Expression of Acetylcholine Receptor α-Subunit mRNA during Differentiation of the BC₃H₁ Muscle Cell Line*

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The accumulation of translatable acetylcholine receptor α-subunit mRNA was examined in the BC₃H₁ muscle cell line in response to serum and cell growth. Relative amounts of α-subunit mRNA were quantitated during differentiation by cell-free translation and immunoprecipitation with an α-subunit-specific monoclonal antibody. Logarithmically growing cells do not possess cell surface acetylcholine receptors; however, a significant amount of α-subunit mRNA is detectable in cells under these conditions. Furthermore, α-subunit is synthesized in growing undifferentiated cells at a rate similar to that of differentiated cultures. Following growth arrest of BC₃H₁ cells, surface receptors are induced to levels greater than 100-fold above that of growing cells. The relative level of translatable α-subunit mRNA in differentiated cells, however, is only approximately 4-fold greater than in growing cultures. Induction of α-subunit mRNA appears to be reversible since reinitiation of growth in quiescent differentiated BC₃H₁ cells results in a reduction in relative abundance of this mRNA species to levels comparable to that of undifferentiated cells and the concomitant loss of surface receptors. These results indicate that receptor expression during differentiation is regulated both post-translationally and at the level of receptor subunit mRNA accumulation.

Muscle cell differentiation involves the fusion of mononucleated myoblasts to form multinucleated myotubes, a transition which is accompanied by the permanent withdrawal of muscle cells from the cell cycle. The process of myoblast fusion has been shown to be temporally associated with the expression of a variety of muscle-specific gene products (for review see Ref. 1); however, it is clear from studies in tissue culture that fusion is not an absolute prerequisite for differentiation. In studies of primary cultures of embryonic chick and quail muscle cells in medium containing low concentra-

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† The abbreviations used are: ACh receptor, acetylcholine receptor; NaCl/P₄, phosphate-buffered saline, 0.14 M NaCl, 10 mM sodium phosphate, pH 7.2; EDTA, ethylenediaminetetraacetic acid; NaDodSO₄, sodium dodeyl sulfate.

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of serum to quiescent cells, the rate of α-subunit synthesis was not significantly reduced and in many experiments was actually increased compared to quiescent cultures.

In the present study, the effects of serum and cell division on the accumulation of functional ACh receptor α-subunit mRNA were examined. The results demonstrate that logarithmically growing cells contain functional α-subunit mRNA which is translated in vivo at a rate similar to that of differentiated cells. Cessation of cell division, by exposure to low levels of serum, results in a 4-fold increase in the relative levels of α-subunit mRNA and greater than a 100-fold induction in the level of surface ACh receptors. Reinitiation of cell division in quiescent BC3H1 cells arrests the accumulation of translatable ACh receptor α-subunit mRNA and causes the loss of surface ACh receptors within 24 to 48 h. Under these conditions, the level of α-subunit mRNA is decreased to a level similar to that in undifferentiated cells. These data indicate that during muscle cell differentiation ACh receptor expression is regulated only partially at the level of transcription (or accumulation) of translatable α-subunit mRNA. Recent entry of differentiated cells into the cell cycle not only inhibits the post-translational processing of ACh receptors (20) but also prevents the further accumulation of translatable receptor subunit mRNA.

MATERIALS AND METHODS

Cell Culture—The clonal mouse muscle-like cell line, BC3H1 (18), was grown as described previously (4). Fetal calf serum was obtained from K.C. Biologicals and media were obtained from the Washington University Basic Cancer Center. Cell numbers were determined using a Coulter counter.

Labeling of proteins with [35S]methionine and preparation of cell extracts was performed as described (20).

Assay for Acetylcholine Receptor—Previously published procedures were used to measure the binding of [125I]α-bungarotoxin to cell cultures as described (20).

Preparation of Total Cellular RNA—Total cellular RNA was extracted from BC3H1 cells grown on 10-cm Falcon tissue culture dishes according to the 8 M guanidine HCl procedure of Cox as modified by Deerley et al. (21, 22). Details of the RNA isolation procedure have been described previously (4). 20-20 μg of total cellular RNA/106 cells could generally be obtained from quiescent cells in 1% fetal calf serum. Cells growing in 20% serum gave about a 25% greater yield.

