Synthesis of Rat Myosin Light Chains in Heterokaryons Formed between Undifferentiated Rat Myoblasts and Chick Skeletal Myocytes

WOODRING E. WRIGHT
Department of Cell Biology, University of Texas Health Science Center, Dallas, Texas 75235

ABSTRACT The control of gene expression during terminal myogenesis was explored in heterokaryons between differentiated and undifferentiated myogenic cells by analyzing the formation of species specific myosin light chains of chick and rat skeletal muscle. Dividing L6 rat myoblasts served as the biochemically undifferentiated parent. The differentiated parental cells were mononucleated muscle cells (myocytes) that were obtained from primary cultures of embryonic chick thigh muscle by blocking myotube formation with EGTA and later incubating the postmitotic cells in cytochalasin B. Heterokaryons were isolated by the selective rescue of fusion products between cells previously treated with lethal doses of different cell poisons. 95-99% pure populations of heterokaryons formed between undifferentiated rat myoblasts and differentiated chick myocytes were obtained. The cells were labeled with [35S]methionine, and whole cell extracts were analyzed on two-dimensional polyacrylamide gels. These heterokaryons synthesize the light chain of chick myosin and both embryonic and adult light chains of rat skeletal myosin. Control homokaryons formed by fusing undifferentiated cells to themselves did not synthesize skeletal myosin light chains. Control heterokaryons formed between undifferentiated rat myoblasts and chick fibroblasts also failed to synthesize myosin light chains. These results indicate that differentiated chick muscle cells provide some factor that induces L6 myoblasts to synthesize rat myosin light chains. This system provides a model for investigating the processes by which differentiated cell functions are induced.

Cells expressing different differentiated programs mutually suppress specialized functions when hybridized (1). This observation suggests that during the developmental program cells may produce molecules that inhibit alternate pathways. Previous experiments have not addressed the issue of whether differentiated cells contain molecules that can induce differentiation in cells from earlier stages of the same developmental pathway. The behavior of heterokaryons formed by fusing a differentiated cell to its undifferentiated precursor would resolve this question. We describe here the application of this approach to the study of the control of skeletal myosin light chain synthesis during terminal differentiation.

Skeletal myosin has a molecular weight of ~450,000 and is composed of two 200,000 mol wt heavy chains associated with several different light chains ranging in molecular weight from 15,000 to 30,000 (2). Although both fast and slow light chains can be expressed in cultured muscle cells (3), the principal forms synthesized in short-term cultures are the fast light chains. Because the fast light chain 1 (LC1) is the most prominent form on two-dimensional polyacrylamide gels, LC1 was selected as the protein marker for the expression of differentiated functions. Chick and rat myoblasts were chosen for the present experiments because the skeletal myosin light chains specific to each species can be resolved on two-dimensional polyacrylamide gels, permitting the identification of differentiated markers from each parental genome.

The established line of L6 rat myoblasts (4) was chosen to be the undifferentiated parent because of the ease of maintaining dividing cultures in a biochemically undifferentiated state. These cells are determined for myogenesis, and are capable of differentiating if they are permitted to reach confluency. Differentiated L6 cells synthesize only the embryonic form of
skeletal myosin LC1 (5–7). Embryonic chick skeletal muscle cells served as the differentiated parent. Cultures of freshly isolated chick embryonic myoblasts are morphologically and biochemically undifferentiated. After dividing several times they become postmitotic and spontaneously fuse to form multinucleated myotubes. Myotube formation itself is not a prerequisite for the expression of differentiated functions because muscle-specific products are synthesized in mononucleated cells that have been prevented from fusing by low calcium concentration (8–11), cytochalasin B (12), or other agents (13, 14).

Mononucleated cells were used to avoid gene dosage effects that could occur if multinucleated myotubes were fused to undifferentiated mononucleated myoblasts. Mononucleated differentiated muscle cells are referred to as myocytes to distinguish them from both multinucleated myotubes and undifferentiated myoblasts.

Classic hybridization approaches in which fused cells must divide and produce hybrid colonies were inappropriate for these experiments. Because the difference between these parental cells is only one of several cell divisions, the expression of differentiated functions in hybrid clones isolated many cell generations after fusion would probably not reflect the initial molecular interactions. Furthermore, because differentiated myocytes are postmitotic, restricting the analysis to only those clones capable of cell division would clearly bias results against the expression of the differentiated functions of the postmitotic cells. We thus performed our analysis on heterokaryons, the initial fusion products before cell division has begun. The results of these experiments demonstrate that undifferentiated rat myoblasts are stimulated to synthesize both embryonic and adult rat skeletal muscle light chains when fused to chick myocytes. This suggests but does not fully establish the presence of positive inductive regulatory molecules in the terminally differentiated chick cells that govern the expression of this protein.

