20-Hydroxyecdysone regulates cytoplasmic actin gene expression in Drosophila cultured cells

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ABSTRACT
The steroid hormone 20-hydroxyecdysone (20-OHE) induces, in Kc cultured Drosophila melanogaster cells, important morphological transformations and specific changes of enzymatic activities and of protein synthesis. These changes are accompanied by an increase of synthesis and an accumulation of actin. Specific probes were used to reveal transcripts of each actin gene in mRNA populations isolated from cells at various times of 20-OHE treatment. Only the two cytoplasmic actin genes 5C and 42A are expressed in Kc cells and the hormone induces the accumulation of transcripts of these two genes. We have also taken advantage of S1 mapping and extension procedures to identify the 5' ends of the actin mRNAs from these two genes and to compare their respective levels of expression. The 5C gene is more expressed than the 42A one in untreated and in hormone treated cells. The 5C gene encodes three RNAs that differ in their 3' end. The two genes are interrupted by an intervening sequence immediately upstream of ATG initiation codon but not at the same position. The transcription rate for the two genes is increased up to five fold upon 20-OHE treatment, demonstrating a direct effect of the steroid hormone at the transcriptional level for these genes.

INTRODUCTION
The contractile protein actin, found in all eukaryotic cells, has been implicated in a variety of developmental and cellular processes (for review see ref.1). Moreover, another characteristic of actin is that the genes encoding this protein appear to be members of a multigene family. Temporal and spatial expression of actin gene family members is well documented in metazoans. Two classes of genes were defined according to whether they encode muscle or cytoplasmic isoforms (2). In Drosophila melanogaster there are two cytосkeletal and four muscle actin genes (3).

20-Hydroxyecdysone (20-OHE) induces dramatic morphological changes in Kc Drosophila cultured cells. Subsequently cells acquire motility and then aggregate (4). During this differentiation process there is a sharp increase in the synthesis and accumulated amounts of actin as detected by two-dimensional electrophoresis (5) and by the DNAse I inhibition assay (6). This increase of actin synthesis apparently results from an increased quantity of actin mRNA as assayed by in vitro translation of Kc cells mRNA. In a search of 20-OHE regulated sequences (7) by differential screening of a Drosophila genomic library we have isolated a 20-OHE inducible gene that we have identified as the 5C actin gene. We have used this probe to show that 20-OHE induces an increase of the steady state of actin mRNA in Kc cells (8). In this paper we have examined the organization and the expression of the actin multigene family in Drosophila cultured cells. We have used transcribed...
but untranslated sequences from the actin genes as hybridization probes, making it possible to specifically follow the qualitative expression of these genes during 20-OHE induced differentiation of the Kc cells. Quantitative expression and precise structure of the two cytoplasmic actin genes were established by S1 nuclease protection and primer extension.

**Materials and Methods:**

**Cell culture and hormone treatment**

The Drosophila cell line used in this study was derived from the lines established by Echaller and Ohanessian (9). This line Kc167 was grown at 23°C in D22 medium with 2% foetal calf serum. 20-hydroxy-ecdysone (SIME) was added to cell cultures from a 10mM stock in 95% ethanol, to a final concentration of 1μM.

**DNA isolation and labelling**

Drosophila high molecular weight nuclear DNA was prepared from Kc cells by the method of Gros-Bellard et al (10). Recombinant charon phages were grown as described by Bingham et al (11) and DNA was prepared according to the technique of Maniatis et al (12). Plasmid DNA was prepared as described elsewhere (13).

**PolyA⁺ RNA preparation, electrophoresis and blotting**

RNA was extracted by the SDS-hot phenol-chloroform technique described by Palmiter (14). Subsequently polyA⁺RNA was selected by oligo d(T) chromatography (15). RNAs were size-fractionated on 1.0% agarose / formaldehyde gels (16) and transferred to nitrocellulose. For hybridization with 32P labelled nick translated probes, prehybridation, hybridation and washing procedures were as described by Thomas (17).

