Stimulation of Cytoplasmic Actin Gene Transcription and Translation in Cultured *Drosophila* Cells by Ecdysterone*

Michael P. Vitek†, Christine M. Morganelli, and Edward M. Berger§

From the †Department of Biochemistry, Dartmouth Medical School and §Department of Biology, Dartmouth College, Hanover, New Hampshire 03755

(Received for publication, June 27, 1983)

When cultured *Drosophila* line S3 cells are incubated in the presence of the steroid hormone, ecdysterone, they flatten, elongate, and become motile. We show here that accompanying this morphological transformation there is a 3-fold increase in the rate of actin synthesis and a 2-fold increase in actin content. These increases, in turn, are primarily based on a 9-fold increase in the level of mRNA transcribed from the cytoplasmic actin *A3* gene. We also find that during hormone treatment the cells go into proliferative arrest, accumulating in the G2 phase of the cell cycle. During this period the level of histone mRNA in the cells decreases significantly.

The cytoskeleton participates in both the maintenance of cell shape and the dynamics of cell motility. Two major structural elements involved in these functions are tubulin-containing microtubules and actin-containing microfilaments (1, 2). Advances in protein purification, immunology, and fluorescence microscopy have provided new and important information on the molecular composition of these structural elements and on their three-dimensional organization within the cell. Very little is known, however, about the molecular events which underlie abrupt changes in cell morphology or cell motility.

Recent studies employing high resolution two-dimensional gel electrophoresis have uncovered the existence of structural heterogeneity among actins and tubulins (3–8). Gene-cloning studies indicate that much of this heterogeneity has a genetic basis (6, 7, 9–18). In the case of *Drosophila*, for example, the actin gene family is known to contain at least six unlinked members, while the α and β tubulin gene families contain some four unlinked members each. While evidence for both developmental and tissue-specific expression of individual members of the actin and tubulin gene families has been obtained (6, 7, 13, 14, 16, 18–20), the functional significance of this specificity is largely unknown.

Several years ago Courgeon (21) first showed that when *Drosophila* tissue culture cells are exposed to the molting hormone ecdysterone, they rapidly undergo cell shape changes, acquire motility, and aggregate. Subsequent experiments demonstrated the importance of microtubule and microfilament function in these morphogenetic changes (22–25). We have continued this line of investigation by examining the expression of the actin gene family members in the S3 cell line following hormone treatment. We show here that with the observed morphological transformation, there is a marked increase in the synthesis and accumulation of cytoplasmic actin. These increases, in turn, are based on the preferential induction of one of two cytoplasmic actin genes designated *A3*.

**MATERIALS AND METHODS**

**Cell Culture**—Schneider line 3 (S3) cells were maintained in Schneider's *Drosophila* medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (Gibco). Cells were grown in 150-cm² Corning flasks, at 25 °C, to a density of 4 to 5 × 10⁶ cells/ml. Ecdysterone (Calbiochem) was maintained as a 1 mg/ml aqueous stock and stored at −20 °C. Appropriate cultures were treated by the direct addition of hormone to a final concentration of 1 μM or with an equal volume of solvent (sterile water) for the times indicated.

**Plasmid Purification and Radiolabeling**—Plasmid DNA from selected clones were prepared using the modified cleared lysate procedure described in Ireland et al. (26). Purified plasmid DNAs were radiolabeled with [³²P]dCTP by the nick translation method of Rigby et al. (27). Labeled DNAs were recovered by three successive ethanol precipitations according to Schleif and Wensink (28).

**RNA Extraction and Purification**—Whole cell RNA was prepared from S3 cells using the guanidine HCl, cesium chloride centrifugation method. RNA was recovered by resuspending less than 3 × 10⁶ cells in 8 ml of 0.6 M guanidine HCl, 1 mM dithiothreitol, 0.1 mM Na acetate, pH 5.2, layering this mixture above a 3 M cushion of 7.2 M cesium chloride, 100 mM EDTA, pH 7.0, and centrifuging at 80,000 × g for 30 h at 25 °C in a Beckman J45.1 type rotor. The supernatant was discarded and the clear RNA pellet was dissolved in 0.3 M Na acetate, pH 7.0, phenol extracted, and ethanol precipitated.

