Transcriptional oscillation of Lunatic fringe is essential for somitogenesis

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A molecular oscillator that controls the expression of cyclic genes such as lunatic fringe (Lfng) in the presomitic mesoderm has been shown to be coupled with somite formation in vertebrate embryos. To address the functional significance of oscillating Lfng expression, we have generated transgenic mice expressing Lfng constitutively in the presomitic mesoderm in addition to the intrinsic cyclic Lfng activity. These transgenic lines displayed defects of somite patterning and vertebral organization that were very similar to those of Lfng null mutants. Furthermore, constitutive expression of exogenous Lfng did not compensate for the complete loss of cyclic endogenous Lfng activity. Noncyclic exogenous Lfng expression did not abolish cyclic expression of endogenous Lfng in the posterior presomitic mesoderm (psm) but affected its expression pattern in the anterior psm. Similarly, dynamic expression of Hes7 was not abolished but abnormal expression patterns were obtained. Our data are consistent with a model in which alternations of Lfng activity between ON and OFF states in the presomitic mesoderm prior to somite segmentation are critical for proper somite patterning, and suggest that Notch signaling might not be the only determinant of cyclic gene expression in the presomitic mesoderm of mouse embryos.

[Keywords: Somitogenesis; segmentation clock; Lunatic fringe (Lfng)]

Received September 30, 2002; revised version accepted February 7, 2003.

Somitogenesis is a fundamental patterning process in vertebrate embryos that subdivides the paraxial mesoderm into a metameric series of homologous subunits, the somites. Somites form sequentially by segmentation of tissues at the anterior end of the presomitic mesoderm (psm). A molecular oscillator referred to as “segmentation clock” that has been shown to be coupled with the progression of somite segmentation has been revealed by dynamic and cyclic expression of genes in the psm. Expression of cyclic genes occurs in a tightly coordinated periodicity such that one wave of expression passes through the psm during the formation of one somite [Palmeirim et al. 1997; Forsberg et al. 1998; McGrew et al. 1998; Aulehla and Johnson 1999; Jiang et al. 2000; Jouve et al. 2000]. The segmentation clock is likely to be linked to Notch signaling activity as genes displaying cyclic activity encode components of the Notch pathway. Misexpression of Notch components or disruption of signaling by dominant negative factors disrupts somite formation and patterning in Xenopus and zebrafish embryos [Jen et al. 1997, 1999; Takke and Campos-Ortega 1999; Sawada et al. 2000]. Furthermore, mutations in some Notch pathway components that lead to defects in somitogenesis also affect the expression of cyclic genes [del Barco Barrantes et al. 1999; Jiang et al. 2000; Jouve et al. 2000; Dunwoodie et al. 2002]. It has been proposed that the segmentation clock regulates the periodic activation of Notch [Pourquier 1999], its signaling is required for the synchronization of the clock in neighboring cells [Jiang et al. 2000], and the Notch pathway is part of the oscillator mechanism per se [Holley et al. 2002; Morales et al. 2002; Dale et al. 2003].

In mice, four genes, lunatic fringe (Lfng) and three bHLH genes (Hes1, Hes7, and Hey2), are known to date, which display oscillating expression in the psm [Forsberg et al. 1998; McGrew et al. 1998; Aulehla and Johnson 1999; Jouve et al. 2000; Leimeister et al. 2000; Bessho et al. 2001]. Lfng encodes a glycosyltransferase that modifies Notch in the trans-Golgi network and thereby modulates its receptiveness to various ligands [Hicks et al. 2000], and Notch signaling regulates the expression of the bHLH genes. Loss-of-function analyses have shown that Lfng and Hes7 function are essential for normal somite formation and patterning [Evrad et al. 1998; Zhang and Gridley 1998; Bessho et al. 2001b], whereas the loss of Hes1 and Hey2 does not affect somitogenesis [Ishibashi et al. 1995; Ohtsuka et al. 1999; Jouve et al. 2000; Bessho et al. 2001b; Gessler et al. 2002]. Mice homozygous for null alleles of Lfng and Hes7, respectively, display severe defects in somite compartmentalization, somites are irregular in form and size, and the vertebral column is severely disorganized [Evrad et al. 1998; Zhang and Gridley 1998; Bessho et al. 2001b]. The loss-
of-function studies of Lfng and Hes7 have firmly established essential roles of these genes for somite patterning, and the apparent noncyclic expression of Lfng in Hes7 mutants suggested that cyclic Lfng expression might be essential [Bessho et al. 2001a]. However, thus far the significance of the oscillatory transcription of Lfng for its function during somitogenesis in mouse embryos has not been demonstrated.

To address the functional significance of oscillating Lfng activity in somite patterning, we have generated transgenic mice that express Lfng in a nonoscillating manner in the presomitic mesoderm using a portion of the Delta1 promoter [msd] that directs heterologous gene expression into the paraxial mesoderm [Beckers et al. 2000]. Transgenic msd::Lfng mice had somite and vertebral column defects very similar to Lfng null mutants. Reducing the level of endogenous Lfng did not significantly alter the phenotype caused by the transgene, and the nonoscillating exogenous Lfng did not rescue the loss of endogenous cyclic Lfng. Nonoscillating exogenous Lfng did not block dynamic expression of endogenous Lfng in the posterior psm but lead to a diffuse broad expression domain in the anterior psm. Likewise, dynamic expression of Hes7 was not completely abolished but abnormal expression patterns were obtained. Our data provide direct experimental evidence that oscillations of Lfng transcription between active and inactive states are critical for Lfng function in the paraxial mesoderm and suggest that Notch signaling might not be the sole determinant of cyclic transcriptional activation of oscillating genes in the posterior presomitic mesoderm of mouse embryos.

Results

Generation and skeletal defects of msd::Lfng transgenic mice

To analyze the effect of nonoscillating (“constitutive”) Lfng transcription on somite formation and patterning, we generated transgenic mice by DNA microinjection with Lfng cDNAs encoding an untagged and a C-terminally HA-tagged Lfng protein, respectively [Fig. 1A]. In both cases, the 1.5-kb msd fragment from the Delta1 gene, which directs heterologous gene expression in the presomitic mesoderm and at later stages additionally in newly formed somites and myotomes, was fused to the Delta1 minimal promoter and the 5′ UTR of exon 1 up to the ATG codon [Beckers et al. 2000]. The Lfng cDNAs were fused in frame to the Delta1 ATG. 3′ to the Lfng coding sequence an IRES sequence followed by a destabilized GFP cDNA, and polyadenylation signal was included [see Material and Methods for details].

