



HAL
open science

Myogenesis control by SIX transcriptional complexes

Pascal Maire, Matthieu dos Santos, Rouba Madani, Iori Sakakibara, Camille Viaut, Maud Wurmser

► **To cite this version:**

Pascal Maire, Matthieu dos Santos, Rouba Madani, Iori Sakakibara, Camille Viaut, et al.. Myogenesis control by SIX transcriptional complexes. *Seminars in Cell and Developmental Biology*, 2020, 104, pp.51 - 64. 10.1016/j.semcdb.2020.03.003 . hal-03490399

HAL Id: hal-03490399

<https://hal.science/hal-03490399>

Submitted on 22 Aug 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Myogenesis control by SIX transcriptional complexes

Pascal Maire^{1*}, Matthieu Dos Santos¹, Rouba Madani¹, Iori Sakakibara², Camille Viaut¹, Maud Wurmser³.

1. Université de Paris, Institut Cochin, INSERM, CNRS. 75014 Paris, France.
2. Research Center for Advanced Science and Technology, The University of Tokyo, Japan
3. Department of Integrative Medical Biology (IMB), Umeå universitet. Sweden

*Corresponding author - Phone: 33-1-44.41.24.13, E-mail: pascal.maire@inserm.fr

Key words: Myogenesis, development, genetic network, Pax7, Six1, MyoD, Myogenin, adult myofiber, satellite cell.

Abstract

SIX homeoproteins were first described in *Drosophila*, where they participate in the Pax-Six-Eya-Dach (PSED) network with *eyeless*, *eyes absent* and *dachsund* to drive synergistically eye development through genetic and biochemical interactions. The role of the PSED network and SIX proteins in muscle formation in vertebrates was subsequently identified. Evolutionary conserved interactions with EYA and DACH proteins underlie the activity of SIX transcriptional complexes (STC) both during embryogenesis and in adult myofibers. Six genes are expressed throughout muscle development, in embryonic and adult proliferating myogenic stem cells and in fetal and adult post-mitotic myofibers, where SIX proteins regulate the expression of various categories of genes. *In vivo*, SIX proteins control many steps of muscle development, acting through feedforward mechanisms: in the embryo for myogenic fate acquisition through the direct control of Myogenic Regulatory Factors; in adult myofibers for their contraction/relaxation and fatigability properties through the control of genes involved in metabolism, sarcomeric organization and calcium homeostasis. Furthermore, during development and in the adult, SIX homeoproteins participate in the genesis and the maintenance of myofibers diversity.

- 1- The evolutionary conserved *Pax/Six/Eya/Dach* (PSED) genetic network**
 - 1.1 Overview of the PSED genetic network
 - 1.2 Regulation of SIX activity by EYA and DACH
 - 1.3 *Six* in development and pathophysiology
- 2- *Six* and embryonic myogenesis**
 - 2.1 Expression of *Six* genes and their cofactors in myogenic territories
 - 2.2 STC activity in myogenic compartments
 - 2.3 Signaling molecules controlling STC activity in myogenic progenitors in the dermomyotomal epaxial lips
 - 2.4 Role of *Six* in the myogenic commitment in the hypaxial lip
 - 2.5 Differentiation of myogenic progenitors and *Myogenin* induction
 - 2.6 Craniofacial myogenesis
- 3- *Six* genes in adult myofibers**
 - 3.1 *Six* in the genesis of muscle fiber-type diversity during development
 - 3.2 *Six* in the maintenance of the fast phenotype in adult myofibers
 - 3.3 *Six* in physiology, atrophy and hypertrophy
- 4- *Six* genes in adult myogenic stem cells**
 - 4.1 Role of *Six* genes in self-renewal and genesis of post-natal myogenic stem cells
 - 4.2 *Six* genes in cell reprogramming

1 The evolutionary conserved PSED genetic network

1.1 Overview of the PSED genetic network

The *Pax/Six/Eya/Dach* gene network was initially described in *Drosophila melanogaster*, where *eyeless* (*ey*), *sine oculis* (*so*), *eyes absent* (*eya*) and *dachshund* (*dac*) drive synergistically eye development through genetic and biochemical SO-EYA and EYA-DAC interactions. Consequently, the absence of any of these genes leads to the absence of eye development, while ectopic expression of *ey* in leg imaginal discs gives rise to ectopic eye development (1) through the activation of *so/eya/dach* gene network. This property is conserved during evolution, and it has been shown that ectopic *Six3* (2) or *Pax6* (3) expression in vertebrate embryos can trigger a cascade of events required for retinal induction, through activation of this network.

Ey belongs to the paired homeodomain family of transcription factors for which 9 *Pax* genes of this family have been characterized in vertebrates, reviewed in (4). *Ey* activates *eya* and *so*, which belong to the SIX homeodomain family of transcription factors. Three *Six* genes have been characterized in *Drosophila* (*so*, *optix* and *D-six4*) and six *Six* genes in vertebrates (*Six1* to *Six6*), reviewed in (5). The evolutionary history of *so/Six* genes has been reviewed (6). SIX proteins can act either as a transcriptional repressor when interacting with GROUCHO, a family of non-DNA binding co-repressors, or as a transcriptional activator when interacting with EYA (7, 8). EYA is also a transcriptional cofactor of DACH, as reviewed in (9). The relative level of *so* and *eya* controls the positive feedback loop driving *ey* activation but also its repression (10). Other cofactors of SO have been identified in *Drosophila*, among which Sine Oculis Binding Protein, SOBP/SBP (11) whose interaction with vertebrate SIX proteins is also evolutionary conserved, and may modulate SIX transcription complexes (STC) properties. Many proteins have been identified as SIX cofactors, in *Drosophila* and in vertebrates, so that the nature and relative availability of SIX cofactors present in one cell can modify SIX transcription properties and their related effects on cell behavior (11, 12).

It has been established in *Drosophila* that *ey* and *so* were required to activate directly and indirectly the proneural bHLH determinant gene *atonal* (Figure 1), allowing the

progression and differentiation of eye imaginal disc cells into a neuronal fate and photoreceptor specification (13) (14). Evidence for the control of bHLH determination genes by members of this network has then been established in amniotes. *Six* and *Eya* genes play a role in the determination of neuroblast cell fate by controlling bHLH determination genes of the *NeuroD* family in epibranchial placodes. Forced expression of *Six1* and *Eya1* in fibroblasts is able to activate *NeuroD1* and *NeuroG1* and converts these cells into neurons (15).

While *ey* is transiently expressed in undifferentiated progenitors (10), *so* is expressed all along *Drosophila* eye development in both progenitors and committed neural cells, and is required for the development of the rest of the visual system, including the optic lobes (16). In the mouse, we will see that *Pax3* is expressed transiently in undifferentiated myogenic progenitors while *Six1* and *Eya1* are expressed all along muscle development. Environmental cues play instructing role in the process of eye formation and Hedgehog (HH) and Decapentaplegic (DPP) proteins are required for the ability of the *ey/so/eya/dach* network to drive efficient ectopic eye formation (17). *So*, *eya* and *dach* are not only expressed in the *Drosophila* eye but also in other cell types where they do not control each other expression and where they can act independently. This is the case for *eya* and *so* that are expressed in a spatiotemporal exclusive manner in the mesoderm of *Drosophila* (18). *Eya* expression is detected transiently in the ventral and lateral mesoderm, and its mutation leads to a loss of somatic muscles (18, 19). Interestingly, *D-six4* has a similar mesodermal expression as *eya*, and *D-six4* mutants partially phenocopy the defects observed in *eya* mutants (20), suggesting that a D-SIX4-EYA transcription complex can activate downstream targets leading to somatic muscle fate. We will see that the biochemical interactions between SIX and EYA proteins are conserved in mammals, and that the STC is active during embryonic myogenesis and in adult myofibers. Table 1 recapitulates the different players of the PSED discussed in this review.

1.2 Regulation of SIX activity by EYA and DACH

The SIX transcription cofactors EYA belong to the haloacid dehalogenase protein family (21-23), a phosphatase family subgroup. Four genes (*Eya1* to *Eya4*) have been identified in vertebrates (24). While in *Drosophila* EYA proteins are mainly nuclear, due to the presence of nuclear localization signal (nls) motifs, they are found in the cytoplasm and in the nucleus in vertebrate cells. Nuclear EYA transcriptional activity is increased by phosphorylation by NEMO (25, 26) and MAPK (27), and decreased by Abelson tyrosine kinase (28) that relocates EYA into the cytoplasm in *Drosophila* cells. EYA Tyr-phosphatase activity seems dispensable for *Drosophila* eye development (29).

In vertebrates EYA are associated in the cytoplasm with several proteins among which G α i proteins, sequestering them at the membrane. SIX homeoproteins, interacting with EYA, are required to trigger their translocation to the nucleus (30, 31). Cytoplasmic Tyr-phosphatase activity of EYA proteins increases the levels of active RAC1 and CDC42 (32), and modifies WDR1 phosphorylation (33) leading to Actin cytoskeleton remodeling. EYA nuclear Tyr-phosphatase activity is required during DNA double strand repair through dephosphorylation of phosphoryl-H2AX (34). A mode of transcriptional activation by EYA has been characterized. It was shown that nuclear EYA can interact directly with BRG1 and BAF170, two components of the nucleosome-remodeling SWI/SNF protein complex, allowing the SIX-EYA transcription complex to remodel chromatin at precise loci, therefore contributing to activate and maintain specific cellular identities (15). Such interactions remain to be established in the myogenic lineages in vertebrates.

DACH proteins are positive or negative regulators of the SIX-EYA transcription complexes (23). They contain a DNA binding domain characteristic of winged helix/forkhead of transcription factors (35). Four members of this family (*Dach1*, *Dach2*, *Ski*, *Skil/SnoN*)

have been characterized in vertebrates (36). Both DACH and EYA activities are modulated by and modulate DPP/BMP/TGF β signaling in *Drosophila* and vertebrates (37-39). SKI interacts with SMAD2, SMAD3 and SMAD4 and inhibits TGF- β and BMP signaling via its interaction with phospho-SMAD and the recruitment of HDACs to the DNA binding complexes, thus repressing transcription of target genes (40, 41). SKI also interacts with ubiquitous Nuclear Factor-1 (NF-1/Nfi) proteins (42) known to control expression of many genes in skeletal muscle (43, 44). While DACH proteins can interact with NCoR, HDAC, SIN3A/B transcriptional repressor components (23, 45), they can also associate with SIX-EYA complexes, allowing interaction with the histone acetyl transferase CBP which leads to activation of downstream targets, among which *Myogenin*, in myogenic cells (30). This positive synergy between DACH and EYA during myogenesis has also been demonstrated by their forced expression in pluripotent presomitic explants, where expression of both factors can induce a myogenic fate of the cells of these explants by activating *MyoD* and *Myh* expression (46). Positive synergy between EYA and SKI also leads to *Myogenin* activation (47). Last, deregulation of *Dach* genes expression is involved in abnormal cell growth and tumorigenesis of breast, prostate and ovarian cancer, reviewed in (48). The recruitment of a repressive or activating complex by DACH/SKI protein complexes may depend on external cues, and of the nature of the protein complex (directly through DACH DNA binding, or indirectly through STC, NF1 complex, SMAD complex).

1.3 Six in development and pathophysiology

In vertebrates orthologues of the PSED genes are expressed and required for eye development, among which *Pax6*, *Six3*, *Eya1* and *Dach1* (49-53). Members of these genes families are also expressed in many other cell types in the embryo, and required for the genesis of the thymus, of the kidney, of dorsal root ganglia and of most sensory organs during embryogenesis, suggesting that mechanisms deployed by the combined expression of these genes has been selected and conserved in many species during evolution (18, 54-56) and presides multiple types of organogenesis (4, 5, 24, 57, 58) reviewed in (59). Among downstream targets of this network are genes that control proliferation and cell fate decision (60, 61).

Abnormal expression of these genes is associated with human developmental malformations. This is the case for mutations of *Six1* or *Eya1* that are responsible of the Branchio Otho Renal syndrome (62, 63). Mutation of the *Eya4* gene is responsible for dilated cardiomyopathy and sensoryneural hearing loss (64), while *Six3* mutation induces holoprosencephaly (65). In addition, several mutations in the *Six* and *Eya* genes have been associated with adult pathologies and particularly cancers. Increased expression of *Six1* is associated with increased proliferation with aggressive and metastatic properties of adult human cancer from different origin (66), like liver (67), breast (68), kidney (69), colon, through WNT/ β -Catenin activation (70), or skeletal muscle (71), reviewed in (72). Increased expression of *Eya2* is associated with breast and ovarian cancer (38, 73). SIX1 in cancer cells regulates many glycolytic genes through interactions with HBO1 and AIB1 histone acetyltransferases, promoting the Warburg effect and tumor growth (74). *Six1* over expression appears to control several signaling pathways favoring EMT and invasiveness, including TGF β (38), ZEB1 (75), Ezrin (76), HH signaling in meduloblastoma (77) or VEGFC (78), reviewed in (36, 72). *Six1* and *Six2* are components of non-canonical NF κ B signaling, and in macrophages control inflammatory gene expression (79). Furthermore, SIX1 in human fibroblasts represses senescence and stemness through the control of *p16INK4a* and of the transcription factor *Sox2* respectively (80, 81). Last, SIX1 directly interacts with EBF2, C/EBP α and C/EBP β , and promotes brown adipogenesis of C3H10T1/2 cells committed to the brown lineage (82).

2- Six and embryonic myogenesis

2.1 Expression of Six genes and their cofactors in myogenic territories

Myogenesis in vertebrates is initiated during embryogenesis by the expression of bHLH transcription factors of the *MyoD* family called Myogenic Regulatory Factors (MRF). Three members of this family, *Myf5*, *MyoD* and *Mrf4* act as determination genes, and are able to commit pluripotent cells in the embryo into a myogenic fate, while the fourth, *Myogenin* is expressed in committed cells to initiate their differentiation (83, 84). At the axial level myogenic cells originate from embryonic structures called somites, segmented balls of epithelial cells that arise from the unsegmented paraxial mesoderm, while at the head level unsegmented craniofacial mesoderm gives rise to skeletal muscles of the face and of the oesophagus (85), and at the neck level both craniofacial mesoderm and rostral somites are involved (86). Reviews on the genetic cascades and signaling pathways that preside somitic, limb, craniofacial and adult myogenesis in vertebrates have been published (87-92). *Six1* and *Six4* genes lie on the same locus on chromosome 12 in the mouse (93, 94) and show similar patterns of expression during embryogenesis (5, 95). *Six1/2/4/5* and their associated cofactors are expressed dynamically in the dermomyotomal cells of the somites and are good candidates to activate (MRF) expression.

