PERSPECTIVE

From insect eye to vertebrate muscle: redeployment of a regulatory network

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The redeployment of the same signaling systems at different places and times during embryogenesis, and the conservation of these systems across species, from invertebrates to vertebrates, has emerged as a major theme of biology. This conservation also applies to transcriptional regulation; the Hox genes are a classic example. More recently a striking illustration of the phenomenon was provided by the demonstration that homologous regulatory genes are responsible for the formation of the ommatidia, which constitute the eye of the fruit fly *Drosophila* and of the mammalian eye. Furthermore, the transcription factor Pax6, like its *Drosophila* homolog *eyeless*, which is at the top of the regulatory cascade, can induce ectopic eye formation in the fly (Halder et al. 1995). The article by Heanue and colleagues in this issue of *Genes & Development*, demonstrates that the same combination of transcriptional regulators required for eye formation is redeployed elsewhere during vertebrate embryogenesis, in this case in the somite and its skeletal muscle derivatives.

The network of genes implicated in *Drosophila* eye formation

In *Drosophila*, the *eyeless* gene activates a cascade of genes (Halder et al. 1998), including *eyes absent*, *dachshund*, and *sine oculis*, with subsequent feedback to form a regulatory network (Fig. 1A), such that ectopic expression of *dachshund* (Shen & Mardon 1997) or *eyes absent* (Chen et al. 1997; Pignoni et al. 1997), also leads to ectopic eye formation. *sine oculis* (Pignoni et al. 1997) and *dachshund* (Chen et al. 1997) are both capable of synergizing with *eyes absent* to promote this process and the proteins have been shown to form molecular complexes. While *Sine oculis* (So) and *Eyeless* (Ey) are homeodomain proteins and bind DNA, this is not the case for *Eyes absent* (Eya) and *Dachshund* (Dac) which probably act as transcriptional cofactors. In addition to these genes that are essential for eye formation, another gene, *twin of*

eyeless, has been identified recently (Czerny et al. 1999). This gene appears to correspond to a duplication of *eyeless* that occurred during the radiation of insects. It acts upstream of *eyeless* but is not regulated by a feedback loop (Fig. 1A). This regulatory network does not necessarily function at other sites in the *Drosophila* embryo, in which these genes show different expression patterns and have different mutant phenotypes; for example, *sine oculis* expression elsewhere in the embryonic head is independent of *eyeless* and *twin of eyeless* (Halder et al. 1998).

Vertebrate homologs of the *Drosophila* eye network

Vertebrate homologs of these genes have now been identified (Fig. 1B), and as is often the case, they are present as multigene families with distinct if overlapping expression patterns. A subset is involved in eye development. Pax6 plays a similar role to that of *eyeless* (or *twin of eyeless*) and is a member of a large family of paired-domain proteins, many of which also have a homeodomain (for review, see Tremblay and Gruss 1994). Paired-domain proteins bind DNA, and different regions of the proteins may show activator or repressor functions in transcriptional assays. Pax genes are expressed, often in pairs, at different sites in the embryo. Mutations of many of them have now been analyzed in the mouse, in which they have been shown to play key roles in the development of tissues and organs. Outside their normal in vivo context, Pax genes also act as proto-oncogenes and a number of translocations involving human Pax genes may give rise to tumors (for review, see Tremblay and Gruss 1994). A striking feature of PAX genes is the number of spontaneous mutations that exist, illustrated by Pax6, which has been shown to be responsible for *Small eye* in the mouse and *ANIRIDIA* in humans. As in the *Drosophila* paradigm, ectopic expression of Pax6 can lead to eye formation (Jean et al. 1998; Chow et al. 1999).

