Transcriptional and Translational Regulation of Ribosomal Protein Formation during Mouse Myoblast Differentiation*

(Received for publication, August 4, 1986)

Mamta G. Agrawal and Lewis H. Bowman

From the Department of Biology, University of South Carolina, Columbia, South Carolina 29208

The metabolism of ribosomal proteins (r-proteins) and r-protein mRNAs was examined during mouse myoblast differentiation to identify the levels at which r-protein accumulation is regulated. Pulse-chase analyses of r-proteins in myoblasts and fibers indicate that the synthesis of r-proteins is coordinately reduced 2-6-fold following myoblast differentiation and that newly synthesized r-proteins do not turnover. This decreased synthesis of r-proteins in fibers is due to both a reduction in the steady-state levels of r-protein mRNAs and a decrease in the translational efficiency of r-protein mRNAs. Northern analyses of r-protein mRNA indicate that the steady-state levels of r-protein mRNAs S16, L18, and L32 are decreased 1.5–2.0-fold in fibers as compared to myoblasts. Analyses of the distribution of r-protein mRNAs in polysome gradients indicate that their translational efficiencies are reduced 1.3–1.6-fold in fibers as compared to myoblasts. To determine if the decrease in the steady-state levels of r-protein mRNAs is regulated at the level of transcription, the transcription of these genes was measured in isolated nuclei. These experiments show that the transcription of these r-protein genes is reduced 2–6-fold following myoblast differentiation. Thus, the production of r-proteins is regulated both at the level of transcription and translation during mouse myoblast differentiation.

The rate of ribosome accumulation usually decreases several fold in nondividing cells as compared to their rapidly dividing counterparts. This decrease occurs because nondividing cells only need to produce enough ribosomes to balance ribosome degradation while dividing cells must produce ribosomes to balance degradation as well as to keep pace with cell division. As ribosomes are comprised of both rRNA and ribosomal proteins (r-proteins), changes in the rate of ribosome accumulation must involve changes in the metabolism of both rRNA and r-proteins (Hadjiolov and Nikolaev, 1976; Perry, 1976; Warner et al., 1980; Wool, 1980; Meyuhas, 1984).

When mammalian cells stop dividing, the accumulation of both rRNA and r-proteins can be regulated at many different levels. rRNA can be regulated both transcriptionally (Mauck and Green, 1973; Johnson et al., 1976; Drummt et al., 1977; Krauter et al., 1979; Dabeva and Dudov, 1982; Cavanaugh and Thompson, 1983; Jacobs et al., 1985) and post-transcriptionally (Cooper, 1973; Clissold and Cole, 1973; Abelson et al., 1974; Bowman and Emerson, 1977; Wolf et al., 1980). The accumulation of r-proteins is frequently regulated post-translationally, either by the turnover of newly synthesized r-proteins (Krauter et al., 1979; Jacobs et al., 1985) or by a decrease in the translational efficiency of r-protein mRNAs (Geyer et al., 1982). However, during liver regeneration the levels of r-protein mRNAs increase, but it is not known whether this is due to changes in the transcription of r-protein genes (Faliks and Meyuhus, 1982). This diversity in the mechanisms regulating ribosome formation suggests that there are several different intracellular pathways and extracellular signals that coordinate ribosome formation. The basic elements of these pathways and signals are not known.

Mouse myoblast cell culture is an excellent system for identifying the extracellular signals and dissecting the intracellular mechanisms that regulate ribosome formation. This is because cell division and differentiation are easily manipulated in cell culture by altering the concentrations of mitogens in the culture medium (Hauschka et al., 1979; Linkhart et al., 1981). In medium containing a high concentration of fibroblast growth factor and serum, myoblasts divide rapidly. After they deplete the medium of fibroblast growth factor, they permanently withdraw from the cell cycle and fuse to form multinucleated muscle fibers which actively synthesize contractile proteins and other proteins (Emerson and Becker, 1975). As a first step in elucidating the extracellular signals and intracellular mechanisms that regulate ribosome formation in differentiating mouse myoblasts, we have shown that the rate of rRNA formation is reduced 2–3-fold following myoblast differentiation due to a decrease in the rate of rDNA transcription (Bowman, 1987).

In this paper, we report measurements of the synthesis and stability of r-proteins and measurements of the steady-state levels, transcriptional rates, and translational efficiencies of r-protein mRNAs during mouse myoblast differentiation. These experiments indicate that both the transcription of r-protein genes and the translational efficiencies of r-protein mRNAs decrease following myoblast differentiation. These results establish the mouse myoblast system as an excellent system for studying the mechanisms and signals for the transcriptional coupling of r-protein and rRNA genes.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

**Synthesis and Stability of r-Proteins**—The relative rate of r-protein synthesis in myoblasts and fibers was measured by

1 Portions of this paper (including "Experimental Procedures") are presented in miniprint at the end of this paper. The abbreviation used is: MD, dithiothreitol.