**RNA Dot Blot and Northern Blot Hybridizations**—10 μg of undenatured RNA was dot blotted onto nitrocellulose according to the method of Thomas (29). For Northern blots, 10 μg of RNA was denatured with glyoxal and electrophoresed on 2% agarose gels in 10 mM phosphate buffer pH 6.5, exactly as described by Baringa et al. (30). Radiolabeled DNAs were hybridized to blots as described by Baringa et al. (30) in a buffer containing 10% dextran sulfate, 50 mM Tris HCl, pH 7.4, 1 M NaCl, 100 μg/ml of sonicated salmon sperm DNA, 0.1% NaPP₃, pH 7.0, 50% formamide, 10 × Denhardt's solution (31), and 0.1% SDS. The blots were washed for 15 min in each of the following buffers: twice in 2 × SSC/0.1% SDS; twice in 0.1 × SSC/0.1% SDS, and twice at 55 °C in 0.1 × SSC/0.1% SDS. Air-dried blots were autoradiographed at −70 °C with intensifying screens. In quantitative experiments, dots were excised with a No. 5 cork borer, placed in glass vials with Econofluor (New England Nuclear), and analyzed by scintillation counting.

**Protein Labeling**—Cells were concentrated 10-fold in Schneider's medium lacking methionine and incubated for 60 min in the presence of 100 μCi/ml of [³⁵S]methionine (Amersham Corp.). Labeled proteins were extracted (32) and electrophoresed in a 17.5% acrylamide/0.1% SDS gel as described (33). Two-dimensional gel electrophoresis was carried out as described previously (34). Dried gels were autoradiographed at −70 °C with intensifying screens. In quantitative experiments, dots were excised with a No. 5 cork borer, placed in glass vials with Econofluor (New England Nuclear), and analyzed by scintillation counting.

*This work was supported in part by Grant GM22866 from the National Institutes of Health. The cytofluorograph used was a gift of the Fannie E. Rippel Foundation and is partly supported by the core grant of the Norris Cotton Cancer Center (CA31981). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom all correspondence should be addressed.

1 The abbreviation used is: SDS, sodium dodecyl sulfate.
Drosophila Actin Genes

We first examined the pattern of protein synthesis in S3 cells incubated for 12 h in either the presence or absence of ecdysterone. Cells were allowed to incorporate \[^{35}S\]methionine for an hour, and the extracted proteins were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. A visual comparison of the autoradiographs (Fig. 2) revealed several striking differences between control and hormone-treated cells. In the ecdysterone-treated cells, there was a marked increase in the relative labeling intensity of a 43-kDa polypeptide. We have previously identified this band as actin in both control and hormone-treated cells on the basis of its affinity to immobilized DNase and its comigration with purified chick brain cytoplasmic actin (23). In high resolution two-dimensional gels (Fig. 3) the labeled actin band from both control and hormone-treated cells is resolved into two spots, differing only in isoelectric point (3-5, 8). One of these, designated actin III, with an isoelectric point of 5.84 is the metabolic precursor of the other, actin II (3) with an isoelectric point of 5.77; processing appears to involve an acetylation at, or near, the NH\(_2\) terminus (18, 39, 40). Both actin II and actin III are selectively retained on an affinity column containing immobilized DNase.

The two low molecular weight polypeptides designated hsp 23 and hsp 26, in Fig. 2, are also synthesized at elevated rates in hormone-treated cells. Their induction has been described in detail previously (26, 34, 41) and is apparent in two-dimensional gels (Fig. 3). The band designated T (Fig. 2) contains the \(\alpha\) and \(\beta\)-tubulin subunits (24). There is no obvious change in the relative rate of tubulin synthesis in

**RESULTS**

**Actin Synthesis and Content**—During the first 12 h of continuous exposure to 1 \(\mu\)M ecdysterone, Drosophila line S3 cells develop an extensive array of moderately birefringent filopodia (Fig. 1). Time lapse cinematography studies indicate that over 99% of the cells display this response synchronously. Simultaneously the cells go into proliferative arrest and accumulate in the G2 phase of the cell division cycle (Ref. 36 and data not shown). DNA synthesis steadily declines during this period (32). Label incorporation studies (37) indicate that the rates of both RNA and protein synthesis remain essentially unchanged. During the next 20 h, the cells become motile and aggregate into randomly spaced foci on the substrate, in a fashion quite similar to the Kc cell line (21, 24, 25, 35, 38).

