Development of Extraocular Muscles Requires Early Signals From Periocular Neural Crest and the Developing Eye

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**Objectives:** To identify and explain morphologic changes of the extraocular muscles (EOMs) in anophthalmic patients.

**Methods:** Retrospective medical record review of patients with congenital anophthalmia, using magnetic resonance imaging and intraoperative findings to characterize EOM morphology. We then used molecular biology techniques in zebrafish and chick embryos to determine the relationships among the developing eye, periocular neural crest, and EOMs.

**Results:** In 3 human patients with bilateral congenital anophthalmia and preoperative orbital imaging, we observed a spectrum of EOM morphologies ranging from indiscernible muscle tissue to well-formed, organized EOMs. Timing of eye loss in zebrafish and chick embryos correlated with the morphology of EOM organization in the orbit (eye socket). In congenitally eyeless Rx3 zebrafish mutants, or following genetic ablation of the cranial neural crest cells, EOMs failed to organize, which was independent of other craniofacial muscle development.

**Conclusions:** Orbital development is dependent on interactions between the eye, neural crest, and developing EOMs. Timing of the ocular insult in relation to neural crest migration and EOM development is a key determinant of aberrant EOM organization. Additional research will be required to study patients with unilateral and syndromic anophthalmia and assess for possible differences in clinical outcomes of patients with varied EOM morphology.

**Clinical Relevance:** The presence and organization of EOMs in anophthalmic eye sockets may serve as a markers for the timing of genetic or teratogenic insults, improving genetic counseling, and assisting with surgical reconstruction and family counseling efforts.


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The volume of the adult human orbit (eye socket) is 30 mL and contains the ocular globe and a surrounding complex set of neuronal, vascular, muscular, and glandular structures. These include 6 extraocular muscles (EOMs) that differentiate contemporaneously with the eye vesicle, form precise anatomic attachments to sclera and bone, and are innervated by cranial nerves 3, 4, and 6. Vertebrate EOMs are required for the precise control of eye movements to achieve directionality of gaze. In binocular animals with overlapping visual fields (eg, primates, birds), eye movement control is required for stereopsis and fusion. Extraocular muscles are skeletal muscles with unique properties, including patterns of gene expression different from skeletal muscle and altered susceptibility to systemic myopathies.6,15 Vertebrate EOMs are derived from 2 populations of mesenchymal cells: the unsegmented paraxial head mesoderm, which likely contributes to the lateral rectus and superior oblique muscles (innervated by the abducens and trochlear nerves, respectively); and the prechordal head mesoderm, which likely gives rise to the superior, inferior, and medial rectus and inferior oblique muscles (innervated by the oculomotor nerve). These presumptive origins of the EOMs are based on ablation, transplantation, and genetic data using mostly chick and rodent models.6,11 Extraocular muscle development appears to initiate caudal to the eye field, followed by anterior migra-
tion of mesodermal cells and subsequent muscle morphogenesis.

The mesodermal mesenchyme that forms the EOMs is intermingled with cells of the cranial neural crest that will form the sclera and part of the choroidal layer of the eye. Experiments that removed the neural crest suggest that interactions between mesoderm and neural crest may be involved in EOM formation. However, owing to differences in animal models, the involvement of periorcular neural crest in EOM development and organization is somewhat controversial. This may be owing to the use of different techniques in the ablation of the neural crest. For example, surgical ablation may fail to completely and reproducibly ablate the entire cranial neural crest, whereas genetic ablation may cause unanticipated collateral damage to surrounding tissues, including the developing EOMs directly. Still, given that cranial neural crest cells contribute to craniofacial bone, connective tissue, nerves, and the developing eye, it is likely that cranial neural crest plays an important role at least in the organization of the EOMs, if not the induction of differentiation.8,9,11-14

Anophthalmia and microphthalmia are rare conditions, with a combined birth prevalence of up to 30 per 100,000 persons.15 These are caused by genetic mutations or teratogenic insults that interfere with differentiation of the neuroectodermal optic tissues and surrounding structures, resulting in variable attenuation of eye development (unilateral or bilateral). The genetic or teratogenic insult may primarily disrupt eye development and secondarily affect surrounding structures. Alternatively, the primary insult may disrupt orbitofacial mesenchymal development, secondarily affecting eye development. Regardless of their etiologies, studies of anophthalmia and microphthalmia have been fruitful in elucidating the genetics and complex tissue interactions that are required for eye formation.16 These conditions likely represent a phenotypic continuum and are commonly associated with systemic phenotypes as part of a syndrome. As a consequence, it is often difficult to determine the precise timing of the ocular insult retrospectively.