Cell-free Protein Synthesis—Total cellular RNA was translated in 30 μl translation reactions in a micrococcal nuclease treated rabbit reticulocyte lysate translation system (Bethesda Research Laboratories) containing [35S]methionine at 1.65 mCi/ml (>1000 Ci/mmol, Amersham Corp.) as described previously (23, 24). Following incubation for 60 min at 30 °C, reactions were terminated by addition of 200 μl of NaCl/P, containing 1% (w/v) Triton X-100, 1 mM phenyl methylsulfonyl fluoride, 5 mM EDTA, and 0.1-0.2 unit/ml of bovine lung aprotinin (Sigma). Total [35S]methionine incorporation was determined by applying a 5-μl sample of the total translation reaction to a 3-cm disk of Whatman filter paper followed by boiling for 10 min in 1% trichloroacetic acid and washing in 95% ethanol. Filters were counted in a scintillation spectrometer.

Immunoprecipitation—Immunoprecipitation of nascent α-subunit mRNA from cell-free translations and of α-subunit from cell extracts was performed using mAb61 (an α-subunit-specific monoclonal antibody) exactly as described (20, 24).

Electrophoresis—NaidodSO4-polyacrylamide gel electrophoresis was performed according to Laemmli (25) as described previously (23). Following electrophoresis, gels were processed for fluorography as described (23), and the films were analyzed by densitometry. Peak height was found to be proportional to peak area and, therefore, was employed as a measurement of band intensity.

RESULTS

Cell Growth and Acetylcholine Receptor Induction—BC3H1 cells grow logarithmically with a doubling time of approximately 18 h in medium containing 20% fetal calf serum (17). Transfer of subconfluent cultures to medium containing 1% fetal calf serum results in the arrest of cell division within approximately 24 h (Fig. 1A). Following growth arrest, ACh receptors appear on the cell surface and continue to increase to a maximum level after about 5 days (Fig. 1, B and C). As described previously (20), exposure of subconfluent differentiated cultures to 20% serum results in the rapid loss of surface ACh receptors following reinitiation of cell division. In the experiment presented in Fig. 1, the number of surface α-bungarotoxin-binding sites decreased approximately 85% within 24 h of serum stimulation (compare day 5 (1% serum) with day 6 (20% serum)). The culture dishes used in these experiments contain approximately 1 × 105 cells/dish at confluent cell densities; therefore, at the time of growth arrest in 1% serum the cells are relatively sparse (9 × 104 cells/dish). By day 8 in 20% serum, areas of confluent cells can be observed. These regions of high cell density may contribute to the slight reinduction of α-subunit mRNA observed on day 8 in serum-stimulated cultures (see below).

Cell-free Translations and Immunoprecipitations—In order to determine whether exposure of differentiated cells to 20% serum influenced the accumulation of mRNA coding for the

![Fig. 1](http://www.jbc.org/)

**Fig. 1. Effect of serum on cell growth and acetylcholine receptor expression.** BC3H1 cells were plated or 10-cm Falcon dishes at 1.5 × 105 cells/dish in Dulbecco’s minimal essential medium (Grand Island Biologicals) containing 20% fetal calf serum, 0.1 mg/ml of glutamine, 100 units/ml of penicillin, 100 μg/ml of streptomycin. On day 1, designated by the arrow, cultures were transferred to medium containing 1% fetal calf serum. On day 5, half of the cultures were transferred to medium containing 20% fetal calf serum (C→D), and the remaining cultures were refed with medium containing 1% serum (B). Cell numbers and numbers of toxin-binding sites/dish were determined as described under "Materials and Methods." Note that following transfer of quiescent cultures to medium containing 20% serum, the number of toxin-binding sites/dish (C) decays almost completely within about 24 h and remains at this low level in cultures which have reinitiated cell division.
Regulation of ACh Receptor \( \alpha \)-Subunit mRNA during Differentiation

subunits of the ACh receptor, total cellular RNA was extracted from BC3H1 cells and translated in a micrococcal nuclease-treated rabbit reticulocyte lysate system. The primary translation product of \( \alpha \)-subunit mRNA was then immunoprecipitated from total translation reactions with mAb61 (an \( \alpha \)-subunit-specific monoclonal antibody prepared against NaDodSO\(_4\)-denatured purified Electrophorus electricus ACh receptor) (26–29) and analyzed by NaDodSO\(_4\)-polyacrylamide gel electrophoresis (Fig. 2). As described previously, the \textit{in vitro} translation product of \( \alpha \)-subunit mRNA demonstrated an apparent molecular weight of 41,000 daltons which is approximately 2,000 daltons larger than the nonglycosylated \( \alpha \)-subunit (24). The identity of the \( \alpha \)-subunit has been confirmed previously by peptide mapping (24).