**S1 nuclease mapping and primer extension analysis**

S1 nuclease mapping was carried out according to methods developed by Berk & Sharp (18). Poly A⁺ Kc cell RNA and DNA fragments to be hybridized were first mixed and coprecipitated with ethanol. The pellet was dried under vacuum and dissolved in 13μl of a buffer containing 62% (v/v) formamide, 0.4M NaCl, 40 mM PIPES (pH 6.5) and 1mM EDTA. The solution was boiled for three min. and incubated at 55°C for 15h. The DNA-RNA hybrids were diluted with 300μl in S1 digestion buffer containing 4 mg of denaturated calf thymus DNA/ml and 300 u of S1 nuclease (Boehringer). The mixture was incubated at 37°C for 1h and the digestion products analyzed on a 5% polyacrylamide sequencing gel.

End-labeled double stranded primer (approximately 5000 counts/min.) was mixed with 0.1μg of poly A⁺ RNA, precipitated with ethanol and suspended in 13μl of 80% formamide, 40 mM PIPES (pH 8.4), 1mM EDTA and 0.4M NaCl. The samples were heated to 85°C for 3min. and then annealed for 15h at 55°C. The hybrids were digested fivefold with stop buffer to give a final salt concentration of 0.1 M NaCl, and the samples were precipitated with ethanol, dried and suspended in 80μl of reverse transcriptase buffer containing 50mM Tris (pH 8.3), 50mM KCl, 6 mM MgCl₂ and 2mM dithiothreitol. The reaction was started with the addition of reverse transcriptase , 12 to 15 units per microliter and incubated at 37°C for 1h. The reaction was stopped by addition of 80μl of stop solution to give a final concentration of 10mM EDTA, 0.1% SDS and 1M ammonium acetate. DNA was extracted once with phenol and once with chloroform and ethanol precipitated. Samples were loaded on a 5% polyacrylamide sequencing gel.

Appropriate exposures were scanned densitometrically on a Vernon integrating densitometer. A series of trial experiments with various amounts of poly A⁺RNA known to contain actin mRNA showed that for low film exposures there was a linear relationship between the amount of actin mRNA and the darkening of the film.

**In vitro transcription**

Nuclei were isolated by poterisatlon of Kc cells in the presence of an hypotonic buffer (TNM) plus detergent(10mM Tris-HCl,pH7.4/ 10mMNaCl/ 3mM MgCl₂/0.1mM PMSF and 0.5% Nonidet P40). Nuclei were pelleted by a rapid centrifugation(2 min/800g/4°C). The nuclear pellet was washed once with TNM without Nonidet P40. The washed nuclear pellet was resuspended in 40% (vol/vol) glycerol/ 50mM Tris-HCl,pH8/ 5mM MgCl₂/ 0.1mM EDTA/ 5mM DTT and 5mM PMSF. In vitro transcription reactions were performed by incubating an aliquot of nuclei(5x10⁸ nuclei per ml) for 30min at 32°C in a 60μl reaction mixture containing 10% glycerol (vol/vol)/
350mM ammonium sulfate/ 10mM MgCl2/ 1.25mM DTT/ heparin sulfate at 1 mg/ml/ 0.5% Sarkosyl/ 0.5mM GTP/ATP/CTP and 2μM [α32P]UTP(410Ci/mmol). RNAs were purified essentially as described (19) except that the first phenol/chloroform extraction was omitted. For hybridization of newly transcribed RNA to plasmid DNA dots, filters were prehybridized for 12h and hybridized for 72h at 40°C in hybridization solution as described by Thomas (17). The same number of counts per minute, from 1-5x10^6 cpm/ml, were added to each hybridization reaction.

Sequencing of fragments cloned in phage M13
Suitable restriction fragments of purified plasmid DNA were cloned into phage M13mp8 (20). Sequence from the restriction sites shown in Fig. 1 was determined using the dideoxy chain termination method of Sanger et al (21). Single strand template preparations and synthesis reactions were carried out as specified on the Phamacia data sheet.

Biohazards associated with the experiments described in this publication have been examined previously by the French National Control Committee.

