XLA results in an enlargement of the pro–B cell population and inefficient development of pre–B cells in the bone marrow, resulting in very few mature peripheral B cells (1% of normal) and severe hypogammaglobulinemia of all immunoglobulin isotypes (*24*, *25*). The *xid* (and XLA) phenotype is the result of a point mutation in the pleckstrin homology (PH) domain of Btk. However, *Xid* mice have a less dramatic decrease in the number of peripheral B cells than do XLA patients, but many of these peripheral cells appear immature in nature. *Xid* mice have relatively normal serum concentrations of IgG1, IgG2a, and IgG2b but markedly reduced serum concentrations of IgM (*26*, *27*). Mice with a targeted deletion of Btk (*28*, *29*) show similar characteristics. The differences in severity of phenotypes observed between humans (XLA) and mice (*xid*) might be due to the presence in the latter of the molecule Tec, which may partially compensate for defective Btk activity (*30*).

CD19 is a B cell coreceptor that augments signals delivered through the BCR by lowering the signaling threshold for B cell activation. Although CD19 is expressed throughout B cell development, no absolute requirement for this molecule is evident until the mature B cell stage, where lack of CD19 causes a substantial decrease in the number of mature splenic B cells (*31*). In contrast, transgenic mice in which CD19 is overexpressed exhibit a hyperresponsive B cell phenotype with a predisposition for autoimmunity (*31*, *32*).

Another key pathway activated after BCR activation involves phosphoinositide 3-kinase (PI3K), a lipid kinase that mediates production of phosphatidylinositol 3,4,5-trisphosphate [$PI(3,4,5)P_3$] from phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$]. Disruption of PI3K expression by genetic deletion (*33*, *34*) results in impaired B cell development, vastly decreased numbers of pre–B cells and mature peripheral B cells, and reduced serum Ig concentrations. The observed defects are reminiscent of those found in *xid* mice. This is consistent with the requirement for $PI(3,4,5)P_3$ generation in BCR-mediated activation of Btk (*35*).

Further elucidation of the signaling cascades initiated by BCR aggregation will aid our understanding of both immunodeficiency and autoimmune disorders resulting from aberrant BCR signaling.

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VIEWPOINT

Connections and Regulation of the Human Estrogen Receptor

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Estrogen regulates a plethora of functionally dissimilar processes in a broad range of tissues. Recent progress in the study of the molecular mechanism of action of estrogen(s) has revealed why different cells can respond to the same hormone in a different manner. Three of these findings are of particular importance: (i) There are two genetically and functionally distinct estrogen receptors that have distinct expression patterns in vivo; (ii) the positive and negative transcriptional activities of these receptors require them to engage transcription cofactors (coactivators or corepressors) in target cells; and (iii) not all cofactors are functionally equivalent, nor are they expressed in the same manner in all cells. Thus, although the estrogen receptor is required for a cell to respond to an estrogenic stimulus, the nature and extent of that response are determined by the proteins, pathways, and processes with which the receptor interacts.

The ovarian steroid hormone estrogen has a primary role in the establishment and maintenance of reproductive function. However, the widespread use of estrogen-containing medicines as contraceptives and as components of hormone replacement therapies in postmenopausal women has highlighted additional functions for estrogens in the skeleton, the cardiovascular system, and the nonreproductive centers of the brain (*1*). In addition to these normal homeostatic functions, inappropriate responses to the mitogenic actions of estrogens occur in the majority of malignant breast tumors. Hence, it is not surprising that there is intense interest in defining the molecular mechanism(s) of action of this hormone, so as to clarify how it can participate in a wide variety of seemingly unlinked biological processes.

The biological actions of estrogens are manifest only in cells expressing a specific high-affinity estrogen receptor (ER) (*2*). The ER is in fact a ligand-dependent transcription factor, which accounts for the latency of most estrogenic responses in target tissues (*3*). Recent genetic, biochemical, and pharmacological dissection of the estrogen signal transduction pathway has led to the identification of numerous proteins and processes that impinge on ER function, revealing an unexpect-

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ed level of complexity in the actions of this hormone (*1*) [See Estrogen Receptor Pathway, http://stke.sciencemag.org/cgi/cm/CMP_7006 (*4*)]. Adding to this complexity was the discovery of a second genetically distinct estrogen receptor, ERβ (*5*). This review emphasizes ERα, the subtype that appears to be required for most of the known estrogenic responses (*6*).

In the absence of ligand, ERα is sequestered in target cell nuclei within a large inhibitory heat shock protein complex. Upon binding an estrogen, the receptor undergoes a conformational change that enables the displacement of heat shock proteins and facilitates the interaction of a receptor dimer within the regulatory regions of target genes (*7*). The interaction of ERα with target gene promoters can occur either directly, through specific estrogen response elements (EREs), or indirectly through contacts with other DNA bound transcription factors such as AP1, SP1, or NF-κB. Once tethered to DNA, the receptor can either positively or negatively regulate target gene transcription. The specific domains within ERα that are required for each of these functions—ligand binding, dimerization, DNA binding, and transactivation—have been described in detail (*8*).

Of late, attention has focused on defining the events downstream of ER enhancer association that enable the receptor to activate transcription. ERα contains two distinct transactivation domains, AF-1 (within the NH_2-terminus) and AF-2 (contained within the ligand binding domain). In some cells both AFs are required for maximal transcriptional activity, whereas in others only one is required (*9, 10*). This finding indicates that ERα does not interact with the transcription apparatus in the same manner in all cells. The identification of a large number of ERα-interacting proteins, some of which show preferences for AF-1 or AF-2, supports this hypothesis (*11*). Protein-protein interaction screens have revealed a large group of proteins classified as coactivators on the basis of their ability to interact with the ER and to enhance ERα action when overexpressed in target cells (*11*). Some of these proteins have an important role in ERα action and appear to provide functional and physical links between the receptor and the transcription apparatus. The roles of most of these proteins remain to be determined. But despite this complexity, the semblance of a cohesive model of ER action is beginning to emerge that describes how this receptor recruits and discriminates between the different coactivators available within target cells (Fig. 1).

