The winged helix transcription factor *Foxc1a* is essential for somitogenesis in zebrafish

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Previous studies identified zebrafish *foxc1a* and *foxc1b* as homologs of the mouse forkhead gene, *Foxc1*. Both genes are transcribed in the unsegmented presomitic mesoderm (PSM), newly formed somites, adaxial cells, and head mesoderm. Here, we show that inhibiting synthesis of *Foxc1a* (but not *Foxc1b*) protein with two different morpholino antisense oligonucleotides blocks formation of morphological somites, segment boundaries, and segmented expression of genes normally transcribed in anterior and posterior somites and expression of *paraxis* implicated in somite epithelialization. Patterning of the anterior PSM is also affected, as judged by the absence of *mesp-b*, *ephrinB2*, and *epha4* expression, and the down-regulation of *notch5* and *notch6*. In contrast, the expression of other genes, including *mesp-a* and *papc*, in the anterior of somite primordia, and the oscillating expression of *deltaC* and *deltaD* in the PSM appear normal. Nevertheless, this expression is apparently insufficient for the maturation of the presumptive somites to proceed to the stage when boundary formation occurs or for the maintenance of anterior/posterior patterning. Mouse embryos that are compound null mutants for *Foxc1* and the closely related *Foxc2* have no morphological somites and show abnormal expression of Notch signaling pathway genes in the anterior PSM. Therefore, zebrafish *foxc1a* plays an essential and conserved role in somite formation, regulating both the expression of *paraxis* and the A/P patterning of somite primordia.

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Somite formation—the reiterated subdivision of paraxial mesoderm into paired, epithelial spheres of cells on either side of the midline—is fundamental to vertebrate development. Given this importance, many genetic and embryological studies have been carried out in mouse, chick, frog, and zebrafish to gain insight into the cellular and molecular mechanisms involved. These studies have led to the conclusion that before morphologically discrete pairs of somites appear there is a prepattern of presumptive somites in the anterior of the unsegmented or presomitic mesoderm (PSM). Moreover, cells in the anterior and posterior halves of these presumptive somite already express genes characteristic of anterior and posterior somites, and this polarization is necessary for boundary formation to occur (for reviews, see Pourquie 1999; Dale and Pourquie 2000; Holley and Nusslein-Volhard 2000; Pourquie 2000a,b; Stickney et al. 2000).

There is at present no single model that completely

accounts for the entire process of somite formation (Meinhardt 1986; Stern and Keynes 1988; Primm et al. 1989). The “clock and wavefront” postulated by Cooke and Zeeman (1976) is one of the first models aimed at explaining the formation of patterned segment primordia. This model proposes that cells in the PSM oscillate from one state that is permissive for somite formation to another that is not. The model also requires that neighboring cells are synchronized in their cycles and that the oscillations are slower in the anterior of the PSM than in the posterior. Consequently, changes in gene expression associated with the alternate states manifest themselves as repeated waves that sweep through the PSM from posterior to anterior. All of the genes identified so far that show this behavior are related to the Notch pathway, although the specific genes involved vary among species. For example, dynamic expression is seen for chick *c-hairy1*, a vertebrate homolog of the *Drosophila hairy* gene (Palmeirim et al. 1997), mouse *lunatic fringe* (*Lfng*) (Forsberg et al. 1998; McGrew et al. 1998), and zebrafish *her1* (Holley et al. 2000) and *deltaC* (Smithers et al. 2000). The synchronization of oscillations between neighboring cells in the PSM cells also appears to involve

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the Notch pathway (Jiang et al. 2000). Opposing the segmentation clock is a hypothetical wave front activity that proceeds posteriorly, slowing and then halting the oscillation cycles and inducing somite maturation. When anterior PSM cells receive this putative wave front signal, they give rise to bands or cohorts of cells one somite wide with the same gene expression profiles. Signaling within and between these stabilized cohorts of cells subsequently refines the anterior and posterior domains of the presumptive somite. Formation of these stripes is manifested in domain-specific expression of genes such as mouse *Mesp1* and *Mesp2* (Takahashi et al. 2000), zebrafish *mesp-a* and *mesp-b* (Durbin et al. 2000; Sawada et al. 2000a), and mouse *Delta-like1* (*Dll1*) (Bettenhausen et al. 1995) or *ephrinB2* (Holder and Klein 1999; Holder et al. 2000). The helix-loop-helix transcription factor *Mesp* appears to be important for the anterior-posterior regionalization of somite primordia, and this process is thought to be essential for boundary formation (Durbin et al. 2000; Sawada et al. 2000b; Takahashi et al. 2000).

Formation of the boundary between the posterior of the forming somite and the anterior of the next-to-be-formed presumptive somite involves the correct expression in the mesoderm of a number of evolutionarily conserved genes, as well as signals, not yet identified, from the ectoderm (Sosic et al. 1997; Correia and Conlon 2000). Among the mesodermally expressed genes are members of the *ephrin* and *Eph* receptor gene families, encoding components of the Eph cell communication pathway. A role for *EphA4* and *ephrinB2* in somite boundary formation has been demonstrated in zebrafish. Both genes are expressed in alternating anterior and posterior domains in somite primordia, and overexpression of dominant negative constructs of Eph receptors and ephrins results in strong defects in boundary formation (Durbin et al. 1998, 2000). Inactivation of function of the basic-helix-loop-helix (bHLH) transcription factor *paraxis* in the mouse embryo impairs epithelialization and formation and maintenance of morphological boundaries, but does not affect the initial specification and patterning of the somite primordia within the anterior PSM (Johnson et al. 2000). Therefore, several genetic pathways appear to act in parallel within the PSM to orchestrate the formation of morphologically distinct somites.

Roles in somitogenesis have been postulated for several bHLH genes, both those in the Notch signaling pathway such as *mesp*, and *hes/her1* (Takke and Campos-Ortega 1999; Takahashi et al. 2000) and Notch-independent genes such as *paraxis* (Johnson et al. 2000). However, little is known about the function of other classes of transcription factors. We have shown previously that genes encoding two evolutionarily conserved forkhead/winged helix transcription factors, *foxc1a* and *foxc1b*, are expressed in the PSM and somites. Based on this evidence, we proposed that these closely related genes play a role in somite formation and differentiation (Topczewska et al. 2001). In the present study, we tested this proposition by taking advantage of a recently estab-

lished and effective targeted gene morpholino “knock-down” technology (Nasevicius and Ekker 2000). The data we obtained provide the first evidence of an essential role for the forkhead gene *foxc1a* in zebrafish somitogenesis. Significantly, our data indicate that *Foxc1a* is essential for the formation of the most anterior somites, whereas the requirement for most other genes becomes manifested later, for posterior somites. Analysis of gene expression in the anterior PSM of *foxc1a*-morpholino-injected embryos suggests that *Foxc1a* protein is a key component of the complex molecular circuitry that is centered on the Notch pathway, and initially establishes and then stabilizes the anterior/posterior (A/P) domains of the presumptive somites. In the absence of *Foxc1a* protein, A/P patterning of somite primordia is not completed, boundaries and epithelial somites are not formed, and presumptive somites fail to complete their maturation.