When the results from the cell-free translations are compared with the data in Fig. 1, a discrepancy is observed between the induction of translatable \( \alpha \)-subunit mRNA and surface ACh receptors. During differentiation, the level of surface receptors increases by over 100-fold. In contrast, the relative abundance of translatable \( \alpha \)-subunit mRNA increases only about 3- to 4-fold (Fig. 2, compare lanes 2-6, 8, and 10 and Fig. 3). These findings indicate that ACh receptor induction is regulated only partially at the level of accumulation of translatable \( \alpha \)-subunit mRNA and suggest the possibility of some form of post-transcriptional control in the regulation of receptor expression.

As reported previously (4, 24), other differences in cell-free translation products can be observed between RNA samples isolated from cultures at different stages of differentiation (Fig. 2A). For example, mRNAs directing the synthesis of polypeptides with apparent \( M_r \approx 50,000 \) and 60,000 appear to

![Fig. 2. Cell-free translation and immunoprecipitation of \textit{in vitro} translation product of \( \alpha \)-subunit mRNA. Total cellular RNA from BC3H1 cultures grown on 10-cm Falcon dishes in 16 ml of media/dish was isolated and translated in a micrococcal nuclease-treated rabbit reticulocyte lysate system, as described under "Materials and Methods." RNA was isolated from cultures grown under conditions identical to those described in Fig. 1. For relative quantitation of \( \alpha \)-subunit mRNA, 30-\( \mu \)l translation reactions were immunoprecipitated with mAb61 as described under "Materials and Methods." Immunoprecipitates were denatured in NaDodSO\(_4\) sample buffer, and polypeptides were separated on 10% polyacrylamide gels followed by fluorography (exposure time \( \approx 3 \) days for total translation products and 6 days for immunoprecipitations). Each lane represents the results obtained from translation of 2 \( \mu \)g of RNA from each culture condition. A, total cell-free translation products. Lanes: 1, day 1 (20% serum); 2, day 2 (1% serum); 3, day 3 (1% serum); 4, day 4 (1% serum); 5, day 5 (1% serum); 6, day 6 (1% serum); 7, day 7 (1% serum); 8, day 8 (1% serum); 9, day 9 (1% serum); 10, day 10 (20% serum); 11, endogenous translation without added RNA. B, \( \alpha \)-subunit immunoprecipitated from translation reactions with mAb61 (anti-\( \alpha \)) or nonimmune serum (where indicated). Lanes: 1, day 1 (20% serum immunoprecipitated with nonimmune serum); 2, day 2 (1% serum); 3, day 3 (1% serum); 4, day 4 (1% serum); 5, day 5 (1% serum); 6, day 6 (1% serum); 7, day 7 (1% serum); 8, day 8 (1% serum); 9, day 9 (1% serum); 10, day 10 (20% serum); 11, day 11 (20% serum); 12, day 12 (1% serum immunoprecipitated with nonimmune serum). The relative decrease in concentration of \( \alpha \)-subunit mRNA following addition of 20% serum can be observed by comparing lane 5 in B, which represents \( \alpha \)-subunit mRNA at the time of addition of 20% serum to differentiated cultures, with lanes 11, 9, and 12, which represent \( \alpha \)-subunit mRNA following exposure to 20% serum for 24, 48, and 72 h, respectively. The relative amounts of \( \alpha \)-subunit mRNA during each day in 1 and 20% serum can be directly compared with the data in Fig. 1 for comparison of rates of cell growth and levels of ACh receptor expression under each culture condition.
be more abundant in growing cells, while other mRNAs such as those directing the synthesis of polyphenylides with apparent $M_r \sim 35,000, 40,000,$ and $200,000$ are more prevalent in differentiated cultures. The identities of these developmentally regulated mRNA species remain to be determined.

