Review

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Spatial and temporal organization of signaling pathways

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The development and maintenance of the many different cell types in metazoan organisms requires robust and diverse intercellular communication mechanisms. Relatively few such signaling pathways have been identified, leading to the question of how such a broad diversity of output is generated from relatively simple signals. Recent studies have revealed complex mechanisms integrating temporal and spatial information to generate diversity in signaling pathway output. We review some general principles of signaling pathways, focusing on transcriptional outputs in *Drosophila*. We consider the role of spatial and temporal aspects of different transduction pathways and then discuss how recently developed tools and approaches are helping to dissect the complex mechanisms linking pathway stimulation to output.

Signaling pathways have complex effects on cellular output

The development and homeostasis of multicellular organisms requires the coordinated activity of many different cell types. Dependable mechanisms allowing communication between cells within and between tissues are required to ensure the correct assignment of cell types during development and repair. Strikingly, although hundreds of cell types exist, relatively few signaling pathways have been identified [1], raising the question of how such a limited number of pathways can provide sufficient information to produce and maintain the diversity of cell types present in metazoans.

One aspect of signaling pathway function is that stimulation of a given pathway does not have a predefined outcome. This is illustrated by countless examples throughout development where activation of one pathway can lead to proliferation, senescence, differentiation (into multiple cell types), morphological changes, or cell death, with little obvious difference between signaling inputs in each case. However, despite this repertoire of responses, the correct outcome is invariably achieved.

Elucidating the mechanisms responsible for this variability has proved to be far from simple, partly due to the complexity of the transduction mechanisms used by signaling pathways. Furthermore, signaling pathways do not act in isolation, with different pathways sharing components and an increasing number of interactions being identified between components of different pathways. Finally, although activation of a signaling pathway has historically been considered as a binary switch, it is now clear that the dynamics of signaling pathway activation and transduction play important roles in determining signaling outcome. After reviewing some general principles of signal transduction, we describe here the importance of understanding signaling dynamics and context to tease apart the complex mechanisms leading to the selection of appropriate signaling outputs.

Signaling context and cellular history generate diversity in outputs

One of the purposes of intercellular signaling systems is to produce different transcriptional profiles in different cell types. The simplest way to achieve this, in theory, would be to induce each cell type with a different signaling pathway, each regulating different target genes. However, the discrepancy between the number of cell types and the number of signaling pathways indicates that this mechanism cannot explain the diversity of cell types identified. Signaling through different pathways does however lead to diverse transcriptional responses, such that the differential use of these pathways goes some way towards generating different cell types. The basic mechanism by which this occurs is relatively simple, with different signaling pathways regulating different transcription factors [1] (Figure 1, Table 1). For example, the BMP (bone morphogenetic protein) pathway regulates Mad, and the JNK (Jun N-terminal kinase) pathway regulates Jun and Fos.

Given that the differences in the transcriptional outputs between signaling pathways are insufficient to produce all of the diversity of responses, other mechanisms must exist that further diversify the outputs from signaling pathways. One way in which this occurs is via the combined effects of multiple signals, which generally produce non-additive effects compared to activation of the pathways in isolation [2–4]. Therefore, the signaling context in which pathway activation occurs can lead to regulation of a subset of genes distinct from activation of each pathway alone (Figure 1). For example, correct expression of the *pax2* gene in the *Drosophila* eye requires direct inputs from Su(H) and Pnt, that are downstream of the Notch and EGFR (epidermal growth factor receptor) pathways, respectively, neither of which is sufficient alone [5].

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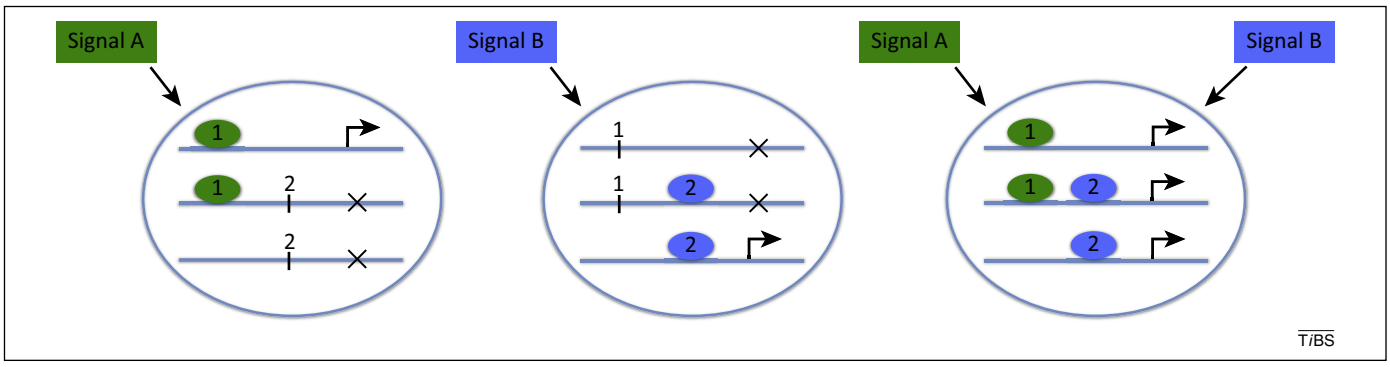