Because the bony orbits of congenitally anophthalmic patients are abnormally small, eye socket reconstruction includes bony expansion (nonsurgically using conformers or surgically using implants, expanders, or osteotomies) before placing a permanent orbital implant and fashioning a custom ocular prosthesis.17 The organization and functionality of EOMs in congenitally anophthalmic patients is critical to reconstructive surgical efforts; functional EOMs can be attached to orbital implants, providing the ocular prosthesis with movement characteristics that create a more natural appearance. Even nonfunctional EOMs can be helpful in securing the implant to prevent postoperative migration and reduce the risk of implant exposure or extrusion.

Based on our clinical work with anophthalmic patients, we investigated the temporal dependence of EOM development on the presence of the ocular globe in zebrafish and chick embryos. Our findings reveal a strong correlation between the extent and organization of remnant EOMs and the timing of the ocular insult. This correlation merits further study because it could serve as a longitudinal marker for anophthalmia genetic research and aid in clinical care and the development of new therapies.

**METHODS**

**HUMAN PATIENTS**

A retrospective medical record review of 22 patients with unilateral or bilateral congenital anophthalmia treated at the University of Michigan Kellogg Eye Center was performed with the approval of the University of Michigan institutional review board (No. HUM00040783). We identified 3 patients with congenital bilateral anophthalmia who were well matched by age (1-2 years) and clinical examination findings and who under-

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**Figure 1.** Preoperative magnetic resonance image of patient 1 with congenital bilateral anophthalmia but well-defined extracocular muscles (EOMs). The image reveals the presence of relatively organized EOM groups. The EOMs (arrows) are labeled according to position in the anophthalmic eye socket. This patient wore small custom socket conformers fitted by an ocularist. Note the absence of an optic nerve. Parts A and B show fat suppression images. IR indicates inferior rectus; LR, lateral rectus; MR, medial rectus; SO, superior oblique; and SR, superior rectus.
went orbital magnetic resonance imaging (MRI) prior to any surgical intervention. Their MRIs were carefully reviewed for the presence and organization of EOMs and correlated with intraoperative findings. In one case, a unilateral orbital cyst was excised and the paraffin-embedded tissue block was resectioned and stained for microscopic evaluation. Mosaics of microscope images were obtained using Photoshop CS4 (Adobe Systems Inc, San Jose, California).

**ZEBRAFISH**

Zebrafish (*Danio rerio*) were raised in a laboratory breeding colony on a cycle of 14 hours of light followed by 10 hours of dark at 28.5°C and staged as described using hours after fertilization (hpf). The Tg(α-actin::EGFP) strain was a generous gift of Simon Hughes, PhD, King’s College, London, England. The Rx3/chokh mutant strain was the generous gift of Herwig Baier, PhD, University of California, San Francisco. When noted, embryos were treated with 0.003% (200µM) propylthiouracil at 16 to 20 hpf to inhibit pigmentation. The protocols have met guidelines established by the University of Michigan Committee on the Use and Care of Animals.

**ZEBRAFISH ENucleATION EXPERIMENTS**

Embryos from wild-type and Tg(α-actin::EGFP) zebrafish were dechorionated and enucleated at 16 to 18 or 26 hpf using sharp tungsten needles and a dissecting microscope. Care was taken not to injure the cranium or the surrounding orbit during the procedure. Embryos were then returned to zebrafish growth medium and allowed to recover at 28.5°C. Live Tg(α-actin::EGFP) embryos were imaged at 72 and 96 hpf and harvested in 4% paraformaldehyde. Additional embryos were raised to adulthood using standard aquarium, light cycle, and feeding protocols. At 4 to 5 months, they were killed by deep anesthesia and immersion in 10% buffered formalin. Adults were processed for histology as described below.

