Single-fiber myosin heavy chain polymorphism during postnatal development: modulation by hypothyroidism

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Di Maso, Nick A., Vincent J. Caiozzo, and Kenneth M. Baldwin. Single-fiber myosin heavy chain polymorphism during postnatal development: modulation by hypothyroidism. Am J Physiol Regulatory Integrative Comp Physiol 278: R1099–R1106, 2000.—The primary objective of this study was to follow the developmental time course of myosin heavy chain (MHC) isoform transitions in single fibers of the rodent plantaris muscle. Hypothyroidism was used in conjunction with single-fiber analyses to better describe a possible linkage between the neonatal and fast type IIB MHC isoforms during development. In contrast to the general concept that developmental MHC isoform transitions give rise to muscle fibers that express only a single MHC isoform, the single-fiber analyses revealed a very high degree of MHC polymorphism throughout postnatal development. In the adult state, MHC polymorphism was so pervasive that the rodent plantaris muscles contained ~12–15 different pools of fibers (i.e., fiber types). The degree of polymorphism observed at the single-fiber level made it difficult to determine specific developmental schemes analogous to those observed previously for the rodent soleus muscle. However, hypothyroidism was useful in that it confirmed a possible link between the developmental regulation of the neonatal and fast type IIB MHC isoforms.

MYOSIN HEAVY CHAIN (MHC) protein isoforms found in rodent hindlimb skeletal muscle are encoded by a highly conserved multigene family (12, 14, 18) that is responsible for the expression of six known isoforms. These have been identified as embryonic (Emb), neonatal (Neo), slow type I, fast type IIA, fast type IIX, and fast type IIB MHC isoforms. Expression of the various MHC genes is highly plastic and regulated in a developmental manner (7, 8, 11–13).

The neonatal MHC isoform profile of rodent skeletal muscles shortly after birth is primarily characterized by a predominance of Emb and Neo MHC protein isoforms (1, 2, 7, 8, 15, 17). Between ~10–20 days of age, there is a rapid transition from the Emb and Neo MHC isoforms to the adult patterns of MHC expression (1, 7, 8).

Within this general framework, however, a number of fundamental issues are yet to be resolved. Of central importance, the developmental origin of muscle fiber type diversity in adult rodent slow and fast skeletal muscle remains poorly described. Butler-Browne and Whalen (2) found two major pools of fibers in postnatal (1 wk neonates) rodent soleus muscle. One pool (~50% of the fibers) consisted of fibers that coexpressed both Emb and slow myosin isoforms. The other pool (the remaining 50%) of fibers coexpressed both the Emb and Neo myosin isoforms. Because the adult rodent soleus muscle contains ~80–90% so-called slow type I fibers, it is clear that both pools of fibers must give rise to slow type I fibers. Given the preponderance of slow type I fibers in the adult state, Butler-Browne and Whalen (2) proposed a relatively simple developmental model (Fig. 1) uncomplicated by the diversity of fast MHC isoforms (i.e., fast type IIA, IIX, and IIB MHC isoforms).

At the single-fiber level, much less is known about developmental MHC isoform transitions in rodent fast skeletal muscles, especially those that express all four MHC isoforms. At the whole muscle level, we recently used hypothyroidism (−T3) as a tool to modulate developmental transitions in the rodent plantaris muscle (1). Interestingly, we found what appears to be an inverse stoichiometric relationship between the Neo and fast type IIB MHC isoforms during development. However, it is not clear how such a relationship is manifested at the single-fiber level. Additionally, the whole muscle analyses suggested that there was also a relationship, although less distinct, between transitions in the Emb and IIA/IIX isoforms (1).

Given these considerations, there were two key objectives of this study. First, we employed electrophoretic analyses to follow the developmental time course of MHC isoform transitions at the single-fiber level. In so doing, we were interested in 1) identifying possible developmental schemes that give rise to the adult pattern of fiber types and 2) determining the degree of monop/ polymorphism at each time point examined. Second, we used −T3 in conjunction with single-fiber analyses to better delineate a possible linkage between the Neo and fast type IIB MHC isoforms during postnatal development.