Results

Antisense morpholino-modified oligonucleotides specific for foxc1a, but not foxc1b, inhibit formation of somites

To test the hypothesis that *foxc1a* and *foxc1b* are required for somitogenesis, we blocked translation of the respective mRNAs using antisense morpholino-modified oligonucleotides (MOs). Recent reports have shown that MOs injected into early embryos inhibit the translation of specific mRNAs even many hours later, mimicking a null mutant phenotype (Nasevicius and Ekker 2000). For each *foxc1* gene we designed two different MOs, one against 5' untranslated sequences (MO-1), the other against sequences overlapping the translation start site (MO-2). The same phenotypes were observed when these MOs are injected either singly or in combination, confirming the specificity of our results.

Coinjection of MO-1 and MO-2 against *foxc1a* at a total concentration of ~6 ng/embryo inhibited the formation of morphologically distinct anterior somites without significantly affecting either the rate of development of the embryos or their overall size (Fig. 1A–D). Confocal microscopy after whole-mount staining with β -catenin antibody also showed no evidence of segmental organization of the paraxial mesoderm cells (Fig. 1H,I). Both the severity and penetrance of the observed phenotype depended on the dose of MOs. As shown in Table 1, ~80% of the embryos injected with ~6 ng of *foxc1a*-MOs lacked anterior somites. The inhibition was usually seen up to the stage when control embryos have developed 6–7 somites, after which there was a gradual recovery of somitogenesis with morphologically distinguishable somites forming at the anterior of the PSM. Embryos injected with higher doses of *foxc1a*-MO (~10 ng) displayed a more extensive loss of somites (up to nine), but they also showed a general delay in development. Lower doses caused only partial loss of somites and a more rapid recovery of somitogenesis.

Different combinations of two MOs against *foxc1b* did

Figure 1. Distribution of Foxc1 protein in zebrafish embryos and effect of inhibition of translation. (A–F) Images of live embryos using Nomarski optics. Lateral view of wild-type (A) and *Foxc1a*-MO injected (B) embryos at 9-somite stage. (C and D) Dorsal views of 7-somite stage wild-type (C) and injected (D) embryos corresponding to the regions boxed in A and B. (E,F) Dorsal view of embryo injected with both *foxc1a*-MOs and zebrafish modified *foxc1a* RNA (E) and MOs together with mouse synthetic *Foxc1* RNA (F). Control embryos were at the 7-somite stage. Arrowheads indicate intersomitic borders. (G–K) Confocal images of embryos after immunohistochemistry. Cell boundaries are visualized using β -catenin antibody (green). (G) Localization of Foxc1a protein (red) in nuclei of 17-somite stage wild-type embryo. Arrowhead indicates migrating slow muscle precursors. Dorsal view of wild-type (H) and MO-injected (I) embryos at 5-somite stage after immunohistochemistry with Foxc1 antibody (red). (J,K) Dorsal view of wild-type and MO-injected embryos after immunohistochemistry with Myf5 antibody (red). (L) Western blot analysis with mouse Foxc1 specific antibody. Columns a–g represent protein extracts from: (a) COS cells transfected with zebrafish pCS2-Foxc1a expression vector; (b) *foxc1a*-MO-injected zebrafish embryos (MO) at 5-somite stage; (c) wild-type embryos at 5-somite stage; (d,e) wild-type embryos and *foxc1a*-MO-injected embryos at 12-somite stage; (f) COS cells; (g) COS cells transfected with mouse pCS2-Foxc1 expression vector. In H–F arrowheads indicate adaxial cells. nt, Notochord; psm, presomitic mesoderm; tb, tail bud.

not inhibit somite formation at any concentrations tested (2–10 ng/embryo, Table 2) but did affect the development of the head mesoderm, including the presumptive branchial arch region where the gene is strongly expressed (Topczewska et al. 2001).

Foxc1a-MOs specifically inhibit synthesis of *Foxc1a* protein

To determine the effectiveness of MOs in blocking the translation of *foxc1a*, we used an affinity-purified, polyclonal rabbit antibody raised against a mouse Foxc1 peptide. The sequence of the peptide used for immunization

differs by two and three amino acids from the corresponding predicted sequences of zebrafish Foxc1a and Foxc1b, respectively (see Materials and Methods). Western blot analysis showed that the antibody recognizes a protein made in COS cells transfected with a mouse *Foxc1* expression vector (Fig. 1L, column g) or transfected with zebrafish *foxc1a* (Fig. 1L, column a) but not *foxc1b* expression vector (data not shown). The antibody also reacts with a protein in extracts of wild-type zebrafish embryos (at the 5-somite stage; Fig. 1L, column c) and embryos injected with zebrafish *foxc1a* synthetic RNA (data not shown), further confirming antibody specificity. The difference in electrophoretic mobility

Table 1. The effect of *foxc1a*-specific MOs on the development of wild-type embryos

Observed phenotypes	Total amount of <i>foxc1a</i> -MOs (1 + 2) injected		
	4 ng	6 ng	10 ng
Partial loss of somites	78%	12%	2%
No somites	9%	77%	24%
No somites, retarded development	0%	2%	59%
Not affected	13%	9%	15%
Total embryo number	102	127	113

Results from two independent experiments carried out for the purpose of establishing dose response. All subsequent experiments for gene expression studies used 6 ng *foxc1a*-MOs (1 + 2) per embryo.

between the zebrafish and mouse proteins may be due to differences in their overall predicted amino acid sequences (476 residues vs. 553, respectively). Moreover, the zebrafish protein lacks long stretches of alanine, glycine, and serine residues that may decrease the mobility of the mouse protein (Topczewska et al. 2001).

To study subcellular localization of Foxc1a protein in the intact embryo, we used immunohistochemistry and confocal microscopy. As shown in Figure 1G and H, during the segmentation period Foxc1a protein is localized in the nucleus of cells in the PSM, but not in the tail bud. Significantly, protein expression in the PSM appears uniform, even in the anterior region where *foxc1a* transcript levels are higher in two distinct stripes (Fig. 2A). These stripes overlap with the transcription domains of *mesp-b* gene in the most anterior cells of S-1 and S-2 (where S0 is the forming somite), but are broader and extend more posteriorly (Fig. 2B). Foxc1a-positive nuclei were also observed in adaxial cells, which are organized into a single epithelial-like layer on either side of the notochord and give rise to slow muscles (Fig. 1H) (Devoto et al. 1996). The newly formed epithelial somites, including dorsal and ventral layers, intersomitic border cells, and the few internal mesenchymal cells also accumulate Foxc1a in the nucleus. When young somites start to differentiate, immunoreactivity declines in most of the cells but remains high in migrating adaxial cells and presumptive sclerotome cells (Fig. 1G; data not shown).