**ZEBRAFISH MICROINJECTIONS**

Morpholino oligonucleotide (MO) (Gene Tools, Cowallis, Oregon) were reconstituted in deionized water to concentrations of 0.1 to 0.3 mM. Sequences of MO were previously published and are available on request.

Embryos obtained from timed matings were injected with 0.2 to 1 pmol MO at the 1- to 2-cell stage. Morpholino oligo-
nucleotide against a control sequence (β-globin, Gene Tools) was also injected for each experiment. Microinjections were done at multiple doses to establish concentrations at which consistent and reproducible phenotypes were yielded. Live embryos were imaged and harvested at 72 and 96 hpf for further analysis, as described below.

IN SITU HYBRIDIZATION

Whole-mount in situ hybridization was performed as described using digoxigenin-labeled RNA antisense probes. The RNA probes were generated by transcribing a polymerase chain reaction product of a complement DNA fragment using a 3′ primer that included a T3 or T7 promoter. The only exception was the crestin probe, which was obtained from a plasmid digested with EcoRI and transcribed with T7 RNA polymerase (the crestin plasmid was a generous gift from Mary Halloran, PhD). Tissue was fixed in 4% buffered paraformaldehyde, treated with proteinase K, acetylated, and then prehybridized. Tissue was incubated with probe in hybridization buffer overnight at 65°C. Probes were detected using antidigoxigenin alkaline phosphatase–conjugated antibody and visualized with 4-nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Roche Molecular Biochemicals, Indianapolis, Indiana). Embryos were cryoprotected and embedded in OCT for sectioning.

ZEBAFISH HISTOLOGY AND MICROSCOPY

Zebrafish embryos were fixed in 10% buffered formalin overnight at room temperature and embedded in paraffin. Adult zebrafish specimens were fixed in 10% formalin for at least 48 hours at room temperature, then decalcified for 1 hour in 5% nitric acid solution, neutralized, washed, and embedded in paraffin. All sections were sectioned at 5 µm and mounted on slides. Sections were stained with hematoxylin-eosin or Masson trichrome using standard techniques. Permanent coverslips were placed using CytoSeal (Richard-Allan Scientific, Kalamazoo, Michigan).

Live embryos were embedded in methylcellulose, and images were obtained using a Leica M205FA Combi microscope using Leica DFC290 (brightfield) and Hamamatsu ORCA-ER (fluorescence) cameras (Hamamatsu Photonics, Hamamatsu City, Japan). Animals processed for in situ hybridizations were imaged with a Leica DM6000B microscope using a Leica DFC500 camera (Leica Microsystems CMS GmbH, Wetzler Germany). Images were processed using Photoshop (Adobe Systems, San Jose, California) and Leica LAS AF software.

CHICK ENUCLEATION AND ANALYSIS

Chick research protocols followed Royal Veterinary College guidelines. Fertile white leghorn chicken eggs (Henry Stewart and Co Ltd, Lincolnshire, England) were incubated at 37°C. Embryos were staged according to Hamburger and Hamilton (HH).21 Enucleations were performed as described, collected at embryonic day 14 (HH stage 40-42), and fixed in 4% paraformaldehyde in phosphate-buffered saline. Embryos were embedded in paraffin and sectioned at 8 µm and mounted on slides. Staining with hematoxylin-eosin or Masson trichrome was performed using standard techniques. For immunostaining, sections were dewaxed, rehydrated, and treated with 0.05% hydrogen peroxide before incubating overnight in antimouse myosin (1:10; DSHB, University of Iowa, Iowa City) at 4°C. Slides were then incubated in goat antimouse horseradish peroxidase secondary antibody (1:250; Invitrogen, Paisley, England), and the peroxidase reaction product was generated using diaminobenzidine. Labeled muscles were imaged using a Leica DM4000B microscope. In situ hybridizations for RXR-γ were performed as previously described.28