While *Six1* and *Six2* expression is not detected in the presomitic mesoderm (94, 96), *Six4* (97) and *Six5* (unpublished data) are expressed in this transient structure. The somite gives rise to multiple cell lineages, among which the sclerotomal and endothelial tissue, brown fat, back dermis, smooth muscle, and skeletal muscles (98). In the epithelial somites, *Pax3* and *Six1* genes are coexpressed in most of the cells of their dermomyotome (96). *Pax3/7* are required for proliferation, cell survival and delamination of dermomyotomal cells, and control bHLH determination gene expression in the myogenic lineage (88). *Pax3* forced expression in somitic explants of chick embryos can activate myogenesis (99), and double *Pax3/Pax7* mutant embryos show only early residual epaxial myogenesis that stops prematurely by exhaustion of progenitors (100). Myogenic progenitors arise first during development from the four lips of the somitic dermomyotome, the epaxial/dorsomedial lip, the ventrolateral/hypaxial lip, the anterior/caudal lip and the posterior/rostral lip (101). At these early stages, other *Six* genes than *Six1* are expressed in the somite, among them *Six2* (94, 102), *Six4* (97) and *Six5* (103), that may influence the fate of somitic cells in synergy with *Pax3/7*. Accordingly, PAX3 in cooperation with SIX4 and TEAD2 increases chromatin accessibility of downstream targets favoring myogenic fate specification (104).

Dynamic expression of *Eya1* and *Eya2* has been detected in somites, dermomyotomes, dermomyotomal lips, myotomes of xenopus (105), zebrafish (106), chick (107) and mouse (108). Expression of *Eya1* and *Eya2* is high in hypaxial dermomyotomal progenitors of the somite (109), and in migrating myogenic progenitors into the limbs (108). Expression of *Eya4* is high in the dermomyotome of the somite (110), while expression of *Eya3* is low (111, 112). Comparison of the expression pattern of *Pax*, *Six* and *Eya* genes in the embryo revealed that they are coexpressed in somites, mainly in the lips of the dermomyotomes, suggesting that they may control each other's transcription, and may synergize to instruct the behavior of these cells in a myogenic fate through activation of bHLH MRF expression. How the signaling pathways modulating EYA subcellular localization in *Drosophila* influence EYA activity in myogenic cells of vertebrates remains to be established. Interestingly, in vertebrates Nemo like kinase (NLK) plays a crucial role in controlling spinal and bulbar muscular atrophy (113), MAPK activity participates in muscle fiber-type modulation (114), and Abelson tyrosine kinase regulates myoblast proliferation and muscle fiber length (115); the involvement of EYA proteins in these signaling has not yet been evaluated.

Expression of *Dach1* and *Dach2* has been analyzed during mouse and chick embryogenesis, revealing expression of *Dach2* in the dermomyotome and its ventral and dorsal lips (46, 116). *Dach1* expression is high in the newly formed somites, and then is restricted to the medial and lateral somites (116, 117). *Ski* and *SnoN* are expressed in the presomitic mesoderm, and later on between E12.5 and E16.5 during fetal myogenesis (118, 119). Forced expression of *Ski* in quail embryonic cells has the property to induce their myogenic differentiation (120). Accordingly, *Ski* KO embryos show fetal myogenesis defects (121), while *Dach1/Dach2* mutant embryos have no major myogenic defects (122). While *SnoN* efficiently represses *Myogenin* transcription (123), *SKI* activates this gene in association with *SIX* and *EYA* proteins (47).

In conclusion, several genes of the PSED network are expressed in myogenic territories of the embryo, where they contribute to restrict the pluripotent state of these embryonic cells to favor a myogenic fate.

2.2 STC activity in myogenic compartments

SIX1, *SIX2*, *SIX4* and *SIX5* homeoproteins bind the same DNA sequence, (5, 124, 125) (126, 127), a non-palindromic sequence of DNA (TCAGGTTTC) called MEF3 (muscle enhancer 3). This sequence has been identified in the regulatory sequences of numerous genes expressed in myogenic cells; the *TnnC* gene (128), the *Na,K-ATPase alpha1* gene (129), the *Myogenin* promoter (124), the muscle *creatine kinase* enhancer (130), the hypaxial *Myf5* enhancer (131), the two identified *MyoD* enhancers (102, 132), the muscle specific *Aldolase A* promoter (43), in the fast *Myosin heavy chain* (*Myh*) locus (133). More recently, ChIP experiments with antibodies directed against *SIX1* and *SIX4* identified their genome wide targets in myogenic cells (126, 127, 134). To follow the STC activity in the mouse embryo and in the adult, a *PolyMEF3-LacZ* mouse transgenic line has been studied. *LacZ* expression was detected in the mouse embryos mainly in epaxial and hypaxial lips of the dermomyotome, in craniofacial myogenic cells of E10-E13.5 embryos (109), and at later stage in adult fast MYH4+ myofibers (135). These experiments demonstrate that STCs are highly active during embryogenesis in proliferating cells of the dermomyotomal lips that give rise to yet uncommitted myogenic progenitors, and in adult post mitotic muscle fibers. Accordingly, *SIX* proteins accumulate in dermomyotomal lips and in the nuclei of adult MYH4+ myofibers. In addition, transgenic analysis of the activity of wild type or MEF3 mutant *Myogenin* promoters (124), *Myf5* hypaxial enhancer and *MyoD* enhancers during embryogenesis (102, 131) and *AldolaseA* promoter in adult myofibers (136) shows a strong reduction of the expression of all mutant transgenes, demonstrating the requirement of STC at all stages in the myogenic lineage to activate downstream targets *in vivo* in a chromatin environment. Like in the *Drosophila* eye for *so* expression, *SIX* proteins (at least *SIX1*) are present in the muscle lineage at all stages of development during embryogenesis and in the adult: in axial and head embryonic and adult proliferating progenitors; and in embryonic and adult post mitotic differentiated fibers where they may control, in synergy with other TFs the activation of distinct networks of genes, specific of each cell fate.

We will illustrate by several examples the spatio-temporal activity of the STC in the muscle lineage.

2.3 Signaling molecules controlling STC activity in myogenic progenitors in the dermomyotomal epaxial lips.

Embryonic cells of the epaxial lip of the dermomyotome give rise to epaxial back muscles. During this process, these cells undergo an epithelial to mesenchyme transition (EMT), invaginate under the dermomyotome, activate *Myf5* expression and adopt a myogenic fate. Highest *PolyMEF3-LacZ* expression is found in epaxial dermomyotomal cells before/

and during the EMT (109), along with *Eya1* and *Eya2* mRNA accumulation. Accordingly, *polyMEF3-LacZ* expression is lost in *Eya1*^{-/-} embryos, although *Six1* expression is still detected and epaxial cells still present in these mutants. Induction of myogenesis in epaxial dermomyotomal cells is controlled by WNT1 and WNT3a molecules secreted by the dorsal neural tube and the dorsal ectoderm (137-139), but also by SHH signaling (140, 141) and by transient NOTCH signaling from migrating neural crest cells (142) that activate *Myf5* expression. Accordingly *Myf5* epaxial enhancer activity is under the control of GLI and LEF transcription complexes (138). On the contrary, Noggin and Follistatin are required to inhibit negative BMP signaling coming from the lateral mesoderm, and promote induction of myogenic fate (143). Whether these signaling molecules impact on *Six* and *Eya* expression and on STC activity to favor epaxial cells myogenic commitment, at the expense of their self-renewal as pluripotent dermomyotomal cells, is unknown. E9.5 *Six1*^{-/-}*Six4*^{-/-} embryos show decreased epaxial *Myf5* expression, that may be due to the direct involvement of these two homeoproteins in the control of early epaxial *Myf5* expression (95). On the other hand, ChIP experiments performed on C2C12 myogenic cells reveal SIX1 binding in the upstream regions of the *Fzd7*, *Fzd3*, *Rbpj*, and *Notch3* genes (144). Thus, alternatively, modulation of the expression level of these molecules by STC could orient the fate choice of dermomyotomal cells during myogenic commitment in the somite, resulting in decreased epaxial *Myf5* expression in *Six1*^{-/-}*Six4*^{-/-} mutant embryos. The existence of regulatory feedback loops between *Six* and the major signaling pathways orchestrating embryonic stem cells commitment at the onset of myogenesis remain to be established but, interestingly, MRF participate in *Six1* activation through a positive feedback loop, that may be important to reinforce the myogenic fate acquisition (145).

2.4 Role of *Six* in the myogenic commitment in the hypaxial lip

Most of the mammalian musculature derives from the hypaxial lips of the somitic dermomyotome that give rise to limb, abdominal and thoracic muscles, as well as the tongue and the diaphragm. At the interlimb level, the epithelial hypaxial lip moves ventrolaterally into the flank somatopleure of the growing embryo (146, 147), some hypaxial cells ingress and settle as myocytes, constituting the growing hypaxial myotome (with loss of *Pax3* expression but maintenance of *Six1* expression), while other cells self-renew in the lip, maintaining high *Pax3*, *Paraxis*, *Six1* and *Eya1* expression. This ventral hypaxial dermomyotomal lip wave front, leaving committed myogenic cells behind, is reminiscent of the morphogenetic furrow in the *Drosophila* eye, with *ey* positive retinal precursors cells ahead of the progression of the furrow and more differentiated *ey* negative cells behind the furrow (6). Signals from the intermediate and lateral mesoderm synergize with dorsalizing signals from the surface ectoderm to induce the initiation of the hypaxial musculature (148). Multiple signals orient pluripotent dermomyotomal cells toward a myogenic fate. BMP signaling from the lateral plate and non-canonical WNT signaling from the surface ectoderm modulate dermomyotomal growth and myogenic commitment by increasing *Pax3* expression (149, 150) and NOTCH signaling (151, 152). BMP signaling impairs *MyoD* activation (153), and favors endothelial commitment (151, 154) of hypaxial dermomyotomal cells, while high NOTCH signaling favors smooth muscle fate commitment (151). High *polyMEF3-LacZ* expression is also observed in hypaxial dermomyotomal cells expressing *Pax3*, *Six1* and *Six4* as well as *Eya* and *Dach* cofactors, allowing their progression into a myogenic transition fate.

In Figure 1, we summarize the genetic network that drives eye development and the activation of *atonal* in *Drosophila*. If this genetic network hierarchy is conserved and presides to hypaxial myogenesis in mouse embryos, *Pax3* may control the expression of *Six1* and *Eya1* in hypaxial dermomyotomal cells (Figure 1, hypothesis).

At the limb level, competent hypaxial dermomyotomal cells expressing *Lbx1* and *cMet* delaminate from the dermomyotome and migrate into the limb buds under the influence of FGF 4/8 and HGF (155) produced in the limb buds, as well as of uncharacterized molecules produced in endothelial cells (154). These myogenic progenitors express *Pax3* and *Six1*. *Pax3* and *Six1/4* are responsible for *cMet* expression, allowing delamination and myogenic progenitors migration. After their migration into the limb, these PAX3/SIX1+ myogenic progenitors proliferate, self-renew and activate *Myf5* under the influence of WNT, BMP and NOTCH signaling (156). Activation of limb *Myf5* expression is controlled by PAX3 (157), SIX (131), MEOX2, MSX1 (158) and yet uncharacterized transcription factors that bind the *Myf5* limb specific enhancer. Absence of *Six1/Six4* during embryogenesis as well as of *Eya1/Eya2* impairs hypaxial *Pax3* expression. *Six1/Six4* and *Eya1/Eya2* mutant dermomyotomal cells conserve, respectively, *Eya1* and *Six1* expression, and are rerouted ventromedially instead of migrating laterally in the limb bud. No major proliferation defects have been observed in these mutant cells, unable to activate *Pax3*, *MyoD* or *Myf5* (95), that probably adopt a non-skeletal myogenic fate, further demonstrating the instructive role of the SIX/EYA complex for acquisition of a myogenic fate identity (109). Absence of *Pax3* during embryogenesis impairs the progression of hypaxial dermomyotomal cells toward a myogenic fate which die by apoptosis, despite maintained expression of *Six1* and *Eya1*. Hypaxial *Pax3* expression is controlled by a specific hypaxial enhancer (159), bound by SIX homeoproteins on three adjacent MEF3 sites, and *Pax3* hypaxial expression is lost in *Six1/4* mutants, showing the requirement of STC to activate *Pax3* in this subset of hypaxial dermomyotomal cells (109). These findings indicate that the *Pax/Six/Eya* genetic hierarchy operating in hypaxial dermomyotomal cells has been rewired compared to the one operating in *Drosophila* eye development (Figure 1, right).

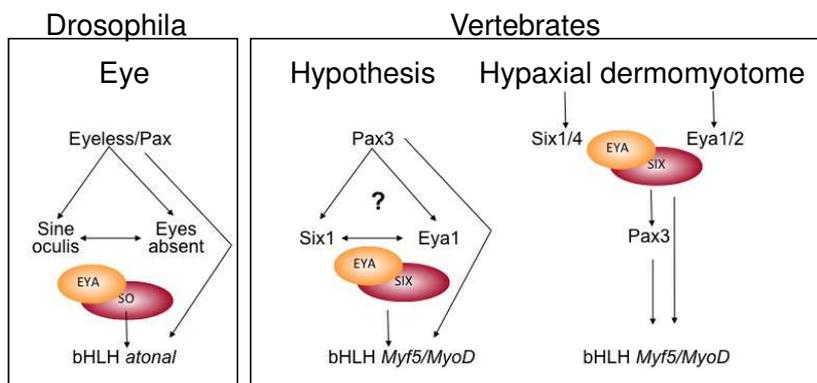


Figure 1. Six and Eya are required to activate hypaxial myogenesis through direct activation of Pax3 and MRF genes. Left: in *Drosophila* *ey* controls the expression of *so* and *eya* during eye development. *So* and *eya* proteins interact and control the expression of *atonal* in synergy with *ey*. Middle, hypothesis: if the genetic hierarchy is conserved through evolution and operates in hypaxial somites, *Pax3* should control the expression of *Six1* and *Eya1* in hypaxial lips cells. Right: hypaxial dermomyotomal expression of *Pax3* is under the control of both *Six1* and *Eya1*, and expression of *Myf5* and or *MyoD* is under the control of these protein families in hypaxial dermomyotomal cells of the somite.