Five transgenic founder mice with shortened and kinked tails were obtained [Fig. 1B, panels a,b, data not shown]. Three carried the C-terminally HA-tagged and two carried the untagged Lfng transgene. Two male founders carrying the tagged version of Lfng and one female founder carrying the untagged Lfng cDNA bred and transmitted the transgene to the offspring. Two transgenic lines referred to as msd::LfngHA2 and msd::LfngHA3 were established from the founders carrying the tagged Lfng. The female founder carrying the untagged transgene [Fig. 1B, panel b] gave rise to only one litter of stillborns with severe skeletal malformations [Fig. 1C, panel a] precluding the establishment of a stable transgenic line and the analysis of transgenic embryos. However, two phenotypically inconspicuous founders, referred to as msd::Lfng11 and msd::Lfng12, were obtained from an additional series of microinjections with the untagged Lfng transgene and gave rise to transgenic offspring with shortened and kinked tails [Fig. 1B, panel c, data not shown]. The female founder msd::Lfng12 produced four litters with 31 offspring, only two of which were transgenic and did not breed thus far. In contrast, the male founder msd::Lfng11 transmitted the transgene consistently allowing us to collect and analyze transgenic embryos for somite defects.

Transgenic founders and hemizygous mice carrying both the HA-tagged and untagged Lfng transgene displayed similar external phenotypes and axial skeleton defects [e.g., Fig. 1B, cf. panels b and f, C, cf. panels a and e]. The segmental pattern of the vertebral column was disrupted; irregularly shaped vertebral bodies and fusions of adjacent neural arches and proximal ribs were consistently obtained [Fig. 1C, data not shown]. However, the severity of the phenotype varied between different founders and transgenic lines carrying either transgene. Hemizygous msd::LfngHA3 mice [n = 25] displayed vertebral malformations and severe shortening of the body axis [Figs. 1B, panel f, C, panel e, 4A, panel c, below] similar to Lfng null mutants [Ervard et al. 1998; Zhang and Gridley 1998]. In contrast, hemizygous msd::LfngHA2 mice showed milder defects [n = 20; Fig. 1B, panel d, C, panel c]. Founders msd::LfngID9 [Fig. 1B, panel b, C, panel a] and ID10 [Fig. 1B, panel a] were virtually indistinguishable from msd::LfngHA3 mice [Fig. 1B, panel f, C, panel e], whereas hemizygous msd::Lfng11 mice [n = 14] had a phenotype that was slightly less severe and more similar to homozygous msd::LfngHA2 mice [Fig. 1B, cf. panels c and e, C, cf. panels b and d]. The similar external and skeletal phenotypes, as well as the irregular somites and somite patterning defects (see below) suggested that the patterning defects in msd::LfngHA embryos and mice are not caused by the HA tag and both the tagged and untagged transgene functioned equivalently.

Transgene expression and phenotypic outcome

To address whether different levels or a different timing of exogenous Lfng transcription could account for the different severity of defects in msd::LfngHA2, msd::LfngHA3, and msd::Lfng11 mice, respectively, transgene expression was assessed in day 7.5–10.5 embryos by mRNA in situ hybridization using a GFP riboprobe that detects the transgenic Lfng-GFP fusion transcripts. Fluorescence of the destabilized GFP protein was not detected. In msd::LfngHA3 embryos, transgene expression was evident already at the 1-somite stage and
was found in the psm but not in somites of hemizygous day 8–8.5 [2–6-somite stage] embryos [Fig. 2A, panels e, B, panels a–d]. Subsequently, the transgene was expressed in the psm and newly formed and differentiating somites, which were irregular in size and shape [Fig. 2A, panels f, g]. After day 9.5, the expression domain in the psm had a sharp anterior border with lower levels of expression anteriorly [Fig. 2A, panel g, arrowheads; data not shown], which was more clearly seen in embryos after less extensive staining [Fig. 2A, panel g, inset]. In contrast, hemizygous msd::Lfngha2 embryos showed only weak transgene expression in the psm but not in the somites at day 8–8.5 [Fig. 2A, panel a]. Higher levels in the paraxial mesoderm were only found later in day 9.5 and older embryos [Fig. 2A, panels b, c; data not shown] in a pattern very similar to, but with levels always lower than in msd::Lfngha3 embryos. In msd::Lfngh11 emb-

Figure 1. Structure of transgenes and external and skeletal phenotype of transgenic mice. (A) Structure of the msd::Lfngh and msd::Lfngha transgenes. msd refers to the portion of the Delta1 promoter directing heterologous gene expression into the paraxial mesoderm (Beckers et al. 2000). (B) Transgenic founder mice obtained with msd::Lfngh (panels a, b), a hemizygous msd::Lfngh1 mouse (panel c), hemizygous (panel d) and homozygous (panel e) transgenic msd::Lfngha2 mice, and a hemizygous msd::Lfngha3 mouse (panel f). (C) Skeletal preparations of a stillborn transgenic mouse obtained with female msd::Lfngh founder ID9 (panel a), a hemizygous d16.5 msd::Lfngh11 fetus (panel b), hemizygous (panel c) and homozygous (panel d) transgenic msd::Lfngha2, and hemizygous msd::Lfngha3 (panel e) newborn mice. Arrowheads and arrows point to fusions of neural arches and ribs, respectively.
and a similar intensity of staining was found in wild-type, msd::LrngHA3, and msd::Lrng11 embryos, suggesting that endogenous and exogenous Lrng are expressed at similar levels in this transgenic line.

To further analyze the effect of timing and level of transgene expression on phenotypic outcomes, we attempted to generate homozygous transgenic lines by interbreeding hemizygous msd::LrngHA2 and msd::LrngHA3 mice, respectively. Transgenic msd::LrngHA3 females did not reproduce, precluding the analysis of homozygous msd::LrngHA3 transgenic mice. Interbreeding of hemizygous msd::LrngHA2 transgenic mice produced some offspring with a severe phenotype similar to msd::LrngHA3 mice (Fig. 2B, cf. panels d and e, C, cf. panels c and d), suggesting that they are homozygotes. Severely affected msd::LrngHA2 females, like hemizygous msd::LrngHA3 females, did not reproduce. Homozygous msd::LrngHA2 males (n = 4, displaying a severe phenotype and homozygosity ascertained by test matings with wild-type females) mated with msd::LrngHA2 females produced embryos half of which showed stronger transgene expression [Fig. 2A, panel d] than hemizygous msd::LrngHA2 embryos [Fig. 2A, panel b], but significantly lower Lrng expression than hemizygous msd::LrngHA3 embryos [Fig. 2A, cf. panels d and f]. These findings suggest that constitutive expression of Lrng that moderately elevates the level of endogenous Lrng is sufficient to disrupt normal somitogenesis, and a further increase of constitutive expression has little additional effect on Notch activity.

**Disrupted somite patterning in msd::Lrng transgenic embryos**

Complete loss of Lrng causes irregular somite borders and disrupts somite compartmentalization as evidenced by a severely disorganized pattern of markers for anterior and posterior somite halves and affects expression of Notch pathway components [Evrard et al. 1998; Zhang and Gridley 1998].

