Visual system maldevelopment disrupts extraocular muscle-specific myosin expression

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The extraocular muscles (EOMs) of mammals represent a functionally heterogeneous group of skeletal muscles that are responsible for generating not only rapid saccadic eye movements but slow vergence and pursuit movements as well. The functional diversity of this muscle group is reflected in its contractile protein composition. These studies addressed the expression and regulation of one of these muscle proteins, myosin heavy chain (MHC), in rat extraocular muscle. Mature EOMs exhibit a unique myosin isoform phenotype, including the retention of developmental isoforms, expression of a tissue-specific isoform, and longitudinal variations in the expression of some developmental and fast myosins. The regulation of this unique myosin phenotype is not well understood, although roles for both genetic and epigenetic components have been considered. Given the functional requirements associated with this muscle group, epigenetic influences may be key in understanding how the EOM phenotype is established.

The diversity of the EOM properties may result from the extreme demands placed on them by the wide dynamic range of the oculomotor system. Little is known about the role played by the visual system in modulating development of extraocular motor units. By contrast, the development of the visual afferents that drive eye movements proceeds under the regulation of well-established activity-dependent mechanisms. The visual cortex of mammals is anatomically and physiologically immature at birth, with key properties such as binocularity and depth perception developing during a species-specific postnatal window. This process depends on the visual experience obtained during an early period of plasticity known as the critical period. Alterations in sensory experience disrupt the maturation of neural connections, but only if they occur during the critical period. Perturbations occurring either before or after this period do not have consequences for visual development.

Two experimental paradigms, dark rearing (DR) and monocular deprivation (MD), are commonly used in the study of visuomotor development. DR during the visual critical period influences the properties of visual cortical neurons. DR delays visual cortical development but prolongs the critical period, so its effects can be largely reversible (12, 14, 36, 59). By contrast, MD permanently disrupts the development of binocularly driven neurons in the primary visual cortex by creating an asymmetry in visual inputs, thereby inhibiting the activity-dependent maturation of binocular cells in the visual cortex. Both paradigms directly affect the oculomotor system, inducing profound visuomotor deficits (2, 9, 10, 11, 15, 19–22, 34, 43, 45, 48, 50, 55, 56) and consequences for the EOM motor unit (29, 32). In summary, maldevelopment of the visuomotor system by visual deprivation, as in untreated strabismus or amblyopia, likely has consequences for the contractile phenotype of the EOMs. Knowledge of the interactions between visual inputs and EOM properties is important for an understanding of both muscle regulation and response in ocular motility disorders.

In these studies, the role of the visual system in directing EOM maturation was assessed by using two visual deprivation paradigms, DR and MD. Isoforms of the contractile protein MHC were used as an index of phenotypic change in the EOMs after these visual insults.
**MATERIALS AND METHODS**

DR experiments. Juvenile and adult rats were housed in a lightproof box equipped with a ventilation system to maintain oxygen levels and ambient temperature. Three experimental groups of rats were dark reared. Rats in group 1 were raised from birth to 45 days postnatal (P45) in complete darkness. Age-matched controls (P45 rats) were used for comparison with this group. Rats in group 2 were dark reared from birth as in group 1, but at P45 they were returned to 12:12-h light-dark conditions for 45 additional days and were euthanized on P90. Age-matched controls (P90 rats) were used for comparison with this group. Group 3 consisted of adult rats that were dark exposed for 45 days. Age-matched controls (5-mo-old rats) were used for comparison with this group. P45 was chosen as the temporal end point in these studies because it represents the end of the visual critical period in the rat. At the time of death, the EOMs from rats in each group, as well as age-matched controls, were processed for protein analysis (immunocytochemistry and Western blots) and RNA analysis (in situ hybridization and Northern blots).

For all groups, a single light (Kodak 3A filter) was used when cages were changed and was kept at a distance of at least 6 ft from the rats (1). Retinal rods are barely sensitive to the extreme end of this spectrum so that the dark-adapted eye may be exposed to fairly high luminance levels of deep red light without serious loss of dark adaptation (13). Unexposed photographic paper was left in the box and subsequently developed to ensure that no light leaks occurred.