MATERIALS AND METHODS

Cell Lines

The established line of L6 rat myoblasts (4) was cultivated in medium composed of four parts Dulbecco's modified Eagle's medium to one part medium 199 supplemented with 15% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. Although this line will grow in lower concentrations of fetal bovine serum or horse serum, high concentrations of FBS were chosen to ensure that the cells remained in an undifferentiated rapidly proliferating state. Cells were subcultivated three times weekly before confluency was reached. The subclone of L6 is the same as the one used in the studies of Whalen et al. (5, 6). It is near diploid and has a modal number of 41 chromosomes (15).

Primary Embryonic Chick Cultures

Skeletal myoblasts were obtained from the thigh muscle of 12-d embryonic chickens. Thigh muscles were dissected, minced, then aspirated vigorously in FBS conditioned by a 24-h exposure to confluent dishes of chick fibroblasts. The cells were pelleted and washed. 10⁶ cells that had been treated with diethylpyrocarbonate were made in absolute ethanol. The final dilution into diethylpyrocarbonate is on the order of 15 min at 0°C, all stock dilutions of diethylpyrocarbonate for L6 rat myoblasts. Because the aqueous half-life of diethylpyrocarbonate is on the order of 15 min at 0°C, all stock dilutions of diethylpyrocarbonate were made in absolute ethanol. The final dilution into Hanks' BSS coincided with the start of the treatment time. After the 30-min incubation, cells were pelleted and washed. 10⁶ cells that had been treated with iodoacetamide were mixed with 10² diethylpyrocarbonate-treated cells, the mixture was pelleted, the supernate discarded, and then the cells were centrifuged a final time to remove traces of saline from the walls of the test tube. The pellet was then aspirated to dryness and resuspended in polyethylene glycol 1000 (PEG) (20), which was diluted with medium containing 15% dimethylsulfoxide (21). Under these conditions, 35% PEG (vol/vol) fused 10–15% of the cells with iodoacetamide treated myocytes, which were labeled at earlier or later time-points. Using this approach, 19 of 20 experiments showed the induction of rat myosin light chain synthesis by PEG, and 16 of 19 showed the induction of rat skeletal muscle myosin heavy chain synthesis. These results were then confirmed as well as others (19). The general procedure we have developed for the biochemical selection of heterokaryons has been described in detail elsewhere (19). The specific values for doses and cell number given below are representative, but were adjusted according to the needs of the individual experiment. Cultures were treated for 30 min at 0°C in Hanks' balanced salt solution (BSS) containing either iodoacetamide or diethylpyrocarbonate (Sigma Chemical Co.). Typical doses of the irreversible inhibitors used to kill parental cells were 4.5 mM iodoacetamide or 0.004% diethylpyrocarbonate for chick myocytes and 5.5 mM iodoacetamide or 0.0005% diethylpyrocarbonate for L6 rat myoblasts. Because the aqueous half-life of diethylpyrocarbonate is on the order of 15 min at 0°C, all stock dilutions of diethylpyrocarbonate were made in absolute ethanol. The final dilution into Hanks' BSS coincided with the start of the treatment time. After the 30-min incubation, cells were pelleted and washed. 10⁷ cells that had been treated with diethylpyrocarbonate for 45 s, then diluted with 1 ml of complete medium. The cells were then plated at 2 x 10⁵ cells/cm² in 15% FBS. Cells were plated in high serum to prevent heterokaryons or control cells from differentiating as a response to culture conditions rather than to the interactions occurring as a result of cytoplasmic mixing.

