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Developmental ERK Signaling Goes Digital

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Reporting in *Developmental Cell*, de la Cova et al. (2017) present a biosensor to measure ERK activity dynamics in *C. elegans* larvae. They find that fate decision signaling involves frequency-modulated, digital ERK activity pulses. These findings may explain how graded morphogen signals are converted into precise and robust cell fate patterns.

A contemporary question is how signaling networks decode extracellular inputs to robustly induce specific cell fates in space and time during development. Three decades of genetic analysis have made the *C. elegans* vulva system a central model for signaling and cell fate determination during organogenesis. In this system, the EGFR/Ras/ERK, NOTCH, and WNT signaling networks cooperate to induce a specific and robust pattern of cell fates in the five vulval precursor cells (VPCs). Ultimately, this leads the P6.p VPC to exhibit primary VPC fate and the two neighbor VPCs cells, P5.p and P7.p, to display a secondary VPC fate, whereas the P4.p/P8.p VPCs display a tertiary fate (Figure 1). Previous genetic studies proposed that an EGF gradient secreted by the anchor cell is important for fate specification through EGFR/Ras/ERK signaling (Schmid and Hajnal, 2015). This model implies that there must be a mechanism to interpret this EGF gradient and convert it into discrete ERK signaling states, specifying the different fates within this cell community. An ability to monitor ERK activity *in vivo* in single cells is thus necessary to refine our understanding of this process.

Reporting in the current issue of *Developmental Cell*, de la Cova et al. (2017) adapt a pre-existing genetically encoded ERK biosensor (Regot et al., 2014), ERK-KTR, for *in vivo* imaging in worms. ERK-KTR consists of a synthetic ERK substrate that monitors ERK kinase activity through a nucleocytoplasmic translocation event. Thus, when ERK activity is either low or high, the reporter will localize to the nucleus or the cytosol, respectively. The ratio of cytosol/nuclear ERK-KTR fluorescence can be used as a proxy for ERK activity. Although this biosensor allows measurement of ERK activity in single cells cultured in a dish, its application to *in vivo* imaging of complex 3D tissues is difficult because of cell-shape segmentation issues. For that purpose, the authors designed the ERK-nKTR sensor. This modified sensor consists of a bicistronic operon expressing ERK-KTR-mClover and a mCherry-tagged nuclear marker. ERK activity is then monitored by calculating the ratio of nuclear (red) to ERK-KTR (green) fluorescence, allowing for a readout that is independent of cell shape or biosensor expression levels. Furthermore, nuclei can easily be tracked automatically to follow signaling dynamics. Finally, ERK-nKTR displays a larger dynamic range compared to fluorescence resonance energy transfer (FRET)-based biosensors, and ERK activity can be detected visually without any image processing.

The authors use this biosensor to visualize ERK activity patterns in different *C. elegans* developmental contexts at the single-cell level. They validate that ERK-nKTR responds to genetic perturbations of ERK signaling network components and focus on EGF gradient-triggered ERK activity dynamics in VPCs. Surprisingly, they observe ERK activity pulses of similar amplitude and duration in all the VPCs. That is, ERK activity appears digital. Strikingly, however, the frequency of these digital pulses differs in VPCs adopting the different fates (Figure 1). Thus, the P6.p VPC, which will adopt the primary fate, displays a high frequency of these digital ERK activity pulses, whereas the adjacent VPCs, which will adopt secondary/tertiary fates, display much lower pulse frequencies (Figure 1). These results imply that the VPCs convert the graded EGF gradient into a frequency-modulated, digital ERK signaling response, which specifies the different vulval fates.

This feature of developmental ERK signaling brings up several questions. First, how common is the phenomenon of frequency-modulated digital EGFR/Ras/ERK signaling? Similar digital ERK activity pulses have been documented in EGF-stimulated cultured epithelial cells (Albeck et al., 2013). Here, the EGF dose controls the frequency of ERK activity pulses of fixed amplitude and duration. Moreover, the time ERK remains ON correlates with cell-cycle entry, providing a link between the EGF dose and cell-cycle entry efficiency. Another example is the PC-12 cell fate determination system. In this system, EGF-triggered transient ERK activity leads to a proliferative fate, whereas NGF-triggered, sustained ERK activity leads to differentiation (Ryu et al., 2015). However, pulsed EGF stimulations used to artificially evoke multiple digital ERK activity pulses at a specific frequency lead to a differentiation response (Ryu et al., 2015). These lines of evidence emphasize the widespread ability of EGF to encode cell fate decisions through frequency modulation of ERK signaling. Second, what is the mechanism that generates digital ERK signaling, and what are the benefits associated with such a system? The ability to translate a graded EGF signal into a digital ERK signal of fixed amplitude and duration is most likely dependent on a negative feedback amplifier signaling network topology (Birtwistle and Kolch, 2011). This involves the sequential Raf-MEK-ERK phosphorylation cascade, combined with negative feedback from ERK to Raf, or other upstream signaling components. Beyond the ability to perform such highly non-linear signal transmission, this network topology also provides several benefits. Theoretical studies have shown that such "bursty" signaling responses