Note that a large number of proteins are nonspecifically immunoprecipitated from the translation reactions with mAb61; however, the $\alpha$-subunit is among the most abundant. The nonspecific background bands in the gel are also precipitated with nonimmune serum (Fig. 2B, lanes 1 and 12). This background is not unexpected considering that $\alpha$-subunit mRNA constitutes less than 0.1% of total mRNA in maximally differentiated BC3H1 cells.

Assay by cell-free translation of RNA from cells which were transferred from 1 to 20% serum, followed by immunoprecipitation of the protein product of $\alpha$-subunit mRNA, indicates that re-entry of quiescent cells into the cell cycle is associated with a decrease in $\alpha$-subunit mRNA of 3- to 4-fold, resulting in a basal $\alpha$-subunit mRNA level characteristic of undifferentiated cells. At the time of addition of 20% serum to differentiated cultures, $\alpha$-subunit mRNA represented approximately 205 relative units of translatable mRNA based upon densitometry of immunoprecipitable $\alpha$-subunit from translation reactions (Fig. 2B, lane 5, Fig. 3, and Table I). The fraction of total translatable mRNA that is represented by $\alpha$-subunit mRNA decreased to 133 and 106 relative units following exposure of cultures to 20% serum for 24 and 48 h, respectively (Fig. 2B, compare lane 5 with lanes 7 and 9, Fig. 3, and Table I). A slight increase in the relative abundance of $\alpha$-subunit mRNA was observed after 3 days growth in 20% serum (Fig. 2B, lane 11). This may be due to reinduction of this mRNA since these cultures are beginning to reach high cell density. A minor species which comigrates with $\alpha$-subunit was immunoprecipitated with nonimmune serum from a translation of mRNA extracted from cells on day 7 in 1% serum (Fig. 2B, lane 12). We are uncertain as to whether this is actually $\alpha$-subunit or a nonspecific contaminant. The relative intensity of the peak corresponds to 10 relative units on the scale in Fig. 3. The intensity of this band has not been subtracted from the intensities of $\alpha$-subunit bands immunoprecipitated with mAb61. Because the translation efficiencies of the different RNAs were not absolutely identical for all samples, $\alpha$-subunit mRNA is expressed in Fig. 3 as relative units which are equivalent to the relative peak heights of the $\alpha$-subunit bands in the gel in Fig. 2 and gels from 2 additional sets of translations (gels not shown) divided by the amount of radioactivity incorporated into total protein in each translation reaction. The differences in translation efficiency of the different RNA samples are due to variations between RNA samples rather than differences in the translation reactions. Similar values for the relative abundance of $\alpha$-subunit mRNA are obtained if the $\alpha$-subunit intensities are normalized to one of the nonreceptor polyphenylides such as actin.

The apparent reduction in the concentration of $\alpha$-subunit mRNA following reinitiation of cell division can probably be attributed to at least two factors. 1) The steady state level of a given mRNA species is governed by the relative rates of transcription and degradation. Therefore, if as appears to be the case, addition of 20% serum prevents further accumulation of $\alpha$-subunit mRNA, then pre-existing $\alpha$-subunit mRNA will decay with time and thus comprise a smaller fraction of total cellular RNA/culture. 2) Addition of 20% serum to quiescent cultures results in reinitiation of cell division which is accompanied by an increase in total RNA content/culture. The transition of cells from the resting to the growing state has been reported to result in a rapid and significant increase in mRNA content/cell (30). Thus, under these conditions the amount of $\alpha$-subunit mRNA will appear to decrease as a fraction of total cellular RNA due to dilution by the newly transcribed RNA from cells as they re-entered the cell cycle. The actual reduction in abundance of $\alpha$-subunit mRNA/culture is, therefore, not as dramatic as indicated from cell-free translation assays of an equivalent amount of total RNA per translation from each culture condition. Currently, we are unable to examine the effects of growth-arrest and reinitiation of cell division on the $\beta$, $\gamma$, or $\delta$ ACh receptor subunit mRNAs due to the high degree of protease sensitivity of these subunits, and the lack of sufficiently characterized antisera which recognize the primary translation products of the individual subunit mRNAs from mouse muscle.

