Expression of *Msgn1* in the presomitic mesoderm is controlled by synergism of WNT signalling and *Tbx6*

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The vertebral column and skeletal muscles of vertebrates are derived from the paraxial mesoderm, which is laid down initially as two stripes of mesenchymal cells alongside the neural tube and subsequently segmented. Previous work has shown that the wingless-type MMTV integration site family (WNT), fibroblast growth factor- and Delta–Notch signalling pathways control presomitic mesoderm (psm) formation and segmentation. Here, we show that the expression of *mesogenin 1*, a basic helix–loop–helix transcription factor, which is essential for psm maturation, is regulated by synergism between WNT signalling and the T-box 6 transcription factor, involving a feed-forward control mechanism. These findings emphasize the crucial role of WNT signalling in the control of psm formation, maturation and segmentation.

Keywords: WNT signalling; transcriptional regulation; somitogenesis; T-box genes; presomitic mesoderm

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INTRODUCTION

Paraxial mesoderm, the mesodermal tissue located adjacent to the neural tube, is generated in the primitive streak and tail bud of amniote embryos. On formation, the paraxial mesoderm has a mesenchymal appearance and is called presomitic mesoderm (psm). After maturation, the psm becomes segmented, resulting in two rows of epithelial spheres, the somites, alongside the neural tube. Later in development, the somites undergo further differentiation along the dorsal–ventral axis into dermomyotome and sklerotome, which give rise to dermis and skeletal muscles, and to the axial skeleton, respectively (Gossler & Tam, 2002).

The wingless-type MMTV integration site family (WNT), fibroblast growth factors (FGF), and Delta–Notch signalling cascades control psm formation and segmentation in the trunk and tail of mouse embryos (Aulehla & Herrmann, 2004). The wingless-related MMTV integration site 3A (*Wnt3a*) gene, FGF receptor 1 (*Fgfr1*) and the T-box transcription factor brachyury (*T*) are essential for epithelial–mesenchymal transition, the first stage of psm formation. Mutants lacking any of these factors show an early arrest of trunk elongation at approximately the 8- to 12-somite stage (Wilkinson *et al*, 1990; Herrmann, 1995; Ciruna *et al*, 1997; Yoshikawa *et al*, 1997). *T* is a target gene of the WNT and FGF signalling cascades, and acts upstream of the T-box 6 transcription factor (*Tbx6*; Yamaguchi *et al*, 1999b; Hofmann *et al*, 2004). *Tbx6* mutants undergo psm formation but fail to maintain cell type identity, resulting in embryos with two additional ectopic neural tubes (Chapman & Papaioannou, 1998).

In addition to the WNT and FGF signalling pathways, the segmentation of the psm involves the Delta–Notch signalling cascade. WNT and Notch signalling are important components of a molecular oscillator, which, in conjunction with the *Wnt3a* protein and an *Fgf8* RNA gradient, determines the position of the segment boundaries (Aulehla *et al*, 2003; Dubrulle & Pourquie, 2004). Notch signalling is dispensable for segmentation; however, it seems to be essential for the separation of intermingled psm cells belonging to neighbouring segments and for intrasomitic anterior–posterior patterning (Conlon *et al*, 1995; Hrabe de Angelis *et al*, 1997; Sato *et al*, 2002). Recently, we have shown that the expression of Delta-like 1 (*Dll1*), a crucial activator of Notch gene homologue 1 (*Notch1*) in the psm, is synergistically controlled by WNT signalling and *Tbx6* (Galceran *et al*, 2004; Hofmann *et al*, 2004). In addition, Notch signalling in the oscillator is dependent on *Wnt3a* (Aulehla *et al*, 2003; Galceran *et al*, 2004; Hofmann *et al*, 2004).