RESULTS

HUMAN ANOPHTHALMIA AND EOMs

While providing surgical care to patients with congenital anophthalmia and microphthalmia, we noted that EOM anatomy could vary widely. Variations included the presence or absence of EOMs, variable numbers of EOMs, and alterations in the spatial organization of the muscles within the orbit. Twenty-two patient medical records were reviewed, including patients with bilateral and unilateral clinical anophthalmia, often associated with hemifacial microsomia and other craniofacial abnormalities. We identified 3 patients with congenital bilateral anophthalmia who were well matched by age and clinical examination and who underwent orbital MRI prior to any surgical intervention. A careful assessment of the preoperative MRI scans and intraoperative findings suggested that preoperative MRI accurately represented the presence and organization of EOMs (patient 1). The MRIs of patients 2 (Figure 2) and 3 (Figure 3) showed less muscle mass and reduced
organization, respectively. Patient 2 had a 6-mm orbital cyst removed from the right orbit, and histopathology (Figure 2B) revealed an extremely microphthalmic remnant with minimal retinal development, small and malformed lens, fibrovascular scar, and irregular muscle insertions. Of note, the EOMs of patient 2 were identified intraoperatively. The EOMs were attached to the orbital implant during socket reconstruction to stabilize the implant and facilitate prosthesis movement. Patient 3 underwent reconstructive eye socket surgery, during which EOMs were barely identifiable on the right and could not be identified on the left. The intraoperative report noted the presence of fibrovascular connective tissue where the EOMs would normally be found, consistent with the preoperative MRI findings that lacked identifiable EOM structures (Figure 3).

To explore the correlation between anophthalmia and EOM organization, we turned to a zebrafish experimental model. In situ hybridization for myh2, the gene encoding myosin heavy chain, labeled the 6 EOMs at 72 hpf with anatomic positions similar to that found in humans (Figure 4, G-I). In the Rx3/chokh mutants, muscles of the jaw and pharyngeal arch differentiate and form normally (purple arrows). Schematics of the rx3/chokh mutant (D-F) and wild-type (J-L) show the relation of the EOMs (red) to the eye (blue) and jaw musculature (purple). The dotted line in the lateral view corresponds to the coronal cut shown on the sections shown in Figure 5 (original magnification ×200). IO indicates inferior oblique; IR, inferior rectus; LR, lateral rectus; MR, medial rectus; P, pyramidalis; SO, superior oblique; SR, superior rectus; and WT, wild-type.

MODEST EOM DEVELOPMENT WITH POOR ORGANIZATION IN ZEBRAFISH rx3/chokh EYELESS MUTANTS

Figure 4. Genetic ablation of the optic vesicle in Rx3/chokh −/− mutant embryos results in poorly formed extraocular muscles (EOMs). A-C, Whole-mount in situ hybridization for myh2, the gene encoding myosin heavy chain, in Rx3−/− mutants at 72 hours after fertilization (hpf) demonstrates the presence of differentiated muscle in the anophthalmic sockets (red arrows). However, the muscles are not properly organized into 6 EOMs, as seen in wild-type embryos (G-I). In the Rx3/chokh mutants, muscles of the jaw and pharyngeal arch differentiate and form normally (purple arrows). Schematics of the rx3/chokh mutant (D-F) and wild-type (J-L) show the relation of the EOMs (red) to the eye (blue) and jaw musculature (purple). The dotted line in the lateral view corresponds to the coronal cut shown on the sections shown in Figure 5 (original magnification ×200). IO indicates inferior oblique; IR, inferior rectus; LR, lateral rectus; MR, medial rectus; P, pyramidalis; SO, superior oblique; SR, superior rectus; and WT, wild-type.
sal muscle groups (Figure 5, A and B) that did not further organize into rectus and oblique muscles. In contrast, the jaw and pharyngeal arch muscles differentiate and form normally (Figure 4, A-C) in the rx3/chokh mutants.

