Lighting Up ERK Activity

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Activation of extracellular signal regulated kinase (ERK) is used by many signaling pathways to control tissue patterning in a broad range of multicellular organisms. In this issue of *Developmental Cell*, Johnson et al. (2017) provide an optogenetic approach to manipulate this pathway with high precision and explore its signaling code.

The ERK pathway is typically activated by receptor-tyrosine kinases (RTKs) that are triggered by tightly regulated presentation of ligands. ERK is a broad specificity kinase, and its predominant targets are transcription factors that induce or repress gene expression. The "bow-tie" configuration of the ERK pathway poses a conceptual problem. Despite the tight and distinct regulation of the ligands triggering the respective RTKs, all signals funnel through ERK (Citri and Yarden, 2006). How, then, are the distinct identities of the different signals activating the ERK pathway encoded (Simon, 2000)? The mode of ERK signaling, such as the strength of activation or its temporal dynamics, may convey additional information on the nature of the respective RTK pathway.

The central developmental roles of ERK signaling were demonstrated by the phenotypes of loss-of-function mutations for various components of the pathway in model organisms. Moreover, gain-of-function mutations also result in severe phenotypes (Brunner et al., 1994), suggesting that the pathway is not only an essential switch but also conveys instructive spatiotemporal information. However, to study the ERK pathway at high resolution, the position, level, and duration of signaling must be manipulated with a precision beyond that provided by traditional approaches relying on ectopic, or tissue-specific, expression of gain-of-function mutants. This is now achieved in a paper by Johnson et al. (2017), who present a novel optogenetic approach for ERK activation, thereby providing new avenues to explore the signaling code of this pathway.

The normal pattern of ERK activation has been well characterized using antibodies that specifically recognize its active, double-phosphorylated form (termed dpERK) and hence mark all tissues and cells in which RTK signaling is activated. The use of such antibodies revealed the diversity of ERK regulation by RTKs throughout *Drosophila* embryonic and larval development (Gabay et al., 1997a, 1997b). Interestingly, signals from different RTKs do not converge in space and time in this setting, so each aspect of the dpERK pattern could be assigned to a distinct receptor. Thus, it was determined that normal activation of some RTK receptors is highly restricted (e.g., Torso), while in other cases—most notably the epidermal growth factor receptor (EGFR) homolog—activation occurs numerous times over the course of embryogenesis and in multiple tissues.

The approach taken by Johnson et al. (2017) utilizes regulated expression of a light-switchable membrane anchor that can recruit the RAS activator protein Son-of-Sevenless (SOS), a key pathway mediator, to the plasma membrane, thereby initiating constitutive activation of ERK signaling. This activation is rapid and reversible within a time frame of 1–2 min and can be tuned by the duration and intensity of illumination. With this sensitive tool in hand, ERK activation can be precisely manipulated in developing *Drosophila* embryos.

Utilization of the optogenetic switch was initially applied in early embryos, a phase in which RTK (Torso) activation is restricted to the embryonic termini, to ask whether hyperactivation of ERK in the correct time and place would have an effect. This manipulation did not disrupt normal development, indicating that at this early phase and in this particular position, the only requirement for the signal is to reach a critical threshold. The benign outcome of hyperactivation in this setting is to be expected, because Torso-mediated activation of ERK leads to phosphorylation and inactivation of the transcriptional repressor Capicua (Jiménez et al., 2000), and the added surplus of activation should not have an effect. In contrast, activation of the construct at the same time in other parts of the embryo, where no RTK is active, had deleterious effects, even in illumination stripes as narrow as 40 μm. These ectopic effects indicate that all cells can respond to ERK activation at this phase and that it is the localized activation of Torso that determines which cells will give rise to the termini of the embryo.

Light activation of SOS at later stages of embryogenesis gave rise to very different responses than those observed following early stimulation. Uniform illumination of the embryo at different phases resulted in a corresponding elevation in ERK activation that was superimposed on the normal dynamic pattern. Surprisingly, despite the marked elevation of activated ERK levels, this manipulation did not lead to phenotypic abnormalities, and normal larvae were recovered. This result was unexpected and begs the question of how it can be reconciled with what we know about the instructive nature of RTK signaling throughout development. Why was transient activation of the ERK pathway by light not able to recapitulate and harness the inductive potential of the pathway?

RTK signaling may be a simple switch that is sufficiently confined by other overlapping pathways and that therefore cannot elicit ectopic fates on its own. However, the established consequences of pathway hyperactivation upon removal of inhibitors would tend to rule out this possibility. Thus, in the absence of

endogenous inhibitors of RTK signaling, ERK-induced cell fates were expanded, arguing for an instructive role of ERK signaling (Freeman et al., 1992; Kramer et al., 1999). An alternative possibility is that RTK signaling pathways branch downstream of the receptor, such that ectopic SOS/RAS activation on its own would not suffice to trigger a biological response. This, again, is not consistent with what we know about the consequences of constitutively activated RAS or ERK, which are highly potent and capable of inducing ectopic cell fates in various tissues (Brunner et al., 1994).

Perhaps one of the primary features of the optogenetic method—its immediate and confined activation—is actually the reason for its limited biological effect. The normal activation of each RTK pathway represents a temporal succession, with a typical time window of activation, and possibly also a stereotypic profile of activation magnitude during this window. We can envision, for example, a situation in which an initial wave of low-level activation leads to modification of the chromatin in certain regions of the genome, creating the competence for activation by transcription factors at a subsequent stage. A short pulse of activation driven by light that is restricted in time may not give rise to aberrant phenotypes in the absence of the initial preparatory stage. A case in point is the Sonic hedgehog (Shh) pathway, in which a stereotypic alteration in the intensity of the signal over time leads to distinct zones of target-gene expression in the vertebrate ventral nerve cord (Balaskas et al., 2012). The cells are thus able to integrate the level and duration of Shh signaling.

In conclusion, the availability of a new tool to trigger the ERK pathway at will provides a potent way to manipulate the cascade in a variety of model organisms at high temporal and spatial resolutions. This approach may reveal the underpinnings of the signaling code and its specificity, not only in situations where it leads to ectopic consequences, but also—perhaps even more interestingly—in those cases where it is not able to elicit the expected response. This new optogenetic approach may now provide a handle to manipulate and explore the spatiotemporal code of the ERK signal.

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Mis-placed Congeniality: When Pathogens Ask Their Plant Hosts for Another Drink

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Plants control nutrient availability in intercellular spaces (the apoplast) via transporters, channels, and vesicular transport. Recent papers in *Science* and *Nature* from two groups have highlighted how plants control sugar to restrict bacterial growth (Yamada et al., 2016) and how increased water availability enhances pathogenesis (Xin et al., 2016).

The field of plant/microbe interactions, like Christmas, contains elements of ritual. The plant immunity response is routinely referred to as the "Zig-Zag-Zig" of pattern-triggered immunity (PTI) via cell-surface receptors, suppression of PTI by pathogen effectors, and recognition of effectors (directly or indirectly) by intracellular Nucleotide-binding, Leucine-rich Repeat (NLR) immune receptors (Dodds and Rathjen, 2010; Jones and Dangl, 2006; Jones et al., 2016). But two recently published papers (Yamada et al., 2016; Xin et al., 2016) have started to identify how incomplete this picture is and to fill in the gaps.

After successful entry into the host, plant pathogens require water and nutrition, including sugars, for growth. Plant sugar transporters regulate sugar levels in different compartments (Lalonde et al.,