Received for publication, August 4, 1986

Printed in U.S.A.
pulse labeling techniques. Warner (1977) showed that most pulse-labeled r-proteins could be identified from whole cell extracts by two-dimensional gel electrophoresis, although some nonribosomal proteins are highly labeled and do obstruct the analysis of some r-proteins. To determine whether any highly labeled nonribosomal proteins obstruct the analysis of pulse-labeled r-proteins from myoblast cultures, myoblasts were labeled for 30 min with [3H]lysine, and the homogenate was mixed with stainable amounts of r-proteins isolated from 80 S ribosomes. Proteins were extracted from whole cell extracts and fractionated by a two-dimensional gel electrophoresis system optimized for r-proteins (Lastick and McConkey, 1976). Comparison of the staining profile of the gel (Fig. 1A) and the fluorogram (Fig. 1B) indicates that many of the spots on the fluorogram co-migrate with authentic r-proteins. However, several non-r-protein spots are also present in the fluorogram (labeled A–E). Two of these spots are much more highly labeled than the r-protein spots and obstruct the analysis of the r-protein spots that migrate in their immediate vicinity. Similar results were obtained when pulse-labeled fiber proteins were analyzed (data not shown). However, this analysis does indicate that the radioactivity incorporated into many r-proteins can be unambiguously measured.

To determine the relative rate of r-protein synthesis, cultures of myoblasts and fibers were labeled for 30 min with [3H]lysine. After harvesting the cells, stainable amounts of unlabeled r-proteins were added to the homogenate to facilitate identification of r-proteins on the two-dimensional gels. In addition, a known amount of [14C]-labeled r-protein was added to the homogenate to control for the recovery of r-proteins during extraction and electrophoresis. Proteins were then extracted and fractionated on two-dimensional gels and stained with Coomassie Blue. The stained r-protein spots were punched out of the gel and the [3H] and [14C] radioactivity of the spot measured. The [3H]/[14C] ratio for each spot was divided by the [3H]/[14C] ratio in total protein. This normalization controls for differences in the total number of cells labeled and the total incorporation of radioactivity into proteins. This normalized ratio is a measure of the fraction of total protein synthesis devoted to an r-protein. Table I shows these values for 11 small subunit r-proteins and 16 large subunit r-proteins. On the average, the relative rate of r-protein synthesis is 2-fold higher in myoblasts than in fibers. There is some variation from r-protein to r-protein as noticed previously (Warner, 1977). In particular, the synthesis of L7/L7A is not significantly reduced in fibers.

Although these experiments measure the fraction of total protein synthesis devoted to r-proteins, we believe that the measured 2.0-fold decrease in the relative rate of r-protein synthesis following myoblast differentiation reflects a decrease in the absolute rate of r-protein synthesis. This is because the fraction of ribosomes translating mRNA (Fig. 3) and total number of ribosomes (Bowman, 1987) are similar in myoblasts and fibers, suggesting that the total rate of protein synthesis is similar in myoblasts and fibers. Further, the measured 2.0-fold decrease in the rate of r-protein synthesis is similar to the measured absolute decrease in the rate of mature rRNA formation (Bowman, 1987).

The stability of newly synthesized r-proteins was measured in pulse-chase experiments to determine if the accumulation of r-proteins is also regulated by the turnover of newly synthesized r-proteins. Cultures of myoblasts and fibers were labeled for 30 min with [3H]lysine and then chased in medium containing unlabeled lysine. As whole cell extracts were used in these studies, possible nuclear-cytoplasmic compartmen-
talization of newly synthesized r-proteins would not influence the results of these experiments. Table I shows that there is no net incorporation of radioactivity into total protein during the chase. In fact, the radioactivity in total protein decreases slightly during the chase, suggesting that the chase is reasonably effective. Table I indicates that on the average there is little turnover of newly synthesized r-proteins in either myoblasts or fibers. However, some individual r-proteins such as L9 and S20 do turn over in myoblasts but not in fibers. Other proteins such as L28 turn over more in fibers. However, the overall results clearly indicate that the turnover of newly synthesized r-proteins is not a major control point in regulating r-protein accumulation in fibers. Rather, the accumulation of r-proteins in fibers is regulated by a reduced rate of r-protein synthesis.

Steady-state Concentrations of r-Protein mRNAs Decrease during Mouse Myoblast Differentiation—The reduced synthesis of r-proteins in fibers could be due to decreased levels of r-protein mRNAs or to a decreased efficiency of r-protein mRNA translation. To determine if the reduced rate of accumulation of r-proteins is regulated by a decrease in r-protein mRNA concentrations, the steady-state levels of three r-protein mRNAs were measured in myoblasts and fibers using Northern and dot blot filter hybridization techniques ( McMaster and Carmichael, 1977; Thomas, 1983). The hybridization probes used in these studies were cDNA plasmids containing sequences complementary to two large subunit r-protein mRNAs, L18 and L32, and one small subunit r-protein mRNA, S16 (Meyuhas and Perry, 1980). In these and subsequent experiments, we have also monitored the mRNA for a small basic protein of unknown function, p31 (Theodor et al., 1985). These measurements of p31 mRNA have served as a convenient control for our analysis of the metabolism of r-protein mRNAs.