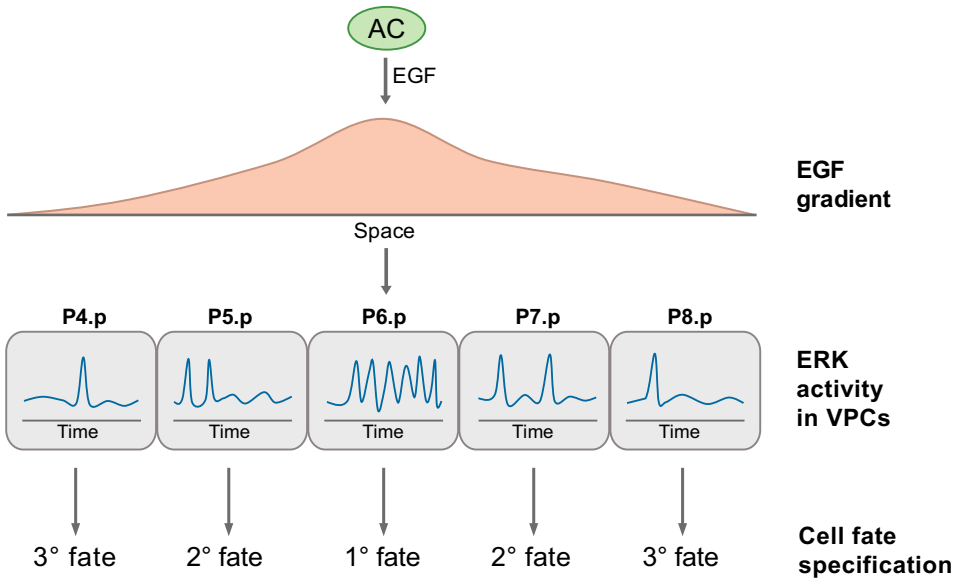

Figure 1. Converting a Graded into a Digital Signal
As a consequence of their distinct spatial locations, each of the five VPCs perceives a different level of EGF released from the anchor cell (AC). In response, the VPCs display ERK activity of fixed amplitude and duration but with different frequencies, corresponding to their location in the EGF morphogen gradient.

are highly accurate for fast signaling events (Micali et al., 2015). Further, this specific network architecture has been shown to provide resilience against external perturbations (Birtwistle and Kolch, 2011). The network topology underlying digital ERK signaling may thus have evolved to ensure robust developmental signal transduction in the presence of environmental perturbations. Finally, how is the digital signaling response integrated into a transcriptional output? Conversion of a graded EGF input into discrete cell fates thanks to frequency-modulated digital ERK signaling suggests the existence of mechanisms that can interpret the ERK signal (e.g., the number of ERK activity pulses) to produce fate-relevant transcriptional programs. In mammalian cells, it is known that the transcription factor FOS serves as a sensor for ERK signal duration (Murphy et al., 2002). Upon transient ERK activation, ERK-mediated transcriptional activation allows FOS to be rapidly synthesized but then immediately degraded, resulting in limited FOS transcriptional activity. In contrast, persistent ERK activity leads to ERK-mediated FOS phosphorylation that prevents its degradation, sustaining the ability of FOS to induce a fate-relevant, transcriptional program. In the *C. elegans* vulva, the LIN-1 transcription factor represses differentiation and is inactivated by ERK phosphorylation leading to the primary fate in P6.p (Schmid and Hajnal, 2015). The ability of each VPC to produce robust ERK activity pulses suggests that there might be a similar mechanism to interpret the frequency-modulated ERK response.

To summarize, the robust biosensor technology presented by de la Cova et al. (2017) enables the resolution of dynamic (rather than steady-state) *in vivo* signaling states. Such a biosensor is essential for understanding features of ERK signaling systems and for providing clues into how accurate spatial signal transmission is achieved. Systematic ERK activity measurements in response to genetic perturbations, combined with modeling studies, have the potential to provide new insights into how EGFR/Ras/ERK signaling regulates developmental fate patterning.

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