

# Shooting from the hip: Spatial control of signal release by intracellular waves

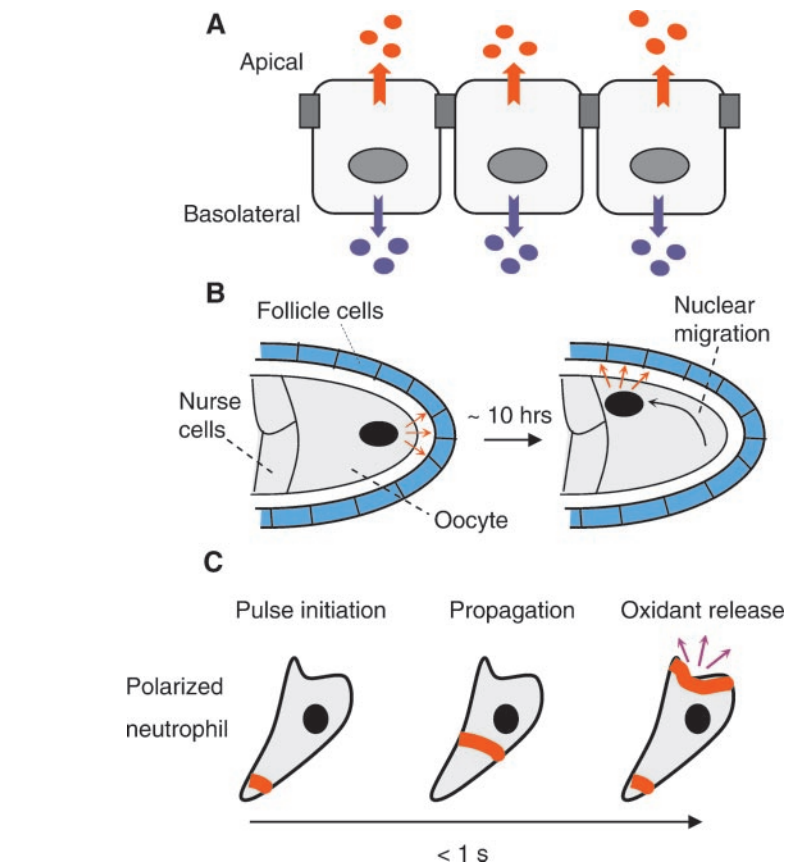
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The versatility of cell–cell communication relies on sophisticated modules for signal generation, transmission, detection, and processing (1). Compared with signal detection and intracellular processing, signal release is relatively poorly understood. For instance, even though the epidermal growth factor receptor (EGFR) signaling network has been extensively studied over the past 4 decades, the molecules that mediate release of EGFR ligands are being identified only now (2–5). The outstanding issues in signal release are similar to the ones studied in the context of signal detection and processing (6). What are the basic patterns in the dynamic release of small molecules, ions, and hormones? What are the constraints on the time scales of signal release imposed by the corresponding time scales of the signal transmission and detection? Finally, can a cell point its signals toward its neighbors, in a way similar to aiming a gun at a target or to directing electromagnetic and acoustic signals in man-made communications networks?

Spatially directed fluxes of soluble signals can be generated by mechanisms that restrict secretion to a specific region of the cell surface. This mode of release can be accomplished by a “static” design, in which a signal is always released in the same direction. By using this strategy, epithelial cells can direct secretion of soluble molecules to their apical or basolateral membranes, Fig. 1A. Several aspects of epithelial physiology, such as release of digestive enzymes and wound healing, critically depend on the polarized secretion of soluble signals (7). Although this static design is a natural consequence of the static epithelial polarity, a different strategy is required for a developing cell or for a cell that finds itself under rapidly changing conditions. Under these circumstances, it might be necessary to redirect fluxes of secreted signals.

Signal release can be redirected by rearranging the intracellular architecture. An example of such design is found in fruit fly development. The body axes of *Drosophila* embryo are determined during egg development by the localized interaction



**Fig. 1.** Static (A) and dynamic (B and C) strategies for the generation of directed fluxes of extracellular signals. (A) Signal direction is fixed by cell orientation. (B) Nuclear migration redirects signal release from the *Drosophila* oocyte. (C) Signal direction is dynamically regulated by propagating intracellular waves.