Therefore, psm formation, maintenance and segmentation are controlled by a hierarchy of factors including *Wnt3a*, *Fgfr1*, *T* and *Tbx6*. *Wnt3a* and *Fgfr1* control *T* expression, whereas *T* regulates *Tbx6*. Furthermore, *Tbx6* and WNT signalling synergistically control *Dll1*, the ligand of *Notch1*, which is essential for somite formation (Hrabe de Angelis *et al*, 1997; Hofmann *et al*, 2004).

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Psm maturation requires the function of mesogenin 1 (*Msgn1*), a basic helix–loop–helix transcription factor specifically expressed in the psm. Mouse embryos lacking *Msgn1* show severely reduced psm and the absence of somites in the trunk (Yoon & Wold, 2000). The transcriptional control of this important factor has not been investigated. Here, we show that *Msgn1* is a direct target of WNT signalling and *Tbx6*, placing *Msgn1* downstream of these factors in the hierarchy of regulators controlling paraxial mesoderm development in the mouse.

RESULTS AND DISCUSSION

Phenotypic analysis of *Msgn1* mutant embryos suggested a role for this gene in the specification and maturation of the paraxial mesoderm downstream of *Wnt3a* (Yoon & Wold, 2000; Aulehla et al, 2003). To analyse whether *Msgn1* expression in the psm depends on *Wnt3a* function, we used the *Wnt3a* hypomorphic allele vestigial tail (*vt*). Homozygous *vt* embryos are viable but show failure of axial elongation in the tail region, resulting in a vestigial (short) tail phenotype. Psm formation in these mutants is arrested at embryonic day (E) 10.5 in conjunction with an overgrowth of neural tissue (Greco et al, 1996). The levels of *Wnt3a* messenger RNA are reduced in the tailbud of such embryos at E9.5–E11.5 (Greco et al, 1996). Our expression analysis at E10–E11 (Theiler stage (TS)16–TS18), by *in situ* hybridization, showed that *Msgn1* also is downregulated in these mutants (Fig 1A–L). At E11, residual *Msgn1* expression is detected only in a subset of mesodermal cells on the ventral side (Fig 1L), strongly suggesting that *Msgn1* is downstream of *Wnt3a*. *Wnt3a* directly controls *T*, which is essential for early mesoderm formation and migration (Wilson & Beddington, 1997; Yamaguchi et al, 1999b; Arnold et al, 2000). Therefore, we analysed *Msgn1* expression in *T/T* embryos at the four- to six-somite stage, just before the arrest of axial elongation in this mutant. *Msgn1* expression was completely absent in *T/T* embryos (Fig 1S,T), suggesting that *Msgn1* expression is controlled by *T*. As *T* acts upstream of *Tbx6* (Hofmann et al, 2004), *Tbx6* expression should also depend on *Wnt3a*. This was confirmed by the significant downregulation of *Tbx6* in *vt/vt* embryos (Fig 1M–R). To relate *Msgn1* to *Tbx6*, we analysed *Msgn1* expression in embryos lacking *Tbx6* function, using the *Tbx6*-knockout allele described previously (Chapman & Papaioannou, 1998). *Msgn1* expression is strongly downregulated in *Tbx6*-null mutant embryos, indicating that *Tbx6* acts upstream of *Msgn1* (Fig 1U,V). Residual expression of *Msgn1* in the caudal end of mutant embryos might be due to remaining *Wnt3a* and *T* activity in this region. In summary, these data place *Msgn1* downstream of *Wnt3a*, *T* and *Tbx6* in the regulatory network controlling psm formation and maturation.

Analysis of mutants allowed us to place *Msgn1* in the functional hierarchy, but it did not distinguish between direct and indirect control. Therefore, a detailed analysis of the *Msgn1* promoter was initiated (Fig 2). First, we mapped the transcriptional start site of *Msgn1* 55 bp upstream of the translation start, using rapid amplification of 5' complementary DNA ends (5'-RACE; data not shown). This allowed us to define the promoter region of *Msgn1* for which only a single exon is known (ENSEMBL database; Yoon & Wold, 2000). Next, we cloned a genomic fragment covering 7 kb upstream of the translation start and tested its activity in regulating the expression of the lacZ reporter in transgenic embryos. The reporter construct produced strong