Immunohistochemistry showed the complete absence of Foxc1 protein in MO-injected embryos at the time

Table 2. The effect of *foxc1b*-specific MOs on the development of wild-type embryos

Observed phenotype	<i>foxc1b</i> -MOs injected			
	MO-1	MO-2	MO-1	MO-2
	4–6 ng		6–8 ng	
Embryos with somites	100%	100%	100%	100%
Total embryo number	42	38	30	26

Results from two independent experiments.

Figure 2. Gene expression in wild-type and *foxc1a*-MO-injected embryos revealed by whole-mount in situ hybridization. (A) *foxc1a* RNA in wild-type embryo. (B) Double color in situ hybridization of *foxc1a* (purple) and *mesp-b* expression (red) in wild-type embryo at 5-somite stage. (C,D) *deltaC* (purple) and *pax2.1* (red) expression in 5-somite stage of wild-type and MO-injected embryos. (E,F) In situ hybridization with *paraxis* (*par*), *krox20* (*krox*), *no tail* (*ntl*), and *pax2.1* probes in wild-type and MO-injected embryos. (G–J) In situ hybridization with probe for *myoD* (G,H) and double color in situ hybridization with probes for *myoD* (red) and *mesp-b* (blue) at the 8-somite stage. Arrowheads in C and E indicate *pax2.1*- and *deltaC*-positive pronephros primordium present in wild-type but not MO-injected embryos.

when uninjected embryos are at the 5-somite stage (Fig. 1I). In contrast, adaxial cells of injected embryos stained strongly with antibody against Myf5, indicating that the MOs do not inhibit expression of an unrelated protein (Fig. 1J,K). Interestingly, in MO-injected embryos there appears to be somewhat more Myf5 protein-positive cells than in the wild-type, and they extend more than one cell diameter from the notochord (Fig. 1J,K), but this effect has not been quantitated or explored further.

The absence of Foxc1a protein at the 5-somite stage in MO-injected embryos was also confirmed by Western blot analysis (Fig. 1L, column b). However, protein was detected in extracts of MO-injected embryos collected at the stage when wild-type embryos have reached the 12-somite stage (Fig. 1L, columns d and e). Taken together, these results indicate that *foxc1a*-MOs antisense oligo-

nucleotides specifically inhibit the translation of Foxc1a. Moreover, the failure of early somites formation is accompanied by loss of Foxc1a protein, and the subsequent recovery of somitogenesis is associated with its resynthesis.

The inhibitory effect of foxc1a-MO on segmentation is selective

To test whether the effect of *foxc1a*-MO is specific for somitogenesis, we assayed the expression of a number of genes normally expressed in somites and other tissues. As shown in Figure 2C–F, the expression of *no tail (ntl)* in the forming notochord, *krox20* in rhombomeres 3 and 5, and *pax2.1* in the midbrain–hindbrain boundary, otic primordia, and pronephric ducts were all unaffected compared to wild-type embryos at the 5-somite stage. However, expression domains of *deltaC* and *pax2.1* in the pronephric primordia, which are located lateral to somites 2 to 4 (Smithers et al. 2000) were missing in MO-injected embryos, even though the duct primordia were unaffected (Fig. 2C,D). This is consistent with the fact that only primordia of the pronephros and not the ducts express *foxc1a* (Topczewska et al. 2001) and suggests that the gene is specifically required for the development of the pronephros. In addition, the expression of

Table 3. The effect of mouse synthetic foxc1 RNA and rescue, modified foxc1a RNA on the foxc1a-MO phenotype

Gene analyzed	No expression ^a	Partial expression ^b	Wild-type expression ^c	Total no. of injected embryos
Injection of 6 ng <i>foxc1a</i> -MO				
<i>deltaC</i>	86%	8%	6%	140
<i>paraxis</i>	87%	5%	8%	117
Coinjection of 6 ng of <i>foxc1a</i> -MO and 10 pg of mouse <i>foxc1</i> synthetic RNA				
<i>deltaC</i>	45%	51%	5%	164
<i>paraxis</i>	58%	38%	3%	178

Results from three independent experiments. Embryos were fixed at the 5 somite stage and examined by whole amount *in situ* hybridization for *deltaC* and *paraxis*.

^aNo expression in region of first 5 somites.

^bOne to three stripes of *deltaC* or segments of *paraxis* expression.

^c5 bands of expression.

myoD was strongly reduced in the somite region of MO-injected embryos, but not in the adaxial cells (Fig. 2G–J).

The foxc1a-MO induced phenotype can be rescued

Further evidence of the specificity of the *foxc1a*-MO phenotype comes from rescue experiments with the mouse homolog, *Foxc1*, which differs from zebrafish *foxc1a* in the nucleotide sequence targeted by MO. Preliminary experiments indicated that zebrafish embryos are very sensitive to the dose of zebrafish or mouse *Foxc1* RNA injected and that high levels impair gastrulation (data not shown). This finding is consistent with the observation that mouse embryos are sensitive to the gene dosage of *Foxc1* and *Foxc2* (Kume et al. 2001). A series of different concentrations of mouse *Foxc1* RNA was therefore injected together with 6 ng of *foxc1a*-MOs. At a dose of about 10 pg of *Foxc1* RNA, partial rescue of morphological somites and recovery of expression of *deltaC* and *paraxis* was observed compared to embryos injected with *foxc1a*-MO alone (Figs. 1E, 3; Table 3). Because mouse synthetic *Foxc1* RNA only weakly suppressed the MO phenotype, another construct was prepared in which the 5' UTR sequences of zebrafish *foxc1a* mRNA recognized by *foxc1a*-MOs was deleted. Injection of this modified *foxc1a* RNA together with *foxc1a*-MO (10 pg of RNA and 8 ng of MOs) increased by 60% the frequency of embryos with morphologically distinguishable somites compared to MOs injection alone ($n = 84$; Fig. 1D,E).