The Six genes are homologs of *sine oculis*. To date, the number of Six gene family members is, coincidentally, six, and includes the related sequence *Optx2* (Toy et al. 1998), or *Six6* and *Six3*, which are closer homologs of the *Drosophila sine oculis*-related gene *optix*. Six genes are

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Figure 1. A network of regulatory genes involved in *Drosophila* eye formation conserved between insects and vertebrates. (A) The network of genes that regulates eye formation in *Drosophila* (see text for details). (B) Vertebrate homologs of these *Drosophila* genes. The number of related genes identified to date in vertebrates is indicated in parentheses. In vertebrates, two *Dach* genes have been described, as well as two *Dach*-related genes, *Ski* and *Sno* (+2). (C) Putative models for transcriptional complexes formed by the Pax/Six/Eya/Dach proteins. Among these proteins, Six and Pax have DNA-binding domains. Biochemical interactions have been shown between Six/Eya and Eya/Dach through conserved domains. Dach may be part of an Eya/Six transcription complex, potentially interacting (arrows) with another adjacent transcription factor/complex (Y), or it may interact with Eya as part of a complex with another DNA binding transcription factor (X). Pax appears to bind to an independent site, possibly in association with cofactors (Z). The angled arrows indicate transcriptional initiation sites.

also expressed at many different sites in the embryo, again often in pairs. Ectopic expression of *Six3* in fish leads to changes in cell fate, with expression of lens and retinal markers (for review, see Jean et al. 1998; Loosli et al. 1999), whereas *Optx2*, in addition to affecting cell fate

(Toy et al. 1998), has also been implicated in the proliferative control of retinal cells (Zuber et al. 1999). Human *SIX1*, isolated in a screen for cell cycle-regulated genes from mammary carcinoma cells, abrogates the G₂ cell cycle checkpoint (Ford et al. 1998), pointing to a role for this gene family in growth control. Six proteins bind DNA and were identified independently of their homology to So, as transcription factors involved in the regulation of the ARE element of the ubiquitously expressed Na⁺/K⁺ ATPase-1 subunit gene (Kawakami et al. 1996) and of the MEF3 element necessary for the expression in skeletal muscle of the *Aldolase A* (Spitz et al. 1997) and *Myogenin* (Spitz et al. 1998) genes. An indication of the possible consequences for skeletal muscle tissue of *Six* mutations is provided by the human disease Steinert's myotonic dystrophy, in which amplification of a trinucleotide repeat may perturb the regulation of *Six5*. This disease is accompanied by the down-regulation of genes that have MEF3 motifs in their regulatory elements (Spitz et al. 1997). As in the *Drosophila* eye, Six proteins cooperate with homologs of Eya to activate their target genes (Ohto et al. 1999) (Fig. 1C).

Four *Eya* genes have been identified (Xu et al. 1997b; Borsani et al. 1999) and are expressed in partially overlapping patterns at different sites in the mouse embryo, including the lens and nasal placode where *Eya1* and *Eya2* transcripts overlap with and depend upon expression of *Pax6* (Xu et al. 1997b). Haploinsufficiency for human *EYA1* results in bronchio-oto-renal (BOR) syndrome (Abdelhak et al. 1997), and targeted mutation of the mouse *Eya1* gene results in failure of ear and kidney development in homozygotes (Xu et al. 1999). *Pax* and *Six* genes are also expressed at these sites, and *Six* but not *Pax* gene expression is affected in the mutant. This observation is in keeping with the suggestion that *Pax* genes (*Pax6*) may be more similar to *Drosophila* *twin of eyeless*, rather than *eyeless* (Fig. 1; Czerny et al. 1999). The *Eya1* mutation does not affect eye development, presumably because of redundancy with *Eya2*. The loss of organs in this mutant has been attributed to a defect in inductive tissue interactions. Interestingly, the molecular interaction demonstrated for Six and Eya (Ohto et al. 1999) promotes nuclear translocation of Eya from the cytoplasm, reminiscent of what happens to β -catenin on reception of Wnt signalling. Their cooperative effect on transcription varies with different Eya/Six combinations, suggesting some specificity of interaction between isoforms. It is not yet clear by what mechanism transcription is activated. Six4 has an intrinsic activation domain in the carboxyl terminus (Kawakami et al. 1996), while amino-terminal portions of Eya1–Eya3 show transactivation activity (Xu et al. 1997a). Six1 and Six4 alone can activate transcription through a multimerized MEF3 site from the *Myogenin* promoter, but this activation is weak (Spitz et al. 1998) and is only clearly evident in the presence of Eya binding to Six (Ohto et al. 1999).