RESULTS

Construction of specific probes for the cytoplasmic actin genes:
We have previously isolated and characterized a 20-OHE inducible gene in Kc cells (8) and identified this gene as the 5C actin gene. The actin gene family has been isolated by Fyrberg et al (22) and Tobin et al (23). They have shown that the protein coding regions of these genes were approximately 85% homologous and so crosshybridized efficiently. This property allows us to isolate with the actin 5C probe other phages containing actin genes from a recombinant λDm library (12). In each isolated phage the actin gene was identified by restriction mapping and chromosomal assignment. The EcoRI fragments containing the entire cytoplasmic actin genes, 5C and 42A, were subcloned in pBR328 and analysed in detail. Because of the conservation of aminoacid encoding nucleotide sequences in actin genes, these clones cannot be used to detect the transcripts of anyone individual gene. Since only about 1.1kb is needed to encode an actin protein, it is clear that even the smallest actin RNA transcripts observed (8) must contain approximately 0.5 kb of untranslated regions at either one or both ends of the transcript. Moreover it is well known that the 5' and 3' ends of conserved multigene families have diverged and do not crosshybridize. We therefore isolated specific probes from the transcribed but nontranslated 5'(5C) and 3'(5C and 42A) portions of these actin genes whose position is indicated in Fig. 1. The specificity of each probe was tested by hybridization to EcoRI genomic blots of Kc cell DNA. In our moderate conditions of hybridization, each of these probes hybridizes only to a DNA fragment of the same molecular weight as that from which it was derived (Fig. 2b, 2c, 2d), contrasting with the hybridization of a probe containing nucleotides encoding the actin protein (Fig. 2a). Such a probe hybridizes to fragments of six different sizes in an EcoRI digest of Kc cell DNA. 3' specific probes were also isolated from the four other actin genes 57B, 79B, 87E and 88F (data not shown). Thus all these specific probes will hybridize only to the transcripts of the actin gene from which they were derived and could be used to know what actin genes are expressed in Kc cells and whether 20-OHE induced accumulation of specific actin transcript(s).

Expression of the two cytoplasmic actin genes is regulated by 20-OHE:
To study variation in steady state levels of cytoplasmic actin gene transcripts, poly A+
Fig. 1: Cytoplasmic actin gene restriction maps.

The restriction maps of the genomic fragments containing the cytoplasmic actin genes were determined for EcoRI (E), HindIII (H'), SalI (S), HpaII (H'), BglII (B), Hinfl (H) and TaqI (T). The site (EcoRI) is a synthetic site introduced during cloning. The location of the fragments used as probes for Northern blots are indicated by a full line. For the 5C gene, the probes A, B and C are respectively the 5', coding and 3' specific probes. For the 42A gene, the probes A and B are respectively the coding and the 3' specific probes. The probes for S1 mapping and RTase extension experiments are indicated under each map by open boxes and closed boxes respectively. The clones used in the DNA sequence determination of the 5' end of the 42A gene are indicated by horizontal arrows.

Fig. 2: Southern blot analysis of actin specific probes.

To test the specificity of each probe, 10µg of Kc cell DNA were digested by EcoR1, fragments were size separated and transferred to nitrocellulose. Each filter was hybridized with the 5C coding region (a), the 5' specific 5C probe (b), the 3' specific 5C probe (c) and the 3' specific 42A probe (d). Hybridizations were in 50% formamide, 42°C and washed in 0.5X SSC, 0.1% SDS, 42°C.
Fig 3: Analysis of 5C actin mRNA in polyA⁺ RNA of Kc cells.
1.5 micrograms of polyA⁺ RNA from untreated (0) and 6, 16 and 22 hours 20-OHE treated cells were separated by agarose formaldehyde gel electrophoresis, blotted onto nitrocellulose filters and hybridized with the 5’ non-coding probe (probe A, Fig.1)(A), the coding 5C probe (probe B, Fig.1)(B) and the 3’ noncoding 5C probe (probe C, Fig.1) (C). Origin of the migration is in the top of the figure. Size of the different actin mRNAs were indicated in Kb at the left of the figure.

RNAs were prepared from Kc cells untreated and treated with 20-OHE for different time. Equal amounts of RNA were subjected to agarose-formaldehyde gel electrophoresis, transferred to nitrocellulose filters and hybridized to nick-translated unspecific or specific probes (Fig. 3 and 4).