![Figure 2. Transgene expression in msd::LrngHA2, msd::LrngHA3, and msd::Lrng11 embryos.](image-url)
patterning, we analyzed expression of *Uncx4.1* and *Tbx18,* which mark posterior and anterior somite halves, respectively. Expression of *Uncx4.1* and *Tbx18* was no longer confined to anterior and posterior somite compartments, respectively, but spread throughout the somite [Fig. 3] similar to the expression in *Lfng−/−* mutants [Evrard et al. 1998; Zhang and Gridley 1998]. In addition, the regularly spaced expression of *Dll1* in posterior somite halves was disrupted, the distinct expression of *Dll3* and *Notch2* in anterior portions of newly formed somites was lost, and the anterior expression border of *Notch1* was poorly defined [Fig. 3], closely resembling the somite defects found in homozygous *Lfng* mutant embryos (Evrard et al. 1998; Zhang and Gridley 1998). Likewise, similar to *Lfng* mutant embryos, *Pax9* expression was diffuse, and myogenin expression domains were frequently fused in *msd::LfngHA3* transgenic embryos [Fig. 3ze, data not shown]. Expression of *pMeso1, Mesp1,* and *Mesp2* were not altered in transgenic embryos [data not shown]. Consistent with the significantly lower levels of transgene expression in *msd::LfngHA2* embryos, disruptions of expression patterns were less severe and began at more posterior axial levels (i.e., in older embryos, Fig. 3j–o] than in *msd::LfngHA3* or *msd::Lfng11* embryos. Because transgene expression was confined to the psm of day 8.5 embryos [Fig. 2A, panel e] but somite patterning was already disrupted in *msd::LfngHA3* embryos at this stage [Fig. 3p, q, r], nonscillating *Lfng* expression in the psm is sufficient to disrupt anterior-posterior somite patterning, and the ectopic somitic expression at later stages is unlikely to cause these defects.

**Failure of exogenous *Lfng* to compensate for the loss of cyclic endogenous *Lfng***

To test whether a reduction of cyclic endogenous *Lfng* modulates the phenotype caused by constitutive exogenous *Lfng* expression, and whether a sustained level of *Lfng* expression can compensate for the loss of endogenous cyclic *Lfng*, *msd::LfngHA3* transgenic mice also carrying the recessive *LfnglacZ* null allele [Zhang and Gridley 1998] were generated. Mice hemizygous for *msd::LfngHA3* and one copy of *LfnglacZ* (*LfnglacZ+/msd::Lfng*) resembled hemizygous transgenic *msd::LfngHA3* mice both in their external morphology and skeletal defects (26 adults and n = 36, day 15.5–16.5

![Figure 3](https://example.com/figure3.png)

*Figure 3.* Somite patterning defects in *msd::Lfng* and *msd::LfngHA* transgenic mice. In situ hybridization of wild-type [a–i], *msd::LfngHA2* [j–o], *msd::LfngHA3* [p–ze], and *msd::Lfng11* [y–zd] embryos. Probes and stages are indicated above each column. In *msd::LfngHA3* embryos *Uncx4.1, Tbx18,* and *Dll1* expression is essentially normal in the prospective cervical somites of day 8.5 embryos but disrupted in more posterior somites of day 9.5 embryos, whereas in *msd::LfngHA3* embryos expression patterns are abnormal already in day 8.5 embryos. Day 9.5 *msd::Lfng11* embryos show patterning abnormalities similar to *msd::LfngHA3* embryos. Arrows in o and r point to *Dll1* expression domains out of register with the contralateral side. In *msd::LfngHA3* embryos, myotome fusions [arrowheads in ze] were frequently observed. [v–x, z, zd] Altered expression boundaries of Notch pathway components in day 9.5 *msd::LfngHA3* and *msd::Lfng11* embryos. The sharp anterior expression borders of Notch1, *Dll3,* and Notch2 in wild-type embryos [arrowheads in g–i] were indistinct and fuzzy in transgenic embryos. Expression of *Dll3* in anterior somite portions of wild-type embryos [arrows in g] was not detected in transgenic embryos. The two distinct expression domains of Notch2 in wild-type embryos [arrowheads in i] were no longer discernable [brackets in x, zd].
embryos; Fig. 4A, panels c,d,i,j). Thus, the reduction of the endogenous cyclic Lfng did not significantly alter the skeletal phenotype caused by a sustained level of exogenous Lfng. Hemizygous msd::LfngHA3 mice homozygous for LfnglacZ/LfnglacZ/msd::Lfng displayed a phenotype virtually identical to homozygous Lfng null mutants (n = 5, Fig. 4A, panels e,f,k,m). Consistent with the comparable skeletal defects amongst msd::Lfng, msd−::LfngHA3/LfnglacZ/lacZ, and LfnglacZ/lacZ/msd::Lfng mice, expression patterns of Uncx4.1 andDll1 were similarly altered in all these embryos [Fig. 4B, data not shown], indicating that nonoscillatory Lfng expression cannot rescue vertebral and somite malformations caused by the loss of cyclic Lfng activity. The inability of the transgene to rescue the Lfng null phenotype was not because of its lack of expression because msd::LfngHA expression in LfnglacZ/lacZ/msd::LfngHA3 mice was similar to that of msd::LfngHA3 mice [Figs. 2C, panels i–l, 4A, panel l]. Thus, constitutive expression of Lfng and absence of Lfng have a similar impact on somite formation and patterning.

**Effect of exogenous Lfng on cyclic gene expression in the psm**

If cyclic Lfng expression were central to the segmentation clock, noncyclic Lfng expression should disrupt oscillating gene expression. To address whether the constitutive expression of exogenous Lfng in the psm affects the periodic transcription of the endogenous Lfng gene,
we used an intron probe that only detects unprocessed nuclear endogenous transcripts [Morales et al. 2002]. In msd:\(\text{-}\)LfgHA2 (n = 16) and msd:\(\text{-}\)LfgHA3 day 9.5 embryos (n = 27) essentially two types of endogenous Lfg expression patterns were detected in the psm. In the first type, no endogenous Lfg transcripts were detected in the posterior psm, but transcripts were present in the anterior psm in a broad band or poorly delineated stripes (msd:\(\text{-}\)LfgHA2, 11/16, Fig. 5A, panels d,e, msd:\(\text{-}\)LfgHA3, 20/27, Fig. 5A, panels l,m). In the second type, endogenous Lfg transcripts were present in the posterior psm, and in the anterior psm in a broad domain similar to the first group (msd:\(\text{-}\)LfgHA2, 5/16, Fig. 5A, panels f,g, msd:\(\text{-}\)LfgHA3, 7/27; Fig. 5A, panels n,o). Embryos with two distinct bands of expression in the anterior psm clearly resembling late phase II or phase III of the endogenous expression cycle were only observed in two embryos of msd:\(\text{-}\)LfgHA2 (Fig. 5A, panel d, data not shown). This suggested that Lfg transcription is still cyclic, but the progression of Lfg oscillations and the refinement of anterior expression domains into distinct stripes are disrupted by ectopic Lfg transcripts in transgenic embryos. The broad anterior expression domain of endogenous Lfg in msd:\(\text{-}\)LfgHA transgenic mice was similar to the expression pattern of the lacZ mRNA in homozygous Lfg\(^{lacZ}\) mutant embryos (n = 5; Fig. 5A, panel t; Zhang and Gridley 1998). However, in contrast to transgenic embryos, lacZ transcripts derived from the Lfg\(^{lacZ}\) allele, which reflect transcription of the endogenous locus, appeared down-regulated and diffuse in the posterior psm of different embryos homozygous for the Lfg\(^{lacZ}\) null allele (Fig. 5A, panel t; Zhang and Gridley 1998).

