The division investigates how embryonal cell fate is determined during early vertebrate development. The frog embryo (Xenopus) is used as embryonic system, allowing a combination of classical transplantation and explantation experiments with modern molecular biology. Gene function is studied in the context of a developing organism by microinjection of mRNAs and gene expression in primordial tissues is followed by nucleic acid hybridisation. The aim is a molecular understanding of the mechanisms underlying progressive cell differentiation of embryonal mesoderm, which is studied in the department as a paradigm, by identifying developmental control genes. Such control genes are typically highly conserved in evolution and regulate growth and differentiation in normal and neoplastic cells in all metazoans including man.

will continue this screen with the primary aim of identifying novel members synexpression groups, which may reveal entire molecular pathways. Further, we will analyze the regulation of genes expression in synexpression groups.

3. Characterizing Components of the BMP Signalling Pathway

R. Dosch, E. Karaulanov, D. Onichtchouk, M. Reichelt

The BMP pathway plays an important role in antagonizing the Spemann organizer [9]. We have cloned a transmembrane protein, BAMBI, which is related to TGF-β family type I receptors but lacks an intracellular kinase domain [6]. BAMBI is coexpressed with the ventralizing morphogen BMP4 during Xenopus embryogenesis and it requires BMP signalling for its expression. In collaboration with Joan Massagué (Sloan Kettering, New York) it was shown that the protein stably associates with TGF-β family receptors and functions as an inhibitor of BMP, activin as well as TGF-β signalling. BAMBI’s inhibitory effects are mediated by its intracellular domain which resembles a type I receptor homodimerization interface and prevents receptor complex formation. Thus, BAMBI negatively regulates TGF-β family signalling by a novel regulatory mechanism involving interaction of signalling receptors with a pseudoreceptor.

Another BMP studied is Anti-Dorsalizing Morphogenetic Protein (ADMP) because it is paradoxically expressed in the trunk organizer but acts as a potent organizer antagonist, as shown previously. We found that ADMP represses head formation during gastrulation and that its expression is activated by BMP antagonists (Fig.1). A specifically acting dominant-negative ADMP anteriorizes embryos and its coexpression with BMP antagonists induces secondary embryonic axes with heads as well as expression of head inducers. Unlike other BMPs, ADMP is not inhibited by a dominant-negative BMP type I receptor, Noggin, Cerberus and Chordin but by Follistatin, suggesting that it utilizes a distinct TGF-β receptor pathway and displays differential sensitivity to BMP antagonists. This indicates that ADMP functions in the trunk organizer to antagonize head formation, thereby regulating organizer patterning [17].

Two other extracellularly acting proteins that have been implicated in antagonizing BMP signalling are one eyed pinhead (oep) and PACAP peptide. Oep has been identified previously in zebrafish and is an essential cofactor in signalling by the TGF-β growth factor Nodal. Our experiments revealed that in addition oep antagonizes BMP signalling, because is neuralizes animal caps in Xenopus and dorsalizes ventral mesoderm. Furthermore, in zebrafish, misexpression of Smad1 in oep mutant embryos also reveals an interaction of oep with BMP signalling [13]. In collaboration with Günther Schütz we have analyzed the function of a highly conserved neuropeptide PACAP (pituitary adenylate cyclase activating polypeptide) which has been implicated in a broad variety of physiological processes. The PACAP precursor protein gives rise to three different peptides, the cryptic peptide, GHRH, and PACAP.

Figure 1: Expression of anti dorsalizing morphogenetic protein (ADMP). Lateral view of an in situ hybridisation of a Xenopus neurula showing the expression of ADMP in the chordamesoderm. ADMP prevents formation of the head organizer in chordamesoderm.
respectively. PACAP and GHRH but not the cryptic peptide directly neutralize animal caps. In contrast to GHRH, the neutralizing effect mediated by PACAP is independent of the PKA pathway. Moreover, PACAP but not GHRH behaves like a BMP-4 antagonist. Blastocoel injection of PACAP-38 but not of the closely related peptides PACAP-27 and VIP leads to strong anteriorization of the injected embryos suggesting the possible involvement of a novel PACAP-prefering receptor [12]. Another component of the cAMP signalling pathway, CREB, may be involved in antagonizing the organizer as its overexpression affects gastrulation movements [2].

4. Wnt-Signalling, Wnt Inhibitors and Spemann’s Head Organizer

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In Xenopus embryogenesis, Wnt-signalling is involved in dorso-ventral axial patterning at two stages, before and after midblastula transition (MBT), referred to as early and late Wnt-signalling. The early Wnt signalling pathway is thought to mediate the dorsalizing function of the Nieuwkoop center. Late Wnt-signalling is thought to be involved in the formation of ventro-lateral mesoderm [5].