The work on the *Drosophila* proteins showed that Eya also interacts with Dac (Fig. 1C; Chen et al. 1997). A mammalian homolog of the *dachshund* gene, named *Dach* (Hammond et al. 1998; Kozmik et al. 1999) or *Dac*

(Caubit et al. 1999) has been isolated. It has a dynamic expression pattern during embryogenesis which includes the eye, limb, neural crest, and neocortex. *Dach* maps to the mouse *Piebald* locus and its absence may be responsible for the phenotype seen in some deletions of this region. The human *DAC* gene maps to a syntenic region on human chromosome 13 also associated with neural crest deficiencies, mental retardation, and occasional deficiencies in limb and central nervous system (CNS) development (Caubit et al. 1999). *Pax6* and *Dach* have overlapping domains of expression in the eye. However *Dach* expression is not affected in the mouse *Small eye* (*Pax6*) mutation (Hammond et al. 1998). *Dach* shows partial homology to the Ski proto-oncoprotein and related Sno proteins. Targeted disruption of the mouse *Ski* gene (Berk et al. 1997) demonstrates its importance for normal morphogenesis of the face and brain. The skeletal muscle mass is also reduced in the mutant. Over expression of *Ski* results in skeletal muscle hypertrophy (Sutrave et al. 1990), with fast fibers particularly implicated (Lana et al. 1996). *Sno*, like *Ski*, is expressed in many tissues, including neonatal and adult skeletal muscle (Pearson-White and Crittenden 1997). Although they have transforming activity, both Ski and Sno can convert muscle cells to myogenesis (Colmenares and Stavnezer 1989; Zheng et al. 1997), and Ski potentiates transcription from the *Myogenin* promoter in differentiating muscle cells (Ichikawa et al. 1997). Recently, it has been shown that Ski is a component of a histone deacetylase complex, modulating the effect on transcription of Mad and nuclear hormone receptors (Dahl et al. 1998; Nomura et al. 1999). In the myogenic context, Ski may inhibit transcriptional repression by such receptors. Another model, based on interaction between NF1 transcription factors and Ski (Tarapore et al. 1997), is suggested by studies on the muscle *Aldolase A* promoter, which is regulated by adjacent MEF3 and NF1 sites (Spitz et al. 1997). In this case, Eya2-Ski/Dach interaction would be facilitated by their binding as cofactors to Six and NF1, respectively (see Fig. 1C). In such a model it is more evident to envisage Ski as a transcriptional activator of the *Aldolase* gene, which is expressed in fast muscle fibers, in which particular NF1 isoforms are present (Spitz et al. 1997). Part of the transforming activity of Ski, may be exercised via repression of Smad transcriptional activity and, hence, of transforming growth factor β (TGF β)-induced growth arrest (Luo et al. 1999).

A novel *Dach* gene and expression of the regulatory network in somites

In this issue, Heanue et al. (1999) report the isolation of a second vertebrate *dachshund* homolog, *Dach2*. This gene, isolated from chicken, can rescue the eye phenotype in *dachshund* mutant flies. Two protein domains are conserved between fly *Dac* and the vertebrate *Dach*, *Dach2*, *Ski*, and *Sno* proteins: a carboxy-terminal region which is involved in the formation of helical structures with other proteins, including *Ski* itself, which acts as a dimer; and an amino-terminal domain that is necessary for the transforming and myogenic properties of *Ski* and *Sno* proteins. In *Drosophila dachshund* this region is associated with transcriptional activation (Chen et al. 1997). *Dach2*, together with members of the *Pax*, *Six*, and *Eya* families are expressed in somites, in the domain that gives rise to skeletal muscle progenitors and subsequently in these cells. Indeed, it was the presence of *Pax*, *Six*, and *Eya* transcripts, and the analogy with the situation in the *Drosophila* eye, which led Heanue et al. (1999) to look for expression of a *dachshund* homolog in the somite.