Figure 1. Signaling pathways produce distinct transcriptional outputs. Signaling pathways can produce distinct transcriptional outputs by binding to the regulatory sequences of different genes. Oval shapes labeled 1 and 2 represent transcription factors regulated downstream of signals A and B, respectively, and their effects on three classes of genes (blue lines) are illustrated. Arrows indicate gene activation and crosses represent the lack of activation. In some cases a single signal may be sufficient; in the first two examples, binding of transcription factor 1 or 2 is sufficient to activate the top or bottom target gene respectively. In other cases combinatorial inputs may be required for activation; in the third example, both transcription factors must be bound for activation of the middle gene. This leads to diversity of signaling outputs depending on the signaling contexts in which a pathway is activated.

Another way in which context can alter the output of signaling pathways is through the presence or absence of specific transcription factors. This is predetermined by the history of the cell, including previous signaling events, and provides a transcriptional background in which a signal is received. Therefore, integration of signaling pathway transcription factors with context-specific transcription factors can alter the response (Figure 2).

One example of this is the interaction between Notch and EGFR signaling pathways in different cell types within the developing wing disc. Notch and EGFR pathways have an antagonistic relationship in the wing pouch, with EGFR promoting wing vein formation and Notch inhibiting it [6–12]. EGFR activity in the vein tissue stimulates expression of Delta [6], which activates Notch signaling in the neighboring intervein tissues. Notch, in turn, inhibits the expression of EGFR components including *argos* and *rhomboid* in the intervein tissues [6,12]. This leads to exclusive activity of the two pathways in their respective tissues and is essential for correct patterning of the wing veins. However, a recent investigation into the role of Notch signaling in the adult muscle progenitor cells (AMPs) in the wing disc identified a different interaction between these pathways, with Notch signaling activating *argos* and the two pathways playing cooperative roles in maintaining the undifferentiated state of the cells [13]. How, then, could Notch inhibit *argos* in intervein cells and activate it in AMPs? Further investigation into the regulation of *argos* by Notch in these two contexts demonstrated that different enhancer sequences are used in each tissue. One mediates direct activation of *argos* by Su(H) in the AMPs, whereas the other mediates its indirect repression via HLHmβ [14], a direct target of Notch signaling in the wing pouch. These results suggest that enhancers are selected based on the expression of tissue-specific transcription factors that modulate enhancer accessibility. In cultured cells derived from the AMPs, expression of the transcription factor Twist is required for Su(H) binding to the relevant *argos* enhancer, and overexpression of Twist in the wing pouch can convert the regulation of *argos* downstream of Notch from repression to activation [14,15]. A similar factor (Vvl) was identified that may play a role analogous to that of Twist in regulating enhancer activity in the wing pouch [14].

Table 1. Key components of Drosophila canonical signaling pathways

Pathway	Receptor	Ligand	Transcription factor
Hormone receptors	e.g., EcR	e.g., Ecdysone	EcR
Notch	Notch	Delta, Serrate	Su(H)
JAK STAT	Domeless	Upd, Upd2, Upd3	STAT92E
RTK	EGFR	Spitz, Kerren, Gurken, Vein	Pointed, Yan, Cic
	FGFR (breathless)	Branchless, Heartless, Thisbe, Pyramus	Pointed,Yan
	InR	Dilp1-Dilp7	Pointed, Yan, Foxo
	PVR	Pvf1-3	Pointed,Yan
	Torso	Trunk, PTTH	Pointed,Yan
	ALK	Jelly belly	Pointed,Yan
	Sev	Boss	Pointed,Yan
Hh	Patched	Hh	Ci
TGFβ (BMP)	Thickveins, Saxophone, Baboon, punt	Dpp, Gbb	Mad, Medea, Smox
Hippo	Fat	Dachsous	Yorkie
NF-κB	Toll	Spatzle	Dorsal
JNK	Eiger	Wengen	Jun, Fos
Wnt	Fz, Fz2, Fz3	Wg, Wnt1-10	Armadillo, Pan (TCF)