Biochemical Selection for Heterokaryons

The general procedure we have developed for the biochemical selection of heterokaryons has been described in detail elsewhere (19). The specific values for doses and cell number given below are representative, but were adjusted according to the needs of the individual experiment. Cultures were treated for 30 min at 0°C in Hanks' balanced salt solution (BSS) containing either iodoacetamide or diethylpyrocarbonate (Sigma Chemical Co.). Typical doses of the irreversible inhibitors used to kill parental cells were 4.5 mM iodoacetamide or 0.004% diethylpyrocarbonate for chick myocytes and 5.5 mM iodoacetamide or 0.0005% diethylpyrocarbonate for L6 rat myoblasts. Because the aqueous half-life of diethylpyrocarbonate is on the order of 15 min at 0°C, all stock dilutions of diethylpyrocarbonate were made in absolute ethanol. The final dilution into Hanks' BSS coincided with the start of the treatment time. After the 30-min incubation, cells were pelleted and washed. 10⁶ cells that had been treated with iodoacetamide were mixed with 10² diethylpyrocarbonate-treated cells, the mixture was pelleted, the supernate discarded, and then the cells were centrifuged a final time to remove traces of saline from the walls of the test tube. The pellet was then aspirated to dryness and resuspended in polyethylene glycol 1000 (PEG) (20), which was diluted with medium containing 15% dimethylsulfoxide (21). Under these conditions, 35% PEG (vol/vol) fused 10–15% of the cells with iodoacetamide treated myocytes, which were labeled at earlier or later time-points. Using this approach, 19 of 20 experiments showed the induction of rat myosin light chain synthesis by PEG, and 16 of 19 showed the induction of rat skeletal muscle myosin heavy chain synthesis. These results were then confirmed as well as others (19). The general procedure we have developed for the biochemical selection of heterokaryons has been described in detail elsewhere (19). The specific values for doses and cell number given below are representative, but were adjusted according to the needs of the individual experiment. Cultures were treated for 30 min at 0°C in Hanks' balanced salt solution (BSS) containing either iodoacetamide or diethylpyrocarbonate (Sigma Chemical Co.). Typical doses of the irreversible inhibitors used to kill parental cells were 4.5 mM iodoacetamide or 0.004% diethylpyrocarbonate for chick myocytes and 5.5 mM iodoacetamide or 0.0005% diethylpyrocarbonate for L6 rat myoblasts. Because the aqueous half-life of diethylpyrocarbonate is on the order of 15 min at 0°C, all stock dilutions of diethylpyrocarbonate were made in absolute ethanol. The final dilution into Hanks' BSS coincided with the start of the treatment time. After the 30-min incubation, cells were pelleted and washed. 10⁶ cells that had been treated with diethylpyrocarbonate for 45 s, then diluted with 1 ml of complete medium. The cells were then plated at 2 x 10⁵ cells/cm² in 15% FBS. Cells were plated in high serum to prevent heterokaryons or control cells from differentiating as a response to culture conditions rather than to the interactions occurring as a result of cytoplasmic mixing.

The following protocol was adopted to reduce the toxicity of dying parental cells. The cells were treated and fused on day 0 and initially plated at low density in bacteriological-grade petri dishes. Heterokaryons take 24–36 h to recover from the trauma of biochemical selection before they are able to reattach. Bacterial dishes were used to ensure that the cells remained in suspension during the 24–48 h before final plating (see below). On day 1 aliquots of the floating cells were transferred to tissue-culture petriwells, then fixed, stained, and counted the next day (day 2). Experiments in which >95% of the nuclei were in heterokaryons were then performed as follows. Based upon the specific rescue obtained, the surface area necessary to give a surviving heterokaryon density of 60,000 cells/cm² was calculated. The remaining cells were then centrifuged on a Ficoll–sodium diatrizoate cushion (lymphocyte sedimentation medium, density 1.08, Litton Bionetics). Most of the dead cells were unable to exclude the Ficoll and therefore pelleted, whereas most of the viable heterokaryons remained at the Ficoll-medium interface. The interface cells were then harvested, washed, and plated in the calculated surface area in gelatinized Falcon microtiter II dishes. The cells were washed and fed [³⁵S]methionine containing medium on the afternoon of day 3. After labeling overnight, the cells were harvested the morning of day 4. In some cases cells were labeled at earlier or later time points. Using this approach, 19 of 20 experiments showed the induction of rat myosin light chains. Homokaryon controls were also performed using this procedure.

In all experiments, aliquots of chick myocytes or L6 rat myoblasts were plated on bacterial dishes for the first 2 d, then replated at equivalent densities in microwell plates and labeled along with the heterokaryons. These controls verified the differentiated or undifferentiated status of the parental cells.

Cell Labeling and Two-Dimensional Gels

Methionine free Eagle's minimal essential medium containing 10% undialyzed FBS was conditioned by a 24-h exposure to confluent dishes of chick fibroblasts.
Conditioning produced a twofold increase in subsequent [35S]methionine incorporation, possibly by reducing the cold methionine contributed by the serum. The use of dialyzed serum produced inferior results. Heterokaryons were washed once with methionine-free medium and then labeled overnight in conditioned methionine-14C medium containing 300 μCi/ml of [35S]methionine. The next morning cells were washed twice in Hanks' BSS, rinsed very quickly with distilled water to remove salts, and extracted directly into lysis buffer (22).