Collectively, the findings of this study are contrary to the general concept that postnatal development gives rise to muscle fibers that express only a single MHC isoform. Rather, the single-fiber analyses revealed a very high degree of MHC polymorphism throughout postnatal development. In the adult state, polymorphism was so pervasive that the rodent plantaris...
muscles contained ~15 different pools of fibers (i.e., fiber types), as defined by MHC isofrom composition. The degree of polymorphism observed at the single-fiber level made it difficult to determine specific developmental schemes analogous to that described by Butler-Browne and Whalen (2). However, -T3 appeared to confirm a possible link between the developmental regulation of the Neo and fast type IIB MHC isoforms.

**MATERIALS AND METHODS**

Animal care and experimental design. Twelve pregnant Sprague-Dawley rats (Taconic Farms, Germantown, NY) were assigned to one of two groups: control (Con; n = 6) or -T3 (n = 6). The adult animals assigned to the -T3 group were thyroidectomized by the vendor before pregnancy and given supplemental daily injections of propylthiouracil (PTU) starting immediately after giving birth. PTU was administered intraperitoneally at a dosage of 12 mg/kg for 40 days.

Pups delivered by the thyroidectomized dams received daily injections of PTU (12 mg/kg) until the time of death. For each group (i.e., Con and -T3), one to two pups from each litter (total n = 7 per time point) were killed at 5, 10, 20, or 40 days of age.

The PTU doses for the adult rats were selected based on previous studies demonstrating that 12 mg/kg is a dose three times that needed to completely block conversion of T4 to T3 (2). However, -T3 appeared to confirm a possible link between the developmental regulation of the Neo and fast type IIB MHC isoforms.

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Table 1. Plasma levels of thyroid hormone at 40 days postpartum

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<th>Control</th>
<th>Hypothyroid</th>
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<tr>
<td>T₄, µg/dl</td>
<td>4.41 ± 0.55</td>
<td>0.32 ± 0.05†</td>
</tr>
<tr>
<td>T₃, ng/dl</td>
<td>113.97 ± 16.55</td>
<td>67.77 ± 8.47*</td>
</tr>
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Values are means ± SE. T₄ and T₃ levels for control calculated with n = 7; T₂ and T₃ levels for hypothyroid group calculated with n = 11. Significantly different from control group. *P ≤ 0.01, †P ≤ 0.001.

quantified using a Molecular Dynamics Densitometer (Sunnyvale, CA) to determine the relative percentage of each isoform in a sample.

Single-fiber determination of MHC isoform composition. Approximately 50 single fibers per muscle sample (total n = 2,191 fibers) were dissected using microsurgical forceps (Super Fine Dumont tweezers, Biomedical Research Instruments, Rockville, MD) and a dissecting microscope (Technival 2, aus Jena, Germany). Single fibers were placed into 500-µl polypropylene microcentrifuge tubes (1 fiber per tube) that contained 30 µl of the sample buffer mentioned above (3). Each single-fiber solution was then heated at 100°C for 2 min. The entire 30 µl of this solution were loaded into the well of a gel, and electrophoresis was performed as described above. Relative distributions of each MHC protein isoform within a given single fiber were determined using a densitometer after the gel was stained using a silver stain kit (see Fig. 2).

Statistical analyses. All data except for the fiber type distribution data are reported as the means ± SE. All statistical analyses were performed using a computer program (Systat, Evanston, IL). The whole muscle MHC data were analyzed using two-way ANOVA. The data for each MHC isoform were analyzed independently of the other isoforms. The Tukey test was used to determine differences between the two groups at each time point. Correlation coefficients between single-fiber data and whole muscle data were determined via linear regression analyses. Differences between population distributions were determined using chi-square analysis. Only those chi-square tests that were statistically significant are reported in the RESULTS section. Statistical significance was defined as P ≤ 0.05.