The *Pax3* embryonic mutant cells that escape apoptosis have increased *Foxc2* expression and are rerouted toward a vascular cell fate (160). Analysis of *Pax3*, *Six1/4* and *Eya1/2* mutant embryos show that all three genes families are required in the hypaxial dermomyotome to commit progenitors into a myogenic fate, and that PAX3 directly competes with FOXC2 for cell fate decision (161). It will be interesting to investigate the level of P-SMAD, NICD and P-CREB in these mutant hypaxial cells to establish the existence of potential cross-talks between BMP, NOTCH and non-canonical WNT signaling, hypaxial

cells behavior and the core genetic PSED network that triggers their myogenic commitment (98, 104). Such a study would inform on the transcription factors present in these cells that distinguish them from other embryonic cells expressing the *Pax3/Six1/Eya1* genes, such as neural crest cells for example, that adopt a non-muscle fate.

2.5 Differentiation of myogenic progenitors and *Myogenin* induction

Dermomyotomal progenitors become myoblasts when SIX1 and PAX3 activate *Myf5* or *MyoD* expression (102, 131, 157, 162). Depending on external cues, these embryonic myoblasts have the ability to proliferate, or to withdraw from the cell cycle, activate *Myogenin* expression and differentiate and fuse into multinucleated myofibers (163). Several signaling pathways have been identified that modulate the differentiation process, including the FGF, NOTCH and BMP/MYOSTATIN pathways (reviewed in (98)). The regulatory elements of the *Myogenin* gene that allow its embryonic expression have been delimited to a 184bp promoter element that can drive the expression of a *LacZ* transgene mimicking endogenous embryonic *Myogenin* expression. Among the transcription factors that bind this promoter are MYF5/MYOD/MRF4, MEF2, NF1/NF κ B, PBX/MEIS, MSY-3, NFATc and SIX proteins (Review in (164)). Mutation of *Mef2*, *Myf5* or *Six* DNA binding elements prevents mutant transgene expression (124, 165, 166), demonstrating that binding of these transcription factors is absolutely required to achieve embryonic *Myogenin* activation. EYA proteins interact with SIX proteins in an active transcription complex at the *Myogenin* promoter (30). STCs recruit the histone demethylase UTX to eliminate H3K27me3 (134, 167), CBP (30), and potentially the SWI/SNF complex, since both SIX1 and EYA1 interacts with BRG1 (15), and SIX4 with BAF60c (168). STCs may also be directly involved in the recruitment of the DNA demethylation machinery (169). Absence of SIX binding to the *Myogenin* promoter leads to the absence of its embryonic expression and probably reflects the synergy required between SIX, PBX, NF1, MyoD and MEF2 proteins to efficiently remodel the chromatin and activate transcription. The involvement of PBX/MEIS to attract MYOD to the *Myogenin* promoter, followed by recruitment of a p300 and SWI/SNF chromatin remodeling complex, then of MEF2 was demonstrated in *ex vivo* cell culture models (reviewed in (170)). Synergy between SIX and MYOD to activate downstream targets without direct interactions between these proteins was established (167). Synergy between SIX and NF1 was also demonstrated in transgenic mouse models (43). The synergy between SIX and MEF2 and PBX proteins remains to be thoroughly studied. Whether PBX, NF1, MYOD and MEF2 are able to bind MEF3 mutant *Myogenin* promoter remains to be determined, to better characterize the involvement of SIX homeoproteins in 3D chromatin reorganization. In summary, *Six* genes are expressed and required at all steps that govern ventral trunk and limb myogenesis in the embryo (Figure 2).

2.6 Craniofacial myogenesis

Little is known concerning the role of *Six* and *Eya* genes in craniofacial muscles. *Pax3* gene is not expressed in craniofacial muscles and *Pax7* is expressed at a later stage than *Myf5/MyoD*, showing that the PSED does not control craniofacial myogenic fate. *Myf5/MyoD* expression in craniofacial muscles is controlled by *Pitx2/Tbx1/MyoR/Capsulin* genes (91, 171-173) but expression of *Six1*, *Six2*, *Six4* and *Six5* in craniofacial mesoderm and their role in the control of *MyoD* (102) and *Myogenin* promoter (124) in craniofacial myogenic cells suggests that they participate to craniofacial myogenesis (174). Analysis of *Six1*, *Six2*, *Six4* and *Six5* single and of *Six1/Six4* mouse double mutants failed to reveal a strong craniofacial muscle phenotype. Nevertheless, analysis of E17.5 *Six1^{-/-}Eya1^{-/-}* fetuses (175) showed that these double mutant fetuses present a hypoplasia of extraocular muscles (EOM) and of branchiomeric skeletal muscles, including the first and second arch-derived muscles, showing

the involvement of the STC activity during craniofacial myogenesis. Furthermore, analysis of *Tbx1* expression in *Six1^{-/-}Eya1^{-/-}* mutant and of *Six1* and *Eya1* in *Tbx1* mutant revealed that *Tbx1* is genetically upstream of *Six1* and *Eya1* during cardio- craniofacial morphogenesis. In zebrafish, *Six1a* is expressed after *Myf5* in all craniofacial muscles, contrary to what was observed in the mouse, where *Six1* is expressed before *Myf5*. Absence of zebrafish *Six1a* leads to decreased *MyoD* and *Myog* expression and loss of specific EOM (176). Last, in *Amphioxus*, expression of transdominant negative SIX1/2-engrailed proteins abrogates head muscle formation (177) showing that SIX1/2 binding on their targets in specific cells impacts on their ability to activate myogenesis.

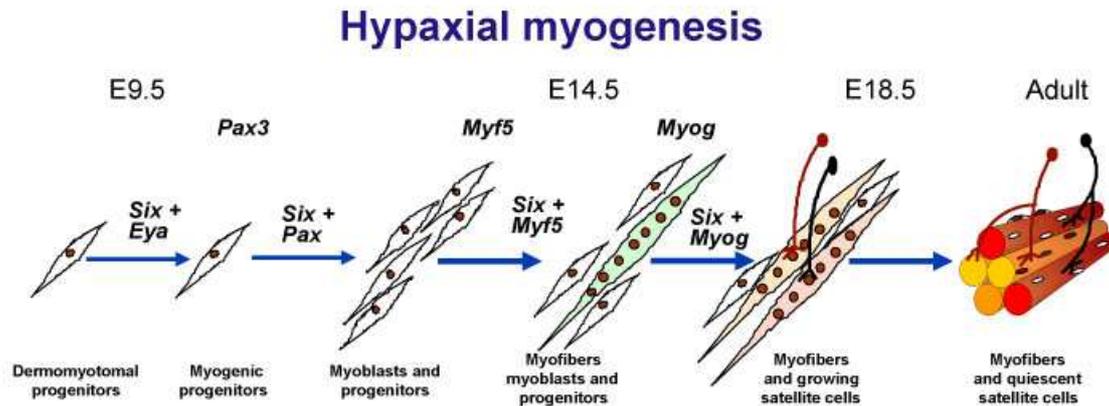


Figure 2. Multiple steps that govern the formation of adult myofibers are controlled by the STC. SIX + EYA in dermomyotomal cells with high P-CREB allow hypaxial *Pax3* activation. SIX + PAX3 in the presence of WNT6 signaling allow hypaxial/ limb *Myf5* expression. SIX + MYF5 + PBX1 + NF1 + MEF2 and low NOTCH activity are required for *Myogenin* expression and differentiation. SIX + Myogenin/MRF are required to activate a network of muscle specific genes (*Myosin*, *Troponin*). SIX1 accumulates at high levels in brown myonuclei along embryonic and adult myogenesis, accumulates less robustly in myonuclei of adult slow-type myofibers (white nuclei), and is expressed in quiescent and activated SC, represented on the top of adult myofibers.

3- Six in myofibers

3.1 Six in the genesis of muscle fiber-type diversity during development.

In amniotes, somitic myocytes have a mixed slow/fast phenotype (Figure 3, model 1). In the chick embryo, myotomal fibers express both fast and slow MYH proteins (178), and in the mouse embryo, at E10.5, the primary myotome expresses both slow (*Tnnc1*, *Tnnt1*, *Tnni1*, *Myl6b*) and fast (*Tnnc2*, *Tnnt3*, *Myh7*, *Atp2a1*) genes. SIX1 and SIX4 proteins accumulate in the nuclei of dermomyotomal progenitors and remain robustly expressed in the nuclei of post-mitotic differentiated myocytes. The primary myotome of *Six1^{-/-}Six4^{-/-}* mutant embryos is reduced in size and unable to activate fast-type genes (*Tnnt3*, *Myh7*, *Atp2a1*) while slow-type genes are still expressed (*Tnnt1*, *Tnni1*, *Myl6b*) (179), indicating that SIX homeoproteins are required for the expression of fast muscle genes in mixed slow/fast myocytes.

During fetal development, SIX1 and SIX4 proteins are still detected in the nuclei of cycling undifferentiated myogenic progenitors, and in the nuclei of differentiated myofibers. During fetal development mixed slow/fast myofibers acquire a slow or a fast specific phenotype, and at E18.5 most myofibers express either slow MYH7, or fast-type Actinin- α 3. At E18.5, *Six1^{-/-}Six4^{-/-}* mice have no limb, no thoracic or abdominal musculature, but present hypoplastic and disorganized back muscles, without expression of the fast Actinin- α 3 protein, and expressing slow MYH7 and embryonic MYH3 in most fibers (180). These findings underpin the absolute requirement of *Six1/4* for the activation of the subset of fast muscle

genes in growing myofibers. Furthermore, the expression of adult fast *Myh1*, *Myh2* and *Myh4* genes is severely impaired in E18.5 *Six1*^{-/-}*Six4*^{-/-} fetal muscles, showing that these homeoproteins are also required for fast-myofiber subtype specialization. Affymetrix transcriptome analysis of residual back muscles of E18.5 *Six1/4* KO identified a specific down-regulation of several fast-type genes either involved in Ca²⁺ homeostasis regulation (*Smtnl1*, *Pvalb*, *Casq1*, *Atp2a1*), coding for structural genes (*Myh4*, *Myh1*, *Actn3*), or involved in specific signaling pathways (*Hrasls*, *Wnt4*), defining the network of genes under the control of *Six1* and *Six4* in fetal myofibers (180). ChIP experiments revealed that the regulatory elements of most of those genes bind SIX homeoproteins, suggesting a direct control of their expression by STC (144, 179).

How do mixed slow/fast myofibers becoming fast myofibers extinguish the slow-type program during fetal development, and how are SIX1/4 homeoproteins involved in this process? It has been shown in zebrafish that the nuclear repressor SOX6 binds directly the regulatory regions of numerous slow-type muscle genes (181). This feature is conserved in mammals, and mouse SOX6 can repress slow *Myh* and other slow-type muscle genes (182, 183). *Sox6* is expressed during mouse embryonic myogenesis, but unable to inhibit slow muscle genes in mixed myofibers before E15, suggesting that SOX6 nuclear accumulation is prevented in myofibers before E15. However, SOX6 binds and activates the promoter of *Mef2c* during embryonic myogenesis, and increased MEF2c leads to the activation of the slow *Myh7* gene before E15. Association of SOX6 with NFIX after E15, during fetal myogenesis, leads to their binding to the *Myh7* promoter and its repression (184). Accordingly, E18.5 *Sox6*^{-/-} myofibers show an increased expression of the slow muscle genes, such as *Myh7* (185). Contrary to wild type myofibers, E18.5 *Six1*^{-/-}*Six4*^{-/-} myofibers present SOX6 cytoplasmic accumulation and decreased nuclear SOX6 accumulation that probably participates in the increase of slow-type myofibers observed in *Six1*^{-/-}*Six4*^{-/-} fetuses. How do SIX proteins contribute to nuclear accumulation of SOX6 during fetal myogenesis has to be clarified. Interestingly, SIX1 and EYA1 directly interact with the SOX2 protein in the sensory progenitors of inner ear (15, 186); it would be interesting to test if a direct interaction between SIX1 and SOX6 proteins occurs in myogenic cells and whether this interaction impacts on SOX6 subcellular localization. HDAC4, known to bind MEF2 proteins and inhibit their activity (187), is also retained in the cytoplasm of *Six1*^{-/-}*Six4*^{-/-} myofibers. MEF2 proteins without nuclear HDAC4 are free to activate slow muscle genes in this *Six* double mutant background (180). Most E18.5 *Six1*^{-/-}*Six4*^{-/-} myofibers are not innervated (180). Among the downregulated genes in E18.5 *Six1*^{-/-}*Six4*^{-/-} mutant myofibers figures *Wnt4*. As *Wnt4* expression is required during development for neuromuscular junctions formation (188), it is tempting to speculate that *Wnt4* expression defects in the mutant myofibers may contribute to the innervation deficit observed. The influence of innervation in fast myofiber specialization remains to be precisely evaluated. It is important to note that no major myofiber hypotrophy is observed in E18.5 residual fetal muscles of *Six1/4* double mutant fetuses (180), suggesting that at this stage these homeoproteins do not control myofiber growth, and that absence of innervation does not lead to atrophic effects as in the adult. SIX1 in myogenic C2C12 cells also enhances expression of fast-type muscle genes (189) as well as many other genes expressed in muscle cells (144), showing that some of the *in vivo* SIX targets are found also in cell cultures (190).

Six1/Six4 genes are also expressed in myogenic lineages of zebrafish and involved in long-range myogenic cell migration during embryogenesis (191). As in amniotes (192), *Six* genes in zebrafish are required for fast-type muscle specialization (193).