MD experiment. Litters were divided equally into experimental and control groups. On P12, the experimental animals were anesthetized with metofane (Pitman Moore) and their left eyelids were sutured with polypropylene 7–0 suture (Ethicon) by using a running stitch. Rats were maintained in a 22°C room with a 12:12-h light-dark cycle and unlimited access to standard chow and water. Animals from both groups were euthanized on P45, and the EOMs were processed for protein analysis (immunocytochemistry and Western blots) and RNA analysis (in situ hybridization). Brains were removed from experimental and control rats and were quick-frozen for parvalbumin immunocytochemistry.

Immunocytochemistry. Monoclonal MHC antibodies were used in immunocytochemical analysis. Primary antibodies included developmental MHC (1:50; Novocastra Laboratories), fast MHC (1:50; Novocastra Laboratories), and MHC (A4.74; 1:20; Developmental Studies Hybridoma Bank), and a slow MHC (A4.840; 1:5; Developmental Studies Hybridoma Bank). The combined use of the generic fast MHC antibody, which recognizes all fast myosins, and the MHC antibody allows for evaluation of fast isoforms other than MHC (3). The unavailability of a suitable antibody for this isoform precluded the protein analyses performed on the other MHC isoforms. The probe's sequence was derived from the coding region (amino acids 1705–1719) and consisted of 44 bases as follows: 5'-ACAGCCCTGCGGTCCGCTCTGTCGTCGGGARCCTTTCATT-3'. The specificity of this probe for MHC was tested previously (3). The corresponding sense oligonucleotide was used as a negative control. An 18S oligonucleotide probe was used as a loading standard for the Northern blots. The sequence of the 18S probe was as follows: 5'-ACGGATCTGATCGTCTTCG-3'. All probes were synthetic oligonucleotides (Integrated DNA Technologies). The EOM-specific MHC and 18S probes were labeled at their 3’ ends by terminal deoxynucleotidyl transferase (Boehringer Mannheim) with 32P (New England Nuclear) for in situ hybridization or with 32P (Amersham) for Northern blotting.

In situ hybridization. In situ hybridization was used to study the cellular localization of the EOM-specific MHC RNA expression. The orbital contents of rats were dissected, frozen, and sectioned as described above (see Immunocytochemistry). In situ hybridization was performed as described previously (3). After hybridization and posthybridization washes, slides were dipped in NTB2 emulsion (Kodak) and exposed for 2–4 wk. Slides were dehydrated and coverslipped for densitometric grain analysis. The BioQuant imaging system (R and M Biometrics) was used to determine the percentage of the field covered by grains.

Northern blots. Northern blot analysis was used to study the levels of EOM-specific MHC mRNA expression. EOMs were quickly dissected and total RNA was isolated from 50 mg of tissue by phenol-chloroform extraction by using Trizol (GIBCO). RNA samples were denatured and separated by electrophoresis, transferred to nylon, hybridized, and washed as previously described (3). After hybridization, membranes were exposed to a storage phosphor screen (Molecular Dynamics) for 10–12 h, the screen was scanned into a phosphorimaging system (Storm, Molecular Dynamics), and densitometric analysis was performed by ImageQuant software (Molecular Dynamics). Membranes were hybridized subsequently with an 18S probe. Densitometric analysis of the 18S rRNA subunit was used to correct for loading.

Statistical analysis. Data are reported as the means ± SE. Statistical differences were determined by using t-tests and ANOVA measures. Differences were considered statistically significant at P < 0.05.

**RESULTS**

Effects of DR on MHC expression in developing and adult rat EOM. To determine the role, if any, of the developing visual system in eye muscle maturation, all visual cues were blocked by using a DR paradigm. MHC isoform composition was used as a phenotypic marker. In rats that were dark reared from birth to P45, the proportion of EOM fibers immunoreactive for each MHC antibody was determined in the EOMs of experimental and control rats (Fig. 1). Four MHC antibodies (specific for isoforms expressed in other skeletal muscles) were used to detect any changes in EOM phenotype after DR. Previous work has shown that orbital layer fibers are atypical in retention of...
developmental myosins into adulthood (3, 26). Developmental MHC isoforms, however, do not appear to be regulated by visual cues because the proportion of fibers expressing developmental MHCs was not significantly altered after DR (Fig. 1A). Conversely, slow MHC was significantly decreased in the middle and distal regions of EOM from dark-reared rats (Fig. 1B). The proportion of fibers expressing fast MHCs also was significantly decreased in the proximal region of EOMs from dark-reared rats (Fig. 1C), whereas the percentage of fibers immunoreactive for the IIA/IIX antibody remained unaltered in these muscles from dark-reared animals (Fig. 1D). These shifts in fiber type-specific expression of MHC indicate that DR sufficiently alters activity levels to elicit a phenotypic change in the contractile apparatus of EOM.