Loss of morphological somites in MO-injected embryos is accompanied by loss of molecular markers in paraxial mesoderm

Although microscopy analyses (Nomarski and confocal) have shown that there is no morphological segmentation

Figure 3. Mouse synthetic *Foxc1* RNA can partially rescue *deltaC* and *par* expression in MO-injected embryos. (A–C) *deltaC* expression; (D–F) *par* expression. (A,D) wild-type embryos; (B,E) *foxc1a*-MO-injected embryos; (C,F) *foxc1a*-MO-injected embryos coinjected with mouse *Foxc1* RNA.

in *foxc1a*-MO-injected embryos, we nevertheless asked whether genes associated with anterior or posterior somite identity are expressed in the anterior of the PSM of MO-injected embryos. Anterior markers tested were *deltaD*, *notch6*, *ephA4*, and *papc*, and the posterior markers were *deltaC*, *notch5*, *ephrinB2*, and *myoD* (Figs. 2,4,5; data not shown). In all cases, no stripes of RNA expression were seen in *foxc1a*-MO-injected embryos in the region where somites should have been present. These results suggest that Foxc1a is required for somitogenesis and functions prior to the formation of morphological somites.

Figure 4. Effect of inhibiting Foxc1a protein synthesis on expression of *deltaC*, *notch 5*, *notch 6*, *mesp-a*, and *mesp-b*. (A) Wild-type embryo hybridized with probe for *deltaC*. Only one example of the variable pattern of expression is shown for the wild-type. (B–D) Representative *foxc1a*-MO-injected embryos showing normal variation in pattern of *deltaC* expression. (E,F) Wild-type at 5-somite stage and MO-injected embryos hybridized with probe for *notch5* and (G,H) for *notch6*. Arrowheads indicate PSM domains of *notch* genes; S-1 indicates presumptive somite posterior to forming somite. (I,J) Double color in situ hybridization with *myoD* and *mesp-b* probe in wild-type and MO embryos at 3-somite stage, and (K–N) the same with *mesp-a* and *mesp-b* probes at 5-somite stage. (K,M) Viewed under visible light. (L,N) The same embryos viewed with rhodamine filter. Note strong expression of *myoD* in the adaxial cells and complete absence of *mesp-b* transcripts in MO-injected embryos.

Figure 5. Inhibition of Foxc1a translation by *foxc1a*-MOs selectively reduces expression of *ephrinB2* in PSM and region where somites should be present. (A–D) Double color in situ hybridization with *ephrinB-2* (purple) and *papc* (red) probes at 3-somite stage in wild-type and MO embryos. (C,D) The same embryos at higher magnification. Arrowheads indicate PSM domains of *papc* (red) and *ephrinB2* (purple). (E,F) Double in situ hybridization with *par* and *myoD* probes at 6-somite stage showing that expression of *par* is strongly down-regulated in MO-injected embryos. Arrowhead indicates *myoD* expressing adaxial cells. (G,H) *Foxc1a* synthetic RNA induces ectopic expression of *par* at 40% epiboly. In situ hybridization with *par* in wild-type embryos (G) and embryos injected with *Foxc1a* RNA (H). Animal pole views.

Delta genes show normal expression in the presomitic mesoderm of *foxc1a*-MO injected embryos

The models of somitogenesis postulate that cells in the PSM oscillate from one mutually exclusive state to another. This is manifested in the zebrafish embryo as periodic waves of expression of *her1*, *deltaC*, and *deltaD* that appear to sweep anteriorly from the tail bud (Jiang et al. 2000; Keller 2000; Kerszberg and Wolpert 2000; Pourquie 2000b). An example of this dynamic behavior is the characteristic pattern of broad and narrow stripes of *deltaC* gene expression seen in the PSM of a population of wild-type embryos all examined at the same stage. As shown in Figure 4A–D, the wild-type pattern of *deltaC* expression is also seen in a group of MO-injected embryos, and transcripts did become localized into tight bands in the presumptive somite region. Similar dynamic expression in the PSM of MO-injected embryos was observed for *her1* and *deltaD* (data not shown). However as mentioned before, the striped expression of *deltaC* was not maintained in the region where the somites should have been present. Taken together, these results suggest that the first step of segmentation, establishment of segmental prepatter, is not affected by

Foxc1a inhibition and that synchronized oscillatory behavior does occur in the PSM of MO-injected embryos.

Some aspects of the anterior/posterior identity of cells in the anterior presomitic mesoderm are affected in MO-injected embryos

Before epithelial somites are formed, precise A and P domains are established in the somite primordia, a step that is thought to require Notch-dependent signaling (Buchberger et al. 2000; Johnson et al. 2000; Sawada et al. 2000a; Takahashi et al. 2000). Studies in both mouse and zebrafish have identified Mesp proteins as specific bHLH transcription factors required for effecting this Notch signaling in the anterior PSM. Both *mesp-a* and *mesp-b* genes are expressed in overlapping domains in the anterior of the somite primordia (S-1 and S-2) (Fig. 4I–N; Durbin et al. 2000; Sawada et al. 2000a). In addition, a transient band of *mesp-b* expression is seen in the forming somite, S0. We have consistently observed that the expression of *mesp-b* is strongly reduced in MO-injected embryos in S0, S-1, and S-2 (Fig. 4I,J). In contrast, the expression of *mesp-a* appears to be unaffected. This result is clearly illustrated in experiments in which the same MO-injected embryos were hybridized simultaneously with probes for the two genes, one labeled with DIG and the other with fluorescein (Fig. 4K,N). Our data suggest a differential requirement of *Foxc1a* protein for expression of *mesp-a* and *mesp-b*. Additionally, the expression of *protocadherin C* (*papc*), which is localized in the same regions of the PSM (S-1 and S-2) as the *mesp* genes was not affected in MO-injected embryos (Fig. 5; data not shown).

We next examined the effect of absence of *Foxc1a* protein on the expression of two genes encoding Delta receptors, *notch5* and *notch6* (Fig. 4E–H). Both genes are segmentally expressed in the PSM of wild-type embryos. In addition, *notch5* and *notch6* transcripts are present in the posterior and anteromedial parts of the formed somites, respectively (Bierkamp and Campos-Ortega 1993; Westlin and Lardelli 1997). This segmented expression was strongly down-regulated in MO-injected embryos, but weak, uniform expression of *notch6* persisted throughout the PSM. These observations indicate that only an incomplete A/P polarization of somite primordia is established in the absence of *Foxc1a* protein.

Expression of ephrin2B and eph4A, required for formation of somite boundaries, is abnormal in MO-injected embryos

Because the formation of intersomitic boundaries requires intercellular signaling mediated by cell surface molecules of the Eph/ephrin family (Durbin et al. 1998, 2000), we examined the expression of *ephrinB2* and *ephA4* in MO-injected embryos. As described earlier, stripes of expression of both genes were absent from the region where somites should be present (Fig. 5A,B; data not shown). To assess expression in the anterior PSM,

double staining using *papc* and *ephrin-B2* probes was carried out at the 3–4-somite stage (Fig. 5C,D). While the *papc* expression domains located in S-1 and S-2 were still present in MO-injected embryos, the stripes of *ephrin-B2* RNA were lost. This analysis shows that the expression of Eph/ephrin signaling molecules is perturbed in MO-injected embryos, and this may account, at least in part, for the defect in somite border formation.