Histologic sections taken at 120 hpf from the rx3/chokh mutants (Figure 6, A and B) revealed that there was cartilage demarcating the anophthalmic socket that contained some pigmented epithelium. In addition, there were small clusters of muscle tissue in the erstwhile orbits. However, no discrete muscles could be identified as in the wild-type (Figure 6, C and D). Thus, genetic ablation of the developing eye results in EOM differentiation but impaired EOM organization.

TIME-DEPENDENT INTERACTION BETWEEN THE DEVELOPING EYE AND THE DEVELOPING EOMs IN SURGICALLY REMOVED ZEBRAFISH OPTIC VESICLE

The rx3 gene mutation may directly cause EOM maldevelopment. Alternately, it may prevent EOM development through its effects on the interactions between the eye and periocular mesenchyme. Failure of eye development, including in the Rx3/chokh mutants, was found to cause abnormal neural crest cell migration to the orbit as evidenced by crestin expression, supporting an eye-mediated effect on the surrounding mesenchyme. We then tested whether surgical removal of the optic vesicle would interfere with EOM development in a similar manner to genetic ablation. To visualize differentiated muscles with fluorescence microscopy, we unilaterally enucleated Tg(\textit{actin::EGFP}) embryos, which express green fluorescent protein under the control of the \textit{\alpha}-actin promoter. Enucleations were performed at 16 hpf prior to cranial neural crest migration to the orbit (Figure 7A).32 Removal of the optic vesicle at 16 to 18 hpf resulted in minimal EOM development at 96 hpf (data not shown). Wild-type embryos that were unilaterally enucleated at 16 hpf were also raised to adulthood. Transverse sections stained with hematoxylin-eosin (Figure 7B) confirmed the presence of a small rectus muscle remnant and the complete absence of oblique muscles in the anophthalmic socket. In contrast, enucleation of embryos at 26 hpf, which is after migration of the cranial neural crest (Figure 7A) but prior to the earliest signs of EOM differentiation, revealed well-developed EOMs at 72 hpf (data not shown) and 96 hpf (Figure 8, C, E, G, and H). Despite the absence of the eye and the relatively late development of the EOMs, individual rectus and oblique muscles could be identified (Figure 8, C, E, G, and H) with normal anatomic positions. Together, these studies reveal a critical period for the interaction between the developing eye and the periocular mesenchyme that is required for EOM development and morphogenesis.

Figure 5. Rx3/chokh −/− mutant embryos express myosin but fail to form distinct extraocular muscle (EOM) structures. Cryosection in situ hybridization for the myosin gene \textit{myhz} of Rx3/chokh mutants 72 hours after fertilization (hpf) (A and B) and wild-type (C and D) embryos demonstrates that the EOMs in the Rx3/chokh −/− mutant form dorsal and ventral clusters (double arrows) but do not organize into distinct rectus and oblique muscles (original magnification: A and C, ×200; B and D, ×400). IR indicates inferior rectus; MR, medial rectus; SR, superior rectus; and WT, wild-type.
The interaction between the eye vesicle and the EOMs occurs in a developmental window that overlaps with cranial neural crest migration into the orbit. The role of the cranial neural crest in EOM development is somewhat controversial, but there are significant data to suggest an important relationship between the cranial neural crest and the mesodermal mesenchyme that forms the EOMs. In zebrafish, the eye is required for proper migration of neural crest cells to the orbit; therefore, we investigated whether the interactions between the developing eye and EOMs are mediated by the cranial neural crest.