Fig 1 | Expression analysis of mutant embryos places mesogenin 1 downstream of *Wnt3a*, *T* and *Tbx6*. RNA transcription was detected by *in situ* hybridization of wild-type (wt) (A–C,G–I,M–O,S), *vt/vt* (D–F, J–L, P–R), *T*^{−/−} (T), *Tbx6*^{+/−} (U) and *Tbx6*^{−/−} (V) embryos. Gene probes are indicated on the left; the position of the sections shown in (C,F,I,L,O,R) are indicated on the image of the corresponding embryo. Staging is according to Theiler (1989); TS16 corresponds to embryonic day (E) 10 and TS18 to E11. *Msgn1*, mesogenin 1; np, neural plate; nt, neural tube; nt*, ectopic neural tissue; psm, presomitic mesoderm; *T*, brachyury; *Tbx6*, T-box 6 transcription factor; *vt*, vestigial tail; *Wnt3a*, wingless-related MMTV integration site 3A gene.

β-galactosidase activity in the paraxial mesoderm and weaker staining in the lateral plate mesoderm (Fig 2B, b,f,j,n). A search of the −7 kb promoter for putative binding sites for lymphoid enhancer binding factor/transcription factor (*Lef/Tcf*) regulators, the effectors of WNT signalling and for T-box transcription factors using consensus sequences, identified seven putative binding sites for each type of factor. These binding sites are located in three clusters, two of which are conserved between multiple mammalian species, and partly between mouse and chick (Fig 2A; data not shown; see the supplementary information online). Most of the

Fig 2 | A 1.2-kb mesogenin 1 promoter fragment is sufficient to drive reporter expression in the presomitic mesoderm. (A) Schematic representation of the reporter constructs analysed in the embryos shown in (B); triangles indicate consensus *Leif/Tcf*-binding sites and circles indicate T-box-binding sites. The positions of the consensus sites relative to the transcription start site are given below. Asterisks indicate *Leif/Tcf*-binding sites that are less stringent to the consensus but were integrated in the analysis. (B) Reporter gene (β -galactosidase, LacZ whole-mount *in situ* hybridization (WISH)) assays of both a 7-kb (b,f,j,n) and a 1.2-kb promoter fragment (c,g,k,o) driving lacZ expression in transgenic embryos show strong β -galactosidase activity in the psm and significant activity in lpm, whereas lacZ transcription is restricted to paraxial mesoderm (d,h,l,p). *Msgn1* transcripts are detected only in the psm (a,e,i,m). Hg, hindgut; *Leif/Tcf*, lymphoid enhancer binding factor/transcription factor; lpm, lateral plate mesoderm; *Msgn1*, mesogenin 1; np, neural plate; nt, neural tube; psm, presomitic mesoderm; wt, wild type.

putative binding sites are located in the cluster extending 1.2 kb upstream of the transcription start. We tested this fragment for promoter activity in transgenic embryos, which showed the same pattern of β -galactosidase activity as transgenic mice carrying the -7 kb promoter, including the ectopic activity in the lateral plate mesoderm (Fig 2B,c,g,k,o). By contrast, transcription of the reporter construct was restricted to the psm and not detected in the

Fig 3 | The mesogenin 1 promoter is synergistically activated by *Lef1* and *Tbx6* *in vitro*. (A) Schematic representation of the wild-type (wt) and mutant (mut) reporter constructs used for *in vitro* (B) and *in vivo* (see Fig 4) analysis; mutated sites are indicated by crosses. (B) *In vitro* transactivation assays: transfection of plasmids expressing *Tbx6*-VP16, *T*-VP16 or *Lef-bcat* fusion proteins induce only moderate activation of the wt -1.2-kb *Msgn1* promoter, whereas a combination of *Tbx6*-VP16 and *Lef-bcat* caused synergistic activation of the reporter, which is prevented by point mutations in either the T-box-binding site (1.2 kb-mut-T) or (three out of four) *Leif/Tcf*-binding sites (1.2 kb-mut L1,3,4). bcat, β -catenin; *Lef*, lymphoid enhancer binding factor; *Msgn1*, mesogenin 1; T, brachyury; *Tbx6*, T-box 6 transcription factor; *Tcf*, transcription factor.