Expression of paraxis depends on foxc1

Mouse embryos lacking the bHLH gene *paraxis* (*par*) fail to form epithelial somites, although segmentation and initial anteroposterior specification of somite primordia do occur (Burgess et al. 1996). As shown in Figures 2E,F and 5E,F, *par* expression is severely down-regulated in both the PSM and paraxial mesoderm of MO-injected embryos. As in the case of *mesp-a*, this effect is selective, because *myoD* expression in adaxial cells of injected embryos was normal or even elevated (Fig. 5E,F).

The down-regulation of *par* expression in MO-injected embryos raised the possibility that *Foxc1a* protein is a positive regulator of *par* gene. To test this hypothesis, we injected capped synthetic *foxc1a* RNA (40–60 pg) into one cell stage zebrafish embryos. As shown in Figure 5G,H, the overexpression of *foxc1a* induces premature transcription of *par* at 40% epiboly, whereas during normal development, expression of *par* RNA was first detected at about 65% epiboly (Shanmugalingam and Wilson 1998). These results show that *foxc1a* is both essential and sufficient for *par* expression.

The phenotype of foxc1a-MO-injected embryos is more severe than that of fss mutants

Several zebrafish mutants have been described that are defective in somitogenesis. In every case except *fused somites* (*fss*), the anterior somites develop normally, before defects are seen in the formation of more posterior somites (van Eeden et al. 1996). In contrast, *fss* mutants, like *foxc1a*-MO-injected embryos, lack all anterior somites. Despite this similarity, *fss* and *foxc1a* affect somitogenesis differently, because *par* is not down-regulated in *fss* as observed for MO-injected embryos (Fig. 6). We also compared the expression of *foxc1a* in *fss* and MO-injected embryos. Our data indicate that the striped pattern of *foxc1a* expression is lost in the anterior PSM of *fss* mutants (Fig. 6A,C). Similarly, no striped expression of *foxc1a* was seen in MO embryos, where inhibition of *foxc1a* translation resulted in uniform expression of the gene throughout the PSM and into the region where somites should have been present. We conclude that *foxc1a* acts in parallel to the *fss* genetic pathway as an essential factor for the complete anterior–posterior patterning of the PSM and the formation of epithelial somites.

Discussion

The findings reported here establish the forkhead/

Figure 6. Phenotypic differences between MO-injected embryos and *fss* mutants. (A,D) Wild-type embryos; (B) *foxc1a*-MO injected embryo, and (C,E) *fss* embryos. (A–C) In situ hybridization with *foxc1a* probe. Note loss of striped expression of *foxc1a* in the anterior PSM of *fss* mutant (D,E) *par* probe. Note that expression of *par* is not down-regulated in *fss* embryos.

winged helix transcription factor, Foxc1a, as a novel and necessary component of the molecular circuitry underlying somitogenesis in the zebrafish. Moreover, taken together with the new findings in the mouse described in the accompanying paper (Kume et al. 2001), it appears that this function of Foxc1, the requirement of the gene for *par* expression, and its involvement with elements of the Notch signaling pathway have all been evolutionarily conserved.

The conclusion that Foxc1a is required for zebrafish somitogenesis is based on findings with embryos in which synthesis of the protein is specifically inhibited by morpholino antisense oligonucleotides (Nasevicius and Ekker 2000). These embryos lack, at least up to about the 6–7-somite stage, morphological somites, segmented paraxial mesoderm, and expression in the somite region of genes characteristic of anterior and posterior somite cell fates. Several lines of evidence argue that the inhibitory effect of the *foxc1a*-MOs is specific and not due to a general toxicity. First, MOs block the synthesis of Foxc1a protein but not that of Myf5. Second, the inhibition of somite formation is dose-dependent, and a similar phenotype is observed with two separate *foxc1a*-MOs, each targeted against different regions of the *foxc1a* RNA. In contrast, somitogenesis proceeds normally in embryos injected with MOs targeted to the closely related gene *foxc1b*. Third, differentiation of other tissues continues on schedule in the absence of somite formation. Fourth, spontaneous recovery of somitogenesis in MO-injected embryos at around the 7-somite stage is accompanied by reexpression of the Foxc1a protein. One explanation for this recovery is that, as shown in Figure 6, the level of *foxc1a* RNA increases significantly in *foxc1a*-MO-injected embryos, probably due to negative feedback by Foxc1a protein on the transcription of its own gene. Consequently, MO oligonucleotides may become saturated. Finally, the phenotype of MO-injected embryos can be reversed by injection of either mouse *Foxc1* or zebrafish *foxc1a* synthetic RNA, which lack the sequences against which the MOs are directed.

Foxc1a protein is not required for the segmentation clock

Analysis of gene expression in *foxc1a*-MO-injected embryos shows that the dynamic expression of *deltaC*, *deltaD*, and *her1* genes in the PSM resembles that of wild-type embryos. Moreover, the anterior boundaries of the *delta* gene expression stripes are sharp and not diffuse, as seen in embryos with mutations in the Notch signaling pathway (Jiang et al. 1998; Holley et al. 2000). These observations suggest that the absence of Foxc1a protein does not affect the oscillation of cells in the PSM from one state to another. Nor does it affect the proposed synchronization of these oscillations between neighboring cells. Although *notch6* expression (Westlin and Lardelli 1997) is strongly down-regulated in MO-injected embryos, and is no longer expressed in stripes in the anterior region, there is still weak uniform expression throughout the PSM. This weak expression may be sufficient to enable synchronization of oscillations in the PSM to occur. Alternatively, other Notch genes such as *notch1a* may be engaged in this process and be unaffected by the absence of Foxc1a.

Foxc1a is required for correct A/P patterning of presumptive somites in the anterior presomitic mesoderm

For morphological boundaries to be generated between forming somites, it appears necessary that each presumptive somite be subdivided into stable anterior and posterior domains (Durbin et al. 2000). Observations initially made in the mouse have indicated a crucial role for Notch/Delta signaling and the basic-loop-helix transcription factor, Mesp2, in establishing this A/P patterning (Takahashi et al. 2000). Thus, Mesp2 in the anterior half of the presumptive somite is thought to inhibit the up-regulation of the *Dll1* gene in response to Notch activation, while the absence of Mesp2 expression in the posterior domain allows *Dll1* induction to proceed. Moreover, Mesp2 is thought to act in an autoregulatory loop with Notch, being both up-regulated by Notch signaling and required for Notch gene expression. A similar role has been suggested for zebrafish *mesp-b* gene (Sawada et al. 2000a). We found that the expression of one *mesp* gene, *mesp-a*, is unaffected in MO-injected embryos, in both the posterior PSM where it is expressed diffusely and in the stripe of high-level expression in the anterior region of the presumptive somites (S-1 and S-2) (Fig. 4). In contrast, transcripts for the *mesp-b* gene, normally localized exclusively in the most anterior part of S-1 and S-2, are completely absent in MO-injected embryos. This deficit is accompanied by the absence of the striped expression of *notch6* and *notch5* genes. We conclude that in zebrafish, the activity of *mesp-b* is required for correct patterning of the presumptive somites. Moreover, the loss of expression of only one *mesp* gene, in this case *mesp-b* due to the absence of Foxc1a protein, is enough to prevent the completion of somite formation, possibly in part because of the down-regulation of *notch5* and *notch6*.