RESULTS

Evidence of −T₃. Plasma concentrations of T₄ and T₃ were significantly lower in the −T₃ group compared with the Con group (Table 1). Consistent with the lower levels of circulating thyroid hormone, body weight, heart weight, and muscle weight were significantly less in the −T₃ group (Table 2). Collectively, these data demonstrate that the approach taken in this study was effective in inducing a hypothyroid state.

Whole muscle MHC protein isoform transitions. As shown in Fig. 2, the techniques used in the present study were capable of identifying the two developmental and four adult MHC isoforms. At 5 days postpartum, the Neo (49%) and Emb (37%) MHC isoforms comprised ~86% of the total MHC pool in the Con group. The remaining 14% of the MHC pool consisted of relatively small proportions of slow type I (9%), fast type IIA (2–3%) and fast type IIB (2–3%) MHC isoforms (Fig. 3).

The Con group demonstrated an essentially complete transition from immature neonatal MHC isoform profile to the adult pattern is largely blunted in −T₃ group. A: embryonic; B: neonatal; C: type I; D: type IIA; E: type IIX; F: type IIB. Two-way ANOVA for each MHC isoform data set demonstrated that there was a significant group effect (P ≤ 0.001), time effect (P ≤ 0.001), and group × time interaction (P ≤ 0.001) with exception that slow type I MHC isoform group effect was not statistically significant (P > 0.05). Significant differences at each time point were determined by using Tukey test. *P ≤ 0.05; †P ≤ 0.07; ‡P ≤ 0.001.

Table 2. Physical characteristics of animals and plantaris muscles

<table>
<thead>
<tr>
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<th>Control</th>
<th>Hypothyroid</th>
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<tbody>
<tr>
<td>5 Day</td>
<td>10 Day</td>
<td>20 Day</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>11 ± 0.3</td>
<td>21 ± 0.7</td>
</tr>
<tr>
<td>Heart wt, mg</td>
<td>59 ± 2.5</td>
<td>104 ± 6.4</td>
</tr>
<tr>
<td>Muscle wt, mg</td>
<td>3.4 ± 0.1</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td>Body/heart wt</td>
<td>5.4</td>
<td>5.0</td>
</tr>
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Values are means ± SE. Significantly different from control group: *P ≤ 0.01, †P ≤ 0.001.
completely repressed by 20 days of age and were concomitantly replaced by the type IIA, IIX, and IIB MHC isoforms. The MHC protein isoform pattern of expression at this time point revealed an MHC profile consisting of type IIB (43%), type IIX (22%), and type IIA (17%) MHC isoforms that comprised ~82% of the total MHC pool. Type I (11%) and Neo (7%) MHC isoforms made up the remaining 18% of the total MHC pool. Essentially the same pattern existed at 40 days of age, with the exception that the Neo isoform became almost fully repressed.

Effects of hypothyroidism on whole muscle MHC protein isoform transitions. At 5 days of age, the MHC isoform composition of the -T3 group was similar to that of the Con group. However, it is evident that the transition to the adult MHC isoform profile was largely blunted in the -T3 group (Fig. 3). It appears that the hypothyroid state effectively prevented the plantaris muscle from progressing through a normal transition process whereby the IIB MHC isoform becomes the predominant adult fast MHC (Fig. 3). This is exemplified by the fact that the phenotypic profile in the hypothyroid group at 20 days of age was nearly identical to that of postnatal day 5. Moreover, the upregulation of the fast type IIB MHC isoform was effectively blunted throughout the 40 days examined in this study (Fig. 3).

Correlation between whole muscle and single-fiber MHC protein isoform expression. The use of single-fiber electrophoretic MHC analyses is only valid if certain conditions are met: 1) a large number of fibers must be sampled and 2) the fibers sampled must be selected at random. Previous studies (3, 4) using this approach demonstrated very high correlation coefficients between whole muscle MHC isoform composition predicted from single-fiber analyses and actual whole muscle samples. Figure 4 represents linear regression analyses of whole muscle MHC isoform composition predicted from single-fiber analyses versus that determined from actual whole muscle samples. With the exception of the slow type I MHC isoform, the coefficients of determination (r² values) were generally >0.87, while the slopes approximated 1.0. From this information, it appears that the single-fiber MHC analyses provided an accurate assessment of whole muscle MHC protein isoform content.

