells from the Falcon culture flasks, washing the pellet twice in 10 mM Tris pH 8, 75 mM NaCl, 24 mM EDTA, 3 mM $CaCl_2$, 2 mM $MgCl_2$ and homogenizing the cells by vortexing in lysis buffer : 5 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM $MgCl_2$, 0.1 mM dithiotreitol, 0.2 mM ATP, 0.5 % Triton X-100. About 10^7 cells were treated in 0.6 ml of lysis buffer. Aliquots were taken for measurement of DNase inhibitor activity according to Blikstad et al. (11). Another aliquot was diluted (1 : 1, v : v) in Tris 20 mM pH 7.5, guanidine hydrochloride 1.5 M, Na^+ acetate 1 M, $CaCl_2$ 1 mM, ATP 1 mM, vortexed, incubated at 0° for at least 15 minutes, and used to quantify total actin.

Protein concentrations were determined as described by Lowry et al. (13), using bovine serum albumin as a standard.

Indirect immunofluorescence staining of fixed cells

Cells grown on glass coverslips were fixed with 4 % formaldehyde in phosphate buffered saline (PBS : Na_2HPO_4 10 mM, KH_2PO_4 1.8 mM, NaCl 170 mM, KCl 3.3 mM) for 60 minutes at room temperature. An appropriate dilution of DNase I (I mg/ml) in PBS was applied to the cells for 30 minutes at 37°. Coverslips were rinsed with PBS once more, and incubated with antiserum to DNase I in a humid atmosphere at 37° C for 45 minutes. After another wash, coverslips were covered with goat-antirabbit FITC*-labeled IgG for 45 minutes at 37°. Preparations were examined by fluorescence microscopy. If DNase I treatment and/or anti-DNase I were omitted, the characteristic positive immunofluorescence staining was not seen.

RESULTS

1. *Determination of actin pools in Kc 0 % Drosophila melanogaster cells :*

The actin pools were assayed by the DNase I inhibition test. This technique is based on the different rate of interaction between DNase I and the

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*Abbreviation : F.I.T.C. fluorescein isothiocyanate

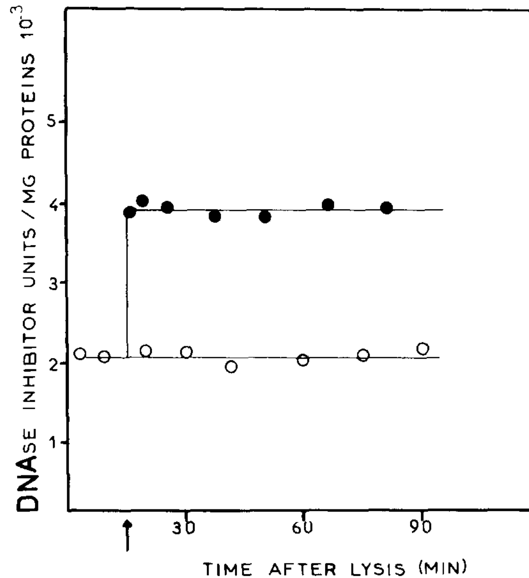

Figure 1. Determinations of actin pools in cell extracts by the DNase inhibition assay.
Cells were grown and lysed as described in Materials and Methods. DNase inhibitor activity in the extracts was determined at different times after lysis (open symbols). At time indicated (arrow) a part of each sample was treated with guanidine hydrochloride and the total amount of actin was determined (closed symbols).

two forms of actin, so that inhibition by monomeric actin is virtually complete before appreciable inhibition from the filamentous form is detected.

The amount of unpolymerized actin was measured at different times after lysis and corresponds to the direct inhibitor activity of the sample. The total amount of actin is determined in the same way after depolymerization of filaments with guanidine-HCl.

The distribution of actin between the two pools was relatively stable for at least 90 minutes at 0° C after the lysis of untreated cells (fig. 1). The result is similar for ecdysterone treated cells (data not shown). Total actin represents about 4 % of total proteins in untreated Kc 0 % cells, and about 54 % are in unpolymerized form.

2. *Modification of actin pools in Kc 0 % cells after ecdysterone treatment :*

The results illustrated on figure 2 show that all the actin pools increase after hormonal treatment of the cells.

Increase of total actin was detectable after only one day of treatment while no morphological transformation of the cells had occured. Then it was linear for two days and reached a maximum between the third and fourth day of treatment. At this time, total actin represented about 9 % of total proteins and therefore was more than twice as high as that in untreated cells.