Among the several zebrafish mutations with defects in somitogenesis, *fss* has the closest phenotype to that of *foxc1a*-MO-injected embryos, because in both sets of embryos all anterior somites are missing (van Eeden et al. 1996). However, there are significant differences between the two phenotypes. For example, the *fss* mutation completely disrupts normal expression of both *mesp-a* and *mesp-b* during the segmentation period (Sawada et al. 2000a). In other *fss*-type mutants such as *aei*, *mesp-b* is expressed in a diffuse “salt and pepper” pattern in the paraxial mesoderm similar to that seen for *deltaC* in the same mutants (Jiang et al. 2000), whereas expression of *mesp-a* is very reduced and limited mostly to adaxial cells. Based on these results, Sawada et al. (2000a) proposed that the two *mesp* genes are differentially regulated, a conclusion supported by our own results. Another difference between the phenotype of *fss* and *foxc1a*-MO-injected embryos is seen in the expression of the *protocadherin* gene, *papc*. In *fss* mutants, the down-regulation of both *mesp* genes is associated with strongly reduced expression of *papc* in the anterior PSM (Jiang et al. 2000). In contrast, *papc* is still expressed normally in *foxc1a*-MO-injected embryos in the PSM, suggesting that the remaining activity of *mesp-a* is sufficient to drive *papc* expression even though segmentation does not proceed to completion. Expression of *mesp-a* and *papc* in the anterior of the presumptive somites also suggests that the hypothetical wave front activity, postulated to be disrupted in *fss* mutants, functions in the absence of Foxc1a activity, and is able to partially stabilize the anterioposterior patterning of the anterior PSM.

Foxc1a is required for expression of ephrinB2 and its receptor ephA4 during somite formation

The formation of intersomitic boundaries requires the expression of Ephrins and their receptors, and manipulation of ephrin signaling genes in zebrafish disturbs somite differentiation (Durbin et al. 1998). We found that expression domains of *ephrinB2* and its receptor *ephA4* are strongly down-regulated in the anterior PSM of *foxc1a*-MO-injected embryos. This result is significantly different from that seen in *fss* mutant embryos, where the posteriorly expressed *ephrinB2* is expanded, and *fss*-like mutants in which *ephrinB2* and *ephA4* are expressed in a salt and pepper pattern (Durbin et al. 2000). The strong phenotype of MO-injected embryos compared to that of *fss*-type mutants raises the possibility that Ephrins and Eph receptors are regulated by Foxc1a directly, or that completed A/P specification of somite primordia is required for *ephrinB2* and *ephA4* expression. The observed lack of *ephrinB2* and *ephA4* expression in our experiments may be one of the reasons why somite boundaries are not formed and further somitic mesoderm differentiation is arrested.

Foxc1a is required for paraxis expression

Analysis of mouse mutants has shown that the gene *paraxis*, encoding a bHLH transcription factor, is re-

quired for the formation of epithelial somites but not for segmentation of paraxial mesoderm (Burgess et al. 1995, 1996). Recent evidence suggests that in the absence of *Par* in the mouse, Notch signaling and expression of *Mesp2*, *EphrinB2*, and *EphA4* in the PSM are initially normal. However, intersomitic boundaries fail to form, and the A/P polarity of the anterior PSM is not maintained in the segmented mesoderm (Johnson et al. 2000). *Paraxis* expression does not depend on the Notch signaling pathway in the mouse (Johnson et al. 2000), and is maintained in *fss* mutant embryos (Fig. 6E). In contrast, in *foxc1a*-MO-injected embryos (and in mouse embryos lacking both *Foxc1* and *Foxc2*) *paraxis* expression is strongly down-regulated. Further, we have shown that injection of *foxc1a* RNA into the zebrafish embryo leads to ectopic and premature *par* expression, suggesting that *par* is a direct or early downstream target of the Foxc1a transcription factor. Nevertheless, the phenotype of *foxc1a*-MO-injected embryos is more severe than that of *par* null embryos and affects the A/P patterning polarity of the anterior PSM. We conclude that loss of Paraxis function cannot be the primary and sole defect in MO-injected embryos, although it may still contribute to the lack of border formation and epithelialization of somites.

Comparison of the roles of foxc1 genes in mouse and zebrafish somitogenesis

Recent evidence in the mouse presented in the accompanying paper indicates that the two closely related winged helix transcription factors, Foxc1 and Foxc2, function combinatorily, and that inactivation of both sets of alleles is required to completely disrupt somite formation and the prepatterning of the anterior PSM (Kume et al. 2001). However, our present results suggest that there are differences in the role of *foxc1* genes in somitogenesis between mice and zebrafish. First, *foxc1b*, although transcribed in the same pattern as *foxc1a* in paraxial mesoderm and probably required for head mesoderm development, cannot substitute for the absence of *foxc1a* in somite formation. Second, if a homolog of mouse Foxc2 exists in the zebrafish, then it, too, does not compensate for the absence of Foxc1a. It therefore appears that the completion of somite formation in the zebrafish embryo is more dependent on the level of *foxc1* gene expression compared to the mouse embryo. Inhibiting Foxc1a protein synthesis alone is sufficient to uncover an evolutionarily conserved requirement for this class of transcription factor in the maturation of the presumptive somites.

Materials and methods

Fish embryos

Fish were maintained as described (Solnica-Krezel et al. 1994). The mutant allele used was *fused somites* (*fss*^{m774}; Driever and Fishman 1996).

In situ hybridization

Whole-mount in situ hybridization was performed essentially as described (Thisse et al. 1993). For two-color in situ hybridization, the digoxigenin- and fluorescein-labeled antisense probes were used simultaneously. After the first color reaction, the alkaline phosphatase-conjugated antibody was inactivated by washing three times for 5 min at room temperature first with 0.1 M glycine-HCl (pH 2.2), 0.1% Tween 20 and then with PBST (PBS at pH 7.4, 0.1% Tween 20). Embryos were then incubated with antifluorescein antibody for 2 h at room temperature or overnight at 4°C. After intense washing in PBST (6 times every 15 min at room temperature), followed by two more washes with 0.1 M Tris (pH 8.2), 0.1% Tween, the second color reaction was performed with Fast Red (Roche) in 0.1 M Tris (pH 8.2), 0.1% Tween, with the addition of 0.4 M NaCl. Depending on the probe, the reaction was continued for a few hours to overnight. For photomicrography, the embryos were mounted flat in 50% glycerol.