The large y-intercept, dramatic departure of the slope from 1.0, and small r² value for the slow type I MHC isoform were probably due to the high degree of homogeneity in the data set for this isoform.

Developmental transitions and MHC polymorphism in singlefibers. At 5 days of age, ~86% of the total MHC pool in the whole muscle was comprised of Neo (49%) and Emb (37%) MHC isoforms (Fig. 5). At the single-fiber level, ~60% of the fibers in the Con plantaris muscles coexpressed both the Emb and Neo MHC isoforms. The other 40% of fibers not only coexpressed both Emb and Neo MHC isoforms but also contained various combinations of adult MHC isoforms (Fig. 5).

Transitions in single-fiber MHC isoform composition were subtle between 5 and 10 days postpartum (data not shown). In contrast, there were dramatic transitions in single-fiber MHC isoform composition at 20 days postpartum. Unlike the earlier time points, the single-fiber MHC isoform composition at 20 days postpartum was highly polymorphic with ~18 different fiber types (Fig. 5). Additionally, unlike earlier time points, none of the fibers at 20 days postpartum coexpressed just the Emb and Neo MHC isoforms. Instead, the fibers were fractionated into two groups: 1) those coexpressing the Neo MHC isoform in conjunction with various combinations of adult MHC isoforms and 2) fibers expressing various combinations of only the adult MHC isoforms (Fig. 5). Importantly, it should be noted that the former group of fibers (i.e., those containing the Neo MHC isoform) contained a relatively small amount of the Neo MHC isoform (Fig. 5B, inset). As
noted from the whole muscle data, the transition to the adult state was almost complete by 40 days postpartum. However, this transition to the adult state did not result in large pools of fibers expressing only a single isoform. Rather, as shown in Fig. 5, the single-fiber MHC isoform profile at 40 days postpartum continued to be highly polymorphic, and the largest pool of fibers seen at this time point was the IIx/IIB fibers (Fig. 5).

Effects of hypothyroidism on single-fiber MHC protein isoform developmental transitions and coexpression patterns. The single-fiber MHC isoform composition of the $-T_3$ group at 5 days postpartum was similar to that seen in the Con group (Fig. 6). At this time point, both groups had significant pools of Emb/Neo, Emb/Neo/I, and Emb/Neo/I/IIB fibers. The transition in single-fiber MHC isoform composition between 5 and 20 days postpartum was relatively minor for the $-T_3$ group, with most fibers continuing to express large...
proportions of both the Emb and Neo MHC isoforms. This is in dramatic contrast to the significant transitions seen in the Con group. The most significant transition observed at 20 days postpartum in the \(-T_3\) group was the large reduction in Emb/Neo fibers and the concomitant appearance of Emb/Neo/IIB fibers (Fig. 6). However, the relative proportion of the fast type IIB MHC isoform was very small in the Emb/Neo/IIB fibers. In contrast to 20 days postpartum, the single-fiber MHC isoform composition at 40 days postpartum was highly polymorphic with \(\sim 19\) different pools of fibers. Whereas the relative content of the Emb MHC isoform declined from 20 to 40 days postpartum, the majority of the fibers continued to coexpress both the Emb and Neo MHC isoforms, but in combination with various adult MHC isoforms (Fig. 6).

**DISCUSSION**

D’Albis et al. (8) published an extensive data set demonstrating that a large number of rodent muscles undergo substantial native myosin isoform transitions between \(\sim 10\) and 20 days postpartum. During this period, the developmental isoforms (i.e., Emb and Neo) are replaced by so-called adult native myosin isoforms.