Somites (Fig. 2) form progressively during embryogenesis along the rostral-caudal axis by condensation of paraxial mesoderm on either side of the neural tube (for review, see Christ and Ordahl 1995; Tajbakhsh and Buckingham 1999). Initially the somite is a sphere of epithelial-type cells, which at this stage are multipotent. Their adoption of a cartilage, muscle or dermal cell fate depends on signals from surrounding tissues. As the somite matures, the dorsal dermomyotome retains an epithelial structure, whereas mesenchymal cells form the sclerotome ventrally. This compartment will give rise to the cartilage and bones of the vertebral column and at least part of the ribs. Skeletal muscle cells derive from the dermomyotome. Epaxial musculature (e.g., deep back muscles) derives from cells situated dorso-medially, adjacent to the neural tube, which migrate under the dermomyotome to form the differentiated muscle of the epaxial myotome (see Fig. 2). This process depends on signals from the notochord (e.g., Sonic hedgehog) and the neural tube (e.g., Wnts). Ventro-laterally, muscle progenitor cells in the dermomyotome will contribute to the hypaxial myotome that participates in the formation of ventral body muscles. The specification of hypaxial muscle cells depends on signals from the surface ecto-

Figure 2. Schema of somite maturation and early muscle formation. (A) The epithelial somite; (B) a more mature somite where cell fate has been specified.

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derm, also a source of Wnt signaling, and is retarded by lateral plate mesoderm, a source of bone morphogenetic proteins (BMP) and fibroblast growth factor (FGF) signals. Muscle progenitor cells also migrate out from this lateral part of the dermomyotome to form hypaxial muscle masses such as those of the limb (Fig. 2).

Pax3 (and *Pax7*) (for review, see Tajbakhsh and Buckingham 1999) is expressed in the dermomyotome, as well as in the presomitic paraxial mesoderm. As the somite matures *Pax3* expression remains high in the hypaxial dermomyotome and in the myogenic progenitor cells that migrate from it. *Pax3* transcripts are also detected later in differentiated muscle masses. In *Spotch* mice which carry mutations in the *Pax3* gene, the hypaxial dermomyotome is foreshortened and migratory muscle progenitor cells do not leave it; limb muscles, for example, do not form. The *Six1* gene in mouse (Oliver et al. 1995) and *Six4* gene in chicken (Esteve and Bovolenta 1999) are also transcribed in presomitic mesoderm and in newly formed somites. As the somites mature, expression is restricted to the dermomyotome and later to the skeletal muscle mass of the myotome. It is also seen in developing limb buds, in which expression in connective tissue can be distinguished from that in muscle cells using *Spotch* mutant mice (Oliver et al. 1995). Both *Six1* and *Six4* transcripts are also detected in adult skeletal muscle (see also Spitz et al. 1997). Expression of three of the four murine *Eya* genes has been documented in the epithelial somite, the dermomyotome, migrating muscle progenitor cells, and differentiated skeletal muscle (Xu et al. 1997b; Michima and Tomarev 1998; Borsani et al. 1999). At least in the case of *Eya1* and *Eya2*, expression was also noted in presomitic mesoderm (Xu et al. 1997b). *Dach* transcripts were not detected in somites, although a recent report suggests that there may be low-level expression (Davis et al. 1999; Kozmik et al. 1999). *Dach* is expressed in a dynamic pattern in limb bud mesenchyme, but there is no evidence for expression in skeletal muscle (Hammond et al. 1998; Caubit et al. 1999).

Heanue et al. (1999) now show that *Dach2* is expressed in the developing avian somite. As in the case of the other genes discussed, *Dach2* expression is dynamic. Expression is not evident in presomitic mesoderm but is seen in the dorsal part of the somite as it forms. As the somite matures, *Dach2* transcripts accumulate in the hypaxial part of the dermomyotome and are seen in migratory muscle progenitor cells and in the premuscle masses of the limb bud. Expression remains detectable in the epaxial dermomyotome immediately adjacent to the neural tube. *Dach2* and *Pax3* expression remains restricted to the dermomyotome, whereas *Six1* and *Eya2* transcripts become concentrated in the myotome. It remains to be seen whether *Dach2* expression is detected later in differentiated skeletal muscle. It is possible that *Ski/Sno*, not *Dach2*, synergize with *Six* and *Eya* to activate and/or maintain transcription of skeletal muscle genes once muscle has formed. *Dach2* is also expressed in lateral plate mesoderm and, like *Pax3*, in the dorsal neural tube.