We suggested a role for late Wnt-signalling in head formation based on the observation that co-ordinate expression of inhibitors of Wnt- as well as BMP-signalling will induce complete secondary axes including a head [5]. This indicates that head organizer activity results from simultaneous repression of BMP- and Wnt- signalling. This suggested a two-inhibitor model for region-specific induction by the Spemann organizer. In this model trunk organizer is generated by antagonizing only BMP signals while head organizer is generated by the coordinate expression of both BMP as well as Wnt inhibitors.

**The dickkopf family**

Based on these findings we have devised an expression screen to isolate Wnt-inhibitors and have identified a novel protein *dickkopf-1 (dkk1)*, member of a new family of secreted proteins [1]. *dkk1* is expressed in the head organizer and mRNA coinjection with BMP inhibitors leads to induction of complete head structures in Xenopus embryos. In contrast, injection of anti *dkk1* antibodies leads to microcephaly. *Dkk1* is thus the first growth factor antagonist of the Spemann organizer required for head formation. *Dkk1* is a potent Wnt antagonist and functions upstream of the first intracellular component of the Wnt pathway, *dishevelled*.

A detailed analysis [14] revealed that *dkk1* unlike other known WNT inhibitors is able to induce functional prechordal plate, which explains its ability to induce secondary heads with bilateral eyes. This may be due to differential WNT inhibition since *dkk1* unlike *frzb* inhibits Wnt3a signalling. Injection of inhibitory anti Dkk1 antibodies reveals that *dkk1* is not only sufficient but also required for prechordal plate- but not for notochord formation. In the neural plate *dkk1* is required for antero-posterior and dorso-ventral patterning between mes- and telenchephalon, where *dkk1* promotes anterior and ventral fates. *Xenopus* embryos posteriorized with bFGF, BMP4 and Smads are rescued by *dkk1*. While the interaction with bFGF is indirect, there is cross-talk between BMP and Wnt signalling until early gastrula stage. Embryos treated with retinoic acid (RA) are not rescued by *dkk1* and RA affects the central nervous system (CNS) more posterior than *dkk1*, suggesting that WNTs and retinoids may act to pattern anterior and posterior CNS, respectively, during gastrulation. We speculate that Dkk either interacts with Wnt-receptors which belong to the frizzled class or that it interacts with Wnt-proteins.

We have analyzed the properties of a related *dkk* family member, *dkk2*. We find that *dkk2* is activating rather than inhibiting the Wnt/β-catenin signalling pathway in Xenopus embryos. *Dkk2* activates both pre- as well as post-midblastula transition Wnt signalling in Xenopus embryos, leading to axis duplication and cyclopia, respectively. *Dkk2* strongly synergizes with *frizzled-2*, *-5 and -8* to induce Wnt signalling responses and its action can be inhibited by *glycogen synthase kinase-3*. Its signalling is specifically antagonized by *dkk1*, but not by other secreted Wnt inhibitors such as secreted frizzled receptor proteins, dominant negative Wnt8 or cerberus. *Dkk2* signals in a cyclohexide-insensitive fashion, indicating a direct activation of the Wnt/β-catenin pathway. This identifies *dkk2* as the first secreted effector able to activate Wnt/β-catenin pathway other than Wnts themselves. The results suggest that a coordinate interplay between inhibiting *dkk1* and activating *dkk2* can modulate frizzled receptor signalling [18].

The later embryonic expression of *dkk1*, *-2* and *-3* was analyzed in mouse. These genes are both temporally and spatially regulated. They define overlapping deep domains in mesenchymal lineages suggesting a coordinated mode of action. All *dkks* show distinct and elevated expression patterns in tissues that mediate epithelial- mesenchyme transformations suggesting that they may participate in heart, tooth, hair and whisker follicle, limb and bone induction. In the limb buds expression of these genes is found in regions of programmed cell death. In a given organ, *dkk1* tends to be the earliest member expressed. Comparison with *Xenopus dkk1* and chicken *dkk3* shows evolutionarily conserved expression patterns. Dkks may hence mediate inductive interactions between epithelial and mesenchymal cells more generally [4]. A human *dkk1* was isolated and its chromosomal localization determined [16].

**XBlimp1 and anterior endoderm formation**

Like *dkk1*, the *XBlimp1* gene is also involved in regulating head organizer function. *XBlimp1* was identified in our *in situ* screen and is a zinc-finger transcriprional repressor coexpressed with *dkk1* in Spemann’s head organizer. *XBlimp1* represses trunk mesoderm and induces anterior endomesoderm in a cooperative manner with the pan-endodermal gene Mix.1. Furthermore, *XBlimp1* can cooperate with the BMP-inhibitor chordin to induce ectopic heads, while a dominant-negative *XBlimp1* inhibits head formation. While *dkk1* does not appear to be regulated by
XBlimp1, the head inducer cerberus is positively regulated by XBlimp1 and cerberus is able to rescue microcephalic embryos caused by dominant-negative XBlimp1. The results indicate that XBlimp1 is required for anterior endomesodermal cell fate and head induction [8, 19].

Publications (* = external co-author)