**Rates of $\alpha$-Subunit Synthesis in Vivo**—Because of the presence of $\alpha$-subunit mRNA in undifferentiated cultures which lacked ACh receptors, we decided to determine whether or not this mRNA species was translated in these cells or whether some form of translational control might explain the apparent discrepancy between receptor induction and $\alpha$-subunit mRNA accumulation. Relative rates of $\alpha$-subunit synthesis were measured in vivo by a 5-min pulse with [35S]methionine followed by immunoprecipitation of labeled $\alpha$-subunits from cell extracts with mAb61. The rate of total cellular protein synthesis, as measured by incorporation of [35S]methionine into trichloroacetic acid-insoluble material, was approximately 2- to 3-fold greater in undifferentiated growing cells compared to quiescent differentiated cultures (Table I, compare log phase (20% serum) and day 5 (1% serum)). The cell densities of the growing cultures and the differentiated cultures (Table I, compare log phase (20% serum) and day 5 (1% serum)) were equivalent in this experiment (1.5 x 10^6 cells/dish). The rates of $\alpha$-subunit synthesis/dish under the two conditions can, therefore, be directly compared as rates of $\alpha$-subunit synthesis/cell. Surprisingly, the rate of $\alpha$-subunit synthesis/culture in growing cultures was approximately 60% that of differentially cultured cultures; however, because of the greater rate of total protein synthesis in growing cells, $\alpha$-subunit comprised a smaller fraction of total proteins synthesized in growing compared to quiescent cultures (Fig. 4 and Table I) (compare the rate of $\alpha$-subunit synthesis/dish with the relative rate of $\alpha$-subunit synthesis). The relative rate of $\alpha$-subunit synthesis represents the rate of $\alpha$-subunit synthesis/dish divided by the total [35S]translation efficiency.
methionine incorporation/dish. As reported previously (20), exposure of differentiated cultures to 20% serum for 24 h resulted in a similar increase in the rate of total protein synthesis (Table I, compare day 5 (1% serum) and day 6 (20% serum)). The rate of α-subunit synthesis/dish increased by 50% following reinitiation of growth in differentiated cells despite the disappearance of ACh receptors during this same period. These findings indicate that α-subunit mRNA is translated in undifferentiated BC,SHl cells and suggests the possibility of post-translational control in the regulation of ACh receptor expression.

**DISCUSSION**

Previously we reported that the surface expression of ACh receptors in the BC,SHl muscle cell line was inhibited following addition of high concentrations of serum to quiescent differentiated cells (20). In the present study, the role of serum and cell growth in the regulation of expression of functional ACh receptor α-subunit mRNA was examined. Particular attention was focused upon the control of accumulation of this mRNA species during differentiation of quiescent cells and in cultures which, once differentiated, were stimulated to re-enter the cell cycle.

The results indicate that ACh receptor expression is regulated during differentiation of BC,SHl cells only partially at the level of accumulation of translatable α-subunit mRNA. Growing cells, which lack receptors, contain translatable α-subunit mRNA, the relative abundance of which increases approximately 3- to 4-fold following cessation of cell division. This change in the level of mRNA is insufficient to account for the greater than 100-fold increase in the level of surface ACh receptors in differentiated compared to growing cells and suggests the possibility of some form of post-translational regulation of receptor expression. Using the C2 fusing muscle cell line originally described by Yaffe and Saxel (31), a similar disparity between the induction of α-subunit mRNA and ACh receptors has also been observed.  

Previously, Sebbane et al. (24) reported a 10- to 12-fold induction of translatable α-subunit mRNA during differentiation of BC,SHl cells at confluent densities. The lower level of induction reported here may reflect the fact that cells used for these studies were differentiated at low cell densities in low concentrations of serum. If, for example, extensive cell-cell contacts are required for maximum α-subunit mRNA induction, then the cells used in these studies may not be fully differentiated. In both cases, however, there is a clear discrepancy between the level of induction of translatable α-subunit mRNA and surface receptors.

Induction of α-subunit mRNA appears to be reversible since conditions which reinitiate cell division in differentiated BC,SHl cultures result in the arrest of further accumulation of this mRNA species and the deinduction of α-subunit mRNA to levels characteristic of growing cells. It is unclear at the present time as to whether the arrest of accumulation of α-subunit mRNA is obligatorily coupled to the reinitiation of cell division or whether these cellular responses can be dissociated by various culture conditions.