lateral mesoderm, as shown by whole-mount *in situ* hybridization (WISH) analysis using lacZ antisense RNA as a probe (Fig 2B,d,h,l,p). These data suggest that the -1.2 kb promoter of *Msgn1* is transiently active in lateral mesoderm progenitor cells in the tailbud, and that the β -galactosidase activity observed in the differentiating lateral mesoderm is due to the high stability of the enzyme. Unlike the wild-type gene, however, reporter gene transcripts were also detected in anterior segmenting psm and in a few somites (Fig 2B,a,e,i,d,h,l). This might be due to either high transcript stability or a lack of timely downregulation of the reporter in psm cells undergoing segmentation. In summary, the -1.2 kb *Msgn1* promoter comprises the regulatory elements driving *Msgn1* expression in the psm.

The presence of putative *Leif/Tcf*- and T-box-binding sites in the *Msgn1* promoter supported the assumption, based on mutant analysis, that *Msgn1* might be a direct target of WNT signalling and/or the T-box transcription factors *T* and/or *Tbx6*. To test this assumption, we examined the effect of all three factors on the activity of the *Msgn1* promoter *in vitro* (Fig 3). A construct

expressing a constitutively active *Lef*- β -catenin fusion protein (*Lef-bcat*; J. Huelsken, unpublished data; see the supplementary information online) was used to assay for WNT activity on the promoter. For *T* and *Tbx6*, fusion proteins with the strong transactivation domain of VP16 were used, as the wild-type factors showed only low transactivation activity in the heterologous human embryonic kidney (HEK)293 T cells. Neither factor alone produced strong luciferase reporter activity, although *T* was the most effective; however, a combination of *Tbx6* and *Lef-bcat* increased the promoter activity more than fivefold above the activity of either single factor. This synergistic activation of the; *Msgn1* promoter was specific for *Lef-bcat* and *Tbx6*, as *T* failed to produce this effect (Fig 3B). The strong upregulation of the *Msgn1* promoter by *Lef-bcat* and *Tbx6* was prevented by point mutations in the binding sites of either factor, showing that *Lef/Tcf*- and T-box-binding sites are essential for strong upregulation of *Msgn1* expression *in vitro* (Fig 3B). Furthermore, these results strongly suggested that *Msgn1* is controlled by synergistic activity of WNT signalling and *Tbx6* *in vivo*.

To test the role of these factors in *Msgn1* expression in the psm, we examined the activity of mutant reporter constructs in mouse embryos. Point mutations in three of the four consensus *Lef/Tcf* sites located in the -1.2 kb promoter resulted in a strong reduction of the reporter activity in the psm (Fig 4A,a,e,i), leaving residual β -galactosidase activity in the psm and lateral plate mesoderm. Mutation in all four *Lef/Tcf* sites reduced the reporter activity further in both the psm and the lateral mesoderm (Fig 4A,b,f,j). Mutations in the four consensus T-box sites also strongly affected the reporter activity throughout the paraxial mesoderm (Fig 4A,c,g,k). Residual expression was observed in scattered cells towards the anterior psm. Not even residual reporter activity was detected in the psm with the construct in which both the *Lef/Tcf* and the T-box sites were mutated (Fig 4A,d,h,l). In summary, the reporter assays *in vivo* show the essential role of WNT signalling and T-box transcription factors in controlling *Msgn1* expression in the psm. These data also support the finding of a synergistic control of *Msgn1* by these factors *in vivo*, as mutations in the binding sites of either factor alone result in strong downregulation of the reporter activity.