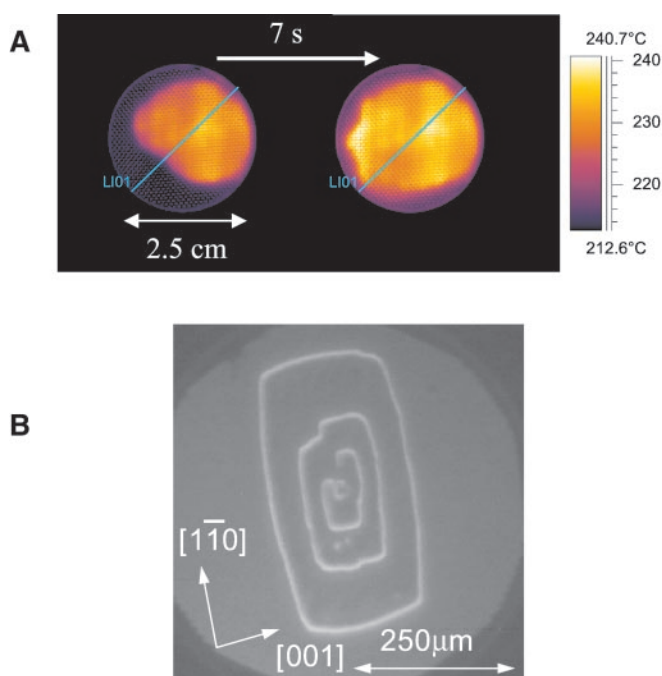
between the oocyte and the overlying epithelial follicle cells (8). This interaction is mediated by the localized release of the *Drosophila* EGFR ligand (Gurken) from the oocyte (ref. 9; Fig. 1B). The anterior-posterior axis is specified when Gurken is secreted from the posterior cortex of the oocyte. Later in egg development, the same signal released from the dorsal-anterior cortex initiates dorsoventral patterning of the eggshell and the embryo. In this system, signal release (Gurken secretion) is dynamically redirected from the posterior to the dorsal-anterior part of the cell as a result of regulated nuclear migra-

tion and localized mRNA processing. The time scale for redirecting the signal release in this system is several hours (8). It is easy, however, to imagine the circumstances under which a much faster strategy might be needed.

For example, a cell moving in a gradient of a chemical released by its neighbor might respond by sending a signal up the gradient. Because both the cell that releases a signal and the target cells can be moving, this type of dialogue requires a

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**Fig. 2.** Combination of autocatalytic chemistry (e.g., calcium-induced calcium release) with diffusion is a basic mechanism for the generation of propagating reaction waves (16, 21). Examples of reaction waves in catalysis on metal surfaces: (A) Temperature wave observed during CO oxidation on the surface of a supported Pd catalyst. The wave arises from the interaction between the autocatalytic heat release and heat conduction. (B) Spirals and target patterns in the isothermal NO reduction on an Rh single-crystal catalyst. The wave arises from the interaction between the autocatalytic generation of empty catalyst sites and adsorbate diffusion. Thus, a combination of autocatalytic chemistry (e.g., calcium-induced calcium release) with diffusion is a basic mechanism for the generation of propagating waves.

dynamic and flexible mechanism for redirecting secreted signals. This strategy would enable a cell to quickly “take aim” and “shoot” directed messages into its microenvironment.

In the paper appearing in this issue of PNAS, Kindzelskii and Petty report that polarized neutrophils periodically release pulses of reactive oxygen metabolites from their leading edge (10). The authors have discovered that both the directionality and the timing of oxidant release are determined by propagating pulses of metabolite (NADPH) concentration (Fig. 1C). NADPH is a substrate for the NADPH oxidase, an enzyme that is uniformly distributed throughout the cell membrane and catalyzes the oxidation of NADPH with oxygen superoxide,  $O_2^-$ , as one of the products (11). By using imaging spectrophotometry and high-speed microscopy, Kindzelskii and Petty have observed that the NADPH pulse is periodically generated at the cell back, travels through the cell at high speed ( $\approx 50 \mu\text{m}/\text{sec}$ ), and disappears when it hits the lamellipodium. The arriving pulse of NADPH concentration supplies NADPH oxidase with its substrate and generates localized release of superoxide. This locally generated oxidant is the starting point in the production of reactive species that initiate deg-

radation of bacteria and other targets of neutrophils.