As previously described, microinjection of anti-sense MO directed against the transcription factor Sox10 at the 1 to 2–cell stage (within 30 minutes of fertilization) effectively knocks down expression of sox10 and results in fish that survive to the early larval stage but with neural crest–derived craniofacial cartilages and hindbrain structures that fail to develop. Nonspecific effects of the Sox10 MO such as heart edema improve with coinjection of p53 MO. Using Sox10 gene knockdown, we tested whether EOM differentiation and organization were affected by inhibition of neural crest development. Inhibition of sox10 expression in the Tg(α–actin::EGFP) strain showed that EOMs failed to organize around the eye by 72 hpf (Figure 9, A-C) and were severely maldeveloped by 96 hpf (Figure 9, G-I). Further, we determined by in situ hybridization that the EOMs as well as the pharyngeal muscles expressed myosin heavy chain in the sox10 morphants (data not shown) and confirmed that the EOMs did not form proper attachments to the eye. The overall α–actin signal was decreased, revealing a smaller muscle mass (Figure 9, A-C and G-I). Similar results were obtained with MO knockdown of FoxD3, another transcription factor required for neural crest development (data not shown). Thus, in the zebrafish embryo, the cranial neural crest is required for proper EOM development and organization, and lack of orbital neural crest cells mimicked the effect of early anophthalmia on EOM development.

CONFIRMATION OF EOM DEVELOPMENT IN THE ABSENCE OF AN EYE THROUGH SURGICAL REMOVAL OF THE CHICK OPTIC VESICLE

We next tested whether our experimental observations using zebrafish can be replicated in another vertebrate model. We used the chick embryo, a well-established model for ocular and craniofacial development, to test whether EOM organization is dependent on the presence of the cranial neural crest and the optic vesicle. The cranial neural crest in the chick embryo migrates into the craniofacial region at HH stage 11/12, which is at an ear-
lier stage (early optic cup) than in zebrafish. In situ hybridization for RXR-\(\gamma\) (Figure 10A), which is expressed in the neural crest in chick embryos, demonstrates the close relationship between the cranial neural crest and the developing eye at HH 12. The right eye of chick embryos was enucleated at HH stage 13, and EOM development was examined at HH stage 40 (embryonic day 14; Figure 10, B-D). Immunohistochemistry for myosin heavy chain demonstrates that the EOMs formed and organized properly in controls (Figure 10, C and D). On the anophthalmic side, large clusters of orbital muscle were identifiable in the area normally occupied by the medial rectus muscle (Figure 10C) and caudally in the lateral rectus field (Figure 10D). These clusters, however, lacked discernible anatomic organization within the orbit. Hence, early enucleation of a chick eye immediately after periocular neural crest cell migration resulted in the development of EOMs with very limited spatial organization.

**COMMENT**

Our evaluations of human patients with clinical congenital anophthalmia revealed significant variability in their EOM anatomy. In zebrafish, microsurgical removal of the developing eye at 16 hpf prior to optic cup evagination or orbital neural crest migration resulted in poorly developed and disorganized EOMs (Figure 11C). Using a genetic eyeless mutant (rx3) in which ocular development ends at 18 to 20 hpf resulted in differentiated EOMs that were small and disorganized. Microsurgical removal of the developing eye in zebrafish at 26 hpf after orbital neural crest migration resulted in discernible EOM
organization (Figure 11D), though expression of early muscle transcription factors such as Myf5 and MyoD in EOMs only begins at 26 to 32 hpf.33,34 In the chick model, the eye was enucleated shortly after the neural crest migrated to the developing eye. Analysis of the resulting chick orbit revealed muscle clusters that did not develop into properly organized EOMs. Our results, based on observations made with human patients as well as experimental data using zebrafish and chick embryos, suggest that the observed clinical variability in EOM development may reflect the onset and extent of ocular maldevelopment.

Previous studies of the relationship between the eye and cranial neural crest show that the developing eye is required for proper cranial neural crest migration.31 Similarly, cells of the cranial neural crest and EOMs show complex interdependence that can vary depending on the tools and experimental models.8,9,11-14 Our results suggest that the developing eye and the cranial neural crest provide early signals that are required to drive the morphogenic process of EOM development.

Based on our results and other published data,6,7,11,14,31 we suggest that the developing eye, migrating cells of the cranial neural crest, and head paraxial and prechordal me-
soderm (Figure 11A) interact to create the functional orbit. Absence or abnormality in one group of cells has a lasting effect on the other groups. For example, patients with neurocristopathies and craniofacial defects will often have ocular manifestations including microphthalmia and/or anophthalmia (eg, Goldenhar syndrome and hemifacial microsomia) and strabismus (eg, Saethre-Chotzen syndrome).