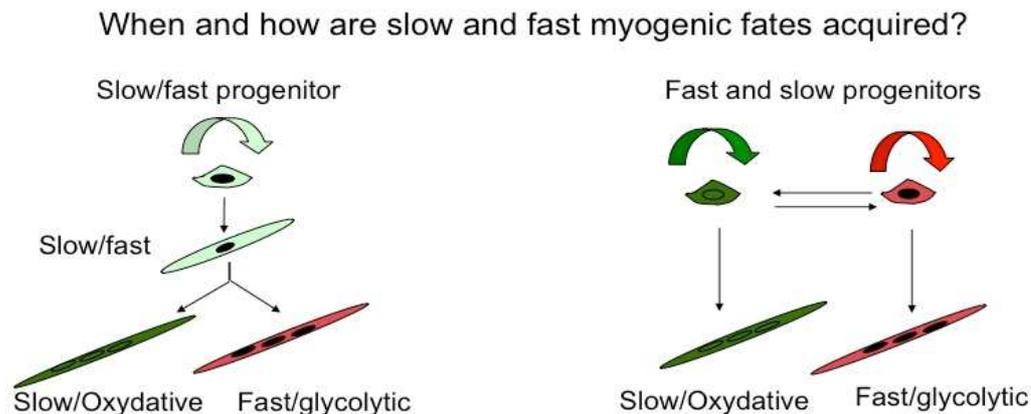


Figure 3. When and how does muscle fiber diversity arise during embryonic development? Two models are presented that may explain the genesis of fast- and slow-type muscle fibers during embryonic development. Left: model 1; embryonic myogenic progenitors are not committed and give rise to mixed slow/fast myocytes, that later acquire a specific slow or fast identity. Right: model 2; slow and fast embryonic progenitors are specified at the onset of myogenic commitment and give rise to slow and fast myofibers, as found in the zebrafish (194).

3.2 Six in the maintenance of the fast phenotype in adult myofibers.

Six1, *Six2*, *Six4* and *Six5* and their associated *Eya* cofactors are not only expressed during embryogenesis, but also in adult skeletal muscles, in post mitotic myofibers and in quiescent and activated PAX7+ myogenic stem cells. Adult skeletal muscles are composed of slow-type fibers expressing *Myh7*, and of fast subtypes expressing *Myh2*, *Myh1* or *Myh4*. Activation in the post-natal period of adult *Myh2*, *Myh1* and *Myh4* in fast myofibers is a process that is poorly understood, and the molecular determinants that preside at the emergence of the three subtypes of fast myofibers remain to be identified (195, 196) in a global neuromuscular context.

Historically, characterization of the transcription complexes responsible for high *Aldolase A* activity in adult fast/glycolytic compared to slow/oxidative myofibers unveiled the involvement of the STC in the control of fast fiber gene expression (135, 136). Interestingly while *Six1* mRNAs and SIX1 proteins are expressed in both fast and slow-type muscles, SIX1 proteins are enriched in the nuclei of fast-type muscles (135, 197). However, the mechanisms decreasing nuclear SIX1 level specifically in slow-types myofibers are unclear. It has been shown that the calcium is an important regulator of fiber-type determination, mediating slow and fast motoneuron firing mainly through the control of Calcineurin activity (198). However, treatment by FK506, a Calcineurin inhibitor which transforms slow fibers into fast-type fibers, does not increase the amount of nuclear SIX1 protein in the soleus (135), suggesting that other mechanisms than those involving the Calcium/Calcineurin pathway control SIX1 subcellular localization.

Forced expression of SIX1 and EYA1 in adult soleus induced a transition from an slow/oxydative to a fast/glycolytic phenotype, showing that the expression of these two proteins can reprogram adult myofibers, and that SIX1 in association with EYA1 orchestrates a complete remodeling of the adult myofiber phenotype, through a direct activation of fast/glycolytic muscle genes and a downregulation of slow-type muscle genes. Interestingly, forced expression of SIX1 and a mutated EYA1 unable to interact with SIX1 impairs this transition (135), demonstrating that the STC involved in fiber typing control contains EYA proteins (135). It is not known whether signaling pathways modulating EYA activity in *Drosophila* (1,2) are also involved in the control of STC activity in adult myofibers in vertebrates. A number of genes are controlled by SIX homeoproteins in adult muscles (197).

Accordingly, hyperacetylated H3K27ac regions in adult fast quadriceps chromatin are enriched for consensus sequences for SIX1, compared to slow soleus (199). ChIP experiments with adult muscle nuclear extracts identified in fast myofibers at least three categories of genes directly relying on SIX activity: the glycolytic *AldolaseA* (43, 136), the calcium binding *Parvalbumin* genes (197) and the fast *Myh* locus (133). This showed that the STC controls several aspects of adult myofiber physiology, including glucose metabolism, calcium flux involved in contraction/relaxation and sarcomeres specialization involved in contraction speed. Furthermore, at the level of the fast *Myh* locus, SIX1 controls not only the expression of *Myh2* in soleus (197), but also the expression of a long non-coding RNA, *linc-Myh*, which is responsible for the downregulation of several slow genes in fast fibers. In line with these findings, the expression of *Sox6*, a repressor of slow muscle genes, is decreased in *Six1* mutant adult myofibers, while the expression of *Prox1*, a repressor of fast muscle genes, is increased (197). The complete substitution of *Myh2* by *Myh7* expression observed in adult *Six1* mutant (*Six1^{flox/flox}; HSA-CRE: Six1^{skm/skm}*) soleus suggests that in this muscle the other SIX proteins are unable to compensate. Analysis of the other limb muscles of the *Six1^{skm/skm}* mutant showed decreased *Myh4* and increased *Myh7* and *Myh2* expression (133, 197) (Figure 4), while the fast *Myh* genes remain expressed in many muscles. Expression of several slow genes is up-regulated in *Six1^{skm/skm}* adult myofibers, while expression of fast genes is downregulated. Consequently, adult *Six1^{skm/skm}* mutant TA fibers present increased fatigue resistance (133). Analysis of double *Six1Six4* mutant adult myofibers should clarify the potential redundancy between these two *Six* genes in adult muscle physiology, as found during development.

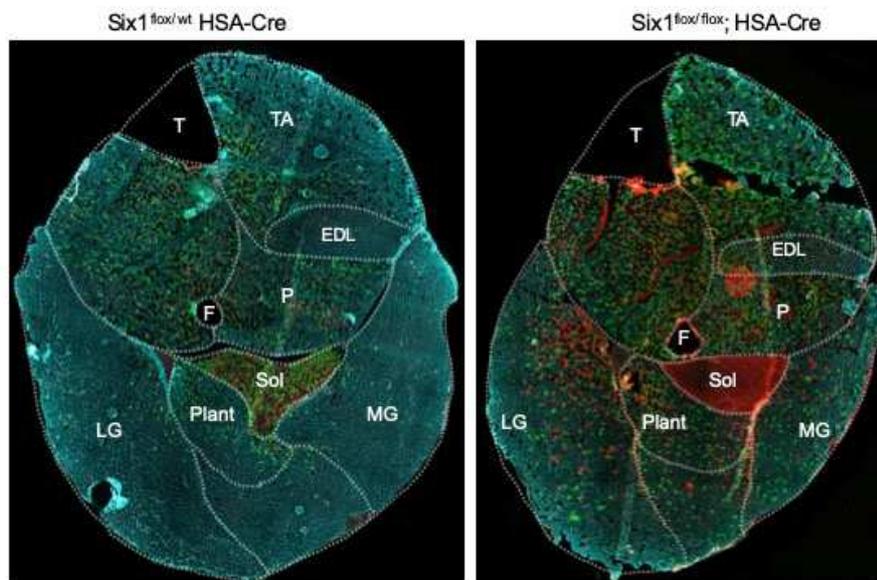


Figure 4. Consequences of *Six1* loss in adult myofibers. Distal hindlimbs of three months old *Six1^{flox/wt}; HSA-Cre* (left) and *Six1^{flox/flox}; HSA-Cre* (right) sections were incubated with antibodies recognizing MYH4 (blue), MYH2 (green) and MYH7 (red) proteins. By default, MYH1 expressing myofibers appear black. T: tibia, F: fibula, EDL: extensor digitorum longus, P: peroneus muscles, Plant: plantaris, Sol: soleus, TA: tibialis anterior, LG: lateral gastrocnemius, MG: medial gastrocnemius.

In conclusion, STCs control all the steps that preside to fast-type myofibers formation and remain major actors in these myofibers at later stages through the direct control of specific muscle genes, as summarized in Figure 5.

Six proteins are master determinants of a genetic cascade that controls all important steps of muscle development as well as major activators for each of these steps.

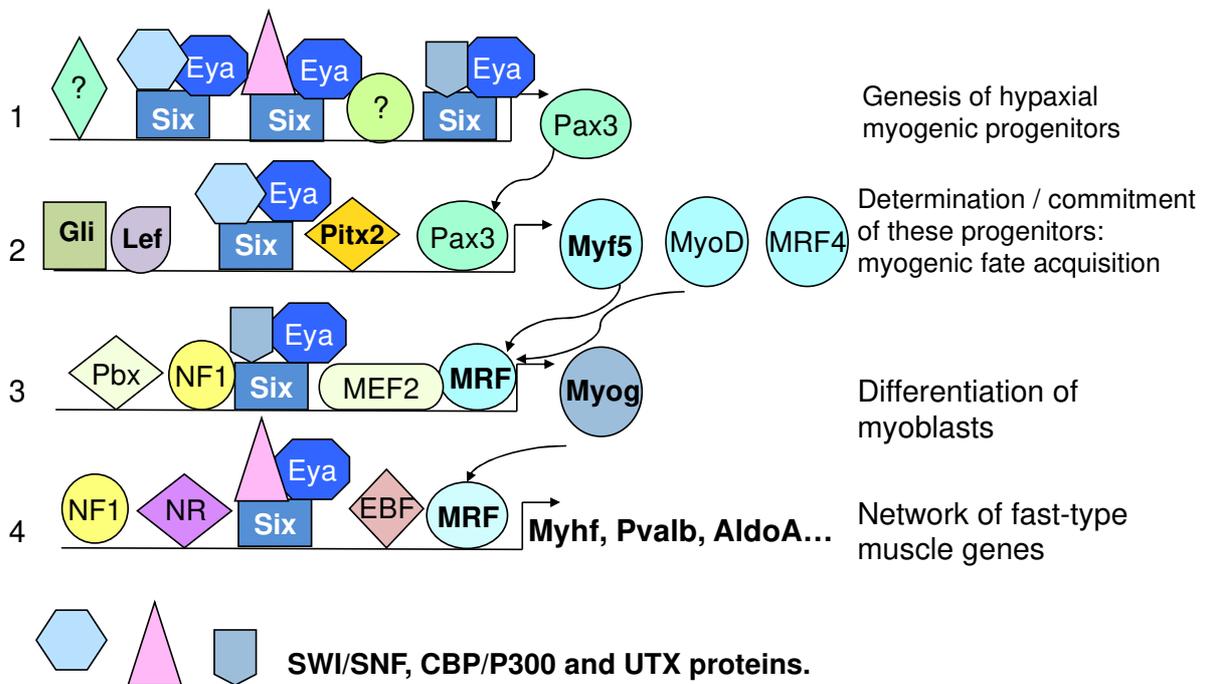


Figure 5. SIX proteins are master determinants of a genetic cascade that controls all important steps of muscle development as well as major activators for each of these steps: feed forward control of myogenesis by SIX homeoproteins. 1- Hypaxial *Pax3* enhancer is bound by SIX1 and yet unidentified proteins. EYA and DACH may participate in the SIX transcriptional complexes and recruit SWI/SNIF, UTX and CBP, allowing hypaxial *Pax3* expression and genesis of hypaxial progenitors. 2- SIX is then recruited with PAX3 to activate hypaxial *Myf5* expression and commit pluripotent dermomyotomal cells into a myogenic fate. GLI and LEF/ β -catenin have been shown to activate early epaxial *Myf5* expression, and SIX and PITX2 directly control *MyoD* expression. 3- SIX is recruited with MYF5 or MYOD, in cooperation with PBX, NF1 and MEF2 to activate *Myogenin*, allowing the differentiation of myogenic cells into multinucleated myofibers. 4- SIX is recruited with Myogenin or another MRF on the regulatory sequences of muscle specific genes, more particularly fast-type specific genes (fast-type *Myosin*, fast-type *Troponin*, *Parvalbumin*, *Serca1*). In adult fast-type myofibers, potential co-regulators of the STC are nuclear receptors among which COUP-TFII, NUR77, RXR, and Nuclear Factor1 (NF1/NFi), TBX1/15, EBF1, MYOD itself (207). Geometric forms with question marks are proteins known to interact with SIX or EYA in other contexts than myogenic embryonic cells or adult muscle fibers. Geometric forms with no indication are putative transcription factors that have not yet been characterized.

3.3 Six in physiology, atrophy and hypertrophy

Although the physiological function of STC in skeletal muscle atrophy and hypertrophy has not been explored, two reports suggest their potential role in heart hypertrophy. *Six1* is expressed transiently in cardiac progenitor cells, then silenced by Polycomb histone methyltransferase EHZ2 (175). *Ezh2* deficient heart exhibited hypertrophy accompanied with increased expression of *Six1/Eya1/Eya2/Eya4* and their known target genes in skeletal muscle (*Tnnt3*, *Tnni2*, *Mylpf*, *Act α 3*) (200). Surprisingly, SIX1 has the ability to

activate skeletal muscle specific target genes in *Ezh2* deficient heart, although MRFs are not expressed. *Six1*, *Six2* and *Eya2* mRNA are up-regulated in heart by swimming exercise, which leads to physiological heart hypertrophy. The heart of transgenic mice overexpressing *Eya2* show hypertrophy, and EYA2 directly activates mTOR, an important physiological hypertrophy-inducing signal (201). These results support the idea that SIX-EYA complexes participate in the control of atrophy/hypertrophy pathways in striated muscles. Accordingly, in conditions of Soleus and Plantaris hypertrophy induced by skeletal muscle overload following synergistic ablation of the gastrocnemius, *Dach2* mRNA expression is reduced, and SIX1 protein and *Eya3* mRNA expression increased (202), suggesting the participation of a SIX1/EYA3 complex in muscle hypertrophy. Accordingly, *Eya3*^{-/-} adult mice show decreased locomotor activity and decreased muscle strength (112). Importantly, *Dach2* expression in adult myofibers is controlled by its innervation status. Lower *Dach2* is observed in adult myofibers after denervation, allowing upregulation of *Myogenin* and *nAChR* expression (203, 204). DACH2 in this adult context also controls negatively *Gdf5* expression in synergy with HDAC9 (205). *Gdf5* is a protein of the BMP family that may exert hypertrophic effects on adult myofibers (206). Upon denervation, *Myogenin* expression is upregulated and activates *Murf1* and *Atrogin1*, driving muscle atrophy (204). The consequences of the STC activity in the control of *Myogenin* expression during muscle atrophy still have to be established. Adult limb myofibers deprived of *Six1* do not present decreased cross section area, indicating that the absence of *Six1* does not lead to myofiber atrophy of distal hindlimb muscles (197). A compensatory mechanism could implicate *Six4*. SIX4 interacts with BAF60c, a subunit of the SWI/SNF chromatin remodeling complex enriched in fast muscles. The SIX4-BAF60c activates *Deptor* expression, AKT signaling and glycolytic metabolism (168). One possibility is that the absence of *Six1* observed in *Six1*^{skm/skm} mutant animals is compensated by *Six4* (or *Six2*), leading to normal myofiber size. Absence of *Six1* in adult *Six1*^{skm/skm} myofibers has a major impact on glucose metabolism (197). How this modified glucose metabolism impacts on the increased fatigue resistance of adult *Six1* mutant TA remains to be further analyzed (133).