Two-dimensional gels were run according to O'Farrel (22). pH 4-6 ampholytes (Bio-Rad Laboratories, Richmond, Calif.) were used for isoelectric focusing and 12.5% polyacrylamide gels separated proteins by molecular weight. The thickness of the second dimension was reduced to 0.6 mm to increase the sensitivity of autoradiographic detection of [35S]methionine without resorting to fluorography. 1-3 x 107 TCA-precipitable counts were loaded for each sample. Dried gels were exposed to Kodak XR-5 medical x-ray film for 9-12 x 108 cpm x d.

Myosin heavy chains aggregate and do not form discrete spots on twodimensional gels. The migration of chick and rat skeletal myosin light chains on two-dimensional gels has been well characterized (3, 5, 7). The location of these light chains in cell extracts was confirmed by their absence from undifferentiated cells, their appearance after differentiation, their comigration with the myosin heavy chains after treatment with anti-myosin heavy chain antibodies.

Partial Purification of Actomyosin

Actomyosin from heterokaryons was purified by a modification of the method of Luzatti and Druegeon (23). Labeled heterokaryons were washed twice in cold Hanks' BSS, once in cold low-salt buffer (30 mM KCI, 10 mM Tris, pH 7.0, 14 mM 2-mercaptoethanol) and then lysed in cold low-salt buffer containing 0.5% Nonidet P-40 (NP-40) and 30 μg of cold carrier chick actomyosin. The extract was centrifuged for 10 min at 4°C in a Beckman microfuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.), the supernate discarded, and the pellet extracted for 3 h in high-salt buffer (600 mM KCI, 10 mM Tris, pH 7.0, 14 mM 2-mercaptoethanol). Insoluble material was removed by a second 10-min centrifugation, and the dissolved actomyosin was diluted 1:20 with distilled water and allowed to precipitate overnight. After a final 10-min centrifugation, the pellet was extracted into lysis buffer and analyzed on two-dimensional gels.

Immune Purification of Myosin

The myosin synthesized in heterokaryons was purified using Staphylococcus aureus to precipitate immune complexes formed between antiamyosin antibodies and heterokaryon cell extracts. Rabbit antibodies prepared against the heavy chain of fetal bovine skeletal muscle were obtained as a gift from Dr. J.-C. Lelong, Paris. Formaldehyde-inactivated Staphylococcus aureus, strain Cowan I, was prepared according to Kessler (24). Labeled heterokaryons were extracted with magnesium salt buffer MSB (1 mM MgCl2, 0.25 M KCl, 50 mM Tris, pH 7.4, 1% NP-40), and the nuclei removed by centrifugation. 25 μl of antiserum was added to an extract from 107 heterokaryons in 100 μl of MSB and incubated at 0°C for 1 h. 250 μl of a 10% solution in MSB of inactivated bacteria was added, incubated for 15 min at 0°C, then pelleted in a Beckman microfuge. The pellet was washed twice in washing buffer (0.15 M NaCl, 0.5% NP-40, 0.5% sodium deoxycholate, 0.1% sodium laureyl sulfate, 5 mM EDTA, 5 mM nonradioactive methionine) and then centrifuged for 15 min at room temperature into O'Farrel's lysis buffer (22) and processed for two-dimensional gels.

RESULTS

Isolation of Heterokaryons

Heterokaryons were obtained by selection with irreversible biochemical inhibitors. In this system, each parental population is treated with a lethal dose of a different irreversible agent. After the unreacted inhibitor is washed away, the two populations are mixed and fused with PEG. Because a different lesion is produced in each population, complementation (rescue) can occur in heterokaryons, whereas parental cells and homokaryons die. Labeling experiments have demonstrated that only authentic heterokaryons are rescued (19). Fig. 1 shows the typical appearance of biochemically isolated heterokaryons between chick myocytes and rat myoblasts. The differential staining properties of the chick (light) and rat (dark) nuclei provide clear confirmation that the selective

FIGURE 1 Heterokaryons between chick myocytes and rat myoblasts. In Giemsa-stained heterokaryons, the nuclei of chick myocytes stain more lightly than the L6 rat nuclei, so the heterokaryon nature of the binucleated cells is evident. These cells were from a 98% pure population of heterokaryons formed by fusing iodoacetamide-treated chick myocytes to diethylpyrocarbonate-treated rat myoblasts. Cells were fixed 3 d after fusion. Bar, 20 μm.
diethylpyrocarbonate (Fig. 3 A) or if chick myocytes treated with diethylpyrocarbonate were fused to rat myoblasts treated with iodoacetamide (Fig. 3 B). The induction of rat myosin is thus not dependent upon the specific biochemical treatment combination employed. This induction was reproducible, and was observed in 19 of 20 experiments.