The rate of α-subunit synthesis/culture was previously shown to remain approximately constant and in many cases to increase, following exposure of differentiated cultures to high concentrations of serum (20). The degree to which α-subunit synthesis increased depended upon the extent of stimulation of total protein synthesis in the presence of serum. Considered in light of the present study, the increased rate of

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**TABLE I**

Comparison of relative abundance of translatable α-subunit mRNA with rates of α-subunit synthesis in *vitro*

| Culture condition (day and serum concentration) | [$^3$S]Methionine incorporation/dish | α-Subunit synthesis/dish | Relative rate of α-subunit synthesis | Relative abundance of α-subunit mRNA |
|-------------------------------------------------|------------------------------------|--------------------------|-------------------------------------|------------------------------------|
| Log phase (20%)                                  | 5.6 × 10⁶                          | 55                       | 10                                  | 86                                 |
| Day 2 (1%)                                       |                                    |                          |                                     | 100                                |
| Day 4 (1%)                                       |                                    |                          |                                     | 184                                |
| Day 5 (1%)                                       | 2.9 × 10⁵                          | 88                       | 30                                  | 205                                |
| Day 6 (1%)                                       | 6.7 × 10⁵                          | 129                      | 19                                  | 238                                |
| Day 6 (20%)                                      |                                    |                          |                                     | 131                                |
| Day 7 (1%)                                       |                                    |                          |                                     | 295                                |
| Day 7 (20%)                                      |                                    |                          |                                     | 114                                |
| Day 8 (1%)                                       |                                    |                          |                                     | 261                                |
| Day 8 (20%)                                      |                                    |                          |                                     | 150                                |

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**FIG. 4. Immunoprecipitation of α-subunit synthesized *in vitro***

The experimental protocol for the cultures is as in Fig. 1. 10-cm culture dishes were pulse-labeled in 1.5 ml of medium containing 20 μM methionine, 1% (day 5) or 20% (day 5 and log phase) dialyzed fetal calf serum, and 150 μCi/ml of [$^3$S]methionine for 5 min. At the end of the labeling period, cells were washed with NaCl/P₅, scraped, and collected by centrifugation at 2000 rpm for 10 min. Pellets were then extracted and immunoprecipitated with mAb6l (anti-tu subunit) as described under "Materials and Methods." Immunoprecipitates were solubilized and radioactive polypeptides were analyzed by 10% NaDodSO₄-polyacrylamide gel electrophoresis. Gels were treated for fluorography as described and exposed for 6 days. Lanes: 1, log phase cultures in 20% serum, 1.5 × 10⁶ cells/dish; 2, day 5 (1% serum, 1.5 × 10⁶ cells/dish); 3, day 6 (1% serum).
Regulation of ACh Receptor α-Subunit mRNA during Differentiation

α-subunit synthesis/culture is not due to an increase in α-subunit mRNA content/culture but is due to the enhanced rate of total protein synthesis/culture. Thus, despite the reduction in translatable α-subunit mRNA in cells following serum stimulation, the increased rate of total protein synthesis under these conditions results in a net increase in the rate of α-subunit synthesis/culture. Similar increases in total protein synthesis have been reported previously following addition of serum to quiescent fibroblasts (32, 33). The increased rate was attributed to an enhanced rate of initiation of translation. The finding that α-subunit is synthesized in cells which have never been differentiated indicates that this protein is constitutively expressed by muscle cells, independent of the degree of differentiation, and is apparently not limiting for assembly of a multisubunit receptor complex. Receptor expression during differentiation, therefore, may be regulated post-translationally, possibly at the level of subunit assembly and/or transport to the surface as we previously reported for differentiated cells which were stimulated to reinitiate growth (20). We are currently investigating these possibilities in an effort to determine whether the lack of receptor expression in growing cells and in differentiated cells which have been stimulated with serum is regulated by common mechanisms. It is also possible that the mRNA encoding the β, γ, and δ subunits is precisely regulated during differentiation of BC3H1 cells and that the accumulation of this mRNA governs the expression of the ACh receptor. Previously, we reported that the inhibitory effects of serum on receptor expression appear to be initiated within a few hours following serum stimulation (20). A rate-limiting subunit mRNA, therefore, would be predicted to exhibit a very short half-life under these conditions in order for the subunit which it encodes to be limiting for receptor assembly within this time period. Examination of the short term effects of actinomycin D on receptor expression in differentiated cells could provide indirect evidence to support this model. Direct examination of the relative levels of the β, γ, and δ subunit mRNAs, however, will require the availability of subunit-specific antibodies or cDNAs.

