BRIEFINGS

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A new trick to tune down TGFβ signal

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Signal transduction in early embryogenesis needs to be properly controlled. A new player involved in tuning down TGF β signaling has now been identified – new evidence that multi-layer control of signaling is essential *in vivo*.

How primary germ layers (ectoderm, mesoderm and endoderm) form has been a fascinating question for developmental biologists for decades. Traditional embryo transplantation experiments in amphibian suggest that initial mesoderm formation is an induction event in blastula by secreted signal(s) from vegetal mass (develop into future endoderm tissues) to equatorial region (develop into future mesoderm tissues). We now believe that maternal transcription factor(s) activate zygotic signals, which in turn trigger mesoderm formation.

Although there are some minor variations between amphibian and zebrafish mesoderm inductions, studies in zebrafish and *Xenopus* indicate that transforming growth factor-beta (TGF-β) family members, especially Nodal and its related factors play essential roles in mesoderm induction and endoderm formation in vertebrates [1]. As in other signaling transduction pathways, TGF-β signaling is tightly regulated. One way to ensure proper amount signaling in cells is to turn down the signaling by negative regulators after initial ligand-receptor engagement. Previously identified such regulators in TGF-β pathway include Antivin/Lefy [2], Tomoregulin-1 [3], Cerberus [4], and Charon [5]. These factors block receptor-ligand association either directly by binding to ligands or receptors or indirectly by binding to co-receptors. Another protein Drap1 [6] prevents transcription factor FoxH1 from binding to the Nodal target genes. Interference by factors in other steps of the pathway can also be envisioned to fine-tune the system.

Dapper2 (dpr2) was initially identified in a whole mount *in situ* hybridization screen for zebrafish tissue specific markers. It has similarity to human DAPPER2 protein. Using reverse genetic approaches, Zhang *et al* [7] now report in October 1st issue of *Science* that zebrafish *dpr2* can also attenuate TGF-β signaling in mesoderm induction in zebrafish. They find that overexpression of *dpr2* in zebrafish partially blocks mesoderm formation. On the other hand, morpholino mediated inhibition of *dpr2* mRNA translation results in opposite phenotype, causing excessive expression of mesoderm markers, such as *goosecoid*, *no-tail*, *snail1* and *sonic hedgehog*, etc. Turning to cell culture systems, Zhang and colleagues find that Dpr2 localizes in late endosomes and that Dpr2 binds to Nodal receptors ALK4 and 5 when they are overexpressed. Furthermore, they find that overexpression of dpr2 significantly reduces the receptor protein level, which is sufficient to attenuate target gene activation in a reporter assay. Pulse–chase labeling and protein degradation inhibition experiments indicate that Dpr2 promotes TGF-β receptors' degradation in a lysosome dependent fashion, not through proteasome pathway. They also find that *dpr2* is absent in *sqt;cyc* double mutant indicating Nodal signaling is essential to activate *dpr2* expression, thus establish that a reciprocal regulation loop between *dpr2* and *sqt/cyc*.

How Dpr2 associates with TGF-β receptors *in vivo*? We do not know, but unlike in overexpressed state in cell culture system, such association shown in *vitro* might result from ligand engagement in *vivo* and might only happen after the receptors endocytosed. *In vitro* data by Zhang and colleagues support such an assumption. Apparently, Dpr2 does not directly participate in initial targeting of endocyosed TGF-β receptors to early endosomes. How Dpr2 facilitates the receptors through late endosomes remains to be investigated. Obviously identification of Dpr2 interaction partners will help elucidate how Dpr2 functions in the pathway. Interestingly, previously in a genetic screen in *Drosophila* for genes that control synapse development, Sweeney and Davis [8] identified a late endosomal transmembrane protein Spinster (Spin), which also negatively regulates TGF-β signaling in synaptic growth. However zebrafish *spinster* homolog *not really started* (*nrs*) [9] has

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different RNA expression profile compared to *dpr2*. It is possible protein(s) with functions similar to Spin may co-express with Dpr2. Interactions between Dpr2 and such protein(s) might influence receptor degradation remain to be examined. Although Dpr2 functions in mesoderm formation is mainly discovered in zebrafish, a low vertebrate, its possible involvement in mesoderm induction in mammals can be tested by targeted *dpr2* gene deletion in mice.

Proteins that are implicated in receptor degradation are not uncommon, but Zhang and colleagues are the first to identify a non-membrane cytoplasmic protein involved in the Nodal pathway. This study provides new information at an intersection of several disciplines, including germ layer induction, membrane traffic, protein degradation, and TGF β signaling.

On the practical side, abnormal functions of TGF-β signaling have been implicated in certain diseases, such as cancer. It is conceivable that *dpr2* mutations in some tissues may cause unwanted cell functions, such as cell proliferation. Uncovering *dpr2* mutations in human cancer patients and even studying Dpr2 as a possible drug target are not remote possibilities.

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