**Photographs of Cells**—Cells were observed under an inverted Nikon phase contrast photomicroscope and photographed at \(x\) 400 magnification.

**Fig. 1.** Line S3 cells incubated in the absence (A) or presence (B) of 1 \(\mu\)M ecdysterone for 24 h. Bar is 10 \(\mu\)m.

3 M. P. Vitek, C. M. Morganelli, and E. M. Berger, unpublished results.

**Fig. 2.** Sodium dodecyl sulfate, 17% polyacrylamide gel electrophoretic analysis of \[^{35}S\]methionine-labeled polypeptides extracted for S3 cells incubated in the absence (a) or presence of ecdysterone at \(10^{-6}\) M (b), \(10^{-8}\) M (c), \(10^{-10}\) M (d) for 12 h prior to a 1-h labeling period. The polypeptides labeled correspond to tubulin (T), actin (A), heat shock protein 26 (26), and heat shock protein 23 (23). Each lane was loaded with 50,000 cpm. The autoradiograph was exposed for 48 h.
FIG. 3. Two-dimensional gel electrophoresis and autoradiography of \[^{35}\text{S}\]methionine-labeled polypeptides synthesized in (a) control S3 cells and (b) S3 cells incubated in the presence of \(10^{-8}\) M ecdysterone, as described in Fig. 2. The induced polypeptides designated 23 and 26 in Fig. 2 are now resolved into four components designated 22, 23, 26, and 27. The two labeled spots above A are the cytoplasmic actin components, actin H (pI = 5.84) and actin I (pI = 5.77). IEF, isoelectric focusing.

FIG. 4. The height of the actin peak measured by densitometric scanning of an autoradiograph in which varying amounts of \[^{35}\text{S}\]methionine-labeled S3 cell protein were analyzed. The autoradiograph was exposed for 48 h.

Response to ecdysterone, confirming earlier studies (24).

The same experimental protocol was then used in a time course study. In these experiments the autoradiographs were also analyzed quantitatively, by densitometric scanning, after first establishing standard curves (Fig. 4) to ensure that the quantitation was being assessed within the linear range of absorbance. The results of replicate experiments, one of which is summarized in Figs. 5B and 6, show that the relative stimulation of actin synthesis is already evident by 4 h and that by 12 h relative actin synthesis had increased by nearly a factor of 5 over control. Between 12 and 18 h the rate decreased substantially and remained at that level until 24 h. In parallel, we analyzed the relative amount of accumulated actin in the cells during this period by scanning the Coomassie blue stained gels. Again, a standard curve was established to verify that our scans were within the linear absorbance range. These results (Figs. 5A and 6) indicate that between 4 and 18 h, relative actin content doubles and subsequently plateaus.

Actin mRNA Content and Synthesis—The increase in actin synthesis and content could be the result of transcriptional control, post-transcriptional control, or some combination of the two. We first examined the level of actin mRNA by Northern blot analysis. There are 6 unlinked actin genes in Drosophila (4, 17). While their protein-coding regions are homologous and cross-hybridize (11), the flanking 3' transcribed but untranslated regions show sequence divergence and do not cross-hybridize (11, 16, 18, 42). We obtained the six gene-specific 3' actin clones from Dr. E. Fyrberg, Biology Dept., Johns Hopkins University (described in Ref. 40 and
used them as probes to analyze the composition of total *Drosophila* cell RNA. We chose not to first select poly(A') sequences, because there is evidence that some proportion of the actin mRNA pool is nonadenylated (20).