Despite this extensive set of data, very little is known about how such transitions to the adult state are manifested at the single-fiber level. In this context, Butler-Browne and Whalen (2) examined the postnatal development of the rodent soleus muscle using immunohistochemical techniques. Their findings suggest that the large proportion of slow type I fibers found in the adult rodent soleus muscle (\(\sim 80-90\%\)) arise mainly from two different pools of neonatal fibers: 1) those coexpressing Emb and slow myosin and 2) those coexpressing Emb and Neo myosin. This rather simple developmental scheme in the soleus muscle benefits from several factors. First, the rodent soleus muscle contains a large proportion of fibers that only express the slow type I MHC isoform (3). Second, the adult rodent soleus muscle expresses basically only two of the four adult MHC isoforms under normal conditions (3). As a result, the developmental scheme in the rodent soleus muscle is not confounded by the presence of the two other fast adult MHC isoforms (i.e., the fast type IIX and type IIB MHC isoforms). Third, the rodent soleus muscle contains a relatively small proportion of polymorphic fibers (3).

In contrast to the study of Butler-Browne and Whalen (2), the current study focused on the postnatal development of a muscle (i.e., the rodent plantaris muscle) that expresses all four MHC isoforms in the adult state. The findings of the current study demonstrate that the developmental transitions in the MHC isoform composition of individual fibers of the rodent plantaris muscle are very complex with a large number of polymorphic fibers evident at each time point. At 5 days of age, seven different pools of polymorphic fibers were observed (Fig. 5). The largest pool of fibers (\(\sim 60\%\)) at this time point coexpressed both the Emb and Neo MHC isoforms. The six remaining pools of fibers coexpressed various combinations of developmental and adult MHC isoforms (Fig. 5). Single-fiber MHC polymorphism was even more complex at the 40 day time point, at which there were at least 15 different MHC isoform profiles. One of the consequences of the extensive polymorphism found in the rodent plantaris muscle was a complexity that made it impossible to derive any clear developmental scheme(s) analogous to that proposed for the rodent soleus muscle (2).

Currently, it is not clear whether the complex postnatal developmental pattern observed in the rodent plantaris muscle represents a scheme common to other muscles that express all four adult MHC isoforms. Interestingly, however, we have recently observed a high degree of polymorphism in other muscles like the vastus intermedius (VI) and the red region of the gastrocnemius (RMG; unpublished observations). On this basis, we postulate that both the VI and RMG muscles will also exhibit a complex pattern of polymorphism during postnatal development. Clearly, however, future studies are required to determine the applicability of the current findings to developmental transitions of other fast muscles.

With regard to the classical concept of fiber type (i.e., monomorphic MHC isoform expression), it should be noted that individual fibers expressing only one MHC isoform represented a small proportion of the total population of fibers observed in the rodent plantaris muscle (Fig. 5). In this context, the two largest pools of fibers evident at the 40-day time point were the IIX/IIB (34% of total pool of fibers) and IIA/IIX/IIB (15% of total pool of fibers) fibers.

As shown in Fig. 6, \(-T_3\) was effective in preventing the development of the adult state. This is evidenced by the fact that at 40 days postpartum, virtually all of the fibers continued to express the developmental MHC isoforms. As noted by the presence of \(\sim 19\) different fiber types at 40 days postpartum, however, \(-T_3\) did not simplify the complexity of developmental transitions, rather it created a different pattern of transition (Fig. 6).

As noted above and on the basis of the Con data shown in Fig. 3, it was difficult to determine specific schemes of MHC isoform transitions during normal development. This was due to the fact that between 10 and 20 days postpartum, there was a rapid decline in the two developmental MHC isoforms that was accompanied by substantial increases in the relative content of each of the fast MHC isoforms. In this regard, \(-T_3\) served as a useful tool, because it completely blunted the downregulation of the Neo MHC isoform and concomitantly prevented the upregulation of the fast type IIB MHC isoform. Hence, \(-T_3\) seemed to uncover a coupling between the regulation of the Neo and fast type IIB MHC isoforms. Concurrent to this effect, there was a significant decrease in the Emb MHC isoform that was accompanied by increases in the slow type I, fast type IIA, and fast type IIX MHC isoforms. On the basis of these observations, it seems reasonable to conclude that the regulation of the Neo and fast type IIB MHC isoforms are somehow coupled to one another and that this process is mediated by thyroid hormone.
In the adult state, the fast type IIB MHC isoform represented the largest pool of MHC isoform (~45% of the total MHC pool; Fig. 3). As shown in Fig. 5, most of the fast type IIB MHC isoform at 40 days postpartum was found in three pools of fibers (listed in order of magnitude): IIX/IIB fibers, fast type IIB fibers, and IIA/IIX/IIB fibers. At the whole muscle level, \(-T_3\) prevented the normal upregulation of the fast type IIB MHC isoform during development. At the single-fiber level, this was manifested by a large reduction in the three fiber types noted above under normal conditions (i.e., IIX/IIB, IIB, and IIA/IIX/IIB) and a reduction in the relative proportion of the fast type IIB MHC isoform found within a given pool of fibers (e.g., Emb/Neo/IIX/IIB).

