tion (22, 23). In addition to hPR-A (the human progesterone receptor A isoform), other proteins such as NCOR (nuclear receptor corepressor), SMRT (silencing mediator of retinoid and thyroid hormone receptors), REA (Repressor of Estrogen Action), SHP (Short Heterodimer Partner), RIP140 (receptor-interacting protein 140), DAX-1 (Dosage-sensitive sex-reversal, Adrenal hypoplasia congenital, X chromosome), and RTA (Repressor of Tamoxifen Activity) negatively regulate ERα- and ERβ-mediated transcriptional activity (1, 11, 24, 25).

Until recently, it was generally believed that coactivators would be expressed in a cell-specific (or cell-selective) manner, and that the pharmacological responses to agonists and antagonists would be determined by the relative and absolute concentrations of these proteins. With few exceptions, however, the majority of cofactors are widely expressed in similar amounts in most cells. It is possible that additional cell-specific cofactors remain to be identified, but it appears likely that differential regulation of coactivator activity rather than control of protein abundance may be more important. Indeed, the recent observations that AIB1 and SRC-1 coactivator activity can be increased by MAPK-mediated phosphorylation, and that TIF2 activity is enhanced by the protein methyltransferase CARM1 (coactivator-associated arginine methyltransferase 1), seem to point in this direction (26–28). Hints about the roles of coactivators in ER action have emerged, but a complete understanding of these proteins and the complex networks in which they participate will occupy investigators in this field for some time.

References

The Promise and Perils of Wnt Signaling Through β-Catenin
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Wnt pathways are involved in the control of gene expression, cell behavior, cell adhesion, and polarity. In addition, they often operate in combination with other signaling pathways. The Wnt/β-catenin pathway is the best studied of the Wnt pathways and is highly conserved through evolution. In this pathway, Wnt signaling inhibits the degradation of β-catenin, which can regulate transcription of a number of genes. Some of the genes regulated are those associated with cancer and other diseases (for example, colorectal cancer and melanomas). As a result, components of the Wnt/β-catenin pathway are promising targets in the search for therapeutic agents. Information about Wnt pathways is available both in canonical terms and at the species level. In addition to the canonical Wnt/β-catenin pathway, information is now available for Drosophila, Caenorhabditis elegans, and Xenopus. The STKE Connections Maps for these pathways provide an important tool in accessing this large body of complex information.

Secreted Wnt ligands activate receptor-mediated signal transduction pathways, resulting in changes in gene expression, cell behavior, cell adhesion, and cell polarity. Investigations of these pathways have been driven for two decades by the knowledge that Wnt signaling is involved in both embryonic development and cancer. This knowledge has fostered a rigorous scientific dissection of Wnt signaling on the basis of genetic studies in the mouse Mus musculus, the fruit fly Drosophila melanogaster, the nematode Caenorhabditis elegans, and the zebrafish Danio rerio, as well as cell biological and biochemical studies in mammalian cultured cells and the frog Xenopus laevis. This worldwide effort has established that multiple Wnt signaling pathways are activated by a multigene family of Wnt ligands.

The first Wnt pathway to be discovered, and the best understood, is the canonical Wnt pathway that activates the function of β-catenin (Fig. 1), with more components, interactions, and target genes described in the canonical STKE Connections Map Wnt/β-Catenin Pathway (http://stke.sciencemag.org/cgi/cm/CMP_5533[J]). Acting through a core set of proteins that are highly conserved in evolution, this pathway regulates the ability of β-catenin to activate transcription of specific target genes. This regulation, in turn, results in changes in expression of genes that modulate cell fate, proliferation, and apoptosis. Components of the β-catenin signaling pathway are also regulated by other signals (Fig. 1), promoting interest in understanding how Wnts can function in combination with other signaling pathways. As more signaling pathways are added to the STKE Connections Maps, it will be possible for both casual users and experts to better understand and predict the outcome of increasingly complex combinatorial signaling.