The Northern blot hybridizations of whole cell RNA indicate that the molecular weights of these mRNAs are the same in both myoblasts and fibers (Fig. 2). Furthermore, dot blot analysis of oligo(dT)-cellulose selected mRNA indicates that greater than 90% of these r-protein mRNAs are polyadenylated in both myoblasts and fibers (data not shown). These experiments indicate that there are no gross changes in the structure of these mRNAs during myoblast differentiation.

The relative concentrations of r-protein mRNAs per μg of total RNA in myoblasts and fibers were determined by densitometric scanning of the autoradiographs from the Northern analyses (Fig. 2) and dot blot analyses (data not shown). This analysis indicates that the steady-state concentrations of these r-protein mRNAs and the p31 mRNA are coordinately reduced 1.5-2.0-fold in fibers. Because myoblasts and fibers contain the same amount of RNA per nucleus (Bowman,
185
185
185
185
285
285
285
285
85
85
85
85
r-Protein Probes

Fig. 2. Northern analyses of total cellular RNA from myoblasts and fibers. 10 μg of total cellular RNA from myoblasts (M) and fibers (F) was denatured with glyoxal, fractionated on 1% agarose gels, and blotted to nitrocellulose filters (Thomas, 1985). Filters were hybridized to the S16, L18, and L32 r-protein probes and the p31 probe. The sizes of individual r-protein mRNAs were measured using 28 S and 18 S rRNAs as molecular weight markers assuming that the molecular weight of 28 S rRNA is 1.5 × 10^6 (Hassouna et al., 1984) and 18 S rRNA is 6.2 × 10^5 (Raynal et al., 1984).

The observed reduction in the fraction of polysomal S16, L18, and L32 mRNAs in fibers is not a consequence of the breakdown of fiber polysomes during cellular fractionation. This is because the slot blot filters hybridized to the p31 probe are identical to those hybridized to S16, L18, and L32 r-protein probes. Thus, if breakdown of polysomes was specifically occurring during cellular fractionation of fibers, the fraction of p31 mRNA associated with polysomes would also be significantly lower in fibers as compared to myoblasts. In fact, about 90% of p31 mRNA is associated with polysomes in both myoblasts and fibers (Fig. 3, C-E). Further, these mRNAs are associated with polysomes containing an average of three ribosomes. This is less than the maximum number of ribosomes (four to six) that can theoretically translate mRNAs of this size (Kafatos, 1972). The remaining 50–70% of these r-protein mRNAs co-sediment with the 40 S ribosomal subunits. Furthermore, the translational efficiencies of these r-protein mRNAs decrease during myoblast differentiation. The fraction of S16, L18, and L32 mRNAs associated with polysomes is 1.3–1.6-fold lower in fibers as compared to myoblasts. The magnitude of this decrease is highly reproducible as indicated by the three experiments shown in Table II. Thus, the translation of S16, L18, and L32 mRNA is coordinately reduced in fibers. This 1.3-1.6-fold decrease in the translational efficiencies of r-protein mRNAs coupled with the 1.5-2.0-fold decrease in the steady-state levels of r-protein mRNAs would serve to lower the rate of synthesis of r-proteins 2-3-fold. This is in reasonable agreement with the measured 2-fold decrease in r-protein synthesis (Table I).

Cycloheximide experiments were performed to determine if the large fraction (50–70%) of r-protein mRNAs that co-sediment with the 40 S ribosomal subunits in myoblasts and fibers are irreversibly sequestered from the translational machinery. Cultures of myoblasts and fibers were treated with low concentrations (0.02 μg/ml) of cycloheximide for 30 min before cell fractionation. This low dose of cycloheximide does not completely block protein synthesis in these cultures, but specifically slows the elongation of nascent peptides (Lodish, 1974; Lodish, 1976). Since the rate of initiation is unaffected, this low dose of drug increases the total number of ribosomes translating an mRNA. This effect of cycloheximide is especially true for mRNAs that are not initiated very frequently. Thus, if the r-protein mRNAs co-sedimenting with 40 S ribosomal subunits are not sequestered from the translational machinery, they should be shifted into polysomes in cycloheximide-treated cultures. Table II shows that 75% of S16, L18, and L32 r-protein mRNAs are associated with polysomes in cycloheximide-treated myoblasts and fibers as compared to 30–50% in the non-cycloheximide-treated cells. Thus, a large fraction of the S16, L18, and L32 mRNAs that co-sediment with the 40 S ribosomal subunits are neither r-protein mRNA degradation products nor sequestered irreversibly from the translational machinery.

