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Meeting report

Highlights of the 1998 Wnt meeting, Cambridge, MA, January 9–11

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Extensive studies in the past few years have illustrated the fundamental roles of Wnt genes in numerous developmental decisions. These include not only the control of cellular proliferation but also the establishment of cell fates. Wnt genes encode a large family of secreted molecules and have been identified in *Drosophila*, *Caenorhabditis elegans*, *Xenopus*, zebrafish, mouse and humans. They act as developmental regulators that can elicit different biological responses, depending upon the cellular context. It is now apparent that they identify a major class of signaling molecules comparable in importance to other secreted peptides such as FGFs, Hedgehogs, and TGF-beta, just to cite a few examples. For a recent review of the functions of Wnt genes see [1] and the Wnt gene homepage (http://www-leland.stanford.edu/~rnusse/wntwindow.html). Approximately 300 people attended the 1998 Wnt meeting held in Cambridge, MA, organized by Norbert Perrimon (Harvard Medical School), Andrew McMahon (Harvard University), and Roel Nusse (Stanford University). Many new exciting results were presented both on the function of these proteins as well as their signal transduction mechanisms.

1. New functions for Wnt proteins

A number of presentations described novel functions associated with Wnt proteins, was known to contain four different Wnt genes [2], but mutations in only one of them, *DWnt-1* or wingless (*wg*), had been previously reported. Two presentations described the characterization of mutants in *DWnt-2* and *DWnt-4*, respectively. K. Kozopas (Nusse lab, Stanford) showed that *DWnt-2* mutants result in male sterility and demonstrated that secreted *D-Wnt-2* is a signal for the specification of pigment cells found in the developing male reproductive tract. B. Wilder (UPenn) and J. Pradel (Marseille) reported that putative mutations in *DWnt-4* [3] exhibit disorganized dorsal embryonic cuticles as well as head defects. *DWnt-4* appears to cooperate with Hedgehog to specify the various cell fates of the dorsal epidermis. The availability of these new *Drosophila* Wnt mutants will allow the question of signaling specificity between different Wnt family members to be addressed. In particular it will be of interest to identify which receptors are activated by these Wnts, as well as to

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determine whether they signal, as Wg, through the Dsh/GSK3/Arm pathway.

C. Murre (UCSD) described the development of human pre-B acute lymphoblastoid leukemias. These leukemias are associated with a translocation that fuses the homeobox gene *Pbx* with the *E2A* gene [4]. In addition to activating a novel member of the FGF family, this chimeric transcription factor activates a novel Wnt, suggesting that this Wnt contributes to leukemia.

P. Salinas (London) discussed an intriguing property of Wnt-7A protein on neuronal growth cones. Soluble Wnt-7a induces axonal spreading and branching in cerebellar neurons. This pathway involves GSK3 since inhibition of GSK-3b results in the loss of stable microtubules, a process associated with the downregulation of a phosphorylated form of the microtubule associated protein, MAP-1B.

Other roles of Wnt on morphogenesis presented at the meeting included a function for Wnt-4 in the induction of epithelial transformation in metanephric mesenchyme [5] (A. Kispert, Freiburg) and a function for Wnt-1 in branching morphogenesis in mouse mammary epithelial cells (J. Kitajewski, Columbia). S. Lee (McMahon lab, Harvard) showed that several mouse Wnt genes are expressed in the developing hippocampus, one of which (Wnt-3A) is required for the formation of this part of the brain because Wnt-3A mutants have defects in hippocampal development.

Wnt genes have now also been implicated in several aspects of *C. elegans* development, including the specification of cell lineages in the early embryo. B. Bowerman (Univ. of Oregon) gave an update on findings showing that many components of Wnt signaling are required for proper EMS cell polarization [6,7], albeit with some complications due to differences in penetrance of the various mutant genes. He also presented evidence for a role for Wnt signaling in spindle orientation in a cell-contact dependent way. Interestingly, several of these Wnt phenotypes seem to be independent of transcriptional activity within the target cells. M. Herman (Kansas State Univ.) used the lin-44 (Wnt)-lin-17(frizzled) interactions and their role in T-cell polarity in the *C. elegans* tail [8] to identify novel components of the signaling pathway. He found one novel gene, *egl-27*, which has homology to a human gene implicated in metastasis. *egl-27* also functions in another Wnt signaling event in the worm, *egl-20* (a Wnt gene), which controls Q cell migration [9].

