PERSPECTIVE | SIGNAL TRANSDUCTION

Are There Close Encounters Between Signaling Pathways?

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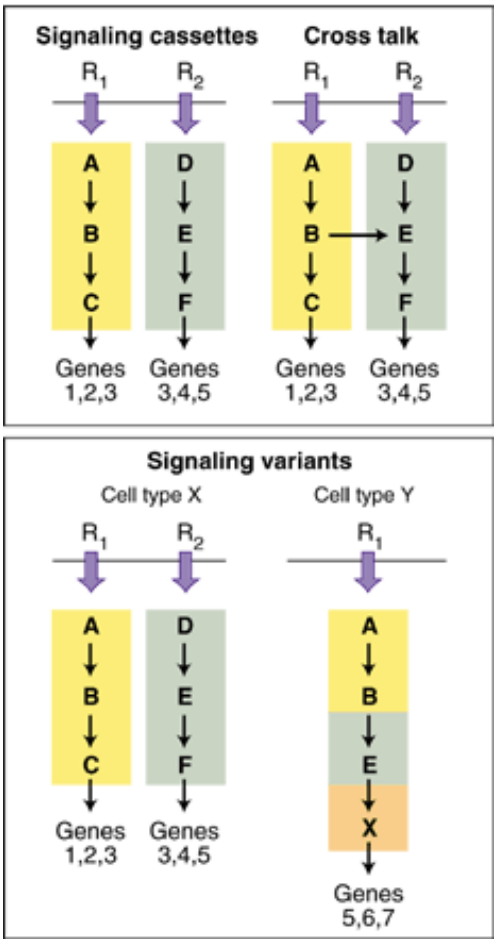
Extracellular signals trigger cascades of molecular changes at the cell's plasma membrane that are then propagated to the nucleus through signal transduction pathways. Families of ligands, when bound to their receptors, activate groups of signaling molecules, suggesting that signal transduction pathways exist as conserved, linear “cassettes” (see the figure). The notion of signaling cassettes, however, is challenged by numerous exceptions where signaling pathways, rather than being discrete and separate units, communicate with each other in a phenomenon called cross talk (see the figure). There seems to be frequent cross talk between signaling pathways in mammalian cells, whereas in cells from model organisms such as yeast, worm, and the fruit fly *Drosophila* cross talk occurs less frequently. We propose that sharing of molecular components between signaling pathways, rather than cross talk between separate signaling cassettes, may account for the apparent differences in signaling between mammalian and invertebrate cells.



Playing simultaneous cassettes.

(**Top**) Cross talk describes the communication between two separate, linear signal transduction pathways in the same cell. One molecule (B) in the signaling pathway (cassette) activated by receptor R_1 regulates the activity of a component (E) in a separate signaling pathway activated by receptor R_2. (**Bottom**) In cell type X, the signaling cassettes activated by R_1 and R_2 remain separate, whereas in another cell type (Y), the R_1 and R_2 signaling pathways share components (signaling variants), resulting in a third pathway that activates an entirely different set of genes.

Many conserved signaling pathways— for example, the Wnt/Wingless, Hedgehog (Hh), transforming growth factor-α (TGF-α) and TGF-β, JAK/STAT, NF-κB, Notch, receptor tyrosine kinase, and c-Jun NH_2-terminal kinase (JNK) pathways—have been dissected through studying embryonic development in *Drosophila*. Mutations in components of the same signaling pathway frequently yield identical phenotypes in a particular tissue or developmental process. For example, mutations in various components of the JAK/STAT signaling pathway such as Hopscotch/JAK and STAT92E/Marelle yield the same aberrant segmentation phenotype in developing fly embryos, suggesting that the JAK/STAT pathway is one linear cassette. Such studies have been instrumental in deciphering relationships between molecules and in ordering components into a linear pathway (1).