In contrast to the constructs containing mutations in the *Lef/Tcf*-binding sites, the construct carrying mutations in the consensus T-box-binding sites still showed considerable β -galactosidase activity in the lateral plate mesoderm, similar to the wild-type promoter. These data suggest that the transient activation of the reporter in the lateral mesoderm is due to WNT signalling, which is expressed throughout the tailbud.

The reporter assays *in vivo*, in conjunction with the mutant analyses, strongly suggest that synergistic activity of WNT signalling and *Tbx6* control *Msgn1* expression in the psm. To test for direct binding of *Tbx6* protein to the *Msgn1* promoter *in vivo*, we carried out chromatin immunoprecipitation (ChIP) experiments with chromatin isolated from the caudal region of E9–E9.5 mouse embryos. A genomic fragment containing two of the four T-box-binding sites in the -1.2 kb *Msgn1* promoter was found to be strongly overrepresented in amplified DNA from ChIP samples obtained with an antibody against *Tbx6*, in comparison with a control amplicon located 6.7 kb downstream (Fig 4B). The data show direct binding of *Tbx6* to the predicted region of the *Msgn1* promoter *in vivo*.

Fig 4 | WNT signalling and *Tbx6* synergistically control mesogenin 1 in the presomitic mesoderm. (A) *In vivo* expression analysis of reporter constructs controlled by the -1.2 -kb *Msgn1* promoter containing point mutations in *Lef/Tcf*- and/or T-box consensus binding sites. Expression of all mutant constructs in the psm is downregulated. The β -galactosidase activity in the lateral plate mesoderm is significantly reduced compared with the wild-type construct (see Fig 2), except for the 1.2-kb-mut-T-lacZ reporter (c,g,k). Mutation of all four *Lef/Tcf*-binding sites nearly abolished β -galactosidase activity in the psm and lateral mesoderm (b,f,j). Expression in the brain is due to positional effects (a). Dashed lines in (e–h) indicate the level of the sections depicted in (i–l). (B) Nuclear material derived from the caudal end of embryonic day (E) 9–E9.5 mouse embryos (caudal to the dashed line) was subjected to chromatin immunoprecipitation (ChIP) assays using *Tbx6* or control (IgG) antibodies. The *Tbx6* amplicon was specifically enriched with respect to a control amplicon located 6.7 kb downstream of it, strongly suggesting specific binding of *Tbx6* protein to the -1.2 kb *Msgn1* promoter. Ce, caudal end; da, dorsal aorta; hg, hindgut; *Lef/Tcf*, lymphoid enhancer binding factor/transcription factor; lpm, lateral plate mesoderm; *Msgn1*, mesogenin 1; np, neural plate; nt, neural tube; psm, presomitic mesoderm; *Tbx6*, T-box 6 transcription factor; WNT, wingless-type MMTV integration site family.

Lef1 and *Tcf7* (previously named *Tcf1*) have previously been shown to be required for psm formation. *Lef1* is a direct target of WNT signalling and shows strong expression in the psm (Galceran

Fig 5 | Functional hierarchy of factors controlling paraxial mesoderm formation, maturation and segmentation, emphasizing the role of canonical WNT signalling—most probably induced by *Wnt3a*—as the crucial control factor of these processes. Solid arrows indicate known direct control and dashed arrows, dependence, based on mutant analysis; for details see text. *Dll1*, Delta-like 1; *Msgn1*, mesogenin 1; psm, presomitic mesoderm; *T*, brachyury; *Tbx6*, T-box 6 transcription factor; *Wnt3a*, wingless-related MMTV integration site 3A gene.

et al, 1999, 2004). Combined with our analysis, this suggests that *Lef1* and/or *Tcf7* might directly bind to the *Msgn1* promoter and control *Msgn1* expression in the psm.

