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Norrin and Frizzled: A New Vein for the Eye

The Wnt signaling network is arguably one of the most complex molecular modules by which cells communicate. Though we become accustomed to novel components, the discovery of an unexpected Wnt receptor ligand, Norrin, is surprising even to the connoisseur.

Wnts bind to cell surface receptors and trigger activation of at least two, likely three pathways (http://www.stanford.edu/~rnusse/wntwindow.html). The best-characterized signaling cascade is the canonical, or Wnt/β-catenin pathway. Activation of the pathway by a Wnt protein results in β-catenin being stabilized; it then enters the nucleus, where, with members of the "high-mobility-group" protein family, it activates the expression of specific genes to control cell fate. Another pathway engaged by Wnts is the planar cell polarity pathway (PCP), which does not involve β-catenin but employs small GTPases of the rho/cdc42 family to activate jun kinase. A third Wnt-triggered pathway, which may be partly overlapping with the PCP pathway, is the Wnt/Ca^{2+} cascade, which mobilizes Ca^{2+} ions within cells and thereby activates certain Ca^{2+}-dependent enzymes, including protein kinase C. One feature that all three pathways have in common is that they require Wnt receptors of the Frizzled (Fz) family of seven-transmembrane proteins. These receptors have N-terminal extracellular domains termed cystein rich domains (CRD), which mediate high-affinity interaction between the 19 or so Wnt and 10 Fz proteins. Different Wnts have different affinities for various Fz and may engage different pathways, generating a great complexity of signaling possibilities.

In addition, Wnts require coreceptors of the lipoprotein receptor related protein 5/6 class (LRP5/6), which are specifically coupling to the Wnt/β-catenin pathway, likely by forming a ternary complex with Wnt and Fz proteins (http://www.stanford.edu/~rnusse/wntwindow.html). Recently, *Drosophila* Wnt5 was shown to engage yet another transmembrane receptor, the tyrosine kinase Derailed (Yoshikawa et al., 2003). Adding to this complexity is the fact that Wnts are not the only secreted factors able to activate the β-catenin pathway. Secreted Frizzled related protein 1 can activate Wnt/β-catenin signaling at low dose while it inhibits at high dose (Uren et al., 2000). Dkk2, a member of the Dickkopf family of secreted Wnt antagonists, binds and activates LRP6 but, in the presence of its coreceptor Kremen, inhibits Wnt/β-catenin signaling (Mao and Niehrs, 2003). A Dkk-unrelated LRP6 ligand, Wise, can also activate or inhibit Wnt signaling in a context-dependent manner (Itasaki et al., 2003).

Enter Xu et al. (2004) who describe in an elegant study in the March 19th issue of *Cell* yet another secreted activator of Wnt/β-catenin signaling, the protein product of the Norrie disease gene (*NDP*), called Norrin (Berger and Ropers, 2001). Their interest in Norrin was triggered by the similarity of phenotypes between *Fz4* mouse mutants and Norrie disease patients. Norrie disease (ND) is a X-linked congenital retinal dysplasia that can be accompanied by hearing loss and mental retardation. Over 70 distinct *NDP* mutations have been identified in patients with ND (Berger and Ropers, 2001). Similar to ND patients, mice mutant for the *NDP* ortholog show absence of intraretinal capillaries and progressive loss of vessels in the cochlea (Rehm et al., 2002).

Congenital retinal defects are also seen in another congenital disease, FEVR (familial exudative vitreoretinopathy), where patients show incomplete vascularization of the retina. Since one FEVR locus has recently been shown to correspond to the human Fz4 gene (Robitaille et al., 2002), Xu et al. went back to their *Fz4*$^{-/-}$ mice and discovered vascular defects in mutant retina which resemble those described in *Ndp* mutant mice. Adding to this the observation that patients and mice with mutations in the Wnt coreceptor *LRP5* also show vascular eye defects, they tested if Norrin might directly play a role in Wnt signaling. The biochemical function of the Norrin protein was unknown, but it encodes a secretory protein containing a cysteine-knot motif. Norrin turns out to be a high-affinity ligand for Fz4, which can activate Wnt/β-catenin signaling. The interaction is of surprisingly high specificity; of six Fz proteins Norrin interacts only with Fz4. Norrin shares a number of properties with Wnt proteins; Norrin binds to the CRD of Fz4; it is poorly secreted and is mostly associated with the extracellular matrix, acting as a short-range signal; Norrin does not bind directly to LRP6. However, unlike Wnts, which robustly activate Wnt/β-catenin signaling with Fz in the absence of cotransfected *LRP*s, Norrin requires cotransfection with *LRP5/6*. This raises the possibility that Norrin interacts in a ternary complex with Fz and LRPs, as has been suggested for Wnts.