The Transcription of r-Protein Genes Decreases during Myoblast Differentiation—The decrease in the concentrations of r-protein mRNAs in fibers can be regulated at the level of transcription, RNA processing, or mRNA stability. To determine if the decrease in mRNA concentrations is regulated at the level of transcription, the transcription of the three r-protein genes and the p31 gene was measured in nuclear run-on transcription experiments (McKnight and Palmiter, 1979; Groudine et al., 1981). Nuclei were isolated from myoblasts and fibers and allowed to continue transcribing in vitro in the
Ribosomal Protein Formation during Myoblast Differentiation

**FIG. 3.** Polysomal distribution of r-protein mRNAs. Panel A shows the optical density profiles of the polysomes isolated from myoblasts and fibers. The RNA isolated from fractions along the gradient were applied to slot blot filters. The filters were hybridized to r-protein probes (S16, L18, and L32) and to the p31 probe. In the cycloheximide experiment, the cells were incubated in 0.02 μg/ml cycloheximide for 30 min before harvesting. The amount of mRNA associated with polysomal fractions was determined by the densitometric scanning of the slot blot autoradiograms.

**TABLE II**

Polysomal distribution of r-protein mRNAs

| Probe | Noncycloheximide-treated cells | Cycloheximide-treated cells | Average of 3 experiments | Ratio of myoblast/fiber |
|-------|---------------------------------|-------------------------------|--------------------------|-------------------------|
|       | Myoblast                        | Fiber                         |                          |                         |
| p31   | 92                              | 95                            | 91 93 93 98              | 1.04                    |
| S16   | Myoblast                        | 73                            | 48 47 40 45              | 1.31                    |
|       | Fiber                           | 73                            | 34 35 34 35              |                         |
| L18   | Myoblast                        | 72                            | 39 39 33 37              | 1.57                    |
|       | Fiber                           | 67                            | 26 21 24 23              |                         |
| L32   | Myoblast                        | 74                            | 53 47 48 50              | 1.49                    |
|       | Fiber                           | 78                            | 34 34 31 33              |                         |

In some transcription reactions heparin was added to prevent RNA breakdown and inhibit the reinitiation of transcription completely (Groudine et al., 1981). The transcriptional values derived from the reactions containing or lacking heparin were identical. The radioactivity incorporated into RNA in the transcription reactions was hybridized to filters containing cDNA plasmids for S16, L18, and L32 r-proteins and for the basic protein p31. The radioactivity that hybridized to the filters was measured by densitometry of autoradiograms and by scintillation counting. The fraction of the total radioactivity that hybridized to these probes is slightly variable as indicated by the standard deviations in Table III. The fraction of total radioactivity incorporated into p31 mRNA is 1.3-fold less in fiber nuclear transcription reactions. However, this difference is not significant. In contrast, the fraction of total radioactivity incorporated into S16, L18, and L32 r-protein mRNAs is 3.1-, 3.3-, and 1.9-fold less in fiber nuclear transcription reactions, and these differences are significant.

Although the available evidence indicates that only one of the 10–20 genes for each r-protein is expressed (Dudov and Perry, 1984; Wiedemann and Perry, 1984; Wagner and Perry, 1985), it is certainly possible that one of the r-protein pseudogenes is located within an intron of a gene whose transcription is regulated during myoblast differentiation. To eliminate this possibility, products of the nuclear transcription reactions were also hybridized to filters containing unique intron sequences from the previously identified expressed S16 and L32 genes. Fig. 4 shows the result of an experiment where equal amounts of myoblast and fiber trichloroacetic acid precipitable radioactive were hybridized to such filters. Densitometric analyses of the autoradiograms (Fig. 4) indicate that the presence of [32P]UTP. In order to compare the relative concentrations of r-protein mRNAs in these fractions, the signal in fraction number 3 (mRNA co-sedimenting with the 40 S subunits) was set equal to one in both myoblasts and fibers, and the signals of the other fractions were changed accordingly. These experiments do not measure the absolute amounts of r-protein mRNAs, but their relative distribution along the polysome gradient.
The fraction of radioactivity incorporated in each mRNA (S16, L18, L32, and p31) during *in vitro* transcription reactions was determined by filter hybridization (see "Experimental Procedures"). Column 3 shows the mean + S.D. There is no statistical difference between the p31 transcriptional values in myoblasts and fibers (\( p = 0.10 \)). However, the difference in the transcriptional values of S16, L18, and L32 are significant with the probability of less than 0.005, 0.005, and 0.025, respectively. The values shown in this table are normalized to the hybridization efficiency in each experiment which was determined by including 1000 cpm of p31 \(^{3}H\)cRNA into each filter. The ratio of radioactivity hybridized to the p31 filter was 0 – 3 cpm above machine background, and the hybridization to the p31 filter was 15 – 40 cpm above background.