2. Mechanism of reception of Wnt signals

The current model for Wnt signaling proposes that, in the absence of Wg signal, the GSK3 serine/threonine kinase, known as Zeste white 3 or Shaggy in *Drosophila* [10], phosphorylates b-catenin, known as Armadillo (Arm) in *Drosophila*, destabilizing the cytoplasmic form of the protein. In the presence of Wnts, the seven transmembrane receptor encoded by the Frizzled (Fz) acts through the Dishevelled (Dsh) protein to antagonize GSK3 activity, and Arm is stabilized, resulting in its interaction with the transcription factor TCF/Lef1, known as Pangolin or DTcf in *Drosophila*, forming a stable transcriptional regulator that can be seen in the nucleus. At the meeting many aspects of this pathway were discussed and new molecules identified, that further our understanding and underscore the complexity, of this pathway (for a previous review of the Wnt signaling pathway see Ref. [11]).

Evidence for interaction between Frizzled receptors and Wnts, first described in *Drosophila*, *Xenopus* and *C. elegans*, is now also emerging from mammalian cell culture assays. M. Semenov (Lab of A.M.C. Brown, Cornell) showed that dominant negative mammalian frizzled species can block the elevation of b-catenin which is normally brought about by Wnt expression. M. Moos (Bethesda) elaborated on his previous findings of secreted forms of Frizzleds (called FrzBs or FRPs [11–14]) which can counteract the activity of Wnts, in particular in *Xenopus*, presumably by direct binding.

In *Drosophila*, there is yet no genetic evidence that Dfz2, a candidate Wg receptor [15], interacts with Wg in vivo but K. Cadigan (Nusse Lab, Stanford) showed that overexpression of Dfz2 mimics Wg signaling in the wing blade, in a *wg*-dependent manner, and furthermore, that expression of Dfz2 is down-regulated by Wg expression. Intriguingly, by uncoupling Dfz2 expression from down regulation, the Wg protein itself becomes stabilized, leading to more Wg protein in areas outside from its site of production.

Two presentations, by X. Lin (Perrimon Lab, Har-

vard Medical School) and F. Reichsman (Cumberledge Lab, Univ. of Massachusetts) presented evidence that heparin sulfate proteoglycans (HSPGs) play a critical role in the activation of Fz receptors by Wnts [16]. These HSPGs may play a role in either the presentation or the increasing of the concentration of ligands to the receptor [17–19]. Progress in the characterization of the role of HSPGs will have to await the characterization of the protein component of the HSPGs. Interestingly, S. Selleck (U. Arizona) presented some evidence that Dally [20], which encodes the fly homolog of Glypican, may encode the protein part of the HSPG.

A. Martinez Arias (Univ. of Cambridge, UK) presented evidence that Wg can associate with Notch [21]. Because genetic interactions can be revealed between Wg and Notch, he proposed that, in some developmental contexts, Notch can modulate or contribute to the integration of a Wnt signal.

3. Signal transduction pathways regulated by Wnts

There is ample evidence that many Wnt proteins regulate the activity of the Dsh/GSK3/b-catenin/Lef signaling cassette. However, it is also becoming apparent that not every Wnt signaling event can be accounted for by this pathway. A number of presentations discussed the ability of Wnts to regulate other pathways.

J. Brown (Moon Lab, U. Washington) presented evidence that Wnt-5A regulate the activity of a signaling pathway that is different from the Wnt-1 pathway. Wnt-5A may increase intracellular calcium, possibly through a pathway that involves a G protein [22]. This pathway also appears to implicate a specific Fz protein.

M. Kengaku (Tabin Lab, Harvard Medical School) presented evidence that, in the limb bud, different Wnts activate different signal transduction pathways. Wnt-3A is expressed in the early limb ectoderm which later forms the AER, while Wnt-7A plays an essential role in limb patterning along the dorsoventral axis [23]. Interestingly, she found that misexpression of b-catenin or Lef1 can mimic the effect of Wnt3A to induce ectopic expression of FGF8, while b-catenin cannot mimic Wnt7A in activating one of its target genes: *Lmx-1* [24].

Most direct evidence that Fz receptors can activate different signaling pathways was obtained in *Drosophila* studies on tissue or planar cell polarity (PCP). In Drosophila, two Fz receptors have been characterized, Fz1 and Dfz2. Only mutations in Fz1 have been identified to date, and they affect the establishment of PCP [25]. PCP signaling orients the cytoskeleton in response to a signal mediated by Dsh, and Fz1, and is easily visualized on the fly adult cuticle by the orientation of the hairs that are secreted by epidermal cells. The mechanism by which Fz1 organize PCP is poorly understood. P. Adler (Univ. of Virginia) discussed the phenotype associated with an unusual allele of prickled. He showed that this gain of function mutant was able to reverse the orientation of PCP, suggesting a key role for this gene in the activation of Fz1. Although the mechanism of activation of Fz1 remains to be characterized, progress has been made at dissecting the downstream events. J. Axelrod (Perrimon Lab, Harvard Medical School) proposed that polarity results from asymmetric Fz1 activation and asymmetric relocalization of Dsh to the membrane, where Dsh effects cytoskeletal reorganization to orient prehair initiation. In support of this model, divergent intracellular pathways are activated by Dsh [26,27]. He found that during Fz signaling, Dsh is selectively recruited to the membrane, and this recruitment depends on a DEP domain in Dsh which has been implicated in the regulation of small GTPases. Similar results were presented by M. Boutros (Mlodzik Lab, EMBL), who in addition presented data suggesting that Fz1/Dsh may regulate the activity of the RhoA [28] and JNK pathway.