This important observation adds an interesting detail to neutrophil biology. In the extended model of target degradation, neutrophils chemotact toward extracellular targets and send directed signals for their destruction. The model is supported by the fact that the intracellular NADPH pulses can be controlled extracellularly by molecules resembling bacterial peptides. This elegant mechanism can be tested by visualizing the oxidant release in a neutrophil approaching one of its natural targets. The state-of-the-art biophysical techniques developed by Kindzelskii and Petty make such measurements possible.

Generation of directed fluxes of extracellular signals by the intracellular concentration waves could be a general phenomenon in cell biology. Another example of this mechanism is the polarized release of digestive enzymes by pancreatic acinar cells. In this case, a calcium wave initiated at the basal pole of the cell propagates to its apical pole, where it triggers exocytosis of secretory granules packed with digestive enzymes (12). It is well established that proteolytic release of a large class of transmembrane proteins can be dynamically regulated by the levels of intracellular calcium (13, 14). Hence, it would be important to test whether re-

lease of other classes of soluble signals, such as peptide growth factors, can be “focused” by the intracellular calcium waves.

By connecting the intracellular waves with the mechanism for regulated signal release, Kindzelskii and Petty have proposed an additional role for the intracellular reaction–diffusion patterns (15). These patterns, which can be both localized and traveling, arise from the interaction between nonlinear chemical reactions and transport of second messengers or metabolites (16). Intracellular patterns formed by reaction and diffusion perform a variety of tasks. For example, robust generation of localized patterns of protein concentration is critical for directional sensing in chemotaxis (17). Propagating calcium waves orchestrate the early development during the zygote and cleavage periods (18). In bacteria, pole-to-pole oscillations of protein concentrations determine the position of the cell division plane (19). These are just a few examples of the physiological role of *intracellular* reaction–diffusion patterns.

Starting with the work by Kolmogorov, Petrovskii, and Piskunov (20), reaction–diffusion waves have been extensively studied by mathematicians, physicists, and chemists (21, 22). Their results can contribute to understanding of the role of these waves in signal transduction. Uniformly propagating fronts and pulses, such as the ones imaged by Petty’s group (23, 24), correspond to the self-similar solutions of the underlying reaction–diffusion equations. The self-similarity comes from the fact that the concentration profile evolves as a wave: in the simplest case, the profile at any given instant is obtained by the spatial shift of the profile at an earlier time point. Thus, a signal can be delivered to an arbitrary distance without changing the amplitude. This mechanism is very different from the one that relies on pure diffusion. The speeds of nonlinear reaction–diffusion waves depend both on transport properties (such as diffusion coefficients) and on the rate constants of chemical reactions involved (16, 21). This combined dependence on chemistry and transport can make the chemical waves move much faster than purely diffusing signals. Furthermore, uniformly propagating self-similar solutions are attracting in the sense that they capture the “intermediate-asymptotic” behavior of solutions under a wide range of initial and boundary conditions (25). These properties make signal delivery by nonlinear reaction–diffusion waves both reliable and quick.

Future biochemical and cellular studies of metabolic waves in neutrophils and their link to oxidant release are needed to formulate quantitative models of these

spatiotemporal patterns (11, 24). Quantitative modeling has been quite successful in complementing the experimental studies of reaction waves in a number of other biochemical and physicochemical settings (16, 21). The models of propagating waves of adsorbate concentrations and temperature on surfaces of solid catalysts are particularly advanced (26). These models show that traveling waves are formed by very simple chemistries coupled to mass and/or heat transfer (ref. 27; Fig. 2). For a long time, engineers have been discussing the possibility of using these catalytic patterns as building blocks of nonstationary regimes in chemical reactors. At this

point, there is a single commercial design that harnesses propagating waves—a reverse-flow reactor used for the oxidation of volatile organic compounds (28).

In addition to their possible applications in chemical engineering, inorganic reaction–diffusion waves can form the basis of man-made communication and computational devices (29–31). This application closely mirrors the role these patterns play in cell signaling. Engineering of reaction–diffusion computers and communication networks naturally leads to a number of new problems at the intersection of dynamical systems and control theories. For example, surprisingly little is

known about the initiation of reaction–diffusion waves or about steering the fronts by external signals. Notably, whereas these problems present a considerable challenge to control theorists, neurophilosophers “solve” them with apparent ease as they control the directionality, the speed, and the number of NADPH pulses in response to extracellular cues (23).

I thank Ronald Imbuhl (University of Hanover) and Moshe Sheintuch (Technion) for providing the images of reaction waves observed in their laboratories, and Anatol Zhabotinsky (Brandeis) for helpful discussion.

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