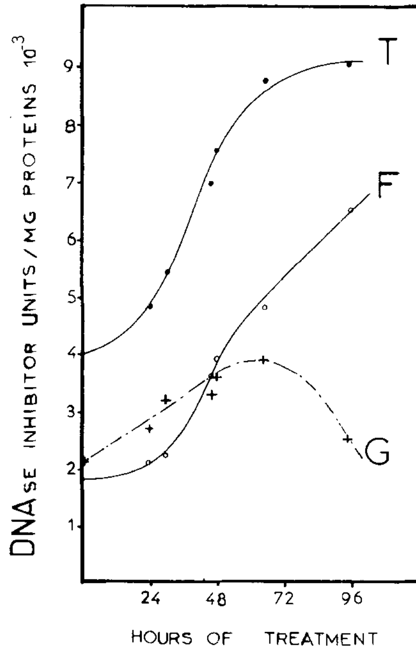

Figure 2. DNase I inhibitor activity in extracts of Kc 0 % cells. Extracts of untreated and ecdysterone treated cells were prepared and assayed for inhibition of DNase I as in section 2. Extracts were kept on ice throughout the experiment. Results are expressed as the DNase inhibitor activity measured directly (+, o, •, representing respectively unpolymerized G-, polymerized F- and total T- actin). In our conditions of experiments 1 unit inhibitor activity is defined as the amount giving 1 % inhibition of the standard amount of DNase I.
Purified chick muscle actin at 0.1 mg/ml corresponds roughly to inhibitor at 1000 units/ml.

G-actin pool increased in the same way as total actin during the first two days of ecdysterone treatment. The ratio of G-actin to total actin was quite stable during this period, but it decreased after two days of treatment. This was correlated with the rapid increase of F-actin in treated cells. So, the F-actin fraction represented more than 70 % of total actin after four days of ecdysterone treatment and its basal level in untreated cell was multiplied by about 3.6.

3. *Immunofluorescence detection of actin :*

Indirect immunofluorescence for actin visualisation was used to reveal changes in the intracellular distribution of actin in ecdysterone treated cells. This technique is based upon detection by indirect immunofluorescence microscopy of exogeneous DNase I binding to actin on fixed cells.

Untreated Kc 0 % cells were rounded in shape. Indirect immunofluorescence studies show a diffuse coloration of the whole cells (fig. 3 A-C), with a more intense fluorescence surrounding the cells.

Two days ecdysterone-treated cells, exhibit drastic morphological transformation especially flattening, elongation, pseudopod extension and finally

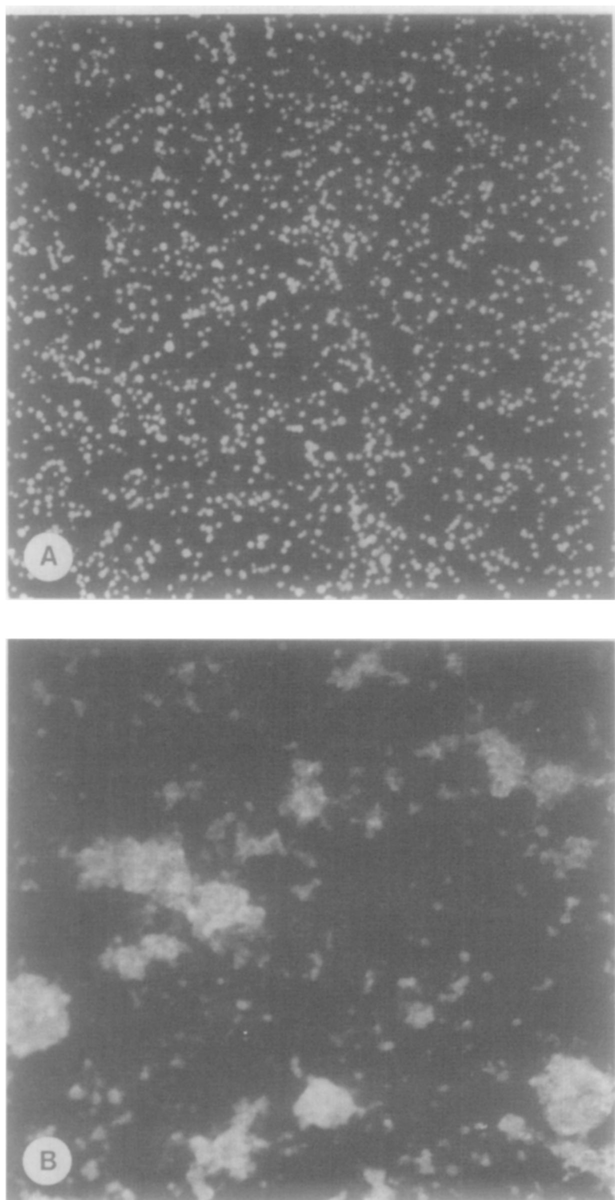

Figure 3. Indirect immunofluorescence studies using DNase I and anti-DNase I. Cells grown on coverslips are fixed and subsequently treated with DNase I, anti-DNase I as described in Materials and Methods. A-C untreated cells, B-D ecdysterone cells treated for 48 hr, A-B magnification 150, C-D magnification 600.

aggregation (fig. 3 B-D). Fluorescence is also distributed all over the cytoplasm including the pseudopod-like formations and the aggregated cells. This technique does not allow the detection of the presence of actin in filamentous structure either in untreated or in ecdysterone treated cells at least up to 72 hrs of treatment, but it indicates that actin is present in the whole cells, and also in the numerous extensions developed by treated cells.