In some experiments, the radioactivity hybridized to the S16, L18, L32, and p31 during *in vitro* transcription reactions was determined by filter hybridization (see "Experimental Procedures"). Column 4 shows the mean + S.D. There is no statistical difference between the p31 transcriptional values in myoblasts and fibers (\( p = 0.10 \)). However, the difference in the transcriptional values of S16, L18, and L32 is significant with the probability of less than 0.005, 0.005, and 0.025, respectively. The values shown in this table are normalized to the hybridization efficiency in each experiment which was determined by including 1000 cpm of p31 \(^{3}H\)cRNA into each hybridization reaction. Input \(^{3}P\)-RNA in these hybridization reactions ranged from 3 to 8 million cpm. The radioactivity bound to control pBR322 filters was 0 – 3 cpm above machine background, and the hybridization to the p31 filter was 15 – 40 cpm above background. In some experiments, the radioactivity hybridized to the S16, L18, and L32 was only 3 – 5 cpm higher than that bound to the pBR322 filters. However, it was possible to accurately measure this small amount of radioactivity by comparing the intensities of their autoradiographical signals to those of known standards and to the p31 filter using a densitometer (see "Experimental Procedures"). This autoradiographical method was more sensitive in accurately measuring small amounts of radioactivity.

### TABLE III

| Probes | Total No. of experiments | cpm hybridized | cpm hybridized fibers |
|--------|-------------------------|----------------|-----------------------|
| p31    |                         |                |                       |
| Myoblast | 4                      | 18.7 ± 3.40    | 1.3                   |
| Fiber   | 3                      | 14.5 ± 3.40    |                       |
| S16    |                         |                |                       |
| Myoblast | 4                      | 5.65 ± 1.37    | 3.1                   |
| Fiber   | 3                      | 1.85 ± 0.59    |                       |
| L18    |                         |                |                       |
| Myoblast | 4                      | 8.84 ± 2.24    | 3.3                   |
| Fiber   | 3                      | 2.66 ± 0.26    |                       |
| L32    |                         |                |                       |
| Myoblast | 4                      | 8.88 ± 2.00    | 1.9                   |
| Fiber   | 3                      | 4.65 ± 1.79    |                       |

The fraction of radioactivity incorporated into p31, S16, and L32 was 1.7-, 5.0-, and 3.0-fold lower in fibers. These results agree with those using cDNA probes, especially when the radioactivity incorporated into r-protein intron probes is normalized to the radioactivity incorporated into p31 and the r-protein/p31 ratio from myoblasts and fibers are compared.

These assays actually underestimate (by a factor of about 2) the difference in the absolute rate of transcription for these genes, because the total rate of transcription is reduced 2-fold in fibers as compared to myoblasts (Bowman, 1987). Thus, the absolute rate of transcription of these genes is reduced in the range of 2–6-fold following myoblast differentiation.

**DISCUSSION**

Our previous experiments indicate that the accumulation of mature rRNAs is reduced 2–3-fold following mouse myoblast differentiation (Bowman, 1987). This result implies that the accumulation of r-proteins is also reduced 2–3-fold following myoblast differentiation. Experiments reported here indicate that the decreased accumulation of r-protein is regulated by a decreased rate of r-protein synthesis, not by the turnover of newly synthesized r-proteins. This decreased synthesis of r-proteins in mouse muscle fibers is controlled by a decrease in the steady state levels of r-protein mRNAs and a decrease in the translational efficiencies of r-protein mRNAs.

The reduced accumulation of r-proteins in rat muscle fibers is due to the turnover of newly synthesized r-proteins which occurs within 30 min of their synthesis (Krauter *et al.*, 1980; Jacobs *et al.*, 1985). In contrast, we could not detect any significant turnover of r-proteins in mouse muscle fibers, even 4 h after their synthesis. Other significant differences between rat and mouse myoblasts have also been noted. For example, the kinetics of myoblast differentiation are much more rapid in mouse myoblast lines than in rat (Linkhart *et al.*, 1981; Bains *et al.*, 1984; Shani *et al.*, 1981). Furthermore, proliferating mouse and rat myoblasts differ in their capacity to express the human cardiac actin gene (Minty *et al.*, 1986).

Previous studies also indicate that r-protein synthesis can be regulated at the level of mRNA transcription during early development in *Xenopus laevis* (Pierandrei-Amaldi *et al.*, 1982), in resting 3T6 cells (Geyer *et al.*, 1982), in insulin-starved chick embryo fibroblasts (Ignotz *et al.*, 1981), and in yeast (Pearson *et al.*, 1982). The best example of translational regulation of r-protein synthesis occurs in bacteria (Nomura *et al.*, 1980; Olsson and Gausing, 1980). When bacterial r-proteins are synthesized in molar excess of rRNAs, some of the excess r-proteins bind to sequences in their own polycistrionic mRNA and inhibit the translation of all the r-proteins encoded by this mRNA.

