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Short Report

The gene *schmalspur* functions in mesoderm formation in zebrafish, and interacts with *notail* and *spadetail*

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ABSTRACT The gene *schmalspur* is involved in the Nodal signalling pathway to maintain the expression of *nodal* genes during zebrafish development. Mutants for nodal-related genes show a partial loss of axial mesoderm, which is also defective in embryos lacking maternal and zigotic expression of *schmalspur*. We have generated double mutants of *schmalspur* with other genes responsible for mesoderm formation and have analyzed the phenotype and the genetic interactions by whole-mount *in situ* hybridization. In addition, cellular transplant experiments were carried out to determine how *schmalspur* functions in mesoderm formation. Our results show a close genetic interaction of *schmalspur* with the *T-box* genes *notail* and *spadetail,* since the axial and paraxial mesoderm of these double mutants were strongly affected, and a non-cell autonomous function for *schmalspur* in mesoderm formation.

Nodal-related proteins are required for the formation of the gastrula organizer, mesoderm induction and specification of the left-right axis (Feldman *et al.,* 1998; Gritsman *et al.,* 2000; Schrier and Shen, 2000). In zebrafish, two *nodal*-related genes have been identified: *squint (sqt)* and *cyclops (cyc).* Homozygous mutants for either *cyc* or *sqt* show only partial loss of axial mesoderm and ventral neuroectoderm because of the overlapping expression and similar activities of both genes, whereas *cyc/sqt* double mutants lack most mesendodermal tissues (Feldman *et al.,* 1998). Embryos lacking maternal and zygotic expression of the gene *one-eyed pinhead (oep)* develop phenotypes very similar to *nodal* mutants. The gene *oep* encodes for an extracellular membrane-associated EGF-CFC protein which is an essential mediator of Nodal signals (Gritsman *et al.,* 1999). It has been recently demonstrated that the gene *schmalspur (sur)* encodes an orthologue of FoxH1, which is expressed maternally and zigotically (Pogoda *et al.,* 2000). FoxH1 is a conserved component of the Nodal signalling pathway, not strictly required to transmit inductive Nodal signals, but necessary for maintained expression of *nodal* genes. The gen *sur* functions in early dorsal specification and organizer formation, and maternal and zygotic mutants for *sur* show some defects in midline and anterior neuroectoderm formation. The purpose of this study is to analyze the genetic interaction of *sur* with other genes responsible for mesoderm formation. Thus, we generated double mutants and focused on those showing a stronger phenotype: *sur/notail (ntl)* and *sur/spadetail (spt)* (Fig. 1*).* The T-box gen *ntl* functions in axial mesoderm formation (Halpern *et al.,* 1993, 1997; Talbot *et al.,* 1995) whereas the T-box gen *spt* does in paraxial mesoderm (Amacher and Kimmel, 1998). All experiments were carried out on 90% epiboly, 5 to 17 somites and 24 h.-stage zebrafish embryos. For whole-mount *in situ* hybridization we used the following riboprobes as markers: *axial, twist, ntl* and *sonic hedgehog (shh)* for midline structures and *papC, myoD, pax2* and *gata 1* for mesodermic precursors or derivatives. In addition, we generated genetic mosaics by cellular transplants in order to demonstrate the possible non-cell autonomous function of *sur* in mesoderm formation. We used wild type (*wt*), maternal and zigotic (*mz*) *sur* and *sur/ntl* embryos as donors of biotin-labelled cells, which were tranferred to *wt, mzsur* and *sur/ntl* blastula-stage host embryos. The host embryos were fixed at the 90% epiboly stage and stained with *papC* by *in situ* hybridization to identify presumptive mesodermic cells. Labelled cells from donor embryos were revealed by using the extravidin-horseradish peroxidase reaction and staining with DAB as the chromogen. The double mutants *sur/ntl* showed a total lack of trunk formation (Fig. 1). Expression of both midline markers such as *twist, axial*, *ntl* and *shh*, and mesodermal markers, was no present in *sur/ntl* mutants since early stages of development (Fig.