Additional support for the notion of linear signaling cassettes has been obtained by directly visualizing the activity of specific signaling molecules. A striking example is the activation of a mitogen-activated protein kinase (MAPK) called Rolled during embryonic development in *Drosophila.* Activated Rolled was detected with an antibody (DpERK) directed against phosphorylated residues (2). Antibody staining revealed the tissue distribution, timing, and duration of the signaling pathway containing Rolled. Most of the antibody staining was attributable to activation of known receptor tyrosine kinases—Torso, epidermal growth factor receptor (EGFR), and fibroblast growth factor receptor (FGFR)—indicating that during normal development other signaling pathways (such as, Wnt, Hh, and Notch) do not contribute to MAPK activation. Interestingly, although certain receptor tyrosine kinase pathways were activated in the same cells, they were activated sequentially so that there was no overlap between them. These findings are reminiscent of those from cDNA microarray analysis of gene transcription during pheromone signaling in yeast (3). Roberts *et al.* (3) have monitored the profile of genes transcribed in response to activation of the Ste2 pheromone receptor and compared it with the profile induced by all known components of the Ste2 signaling pathway, including the Ste12 transcription factor. The exciting finding is that all components affect the pheromone response similarly, thus tightly linking phenotypic and molecular profiling of a group of pheromone-response mutants. These findings demonstrate that, at least from the receptor down to the nucleus, there is no cross talk between the pheromone signaling pathway and other pathways, such as that of the kinase suppressor of SST2 (KSSI) MAPK, which is activated slightly later than the pheromone pathway during the mating response. If cross talk were required to regulate the pheromone response in yeast, then one would have expected variations in signal output depending on which component of the cascade was mutated.

Rather than following a linear path, a signal received by a mammalian cell appears to be transmitted through multiple channels. For example, the activity of SMAD proteins, assigned to the TGF-β signaling cassette, can be regulated by

components of the MAPK pathway (4). Proteins such as Ras, protein kinase C, and Akt/protein kinase B are commonly activated by not one but many extracellular ligands (5). But many of the interactions between signaling pathways in mammalian cells have been identified using overexpression assays or studies in tumor cells, prompting one to wonder whether this level of complexity exists physiologically (5).

It is possible that many of these molecular interactions between signaling pathways are found under pathological but not physiological conditions. Indeed, there are many examples of signaling molecules in tumor cells that are activated by a gain-of-function mutation in a particular protein but are not regulated by the same protein in normal cells. For example, some forms of chondrodysplasia (a disease in which the cartilage of long bones does not form properly) have been linked to mutations in FGFR3, which result in aberrant activation of the transcription factor STAT1 (6). However, STAT1 is not usually activated during embryonic development of cartilage, demonstrating that overexpression of dominant forms of signaling molecules yields misleading information about the organization of signaling pathways. A report of the forced complementation between two MAPK pathways (Fus3 and KSS1) in yeast demonstrates how it is possible to identify cross talk between signaling pathways that does not exist under normal conditions (7).

In mammalian cells, multiple signaling cassettes can be regulated by a single re-ceptor, depending on the activation status of the cell or the cell type. For example, during blood cell development, several growth factors acting through one receptor can simultaneously activate the Ras/Raf, JAK/STAT, and inositol 1,4,5-trisphosphate signaling pathways in hematopoietic progenitor cells. In the fly and worm, a single receptor is able to activate multiple pathways but not simultaneously. For example, in the worm, binding of the EGF ligand, lin3, to its receptor tyrosine kinase Let23 activates separate signaling pathways in two cell types—the Ras/Raf pathway during vulval development and the phosphatidylinositol 3-kinase pathway during ovulation (8). Thus, ovulation is Ras-independent, suggesting distinct pathways controlling differentiation of vulval cells and oocytes despite the ability of Let23 to activate both. It is likely that some of the apparent complexity in mammalian signaling reflects timing differences in the activation of these pathways. Furthermore, experiments in cancer cells indicate that overexpression of signaling molecules may override the mechanisms that establish the spatial or timing differences in pathway activation. Actually, there is little evidence to support extensive cross talk between signaling pathways during normal mammalian development. However, there are many examples of molecules involved in the compartmentalization of signaling components that may prevent cross talk—for example, the scaffolding proteins Ste5 in yeast and JNK interacting protein in mammals (9), which fix components of a signaling complex to a particular location.