The expression patterns of *Dach2* and *Pax3* are similar

in the somite, and both have now been shown to depend on signals from the overlying surface ectoderm (Heanue et al. 1999; for review, see Tajbakhsh and Buckingham 1999). It is not clear whether signals, such as the Wnts, which affect *Pax3*, also activate and/or maintain *Dach2* transcription independently or whether this is dependent on *Pax3*. Heanue et al. (1999) mention that in *Spotch* (*Pax3* mutant) mice, *Dach2* is expressed, although the *Pax3* ortholog, *Pax7*, may be compensating for the loss of *Pax3*. However, in *Pax6* mutants, *Dach* expression is also not affected (Hammond et al. 1998). Interestingly, *Drosophila dachshund*, which, in addition to the eye, is also expressed in the developing wing and limb of the fly, is regulated in the limb by a quantitative balance of the signaling molecules Wingless (Wnts) and Decapentaplegic (BMPs) (Lecuit and Cohen 1997), both present in the vicinity of the somite (for review, see Tajbakhsh and Buckingham 1999). In this context, potential transcriptional repression of BMP signaling by *Dach2* as reported for *Ski* (Luo et al. 1999) may be important for muscle formation. Reciprocal regulation between *Pax3* and *Dach2* has been demonstrated in vitro in somite explant cultures. In these experiments, *Pax3* and *Dach2* transcripts, which are absent or present at a very low level in the explants, accumulate in the presence of *Dach2* or *Pax3*, respectively. *Pax3* expression also increases the level of *Eya2* transcripts, but there is little effect on *Six1*, which is already transcribed at a high level in the explants (Heanue et al. 1999). This is similar to the dependency of *Eya* but not *Six* expression on *Pax* gene expression at other sites in the embryo (Xu et al. 1997b). Heanue et al. (1999) also examine the molecular interactions between these factors and show interactions between *Eya2* and *Dach2* or *Eya2* and *Six1*. The latter interaction is not seen with *Six3*, which is the direct homolog of *Drosophila* Optix, rather than *So* (Toy et al. 1998). The interactions seen between these avian proteins are therefore similar to those described for *Drosophila* *Eya/Dac* (Chen et al. 1997) and *Eya/So* (Pignoni et al. 1997), as well as for murine *Six* and *Eya* proteins, where it was noted that *Six3*, unlike *Six1*, was ineffective in inducing the nuclear translocation of *Eya* (Ohto et al. 1999).

The genetic hierarchy which regulates myogenesis

The formation of skeletal muscle in vertebrates depends on a family of genes that encode basic helix-loop-helix transcription factors. These myogenic regulatory factors transactivate many genes expressed in skeletal muscle and, furthermore, when overexpressed in nonmuscle cells, will convert them to muscle cells (Weintraub et al. 1991). Targeted mutations of the four genes of this family in the mouse (for review, see Tajbakhsh and Buckingham 1999) have demonstrated that Myogenin, which accumulates as myoblasts differentiate, is essential for skeletal muscle differentiation, whereas *Myf5* and *MyoD* together play a role in the determination of myogenic progenitor cells; indeed, in the absence of both of these factors, somitic cells, which in response to exter-

nal signals have activated one of these genes, migrate aberrantly and may assume cartilage or dermal cell fates (Tajbakhsh et al. 1996). More recently, it has been shown genetically that *Pax3* and *Myf5* act upstream of *MyoD* in determining skeletal muscle cell fate (Tajbakhsh et al. 1997); furthermore, ectopic expression of avian *Pax3* will lead to *MyoD* activation in certain cell types (Maroto et al. 1997).