While the authors have shown that Norrin/Fz4 can activate the Wnt/β-catenin pathway in vitro and likely in vivo, one should be open to the possibility that this ligand may also employ the Wnt/PCP or the Wnt/Ca^{2+} pathways, given a matching Fz. A precedent is XWnt5a, which activates Wnt/β-catenin signaling with human Fz5 and the Wnt/Ca^{2+} pathway together with rat Fz2.

It is intriguing that three genes—*NDP*, *Fz4*, and *LRP5*—acting in the Wnt pathway have now been implicated in vascularization of the retina, which suggests that the Wnt/β-catenin pathway may play a wider role in vascular development. Indeed, *Fz5* mutants show defects in placental vasculogenesis (Ishikawa et al., 2001), as do mutants of the lipid phosphatase *LPP3*, which also regulates Wnt/β-catenin signaling (Escalante-Alcalde et al., 2003). The implication of Wnt/β-catenin signaling in

vasculogenesis is not only of relevance to the developmental biologist but may also present novel opportunities for medical intervention in cancer.

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Selected Reading

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Revisiting the Bulge

Two recent papers—one from the group of Elaine Fuchs in *Science* and the other from the group of George Costarelis in *Nature Biotechnology*—on the isolation and multipotentiality of adult hair follicle stem cells catapult us ahead in our understanding of epidermal and hair follicle lineages.

Although hair is presumed to have evolved as a survival vehicle, in contemporary society it clearly also plays a tremendous role in self-esteem and sexual communication. It is therefore not surprising that hair care has become a multi-billion-dollar industry with tremendous stock market consequences. In spite of this socioeconomic prominence, our understanding of the hair follicle lineage and our ability to manipulate it remain rather rudimentary. The identification and characterization of hair follicle stem cells, as well as what regulates their commitment and differentiation, has generally lagged behind other adult stem cells and lineages. However, two recent papers (Tumbar et al., 2004; Morris et al., 2004) move us toward closing this gap.

How did we get here? It has long been known that skin and its appendages, including hair, have the capacity to renew throughout life, implying the existence of a large reservoir of putative stem cells. Over 10 years ago, Cotsarelis et al. (1990) made a seminal contribution by demonstrating the location of the infrequently cycling label-retaining cells (LRCs) in the bulge region of the outer root sheath of the hair shaft (see Figure 1). Not only cells with the highest label-retaining capacity but also those with the highest clonogenicity in vitro in the mammalian epidermis occur in or near the bulge region. Other experimental manipulations provided evidence that the bulge region contains multipotent stem cells that are more proliferative and potent than interfollicular epidermal stem cells. However, these data also raised many questions about bulge cell heterogeneity and the cellular and molecular relationships of the hair follicle to interfollicular stem cells. The two recent papers are nicely complementary in documenting isolation, from the bulge region, of slowly cycling cells with high proliferative capacity, multipotentiality (capacity to contribute to the three distinct cutaneous epidermal lineages), and transplantability. Together they suggest that the long-awaited and elusive hair follicle stem cell has been found.

The two groups reached similar conclusions, but used somewhat different genetic strategies to track and isolate a subpopulation of cells from the bulge. Based on their assumption that bulge stem cells would uniquely be both slow-cycling and active for a keratinocyte-specific promoter, Fuchs' group engineered transgenic mice to express histone H2B–green fluorescent protein (GFP) controlled by a tetracycline (tet)-responsive regulatory element and crossed them to mice harboring a keratin 5 (K5) promoter–tet repressor–VP16 transgene. Four weeks of tet treatment of the double transgenic offspring with tet–controlled regulation restricted to skin epithelium selected for a low frequency ($<1\%$) of slowly cycling bulge cells. Cotsarelis' group, on the other hand, used a keratin 1-15 (K15) promoter fragment to target mouse bulge cells with an inducible Cre recombinase construct or with the gene encoding GFP to mark bulge cells for lineage analysis in vivo and isolation, respectively. Further characterization of either of the GFP-positive populations on the basis of a number of phenotypic and functional properties in vitro and in vivo indicate that the cells meet many of the hallmark definitions of stem cells.

What evidence was provided that any of the cells identified in either study are multipotential and generate or regenerate hair follicles and other cutaneous lineages, a feature attributed to bulge stem cells (Taylor et al., 2000; Oshima et al. 2001)? This is a key question given that there is evidence both for multipotent cells as well as more restricted progenitor cells, i.e., cells that give rise to, for example, only the hair follicle or only the