To directly test if endogenous transcription is dynamic in transgenic day 9.5 msd:\(\text{-}\)Lfg embryos, posterior embryo portions were cut in half along the midline, one half fixed immediately, and the other half cultured for various times prior to fixation and in situ hybridization. Embryo halves from both msd:\(\text{-}\)LfgHA transgenic lines showed clear differences of endogenous Lfg expression in the posterior psm after 60 (n = 4 and n = 3, respectively, Fig. 5A, panels i,g) and 90 (n = 3, respectively, Fig. 5A, panels j,r) min of culture, and similar patterns after 2 h (n = 3, respectively, Fig. 5A, panels k,s). In most cases, caudal Lfg expression was down-regulated, and an anterior band of expression remained after 60 and 90 min, respectively (e.g., Fig. 5A, panel j). In three cases, expression was down-regulated in the posterior and anterior psm, and a new expression domain located caudal to the anterior stripe evident prior to culture was observed (e.g., Fig. 5A, panels i,s). In one culture, Lfg was up-regulated caudally and down-regulated in the anterior psm (Fig. 5A, panel q). These findings confirmed the results of the expression analysis of endogenous Lfg in whole embryos and indicated that in the presence of constitutive Lfg activity endogenous Lfg transcription was still dynamic in the psm.

Hes7 is essential for cyclic Lfg expression in vivo, and Hes7 expression is activated by Notch signaling [Bessho et al. 2001a,b]. In Delta1 mutant embryos, Hes7 expression was restricted to the tail bud and posterior psm, and expression patterns did not vary between embryos (n = 6; Fig. 5B, panel t), suggesting that Delta1 is required for up-regulation and dynamic expression of Hes7 in the psm. Because Lfg potentiates Delta1-mediated Notch signaling in vitro [Hicks et al. 2000], we analyzed Hes7 expression in msd:\(\text{-}\)LfgHA2 and 3 embryos to address how transgene-derived constitutive Lfg affects Hes7 expression. Hes7 expression levels appeared not significantly altered, and variable but abnormal patterns of

Figure 5. Cyclic gene expression in msd:\(\text{-}\)LfgHA2 and msd:\(\text{-}\)LfgHA3 transgenic mice. [A] Endogenous Lfg expression in day 9.5 embryos detected by in situ hybridization with an intron probe. Dorsal (panels a-g,l-o,t) and lateral (panels a’-g’,l’-o’,t’) views of the same embryos are shown. [Panels a-c] The three phases of Lfg expression in wild-type embryos. In transgenic embryos, essentially two types of patterns were observed. In one group of embryos (two examples are shown in panels d,e and l,m, respectively), there was only expression in the anterior psm either in broad domains or in stripes that were in most cases poorly defined and diffuse. In the second group of embryos (two examples are shown in panels f,g and n,o, respectively), there was a broad domain of anterior expression [white arrowheads in panels f,g,n,o, and additional expression in the posterior psm (black arrowheads in panels f,g,n,o) separated by a region of no or low expression [bars in panels f,g,n,o]. [Panels h-k,p-s] Noncultured (0’) and cultured day 9.5 embryo tail halves [culture times indicated in the lower right corners] after in situ hybridization. Lfg expression clearly changed during 60 and 90 min of culture, and similar expression patterns were observed after 120 min. Arrowheads in panels i,j,q,r point to expression domains that changed during culture. In Lfg mutant embryos (panel t) lacZ transcripts derived from the Lfg\(^{lacZ}\) allele, which reflect transcription of the endogenous locus, were present in a broad domain in the anterior psm and appeared down-regulated and diffuse in the posterior psm of different embryos homozygous for the Lfg\(^{lacZ}\) null allele. [B] Hes7 expression in day 9.5 wild-type, Dll1 mutant (panel t), msd:\(\text{-}\)LfgHA2 (panels a-g), and msd:\(\text{-}\)LfgHA3 (panels l-o) transgenic embryos. Dorsal (panels a-g,l-o,t) and lateral (panels a’-g’,l’-o’,t’) views of the same embryos are shown. [Panels a-c] The three phases of Hes7 expression in wild-type embryos. In transgenic embryos essentially two types of patterns were observed. In one group of embryos (two examples are shown in panels d,e and l,m, respectively) there was strong expression in the posterior psm and a domain of homogenous weaker expression extending further anteriorly. In the second group of embryos (two examples in panels f,g and n,o, respectively) there was a domain of strong expression in the posterior psm [black arrowheads in panels f,g,n,o] and a band of strong expression in the anterior psm [white arrowheads in panels f,g,n,o] that were separated by a variable region of weaker expression [bars in panels f,g,n,o]. [Panels h-k,p-s] Noncultured (0’) and cultured day 9.5 embryo tail halves [culture times indicated in the lower right corners] after in situ hybridization. Hes7 expression changed during 60 and 90 min of culture, whereas similar expression patterns were observed after 120 min. For example, expression was up-regulated in the posterior psm [arrowheads in panels i,q], or down-regulated in the posterior psm [arrowheads in panels j,r]. In Dll1 mutant embryos (panel t) Hes7 expression was confined to the posterior psm and appeared similar in all embryos. The number of embryos with each pattern or phase and the total number of analyzed embryos is indicated for each genotype.
Requirement for *Lmg* oscillations in the psm

### A

**+/+**

| Phase | Lmg+/+ | Lmg-/− |
|-------|--------|--------|
| I     | ![Image](image1.png) | ![Image](image2.png) |
| II    | ![Image](image3.png) | ![Image](image4.png) |
| III   | ![Image](image5.png) | ![Image](image6.png) |

**msd::LmgHA2**

| Type 1 Pattern | Type 2 Pattern |
|---------------|---------------|
| (11/16)       | (5/16)        |

**Embryo Halves Cultures**

| embryo halves cultures | embryo halves cultures |
|------------------------|------------------------|
| ![Image](image7.png)   | ![Image](image8.png)   |

**msd::LmgHA3**

| Type 1 Pattern | Type 2 Pattern |
|---------------|---------------|
| (20/27)       | (7/27)        |

**Embryo Halves Cultures**

| embryo halves cultures | embryo halves cultures |
|------------------------|------------------------|
| ![Image](image9.png)   | ![Image](image10.png)  |

### B

**+/+**

| Phase | Lmg+/+ | Lmg-/− |
|-------|--------|--------|
| I     | ![Image](image11.png) | ![Image](image12.png) |
| II    | ![Image](image13.png) | ![Image](image14.png) |
| III   | ![Image](image15.png) | ![Image](image16.png) |

**msd::LmgHA2**

| Type 1 Pattern | Type 2 Pattern |
|---------------|---------------|
| (4/12)        | (8/12)        |

**Embryo Halves Cultures**

| embryo halves cultures | embryo halves cultures |
|------------------------|------------------------|
| ![Image](image17.png)   | ![Image](image18.png)   |

**msd::LmgHA3**

| Type 1 Pattern | Type 2 Pattern |
|---------------|---------------|
| (9/16)        | (9/18)        |