The reduced translation of S16, L18, and L32 mRNAs in fibers may be caused by either of two basic mechanisms. The first possible mechanism is based on a model proposed by Lodish (Lodish, 1974; Lodish, 1976). This model states that the translation of mRNAs that have an inherent low rate of peptide chain initiation will be preferentially reduced under conditions where the overall rate of initiation is reduced. If this model is correct for the translational regulation of r-protein mRNA during mouse myoblast differentiation, then r-protein mRNAs should be inherently poor initiators of polypeptide chain initiation, and fibers should have an overall
reduction in the rate of initiation. Our data are consistent with the idea that these r-protein mRNAs (L18, L32, S16) are inherently inefficient at initiation. However, fibers are actively synthesizing contractile proteins, and there is no evidence that the overall rate of peptide initiation is less in fibers than in myoblasts. Another possible mechanism for the specific inhibition of r-protein mRNA translation is that specific inhibitors bind to some r-protein mRNAs and prevent their translation. In this model, the translation of r-protein mRNA molecules is specifically inhibited in both myoblasts and fibers, but the fraction is larger in fibers than in myoblasts. Further experimentation is necessary to determine the molecular mechanism for the specific inhibition of r-protein mRNA translation. However, our results show that a large fraction of the r-protein mRNAs co-sedimenting with 40 S ribosomal subunits are not irreversibly sequestered from the translational machinery, because they are shifted into polysomes in the presence of low concentrations of cycloheximide.

The reduced steady-state levels of S16, L18, and L32 r-protein mRNAs in fibers are regulated by a 2–6-fold decrease in their rates of transcription. Although there are 5–20 genes for each mouse r-protein, only one gene for each r-protein appears to be expressed, and these expressed genes contain introns (Dudov and Perry, 1984; Wiedemann and Perry, 1984; Wagner and Perry, 1985). Our results are consistent with this data, because similar amounts of radioactivity hybridized to cDNA probes and to unique intron probes for the S16 and L32 genes after normalizing the hybridization signal to the length of the probe. However, this does not eliminate the possibility that some r-protein pseudogenes are transcribed at a low level.

It is not known what role, if any, changes in r-protein mRNA processing or stability have in controlling the 1.5–2.0-fold reduction in r-protein mRNA concentrations in fibers. However, the measured 2–6-fold decrease in transcription rates is sufficient to produce the measured 1.5–2.0-fold decrease in r-protein mRNA levels. In fact, theoretical considerations indicate that a 3.7-fold reduction in the transcription rates would cause a 1.8-fold reduction in the steady-state levels of r-protein mRNAs in fibers, assuming that r-protein mRNAs have a half-life of 11 h in both myoblasts and fibers (see legend to Fig. 4). Thus, it is not necessary to invoke changes in r-protein mRNA processing or stability to account for the decreased levels of r-protein mRNAs in fibers, although this analysis certainly does not eliminate the possibility that such changes may occur. However, our results show that changes in the transcription of r-protein genes do play a major role in regulating r-protein mRNA levels. Previous experiments indicate that the formation of rRNA is also regulated at the level of transcription during mouse myoblast differentiation (Bowman, 1987). Thus, the formation of rRNAs and r-proteins are coordinately regulated at the level of transcription during mouse myoblast differentiation.

The pattern of regulation of the mRNA for the basic, nonribosomal protein p31 is quite different from that for the r-protein mRNAs. Even though the concentration of p31 mRNA is also reduced 1.5–2.0-fold in fibers, its transcriptional rate is not significantly reduced in fibers. Among other possibilities, this may indicate that the stability of p31 mRNA is considerably less than the stabilities of r-protein mRNAs, and it must therefore be transcribed at higher rates in fibers. Furthermore, p31 mRNA is translated very efficiently in both myoblasts and fibers whereas the r-protein mRNAs are not. In addition, the translational efficiencies of the r-protein mRNAs decrease during myoblast differentiation, whereas the efficiency of p31 mRNA translation remains the same. These differences point to the specificity of mechanisms regulating the steady-state concentrations and translational efficiencies of r-protein mRNAs.

Acknowledgments—We thank Karen Wood for her excellent technical assistance and Vicki B. Vance and Mike R. Stallcup for critical reading of the manuscript. S. Hauschka generously sent us mouse myoblasts, and R. Perry and O. Meyuhas generously sent us r-protein cDNA plasmids.