L6 myoblast self x self homokaryons were constructed to verify that the biochemical selection procedure itself did not induce myogenic differentiation. Fig. 3 C shows that L6 homokaryon cell extracts exhibited no radioactivity in the light-chain region of the gels. In every experiment, aliquots of untreated and unfused L6 rat myoblasts were plated, labeled, and analyzed to verify the extent of their undifferentiated state. This control was performed to exclude the possibility that areas of local confluence in the proliferating L6 stock populations might have caused a small percentage of L6 cells to differentiate. Indeed, 5% of the experiments were discarded because the synthesis of small but detectable amounts of rat embryonic myosin light chain was observed in these untreated L6 cells. Adult rat light chains were never observed in these controls.

Chick myocyte homokaryons (Fig. 3 D) did not show radioactivity corresponding to the location of rat myosin light chains, and they continued to synthesize chick skeletal light chains. As an additional control, L6 homokaryons were cocultivated with chick myocyte homokaryons (Fig. 3 E). Extracts of these cells showed the synthesis of only chick myosin light chains, demonstrating that diffusible factors from the chick cells were not able to induce rat myosin synthesis under the culture conditions of these experiments. Thus, the synthesis of adult and embryonic rat skeletal myosin light chains was observed only when the chick myocytes were fused with L6 cells.

Because of the delay imposed by the biochemical selection procedure, the earliest time examined was 2 d after heterokaryon formation. Although both embryonic and adult rat LC1 were synthesized at that time, the relative proportion of adult light chain increased over the next few days.

Heterokaryons between L6 rat myoblasts and chick fibroblasts were constructed to determine if nonspecific interactions in avian-mammalian heterokaryons were responsible for the appearance of differentiated rat proteins. Fig. 3 F shows that
these heterokaryons did not synthesize skeletal myosin light chains.

Lack of Requirement for DNA Synthesis before Rat Myosin Induction

Whether a specific “quantal” cycle of DNA synthesis is necessary before the expression of terminal myogenesis is an unresolved question (25-30). Several approaches were adopted to determine if DNA synthesis was required for the synthesis of rat myosin in heterokaryons between chick myocytes and rat myoblasts. Inhibitors of DNA synthesis and inhibitors of differentiation dependent on DNA synthesis failed to prevent the induction of the rat myosin light chains. Cytosine arabinoside (10^-5 M) reduces DNA synthesis in L6 rat myoblasts by >90% within 8 h of its addition to the culture medium (30). 5-Bromodeoxyuridine (BudR) in concentrations >3 μM inhibits the differentiation of L6 rat myoblasts if it is present during DNA synthesis (30, 31). Heterokaryons exposed to either 10^-5 M cytosine arabinoside (Fig. 3G) or 16 μM BudR (Fig. 3H) for the first 4 d after PEG-induced fusion still showed the induction of both forms of rat myosin LCI. Finally, heterokaryons plated in the presence of [3H]thymidine did not incorporate label during the first 6 d after fusion. Table I demonstrates that only 1% of the heterokaryon nuclei incorporated [3H]thymidine during this period.

Possibility of Gene Dosage Effects

Although differentiated functions are suppressed in 1:1 hybrids, a variety of studies has shown that if the hybrid cell contains twice as many chromosomes from the differentiated parent as from the undifferentiated one it can continue to express differentiated functions (32-40). In some gene dosage combinations the undifferentiated cell can be activated to produce differentiated products (34-37, 40). The majority of heterokaryons between chick myocytes and rat myoblasts in this study are binucleates; however, ~20-30% of the cells are trinucleated or tetrnucleated (Table II). Under the counter hypothesis that undifferentiated myoblasts suppress differentiated functions, myosin synthesis might nonetheless continue in those heterokaryons containing more chick than rat nuclei. The identification of possible gene dosage effects in these heterokaryons will be accomplished once antibodies specific for rat skeletal myosin have been obtained.