**Embryo Halves Cultures**

| embryo halves cultures | embryo halves cultures |
|------------------------|------------------------|
| ![Image](image19.png)   | ![Image](image20.png)  |

(Figure 5 legend on facing page)
Hes7 transcripts were found in msd:\-LfnghA2, and msd:\-LfnghA3 embryos [Fig. 5B, panels d\-g, l\-o, n = 12 and n = 18, respectively]. In one group of embryos, Hes7 transcripts were essentially confined to the posterior half of the psm, and were most abundant in the tail bud and posteriormost psm, similar to phase I expression in wild-type embryos [msd:\-LfnghA2:4/12, Fig. 5B, panels d,c, msd:\-LfnghA3 9/18, Fig. 5B, panels l,m]. In a second group of embryos, Hes7 transcripts were found throughout the psm either in a fairly uniform pattern or with regions of higher expression in the anterior and posterior psm, respectively [msd:\-LfnghA2:8/12, Fig. 5B, panels i,g, msd:\-LfnghA3 9/18, Fig. 5B, panels n,o]. However, no transgenic embryo showed an anterior band of Hes7 expression that was clearly separated from the posterior expression domain by a stripe of Hes7 nonexpressing cells and thus unambiguously resembled a wild-type phase II pattern [Fig. 5B, panel b]. Thus, similar to endogenous Lfngh, Hes7 expression appeared still dynamic but was abnormal in the psm of msd:\-Lfngh transgenic embryos. The dynamic nature of Hes7 expression was further analyzed by culture of embryo halves. Expression patterns in noncultured and corresponding cultured embryo halves showed differences after 60 h [n = 5 and n = 7, respectively, Fig. 5 B, panels i,j] and 90 h [n = 5, respectively, Fig. 5 B, panels j,r] min of culture, and similar expression patterns were observed after 2 h of culture [n = 5 and n = 6, respectively, Fig. 5B, panels k,s]. After 60 and 90 min of culture, Hes7 expression levels were up\-regulated [Fig. 5B, panels i,j] or down\-regulated [Fig. 5B, panels j,r] particularly in the posterior psm of cultured compared to noncultured embryo halves, and the extent of the expression domain in the anterior psm varied. However, the observed differences were less obvious than in the case of Lfngh. Together, these analyses suggested that constitutive Lfngh expression did not abolish dynamic Hes7 transcription but interfered with its normal pattern, implying that in wild-type embryos, cyclic Lfngh is essential for the normal progression of Hes7 expression cycles.  

Discussion  
This study, which addresses the role of cyclic Lfngh transcription in the presomitic mesoderm of mouse embryos, has shown that similar defects in somitic and vertebral patterns are found in mice that either have lost Lfngh function completely or have a sustained Lfngh activity that elevates the basal level and dampens the oscillatory activity of the endogenous Lfngh gene. In LfnghlacZ/lacZ mice that lack endogenous Lfngh activity, the introduction of a noncycling transgenic Lfngh activity does not rescue the developmental defects, indicating that a sustained level of Lfngh expression cannot compensate for the loss of cyclic Lfngh activity. These findings strongly suggest that oscillation of Lfngh expression in the presomitic mesoderm is essential for somite formation and patterning. Furthermore, the maintained dynamic expression of endogenous Lfngh transcription in the presence of constitutive exogenous Lfngh expression suggests that Notch signaling is not the sole determinant of cyclic transcriptional activation in the posterior presomitic mesoderm of mouse embryos.

Cyclic transcriptional activation is an essential parameter of Lfngh function  
Our experiments show that constitutive expression of Lfngh transgenes in the psm of mice causes defects in somite border formation and anterior-posterior somite patterning and is not sufficient to compensate for the loss of cyclic endogenous Lfngh. Based on the similar phenotypes in the different analyzed genotypes, we propose that transcriptional oscillation of Lfngh between an “ON” state and an “OFF” state is critical for Notch function in the psm [Fig. 6]. In wild-type embryos, Lfngh transcription cycles between its full on (ON100\%) and full off (OFF) transcription [Fig. 6A, panel a] leading to transient bursts of Lfngh activity, which may result in waves of increased Notch sensitivity to its ligands and enhanced signaling. The loss of one copy of Lfngh may reduce the amplitude of the oscillations (e.g., ON50\%), but expression is shut off in every cycle, which may still result in cyclic modulations of Notch activity [Fig. 6A, panel b]. In msd:\-Lfngh embryos, transgenic Lfngh generates a constant level of Lfngh superimposed on endogenous oscillating Lfngh expression [Fig. 6A, panel c]. This leads to oscillations of Lfngh expression between two ON levels, ONHIGH and ONLOW, but prevents reaching the OFF state. Similarly, in LfnghlacZ/lacZ/msd:\-Lfngh embryos, endogenous Lfngh oscillations with reduced amplitude are superimposed on exogenous Lfngh expression [Fig. 6A, panel d]. As a consequence, in transgenic embryos with one or two copies of endogenous Lfngh, Lfngh and presumably Notch activity might only alternate between two states of higher activity, ONHIGH and ONLOW, and never would be able to reach below a critical threshold level or be completely OFF. In LfnghlacZ/lacZ/msd:\-Lfngh embryos, where no endogenous Lfngh activity is present, a constant level of exogenous Lfngh expression (ONHIGH) Fig. 6A, panel e) might lead to permanently elevated Notch activity in the psm, whereas in homozygous null mutants there is no Lfngh expression [Fig. 6A, panel f] and presumably there is no Lfngh induced modulation of Notch activity. Thus, a constant low or high level of Notch activity in the psm appears not to be sufficient for somitogenesis. However, because Lfngh activity in the anterior psm has also been implicated in the formation of somite boundaries [Sato et al. 2002], we cannot completely rule out that disruption of normal Lfngh function in the anterior psm contributes to the somite defects.

Lfngh potentiates Delta1-mediated Notch signaling in vitro [Hicks et al. 2000], and Delta1 signals are essential for Notch activity in the paraxial mesoderm [Hrabé de Angelis et al. 1997; Jouve et al. 2000]. Thus, our findings support the idea that cyclic fluctuations of Notch signaling activity are essential for somite formation and patterning [Pourquier 1999], and Notch activity has to fall below a certain threshold during each cycle. Our findings imply that level or activity of the Lfngh protein, which acts as a glycosyltransferase, oscillate in the psm. The
stability and distribution of endogenous Lfng protein in the psm of mice are not known. However, Lfng protein levels fluctuate periodically in the psm of avian embryos, and overexpression of Lfng in the segmental plate of chick embryos resulted in somite defects similar to msd::Lfng transgenic mice (Dale et al. 2003), supporting our interpretation of the phenotypic consequences of constitutive Lfng expression in the psm of mouse embryos. Furthermore, our data suggest that alternating Lfng activity between moderate and high levels is not sufficient for normal somitogenesis. Lfng activity has to reach low or zero levels of activity during each cycle to enable proper somite patterning.