With respect to the large pool of IIX/IIB fibers observed at 40 days postpartum, it is tempting to hypothesize that this pool was derived from two pools of fibers seen at 20 days postpartum: the existing IIX/IIB fibers and Neo/IIX/IIB fibers. Whereas this appears to be the most parsimonious explanation for the presence of the IIX/IIB fibers, a note of caution is warranted given that such a simple additive scheme did not apply to the IIA/IIX/IIB fibers seen at 40 days postpartum.

As noted above, \(-T_3\) completely blunted the developmental transitions normally seen for the Neo and fast type IIB MHC isoforms. In contrast, \(-T_3\) slowed but did not prevent the downregulation of the Emb MHC isoform during postnatal development. Similarly, \(-T_3\) retarded the upregulation of the fast type IIA and type IIX MHC isoforms during postnatal development. In this regard, it would appear as though \(-T_3\) was also effective in uncovering coregulation among developmental transitions involving the Emb, fast type IIA, and fast type IIX MHC isoforms (Fig. 3).

Analyses of MHC isoform transitions during development have been primarily characterized by whole muscle electrophoretic techniques and immunohistochemistry. Clearly, whole muscle analyses of MHC isoform transitions cannot adequately address issues related to the origin of muscle fiber types during development. In this regard, immunohistochemical analyses are clearly more insightful. However, as we have noted previously (3), immunohistochemical analyses of developmental MHC isoform transitions are limited in several respects. First, it is clear that many muscle fibers exhibit polymorphic expression of MHC isoforms during both postnatal development and in the so-called adult state. In this regard, current immunohistochemical techniques can only confirm or reject the presence of a given MHC isoform within a given muscle fiber. Such techniques cannot be used to estimate the relative proportion of a given MHC isoform. In contrast, as shown in this study and others (3, 4), the single-fiber electrophoretic approach is able to address this disadvantage by providing a quantitative estimate of the relative MHC composition within a single fiber. We support the position that the ability to estimate the relative proportions of the various MHC isoforms expressed within a given single fiber could be crucial in understanding the significance of MHC polymorphism.

Second, within the context of the first point, it should be noted that monoclonal antibodies (MAb) vary in their affinity. Hence, it is possible that a single fiber might coexpress two MHC isoforms, but the detection of one of the isoforms might go unrecognized because it is present at a low concentration (e.g., ~15% of the total MHC pool in the fiber) and the MAb specific for that isoform has a low affinity. Third, as noted by us previously (3), the single-fiber electrophoretic technique provides a definitive method for identifying the presence/absence of the fast type IIX MHC isoform within a given fiber. Of the three fast MHC isoforms, the fast type IIX MHC isoform has proven the most difficult to characterize from an immunohistochemical perspective. This is due, in part, to the fact that it has been difficult to produce a monoclonal antibody (MAb) that selectively recognizes the fast type IIX MHC isoform. Additionally, we are not aware of a viable cell line that secretes a MAb that recognizes the fast type IIX MHC isoform. Given that the fast type IIX MHC isoform represents a substantial proportion of the total MHC pool found in a number of fast muscles, the inability to properly characterize the presence of this isoform represents a substantial limitation for any study examining developmental schemes in such muscles.