The 5C actin gene unspecific probe reveals (Fig.3B) two RNA bands of 1.7 and 1.9Kb and a minor one of 2.2Kb. The 5’ specific 5C probe (Fig.3A) reveals also these three actin mRNAs demonstrating that this actin gene encodes in Kc cells at least three different RNAs. This probe reveals also two faint bands of about 3.5Kb that are probably the premessenger RNA since this probe contains a large part of intronic sequences and only a short exon (24,8). Moreover these bands are only present in whole cell RNA and in nuclear RNA and not at all in cytoplasmic RNA (data not shown). The use of the 3’ specific 5C probe shows (Fig.3C) that the heterogeneity of the 5C encoded transcripts is in part due to a 3’ end heterogeneity. Indeed this probe reveals only the 1.9Kb and 2.2Kb mRNA bands. Thus the 3’ end of the 1.7Kb RNA is upstream or near the proximal HindIII site of the probe C and the two others 1.9 and 2.2Kb extends more downstream to this site. The 2.2Kb being revealed rather strongly by this probe it may overlap largely to this HindIII-HindIII fragment. It is noteworthy that the intensity of all the bands, whatever the probe, is increased by 20-OHE treatment and this as soon as six hours of treatment. The level of expression of each class of transcript has been evaluated by measuring the intensity of each band on underexposed films. 20-OHE treatment induces of 3 fold increase of the level of the 1.7 kb and 1.9 kb RNAs and a 10 fold increase of the level of the 2.2 kb and premessenger RNAs according to the intensity of the bands in the uninduced cells.
Fig. 4: Analysis of 42A actin mRNA in polyA+ RNA of Kc cells.

1.5 micrograms of polyA+ RNA from untreated (0) and 9, 19 and 23 hours 20-OHE treated cells were treated as in Fig. 3 and hybridized with the entire 42A actin gene as probe (probe A, Fig. 1)(A) and with the 3' non-coding 42A probe (probe B, Fig. 1)(B). Size of the different actin mRNAs were indicated in Kb at the left of the figure.

The 42A actin gene unspecific probe reveals also the same three RNA bands as the 5C one (Fig. 4A). But the 3' specific 42A probe shows that this gene is transcribed at low level in Kc cells in one RNA of 1.7Kb and that this 42A specific RNA accumulates during 20-OHE treatment to reach a six fold increase after 23 hours of hormonal treatment (Fig. 4B).

Specific 3' end probes for the other actin genes were developed but although they detect transcripts in RNA of pupae (79B, 88F and 87E) and in larvae (57A)(data not shown) each of them failed to detect any in either untreated or treated Kc cells.

So we can conclude that only the 5C and 42A genes were expressed and that 20-OHE increase the steady-state level of each mRNAs of these genes. But comparison of the intensity of the RNA detected by the specific probes of the 5C and 42A genes is very difficult because the length of each probe was very different, although their specific activities were roughly the same. So to compare the relative abundance of transcripts of each cytoplasmic actin gene we decided to use the quantitative technique of S1 nuclease analysis (25).

Quantitation of Individual 42A and 5C actin gene expression:

The 5' end of the Kc transcript(s) from each cytoplasmic actin gene has been mapped by S1 nuclease analysis (18). Poly A+ Kc cell RNA was hybridized to DNA fragments of varying lengths from both Drosophila actin genes. The DNA fragments used in these experiments are indicated in Fig. 1 and drawn in an expanded scale on the side of Fig. 5. For the 5C gene the BgIII-HindIII fragment used as probe is reduced to 265±3 nucleotides in length after hybridization to RNA and digestion with the nuclease S1. This result places the beginning of the protecting RNA at 10±3bp from the
Fig. 5: Time course of accumulation of actin mRNA sequences during 20-OHE treatment of Kc cells.

Separate quantitative S1 nuclease protection reactions were performed without RNA (lane 1 and 12) and with RNA from untreated cells (lane 2, 6 and 11) and from 20-OHE treated cells for 6 hours (lane 3 and 7), for 16 hours (lane 4, 8 and 9) and for 26 hours (lane 5 and 10). In all cases the 20-OHE concentration was 1 μM except for lane 9, 0.1 μM. The probes used are the HindIII-BglII fragment from the 3′ gene (lanes 1-5) and the HindIII-HindIII fragment from the 42A gene (lanes 6-12). M indicates the HindIII cut pBR322 markers (517, 506, 396, 344, 298, 221, 154 bp from top to bottom). A physical map of each actin gene was drawn on each side of the figure. The solid line indicates the translated region and the solid point indicates the labelled site of the probe used in each case. The upstream end of the probes and the end of the protected fragments were also indicated on these maps.