Activation of the Wnt/β-catenin signaling pathway holds both promise and perils for human medicine. The perils have been known for some time—activation of this signaling pathway through loss-of-function mutations in the tumor suppressors adenomatous polyposis coli (APC) protein and axin, or through gain-of-function mutations in β-catenin itself, are linked to diverse human cancers, including colorectal cancers and melanomas (2). This connection has
fueled a search for Wnt/β-catenin pathway antagonists, which may become lead compounds for anticancer drugs. Greater knowledge of the Wnt/β-catenin pathway may benefit patients with other diseases and conditions, because this pathway is involved in regulating angiogenesis (3, 4), adipogenesis (5), and stem cell proliferation (6). For example, in the area of bone density, loss of function of a Wnt/β-catenin pathway co-receptor, low-density lipoprotein receptor-related protein 5 (LRP5), results in low bone mass in children and heterozygous parents (7). Conversely, apparent gain-of-function mutations in the same gene result in an autosomal dominant high bone-mass trait (8). Thus, both antagonists and agonists of components of the Wnt/β-catenin pathway may prove therapeutic in cancer and in stimulating cell and bone replacement, respectively.

Given the clear link between the Wnt/β-catenin signaling pathway and human diseases, and the conservation of molecular functions across many animal taxa, we expect that future advances in understanding the mechanisms of Wnt signaling will benefit substantially from studies in model systems. The specific pathways in the STKE Connections Maps will help to promote the uses of model organisms to understand Wnt/β-catenin signaling. Currently, pathways in Drosophila (http://stke.sciencemag.org/cgi/cm/CMP_6459) (9), C. elegans ([http://stke.sciencemag.org/cgi/cm/CMP_10440]), (http://stke.sciencemag.org/cgi/cm/CMP_10698), (http://stke.sciencemag.org/cgi/cm/CMP_7631)(10–13), and Xenopus (http://stke.sciencemag.org/cgi/cm/CMP_6104), (http://stke.sciencemag.org/cgi/cm/CMP_9763)(10–13), and Xenopus (http://stke.sciencemag.org/cgi/cm/CMP_6031) (14) are available, with possible future additions to include pathways for mouse, chicken, and zebrafish. Supporting this goal of including pathways from more species, much of the earliest work on Wnt signaling and its effects on adhesion and the cytoskeleton was conducted on mammalian cells in culture (15), and subsequent work on the mouse has led to numerous discoveries, including the roles of Wnts as mitogens in the nervous system (16), and as essential signaling factors in formation of the limbs (17), kidneys (18), and female reproductive system (19).

Genetic analyses in Drosophila led to the initial discovery of many Wnt pathway components (20). The first breakthrough in the field was the discovery that mutations at the WINGLESS (WG) locus corresponded to a member of the Wnt family of secreted glycoproteins (21, 22). Mutations of Wg are associated with embryonic segmentation defects. By studying other mutations that affected embryonic segmentation, dishevelled (dsh), zeste white-3 (zw-3) (also known as shaggy and GSK3), and armadillo (arm) (also known as β-catenin) were subsequently affiliated with this pathway and organized epistatically (23, 24). Eventually, many additional components were discovered (25) that define the canonical Wnt/β-catenin pathway. Biochemical studies from several species have confirmed and extended this genetic pathway.

Detailed phenotypic analyses of WG mutants, as well as analysis of other pathway components, have illustrated the developmental roles of this pathway. The list of functions for Wg keeps expanding and includes roles as diverse as embryonic segmentation and patterning, gut patterning, nervous system development, formation and patterning of appendages, and stem cell proliferation. Some of the pathway components, in particular Dsh and one of the Fz receptors, are associated with planar polarity defects not detected with loss of Wg or Arm activity, suggesting that there are other “noncanonical” pathways that share components with the Wnt/β-catenin pathway (26, 27). We expect that analysis of Drosophila will continue to make original contributions to the Wnt field. For example, the Drosophila genome encodes seven Wnts and four Fz receptors (28), and relevant information on many of these proteins is still missing.

Wnt signal transduction is generally thought to influence the transcriptional regulation of target genes in the nucleus of a responding cell, through T cell factor (TCF) and its associated proteins. However, studies of Wnt signaling in C. elegans are also notable for implicating the cytoskeleton as another important target of Wnt signaling.

In early worm embryos, a noncanonical Wnt pathway polarizes endoderm potential within a single embryonic cell that then divides to produce one endoderm and one mesoderm precursor (29). This Wnt signal must be received within 5 minutes of the parent cell’s birth, or two mesoderm precursors are made. Thus, Wnt signaling can polarize developmental potential within a single cell, and this process appears to require microfilaments (30). The same Wnt pathway, up through but not beyond a glycogen synthase kinase 3 (GSK-3) homolog called SGG-1, is required to induce rotation of the forming mitotic spindle during this polarized division (31). Rotation aligns the spindle with the axis of polarization, and the posterior daughter produces endoderm. Rotation can be induced even when gene transcription is blocked, and the single C. elegans TCF homolog POP-1 is not required. Thus, the mitotic spindle appears to be a direct target of Wnt signaling, but the pathway that leads there from SGG-1 remains unknown.