Interestingly, A. Wodarz (Nusse Lab, Stanford) showed that mutant forms of Dsh in which some of the phosphorylation sites [29] are inactivated can be found associated with the centrosomes. Wild type Dsh protein may also locate to centrosomes and stains mitotic spindles in dividing cells. This subcellular localization of Dsh may underlie the mechanism by which Dsh affects polarity.

4. Regulation of GSK3 activity

One of the outstanding issue in the Wnt signaling pathway is the mechanism by which GSK3 activity is regulated by Wnt signaling. The current model is that

Dsh antagonizes GSK3 activity. However, direct proof for this regulatory interaction is lacking. A number of interesting new findings stressed the complexity of GSK3 regulation. In particular, new proteins that either associate or are able to regulate GSK3 activity were reported.

K. Wharton (Lab of M. Scott, Stanford) reported the characterization of the novel protein encoded by the naked (*nkd*) gene. The paradox associated with Nkd is that it exhibits a phenotype similar to *zw3* mutants; however, unlike *zw3*, genetic epistasis has placed it upstream of Wg. He also presented data supporting the model that *nkd* participates in a negative feed back loop. Expression of *nkd* is regulated by Wg signaling and Nkd activity acts between Dsh and GSK3.

C. Yost (Kimelman Lab, U. Washington), in a two-hybrid screen, identified a novel *Xenopus* protein, GBP, that associates with GSK3. GBP stabilizes the level of b-catenin and inhibits the enzymatic activity of GSK3. GPB which is homologous to a proto-oncogene (FRAT-1 [30]) of unknown function defines a new family of GSK3 binding proteins with functions in development and cell proliferation.

Two presentations, by F. Costantini (Columbia) and S. Sokol (Harvard Medical School), described the function of Axin [31] in mice and *Xenopus*, respectively. Axin acts as an essential negative regulator in mice and S. Sokol presented evidence that Axin is associated with GSK3 as well as b-catenin suggesting that Axin may serve as a docking protein mediating negative regulation of b-catenin by GSK3. A possible model is that Dsh also regulates GSK3 activity by acting through Axin.

5. Armadillo and Lef/TCF proteins

Progress was presented in dissecting the chain of events downstream of b-catenin. F. Fagotto (Gumbiner Lab, Sloan–Kettering) discussed recent data that suggest that b-catenin enters into the nucleus by a pathway that does not involve the Importins. b-catenin does not contain a NLS and can enter the nucleus in the absence of Tcf/Lef proteins suggesting that it may acts as its own Importin molecule. Interestingly, the b-importin protein has some homology to b-catenin in that it contains several Armadillo/b-catenin repeats [32]. This novel activity of b-catenin raises the possibility that one of the role of Wnt signaling is to directly regulate the nuclear translocation of b-catenin.

b-catenin is now known to enter the nucleus, an event that also may play a role in Sea Urchin embryogenesis (C. Logan, McClay lab; Duke), and it interacts with the Lef/Tcf transcription factors. R. Cavallo (Peifer Lab, Univ. of North Carolina) reported an interesting competition mechanism between the transcriptional repressor Groucho [33], and the Tcf/Arm complex. As first shown by H. Clevers (Utrecht University, The Netherlands) for mammalian TCFs, TCF can bind to not only to Armadillo [34–36] but also Groucho, such that these two proteins compete for a limited pool of DTcf. R. Cavallo proposed that when there is a low level of Arm in the nucleus (Wg signaling off), Tcf is a repressor of *wg*-target genes because it cooperates with Groucho. H. Clevers showed am interesting knockout phenotype of one TCF members in the mouse, (TCF-4) which is required for intestinal development. An armadillo-related gene in *C. elegans* (called *Bar-1*, D. Eisenmann; Kim Lab, Stanford) is involved in *Hox* gene expression, in conjunction with the *ras* signaling pathway.

Finally, S. Kerridge (Marseille) has identified the Zinc finger protein Teashirt [37], as a new nuclear partner for Arm. Teashirt, which has been previously studied for its role in regulation of homeotic gene, is involved in modulating a subset of Wg functions. The data presented suggest that Teashirt is involved in the autoregulation of Wg and provide the first example of a nuclear protein that modulates the cell type specific response to a Wnt signal.

Given the rapid advances in this field, now emerging from many different systems, the next Wnt meeting (tentatively planned for the summer of 1999) will undoubtedly be as exciting as this one.

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