The detailed analysis of the control of *Msgn1* expression in the psm described here considerably improves our understanding of the regulatory network controlling presomitic mesoderm formation, maturation and segmentation (Fig 5). *Msgn1* acts downstream of *Wnt3a*, the only known WNT signal expressed in the tailbud that acts through the canonical pathway. This strongly suggests a direct control of *Wnt3a* on the activity of *Msgn1*. The effect of WNT signalling is transient in prospective lateral mesoderm cells, but stabilized in the psm by a double feed-forward mechanism involving the *Wnt3a* target *T* and the psm-specific control factor *Tbx6*, acting downstream of *T*. The specification, maturation and segmentation of psm seems to be controlled by the synergism between *Wnt3a*, acting through the effectors *Lef1* and *Tcf7*, and *Tbx6*, regulating target genes involved in these processes such as *Msgn1* and *Dll1* (Galceran *et al*, 2004; Hofmann *et al*, 2004).

The expression of *Notch1* in the psm is dependent on *Msgn1* (Yoon & Wold, 2000), and thus indirectly dependent on *Wnt3a*. Furthermore, Notch signalling in the segmentation clock—an important component of the segmentation mechanism—also indirectly depends on *Wnt3a*, as shown by downregulation of the *Notch1* target *Lunatic fringe* in *vt/vt* mutant embryos (Aulehla *et al*, 2003). Therefore, *Wnt3a* apparently functions as a crucial control factor for psm formation, maturation and segmentation.

Wnt5a, which signals through the non-canonical WNT pathway, is also expressed in the tailbud and is essential for axial development (Yamaguchi *et al*, 1999a) future analyses have to address the role of this factor in more detail.

METHODS

Expression and reporter constructs. A 7-kb genomic region upstream of the *Msgn1* translational start site was obtained by

genomic PCR and cloned into a reporter vector containing the lacZ gene and a simian virus 40 polyadenylation sequence. For luciferase reporter constructs, the promoter fragments were inserted into the pGL3-Basic Vector (Promega, Madison, WI, USA). *Lef1/Tcf* and *T/Tbx6*-binding sites were modified using the QuikChange Multi Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA). The transcriptional start site of *Msgn1* was determined by 5'-RACE PCR (Invitrogen, Carlsbad, CA, USA).

DNA microinjection and staining for β -galactosidase activity.

LacZ reporter constructs were analysed in transient transgenic embryos produced by pronuclear injection of fertilized mouse eggs, followed by β -galactosidase activity assays as described previously (Beckers *et al*, 2000). The numbers of embryos analysed at E9.5–E10.5 for lacZ transcription or β -galactosidase activity are given in the supplementary information online.

In situ hybridization and histology. WISH analyses and the preparation of histological sections were carried out using standard procedures.

Cell culture and reporter gene assays. Reporter gene assays *in vitro* were carried out in HEK293 T cells according to standard procedures (for details, see the supplementary information online).

Chromatin immunoprecipitation. ChIP assays were carried out using tailbud tissue derived from 80 to 100 dissected E9–E9.5 mouse embryos essentially according to the Abcam (Cambridge, UK) ChIP protocol (Abcam), with minor modifications (for details, see the supplementary information online).

Supplementary information is available at *EMBO reports* online (<http://www.emboports.org>).

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REFERENCES

- Arnold SJ, Stappert J, Bauer A, Kispert A, Herrmann BG, Kemler R (2000) Brachyury is a target gene of the Wnt- β -catenin signaling pathway. *Mech Dev* **91**: 249–258
- Aulehla A, Herrmann BG (2004) Segmentation in vertebrates: clock and gradient finally joined. *Genes Dev* **18**: 2060–2067
- Aulehla A, Wehrle C, Brand-Saberi B, Kemler R, Gossler A, Kanzler B, Herrmann BG (2003) Wnt3a plays a major role in the segmentation clock controlling somitogenesis. *Dev Cell* **4**: 395–406
- Beckers J, Caron A, Hrabe de Angelis M, Hans S, Campos-Ortega JA, Gossler A (2000) Distinct regulatory elements direct delta1 expression in the nervous system and paraxial mesoderm of transgenic mice. *Mech Dev* **95**: 23–34
- Chapman DL, Papaioannou VE (1998) Three neural tubes in mouse embryos with mutations in the T-box gene *Tbx6*. *Nature* **391**: 695–697
- Ciruna BG, Schwartz L, Harpal K, Yamaguchi TP, Rossant J (1997) Chimeric analysis of fibroblast growth factor receptor-1 (*Fgfr1*) function: a role for FGFR1 in morphogenetic movement through the primitive streak. *Development* **124**: 2829–2841
- Conlon RA, Reaume AG, Rossant J (1995) Notch1 is required for the coordinate segmentation of somites. *Development* **121**: 1533–1545
- Dubrulle J, Pourquie O (2004) *fgf8* mRNA decay establishes a gradient that couples axial elongation to patterning in the vertebrate embryo. *Nature* **427**: 419–422