During larval development, more canonical pathways influence gene transcription (32–36). However, the prominence of cell polarity in some of these responses again suggests that cytoskeletal regulation is important. Indeed, Wnt signaling and GSK-3 can influence axonal migrations and axonal transport in vertebrate cells (37, 38), and insect and vertebrate β-catenin have dual roles in both signaling and adhesion, suggesting that cytoskeletal regulation by Wnt pathways is widely conserved in evolution.

Studies in eggs and embryos of the amphibian Xenopus laevis have also contributed to our understanding of the mechanisms of Wnt signaling and the roles of Wnt signaling in early development. The contributions to understanding signaling mechanisms include the observation that GSK-3 phosphorylates β-catenin directly and negatively regulates its stability and nuclear accumulation (39); evidence showing that LRP5 and LRP6 function as Wnt co-receptors (40); confirmation of TCF’s role as a transcriptional mediator of β-catenin signaling (41); the discovery of the Wnt antagonists Dick (42), sFRP (43–45), and Cerberus (46); and the identification in vertebrates of β-catenin-independent noncanonical Wnt signaling (47). Interestingly, it is also possible to reconstitute aspects of
β-catenin signaling in vitro in extracts of Xenopus embryos (48). With regard to understanding the roles of Wnts in early development, studies in Xenopus have established that an asymmetry in β-catenin during the first cell cycles correlates with the dorso-ventral axis (49) and is required for axis formation (50). The STKE Specific Pathway on the Xenopus egg Wnt/β-catenin pathway (14) highlights the maternal pathway that is involved in axis specification, and it will be expanded as a consensus is reached regarding the composition and functions of zygotic and noncanonical Wnt and Frizzled pathways.

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Signal Transduction by the TGF-β Superfamily

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Transforming growth factor-β (TGF-β) superfamily members regulate a plethora of developmental processes, and disruption of their activity has been implicated in a variety of human diseases ranging from cancer to chondrodysplasias and pulmonary hypertension. Intense investigations have revealed that SMAD proteins constitute the basic components of the core intracellular signaling cascade and that SMADs function by carrying signals from the cell surface directly to the nucleus. Recent insights have revealed how SMAD proteins themselves are regulated and how appropriate subcellular localization of SMADs and TGF-β transmembrane receptors is controlled. Current research efforts investigating the contribution of SMAD-independent pathways promise to reveal advances to enhance our understanding of the signaling cascade.

The first member of the transforming growth factor-β (TGF-β) superfamily of secreted polypeptide factors, TGF-β1, was discovered approximately 20 years ago. Since then, the family has grown considerably and now comprises over 30 vertebrate members and a dozen or so structurally and functionally related proteins in invertebrates such as worms and flies (1–6). TGF-βs control a plethora of cellular functions, and their activity is critical for regulating developmental and homeostatic processes. Mutations in TGF-β family ligands are responsible for a number of human diseases, including hereditary chondrodysplasia and persistent mullerian duct syndrome (5). In addition, TGF-β itself plays an important role in cancer progression by functioning both as an antiproliferative factor and as a tumor promoter, and numerous components of the signal transduction pathway are tumor suppressors that are functionally mutated in cancer (5, 7). These diverse activities have prompted intense investigations into understanding how TGF-β family members signal their effects.

Parallel work in vertebrates, worms, and flies has revealed a conserved signaling pathway, which at first glance appears to be surprisingly simple (1–5, 7) [see the TGF-β Pathway (6)]. The cell-surface receptor that carries the TGF-β family signal into the cell is a complex of single-pass transmembrane receptors that contain an intracellular kinase domain that phosphorylates serine and threonine residues (Fig. 1). This serine-threonine kinase receptor complex consists of two distinct transmembrane proteins, known as the type I and type II receptors. Ligand binding induces the type I and type II receptors to associate, which leads to a unidirectional phosphorylation event in which the type II receptor phosphorylates the type I receptor, thereby activating its kinase domain. The activated type I receptor then signals to the SMAD family of intracellular mediators. SMAD

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