4- *Six* in adult myogenic stem cells

4.1 Role of *Six* in self-renewal and genesis of post-natal myogenic stem cells

Adult skeletal muscles have the ability to repair after myofiber damage, through adult myogenic stem cells called satellite cells (SC). These SC express the transcription factor *Pax7*, a homeopaired protein, whose absence leads to SC exhaustion in the perinatal period (208). SC are born during fetal development and responsible for fetal and perinatal muscle growth. During fetal development, they are responsible for the genesis of new myofibers, adopting a satellite cell position between the basal lamina and the myofiber at birth when the suitable number of myofibers is reached, and are then recruited by existing myofibers during the perinatal period to increase myonuclei content in these growing myofibers (100, 209, 210). These SC become quiescent at the puberty but can be further recruited after myofiber damage in the adult. The signaling pathways and transcription factors upstream of *Pax7* and responsible for myogenic stem cells homing during development, SC self-renewal, and amplification are far from being characterized (211). It is known that in absence of active NOTCH signaling, SC differentiate prematurely and wear out during development (212). Canonical and non-canonical WNT signaling activate their differentiation and self-renewal, respectively (90). It is also known that all adult SC have expressed myogenic regulatory factors *MyoD* and *Mrf4* during ontogenesis (213, 214). In zebrafish, expansion of PAX7+ SC is under the control of *Six1* that modulates BMP signaling (215). In the mouse, four *Six* homeogenes are expressed in SC: *Six1*, *Six4*, *Six2* and *Six5* (95, 102, 216). While analysis of compound double *Six1/4* mutant revealed their role during embryonic myogenesis (95),

nothing is known concerning the importance of *Six* genes for the genesis of SC in the embryo, nor their potential redundancy in activating common downstream targets.

Analysis of inducible KO for *Six1* in adult SC revealed its role in the differentiation and self-renewal of these cells (216). Adult myogenic stem cells from *Six1^{flox/flox};Pax7-Cre-ERT2 (Six1^{SC/SC})* mutant mice show impaired differentiation properties and decreased *MyoD* and *Myogenin* expression, as observed during embryogenesis. *Six1^{SC/SC}* mutant SC have no major proliferation defects. Thirty days after acute muscle injury, regenerated myofibers from *Six1^{SC/SC}* mutant have a reduced size, with an increased number of associated SC, due to a reduced myogenic fusion and insufficient myonuclei accretion during the repair process. These mutant cells fail to activate *Dusp6* expression (216), a phosphatase involved in the dephosphorylation of P-ERK proteins. Accordingly, muscles lacking *Dusp6* were able to regenerate properly but showed a marked increase in SC number after regeneration (216). Analysis of *Six4* knock-down during adult mouse regeneration revealed that SIX4 proteins participate also in the control of *MyoD* and *Myogenin* expression in activated myogenic stem cells, as well as in the control of the expression of many genes specifically expressed in adult myofibers (*Serca1*, *Tnni1*) (134). In *Six5^{-/-}Six4^{+/-}* mutant mice, differentiation of SC is not affected, but their proliferation capacity is increased while knock-down of *Six4* in SC *ex vivo* leads to a decrease in their differentiation potential (217). Altogether, SC lacking either *Six1*, *Six4* or *Six5* present a phenotype suggesting their involvement in the control of the self-renewal/differentiation equilibrium. The expression of *Six1*, *Six2*, *Six4* and *Six5* in SC and their ability to bind the same MEF3 DNA elements may lead to some functional redundancy, and mutation of these four *Six* genes may be required to observe more severe SC phenotype *in vivo*. According to the ability of SIX to activate *Pax7* gene expression in zebrafish (215), alterations of *Pax7* gene expression, and myogenic fate alterations may be observed in myogenic stem cells of compound *Six* mutant.

Analysis of mdx (X-linked muscular dystrophy) mouse deficient in the *Dystrophin* gene, whose mutation is responsible for Duchenne myopathy, and heterozygous for both *Six4* and *Six5*, demonstrated that reduced expression of *Six* genes improved dystrophic phenotype and prolonged life span (218). In these mdx double *Six4^{+/-};Six5^{+/-}* heterozygotes, a higher proportion of myogenic stem cells expressed MYOD, SIX1 and Myogenin, a modulation that may be responsible for increased efficient regeneration of mdx muscles. This aspect is of interest since Dystrophin controls the division axis of SC. Indeed, decreased Dystrophin expression was shown to decrease apico-basal division and SC differentiation (219). It will be important to determine whether the dosage of the various SIX and EYA proteins expressed in SC can influence their division, and the targets involved.

Contrary to embryonic myogenic progenitors, which give rise to mixed slow/fast myofibers (Figure 3), SC isolated from the fast TA of adult mice and cultured in 3D show an enrichment of SIX4/EYA1, and an increase in fast/glycolytic gene expression (220), compared to SC from the slow soleus. This is in agreement with the intrinsic properties of adult myogenic stem cells associated with slow and fast muscles (221), and the involvement of TBX proteins in SC associated with fast myofibers (222). Whether adult SC associated with fast and slow myofibers show a differential enrichment for SIX1 and SIX4 remains to be established. Nevertheless myogenic stem cells isolated from *Six1^{-/-}Six4^{-/-}* E18.5 fetuses are unable *ex vivo* to activate fast-type muscle genes in differentiated myotubes (180).

4.2 *Six* in cell reprogramming

Transcription factors can be used to reprogram cell fate. This has been shown most spectacularly in the case of MYOD (223) and more recently with the generation of iPS cells (224). *In vivo*, myogenesis is triggered by Master regulatory factors, such as MYOD, which jump-start a transcriptional cascade leading to the acquisition of muscle cell fate. This cellular

reprogramming can be reproduced *in vitro*: transfecting different cell types, such as fibroblasts, with *MyoD*, reprograms these cells into a muscle fate (223, 225). A fundamental question concerns the cues that make cells amenable (or not) to reprogramming (226, 227). SIX appear to be master homeoproteins controlling all successive myogenesis steps leading to the adult myofiber *in vivo* (Figure 5). In cell culture experiments, SIX homeoproteins have been shown to interact with CBP/P300 acetyl transferase proteins (30), with UTX histone demethylase (134, 167) and with SWI/SNF chromatin remodeling complexes (15). Strikingly, *Six1^{-/-};Six4^{-/-}* double KO (*SixdKO*) mouse embryonic fibroblasts (MEF) are completely refractory to MYOD reprogramming and fail to adopt muscle cell fate (127), showing that SIX homeoproteins must synergize with MYOD to allow cell reprogramming. Myogenic cells with decreased *Six1* expression have decreased MYOD binding to DNA, suggesting that SIX1 participates to chromatin opening, which is required for further MYOD DNA binding (132). These results suggest that SIX homeoproteins may act as pioneer factors (228). This property would explain the lack of activity of the mutant MEF3 promoters for *Myogenin*, *MyoD* and *Aldolase A* in embryo or adult muscles of transgenic animals (102, 124, 136). Absence of SIX binding on these mutant MEF3 promoters, impairing binding of other transcription factors, precludes their activation. Accordingly, it has been shown that forced expression of *Six1*, *Eya1*, *Essr1* and *Pax3* can orient mouse embryonic fibroblasts toward the Myogenic Stem Cell pathway (229), activate *Pax7* gene expression, and MRF. *Essr1* is suspected in this context to induce a pluripotent stem cell identity that can be further induced toward a myogenic fate by SIX1, EYA1 and PAX3 (229). This result is in agreement with the involvement of the PSED gene network in myogenic fate acquisition during embryogenesis. In amniotes, although several conserved MEF3 sites associated with MYOD binding have been identified at the *Pax7* locus (127), there is to date no evidence that SIX homeoproteins control the expression of *Pax7 in vivo*, as shown by the analysis of *Six1* mutant SC in mice and of *Six4* knock-down in regenerating adult muscles (134, 216). Understanding the nature of the interactions of SIX homeoproteins at the *Pax7* locus may improve our knowledge of the potential feed-back loops between these two classes of homeoproteins and their consequences on the homeostasis of myogenic stem cells.

Acknowledgements

We thank our colleagues P.Daubas, A.Mayeuf-Louchart A.Sotiropoulos and S.Gautron for helpful critical reading of the manuscript. We acknowledge the fundings from the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, the Idex “Sorbonne Paris Cité”, the Agence Nationale de la Recherche and the Association Française contre les Myopathies.

	Drosophila		Vertebrates		
Six genes	• <i>So</i>	Expressed all along eye development Expressed transiently in ventral and lateral mesoderm	• <i>Six1; Six2</i>		Not expressed in early eye development Expressed in the dermomyotome; not in presomitic mesoderm Expressed in adult myofibers and myogenic stem cells
	• <i>Optix</i>	Expressed in the developing eye	• <i>Six3; Six6</i>		Expressed in the developing eye
	• <i>D-Six4</i>	Expression not reported in the eye Expressed in the mesoderm	• <i>Six4</i> • <i>Six5</i>		Expressed in the developing eye Dermomyotome and presomitic mesoderm Expressed in adult myofibers and myogenic stem cells Expressed in the adult eye Dermomyotome and presomitic mesoderm Expressed in adult myofibers and myogenic stem cells
<i>Eya</i> genes	• <i>Eya</i>	Localization mainly nuclear Expressed all along eye development Expressed transiently in ventral and lateral mesoderm	• <i>Eya1</i> <i>Eya2</i> <i>Eya3</i> <i>Eya4</i>	• <i>Eya1/ Eya2</i> • <i>Eya3</i> • <i>Eya4</i>	Localization nuclear and cytoplasmic Expressed in hypaxial dermomyotome and migrating progenitors to the limb Expressed in myofibers Low expression in the dermomyotome Expressed in myogenic stem cells High expression in the dermomyotome Expressed in myofibers and myogenic stem cells
<i>Dac</i> genes	• <i>Dac</i>	Expressed during eye and leg development	• <i>Dach1</i> <i>Dach2</i>	• <i>Dach1</i> • <i>Dach2</i>	Expressed in newly formed somites Expressed in the dermomyotome
Paired homeo-domain TF	• <i>Ey</i>	Expressed transiently in undifferentiated progenitors of the eye	• <i>Pax1</i> — <i>Pax9</i>	• <i>Pax6</i> • <i>Pax3</i> • <i>Pax7</i>	Expressed during eye development Expressed in pancreatic endocrine cells during development and in the adult Transiently expressed in undifferentiated myogenic progenitors in trunk and neural crest cells Expressed in dermomyotomal myogenic progenitors and adult myogenic stem cells

Table 1. Different players of the PSED genetic network in *Drosophila* and vertebrates.

References

1. G. Halder, P. Callaerts, W. J. Gehring, Induction of ectopic eyes by targeted expression of the eyeless gene in *Drosophila*. *Science* **267**, 1788-1792 (1995).
2. F. Loosli, S. Winkler, J. Wittbrodt, Six3 overexpression initiates the formation of ectopic retina. *Genes Dev* **13**, 649-654 (1999).
3. R. L. Chow, C. R. Altmann, R. A. Lang, A. Hemmati-Brivanlou, Pax6 induces ectopic eyes in a vertebrate. *Development* **126**, 4213-4222 (1999).
4. M. Buckingham, F. Relaix, The role of Pax genes in the development of tissues and organs: Pax3 and Pax7 regulate muscle progenitor cell functions. *Annu Rev Cell Dev Biol* **23**, 645-673 (2007).
5. K. Kawakami, S. Sato, H. Ozaki, K. Ikeda, Six family genes--structure and function as transcription factors and their roles in development. *Bioessays* **22**, 616-626 (2000).
6. J. P. Kumar, Retinal determination the beginning of eye development. *Curr Top Dev Biol* **93**, 1-28 (2010).
7. C. C. Zhu *et al.*, Six3-mediated auto repression and eye development requires its interaction with members of the Groucho-related family of co-repressors. *Development* **129**, 2835-2849 (2002).
8. S. J. Silver, E. L. Davies, L. Doyon, I. Rebay, Functional dissection of eyes absent reveals new modes of regulation within the retinal determination gene network. *Mol Cell Biol* **23**, 5989-5999 (2003).
9. C. Desplan, Eye development: governed by a dictator or a junta? *Cell* **91**, 861-864 (1997).
10. M. Atkins *et al.*, Dynamic rewiring of the *Drosophila* retinal determination network switches its function from selector to differentiation. *PLoS Genet* **9**, e1003731 (2013).
11. K. Kenyon, D. Li, C. Clouser, S. Tran, F. Pignoni, Fly SIX-type homeodomain proteins *Sine oculis* and *Optix* partner with different cofactors during eye development. *Dev Dyn*. **234**, 497-504 (2005).
12. K. M. Neilson, F. Pignoni, B. Yan, S. A. Moody, Developmental expression patterns of candidate cofactors for vertebrate six family transcription factors. *Dev Dyn* **239**, 3446-3466 (2010).
13. T. Zhang, S. Ranade, C. Q. Cai, C. Clouser, F. Pignoni, Direct control of neurogenesis by selector factors in the fly eye: regulation of *atonal* by *Ey* and *So*. *Development* **133**, 4881-4889 (2006).
14. M. Tanaka-Matakatsu, W. Du, Direct control of the proneural gene *atonal* by retinal determination factors during *Drosophila* eye development. *Dev Biol* **313**, 787-801 (2008).
15. M. Ahmed, J. Xu, P. X. Xu, EYA1 and SIX1 drive the neuronal developmental program in cooperation with the SWI/SNF chromatin-remodeling complex and SOX2 in the mammalian inner ear. *Development*, (2012).
16. B. N. Cheyette *et al.*, The *Drosophila sine oculis* locus encodes a homeodomain-containing protein required for the development of the entire visual system. *Neuron* **12**, 977-996 (1994).
17. M. Kango-Singh, A. Singh, Y. Henry Sun, Eyeless collaborates with Hedgehog and Decapentaplegic signaling in *Drosophila* eye induction. *Dev Biol* **256**, 49-60 (2003).
18. Y. H. Liu *et al.*, A systematic analysis of Tinman function reveals Eya and JAK-STAT signaling as essential regulators of muscle development. *Dev Cell* **16**, 280-291 (2009).

