

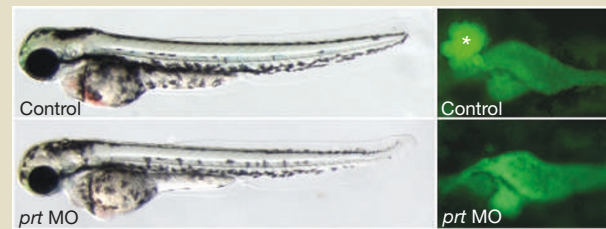
Mesodermal Wnt expression promotes liver specification

Endodermal organs, such as liver and lung, arise at specific locations along the primitive gut tube. Many of the signalling pathways that specify endodermal organs have been determined, but no single gene that exclusively regulates liver specification has been identified. A recent study by Ober *et al.* (Nature, doi: 10.1038/nature04888; 2006) identified zebrafish mutants defective for early liver specification and suggests that mesodermal Wnt expression mediates this specification.

Ober *et al.* performed a genetic screen in a transgenic zebrafish line that expresses GFP in the developing endoderm. This identified a recessive mutant, *prometheus* (*prt*), in which the liver was absent or strongly reduced at 50 hours post fertilization (h.p.f.). By analysing liver markers, they observed that the defect in liver formation occurred very early in liver specification. The mutants had no other morphological phenotype, suggesting that *prt* is important for early liver development.

The group went on to determine where *prt* expression was required using mosaic analysis experiments. When wild-type cells were reintroduced into homozygous mutant embryos the *prt* mutant liver defect was rescued only if the wild-type cells were present in the lateral plate mesoderm (LPM) or the LPM and endoderm, but not in the endoderm alone. This indicates that the *prt* gene acts non-cell autonomously in liver development and is required in the mesoderm adjacent to where the liver, an endodermal organ, arises. This is the first *in vivo* demonstration that a signal from the mesoderm specifies liver fate and indicates that *prt* is required to mediate crosstalk between the LPM and the endoderm during liver formation.

Ober *et al.* then isolated the gene disrupted by the *prt* mutation and identified a *wnt2b* gene that they name *wnt2bb*. All three *prt* mutants that were identified had point mutations in this gene. Using



Knockdown of *wnt2bb* by *prt* MO led to a very strong reduction or complete absence of liver (asterisk in control), whereas the rest of the embryo seemed unaffected at 54 h.p.f.

morpholino oligonucleotide (MO) analysis they confirmed that *wnt2bb* is the gene affected in the *prt* mutants (see figure). The specific expression of *wnt2bb* was investigated using *cloche* mutants (lacking endothelial cells) and *casanova* mutants (lacking all endodermal cells). Analysis of expression in these mutants determined that *wnt2bb* is expressed in the pre-hepatic region of the LPM and that this expression does not depend on signals from the endoderm. Expression in this location is also consistent with the inductive properties of Wnt signalling.

The canonical Wnt signalling pathway, which acts through β -catenin, is known to specify endodermal organs, so the group investigated whether this pathway affects liver specifically. By inhibiting this pathway at various times, using a heat-shock inducible construct, they found that the critical time when β -catenin signalling was required is between 16 and 21 h.p.f., implicating this pathway in early liver development.

Taken together, these results indicate that the spatiotemporal expression of *wnt2bb* is consistent with its function in early liver specification. The work of Ober *et al.*, and other recent studies, indicates that crosstalk between mesoderm and endoderm may contribute to the establishment of endodermal organs along the gut tube and that *Wnt* genes are key determinants for this induction.

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