The molecular mechanisms involved in the regulation of muscle-specific mRNA accumulation during myogenic differentiation have been the subject of considerable controversy. A role for translational control of protein synthesis in muscle cell differentiation was suggested in early studies by Dym et al. (34) and others (35, 36) who demonstrated the presence of myosin heavy chain mRNA in translationally inactive mRNP particles prior to myoblast fusion and the subsequent transfer of this mRNA species to polysomes at the time of fusion. Similarly, Yaffe and Dym (37, 38) reported the presence of muscle-specific mRNAs prior to fusion as evidenced by the ability of cultured rat muscle cells to synthesize these proteins following treatment with actinomycin D at the time of fusion. Transcriptional regulation of muscle gene expression has been suggested in more recent studies by Hastings and Emerson (39) and others (40, 41) who have shown through the use of purified cDNA probes that the accumulation of mRNAs coding for the proteins of the thick and thin filaments of the contractile apparatus is coordinated with myoblast fusion.

The coordination between the growth state of muscle cells and the expression of differentiation-specific mRNAs has been previously reported for other muscle-specific RNA sequences. Nguyen et al. found that the transcription of myosin heavy chain mRNA was obligatorily coupled to the withdrawal of L6E9 myoblasts from the cell division cycle. Furthermore, under nonfusing conditions the accumulation of this mRNA species was reversible during the early stages of differentiation by reinitiation of cell division (42). Accumulation of translatable creatine phosphokinase mRNA in BC3H1 cells has also been shown to be dependent on the withdrawal of cells from the cell cycle. Conversely, re-entry of quiescent differentiated cells into the cell division cycle was found to result in the arrest of accumulation of creatine phosphokinase mRNA in a similar manner to that described for α-subunit mRNA in the present study (4). The low level of induction (3- to 4-fold) of α-subunit mRNA during differentiation of BC3H1 cells, in addition to the presence and functional translation of this mRNA species in undifferentiated cells, however, clearly differs from the characteristics of induction of other muscle-specific proteins.

Whether or not the accumulation of α-subunit mRNA and creatine phosphokinase mRNA is regulated during differentiation of the BC3H1 cell line at the level of transcription or post-transcriptionally, by stabilization or processing of RNA transcripts, for example, will be the subject of future studies. It also remains to be determined whether or not receptor regulation in the developing muscle fiber in vivo takes place by mechanisms similar to those described in the present study.

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REFERENCES

1. Merlie, J. P., Buckingham, M. E., and Whalen, R. G. (1977) Curr. Top. Dev. Biol. 5, 181-234
2. Linkhart, T. A., Clegg, C. H., and Hausekka, S. D. (1981) Dev. Biol. 86, 19-30
3. Emerson, C. P., and Beckner, S. K. (1975) J. Mol. Biol. 93, 431-447
4. Olsoa, E. N., Caldwell, K. C., Gordon, J. I., and Glaser, L. (1983) J. Biol. Chem. 258, 2644-2652
5. Devlin, B. H., and Konigsberg, I. R. (1977) J. Cell Biol. 75, 24a
6. Merrifield, P. A., and Konigsberg, I. I. (1981) J. Cell Biol. 91, 23a
7. Conti-Pessacoli, B. M., and Ratterly, M. A. (1982) Annu. Rev. Biochem. 51, 491-530
8. Fambrough, D. M. (1979) Physiol. Rev. 59, 165-227
9. Anderson, D. J., and Blobel, G. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 5996-8992
10. Claudio, T., Ballivet, M., Patrick, J., and Heinemann, S. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 1111-1115
11. Ballivet, M., Patrick, J., Lee, J., and Heinemann, S. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 4466-4470
12. Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Furutani, Y., Hirose, T., Assai, M., Inayama, S., Mayata, T., and Numa, S. (1982) Nature (Lond.) 299, 793-797
13. Sumikawa, K., Houghton, M., Smith, J. C., Bell, L., Richards, B. M., and Barnard, E. A. (1982) Nucleic Acids Res. 10, 5809-5822
14. Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Kikyotani, S., Hirose, T., Furutani, Y., Takashima, H., Inayama, S., Mayata, T., and Numa, S. (1983) Nature (Lond.) 302, 529-532
15. Devillers-Thiery, A., Giraudrat, J., Bentoulet, M., Char Geoffrey, J.-P. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 2067-2071
16. Merlie, J. P., Sebbane, R., Gardner, S., and Lindstrom, J. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 3843-3847
17. Patrick, J., McMullan, J., Wolson, H., and O'Brien, J. C. (1977) J. Biol. Chem. 252, 2143-2153
18. Schubert, D., Harris, J., Devine, C. E., and Heinemann, S. (1974) J. Cell Biol. 61, 398-43
19. Merlie, J. P., Sebbane, R., Gardner, S., Olson, E. N., and Lindstrom, J. (1983) Cold Spring Harbor Symp. Quant. Biol. 48, in press
20. Olson, E. N., Glaser, L., Merlie, J. P., Sebbane, R., and Lindstrom, J. (1980) J. Biol. Chem. 258, 13946-13950
21. Cox, R. A. (1989) Methods Enzymol. 12, 120-129
22. Deeley, R. G., Gordon, J. I., Burn, A. T. H., Myllinax, K. P., Bina-Stein, M., and Goldberger, R. F. (1977) J. Biol. Chem. 252, 8310-8319
23. Merlie, J. P., Hofler, J. G., and Sebbane, R. (1981) J. Biol. Chem. 256, 6986-6999
24. Sebbane, R., Clokey, G., and Merlie, J. P. and Lindstrom, J. (1983) J. Biol. Chem. 258, 3303-3306
Regulation of ACh Receptor α-Subunit mRNA during Differentiation