In addition to analysis of the expression of the typical MHCs, levels of the EOM-specific MHC were examined in rats dark reared from birth to P45. Expression of this tissue-specific MHC was assessed by using in situ hybridization and Northern blot analysis. A statistically significant decrease in EOM-specific MHC RNA levels in both the orbital and global layers was observed (Fig. 2). Northern blot analysis of these EOMs also demonstrated a statistically significant decrease in bulk EOM-specific MHC RNA expression (Fig. 3). Given these results, this study of EOM-specific MHC RNA expression was extended to rats that were dark reared during the critical period for visual development but were subsequently returned to normally illuminated conditions (group 2) as well as to dark-reared adults (group 3). Group 2 consisted of rats that were dark reared from birth to P45 and then were returned to a 12:12-h light-dark cycle for 45 additional days. Rats raised by using this paradigm exhibit normal visual cortical properties after the period of normal visual experience (14). These rats were euthanized on P90, and their EOMs were analyzed for EOM-specific MHC RNA expression. Bulk RNA analysis was performed by Northern blot analysis, which demonstrated no difference in EOM-specific RNA expression between the recovery and control groups (Table 1). Expression of this RNA species also was analyzed in specific muscle regions, the orbital and global layers.
analysis showed no significant differences among experimental groups for either layer (Table 1). Group 3 consisted of adult rats that were placed in the dark for 45 days. In contrast to the studies with juvenile rats, this period of dark exposure of adult rats produces no changes at the visual cortical level (14). Analysis of EOM-specific mRNA expression in dark-reared adult rat EOMs by in situ hybridization and Northern blot analysis showed no significant changes between experimental and control EOMs (Table 1). The specificity of the effects of DR on EOM-specific MHC to rats deprived solely during the visual critical period raises some interesting questions about the existence of a developmental window for eye muscle maturation that parallels that already established for the visual system.

Effects of MD on MHC expression in rat EOM. To further test the idea of a critical period for eye muscle maturation, a more complex visual deprivation paradigm, MD, was performed during the visual critical period. Before MHC analysis of EOMs from monocularly deprived rats, the efficacy of MD in altering the properties of visual cortical neurons was confirmed. Parvalbumin immunocytochemistry was used as an index of activity-dependent changes in visual cortex as a consequence of MD, as previously described by Cel larino and co-workers (5). The binocular portion of visual cortex contralateral to the sutured eye proved to be devoid of stained neurons and processes (data not shown), establishing that our MD paradigm was effective in disrupting visual cortical properties.

To test the hypothesis that visual cortical changes modulate eye muscle properties, rats were monocularly deprived via eyelid suture of the left eye just before eyelid opening on P13. These animals were euthanized on P45, the end of the visual critical period. The myosin isoform composition of monocularly deprived EOMs was compared with that of control EOMs. The proportion of EOM fibers that were immunoreactive for four typical MHC antibodies were determined for rats that were monocularly deprived for 45 days (Fig. 4). In contrast to results obtained from DR, the proportion of fibers expressing developmental MHCs after MD was decreased significantly in the middle region of orbital fibers in monocularly deprived EOMs (Fig. 4A), suggesting that developmental myosin expression in the EOMs is indeed being regulated by the visual system and may play a role in interocular alignment. The percentage of fibers expressing slow MHC was not altered significantly, whereas the proportion of fibers that were immunoreactive for the generic fast antibody was significantly decreased in the proximal region of visually deprived EOMs (Fig. 4B). This decrease was not consistent among all fast isoforms because the proportion of IIa/IIX fibers was increased significantly in the middle region of deprived EOMs (Fig. 4D).

To test the effect of MD on EOM-specific MHC expression, in situ hybridization analysis was performed (Table 2). No significant changes in grain density were observed in orbital or global layers in EOMs from either the left (sutured) or right (unsutured) eye of monocularly deprived rats.

**DISCUSSION**

These studies tested the hypothesis that the developing visual system modulates the maturation of the
EOMs. A contractile protein, MHC, was used as a sensitive index of skeletal muscle maturation and plasticity. Two visual deprivation paradigms were employed, DR and MD. The results obtained in these studies suggest that these paradigms have distinct and differential effects on myosin expression in developing eye muscle, and they confirm the overall hypothesis that the visual system strongly influences EOM maturation.