Antibody against mouse Foxc1 protein

A peptide from the C-terminal domain of mouse Foxc1 protein was used to raise a polyclonal rabbit antiserum, followed by peptide-affinity purification (Research Genetics). The differences between the predicted mouse and zebrafish protein sequences are underlined: Foxc1, AYPGQQNFHSHVREM FESQRI; Foxc1a, ATPAQQNFHSHVREMFESQRI; Foxc1b, AS PGQQNFHSHVREMFETQRI.

Whole-mount immunocytochemistry

Embryos were fixed in 4% paraformaldehyde, 4% sucrose, 3 mM CaCl₂, and PBS (pH 7.4) overnight at 4°C and permeabilized in acetone for 7 min at -20°C. Blocking was performed using PBST buffer with 5% goat serum, 0.2% BSA, and 2% DMSO. Incubation with primary antibody was overnight at 4°C followed by intense washing in PBST buffer at room temperature. For immunofluorescence microscopy, the secondary antibody was conjugated with Cy-3 (Jackson Labs) or Alexa Fluor 488 (Molecular Probe). Incubation was performed at room temperature for 2–3 h. In double staining experiments, the samples were incubated with the Foxc1 antibody diluted 1:200 overnight at 4°C and subsequently with β -catenin monoclonal antibody (Sigma) diluted in blocking solution 1:500 for 4 h at room temperature.

Transient transfection of COS cells

This procedure was performed using lipofectamine reagent according to the manufacturer's protocol (Life/Technologies-GIBCO BRL).

Western blot

Embryos were dechorionated and washed in Danieau buffer, and the yolk was removed manually. Tissues from 60 embryos were boiled in 60 μ L of Laemmli sample buffer (Laemmli 1990) for 5 min. Following centrifugation, the supernatant equivalent of 20 embryos was electrophoresed on a 10% polyacrylamide gel according to Sambrook et al. (1989). Proteins were electroblotted onto Zeta-Probe membranes (Bio Rad), and antigenic proteins were detected using affinity-purified Foxc1 antiserum at 1:1000 dilution and an ECL kit (Amersham).

Confocal microscopy

Flat mounted embryos were observed initially using epifluorescence with a Zeiss Axiophot microscope and subsequently using a Zeiss LSM410 Confocal Laser Scanning Inverted Micro-

scope with 20 $\times$ or 40 $\times$ Neofluar objectives (facility supported by NIH grants CA68485 and DK20593). For the Alexa 488 and Cy-3 labels, excitation was at 488 and 543 nm, respectively.

Microinjection of zebrafish embryos

Capped mRNA was synthesized by in vitro transcription of linearized plasmid (MessageMachine kit, Ambion). RNA (10–100 pg) was injected into the yolk of one- to two-cell stage embryos.

Morpholino

Morpholino antisense oligonucleotides (Gene-Tools) were designed according to the manufacturer's suggestions. Two different oligonucleotides specific for *foxc1a* and two for *foxc1b* were used for injections, as follows: *foxc1a*-MO-1, 5'-GT CAAGAAGACTGAAGCAATCCACA-3'; *foxc1a*-MO-2, 5'-CCTGCATGACTGCTCTCCAAAACGG-3'; *foxc1b*-MO-1, 5'-GCATCGTACCCCTTCTTCGGTACA-3'; *foxc1b*-MO-2, 5'-AAGTGAATGAAGACTATGCAGACG-3'.

Morpholino oligonucleotides were diluted in 1 $\times$ Danieau buffer, and between 2 and 10 ng was injected into the yolk of embryos at the 1–8 cell stage.

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References

- Bettenhausen, B., Hrabe de Angelis, M., Simon, D., Guenet, J.L., and Gossler, A. 1995. Transient and restricted expression during mouse embryogenesis of Dll1, a murine gene closely related to *Drosophila* Delta. *Development* **121**: 2407–2418.
- Bierkamp, C. and Campos-Ortega, J.A. 1993. A zebrafish homologue of the *Drosophila* neurogenic gene Notch and its pattern of transcription during early embryogenesis. *Mech. Dev.* **43**: 87–100.
- Buchberger, A., Bonneick, S., and Arnold, H. 2000. Expression of the novel basic-helix-loop-helix transcription factor cMespo in presomitic mesoderm of chicken embryos. *Mech. Dev.* **97**: 223–226.
- Burgess, R., Cserjesi, P., Ligon, K.L., and Olson, E.N. 1995. Paraxis: A basic helix-loop-helix protein expressed in paraxial mesoderm and developing somites. *Dev. Biol.* **168**: 296–306.
- Burgess, R., Rawls, A., Brown, D., Bradley, A., and Olson, E. 1996. Requirement of the paraxis gene for somite formation and musculoskeletal patterning. *Nature* **384**: 570–573.
- Cooke, J. and Zeeman, E.C. 1976. A clock and wavefront model for control of the number of repeated structures during animal morphogenesis. *J. Theor. Biol.* **58**: 455–476.
- Correia, K.M. and Conlon, R.A. 2000. Surface ectoderm is necessary for the morphogenesis of somites. *Mech. Dev.* **91**: 19–30.