The finding that the same receptor can activate several different signaling pathways can be attributed to the ability of pathways to recruit (or eliminate) a shared molecule from their signaling cassettes (see the figure). For example, during development of *Drosophila* embryonic ectoderm, signaling components downstream of Hh affect either the anterior or posterior ectoderm depending on the requirement for Fused kinase (10)—anterior ectoderm requires Fused kinase activity whereas posterior ectoderm does not.

If within a single cassette the signaling components vary through borrowing molecules (cassette variants) from other pathways, then one would expect specific mechanisms to exist that would favor emergence and selection of these cassette variants during evolution. A milestone study by Rutherford and Lindquist (11) provides the first example of such a mechanism. These authors studied the complex phenotype associated with loss-of-function mutations in the heat shock protein HSP90 in *Drosophila.* Mutations in HSP90 induce phenotypic variation in nearly all tissues of the adult fly, arguing that this heat shock protein regulates many different signaling pathways. Surprisingly, certain visible phenotypes became independent of the HSP90 mutation. The authors proposed that HSP90 may be part of a molecular buffering system that keeps cryptic signaling cassette variants silent (12). An important consequence of this model is that a reservoir of cryptic variants exists within organisms, providing them with several opportunities during development to adapt their signaling pathways according to their evolutionary needs. It will be important to learn how buffering molecules like HSP83 and HSP90 are regulated. Indeed, if cryptic signaling variants exist in every cell of every organism, one should be careful when interpreting results from studies in which normal or mutant proteins are overexpressed in cultured cells because such manipulations may overcome molecular buffering, inadvertently revealing these cryptic variants.

There are many examples of pathways that do not fit the current definition of signaling cassettes, hence a more elaborate interpretation that encompasses variations in molecular components and their interactions is required. Viewing signaling pathways as an ensemble of separate cassettes that share many components may be appropriate for mammalian cells as well as for those of yeast, fly, and worm. In mammalian cells, functional interactions between signaling components in one cell type are frequently not found in other cell types. Recognizing the flexible nature of signaling pathways in terms of cassette variations should enable us to understand signaling versatility in development and pathology (see the figure). An important prerequisite for the existence of cross talk is the simultaneous activation of at least two signal transduction pathways in the same cell. It is therefore surprising that the notion of cross talk exists in the absence of data describing such simultaneous pathways. To obtain these data, the development of new reagents recognizing activated molecules (such as DpERK antibody) may be necessary. Lessons from development suggest that signaling pathways are organized in a sequential manner, and that proper coordination of development is frequently dependent on the coupling of one

pathway to another through regulating the expression of a second ligand. Thus, the study of normal development may not be the best system in which to study cross talk because coordination between pathways is already integrated into the genetic program. There are, however, clear circumstances in which overlap between signaling pathways could favor cross talk, such as the stress response induced by environmental challenge (infection or chemical/physical insult). For example, MAPK signaling in rat cells can lead to cell death or survival depending on the ratio of JNK and p38 to ERK (all three participate in different pathways that activate MAPK) (13). It will be interesting to see whether the opposing effects of these MAPK signaling pathways involve cross talk. The introduction of engineered molecules or disease mutations into cultured cells may also favor cross talk. In these cases, however, “interference” (the actual meaning of cross talk, as first introduced in radio transmission) may be a more appropriate term as these stresses are not genetically programmed and are likely to compete with existing signaling pathways.

Gene profiling will be important for dissecting the complexity of signaling pathways, and for tracing the signaling circuitry that underlies biological responses. But studying gene transcription alone will not be sufficient; other complementary approaches—including proteomics based, for example, on large-scale two-hybrid methods (12) and mass spectrometry—are needed. Knockout cells deficient in one or more signaling components will help us to determine whether mammalian cells have evolved alternative ways of transmitting extracellular signals or whether they share the common linear signaling pathways of invertebrates.

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