Effects of DR during the visual critical period on MHC expression in rat EOM. DR during the visual critical period yields adult rats with visual cortical functions resembling those of P19-P21 rats (14). Therefore, DR acts to delay the normal maturation of functional properties of visual cortical neurons (14). Consequently, this visual insult impairs development of visuomotor behavior and may have consequences for eye muscle development. Our data show that DR

Table 1. Dark rearing-induced changes in EOM-specific myosin mRNA

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<tr>
<th>Group</th>
<th>Northern Analysis</th>
<th>In Situ Hybridization in Orbital Layer</th>
<th>In Situ Hybridization in Global Layer</th>
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<tr>
<td></td>
<td>Exp</td>
<td>Con</td>
<td>Exp</td>
</tr>
<tr>
<td>Group 1</td>
<td>4,290.3 ± 624.9*</td>
<td>8,027.7 ± 879.4</td>
<td>19.8 ± 2.6*</td>
</tr>
<tr>
<td>Group 2</td>
<td>9,822.0 ± 259.2</td>
<td>9,419.0 ± 82.0</td>
<td>23.0 ± 1.9</td>
</tr>
<tr>
<td>Group 3</td>
<td>6,345.5 ± 563.1</td>
<td>6,195.0 ± 825.9</td>
<td>33.7 ± 0.9</td>
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Values are means of densitometric values ± SE. EOM, extraocular muscle; Exp, experimental group; Con, control; Group 1, eye muscles extracted from rats dark reared during visual critical period (birth to postnatal day 45); group 2, eye muscles extracted from rats dark reared during visual critical period and subsequently returned to a 12:12-h light-dark cycle for 45 additional days; group 3, eye muscles extracted from adult rats dark exposed for 45 days. *P < 0.05 vs. Con.

Fig. 4. Proportion of fibers expressing different MHC isoforms in EOMs from monocularly deprived (solid bars) and control rats (open bars). Four MHC antibodies were used in this study, including developmental (A), slow (B), fast (C), and IIa/IIx (D) MHCs. Immunocytochemical analysis was performed on eye muscle sections from rats monocularly deprived during visual critical period and age-matched controls. Values are means ± SE; n = 12 rats/group. *P < 0.05.
elicited distinct effects on MHC expression in the EOMs. The EOM-specific MHC was uniquely sensitive to changes in visuomotor activity by DR during the critical period for visual development. The downregulation of this isoform after DR during the visual critical period provides strong evidence that it is dependent on visually driven oculomotor activity. The late onset of expression of the EOM-specific MHC during normal development (after the first postnatal week; Ref. 3) may make this isoform acutely sensitive to the effects of DR. These findings are consistent with previous observations that oculomotor activity levels appear to be very important to the proper development of the EOMs (41).

In the rat oculomotor system, eyelid opening occurs soon after the EOM-specific MHC appears, marking the onset of purposeful eye movements and a dramatic increase in oculomotor activity (54). After eyelid opening, the levels of EOM-specific MHC exhibit an increase that parallels the development of visuomotor behavior (3). The levels of the EOM-specific MHC observed after DR during the visual critical period are comparable to those observed in P20 rats (3), suggesting that DR potentially delays full expression of this isoform, as a direct consequence of the effects on visual cortical development. Consistent with this idea, the downregulation of EOM-specific myosin was reversible, as are the effects of DR on the visual cortex (14). Rats dark reared during their visual critical period and subsequently returned to normally illuminated conditions exhibited a normal EOM-specific myosin expression pattern. Moreover, the developmental specificity of the effects of DR becomes clear when it is considered that this visual manipulation had no effect on EOM-specific MHC expression in adults. Together, these data establish strong parallels in the regulation of visual and oculomotor systems, suggesting the existence of a critical period for eye muscle development.