- Dale, K.J. and Pourquie, O. 2000. A clock-work somite. *Bioessays* **22**: 72–83.
- Devoto, S.H., Melancon, E., Eisen, J.S., and Westerfield, M. 1996. Identification of separate slow and fast muscle precursor cells in vivo, prior to somite formation. *Development* **122**: 3371–3380.
- Driever, W. and Fishman, M.C. 1996. The zebrafish: Heritable disorders in transparent embryos. *J. Clin. Invest.* **97**: 1788–1794.
- Durbin, L., Brennan, C., Shiomi, K., Cooke, J., Barrios, A., Shanmugalingam, S., Guthrie, B., Lindberg, R., and Holder, N. 1998. Eph signaling is required for segmentation and differentiation of the somites. *Genes & Dev.* **12**: 3096–3109.
- Durbin, L., Sordino, P., Barrios, A., Gering, M., Thisse, C., Thisse, B., Brennan, C., Green, A., Wilson, S., and Holder, N. 2000. Anteroposterior patterning is required within segments for somite boundary formation in developing zebrafish. *Development* **127**: 1703–1713.
- Forsberg, H., Crozet, F., and Brown, N.A. 1998. Waves of mouse Lunatic fringe expression, in four-hour cycles at two-hour intervals, precede somite boundary formation. *Curr. Biol.* **8**: 1027–1030.
- Holder, N., Durbin, L., and Cooke, J. 2000. Eph receptors and ephrins are key regulators of morphogenesis. *Ernst Schering Res. Found. Workshop* **29**: 123–147.
- Holder, N. and Klein, R. 1999. Eph receptors and ephrins: Effectors of morphogenesis. *Development* **126**: 2033–2044.
- Holley, S.A. and Nusslein-Volhard, C. 2000. Somitogenesis in zebrafish. *Curr. Top. Dev. Biol.* **47**: 247–277.
- Holley, S.A., Geisler, R., and Nusslein-Volhard, C. 2000. Control of *her1* expression during zebrafish somitogenesis by a delta-dependent oscillator and an independent wave-front activity. *Genes & Dev.* **14**: 1678–1690.
- Jiang, Y.J., Aerne, B.L., Smithers, L., Haddon, C., Ish-Horowicz, D., and Lewis, J. 2000. Notch signalling and the synchronization of the somite segmentation clock. *Nature* **408**: 475–479.
- Jiang, Y.J., Smithers, L., and Lewis, J. 1998. Vertebrate segmentation: The clock is linked to Notch signalling. *Curr. Biol.* **8**: R868–R871.
- Johnson, J., Rhee, J., Parsons, S.M., Brown, D., Olson, E.N., and Rawls, A. 2000. The anterior/posterior polarity of somites is disrupted in paraxis-deficient mice. *Dev. Biol.* **229**: 1–12.
- Keller, R. 2000. The origin and morphogenesis of amphibian somites. *Curr. Top. Dev. Biol.* **47**: 183–246.
- Kerszberg, M. and Wolpert, L. 2000. A clock and trail model for somite formation, specialization and polarization. *J. Theor. Biol.* **205**: 505–510.
- Kume, T., Jiang, H.Y., Topczewska, J.M., and Hogan, B.L.M. 2001. The murine winged helix transcription factors, *Foxc1* and *Foxc2*, are both required for cardiovascular development and somitogenesis. *Genes & Dev.* **15**: 2470–2482 (this issue).
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685.
- McGrew, M.J., Dale, J.K., Fraboulet, S., and Pourquie, O. 1998. The lunatic fringe gene is a target of the molecular clock linked to somite segmentation in avian embryos. *Curr. Biol.* **8**: 979–982.
- Meinhardt, H. 1986. Hierarchical inductions of cell states: A model for segmentation in *Drosophila*. *J. Cell Sci. Suppl.* **4**: 357–381.
- Nasevicius, A. and Ekker, S.C. 2000. Effective targeted gene 'knockdown' in zebrafish. *Nat. Genet.* **26**: 216–220.
- Palmeirim, I., Henrique, D., Ish-Horowicz, D., and Pourquie, O. 1997. Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis. *Cell* **91**: 639–648.
- Pourquie, O. 1999. Notch around the clock. *Curr. Opin. Genet. Dev.* **9**: 559–565.
- Pourquie, O. 2000a. Segmentation of the paraxial mesoderm and vertebrate somitogenesis. *Curr. Top. Dev. Biol.* **47**: 81–105.
- Pourquie, O. 2000b. Vertebrate segmentation: Is cycling the rule? *Curr. Opin. Cell Biol.* **12**: 747–751.
- Primmatt, D.R., Norris, W.E., Carlson, G.J., Keynes, R.J., and Stern, C.D. 1989. Periodic segmental anomalies induced by heat shock in the chick embryo are associated with the cell cycle. *Development* **105**: 119–130.
- Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Sawada, A., Fritz, A., Jiang, Y., Yamamoto, A., Yamasu, K., Kuroiwa, A., Saga, Y., and Takeda, H. 2000a. Zebrafish *Mesp* family genes, *mesp-a* and *mesp-b* are segmentally expressed in the presomitic mesoderm, and *Mesp-b* confers the anterior identity to the developing somites. *Development* **127**: 1691–1702.
- Sawada, A., Saga, Y., and Takeda, H. 2000b. Zebrafish somitogenesis-roles of *mesp-* and *hairy-related* genes. *Tanpakushitsu Kakusan Koso* **45**: 2738–2744.
- Shanmugalingam, S. and Wilson, S.W. 1998. Isolation, expression and regulation of a zebrafish paraxis homologue. *Mech. Dev.* **78**: 85–89.
- Smithers, L., Haddon, C., Jiang, Y., and Lewis, J. 2000. Sequence and embryonic expression of *deltaC* in the zebrafish. *Mech. Dev.* **90**: 119–123.
- Solnica-Krezel, L., Schier, A.F., and Driever, W. 1994. Efficient recovery of ENU-induced mutations from the zebrafish germline. *Genetics* **136**: 1401–1420.
- Sosic, D., Brand-Saberi, B., Schmidt, C., Christ, B., and Olson, E. 1997. Regulation of paraxis expression and somite formation by ectoderm- and neural tube-derived signals. *Dev. Biol.* **185**: 229–243.
- Stern, C.D. and Keynes, R.J. 1988. Interactions between somite cells: The formation and maintenance of segment boundaries in the chick embryo. *Development* **99**: 261–272.
- Stickney, H.L., Barresi, M.J., and Devoto, S.H. 2000. Somite development in zebrafish. *Dev. Dyn.* **219**: 287–303.
- Takahashi, Y., Koizumi, K., Takagi, A., Kitajima, S., Inoue, T., Koseki, H., and Saga, Y. 2000. *Mesp2* initiates somite segmentation through the Notch signalling pathway. *Nat. Genet.* **25**: 390–396.
- Takke, C. and J.A. Campos-Ortega, J.A. 1999. *her1*, a zebrafish pair-rule like gene, acts downstream of Notch signalling to control somite development. *Development* **126**: 3005–3014.
- Thisse, C., Thisse, B., Schilling, T.F., and Postlethwait, J.H. 1993. Structure of the zebrafish *snail1* gene and its expression in wild-type, spadetail and no tail mutant embryos. *Development* **119**: 1203–1215.
- Topczewska, J.M., Topczewski, J., Solnica-Krezel, L., and Hogan, B.L. 2001. Sequence and expression of zebrafish *foxc1a* and *foxc1b*, encoding conserved forkhead/winged helix transcription factors. *Mech. Dev.* **100**: 343–347.
- van Eeden, F.J., Granato, M., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.P., Jiang, Y.J., Kane, D.A., et al. 1996. Mutations affecting somite formation and patterning in the zebrafish, *Danio rerio*. *Development* **123**: 153–164.
- Westlin, J. and Lardelli, M. 1997. Three novel Notch genes in zebrafish: Implications for vertebrate Notch gene evolution and function. *Dev. Genes Evol.* **207**: 51–63.

The winged helix transcription factor Foxc1a is essential for somitogenesis in zebrafish

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