ATG codon (Fig. 5). Similar experiments with the HindIII-HindIII fragment from the 5′ of the 42A gene result in a fragment of 179 (±4) bp placing the beginning of the protection at 25 (±4) bp upstream from the initiation site of the translation.

Under the conditions of DNA excess hybridization that we have used in these experiments, the intensity of the autoradiographic signal from a transcript is proportional to the abundance of the complementary RNA. The two primer fragments (5C and 42A) were of similar specific activities.
Fig. 6: Primer extension analysis of the actin isoform mRNA's during 20-OHE treatment.

Quantitative reverse transcription reactions were done with the 5C primer (lanes 1-5) and with the 42A primer (lanes 6-10). A physical map of each actin gene was drawn on each side of the figure. The solid boxes represent the primers labelled at the HindIII site (solid point). The open boxes represent the extended fragments. P designates the primer location. M indicates the HindIII cut pBR322 markers (396, 344, 298, 221, 154, 75 bp from top to bottom). Lanes 1 and 6 do not contain RNA. RNA's are from untreated (lanes 2 and 7) and 20-OHE treated cells for 8hrs (lanes 3 and 8), 16hrs (lanes 4 and 9), 26hrs (lanes 5 and 10). Exposure 24 hours.

and so intensities of each protected band could be compared. In summary, this experiment shows that the 5C and 42A genes are transcribed in Kc cells but that 5C transcripts are more abundant (6-8 fold) than those of the 42A gene (compare slot 2 with slots 6 and 12 in Fig. 5). Moreover, 20-OHE increases the steady-state level of the transcripts 3-5 fold for the 5C transcripts and 6-8 fold for the 42A gene.

It is unlikely that the length of the RNA fragments protected by the 5C and 42A probes corresponds to the 5' end of the transcripts in Kc cells, because this would provide a relatively very short 5' untranslated sequence. Moreover, the 5C gene is known to be split by an intervening sequence near its 5' end (24).
An intron split the 5' untranslated region of the 42A gene:

When S1 mapping experiments were carried out with a 42A probe that is larger than the Hinfl-Hinfl probe used in Fig. 5, two bands were protected. A very strong band that allows to place the beginning of the protection at the position -23bp (±5) upstream from the ATG and a faint band that results from the protection of an mRNA extending to -256bp (±10) upstream from the ATG (data not shown). This band was interpreted as a protection due to premessenger RNA. The presence on the 5C and 42A mRNA of a larger untranslated sequence than that observed by S1 mapping was confirmed by RTase extension experiments using the same fragment BglII-HindIII from each gene as primer. Primer extension experiments (Fig. 6) show that the 42A probe gives one band and the 5C probe three bands, one major and two minor ones. The lengths of the major extended fragments indicate that the cytoplasmic 5C and 42A mRNA's have non-coding leader sequences of approximately 156 (±4) and 102 (±3)bp respectively with a 5' exonic region of 147bp (±4) and 77bp (±3) for each gene respectively. The two other bands of the 5C extension are either unspecific arrests of the reverse transcriptase at critical secondary structures of the RNA or indicate that the 5C gene may possess at least two other minor different 5' ends. Whatever the origin of these bands their ratio is not affected by 20-OHE treatment and this small heterogeneity (30-40 bp) could not explain the heterogeneity observed on Northern blots of the 5C actin mRNAs.

We have determined the sequence at the 5' end of the 42A gene. This was accomplished by sequencing in both directions from the TaqI and SalI sites shown in Fig. 1. This sequence is shown in Fig. 7 and is aligned with the sequences yet available for the 5C gene (24). Six nucleotides immediately upstream the ATG codon are conserved in the untranslated region of the two cytoplasmic actin genes. The 42A intervening sequence is 155 nucleotides in length and the complete nucleotide sequence of it is shown in Fig. 7. The Intervening sequence junctions as determined by S1 mapping and RTase experiments could be aligned perfectly with canonical sequences for such junctions, and with the other intron-exon junction sequences of other Drosophila actin genes (24, 26). No detectable homology by sequence analysis could be detected between all of the 42A actin intervening sequence and that of the other actin genes.