Heanue et al. (1999) now investigate the potential of *Dach2*, *Eya2*, and *Six1* for effecting myogenic conversion. They show that in somite explants in which—in the absence of surrounding tissues—no myogenesis takes place, expression of combinations of *Six1* and *Eya2* or *Dach2* and *Eya2* leads to activation of *MyoD*, *Myogenin*, and *Myosin heavy chain* genes. Again, this is reminiscent of the situation in the *Drosophila* eye. This finding therefore introduces these regulatory factors as key players, together with *Pax3* and *Myf5*, in the genetic hierarchy that regulates myogenesis. *Myf5* expression was not examined in the experiments described by Heanue et al. (1999); and indeed, in birds the relation between *Myf5* and *Pax3/MyoD* has not been established clearly. Expression of either *Dach2*, *Eya2*, or *Six1*, led to a low level of *Pax3* transcripts. Some *MyoD* transcription was detectable but not activation of the muscle differentiation markers *Myogenin* and *Myosin heavy chain*. This probably reflects the low level of *MyoD*; *Six1* alone, present already in quantity in the explants, is not capable of activating *Myogenin* transcription through the MEF3 site in the *Myogenin* promoter under these conditions. The simplest explanation of these observations is that *Pax3*, activated and/or maintained through a *Dach/Eya/Six* feedback loop (Fig. 1A), mediates *MyoD*-driven myogenesis. However, there is no evidence that *Pax3* directly activates *MyoD*; furthermore, expression of *Myogenin* clearly also depends directly on *Six* proteins (Spitz et al. 1998); it remains to be seen whether *MyoD* regulatory sequences also contain MEF3 sites.

Other genes which are potential targets of *Dach/Eya/Six* prior to the onset of muscle differentiation and that may depend on *Pax3* expression include the *c-met* gene, encoding a tyrosine kinase receptor, and the homeobox gene *Lbx1*. These genes are also expressed in the dermomyotome and in migrating muscle progenitor cells. In both cases mutation of the genes (Bladt et al. 1995; Maina et al. 1996; Schäfer and Braun 1999), results in a failure of cells to migrate into the limb and a consequent lack of limb muscles. Interestingly, this is not complete in the case of *Lbx1*-null mice where some forelimb muscles are present (Schäfer and Braun 1999), and a hypomorphic mutation of *c-met* in which Grb2 signaling is uncoupled from the receptor also results in the loss or reduction of some skeletal muscles (Maina et al. 1995). Two other genes encoding homeodomain proteins, *Msx1* (Houzelstein et al. 1999) and *Mox2* (Mankoo et al. 1999), are also expressed in the dermomyotome and in migratory muscle cells. Again, mutation of *Mox2* results in the loss of a subset of muscle masses in the limb (Mankoo et al. 1999). *Mox2* expression does not depend on *Pax3*, and indeed both *Pax3* and *Myf5*, but not *MyoD* are down-

regulated in the limb buds of *Mox2*-deficient embryos (Mankoo et al. 1999). It will be important to see what happens to *Dach/Eya/Six* expression in the *Mox2* mutant. In the absence of *Pax3* this complex may be activating *MyoD* in the limb.

Functional considerations

Formation of limb muscle, and other muscles derived from the hypaxial dermomyotome (see Fig. 2), depends on a number of critical steps: (1) survival and proliferation of myogenic progenitor cells in the dermomyotome epithelium; (2) delamination from this epithelium; (3) migration; (4) proliferation in the premuscle masses of the limb; and (5) activation of the myogenic program through *Myf5* and/or *MyoD*.

Pax3 has been implicated in the first step (Borycki et al. 1999; see also Tajbakhsh and Buckingham 1999) and possibly in the proliferation of the premuscle masses. It is perhaps significant that *Six* proteins (Ford et al. 1998), as well as *Ski/Sno*, can affect cell proliferation. It has been proposed that *Ski*, which is expressed maximally in the skeletal muscle of limbs at embryonic day (E) 13.5–15.5 in the mouse plays a role in the amplification of the secondary myoblast population prior to the large increase in skeletal muscle mass which takes place at this time (see Berk et al. 1997). *c-Met* is necessary for delamination from the epithelium (Yang et al. 1996) and probably for migration (Bladt et al. 1995). Signaling through this receptor also probably affects the proliferation of at least part of the muscle precursor population in the limb (Maina et al. 1995). *Lbx1* plays a role in migration, perhaps through activation of another cell surface receptor involved in this process (Schäfer and Braun 1999). *Mox2* (Mankoo et al. 1999) may also be necessary for the migration and for proliferation of a subpopulation of myogenic progenitor cells. Directly or indirectly it also affects *Myf5* expression and hence activation of the myogenic program.