Based on these results, and to build a more complete biological picture, we propose that the timing of abnormal eye development induces lasting alterations in the development and organization of the EOMs. In turn, clinically observable alterations in EOMs can provide important clues regarding the timing and mechanisms of anophthalmia and microphthalmia. We further suggest that the relationship between the developing eye and surrounding
addition, preoperative evaluation of EOM development within a timeline of ocular and orbital development. In ing of ocular insult and suggest placement of that allele any identified genetic alleles, may help reveal the tim-
EOM development in these patients, and correlation with etation of clinical anophthalmia, and in the context of ge-
sociated syndromes. We suggest that part of the evalu-
and treatment of anophthalmia, microphthalmia, and as-
tween species will help guide future studies on the causes development and the relative difference in timing be-
vergence, an understanding of this temporal window of EOM
EOMs reflects, in part, the timing of cranial neural crest migration. Early loss of the optic vesicle prior to cranial neu-
ral crest migration results in poorly differentiated muscle that fails to organize into discernible EOMs. Later loss of the developing eye, after cranial neural crest cells had mi-
gate migration results in poorly differentiated muscle
neural crest during early stages of orbital development (B and C) affect one
ary crest migration
Normal orbital development
Figure 11. Model of the interactions between the neural crest, mesoderm, and developing eye in orbital development. The developing eye, extracocular muscles (EOMs), and migratory cranial neural crest cells form interdependent relationships that are necessary for the proper development of one another (A and E). Disrupting the development of either the eye or the neural crest during early stages of orbital development (B and C) affect one another and leave a permanent mark on the structural organization of EOMs, whose development requires input signals from both the developing eye and surrounding cranial neural crest cells. Removal of the eye after the migration of the neural crest into the orbit has less effect on EOM development (D).
Whereas results in different animal models may diverge, an understanding of this temporal window of EOM development and the relative difference in timing between species will help guide future studies on the causes and treatment of anophthalmia, microphthalmia, and associated syndromes. We suggest that part of the evaluation of clinical anophthalmia, and in the context of genetic evaluations, an MRI of the orbits be obtained prior to any medical or surgical interventions. The analysis of EOM development in these patients, and correlation with any identified genetic alleles, may help reveal the timing of ocular insult and suggest placement of that allele within a timeline of ocular and orbital development. In addition, preoperative evaluation of EOM development may help with surgical reconstructive and family coun-

**REFERENCES**

1. Zhou Y, Liu D, Kaminski HJ. Myosin heavy chain expression in mouse extraocu-
lar muscle: more complex than expected. Invest Ophthalmol Vis Sci. 2010; 
51(12):6355-6363.
2. Kusner LL, Young A, Tjoe S, Leahy P, Kaminski HJ. Perimysial fibroblasts of ex-
traocular muscle, as unique as the muscle fibers. Invest Ophthalmol Vis Sci. 2010; 
3. Zeiger U, Khurana TS. Distinctive patterns of microRNA expression in extraocu-
5. Yu Wai Man CY, Chinnery PF, Griffiths PG. Extraocular muscles have fundamen-
tally distinct properties that make them selectively vulnerable to certain disorders. 
6. Couly GF, Coltey PM, Le Douarin NM. The developmental fate of the cephalic me-
7. Noden DM, Traxler PA. Relations and interactions between cranial mesoderm and 
8. Noden DM. The embryonic origins of avian cephalic and cervical muscles and 
ning promote the formation of vertebrate head muscle. Genes Dev. 2003;17 
(24):3087-3099.
10. Wachtler F, Jacob M. Origin and development of the cranial skeletal muscles. 
11. von Scheven G, Alvares LE, Mootooosamy RC, Dietrich S. Neural tube derived sig-
als and Fgf8 act antagonistically to specify eye versus mandibular arch muscles. 
Development. 2006;133(14):2731-2745.
12. Gage PJ, Suh H, Camper SA. Dosage requirement of Pitx2 for development of 
interactions in zebrafish jaw development. Development. 1996;122(5):1417- 
1426.