Most of what is known about ERα-coactivator interactions comes from studies of AF-2 function. Crystallographic analysis of the ERα-ligand binding domain occupied with an agonist has indicated that the AF-2 domain is structurally complex (*12*). Specifically, it has been observed that upon binding an agonist, 4 of the 12 α helices that constitute the ligand binding domain of ERα are rearranged to form a hydrophobic cleft with docking sites for the coactivators important for AF-2 function. The most clearly validated AF-2–interacting coactivators are SRC-1 (steroid receptor coactivator–1); TIF2 (transcriptional intermediary factor–2, also called glucocorticoid receptor–interacting protein 1 or GRIP1); and AIB1 [Amplified in Breast Cancer, also called receptor-associated coactivator 3 (RAC3) and ACTR (activator of thyroid and retinoic acid receptors)] (all three are members of the p160 family of coactivators). These proteins have similar affinities for ERα, and thus their relative abundance within target tissues appears to determine which one forms a complex with the receptor (*13, 14*). One of the primary functions of the p160 coactivator is to recruit other transcriptional coactivators and histone acetyltransferases, such as p300, CBP [CREB (cAMP response element–binding protein) binding protein], and pCAF (p300/CBP-associated factor), to ERα-dependent enhancers in target genes (*15*). In this manner, a complex of proteins is assembled at target gene promoters that exhibits potent histone acetyltransferase (HAT) activity, which covalently modifies histone proteins and facilitates local decondensation of chromatin.

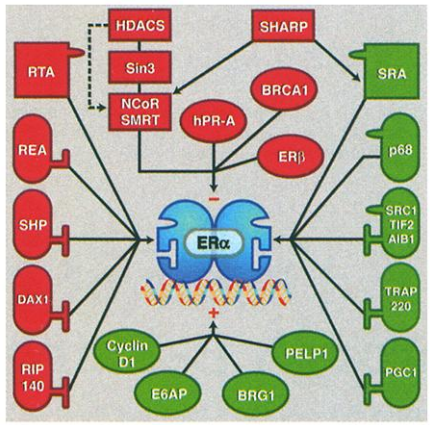

Fig. 1. A connections map for the human estrogen receptor (ER). The ER interacts with a large number of proteins that can either positively or negatively regulate target gene transcription. Most, if not all, of the known ERα cofactors interact with different target proteins linking the receptor to other signal transduction pathways. These proteins can affect ERα signaling through direct or indirect interactions. Some of the key connections that positively (+) and negatively (–) regulate ERα transcriptional activity are shown. Abbreviations not given in text: PGC1, PPARγ coactivator 1; E6AP, E6-associated protein; PELP1, proline–glutamic acid–leucine-rich protein 1; SHARP, SMRT/HDAC-associated repressor protein; BRCA1, Breast Cancer 1; HDACs, histone deacetylases; BRG1, brahma protein homolog.

A caveat to this simple model of complex assembly came with the discovery of the TRAP220 (thyroid hormone receptor–associated protein, also called DRIP205) class of ERα-interacting proteins. These proteins are components of large complexes that appear to be closely related to the SMCC (SRB- and MED-containing cofactor complex) and ARC mediator complexes, suggesting a mechanism by which ERα contacts the general transcription apparatus (*16*). Like the p160 coactivators, TRAP220 protein uses Lew-X-X-Leu-Leu (LXXLL) motifs to interact with the AF-2 domain of ERα. In chromatin immunoprecipitation assays, the p160 proteins and TRAP220-containing complexes are both associated with EREs within target genes, which suggests that these cofactors may have distinct functions in transcription (*17*). It is thought that the primary function of the p160 class of coactivators is to concentrate HAT activity at target gene promoters, and that the role of the TRAP220 class of proteins is to establish a link between ERα and RNA polymerase II. In addition to the p160 and TRAP220 classes of coactivators, there are other AF-2–interacting proteins whose function in ER signaling remains to be determined (*1, 11*).

There is a great deal of interest in defining the mechanism of action of the AF-1 domain because it is required for the partial agonist activity of tamoxifen and because its activity is positively affected by mitogen-activated protein kinase (MAPK)–directed phosphorylation (*18*). The p160 coactivators and CBP interact weakly with the NH_2-terminus of ER (*19*). Other factors, including the RNA coactivator SRA (steroid receptor RNA activator), and the RNA helicases p68 and p72 interact with and regulate AF-1 function (*1, 11*). However, because the activity of AF-1 can vary considerably between cells, it is likely that additional factors important for the function of this domain will be found.

ERα is also subject to negative regulation, in part by ERβ. In response to estradiol, ERβ can activate the same genes as are regulated by ERα, although in general less efficiently. However, ERβ functions as an efficient dominant inhibitor of ERα transcriptional activity in cells in which both receptors are expressed (*20*). The observation that the sensitivity to estradiol is markedly increased in the uteri of ERβ-knockout mice supports this hypothesis (*21*). In the reproductive system, progesterone, acting through the progesterone receptor (PR), is the physiological negative regulator of estrogen action (*22*). PR exists in two distinct isoforms, PR-A and PR-B, within target cells. It now appears that the major role of progesterone-activated PR-A is to modulate estrogen action by preventing ERα from activating transcrip-

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 methyl transferase 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.

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VIEWPOINT

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 cell 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)(*1*)]. 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

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