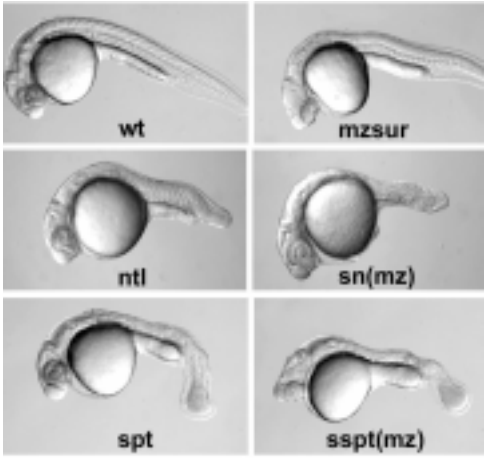

Fig. 1. 24 hour-stage zebrafish embryos. *Abbreviations indicate the type of embryo: wt (wild type); ntl (mutant for notail gene); spt (mutant for spadetail gene); mzsur (mutant for maternal and zigotic schmalspur); sn(mz) (double mutant for ntl and maternal and zigotic smalschpur); sspt(mz) (double mutant for spadetailt and maternal and zigotic schmalspur). Mutants for mzsur lack the floorplate and show a weavy notochord and closer or fused eyes. Note the total lack of trunk in sn(mz) double mutants, whereas the head and the eyes seem to be normal. In sspt(mz) double mutants, there is an absence of somite formation, the notochord is strongly reduced and the eyes are fused. The defect in the tail is maintained as it occurred in spt single mutants.*

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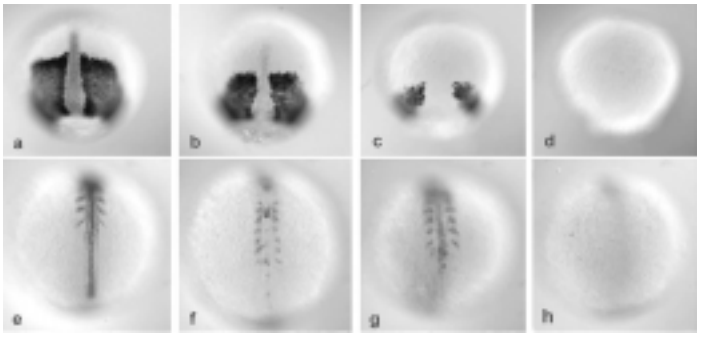

Fig. 2. (a,b,c,d) Expression patterns of *ntl* and *papC* genes in a dorsal view of whole mounts in 90% epiboly-stage embyos. (a) *wt;* **(b)** *mzsur;* **(c)** *ntl mutant;* **(d)** *sur/ntl double mutant. (e,f,g,h) Dorsal views of 10 somite-stage embryos after double staining with shh and myoD.* **(e)** *wt;* **(f)** *mzsur;* **(g)** *ntl;* **(h)** *sur/ntl double mutants. See text for explanations.*