It should be emphasized that electrophoretic analysis of single-fiber MHC isoform composition is also subject to some important technical considerations. As noted above, the type of single-fiber analyses employed in this study can only be considered valid if two criteria are met: 1) random sampling and 2) adequate sample size. The types of analyses presented in Fig. 4 provide strong evidence to suggest that both of these criteria were met in this study. In addition to these issues, the categorization of fiber type based on a highly sensitive technique (as was used in the current study) gives rise to pools of fibers in which a specific MHC isoform may represent a small proportion of the total MHC isoform pool within that fiber. A good example of this is the pool of Emb/Neo/IIX/IIB fibers shown in Fig. 6C, inset. The fast type IIB MHC isoform represented ~4% of the total MHC pool found in these fibers. The question arises as to how such fibers should be classified. Should the presence of a given MHC isoform be ignored because it represents a small fraction of the total MHC pool? If so, what is an acceptable value to ignore? We would argue that a true understanding of MHC isoform plasticity can only be understood by accurately reporting the presence/absence of given MHC isoforms. Hence, in the current study, we did not employ an arbitrary threshold for classifying fiber types, but categorized fibers simply by the presence of MHC isoforms within a given fiber.

Perspectives

The single-fiber results demonstrate that the postnatal development of the rodent plantaris muscle is extremely complex and involves a degree of polymorphism unreported previously. The hypothyroid state inhibits the normal developmental transitions of undiff-
ferentiated fibers to adult phenotype. In so doing, however, $-T_3$ produces a different pattern of polymorphism that retains a high degree of complexity. It is interesting to note that the polymorphic Emb/Neo fibers observed at 5 days must be responsible for a large proportion of the heterogeneity observed at 42 days postpartum. This finding combined with that of Butler-Browne and Whalen (2) suggests that the genetic program of the polymorphic Emb/Neo fibers is capable of producing a wide variety of different fiber types. Within this context, it is interesting to note that hypothyroidism prevents the conversion of Emb/Neo fibers to IIx/IIB fibers presumably by preventing the upregulation of the fast type IIB MHC isoform. The strong influence of hypothyroidism on this population of fibers is in direct contrast to that observed in the adult condition (6a). Further studies will be necessary to understand the mechanistic basis of this differential response of IIx/IIB fibers. Overall, the findings of this study suggest that the Neo type IIB and Emb type IIa/IIX MHC genes appear to be coregulated by some common process. The Neo isoform transition seems to be locked into a coregulatory relationship with only the IIB MHC. Whereas, the Emb isoform appears to be more permissive, transitioning to at least three different MHC isoforms, i.e., type I, IIA, and IIx. Future studies will examine developmental transitions in the white region of the gastrocnemius and vastus lateralis muscles in which the fast type IIB MHC isoform represents ~80–90% of the total MHC pool. If the Neo and IIB MHC isoforms are coregulated as suggested by the findings of this study and that of Adams et al. (1), then hypothyroidism should prevent the upregulation of the fast type IIB MHC isoform in these muscles.

Currently, the functional importance of the high degree of polymorphism found in the plantaris is not clear. There are several intriguing issues that future studies need to address. First, the high degree of polymorphism may be one method for minimizing the functional consequences of MHC isoform transitions (see Ref. 6a for a more detailed discussion on this issue). Second, it is not clear whether the coexpression of MHC isoforms is uniform along the length of a fiber or whether there are segmental differences. Finally, it is not clear how the different pools of polymorphic fibers are distributed across motor unit pools. If the expression of a given MHC isoform is dependent on neural factors, as suggested by a large number of investigators, then how does this mechanism account for fibers that coexpress combinations of IIa/IIx/IIB or IIA/IIX/ IIB MHC isoforms as reported in the current study (see 40-day Con) and in previous studies (3, 4)?

In conclusion, it is apparent that postnatal development of the fast-twitch rodent plantaris muscle, unlike its slow-twitch counterpart, the soleus muscle, is highly complex and does not conform to the prevailing opinion that under steady-state conditions adult fibers express only one MHC isoform.

This research was supported by grants from National Institute of Neurological Disorders and Stroke (NS-33483) and National Aeronautics and Space Administration (NAG5–3741).

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