Notch signaling and cyclic transcription in the psm

Analyses of the Lfng promoter (Cole et al. 2002; Morales et al. 2002) and of Lfng expression in Notch pathway mutants (del Barco Barrantes et al. 1999; Dunwoodie et al. 2002; Zhang et al. 2002) have indicated that Lfng tran-
scription is positively and negatively regulated by Notch signaling. *Lfng* activation being directly, and repression indirectly controlled by Notch (Cole et al. 2002, Morales et al. 2002). Normal transcriptional activation of *Lfng* was disrupted in homozygous *LfnglacZ/lacZ* embryos [Fig. 5A, panel t, Zhang and Gridley 1998] suggesting that *Lfng* itself is part of a feedback loop regulating its own transcription by modulating Notch sensitivity to its ligands [s]. Cyclic *Lfng* expression was also lost in *Hes7* mutant embryos [Bessho et al. 2001b] and in CBF1 (RBPjk) mutant embryos, which are likely to completely lack Notch signaling, only severely reduced noncyclic *Lfng* expression was detected [Morales et al. 2002]. Together, these results suggest that oscillating Notch signaling underlies the segmentation clock and that *Lfng* is part of this regulatory loop. Recent experiments in chick embryos further support this model. *Lfng* overexpression in the segmental plate of chick embryos led to complete down-regulation of endogenous *Lfng* [Dale et al. 2003] similar to overexpression of a dominant-negative version of RBPjk, suggesting that in chick embryos, *Lfng* inhibits Delta-mediated Notch activation and establishes a negative feedback loop that represents a core component of the avian segmentation clock [Dale et al. 2003].

Based on the results of this study, we propose that Notch activity is not the sole determinant of cyclic gene expression in the posterior psm of mouse embryos. Despite constitutive expression of exogenous *Lfng* throughout the psm, we observed cyclic endogenous *Lfng* transcription in the posterior psm of *msd*-/*Lfng* transgenic embryos. Evidence for disrupted *Lfng* cycling was only found in the anterior psm. The down-regulation of *Lfng* expression in mutants with reduced or abolished Notch signaling together with the cyclic endogenous *Lfng* transcription in *msd*-/*Lfng* transgenic embryos suggests that while Notch activity is essential for the expression of oscillating genes, cyclic *Lfng* and presumably cyclic Notch activity is not essential. This implies that some other as yet unidentified cyclic mechanism operates in the posterior psm and interacts with Notch signaling to initiate cyclic gene expression. This mechanism might cyclically generate or activate a component that could directly interact with activated Notch (i.e., the intracellular domain, NICD) and thereby inactivate NICD or prevent it from binding to CBF1 [Fig. 6B, panel a]. An alternative possibility could be that this factor cooperates with activated Notch at the *Lfng* promoter specifically in the posterior psm and acts as a cyclic activator or repressor [Fig. 6B, panel b]. The latter possibility appears conceivable because distinct regulatory elements are required to direct cyclic *Lfng* expression in posterior and anterior expression domains [Morales et al. 2002]. Whereas our data do not allow us to distinguish between these possibilities, either mechanism would be compatible with the mutant phenotypes of Notch pathway components in mice. As long as Notch activity is present in the posterior psm, this oscillating activity could cooperate with activated Notch and induce cyclic activation of target genes. Reducing Notch signaling by removing either individual ligands or receptors would decrease transcript levels of target genes but would not eliminate cyclic initiation of transcription on a low(er) level, as has indeed been observed [Morales et al. 2002]. Total loss of Notch signaling in CBF1 mutant embryos would abolish Notch-dependent transcriptional activation altogether and consequently no cyclic activation of target genes would be possible. Also, the noncyclic expression of *Lfng* in *Hes7* mutant embryos [Bessho et al. 2001b] is compatible with the presence of an oscillating mechanism that acts independently from, but in concert with, Notch. *Hes7* is likely to repress *Lfng*. Thus, in the absence of *Hes7*, cyclic repression after initiation of *Lfng* transcription would no longer occur, leading to the observed expression of *Lfng* throughout the psm in *Hes7* mutant embryos. The effect of the loss of *Lfng* on its own expression is less clear. Loss of *Lfng* appears to lead to down-regulation of *Lfng* caudally, but it cannot be concluded with certainty that transcriptional initiation at the *Lfng* locus in homozygous mutants is no longer dynamic, as the *lacZ* gene has to be used as an indicator of *Lfng* transcription, and that transcription of the *lacZ* allele with the neo gene present in the locus truly reflects the endogenous situation is not known.

Cyclic initiation of transcription of *Lfng* and *Hes7* in the posterior psm could trigger the establishment of a negative feedback loop that generates, reinforces, and maintains periodic Notch activity and controls the oscillating expression of cyclic genes after their initial activation. This feedback loop apparently requires both *Hes7* and *Lfng* because the loss of either gene disrupts oscillations of gene expression, and it can be disrupted by constitutive expression of *Lfng*.

Based on our results, we propose that transcriptional activation of *Lfng* in the posterior psm of mouse embryos depends on activated Notch and an additional cyclic mechanism. This mechanism appears to be functional even with reduced Notch signaling activity, for example, in the absence of *Dll1* [Morales et al. 2002], suggesting that it is independent of Notch activity and might act upstream of Notch to initiate cyclic gene expression in the posterior paraxial mesoderm. Our findings are consistent with and supported by the results of a recent study, which demonstrates that *Wnt3a* plays a major role in the segmentation clock, and which suggests that *Wnt3a* controls intracellular oscillations of Wnt/β-catenin and Notch activity in the psm [Aulehla et al. 2003]. Our data and conclusions concerning the effect of constitutive *Lfng* expression on cyclic gene expression are at odds with the results and conclusions of Dale et al. [2003] that periodic inhibition of Notch by *Lfng* underlies the segmentation clock in chick embryos. Potential experimental differences that might account for the contrasting results could be different expression levels of exogenous *Lfng* in electroporated chick and transgenic mouse embryos, or high levels of expression after electroporation already in the primitive streak or tail bud, where the msd enhancer is not or only weakly active. Future studies disrupting oscillating gene expression in defined regions of the psm and at varying levels will be required to resolve this discrepancy.
Materials and methods

*Constructs and generation of transgenic mice*

The mesoderm-specific promoter msd is a 1495-bp FokI fragment fused with the minimal promoter of *Dll1* containing the first exon as previously described (Beckers et al. 2000). The HA-tagged *Lfng* cDNA was amplified with primer 1 (GGGGTACCC ATGCTCCAGCAGCGCCGCGGC) and primer 2 (GGG TTAACCTAAGCATAATCGATACATATGGATAGAA GATGGCCGAGCGAGCACA) to generate a KpnI site at the 5' end and an HA-tag followed by a stop codon at the 3' end. The 5' KpnI site was used to clone *Lfng* in frame into the first ATG codon of the msd promoter. The untagged version of the *Lfng* cDNA was obtained by amplifying the *Lfng* cDNA with the same primer without the HA sequence. At the 3' end of the *Lfng* cDNA an internal ribosome entry site (IRES) from pIRE2-EGFP (Clontech no. 6029-1) was fused in frame to a destabilized version of GFP (pd1EGFP-N1, Clontech no. 6073-1) followed by a SV40 polyadenylation signal. The whole cloning procedure of the transgene was verified by sequencing. Transgenic mice were generated by injecting the linearized construct without any vector sequences into the pronuclei of FVB fertilized eggs according generated by injecting the linearized construct without any vector sequences into the pronuclei of FVB fertilized eggs according to standard procedures.