Table II also shows that the number of binucleates decreases and the number of multinucleated cells increases during the first 3 d after the cells are plated in tissue-culture dishes. This suggests that binucleated heterokaryons are functionally dif-

| Time after heterokaryon formation* | 1     | 2     | 3     | 4     | 5 or more |
|-----------------------------------|-------|-------|-------|-------|-----------|
| d                                 |       |       |       |       |           |
| 3                                 | 3     | 79    | 13    | 3     | 4         |
| 6                                 | 2     | 51    | 19    | 9     | 19        |

* Heterokaryons were transferred from bacterial to tissue-culture grade petri dishes on the evening of day 2, so day 6 represents the behavior of the cells after ~3.5 d of attached culture.

† Numbers represent percent of total cells. About 250 cells were counted for each determination.

DISCUSSION

Previous somatic cell hybridization experiments between cells of different histiotypic origins have led to the concept that differentiated functions are usually suppressed in hybrids between differentiated and “undifferentiated” (fibroblastoid) cells. The present experiments were designed in part to test the hypothesis that alternate regulatory controls might be exhibited if the “undifferentiated” parent was a precursor cell within the same developmental lineage.

These experiments demonstrate that heterokaryons between chick myocytes and undifferentiated rat myoblasts synthesize both chick and rat skeletal myosin light chains. The phenomenon is independent of the specific drug combination used in the selective system, because inverted treatments give the same results. L6 homokaryons do not show the induction of myosin light chains as a result of the biochemical selection procedure, nor do chick homokaryons show the appearance of new proteins in the light chain region of the gel. Thus this phenomenon is not likely to be an artifact resulting from the selection technique. Simple cocultivation of chick myocytes and L6 cells is not sufficient to induce rat myosin synthesis under the culture conditions of these experiments.

These observations imply that the chick cell is providing a factor that either initiates the rat program of differentiation or at least the synthesis of rat myosin light chains. Although this factor might operate by directly inducing terminal myogenesis, a variety of secondary inductive mechanisms are possible. For example, an inhibition of cell division in the L6 nucleus because of the presence of the chick myocyte cytoplasm or nucleus, the provision by the chick membrane of a receptor that responds to the culture environment with a signal to differentiate, or the presence of a chick enzyme that alters the cells metabolism in a critical fashion all could potentially stimulate L6 to express differentiated functions. Such secondary mechanisms must nonetheless be specific for myogenic cells, because chick fibroblasts do not induce the synthesis of skeletal myosin light chains when fused to L6 cells.

Differenitated L6 myotubes synthesize only the embryonic form of skeletal myosin LCI (5-7). The appearance of adult rat light chain in these heterokaryons suggests either that the chick cells contain factors that release the block of adult light-chain synthesis in L6 myotubes or that the chick cells dilute an L6 suppressive factor.

WRIGHT Myosin Synthesis in Myoblast-Myocyte Heterokaryons
The lack of DNA synthesis in the heterokaryon indicates that the expression of at least some differentiated rat proteins is not dependent upon a specific cycle of DNA synthesis during which "reprogramming" might occur. Similar conclusions were obtained by Nadal-Ginard (30) using a highly aneuploid subclone of L6. He showed that populations in which >90% of the cells were capable of forming clones could also differentiate and form myotubes in the absence of additional DNA synthesis. This demonstrated that G1 cells had the option to differentiate or divide. Our results, using an entirely different approach and a nearly diploid line of L6 myoblasts, confirm the conclusion that DNA synthesis is not a prerequisite for the expression of differentiated functions in L6 rat myoblasts.

These experiments differ in several fundamental aspects from previous somatic cell hybridization studies. The use of purified populations of heterokaryons before the onset of cell division eliminates the possibility of artifacts resulting from the loss of particular chromosomes or bias resulting from the selection of only those heterokaryons capable of division to form hybrid clones. The analysis of tens of thousands of independently fused cells ensures that the observed result is representative of the phenotypic interactions that are occurring. The use of diploid (or near-diploid) cells reduces the possibility that the observations reflect abnormal cell interactions. Finally, the isolation of heterokaryons has permitted the use of cells from defined developmental stages that differ from each other by only a few cell divisions.

We have shown that differentiated chick myocytes are able to induce the synthesis of both fetal and adult rat skeletal myosin light chains when fused to undifferentiated L6 rat myoblasts. This suggests that the control of terminal myogenesis may be under positive inductive regulation.

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