19. M. Boyle, N. Bonini, S. DiNardo, Expression and function of clift in the development of somatic gonadal precursors within the *Drosophila* mesoderm. *Development* **124**, 971-982 (1997).
20. I. B. Clark, J. Boyd, G. Hamilton, D. J. Finnegan, A. P. Jarman, D-six4 plays a key role in patterning cell identities deriving from the *Drosophila* mesoderm. *Dev Biol* **294**, 220-231 (2006).
21. T. L. Tootle *et al.*, The transcription factor Eyes absent is a protein tyrosine phosphatase. *Nature* **426**, 299-302 (2003).
22. J. P. Rayapureddi *et al.*, Eyes absent represents a class of protein tyrosine phosphatases. *Nature* **426**, 295-298 (2003).
23. X. Li *et al.*, Eya protein phosphatase activity regulates Six1-Dach-Eya transcriptional effects in mammalian organogenesis. *Nature* **426**, 247-254 (2003).
24. J. Jemc, I. Rebay, The eyes absent family of phosphotyrosine phosphatases: properties and roles in developmental regulation of transcription. *Annu Rev Biochem* **76**, 513-538 (2007).
25. L. R. Braid, E. M. Verheyen, *Drosophila* nemo promotes eye specification directed by the retinal determination gene network. *Genetics* **180**, 283-299 (2008).
26. S. A. Morillo, L. R. Braid, E. M. Verheyen, I. Rebay, Nemo phosphorylates Eyes absent and enhances output from the Eya-Sine oculis transcriptional complex during *Drosophila* retinal determination. *Dev Biol* **365**, 267-276 (2012).
27. F. C. Hsiao, A. Williams, E. L. Davies, I. Rebay, Eyes absent mediates cross-talk between retinal determination genes and the receptor tyrosine kinase signaling pathway. *Dev Cell* **1**, 51-61 (2001).
28. W. Xiong, N. M. Dabbouseh, I. Rebay, Interactions with the abelson tyrosine kinase reveal compartmentalization of eyes absent function between nucleus and cytoplasm. *Dev Cell* **16**, 271-279 (2009).
29. M. Jin, B. Jusiak, Z. Bai, G. Mardon, Eyes absent tyrosine phosphatase activity is not required for *Drosophila* development or survival. *PLoS One* **8**, e58818 (2013).
30. K. Ikeda, Y. Watanabe, H. Ohto, K. Kawakami, Molecular interaction and synergistic activation of a promoter by Six, Eya, and Dach proteins mediated through CREB binding protein. *Mol Cell Biol* **22**, 6759-6766 (2002).
31. X. Fan *et al.*, The alpha subunits of Gz and Gi interact with the eyes absent transcription cofactor Eya2, preventing its interaction with the six class of homeodomain-containing proteins. *J Biol Chem* **275**, 32129-32134 (2000).
32. R. N. Pandey *et al.*, The Eyes Absent phosphatase-transactivator proteins promote proliferation, transformation, migration, and invasion of tumor cells. *Oncogene* **29**, 3715-3722 (2010).
33. M. Mentel *et al.*, WDR1 is a novel EYA3 substrate and its dephosphorylation induces modifications of the cellular actin cytoskeleton. *Sci Rep* **8**, 2910 (2018).
34. P. J. Cook *et al.*, Tyrosine dephosphorylation of H2AX modulates apoptosis and survival decisions. *Nature* **458**, 591-596 (2009).
35. J. Zhou *et al.*, Transcription elongation regulator 1 is a co-integrator of the cell fate determination factor Dachshund homolog 1. *J Biol Chem* **285**, 40342-40350 (2010).
36. K. L. Christensen, A. N. Patrick, E. L. McCoy, H. L. Ford, The six family of homeobox genes in development and cancer. *Adv Cancer Res* **101**, 93-126 (2008).
37. I. Miguel-Aliaga, D. W. Allan, S. Thor, Independent roles of the dachshund and eyes absent genes in BMP signaling, axon pathfinding and neuronal specification. *Development* **131**, 5837-5848 (2004).
38. S. M. Farabaugh, D. S. Micalizzi, P. Jedlicka, R. Zhao, H. L. Ford, Eya2 is required to mediate the pro-metastatic functions of Six1 via the induction of TGF-beta signaling,

- epithelial-mesenchymal transition, and cancer stem cell properties. *Oncogene* **31**, 552-562 (2012).
39. Y. Liu *et al.*, Interplay of retinal determination gene network with TGF-beta signaling pathway in epithelial-mesenchymal transition. *Stem Cell Investig* **2**, 12 (2015).
 40. T. Nomura *et al.*, Ski is a component of the histone deacetylase complex required for transcriptional repression by Mad and thyroid hormone receptor. *Genes Dev* **13**, 412-423 (1999).
 41. M. Takeda *et al.*, Interaction with Smad4 is indispensable for suppression of BMP signaling by c-Ski. *Mol Biol Cell* **15**, 963-972 (2004).
 42. P. Tarapore *et al.*, DNA binding and transcriptional activation by the Ski oncoprotein mediated by interaction with NFI. *Nucleic Acids Res* **25**, 3895-3903 (1997).
 43. F. Spitz *et al.*, A combination of MEF3 and NFI proteins activates transcription in a subset of fast-twitch muscles. *Mol Cell Biol* **17**, 656-666. (1997).
 44. G. Messina *et al.*, Nfix regulates fetal-specific transcription in developing skeletal muscle. *Cell* **140**, 554-566 (2010).
 45. Y. Kida, Y. Maeda, T. Shiraishi, T. Suzuki, T. Ogura, Chick Dach1 interacts with the Smad complex and Sin3a to control AER formation and limb development along the proximodistal axis. *Development* **131**, 4179-4187 (2004).
 46. T. A. Heanue *et al.*, Synergistic regulation of vertebrate muscle development by Dach2, Eya2, and Six1, homologs of genes required for Drosophila eye formation. *Genes Dev* **13**, 3231-3243 (1999).
 47. H. Zhang, E. Stavnezer, Ski regulates muscle terminal differentiation by transcriptional activation of Myog in a complex with Six1 and Eya3. *J Biol Chem* **284**, 2867-2879 (2009).
 48. V. M. Popov *et al.*, The Dachshund gene in development and hormone-responsive tumorigenesis. *Trends Endocrinol Metab* **21**, 41-49 (2010).
 49. W. Liu, O. Lagutin, E. Swindell, M. Jamrich, G. Oliver, Neuroretina specification in mouse embryos requires Six3-mediated suppression of Wnt8b in the anterior neural plate. *J Clin Invest* **120**, 3568-3577 (2010).
 50. S. Wawersik, R. L. Maas, Vertebrate eye development as modeled in Drosophila. *Hum Mol Genet* **9**, 917-925 (2000).
 51. O. Lagutin *et al.*, Six3 promotes the formation of ectopic optic vesicle-like structures in mouse embryos. *Dev Dyn* **221**, 342-349 (2001).
 52. P. Purcell, G. Oliver, G. Mardon, A. L. Donner, R. L. Maas, Pax6-dependence of Six3, Eya1 and Dach1 expression during lens and nasal placode induction. *Gene Expr Patterns* **6**, 110-118 (2005).
 53. C. L. Salzer, J. P. Kumar, Position dependent responses to discontinuities in the retinal determination network. *Dev Biol* **326**, 121-130 (2009).
 54. J. L. Yanowitz *et al.*, UNC-39, the C. elegans homolog of the human myotonic dystrophy-associated homeodomain protein Six5, regulates cell motility and differentiation. *Dev Biol* **272**, 389-402 (2004).
 55. M. Stierwald, N. Yanze, R. P. Bamert, L. Kammermeier, V. Schmid, The Sine oculis/Six class family of homeobox genes in jellyfish with and without eyes: development and eye regeneration. *Dev Biol* **274**, 70-81 (2004).
 56. I. G. Bebenek, R. D. Gates, J. Morris, V. Hartenstein, D. K. Jacobs, sine oculis in basal Metazoa. *Development genes and evolution* **214**, 342-351 (2004).
 57. W. Zheng *et al.*, The role of Six1 in mammalian auditory system development. *Development* **130**, 3989-4000 (2003).

58. X. Nie, J. Xu, A. El-Hashash, P. X. Xu, Six1 regulates Grem1 expression in the metanephric mesenchyme to initiate branching morphogenesis. *Dev Biol* **352**, 141-151 (2011).
59. G. Schlosser, Vertebrate cranial placodes as evolutionary innovations--the ancestor's tale. *Curr Top Dev Biol* **111**, 235-300 (2015).
60. S. E. Quaggin, Uncapping the potential of renal stem cells. *Cell Stem Cell* **3**, 123-124 (2008).
61. L. L. O'Brien *et al.*, Differential regulation of mouse and human nephron progenitors by the Six family of transcriptional regulators. *Development* **143**, 595-608 (2016).
62. S. Abdelhak *et al.*, A human homologue of the Drosophila eyes absent gene underlies branchio-oto-renal (BOR) syndrome and identifies a novel gene family. *Nat Genet* **15**, 157-164 (1997).
63. R. G. Ruf *et al.*, SIX1 mutations cause branchio-oto-renal syndrome by disruption of EYA1-SIX1-DNA complexes. *Proc Natl Acad Sci U S A* **101**, 8090-8095 (2004).
64. J. Schonberger *et al.*, Mutation in the transcriptional coactivator EYA4 causes dilated cardiomyopathy and sensorineural hearing loss. *Nat Genet* **37**, 418-422 (2005).
65. D. E. Wallis *et al.*, Mutations in the homeodomain of the human SIX3 gene cause holoprosencephaly. *Nat Genet* **22**, 196-198 (1999).
66. J. S. Imam *et al.*, MicroRNA-185 suppresses tumor growth and progression by targeting the Six1 oncogene in human cancers. *Oncogene* **29**, 4971-4979 (2010).
67. K. T. Ng *et al.*, Suppression of tumorigenesis and metastasis of hepatocellular carcinoma by shRNA interference targeting on homeoprotein Six1. *Int J Cancer* **127**, 859-872 (2010).
68. A. L. Smith *et al.*, The miR-106b-25 cluster targets Smad7, activates TGF-beta signaling, and induces EMT and tumor initiating cell characteristics downstream of Six1 in human breast cancer. *Oncogene* **31**, 5162-5171 (2012).
69. C. M. Li *et al.*, Gene expression in Wilms' tumor mimics the earliest committed stage in the metanephric mesenchymal-epithelial transition. *Am J Pathol* **160**, 2181-2190 (2002).
70. W. Song *et al.*, Sine oculis homeobox 1 promotes proliferation and migration of human colorectal cancer cells through activation of Wnt/beta-catenin signaling. *Cancer Sci* **110**, 608-616 (2019).
71. Y. Yu *et al.*, Expression profiling identifies the cytoskeletal organizer ezrin and the developmental homeoprotein Six-1 as key metastatic regulators. *Nat Med* **10**, 175-181 (2004).
72. T. J. Kingsbury, M. Kim, C. I. Civin, Regulation of cancer stem cell properties by SIX1, a member of the PAX-SIX-EYA-DACH network. *Adv Cancer Res* **141**, 1-42 (2019).
73. E. Tadjuidje *et al.*, The EYA tyrosine phosphatase activity is pro-angiogenic and is inhibited by benzbromarone. *PLoS One* **7**, e34806 (2012).
74. L. Li *et al.*, Transcriptional Regulation of the Warburg Effect in Cancer by SIX1. *Cancer Cell* **33**, 368-385 e367 (2018).
75. H. Ono *et al.*, SIX1 promotes epithelial-mesenchymal transition in colorectal cancer through ZEB1 activation. *Oncogene* **31**, 4923-4934 (2012).
76. Y. Yu, E. Davicioni, T. J. Triche, G. Merlino, The homeoprotein six1 transcriptionally activates multiple protumorigenic genes but requires ezrin to promote metastasis. *Cancer Res* **66**, 1982-1989 (2006).
77. A. Eisner *et al.*, The Eya1 phosphatase promotes Shh signaling during hindbrain development and oncogenesis. *Dev Cell* **33**, 22-35 (2015).