REFERENCES

Abelson, H. T., Johnson, L. F., Penman, S., and Green, H. (1974) Cell 1, 161–165
Bowman, L. H. (1987) Dev. Biol. 119, 152–163
Bowman, L. H., and Emerson, C. F., Jr. (1977) Cell 10, 587–596
Bowman, L. H., and Emerson, C. F., Jr. (1980) Dev. Biol. 80, 146–156
Bowman, L. H., and Schlessinger, D. (1981) Nucleic Acids Res. 9, 4951–4966
Cavanaugh, A. H., and Thompson, E. A., Jr. (1983) J. Biol. Chem. 258, 9768–9773
Clackson, T., and Cole, R. J. (1973) Exp. Cell Res. 80, 159–169
Cooper, H. L. (1973) J. Cell Biol. 59, 250–254
Dabea, M. D., and Dudov, K. P. (1982) Biochem. J. 208, 101–108
Denhardt, D. T. (1966) Biochem. Biophys. Res. Commun. 23, 641–646
Drumm, F., Paul, D., and Gumm, I. (1977) Eur. J. Biochem. 76, 7–12
Dudov, K. P., and Perry, R. P. (1984) Cell 37, 457–468
Emerson, C. P., Jr., and Beckner, S. K. (1975) J. Mol. Biol. 93, 431–447
Faliks, D., and Meyuhas, O. (1982) Nucleic Acids Res. 10, 789–801
Gevert, P. K., Meyuhas, O., Perry, R. P., and Johnson, L. F. (1982) Mol. Cell. Biol. 2, 685–693
Groudine, M., Peretz, M., and Weintraub, H. (1981) Mol. Cell. Biol. 1, 281–288
Hadjilov, A. A., and Nikolaev, N. (1976) Prog. Biophys. Mol. Biol. 31, 93–144
Hardy, S. J. S., Kurland, C. G., Voynow, P., and Mora, G. (1969) Biochemistry 8, 2897–2905
Hassouna, N., Michot, B., and Bachellerie, J. (1984) Nucleic Acids Res. 12, 3563–3583
Hruschka, S. D., Linkhart, T. A., Clegg, C. H., and Merrijl, G. M. (1979) in Muscle Regeneration (Mauro, A., ed), pp. 311–322, Raven Press, New York
Ignoz, G. G., Hokari, S., DePhilip, R. M., Tsukada, K., and Lieberman, I. (1981) Biochemistry 20, 2550–2558
Jackson, A. E., Bird, R. C., and Sells, B. H. (1985) Eur. J. Biochem. 150, 255–263
Johnson, L. F., Levis, R., Abelson, H. T., Green, H., and Penman, S. (1976) J. Cell Biol. 71, 933–938
Kafatos, F. C. (1972) Curr. Top. Dev. Biol. 3, 127–191
Konigsberg, W. R. (1979) Methods Enzymol. 58, 511–527
Krauter, K. S., Soeiro, R., and Nadal-Ginard, B. (1979) J. Mol. Biol. 134, 727–741
Krauter, K. S., Soeiro, R., and Nadal-Ginard, B. (1980) J. Mol. Biol. 142, 145–159
Lestick, M., and McConkey, E. H. (1976) J. Biol. Chem. 251, 2967–2975
Linkhart, T. A., Clegg, C. H., and Hauschka, S. D. (1981) Dev. Biol. 86, 19–30
Lodish, H. F. (1974) Nature 251, 385–388
Lodish, H. F. (1976) Annu. Rev. Biochem. 45, 29–72
Maniatis, T., Frisch, E. F., and Sambrook, J. (1982) Molecular Cloning, p. 206, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Mauck, J. C., and Green, H. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 2819–2822
McConkey, E. H., Bielka, H., Gordon, J., Lestick, S. M., Lin, A., Ogata, K., Reboud, J-P., Traugh, J. A., Traut, R. R., Warner, J. R., Welle, H., and Wool, I. G. (1979) Mol. & Gen. Genet. 169, 1–6
McKnight, G. S., and Palmiter, R. D. (1979) J. Biol. Chem. 254, 9060–9068
Ribosomal Protein Formation during Myoblast Differentiation

McMaster, G. K., and Carmichael, G. G. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 4835-4838

Meyuhas, O. (1984) in Recombinant DNA and Cell Proliferation (Stein, G. S., and Stein, J. L., eds), pp. 243-269, Academic Press, Orlando, FL

Meyuhas, O., and Perry, R. P. (1980) Gene 20, 113-129

Minty, A., Blau, H., and Kedes, L. (1986) Mol. Cell. Biol. 6, 2137-2146

Nomura, M., Yates, J. L., Dean, D., and Post, L. E. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 1086-1089

Olsson, M. O., and Gausing, K. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 7084-7087

Palmiter, R. D. (1973) J. Biol. Chem. 248, 8260-8270

Penman, S., Vesco, C., and Penman, M. (1968) J. Mol. Biol. 34, 49-69

Perry, R. P. (1976) Annu. Rev. Biochem. 46, 605-629

Palmiter, R. D. (1973) J. Biol. Chem. 248, 8260-8270

Perry, R. P. (1980) Nature 283, 599-600

Perry, R. P. (1985) Mol. Cell. Biol. 5, 3559-3568

Raynal, F., Michot, B., and Bacheiller, S. (1984) FEBS Lett. 187, 263-268

Rogers, T. D., and David, I. B. (1983) Science 222, 135-139

Shani, M., Zevin-Sonkin, D., Saxol, O., Carmon, Y., Katcoff, D., Nudel, U., and Yaffe, D. (1981) Biochim. Biophys. Acta 626, 483-492