25. Laemmli, U. K. (1970) _Nature (Lond.)_ **227**, 680–685

26. Tzartos, S. J., and Lindstrom, J. (1980) _Proc. Natl. Acad. Sci. U. S. A._ **77**, 755–759

27. Tzartos, S. J., Rand, D. E., Einarson, B. L., and Lindstrom, J. M. (1981) _J. Biol. Chem._ **256**, 8635–8645

28. Tzartos, S. J., Seybold, M. E., and Lindstrom, J. M. (1982) _Proc. Natl. Acad. Sci. U. S. A._ **79**, 188–192

29. Tzartos, S. J., and Lindstrom, J. (1982) in _Monoclonal Antibodies-Endocrine Research_ (Fellow, R., and Eisenbarth, G., eds) pp. 69–86, Raven Press, New York

30. Johnson, L. F., Abelson, H. T., Green, H., and Penman, S. (1974) _Cell_ **1**, 95–100

31. Yaffe, D., and Saxel, O. (1977) _Nature (Lond.)_ **270**, 725–

32. Stanners, C. P., and Becker, H. (1971) _J. Cell. Physiol._ **77**, 31–42

33. Rudland, P. S. (1974) _Proc. Natl. Acad. Sci. U. S. A._ **71**, 750–754

34. Dym, H. P., Kennedy, D. S., and Heywood, S. M. (1979) _Differentiation_ **12**, 145–155

35. Heywood, S. M., and Kennedy, D. S. (1976) _Prog. Nucleic Acid Res. Mol. Biol._ **19**, 477–484

36. Robbins, J., and Heywood, S. M. (1978) _Eur. J. Biochem._ **82**, 601–608

37. Dym, H., Turner, D. C., Eppenberger, H. M., and Yaffe, D. (1978) _Exp. Cell Res._ **113**, 15–21

38. Yaffe, D., and Dym, H. (1972) Cold Spring Harbor Symp. Quant. Biol. **37**, 543–548

39. Hastings, K. E. M., and Emerson, C. P. (1982) _Proc. Natl. Acad. Sci. U. S. A._ **79**, 1553–1557

40. Medford, R. M., Wydro, R. M., Nguyen, H. T., and Nadal-Ginard, B. (1980) _Proc. Natl. Acad. Sci. U. S. A._ **77**, 5745–5753

41. Affara, N. A., Daubas, P., Weydert, A., and Gros, F. (1980) _J. Mol. Biol._ **140**, 459–470

42. Nguyen, H. T., Medford, R. M., and Nadal-Ginard, B. (1983) _Cell_ **34**, 281–293
Expression of acetylcholine receptor alpha-subunit mRNA during differentiation of the BC3H1 muscle cell line.
E N Olson, L Glaser, J P Merlie and J Lindstrom

J. Biol. Chem. 1984, 259:3330-3336.

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