Four of the gene-specific actin probes (A1, A4, A5, and A6) failed to hybridize to line S3 cell RNA extracted from either control or hormone-treated cells. At least three of these genes are known to encode muscle-specific actins (11, 16, 18). Both the actin A2 and A3 gene-specific probes hybridized to control cell RNA to approximately equal levels (Fig. 7). The A2 gene probe hybridized to an RNA band slightly larger than the 1631-nucleotide marker, while the A3 gene-specific probe hybridized to an RNA slightly smaller than the 1631-nucleotide marker. In lanes containing RNA from hormone-treated cells, there was a marked and progressive increase in the level of actin gene A3 transcripts during the first 12 h and then a plateau. A much less pronounced increase in the level of actin gene A2 transcript was observed. To exclude the possibility that the increased actin mRNA levels were merely a consequence of an increase in the relative abundance of all mRNAs, we hybridized two of the RNA samples with a DNA probe containing the structural gene for the heat shock protein, hsp 83. The relative rate of its synthesis in S3 cells remains unaffected by ecdysterone treatment (34). The relative abundance of hsp 83 transcripts in control cells and in cell incubated with hormone for a day were approximately equivalent (Fig. 8).

In order to quantitate the level of actin mRNA, a dot blot procedure was used. A constant amount of total cell RNA was immobilized on nitrocellulose paper and challenged with an excess of 32P-labeled nick translated A2-3' or A3-3' probe, adjusted to the same specific radioactivity. Following hybridization and extensive washing the radioactive spots were excised and counted. Several additional filters were prepared and hybridized with nick translated probes containing the coding region actin (actin A2 clone), the genes for heat shock protein, hsp 23 (JIPR3 clone described in Ref. 26 and 34) and hsp 70, and the *Drosophila* histone gene cluster (clone Dm 500 described in Ref. 43). The results of these experiments are summarized in Table I. As predicted, the levels of actin A2 and actin A3 transcripts were approximately equal in control cells. Over the course of 12 h of ecdysterone treatment, the level of actin A2 mRNA more than doubled and then declined slightly during the following 12-h period. During this period, the level of actin A3 mRNA increased 8- to 9-fold and then declined slightly. The relative level of total actin mRNA, determined by using the actin coding region probe (A2), rose to an intermediate level. In contrast, the relative level of histone mRNA rose only slightly during the first 12 h, but then fell dramatically, reaching a level less than 50% of the control by 24 h. This decline parallels the onset of proliferative arrest. The dramatic increase in the level of hsp 23 transcripts seen confirms previous studies (26, 34). The levels of hsp 70 transcripts remained at the limits of resolution.

**DISCUSSION**

*Drosophila* cell lines have become useful models for investigating the molecular mechanism of steroid hormone action.

---

**TABLE I**

<table>
<thead>
<tr>
<th>Designation of probe</th>
<th>mRNA product</th>
<th>Corrected counts per min bound to RNA from cells incubated in ecdysterone for the hours indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2-3'</td>
<td>Actin A2</td>
<td>20 40 49 45 37</td>
</tr>
<tr>
<td>A3-3'</td>
<td>Actin A3</td>
<td>16 24 51 127 142 124</td>
</tr>
<tr>
<td>A2</td>
<td>Actin</td>
<td>103 175 200 464</td>
</tr>
<tr>
<td>Dm500</td>
<td>Histones</td>
<td>1121 1305 1314 1306 1036 537</td>
</tr>
<tr>
<td>B8</td>
<td>hsp 70</td>
<td>5 8 0 3 4 7</td>
</tr>
<tr>
<td>JIPR3</td>
<td>hsp 23</td>
<td>36 173 348 464 627 895</td>
</tr>
</tbody>
</table>

**FIG. 7.** Northern blots of total RNA from cells treated with 1 μM ecdysterone for 0 h (lanes B and I), 4 h (lanes C and J), and 8 h (lanes D and K), 12 h (lanes E and L), 18 h (lanes F and M) or 24 h (lanes G and N) probes with nick translated A2-3' gene DNA (lanes A-G) or A3-3' gene DNA (lanes H–N). Denatured pBR 322 restriction fragment size markers of lengths indicated on the left are shown in lanes A and H.