78. C. A. Wang *et al.*, SIX1 induces lymphangiogenesis and metastasis via upregulation of VEGF-C in mouse models of breast cancer. *J Clin Invest* **122**, 1895-1906 (2012).
79. Z. Liu *et al.*, A NIK-SIX signalling axis controls inflammation by targeted silencing of non-canonical NF-kappaB. *Nature* **568**, 249-253 (2019).
80. I. Adrados *et al.*, The homeoprotein SIX1 controls cellular senescence through the regulation of p16INK4A and differentiation-related genes. *Oncogene* **35**, 3485-3494 (2016).
81. C. De Lope *et al.*, SIX1 represses senescence and promotes SOX2-mediated cellular plasticity during tumorigenesis. *Sci Rep* **9**, 1412 (2019).
82. R. Brunmeir *et al.*, Comparative Transcriptomic and Epigenomic Analyses Reveal New Regulators of Murine Brown Adipogenesis. *PLoS Genet* **12**, e1006474 (2016).
83. D. A. Bergstrom, S. J. Tapscott, Molecular distinction between specification and differentiation in the myogenic basic helix-loop-helix transcription factor family. *Mol Cell Biol* **21**, 2404-2412 (2001).
84. L. Kassam-Duchossoy *et al.*, Mrf4 determines skeletal muscle identity in Myf5:MyoD double-mutant mice. *Nature* **431**, 466-471 (2004).
85. S. Gopalakrishnan *et al.*, A Cranial Mesoderm Origin for Esophagus Striated Muscles. *Dev Cell* **34**, 694-704 (2015).
86. E. Heude *et al.*, Unique morphogenetic signatures define mammalian neck muscles and associated connective tissues. *Elife* **7**, (2018).
87. T. Braun, M. Gautel, Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis. *Nat Rev Genet* **12**, 349-361 (2011).
88. M. Buckingham, P. W. Rigby, Gene regulatory networks and transcriptional mechanisms that control myogenesis. *Dev Cell* **28**, 225-238 (2014).
89. P. Feige, C. E. Brun, M. Ritso, M. A. Rudnicki, Orienting Muscle Stem Cells for Regeneration in Homeostasis, Aging, and Disease. *Cell Stem Cell* **23**, 653-664 (2018).
90. L. Giordani, A. Parisi, F. Le Grand, Satellite Cell Self-Renewal. *Curr Top Dev Biol* **126**, 177-203 (2018).
91. R. Diogo *et al.*, A new heart for a new head in vertebrate cardiopharyngeal evolution. *Nature* **520**, 466-473 (2015).
92. G. Comai, S. Tajbakhsh, Molecular and cellular regulation of skeletal myogenesis. *Curr Top Dev Biol* **110**, 1-73 (2014).
93. H. Ozaki *et al.*, Structure and chromosome mapping of the human SIX4 and murine Six4 genes. *Cytogenet Cell Genet* **87**, 108-112 (1999).
94. G. Oliver *et al.*, Homeobox genes and connective tissue patterning. *Development* **121**, 693-705 (1995).
95. R. Grifone *et al.*, Six1 and Six4 homeoproteins are required for Pax3 and MRF expression during myogenesis in the mouse embryo. *Development* **132**, 2235-2249 (2005).
96. C. Laclef, E. Souil, J. Demignon, P. Maire, Thymus, kidney and craniofacial abnormalities in Six 1 deficient mice. *Mech Dev* **120**, 669-679 (2003).
97. H. Ozaki *et al.*, Six4, a putative myogenin gene regulator, is not essential for mouse embryonal development. *Mol Cell Biol* **21**, 3343-3350 (2001).
98. M. Buckingham, S. D. Vincent, Distinct and dynamic myogenic populations in the vertebrate embryo. *Curr Opin Genet Dev* **19**, 444-453 (2009).
99. M. Maroto *et al.*, Ectopic Pax-3 activates MyoD and Myf-5 expression in embryonic mesoderm and neural tissue. *Cell* **89**, 139-148 (1997).
100. F. Relaix, D. Rocancourt, A. Mansouri, M. Buckingham, A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. *Nature* **435**, 948-953 (2005).

101. J. Gros, M. Scaal, C. Marcelle, A two-step mechanism for myotome formation in chick. *Dev Cell* **6**, 875-882 (2004).
102. F. Relaix *et al.*, Six homeoproteins directly activate Myod expression in the gene regulatory networks that control early myogenesis. *PLoS Genet* **9**, e1003425 (2013).
103. T. R. Klesert *et al.*, Mice deficient in Six5 develop cataracts: implications for myotonic dystrophy. *Nat Genet* **25**, 105-109 (2000).
104. A. Magli *et al.*, Time-dependent Pax3-mediated chromatin remodeling and cooperation with Six4 and Tead2 specify the skeletal myogenic lineage in developing mesoderm. *PLoS Biol* **17**, e3000153 (2019).
105. R. David, K. Ahrens, D. Wedlich, G. Schlosser, Xenopus Eya1 demarcates all neurogenic placodes as well as migrating hypaxial muscle precursors. *Mech Dev* **103**, 189-192 (2001).
106. I. Sahly, P. Andermann, C. Petit, The zebrafish eya1 gene and its expression pattern during embryogenesis. *Dev Genes Evol* **209**, 399-410 (1999).
107. T. Ishihara, K. Ikeda, S. Sato, H. Yajima, K. Kawakami, Differential expression of Eya1 and Eya2 during chick early embryonic development. *Gene Expr Patterns* **8**, 357-367 (2008).
108. P. X. Xu, J. Cheng, J. A. Epstein, R. L. Maas, Mouse Eya genes are expressed during limb tendon development and encode a transcriptional activation function. *Proc Natl Acad Sci U S A* **94**, 11974-11979 (1997).
109. R. Grifone *et al.*, Eya1 and Eya2 proteins are required for hypaxial somitic myogenesis in the mouse embryo. *Dev. Biol.* **302**, 602-616 (2007).
110. G. Borsani *et al.*, EYA4, a novel vertebrate gene related to Drosophila eyes absent. *Hum Mol Genet* **8**, 11-23 (1999).
111. P. X. Xu, I. Woo, H. Her, D. R. Beier, R. L. Maas, Mouse Eya homologues of the Drosophila eyes absent gene require Pax6 for expression in lens and nasal placode. *Development* **124**, 219-231 (1997).
112. T. Soker *et al.*, Pleiotropic effects in Eya3 knockout mice. *BMC Dev Biol* **8**, 118 (2008).
113. T. W. Todd *et al.*, Nemo-like kinase is a novel regulator of spinal and bulbar muscular atrophy. *Elife* **4**, e08493 (2015).
114. H. Shi *et al.*, Modulation of skeletal muscle fiber type by mitogen-activated protein kinase signaling. *The FASEB journal*, (2008).
115. J. K. Lee, P. T. Hallock, S. J. Burden, Abelson tyrosine-protein kinase 2 regulates myoblast proliferation and controls muscle fiber length. *Elife* **6**, (2017).
116. R. J. Davis, W. Shen, Y. I. Sandler, T. A. Heanue, G. Mardon, Characterization of mouse Dach2, a homologue of Drosophila dachshund. *Mech Dev* **102**, 169-179 (2001).
117. T. A. Heanue *et al.*, Dach1, a vertebrate homologue of Drosophila dachshund, is expressed in the developing eye and ear of both chick and mouse and is regulated independently of Pax and Eya genes. *Mech Dev* **111**, 75-87 (2002).
118. S. Namciu *et al.*, Enhanced expression of mouse c-ski accompanies terminal skeletal muscle differentiation in vivo and in vitro. *Dev Dyn* **204**, 291-300 (1995).
119. T. Pelzer, G. E. Lyons, S. Kim, R. W. Moreadith, Cloning and characterization of the murine homolog of the sno proto-oncogene reveals a novel splice variant. *Dev Dyn* **205**, 114-125 (1996).
120. C. Colmenares, E. Stavnezer, The ski oncogene induces muscle differentiation in quail embryo cells. *Cell* **59**, 293-303 (1989).

121. M. Berk, S. Y. Desai, H. C. Heyman, C. Colmenares, Mice lacking the ski proto-oncogene have defects in neurulation, craniofacial, patterning, and skeletal muscle development. *Genes Dev* **11**, 2029-2039 (1997).
122. R. J. Davis, M. Harding, Y. Moayedi, G. Mardon, Mouse Dach1 and Dach2 are redundantly required for Mullerian duct development. *Genesis* **46**, 205-213 (2008).
123. Y. H. Hsu *et al.*, Sumoylated SnoN represses transcription in a promoter-specific manner. *J Biol Chem* **281**, 33008-33018 (2006).
124. F. Spitz *et al.*, Expression of myogenin during embryogenesis is controlled by Six/sine oculis homeoproteins through a conserved MEF3 binding site. *Proc Natl Acad Sci U S A* **95**, 14220-14225 (1998).
125. T. Pauli, M. Seimiya, J. Blanco, W. J. Gehring, Identification of functional sine oculis motifs in the autoregulatory element of its own gene, in the eyeless enhancer and in the signalling gene hedgehog. *Development* **132**, 2771-2782 (2005).
126. Y. Liu *et al.*, Discovery, optimization and validation of an optimal DNA-binding sequence for the Six1 homeodomain transcription factor. *Nucleic Acids Res* **40**, 8227-8239 (2012).
127. M. Santolini *et al.*, MyoD reprogramming requires Six1 and Six4 homeoproteins: genome-wide cis-regulatory module analysis. *Nucleic Acids Res* **44**, 8621-8640 (2016).
128. M. S. Parmacek *et al.*, A novel myogenic regulatory circuit controls slow/cardiac troponin C gene transcription in skeletal muscle. *Mol Cell Biol* **14**, 1870-1885. (1994).
129. K. Kawakami, H. Ohto, K. Ikeda, R. G. Roeder, Structure, function and expression of a murine homeobox protein AREC3, a homologue of Drosophila sine oculis gene product, and implication in development. *Nucleic Acids Res* **24**, 303-310 (1996).
130. C. L. Himeda *et al.*, Quantitative proteomic identification of six4 as the trex-binding factor in the muscle creatine kinase enhancer. *Mol Cell Biol* **24**, 2132-2143 (2004).
131. J. Giordani *et al.*, Six proteins regulate the activation of Myf5 expression in embryonic mouse limbs. *Proc Natl Acad Sci U S A*. **104**, 11310-11315 (2007).
132. Y. Liu *et al.*, Six1 regulates MyoD expression in adult muscle progenitor cells. *PLoS One* **8**, e67762 (2013).
133. I. Sakakibara, M. Santolini, A. Ferry, V. Hakim, P. Maire, Six homeoproteins and a linc-RNA at the fast MYH locus lock fast myofiber terminal phenotype. *PLoS Genet* **10**, e1004386 (2014).
134. I. Chakroun *et al.*, Genome-wide association between Six4, MyoD, and the histone demethylase Utx during myogenesis. *FASEB J* **29**, 4738-4755 (2015).
135. R. Grifone *et al.*, Six1 and Eya1 expression can reprogram adult muscle from the slow-twitch phenotype into the fast-twitch phenotype. *Mol Cell Biol* **24**, 6253-6267 (2004).
136. M. Salminen, S. Lopez, P. Maire, A. Kahn, D. Daegelen, Fast-muscle-specific DNA-protein interactions occurring in vivo at the human aldolase A M promoter are necessary for correct promoter activity in transgenic mice. *Mol Cell Biol* **16**, 76-85 (1996).
137. J. Gros, O. Serralbo, C. Marcelle, WNT11 acts as a directional cue to organize the elongation of early muscle fibres. *Nature* **457**, 589-593 (2009).
138. U. Borello *et al.*, The Wnt/beta-catenin pathway regulates Gli-mediated Myf5 expression during somitogenesis. *Development* **133**, 3723-3732 (2006).
139. S. Tajbakhsh *et al.*, Differential activation of Myf5 and MyoD by different Wnts in explants of mouse paraxial mesoderm and the later activation of myogenesis in the absence of Myf5. *Development* **125**, 4155-4162 (1998).

140. A. E. Munsterberg, J. Kitajewski, D. A. Bumcrot, A. P. McMahon, A. B. Lassar, Combinatorial signaling by Sonic hedgehog and Wnt family members induces myogenic bHLH gene expression in the somite. *Genes Dev* **9**, 2911-2922 (1995).
141. A. Borycki, A. M. Brown, C. P. Emerson, Jr., Shh and Wnt signaling pathways converge to control Gli gene activation in avian somites. *Development* **127**, 2075-2087 (2000).
142. A. C. Rios, O. Serralbo, D. Salgado, C. Marcelle, Neural crest regulates myogenesis through the transient activation of NOTCH. *Nature* **473**, 532-535 (2011).
143. R. Reshef, M. Maroto, A. B. Lassar, Regulation of dorsal somitic cell fates: BMPs and Noggin control the timing and pattern of myogenic regulator expression. *Genes Dev* **12**, 290-303 (1998).
144. Y. Liu, A. Chu, I. Chakroun, U. Islam, A. Blais, Cooperation between myogenic regulatory factors and SIX family transcription factors is important for myoblast differentiation. *Nucleic Acids Res* **38**, 6857-6871 (2010).
145. J. Ishibashi, R. L. Perry, A. Asakura, M. A. Rudnicki, MyoD induces myogenic differentiation through cooperation of its NH₂- and COOH-terminal regions. *J Cell Biol* **171**, 471-482 (2005).
146. B. Christ, M. Jacob, H. J. Jacob, On the origin and development of the ventrolateral abdominal muscles in the avian embryo. An experimental and ultrastructural study. *Anat Embryol (Berl)* **166**, 87-101 (1983).
147. J. Wilson-Rawls, C. R. Hurt, S. M. Parsons, A. Rawls, Differential regulation of epaxial and hypaxial muscle development by paraxis. *Development* **126**, 5217-5229 (1999).
148. S. Dietrich, F. R. Schubert, C. Healy, P. T. Sharpe, A. Lumsden, Specification of the hypaxial musculature. *Development* **125**, 2235-2249 (1998).
149. S. Kruck, M. Scaal, Divergent regulation of Wnt-mediated development of the dorsomedial and ventrolateral dermomyotomal lips. *Histochem Cell Biol* **138**, 503-514 (2012).
150. A. E. Chen, D. D. Ginty, C. M. Fan, Protein kinase A signalling via CREB controls myogenesis induced by Wnt proteins. *Nature* **433**, 317-322 (2005).
151. R. Ben-Yair, C. Kalcheim, Notch and bone morphogenetic protein differentially act on dermomyotome cells to generate endothelium, smooth, and striated muscle. *J Cell Biol* **180**, 607-618 (2008).
152. A. Mayeuf-Louchart *et al.*, Notch regulation of myogenic versus endothelial fates of cells that migrate from the somite to the limb. *Proc Natl Acad Sci U S A* **111**, 8844-8849 (2014).
153. O. Pourquie *et al.*, Lateral and axial signals involved in avian somite patterning: a role for BMP4. *Cell* **84**, 461-471 (1996).
154. L. Yvernogeu, G. Auda-Boucher, J. Fontaine-Perus, Limb bud colonization by somite-derived angioblasts is a crucial step for myoblast emigration. *Development* **139**, 277-287 (2012).
155. L. E. Alvares *et al.*, Intrinsic, Hox-dependent cues determine the fate of skeletal muscle precursors. *Dev Cell* **5**, 379-390 (2003).
156. P. Geetha-Loganathan *et al.*, Ectodermal Wnt-6 promotes Myf5-dependent avian limb myogenesis. *Dev Biol* **288**, 221-233 (2005).
157. L. Bajard *et al.*, A novel genetic hierarchy functions during hypaxial myogenesis: Pax3 directly activates Myf5 in muscle progenitor cells in the limb. *Genes Dev.* **20**, 2450-2464 (2006).
158. P. Daubas *et al.*, Fine-tuning the onset of myogenesis by homeobox proteins that interact with the Myf5 limb enhancer. *Biol Open* **4**, 1614-1624 (2015).