*Genotyping of transgenic and *Lfng* mutant mice*

Genomic DNA was isolated either from tails of adult mice or from yolk sacs of embryos at different developmental stages. The presence of the msd::*Lfng*HA or msd::*Lfng* transgene was verified by PCR using the following primers: *Lfg*-F7 (CCT GTCCACCTTCTTGTTTCG) and *Lfg*-B13 (CAGAGAATGCT CCGTTGATG). Lunatic fringe mutant mice and embryos were identified with an allele-specific PCR resulting in a 500-bp PCR product for the wild-type allele with primer pair *lfhs*1 (GAACAAATATGGGCA ATG) and a 450-bp PCR product for the mutant allele with primer pair *lfhs*1 (GAACAAATATGGGGCA ATG) and *lfgw*F3 (GGTGCGCTTCTCGCCAGGGC). The presence of the msd promoter was determined by Southern blotting of genomic DNA with a probe that was derived from the luciferase cassette that had been excised from pBluescriptII KS (Stratagene) using T7 RNA polymerase.

*Whole-mount in situ hybridization*

Whole-mount in situ hybridizations were performed following a standard procedure with digoxigenin-labeled antisense riboprobes (Wilkinson 1992) with minor modifications using an In situPro (Intavis AG no. 10.000) for automated in situ detection. The probes used were *Dll1* (Bettenhausen et al. 1995), *Dll3* (Kumari et al. 1998), *Hes7* (Bessho et al. 2001b), *Hey2* (Leimeister et al. 2000), *Lfg* (Zhang and Gridley 1998), *Lfg* Intron (Morales et al. 2002), pMesogenin (Yoon and Wold 2000), *MesP1* (Saga et al. 1996), *MesP2* (Saga et al. 1997), *Myogenin* (Montarras et al. 1991), Notch1 (Conlon et al. 1995), Notch2 (Weinmaster et al. 1992), paraxis (Burgess et al. 1995), *Ftx*1 (Deutsch et al. 1988), *Ftx*9 (Neubüser et al. 1995), *Tbx18* (Kraus et al. 2001), and *Uncx4.1* (Mansouri et al. 1997). A GFP-specific riboprobe was generated from a BamHI/NotI fragment from pd1EGFP-N1 subcloned into pBluescript II KS (Stratagene) using T7 RNA polymerase.

*Embryo culture*

Using a fine tungsten needle the caudal part of day 9.5 mouse embryos was divided into two halves along the midline in 100% FCS as previously described (Aulehla and Johnson 1999). One half was fixed immediately, the other was cultured in a hanging drop of DMEM/F12 medium 1:1 containing 10% fetal bovine serum at 37°C and 5% CO₂ for 60, 90, and 120 min, respectively. After overnight fixation in 4% paraformaldehyde, explants were processed for whole-mount in situ hybridization as described above.

*Skeletal preparation of newborn and day 15.5 embryos*

Staining of the skeletons of newborn mice was performed as previously described (Zachgo et al. 1998). Day 15.5 embryos were fixed in 95% ethanol at least overnight. Then, the cartilage staining was done for 2 d in Alcian blue solution [150 mg/L Alcian blue 8GX in 80% ethanol/20% acetic acid]. Embryos were rinsed and postfixed overnight again in 95% ethanol. Initial clearing was done with 2% KOH for 1–2 h at room temperature. With Alizarin red [50 mg/L Alizarin red S in 5% KOH] bones were stained overnight at room temperature. A second clearing was performed using 1% KOH until the soft tissues became transparent. Following this incubation, the embryos were stepwise transferred to 40% glycerol.

Acknowledgment

We thank Tom Gridley for the generous gift of *Lfng* mutant mice; David Ish-Horowicz and Aixa Morales for the *Lfng* intron probe; Olivier Pourquié for communicating results prior to publication; Andreas Kispert, Tom Gridley, Barbara Wold, Ryochiro Kageyama, and Manfred Gessler for probes; and Johannes Beckers, Tom Gridley, Bernhard Herrmann, Andreas Kispert, and Patrick Tam for critical comments and discussion. This work was supported by the German Research Council (DFG; SFB271).

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References

Aulehla, A. and Johnson, R.L. 1999. Dynamic expression of lunatic fringe suggests a link between notch signaling and an autonomous cellular oscillator driving somite segmentation. *Dev. Biol.* 207: 49–61.

Aulehla, A., Wehrle, C., Kemler, R., Mallo, M., Gossler, A., Kanzler, B., and Herrmann, B.G. 2003. Wnt acts upstream of Notch in the segmentation clock controlling somitogenesis. *Dev. Cell* [In press].

Beckers, J., Caron, A., Hrabé de Angelis, M., Hans, S., Campos-Ortega, J.A., and Gossler, A. 2000. Distinct regulatory elements direct Delta1 expression in the nervous system and paraxial mesoderm of transgenic mice. *Mech. Dev.* 95: 23–34.

Bessho, Y., Miyoshi, G., Sakata, R., and Kageyama, R. 2001a. *Hes7*: A bHLH-type repressor gene regulated by Notch and expressed in the presomitic mesoderm. *Genes Cells* 6: 175–185.

Bessho, Y., Sakata, R., Komatsu, S., Shiota, K., Yamada, S., and Kageyama, R. 2001b. Dynamic expression and essential functions of *Hes7* in somite segmentation. *Genes & Dev.* 15: 2642–2647.

Bettenhausen, B., Hrabé 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.

Burgess, R., Cserjesi, P., Ligon, K.L., and Olson, E.N. 1995.
Serth et al.

Paraxis: A basic helix-loop-helix protein expressed in paraxial mesoderm and developing somites. Dev. Biol. 168: 296–306.

Cole, S.E., Levorse, J.M., Tilghman, S.M., and Vogt, T.F. 2002. Clock regulatory elements control cyclic expression of lunitatic fringe during somitogenesis. Dev. Cell 3: 75–84.

Conlon, R.A., Reaume, A.G., and Rossant, J. 1995. Notch1 is required for the coordinate segmentation of somites. Development 121: 1533–1545.

Dale, J.K., Maroto, M., Dequest, M.-L., Malapert, P., McGrew, M., and Pourquie, O. 2003. Periodic Notch inhibition by lunitatic fringe underlies the chick segmentation clock. Nature 421: 275–278.

del Barco Barrantes, ´I., Elia, A.J., Wu¨nsch, K., Hrabé de Angelis, M., Mak, T.W., Rossant, R., Conlon, R.A., Gossler, A., and de la Pompa, J.-L. 1999. Interaction between L-fringe and Notch signalling in the regulation of boundary formation and posterior identity in the presomitic mesoderm of the mouse. Curr. Biol. 9: 470–480.