The EOM-specific MHC was more dramatically affected than were the expression patterns of other fast myosin isoforms in DR EOM. The proportion of fibers that were immunoreactive for the specific fast MHC antibody was decreased significantly, indicating that this deprivation paradigm has a selective effect on fast myosin expression. The decrease observed in generic fast MHC, however, was not due to a change in IIA or IIX, as demonstrated by our immunocytochemical data, but instead may be the result of a change in IIB expression, although the lack of a IIB-specific MHC antibody in this study precludes its definitive identification. DR also elicited changes in slow myosin expression. A statistically significant reduction in the proportion of fibers expressing slow MHC was observed after DR. This preferential effect on the slow fiber population in EOM was not seen in MD, suggesting that DR may have a preferential effect on myosin expression in the multiply innervated fibers that contain this isoform (3). These findings are not unexpected, given that the global multiply innervated fibers have been implicated in the maintenance of ocular fixation (51), a visuomotor property that is maldeveloped in dark-reared animals (10, 21). In sum, the changes observed in myosin expression after DR indicate that this visual insult preferentially targets myosins that are associated with very specialized fiber types that are unique to EOM.

Effects of MD during the visual critical period on MHC expression in rat EOM. After establishing that visual cues modulate the EOM phenotype by using the DR paradigm, we evaluated the impact of a higher-order visual insult, MD. MD challenges the visual system differently than does DR. Whereas DR only delays visual maturation, MD permanently alters visual cortical properties. When myosin expression in EOMs was assessed after MD, however, the results observed were consistent with an insult that only partially compromised visuomotor system development. Minimal effects on the expression of typical myosin isoforms were noted, whereas the EOM-specific MHC was unaltered.

The lack of response of the EOM-specific MHC after MD may be due to the retention of activity levels sufficient for expression of this unique isoform. It must be considered that, after MD, eye movements, including saccades, still occur, although they are abnormal (6, 46). Thus sufficient oculomotor activity may exist to support EOM-specific MHC expression. Alternatively, rodents have laterally placed eyes and thus have a more limited binocular segment than do primates or carnivores. Disparity cues may be less important in rats because this species places more emphasis on visuomotor mechanisms for generating depth cues (16). We predict that MD in species with more frontally positioned eyes and a larger binocular field may yield a more dramatic change in EOM-specific myosin expression.

Minimal changes were detected in the expression of other myosin isoforms. The orbital localization of developmental myosins may provide insight into its downregulation after MD. Developmental myosins may act as a damper in normal mature EOMs, functioning to reduce the net force generated. MD during the visual critical period interferes with the development of most eye movement control systems (6, 7, 28, 46, 47), resulting in deficiencies in ocular movement and stability. Consequently, there may be a reduced need for developmental myosins in MD EOM. The specific effects of MD on developmental myosin expression in the orbital layer supports the idea that the retention of this myosin in normal adult rat EOM is important for some of the fine, adaptive aspects of oculomotor control.

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<thead>
<tr>
<th>Group</th>
<th>MD</th>
<th>Con</th>
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<tbody>
<tr>
<td>EOMorbital</td>
<td>34.3 ± 1.5</td>
<td>35.4 ± 1.1</td>
</tr>
<tr>
<td>EOMglobal</td>
<td>32.6 ± 2.8</td>
<td>31.2 ± 3.7</td>
</tr>
<tr>
<td>EOMorbital</td>
<td>18.1 ± 4.4</td>
<td>23.5 ± 1.8</td>
</tr>
<tr>
<td>EOMglobal</td>
<td>16.0 ± 3.4</td>
<td>19.6 ± 4.0</td>
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Values are means of densitometric values ± SE (%field covered by silver grains). MD, monocular deprivation; os, oculus sinister (left eye); od, oculus dexter (right eye).
MD also had consequences for the expression of some fast myosin isoforms. The proportion of fibers immunoreactive for the generic fast myosin antibody decreased after MD. These results are consistent with the physiological data of Lennerstrand and Hanson (32), who demonstrated a decrease in contractile velocity in cats EOM after MD. This contractile slowing is a normal response after reduced activity levels in other fast-twitch muscles (27, 30, 31). In fact, this is the same response evoked in limb muscle by upper motor neuron lesions in the spinal cord (53). Indeed, a compromise of visually driven eye movements may be the closest possible approximation of an upper motor neuron lesion in visuomotor systems. The observed decrease in levels of the fast myosin isoforms recognized by the generic fast antibody probably reflects levels of the IIB MHC isoform because the proportion of fibers immunoreactive for IIA and IIX increased after MD. The increased proportion of fibers immunoreactive for the IIA/IIX antibody is consistent with a generalized contractile-slowing scheme. Generally, such a shift in contractile properties proceeds through a series of myosin isoforms (8, 18, 35); in studies, the transition appeared to proceed from IIB to IIX to IIA to I. This would account for the small but significant increase seen in IIA/IIX immunopositive fibers.