**FIG. 8.** Northern blot analysis of total RNA from cells incubated in the absence (a) or presence (b) of 10^-7 M ecdysterone for 24 h. The hybridization probe was a pBR322 clone containing the *Drosophila* heat shock protein 83 (hsp 83) whose expression is constitutive in cell lines and unaffected by ecdysterone (31).
(23–26, 32, 34, 37, 38, 41, 44–47). Several hormone-inducible proteins have been identified, and, in two cases (26, 34, 46), the hormone-responsive genes have been isolated by molecular cloning (46, 48–50). In the case of the small heat shock protein genes an analogous induction has been identified, in vivo, during oogenesis and imaginal disc differentiation (26, 51–53). The results reported here suggest that the actin A3 gene of Drosophila is also cytoskeleton inducible in S3 cells, although we have not ruled out the possibility of regulation also acting at the level of mRNA stability.

An interesting ambiguity in the results involves the lack of correspondence between measured levels of actin mRNA and the relative rate of actin synthesis, during the period 12–24 h after hormone addition. While actin mRNA levels remain quite high, the rate of actin synthesis clearly declines. One possible explanation involves some form of translational control that is exerted as the level of monomeric actin increases. Indeed, Farmer et al. (64) have shown that following suspension of anchorage-dependent mouse fibroblast 3T3 cells, there is a marked and preferential inhibition of actin synthesis, but no change in the level of actin mRNA. A comparable feedback mechanism, involving accumulated subunits, may be responsible for the decreased rate of tubulin synthesis seen in several cell types (55, 56).

The preferential expression of individual actin genes has been observed in developing Drosophila embryos (20) and during larval to adult development (16, 18). However, in both cases the specific induction apparently involves muscle-type actin genes. The preferential utilization of one of the pair of cytoplasmic actin genes is, to our knowledge, a novel observation. We suspect that it reflects some important functional difference between the actin A2 and A3 gene products, associated with the acquisition of motility. A similar explanation may account for the developmental regulation of actin genes in sea urchin (57) and Dictyostelium (58) development.

Finally, we wish to emphasize the striking similarities between the cytoskeleton responsiveness of line S3 cells and imaginal discs. In both systems cytodifferentiation activity is high and is localized primarily within the nucleus (26, 58, 60). In response to hormone both cell types go into proliferative arrest in the G2 phase of the cell cycle (21, 32, 37, 39, 62). We have shown here that in S3 cells proliferative arrest is accompanied by a marked decrease in histone mRNA content. At the molecular level, both S3 cells and imaginal discs show the induction of small heat shock protein gene transcription and translation (26, 51), and the composition of cell surface glycoprotein becomes dramatically altered (47, 62).

At the morphological level, both S3 cells and imaginal disc cells change shape and become motile. In the tissue culture line this leads to aggregation, while in imaginal discs evagination occurs. Evagination results primarily from cell rearrangement, involving coordinated cell movement over short distances (63, 64). Our findings lead us to predict that imaginal disc evagination may be preceded by a burst of actin synthesis resulting from the enhanced and preferential expression of the cytoplasmic actin A3 gene.

Acknowledgments—We are extremely grateful to Dr. Eric Fyrberg for providing us with the six-gene-specific actin clones and to Dr. Ken Burtis for providing us with Drosophila histone clone. We are also grateful to Dr. Michael Young and the members of his lab for teaching us filter hybridization techniques.

Addendum—During the review of this work, Couderc et al. (65) found that the level of A2 gene transcripts increased in Kc cells following treatment with ecdysone.

REFERENCES

Drosophila Actin Genes

Stimulation of cytoplasmic actin gene transcription and translation in cultured Drosophila cells by ecdysterone.
M P Vitek, C M Morganelli and E M Berger


Access the most updated version of this article at [http://www.jbc.org/content/259/3/1738](http://www.jbc.org/content/259/3/1738)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/259/3/1738.full.html#ref-list-1) to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/259/3/1738.full.html#ref-list-1](http://www.jbc.org/content/259/3/1738.full.html#ref-list-1)