Deutsch, U., Dressler, G.R., and Gruss, P. 1988. Pax 1, a member of a paired box homologous murine gene family, is expressed in segmented structures during development. Cell 52: 617–625.

Dunwoodie, S.L., Clements, M., Sparrow, D.B., Sa, X., Conlon, R.A., and Beddington, R.S. 2002. Axial skeletal defects caused by mutation in the spondylocostal dysplasia/pudgy gene DIll3 are associated with disruption of the segmentation clock within the presomitic mesoderm. Development 129: 1795–1806.

Evrard, Y.A., Lun, Y., Aulehla, A., Gan, L., and Johnson, R.L. 1998. Lunitatic fringe is an essential mediator of somite segmentati-

Forberg, H., Crozet, F., and Brown, N.A. 1998. Waves of mouse lunitatic fringe expression, in four-hour cycles at two-hour intervals, precede somite boundary formation. Curr. Biol. 8: 1027–1030.

Gessler, M., Knobeloch, K.-P., Helisch, A., Arnann, K., Schumacher, N., Rohde, E., Fischer, A., and Leimemter, C. 2002. Mouse gridlock: No aortic coartation or deficiency, but fatal cardiac defects in Hey2−/− mice. Curr. Biol. 12: 1601–1604.

Hicks, C., Johnston, S.H., diSibio, G., Collazo, A., Vogt, T.F., and Weinmaster, G. 2000. Fringe differentially modulates Jagged1 and Deltal signalling through Notch1 and Notch2. Nat. Cell Biol. 2: 515–520.

Holley, S.A., Julich, D., Rausch, G.J., Geisler, R., and Nusslein-Volhard, C. 2002. her1 and the notch pathway function within the oscillator mechanism that regulates zebrafish somitogenesis. Development 129: 1175–1183.

Hrabé de Angelis, M., McIntyre II, J., and Gossler, A. 1997. Maintenance of somite borders in mice requires the Delta homologueDll. Nature 386: 717–721.

Ishibashi, M., Ang, S.-L., Shiota, K., Nakanishi, S., Kageyama, R., and Guillemot, F. 1995. Targeted disruption of mammalian hairy and enhancer of split homolog-1 (HES-1) leads to up-regulation of neural helix-loop-helix factors, premature neurogenesis, and severe neural tube defects. Genes & Dev. 9: 3136–3148.

Jiang, Y.J., Aerne, B.L., Smithers, L., Haddon, C., Ish-Horowicz, D., and Lewis, J. 2000. Notch signalling and the synchroni-

Jouve, C., Palmeirim, I., Henrique, D., Beckers, J., Gossler, A., Ish-Horowicz, D., and Pourquie, O. 2000. Notch signaling is required for cyclic expression of the hair-like gene HES1 in the presomitic mesoderm. Development 127: 1421–1429.

Kraus, F., Haenig, B., and Kispert, A. 2001. Cloning and expres-

Kusumi, K., Sun, E.S., Kerrebrouck, A.W., Bronson, R.T., Chi, D.-C., Bulotsky, M.S., Spencer, J.B., Birren, B.W., Frankel, W.N., and Lander, E.S. 1998. The mouse pudgy mutation disrupts Delta homologue Dll3 and initiation of early somite boundaries. Nat. Genet. 19: 274–278.

Leimemter, C., Dale, K., Fischer, A., Klamt, B., Hrabé de Ange-

Neubüser, A., Koseki, H., and Guilmot, F. 1995. Targeted disruption of mouse hairy-related homeobox gene Hairy-1 and development of the presomitic mesoderm. EMBO J. 18: 2196–2207.

Palmeirim, I., Henrique, D., Ish-Horowicz, D., and Pourquie, O. 1997. Avian hairy gene expression identifies a molecular clock linked to somite segmentation in avian embryos. Nat. Genet. 9: 979–982.

Montarras, D., Chelly, J., Bober, E., Arnold, H., Ott, M.-O., Gros, F., and Finset, C. 1991. Developmental patterns in the expression of Myf5, MyoD, myogenin, and MRF4 during myo-

Morales, A.V., Yasuda, Y., and Ish-Horowicz, D. 2002. Periodic lunitatic fringe expression during segmentation is controlled by a cyclic transcriptional enhancer responsive to Notch sign-

Neubüser, A., Koseki, H., and Balling, R. 1995. Characterization and developmental expression of Pax9, a paired-box-contain-

Ohtsuka, T., Ishibashi, M., Gradwohl, G., Nakanischi, S., Guilmot, F., and Kageyama, R. 1999. Hes1 and Hes5 as Notch effectors in mammalian neuronal differentiation. EMBO J. 18: 2196–2207.

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.

Saga, Y., Hata, N., Kobayashi, S., Magnuson, T., Seldin, M.F., and Taketo, M.M. 1996. MesP1: A novel basic helix-loop-helix protein expressed in the nascent mesodermal cells during mouse gastrulation. Development 122: 2769–2778.

Sawada, A., Fritz, A., Jiang, Y., Yamamoto, A., Yasumasa, K., Kuroiwa, A., Saga, Y., and Takeda, H. 2000. Zebrafish Mesp family genes, mesp-a and mesp-b are segmentally expressed in the paraxial mesoderm of Xenopus embryos. Development 124: 1169–1178.

Sawada, A., Gavantka, V., Pollet, N., Niehrs, C., and Kintner, C. 1999. Periodic repression of notch pathway genes governs the segmentation of Xenopus embryos. Genes & Dev. 13: 1486–1499.
in the presomitic mesoderm, and Mesp-b confers the ante-
rior identity to the developing somites. Development 127: 1691–1702.
Takke, C. and Campos-Ortega, J.A. 1999. her1, a zebrafish pair-
rule like gene, acts downstream of notch signalling to con-
trol somite development. Development 126: 3005–3014.
Weinmaster, G., Roberts, V.J., and Lemke, G. 1992. Notch2: A
second mammalian Notch gene. Development 116: 931–941.
Wilkinson, D.G. 1992. Whole mount in situ hybridization of
vertebrate embryos. In In situ hybridization: A practical ap-
proach [ed. D.G. Wilkinson], pp. 75–84. Oxford University
Press, Oxford.
Yoon, J.K. and Wold, B. 2000. The bHLH regulator pMesogenin1
is required for maturation and segmentation of paraxial me-
soderm. Genes & Dev. 14: 3204–3214.
Zachgo, J., Korn, R., and Gossler, A. 1998. Genetic interactions
suggest that Danforth’s short tail (Sd) is a gain-of-function
mutation. Dev. Genet. 23: 86–96.
Zhang, N. and Gridley, T. 1998. Defects in somite formation in
lunatic fringe-deficient mice. Nature 394: 374–377.
Zhang, N., Norton, C.R., and Gridley, T. 2002. Segmentation
defects of Notch pathway mutants and absence of a syner-
gistic phenotype in lunatic fringe/radical fringe double mu-
tant mice. Genesis 33: 21–28.
Transcriptional oscillation of Lunatic fringe is essential for somitogenesis

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*Genes Dev.* 2003, 17: Access the most recent version at doi:10.1101/gad.250603

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