20-OHE regulates actin gene expression at the level of transcription:

We have measured the relative rates of transcription of the 42A and 5C actin genes using a nuclear run on assay and the filter hybridization procedure (27) under conditions of DNA excess. Levels of UTP α 32P incorporation were decreased by > 80% by α-amanitin (1μg/ml), and hybridization with actin probes by more than 95% (data not shown), indicating that the nuclei preparation used are able to perform DNA-dependent RNA synthesis mediated by RNA polymerase II. Moreover there is no difference in total incorporation for nuclei preparations from untreated and 20-OHE treated cells at least up to 24 hours of treatment and only a slight decrease of transcriptional activity for longer periods of treatment (about 20% of decrease for 48 hours 20-OHE treated cells). This indicates that there is not a general effect of the hormone on transcriptional activity in Kc cells.
Fig. 7: DNA sequence of the 5' end of the 42A actin gene.

The sequence of the strand with the same sequence as the mRNA is shown. The sequence of the 5' end of the 42A actin gene is aligned with the published partial sequence of the 5' end of the 5C gene (24). The canonical Intron-exon junction regions were underlined with a broken line. Closed triangle indicates the transcription initiation point. Vertical arrows indicate the extremity of the protection as determined by S1 mapping. The entire sequence of the intron is boxed. The restriction enzymes recognition sites shown in Fig. 1 are underlined. Sequence hyphens have been omitted for clarity.

Fig. 8 shows the result of a typical experiment comparing transcription of the two cytoplasmic actin genes in nuclei of Kc cells cultured in the absence or in the presence of 20-OHE using a 3' specific cloned sequence of each gene. Vector DNA without a recombinant Insert served as a control of background hybridization. Control hybridizations were performed with a plasmid containing a complete copy of the repeated element copia whose expression is not affected by 20-OHE in Kc cells (unpublished data). The hybridization levels using this probe were virtually identical at each time point (data not shown). Transcription of sequences corresponding to the two actin genes was detected in nuclei of untreated cells. In nuclei from 20-OHE treated cells, the level of transcripts of the two actin genes is increased 2 fold after 4 hours of treatment and up to 5 fold after 15 hours of treatment. These results indicate that the transcription rate of the two cytoplasmic actin genes increases up to 5 fold after 20-OHE treatment indicating a direct effect of 20-OHE on actin gene transcription that could account for the observed increased steady state levels of actin mRNA during hormonal stimulation of Kc cells.
Fig. 8: Specific transcription of cytoplasmic actin genes in 20-OHE treated cells.

32P-labelled nuclear RNA run on was hybridized to denatured DNA fragments (10 μg) containing specific cloned 3' sequences of the 5C (probe C, Fig. 1) and the 42A (probe B, Fig. 1) genes and pUC8 vector DNA spotted onto nitrocellulose strips. In this experiment all the hybridization reactions were carried out with inputs of 4 x 10^6 cpm of the labelled RNA sequences. Numbers above the dots indicate the duration of 20-OHE treatment of Kc cells.

DISCUSSION

We have presented a detailed study of the structure and of the expression of the two cytoplasmic actin genes 5C and 42A in Kc cells during 20-OHE induced differentiation by using specific 5' and 3' probes on Northern blots and by quantitative S1 and RTase mapping. These two actin genes are expressed in these cells before 20-OHE treatment and the hormone induces an increase of the steady state of their mRNAs. The four other actin genes are never expressed in Kc cells even in the presence of 20-OHE indicating that the hormone does not switch on the muscle specific actin genes. Moreover this hormonal induction of actin mRNA level of the 5C and 42A genes is hormonal concentration dependent with a half-maximal induction corresponding to a concentration of about 5nM (data not shown). This increase is due, at least in part, to an increase of the transcription after the hormonal treatment of each gene actin gene 42A and 5C. Previous examination of expression of the actin genes of Drosophila has demonstrated temporal and spatial differences in actin mRNA accumulation during development (3). It is interesting to note that the expression of the two actin genes 5C and 42A is regulated during the development and that they are expressed during periods where profound changes occur i.e. early embryogenesis, early pupation and in the ovary of adult females (3). Presumably the expression of these genes is regulated in response to different regulatory signals and so they may also possess hormonally dependant controls during development.