Sargent, T. D., and David, I. B. (1983) Science 222, 135-139

Thomas, P. S. (1983) Methods Enzymol. 100, 255-256

Vieira, J., and Messing, J. (1982) Gene 19, 255-268

Wagner, M., and Perry, R. P. (1985) Mol. Cell. Biol. 5, 3560-3567

Wagner, J. R. (1971) J. Mol. Biol. 115, 315-333

Wiser, J. M., and Weksler, P. S. (1980) in Ribosomes: Structure, Function and Genetics (Chambis, A., ed), pp. 889-902, University Park Press, Baltimore

Wiedemann, L. M. and Perry, R. P. (1980) Mol. Cell. Biol. 11, 2518-2528

Wolf, S., Sameshima, M., Lieblhaber, S. A., and Schlessinger, D. (1980) Mol. Cell. Biochem. 355, 70-80

Wool, I. G. (1980) in Ribosomes: Structure, Function and Genetics (A. Chambis, A., ed), pp. 797-824, University Park Press, Baltimore

**TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION OF RIBOSOMAL PROTEIN FORMATION**

For the analysis of northern blots, 10 μg of total cellular RNA from various tissues were subjected to electrophoresis in 1% agarose gel containing 6% formaldehyde. The size of each band was then estimated and compared to other bands.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**

The myoblast cell line C2C12 was generously supplied by Dr. Harikrishna Nagarajan (1984). Cells were grown in a 5% CO₂ atmosphere at 37°C in DMEM supplemented with glutamine and 10% FCS. The medium was changed every other day by removing the medium and replacing it with fresh medium.

**Preparation and Purification of Ribosomal Proteins**

Ribosomal proteins were isolated from rat liver and kidney tissues as described previously (Raynal et al., 1984). The proteins were solubilized in 8 M urea and 1% SDS and then purified by gel filtration on a column of Sephadex G-75. The purified proteins were concentrated and dialyzed against 50 mM Tris-HCl, pH 7.4, 1 M NaCl, and 1 mM EDTA.

**Transcriptional Analyses**

For the determination of transcriptional activity, RNA was isolated from various tissues by the guanidinium isothiocyanate method and fractionated on formaldehyde-agarose gels. The RNA was then transferred to nitrocellulose filters and hybridized with ribosomal protein probes. The filters were scanned with a Molecular Dynamics densitometer and the intensity of the bands was quantified.

**Northern Blot Analyses**

For the dot and slot blot analyses, RNA was isolated from various tissues by the guanidinium isothiocyanate method and fractionated on formaldehyde-agarose gels. The RNA was then transferred to nitrocellulose filters and hybridized with ribosomal protein probes. The filters were scanned with a Molecular Dynamics densitometer and the intensity of the bands was quantified. The results were compared to those obtained from control tissues.

**Electrophoretic Mobility Shift Assays**

DNA-protein complexes were prepared by incubating purified ribosomal proteins with a labeled DNA probe. The complexes were then analyzed by gel electrophoresis.

**Immunoblot Analyses**

For the detection of ribosomal proteins, cell extracts were prepared by suspending the cells in a buffer containing 20% glycerol, 1 M NaCl, and 10 mM EDTA. The extracts were then subjected to electrophoresis on SDS-PAGE gels and transferred to nitrocellulose filters. The filters were hybridized with ribosomal protein probes and the intensity of the bands was quantified.

**RESULTS**

**Ribosomal Protein Formation during Myoblast Differentiation**

The results presented in this section demonstrate that ribosomal protein synthesis is downregulated during myoblast differentiation. This downregulation is accompanied by a decrease in the expression of certain ribosomal protein genes that are normally upregulated during cell proliferation.

**DISCUSSION**

The results presented in this section demonstrate that ribosomal protein synthesis is downregulated during myoblast differentiation. This downregulation is accompanied by a decrease in the expression of certain ribosomal protein genes that are normally upregulated during cell proliferation. The findings presented in this section provide evidence for a coordinated regulation of ribosomal protein synthesis during myoblast differentiation.

**ACKNOWLEDGMENTS**

The authors wish to thank Dr. Harikrishna Nagarajan for generously supplying the myoblast cell line C2C12 and Dr. Michael Millman for his helpful discussions.

**REFERENCES**

1. Ball, D., and Millman, M. (1984) Mol. Cell. Biol. 4, 1145-1151

2. Ball, D., and Millman, M. (1984) Mol. Cell. Biol. 4, 1145-1151

3. Ball, D., and Millman, M. (1984) Mol. Cell. Biol. 4, 1145-1151

4. Ball, D., and Millman, M. (1984) Mol. Cell. Biol. 4, 1145-1151

5. Ball, D., and Millman, M. (1984) Mol. Cell. Biol. 4, 1145-1151