Activation of the myogenic program at the correct time and place is critical and repressing premature myogenesis may also require specific regulatory strategies. *Pax3* and now *Six* and *Eya* are already expressed in the presomitic mesoderm, as well as in the dermomyotome and the cells that migrate from it. *Dach2* is also expressed at the latter stages. Factors, or a combination thereof, which promote cell division (such as *Pax* and *Six* proteins, potentially) will tend to repress muscle differentiation, which is preceded by cell cycle withdrawal. *Msx1* represses myogenesis; in this case, direct binding of the homeoprotein to the *MyoD* enhancer has been shown (Woloshin et al. 1995). The formation of a complex between *Msx1* and *Pax3* has also been demonstrated in vitro (Bendall et al. 1999), although the in vivo significance in the somite in which *Msx1* expression is low (Houzelstein et al. 1999) is not clear. Signaling from surrounding tissues is an important facet of this scenario (for review, see Cossu et al. 1996), leading to repression or activation of *Myf5* and *MyoD* (for review, see Tajbakhsh and Buckingham 1999). Post-transcriptional

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modification of regulators such as Pax3, Dach/Eya/Six may be introduced as a result of such signaling. In this context, it is intriguing that Eya is subject to translocation from cytoplasm to nucleus (Ohto et al. 1999). At present, information about the expression of these genes is based almost entirely on detection of transcripts.

This discussion has focused on myogenic cells which derive from the hypaxial dermomyotome, where the necessity for Pax3 is evident. However, the role of Pax3 in epaxial myogenesis remains an enigma. *Myf5* is activated initially in the epaxial dermomyotome and it is this myogenic factor that drives early myogenesis in the myotome. Later activation of *MyoD*, as a result of signals from an older neural tube and notochord, is delayed (Tajbakhsh et al. 1997) but occurs in the absence of *Myf5* (for review, see Tajbakhsh and Buckingham 1999). In *Spotch* mice, epaxially derived muscles are present; however, in *Spotch/Myf5* double mutant mice, no muscle forms in the trunk or appendages and *MyoD* does not rescue myogenesis at these sites (Tajbakhsh et al. 1997). Pax3 is required for the activation of *MyoD* in the absence of *Myf5*, although *Pax3* transcripts are down-regulated in the epaxial dermomyotome by this stage in the mouse. *Myf5* is necessary, prior to activation of the myogenic program, for the correct positioning of progenitor cells (Tajbakhsh et al. 1996), presumably via activation of a cell surface receptor. Pax3 plays this role in the hypaxial dermomyotome and it may also play such a role epaxially via a receptor such as c-Met, which is expressed in a few cells in the epaxial dermomyotome. Failure to activate *MyoD* in the *Spotch/Myf5* double mutant would then be due to incorrect positioning of progenitor cells.

The results reported by Heanue et al. (1999) now suggest another explanation. Pax3-dependent activation of *Eya* may be essential for *MyoD* activation. Unlike *Dach2*, *Eya* and *Six* do not appear to be expressed at high levels in the epaxial dermomyotome of the chick embryo, but this remains to be investigated at the time of *MyoD* activation in the mouse. Pax3, acting through Dach/Six/Eya, may therefore regulate transcription of *MyoD* in both epaxial and hypaxial muscle cell derivatives, together with *Myf5*, which is probably situated in another regulatory network. Targeted mutations of the *Dach*, *Six*, and *Eya* genes expressed in the somite should clarify their position in this genetic hierarchy. Compensation by another member of the gene family may make this approach laborious. The manipulation of dominant-negative sequences is another possible approach. Exploring the function of these factors will provide interesting new insights into the regulation of myogenesis. Indeed, the redeployment of this *Drosophila* regulatory network not just for eye and muscle, but in many other developmental contexts in the vertebrate embryo, has widespread functional implications.

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Erratum

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