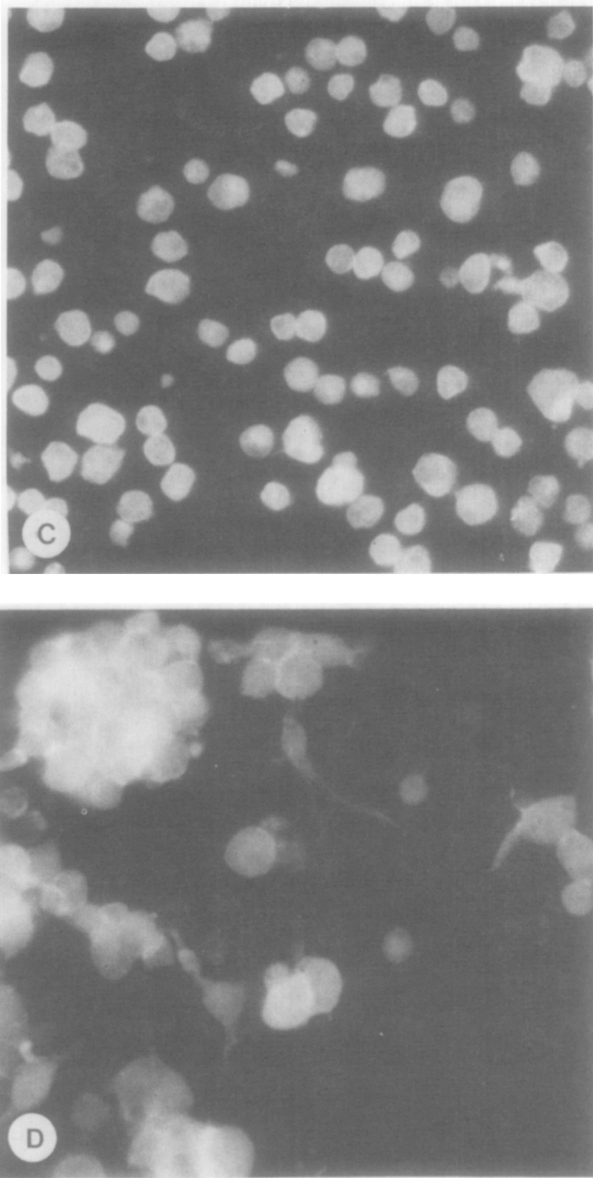

Figure Continued

DISCUSSION

Actin is a major component of eucaryotic cells and is involved in the maintenance of cell shape, movement and in other important cellular functions (6-10). Three electrophoretically distinguishable forms of actin have been reported in *Drosophila melanogaster* (14). Two of the three forms, designated as actin II and III were also found to be synthetized in a continuous non-muscle cell line of *Drosophila* (5). Kc 0 % cells of *Drosophila melanogaster* undergo important

morphological and biochemical changes when they are treated with ecdysterone (1-3). We have recently reported (4) that ecdysterone induces a few specific modifications in the electrophoretic pattern of Kc 0 % cells. The synthesis of only 11 polypeptides was affected upon more than 400 detectable spots. Three polypeptides were newly induced, two were repressed and six had an increased rate of synthesis. Two of these were identified as actins II and III, suggesting that actin synthesis was enhanced after hormone treatment. Using a specific assay for actin (11) which can be used on crude cellular extracts and based on differential inhibition of DNase I by monomeric (G) and filamentous (F) actin, we report here measurements of actin pools before and after ecdysterone treatment in Kc 0 % *Drosophila melanogaster* cells. The results show that total actin represent about 4 % of total proteins in untreated cells. Actin accumulates during ecdysterone treatment and reaches about 9 % of total proteins in 96 hr treated cells. Moreover the proportion of F-actin from untreated cells is about 46 % of total actin and increases during ecdysterone treatment up to 72 % of total actin in 96 hr treated cells. Thus ecdysterone induces some transformation of Kc 0 % cells which involves actin synthesis, accumulation and polymerization.

It is tempting to correlate these variations of actin with the dramatic morphological changes and aggregation of Kc 0 % cells induced by ecdysterone. A similar increase in F-actin measured by DNase I inhibition assay has been previously reported. Comparison of actin pools of mouse embryonal carcinoma and of fibroblast-like cells derived from them (9) suggests that a reorganisation of actin might be a marker of differentiation. Recently (15) it has been shown that platelet activation leads to a very rapid mobilisation of DNase-available actin. In these two cases, as in our study, these changes correlate with important cell shape modification and aggregation. However our work is the first report, where actin pools were measured, that such modifications of actin synthesis and polymerization can be induced after a steroid treatment of cultured cells. Indirect immunofluorescence studies show a diffuse actin distribution in the whole cell, including pseudopods, whether treated or not by ecdysterone, and no filamentous organisation of actin in these cells was detected. However, diffused staining with actin antibodies of motile cells or motile parts of cells have been recently reported (16).

From the present work and from previous data (4) it can be concluded that ecdysterone induces synthesis, accumulation and polymerization of actin in Kc 0 % cells. The question now arises whether this increase of actin synthesis after ecdysterone treatment involves either the synthesis of new actin mRNAs or translational control.

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