In summary, these data show that MD interfered with eye muscle maturation sufficiently to change expression patterns of the typical MHCs but not that of the tissue-specific isofor. The contractile slowing seen in monocularly deprived EOM in these studies corresponds to the observations of Lennerstrand and Hanson (32) in cat EOM. In visually compromised animals, this effect may reflect an adaptive shift toward a more energy-efficient system needed by a reduced oculomotor repertoire. The failure of MD to alter EOM-specific MHC expression indicates that this visual insult may not block visuomotor activity sufficiently to alter the expression of this gene. Finally, the differential effect on expression of the typical myosin isoforms and the EOM-specific myosin further reinforces the idea that the latter is regulated by extrinsic mechanisms distinct from those that modulate expression of other isoforms.

Functional significance of EOM-specific MHC in the visuomotor system. Collectively, our DR and MD data provide strong support for the hypothesis that the tissue-specific myosin may have evolved in eye muscle to help manage the unique functional roles demanded by the oculomotor system. In fact, the fibers that most highly express this isoform under normal conditions, the orbital singly innervated fibers, undergo the most significant phylogenetic changes of any of the EOM fiber types (51), thus presenting an interesting evolutionary perspective. An increase in the mitochondrial content of these fibers is correlated with the phylogenetic development of a fovea, which requires finer positional control, a function achieved by the orbital layer (41). Animals with frontally placed eyes may well be more reliant on the tissue-specific myosin for proper oculomotor function, whereas those with more laterally positioned eyes, such as rodents, are less reliant on a fully developed orbital layer, possibly because of the lack of a fovea and limited binocularity. This might explain the discrepancy observed in levels of EOM-specific myosin after DR and MD. Indeed, primate studies have shown that diminished oculomotor activity levels, induced experimentally by chemical or physical denervation, affect the morphology of the orbital singly innervated fiber type more dramatically than that of the other EOM fiber types (40, 52). The orbital singly innervated fiber type also is preferentially affected in congenitally strabismic monkeys (38). The presence of similar ultrastructural alterations in denervated and congenitally strabismic primate EOMs suggests that changes in visuomotor activity shape the development of orbital EOM fibers and may influence their expression of EOM-specific myosin.

Data from other skeletomotor systems show that an inhibition of the expression of role-specific myosins resulting from interference with the systems they serve is not novel. The developmental program of the laryngeal-specific myosin involved in courtship song and mating behavior in male Xenopus laevis is androgen-dependent and can be blocked with gonadectomy (4). These data reinforce the existence of need-based expression of specialized myosin isoforms, which in this case, is tightly regulated by masculinization. Tissue-specific myosin isoforms can also be blocked by inappropriate neural cues. Expression of the superfast masseteric myosin is intrinsic to the masticatory muscle allotype (25). Transplant of masseteric satellite cells into a fast limb muscle bed yields superfast myosin expression, whereas transplant into a slow muscle bed inhibited superfast myosin expression by the satellite cells (23). Although the synthesis of the superfast myosin is innate to the masticatory allotype, its expression is also clearly modulated by neural activity levels. The nervous system, however, can only modify gene expression within the phenotypic options determined by this allotype (24). That is, at present it appears that only myoblasts of particular lineages can be induced to express the tissue-specific MHC isoforms. Collectively, the existing data support the idea that the expression of the specialized MHC isoforms (EOM-specific, superfast, laryngeal myosins) requires not only specified activity levels but also commitment to a particular cellular lineage as well.

In conclusion, these findings support the hypothesis that the developing visual system modulates eye muscle maturation. Moreover, we propose that visuomotor activity is an important factor in conferring unique myosin expression patterns on EOM. Disruption of an important epigenetic influence, visually driven eye movements, causes dramatic changes in the specialized contractile protein phenotype of the EOMs. In a receptive myoblast pool, visuomotor activity appears to facilitate expression of the EOM-specific myosin isoform. The temporal characteristics of EOM-specific myosin regulation establish that the key elements of a critical period are apparent in EOM. Our data, then, not only have identified one regulatory mechanism for the unique EOM phenotype but also further suggest
that the EOMs maturation state must be considered in the design of therapies for congenital ocular motility disorders.

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