159. C. B. Brown, K. A. Engleka, J. Wenning, M. Min Lu, J. A. Epstein, Identification of a hypaxial somite enhancer element regulating Pax3 expression in migrating myoblasts and characterization of hypaxial muscle Cre transgenic mice. *Genesis* **41**, 202-209 (2005).
160. M. Lagha *et al.*, Pax3:Foxc2 reciprocal repression in the somite modulates muscular versus vascular cell fate choice in multipotent progenitors. *Dev Cell* **17**, 892-899 (2009).
161. A. Mayeuf-Louchart *et al.*, Endothelial cell specification in the somite is compromised in Pax3-positive progenitors of Foxc1/2 conditional mutants, with loss of forelimb myogenesis. *Development* **143**, 872-879 (2016).
162. S. Tajbakhsh, D. Rocancourt, G. Cossu, M. Buckingham, Redefining the genetic hierarchies controlling skeletal myogenesis: Pax-3 and Myf-5 act upstream of MyoD. *Cell* **89**, 127-138 (1997).
163. A. Zalc *et al.*, Antagonistic regulation of p57kip2 by Hes/Hey downstream of Notch signaling and muscle regulatory factors regulates skeletal muscle growth arrest. *Development* **141**, 2780-2790 (2014).
164. C. A. Berkes, S. J. Tapscott, MyoD and the transcriptional control of myogenesis. *Semin Cell Dev Biol* **16**, 585-595 (2005).
165. T. C. Cheng, M. C. Wallace, J. P. Merlie, E. N. Olson, Separable regulatory elements governing myogenin transcription in mouse embryogenesis. *Science* **261**, 215-218 (1993).
166. S. P. Yee, P. W. Rigby, The regulation of myogenin gene expression during the embryonic development of the mouse. *Genes Dev* **7**, 1277-1289 (1993).
167. S. Seenundun *et al.*, UTX mediates demethylation of H3K27me3 at muscle-specific genes during myogenesis. *Embo J* **29**, 1401-1411 (2010).
168. Z. X. Meng *et al.*, Baf60c drives glycolytic metabolism in the muscle and improves systemic glucose homeostasis through Deptor-mediated Akt activation. *Nat Med* **19**, 640-645 (2013).
169. D. Palacios, D. Summerbell, P. W. Rigby, J. Boyes, Interplay between DNA methylation and transcription factor availability: implications for developmental activation of the mouse Myogenin gene. *Mol Cell Biol* **30**, 3805-3815 (2010).
170. S. J. Tapscott, The circuitry of a master switch: Myod and the regulation of skeletal muscle gene transcription. *Development* **132**, 2685-2695 (2005).
171. R. G. Kelly, Core issues in craniofacial myogenesis. *Exp Cell Res* **316**, 3034-3041 (2010).
172. R. Sambasivan, S. Kuratani, S. Tajbakhsh, An eye on the head: the development and evolution of craniofacial muscles. *Development* **138**, 2401-2415 (2011).
173. F. R. Schubert, A. J. Singh, O. Afoyalan, C. Kioussi, S. Dietrich, To roll the eyes and snap a bite - function, development and evolution of craniofacial muscles. *Semin Cell Dev Biol* **91**, 31-44 (2019).
174. F. Berti *et al.*, Time course and side-by-side analysis of mesodermal, pre-myogenic, myogenic and differentiated cell markers in the chicken model for skeletal muscle formation. *J Anat* **227**, 361-382 (2015).
175. C. Guo *et al.*, A Tbx1-Six1/Eya1-Fgf8 genetic pathway controls mammalian cardiovascular and craniofacial morphogenesis. *J Clin Invest* **121**, 1585-1595 (2011).
176. C. Y. Lin *et al.*, The transcription factor Six1a plays an essential role in the craniofacial myogenesis of zebrafish. *Dev Biol* **331**, 152-166 (2009).
177. D. Aldea *et al.*, Genetic regulation of amphioxus somitogenesis informs the evolution of the vertebrate head mesoderm. *Nat Ecol Evol* **3**, 1233-1240 (2019).

178. L. D. Sacks *et al.*, Regulation of myosin expression during myotome formation. *Development* **130**, 3391-3402. (2003).
179. C. Niro *et al.*, Six1 and Six4 gene expression is necessary to activate the fast-type muscle gene program in the mouse primary myotome. *Dev Biol* **338**, 168-182 (2010).
180. A. F. Richard *et al.*, Genesis of muscle fiber-type diversity during mouse embryogenesis relies on Six1 and Six4 gene expression. *Dev Biol* **359**, 303-320 (2011).
181. J. von Hofsten *et al.*, Prdm1- and Sox6-mediated transcriptional repression specifies muscle fibre type in the zebrafish embryo. *EMBO reports* **9**, 683-689 (2008).
182. C. I. An, Y. Dong, N. Hagiwara, Genome-wide mapping of Sox6 binding sites in skeletal muscle reveals both direct and indirect regulation of muscle terminal differentiation by Sox6. *BMC Dev Biol* **11**, 59 (2011).
183. D. Quiat *et al.*, Concerted regulation of myofiber-specific gene expression and muscle performance by the transcriptional repressor Sox6. *Proc Natl Acad Sci U S A* **108**, 10196-10201 (2011).
184. V. Taglietti *et al.*, Nfix Induces a Switch in Sox6 Transcriptional Activity to Regulate MyHC-I Expression in Fetal Muscle. *Cell Rep* **17**, 2354-2366 (2016).
185. N. Hagiwara, M. Yeh, A. Liu, Sox6 is required for normal fiber type differentiation of fetal skeletal muscle in mice. *Dev Dyn* **236**, 2062-2076 (2007).
186. M. Ahmed *et al.*, Eya1-Six1 interaction is sufficient to induce hair cell fate in the cochlea by activating Atoh1 expression in cooperation with Sox2. *Dev Cell* **22**, 377-390 (2012).
187. M. Potthoff *et al.*, Histone deacetylase degradation and MEF2 activation promote the formation of slow-twitch myofibers. *J Clin Invest.* **117**, 2459-2467 (2007).
188. L. Strohlic *et al.*, Wnt4 participates in the formation of vertebrate neuromuscular junction. *PLoS One* **7**, e29976 (2012).
189. W. Wu *et al.*, Overexpression of Six1 gene suppresses proliferation and enhances expression of fast-type muscle genes in C2C12 myoblasts. *Mol Cell Biochem* **380**, 23-32 (2013).
190. W. Wu *et al.*, The role of Six1 in the genesis of muscle cell and skeletal muscle development. *Int J Biol Sci* **10**, 983-989 (2014).
191. J. C. Talbot *et al.*, Muscle precursor cell movements in zebrafish are dynamic and require Six family genes. *Development* **146**, (2019).
192. M. Ma *et al.*, lncRNA-Six1 Is a Target of miR-1611 that Functions as a ceRNA to Regulate Six1 Protein Expression and Fiber Type Switching in Chicken Myogenesis. *Cells* **7**, (2018).
193. D. A. Bessarab, S. W. Chong, B. P. Srinivas, V. Korzh, Six1a is required for the onset of fast muscle differentiation in zebrafish. *Dev Biol*, (2008).
194. J. Yin, R. Lee, Y. Ono, P. W. Ingham, T. E. Saunders, Spatiotemporal Coordination of FGF and Shh Signaling Underlies the Specification of Myoblasts in the Zebrafish Embryo. *Dev Cell* **46**, 735-750 e734 (2018).
195. S. Schiaffino, C. Reggiani, Fiber types in mammalian skeletal muscles. *Physiol Rev* **91**, 1447-1531 (2011).
196. K. Gundersen, Excitation-transcription coupling in skeletal muscle: the molecular pathways of exercise. *Biol Rev Camb Philos Soc* **86**, 564-600 (2011).
197. I. Sakakibara *et al.*, Six1 homeoprotein drives myofiber type IIA specialization in soleus muscle. *Skelet Muscle* **6**, 30 (2016).
198. E. N. Olson, R. S. Williams, Remodeling muscles with calcineurin. *Bioessays* **22**, 510-519 (2000).

199. K. Ramachandran *et al.*, Dynamic enhancers control skeletal muscle identity and reprogramming. *PLoS Biol* **17**, e3000467 (2019).
200. P. Delgado-Olguin *et al.*, Epigenetic repression of cardiac progenitor gene expression by Ezh2 is required for postnatal cardiac homeostasis. *Nat Genet* **44**, 343-347 (2012).
201. S. H. Lee *et al.*, Transcription coactivator Eya2 is a critical regulator of physiological hypertrophy. *J Mol Cell Cardiol* **52**, 718-726 (2012).
202. B. S. Gordon, D. C. Delgado Diaz, J. P. White, J. A. Carson, M. C. Kostek, Six1 and Six1 cofactor expression is altered during early skeletal muscle overload in mice. *J Physiol Sci* **62**, 393-401 (2012).
203. T. J. Cohen *et al.*, The histone deacetylase HDAC4 connects neural activity to muscle transcriptional reprogramming. *J Biol Chem* **282**, 33752-33759 (2007).
204. V. Moresi *et al.*, Myogenin and class II HDACs control neurogenic muscle atrophy by inducing E3 ubiquitin ligases. *Cell* **143**, 35-45 (2010).
205. P. C. Macpherson, P. Farshi, D. Goldman, Dach2-Hdac9 signaling regulates reinnervation of muscle endplates. *Development* **142**, 4038-4048 (2015).
206. M. Traore *et al.*, An embryonic CaVbeta1 isoform promotes muscle mass maintenance via GDF5 signaling in adult mouse. *Sci Transl Med* **11**, (2019).
207. M. Ekmark, Z. A. Rana, G. Stewart, D. G. Hardie, K. Gundersen, De-phosphorylation of MyoD is linking nerve-evoked activity to fast myosin heavy chain expression in rodent adult skeletal muscle. *The Journal of physiology* **584**, 637-650 (2007).
208. P. Seale *et al.*, Pax7 is required for the specification of myogenic satellite cells. *Cell* **102**, 777-786 (2000).
209. L. Kassam-Duchossoy *et al.*, Pax3/Pax7 mark a novel population of primitive myogenic cells during development. *Genes Dev* **19**, 1426-1431 (2005).
210. R. B. White, A. S. Bierinx, V. F. Gnocchi, P. S. Zammit, Dynamics of muscle fibre growth during postnatal mouse development. *BMC Dev Biol* **10**, 21 (2010).
211. D. Brohl *et al.*, Colonization of the satellite cell niche by skeletal muscle progenitor cells depends on Notch signals. *Dev Cell* **23**, 469-481 (2012).
212. E. Vasyutina *et al.*, RBP-J (Rbpsi) is essential to maintain muscle progenitor cells and to generate satellite cells. *Proc Natl Acad Sci U S A* **104**, 4443-4448 (2007).
213. O. Kanisicak, J. J. Mendez, S. Yamamoto, M. Yamamoto, D. J. Goldhamer, Progenitors of skeletal muscle satellite cells express the muscle determination gene, MyoD. *Dev Biol* **332**, 131-141 (2009).
214. R. Sambasivan *et al.*, Embryonic founders of adult muscle stem cells are primed by the determination gene Mrf4. *Dev Biol* **381**, 241-255 (2013).
215. H. Nord, L. Nygard Skalman, J. von Hofsten, Six1 regulates proliferation of Pax7-positive muscle progenitors in zebrafish. *J Cell Sci* **126**, 1868-1880 (2013).
216. F. Le Grand *et al.*, Six1 regulates stem cell repair potential and self-renewal during skeletal muscle regeneration. *J. Cell Biol.* **198**, 815-832 (2012).
217. H. Yajima *et al.*, Six family genes control the proliferation and differentiation of muscle satellite cells. *Exp Cell Res* **316**, 2932-2944 (2010).
218. H. Yajima, K. Kawakami, Low Six4 and Six5 gene dosage improves dystrophic phenotype and prolongs life span of mdx mice. *Dev Growth Differ* **58**, 546-561 (2016).
219. N. A. Dumont *et al.*, Dystrophin expression in muscle stem cells regulates their polarity and asymmetric division. *Nat Med* **21**, 1455-1463 (2015).
220. A. Khodabukus, K. Baar, Contractile and metabolic properties of engineered skeletal muscle derived from slow and fast phenotype mouse muscle. *J Cell Physiol* **230**, 1750-1757 (2015).

221. J. M. Kalhovde *et al.*, "Fast" and "slow" muscle fibres in hindlimb muscles of adult rats regenerate from intrinsically different satellite cells. *J Physiol* **562**, 847-857 (2005).
222. N. Motohashi *et al.*, Tbx1 regulates inherited metabolic and myogenic abilities of progenitor cells derived from slow- and fast-type muscle. *Cell Death Differ* **26**, 1024-1036 (2019).
223. R. L. Davis, H. Weintraub, A. B. Lassar, Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* **51**, 987-1000 (1987).
224. S. Yamanaka, Elite and stochastic models for induced pluripotent stem cell generation. *Nature* **460**, 49-52 (2009).
225. B. W. Schafer, B. T. Blakely, G. J. Darlington, H. M. Blau, Effect of cell history on response to helix-loop-helix family of myogenic regulators. *Nature* **344**, 454-458 (1990).
226. A. P. Fong, S. J. Tapscott, Skeletal muscle programming and re-programming. *Curr Opin Genet Dev* **23**, 568-573 (2013).
227. V. Sartorelli, P. L. Puri, Shaping Gene Expression by Landscaping Chromatin Architecture: Lessons from a Master. *Mol Cell* **71**, 375-388 (2018).
228. M. Iwafuchi-Doi, K. S. Zaret, Cell fate control by pioneer transcription factors. *Development* **143**, 1833-1837 (2016).
229. E. J. Lee *et al.*, Establishment of stably expandable induced myogenic stem cells by four transcription factors. *Cell Death Dis* **9**, 1092 (2018).