The 42A actin gene intervening sequence is 155 nucleotides in length as defined by S1 and RTase mapping and the complete nucleotide sequence is shown in Fig. 7. The extremities of this intervening sequence satisfies the homology to the donor splice site canonical sequence 5'-AG|GTAAGA-3' and fit well the definition of Drosophila 3' splice site defined as a sequence 5'-CAG|3' preceded by a 16 nucleotide region which is free of A-Gs and which is pyrimidine rich (81%) as usually found (28). This splice point (-22bp from the ATG) was not found in other actin genes of Drosophila or the other organisms. It may be recalled that there is no conservation, observed up to
now, whatsoever of intron position between any of the Drosophila actin genes and any deuterostome, despite considerable conservation of intron position within deuterostomes (29). The sole known exception is an identical intron position for the Drosophila cytoskeletal actin 5C (3) and the intron of a chicken cytoskeletal actin (30).

The 5C actin gene produces multiple mRNAs that differ largely at the 3' end and perhaps slightly also at the 5' end. During the increase of the expression of the 5C actin gene induced by 20-OHE the multiple mRNA species were found in about the same proportion which indicates that the multiplicity of the 3' ends is not related to the hormonal regulation. From the data presented we postulated that the various 5C actin mRNA species arise by the utilisation of different polyadenylation sites. We have confirmed by 3' end S1 mapping that the three transcripts of the 5C actin gene differ from each other by about 250bp at the 3' end (data not shown). In the case of the Kc 167 cell line it seems that the first two polyadenylation sites of the 5C gene are strongly preferred. In an other cell line, Kc 0%, growing without fetal calf serum, the third and more distal poly(A) site is virtually not used. The role(s), if any, of such a discrete polymorphism in the 3' end of mRNA of a single gene is (are) totally obscure. However we would like to emphasize that the 5C actin gene encodes for multiple mRNAs during Drosophila development. These mRNAs have the same size as those in Kc cells (3) and are revealed by a 3' probe less distal than our probe and containing a short part of coding region. So they correspond certainly to the same transcripts. Moreover the ratio of these different mRNA species vary at the different stages of the Drosophila development where these genes are expressed, suggesting a different level of the genome expression specific at each stage or a functional role of these transcripts during development.

The DNA sequence of the 5' flanking region of the 42A gene is shown in Fig. 7. Strikingly the typical elements TATAA and CAAT (31) are not present at the position-30 and -65 base pairs of the mRNA start site respectively. This does not induces heterogeneity at the start site of transcription but could explain the lower basal level of expression of this gene as compared to the 5C gene which possess these two typical sequences (32). However at the initiation site of the 42A actin gene is present a sequence 5'-TTCAATC-3' which is analogous to the nucleotide sequence present at the 5' terminus of many other Drosophila mRNAs (5'-ATCAGTC-3')(33). This sequence is also present at the initiation point of the 5C actin gene as 5'-ATCAGTC-3' (32).

When Drosophila Kc cells where exposed to the moulting hormone 20-OHE they rapidly undergo important cell shape changes (4) that implies certainly a complete reorganisation of the cytoskeleton. Two major structural elements involved in the cell cytoskeleton are microtubules and actin microfilaments. We have previously demonstrated that the 20-OHE induces an accumulation and an increase of polymerisation of actin (5,6) that is associated with an increase in actin gene expression (8,34) especially the two cytoplasmic actin genes 5C and 42A (this paper). Moreover we have recently shown (35) that 20-OHE induces the synthesis and the accumulation of a new β tubulin subunit that is the product of the 60C β tubulin gene. All of this demonstrates that the changes in the cytoskeleton of Kc cells during 20-OHE induced differentiation occur by important
changes in the expression of several specific genes encoding cytoskeletal proteins as it has been suggested by Cherbas et al. (36).

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