- Galceran J, Farinas I, Depew MJ, Clevers H, Grosschedl R (1999) Wnt3a^{-/-}-like phenotype and limb deficiency in Lef1^{-/-} Tcf1^{-/-} mice. *Genes Dev* **13**: 709–717
- Galceran J, Sustmann C, Hsu SC, Folberth S, Grosschedl R (2004) LEF1-mediated regulation of Delta-like1 links Wnt and Notch signaling in somitogenesis. *Genes Dev* **18**: 2718–2723
- Gossler A, Tam PPL (2002) Somitogenesis: segmentation of the paraxial mesoderm and the delineation of tissue compartments. In *Mouse Development*. Rossant J, Tam PPL (eds) pp 127–149. San Diego, CA: Academic Press
- Greco TL, Takada S, Newhouse MM, McMahon JA, McMahon AP, Camper SA (1996) Analysis of the vestigial tail mutation demonstrates that Wnt-3a gene dosage regulates mouse axial development. *Genes Dev* **10**: 313–324
- Herrmann BG (1995) The mouse Brachyury (T) gene. *Semin Dev Biol* **6**: 385–394
- Hofmann M, Schuster-Gossler K, Watabe-Rudolph M, Aulehla A, Herrmann BG, Gossler A (2004) WNT signaling, in synergy with T/TBX6, controls Notch signaling by regulating Dll1 expression in the presomitic mesoderm of mouse embryos. *Genes Dev* **18**: 2712–2717
- Hrabe de Angelis M, McIntyre 2nd J, Gossler A (1997) Maintenance of somite borders in mice requires the Delta homologue Dll1. *Nature* **386**: 717–721
- Sato Y, Yasuda K, Takahashi Y (2002) Morphological boundary forms by a novel inductive event mediated by Lunatic fringe and Notch during somitic segmentation. *Development* **129**: 3633–3644
- Theiler K (1989) *The House Mouse: Atlas of Embryonic Development*. Berlin, Heidelberg, New York: Springer
- Wilkinson DG, Bhatt S, Herrmann BG (1990) Expression pattern of the mouse *T* gene and its role in mesoderm formation. *Nature* **343**: 657–659
- Wilson V, Beddington R (1997) Expression of T protein in the primitive streak is necessary and sufficient for posterior mesoderm movement and somite differentiation. *Dev Biol* **192**: 45–58
- Yamaguchi TP, Bradley A, McMahon AP, Jones S (1999a) A *Wnt5a* pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development* **126**: 1211–1223
- Yamaguchi TP, Takada S, Yoshikawa Y, Wu N, McMahon AP (1999b) *T* (Brachyury) is a direct target of *Wnt3a* during paraxial mesoderm specification. *Genes Dev* **13**: 3185–3190
- Yoon JK, Wold B (2000) The bHLH regulator pMesogenin1 is required for maturation and segmentation of paraxial mesoderm. *Genes Dev* **14**: 3204–3214
- Yoshikawa Y, Fujimori T, McMahon AP, Takada S (1997) Evidence that absence of *Wnt-3a* signaling promotes neuralization instead of paraxial mesoderm development in the mouse. *Dev Biol* **183**: 234–242