2) except for *gata 1*, specific for intermediate mesoderm, which showed an abnormal staining pattern. The expression of *ntl* in the notochord splits caudally in *mzsur*, whereas is not present in *ntl* and *sur/ntl* double mutants. The formation of mesoderm is slightly delayed in *ntl* and *sur/ntl* double mutants during gastrulation, when comparing with *wt* embryos (Fig. 2 a,b,c). The gene *myoD* stains not only somites but also adaxial cells, which are not present in *ntl* embryos and strongly reduced in *mzsur* embryos. The double staining with *shh* allow us to dintinguish between both type of embryos, giving rise to a different staining in the midline (Fig. 2 f,g). The gene *shh* is expressed by cells of the notochord and the floorplate. In *ntl* embryos the floorplate is expanded whereas it is almost absent in *mzsur* embryos.The cellular transplant experiments revealed that *wt*-donor cells were able of forming mesoderm in the double mutants *sur/ntl*, and also recruited mutant cells to form it (Fig. 3). When mutant cells from *mzsur* and *sur/ntl* embryos were tranferred to a *wt* environment, they could contribute to mesoderm formation (Fig. 3). Regarding the double mutants *sur/spt*, the characteristic of the phenotype was the absence of somites (Fig. 1), but in addition, the formation of midline structures such as notochord and prechordal plate was severely affected (Fig. 4). As mentioned before, the staining with *ntl* in the midline splits caudally in *mzsur* mutants (Fig. 4b) whereas it is broader than normal in *ntl* mutants because of the lack of adaxial cells (Fig. 4c). In the double mutants *sur/spt* few cells are stained during gastrulation, no in the midline but in a lateral notochord domain, and a reduction of the cells in the margin can be appreciated (Fig. 4d). During gastrulation *sur/spt* double mutants lack expression of *papC* and during somitogenesis there is not staining with *myoD* at all (Fig. 4h). In *spt* mutants some staining is retained (Fig. 4g). In *mzsur* mutants adaxial cells are strongly reduced, and a few somites occupy the midline in those locations where the notochord is missing (Fig. 4f). In parallel to *sur/ntl* embryos, *sur/spt* double mutants showed no staining with the marker *gata 1*, and an irregular staining pattern with *pax2*. In conclussion, our results indicate a close genetic interaction of *sur* with the T-box genes *ntl* and *spt* regarding mesoderm formation during gastrulation and segmentation in zebrafish embryos, and point out for a non-cell autonomous function of *sur* in mesoderm formation, since mutant cells can contribute to form mesoderm in a *wt* environment.

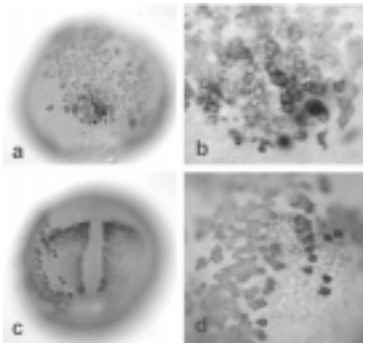

Fig. 3. Dorsal views of 90% epiboly-stage embryos after cell transplantation experiments. (a,b) *wt cells transplanted into sur/ntl mutant embryos. Note the group of cells double-stained with biotin and papC.* **(c,d)** *Biotin-labelled cells from sur/ntl embryos were transferred to wt embryos, and some of them, located near the midline, stained with the mesoderm marker pap C.*

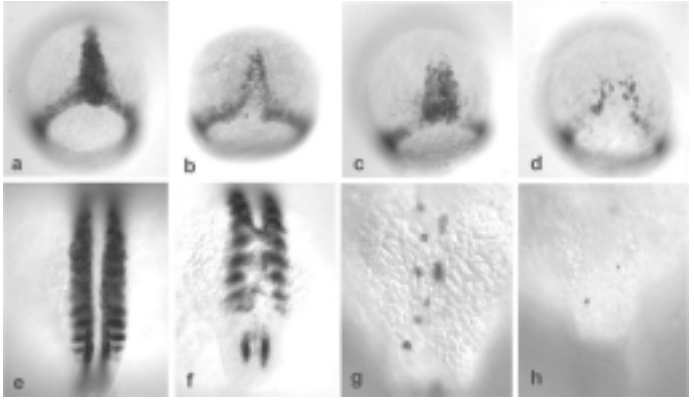

Fig. 4. (a,b,c,d) Expression patterns of the *ntl* gene in a dorsal view of whole mounts in 90% epiboly-stage embyos. (a) *wt;* **(b)** *mzsur;* **(c)** *spt mutant;* **(d)** *sur/spt double mutant. e,f,g,h. Dorsal views of 17 somite-stage embryos after staining with myoD.* **(e)** *wt;* **(f)** *mzsur;* **(g)** *spt;* **(h)** *sur/spt double mutants. See text for details.*

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