

Quantifying collective behavior in mammalian cells

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Collective social behaviors generate one emergent response from many individual actors without central coordination: bees swarm, birds flock, and fish school. Single-celled bacteria and amoebae collectively swarm, detecting chemical signals emitted by close neighbors to synchronize their motion. Within organisms, collective behaviors drive multicellular processes during migration and wound healing. Similar to unicellular organisms, mammalian cells coordinate their responses during these complex processes by detecting changes in concentration of signaling molecules. However, the signaling pathways used by mammalian cells are typically more interdependent than those in unicellular organisms, and gene expression varies with the time-dependent concentration of signaling molecules. Understanding the role of intercellular communications for collective behavior in mammalian cells thus demands sensitive measurements of responses to chemical signals. In PNAS, Sun et al. (1) quantify the spatiotemporal evolution of calcium response to ATP stimulation in fibroblasts. They find that the response of dense colonies of cells to ATP stimulation is faster and more synchronized than that of isolated cells. By quantitatively correlating responses between neighboring pairs of cells, they identify pacemaker cells; by physically separating cells in a hydrogel matrix, they confirm that cells communicate via gap junctions to synchronize their responses. These experiments show that both temporal and spatial dynamics of collective response in mammalian cells depend on intercellular signaling.

In bacteria, transitions from individual to collective behavior are frequently coupled to changes in cell density. For example, bacteria transition from mechanisms of individual motility (2) to collective motility modes, such as twitching (3), when the cell density exceeds a critical threshold. Similarly, initially free-living planktonic bacteria form surface-associated communities called biofilms when the density of cells and the population size exceed critical thresholds, so that the concentration of signaling molecules (autoinducers) in the space between bacteria becomes sufficiently large (4) to initiate changes in phenotype. This process allows bacteria to sustain a single behavior from a collection of noisy and stochastic inputs (5).

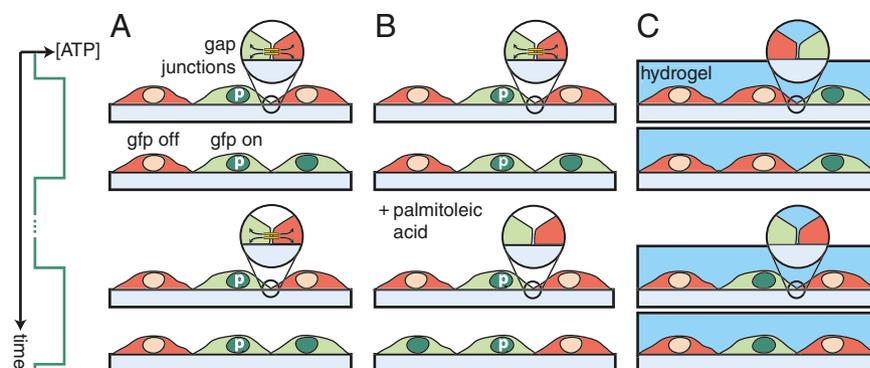


Fig. 1. (A) Fibroblasts use chemical signals, exchanged via gap junctions, to establish a reproducible sequence of responses to ATP stimulation between a pacemaker (labeled p) and its neighbors. (B) Disrupting gap junctions with palmitoleic acid destroys the established temporal order. (C) Preventing formation of gap junctions by encasing cells in a hydrogel matrix eliminates pacemakers.

Changes from individual to social behavior in the unicellular amoeba *Dictyostelium discoideum* are also coupled to cell density. Synchronized waves of a molecular signal (cAMP) direct individual cells to form aggregated fruiting bodies. The proximity of nearby cells in dense colonies plays two important roles in this process. First, random fluctuations in the production of cAMP by neighboring cells below the activation threshold can initiate synchronization throughout the population (6). Second, a subpopulation of hypersensitive first-responder cells can also initiate synchronized waves (7). Studies in unicellular organisms thus highlight the roles of cell density and individual variation in intercellular signaling leading to collective behavior. Direct tests of the importance of these factors for intercellular signaling in mammalian cells, which exhibit tightly controlled signaling pathways because the penalties for deviant behavior are severe, require experiments that carefully measure collective responses.

Here, Sun et al. (1) develop techniques to quantify spatiotemporal variations in chemosensing in fibroblasts, which synthesize the structural components of the ECM. When the ECM is damaged, fibroblasts must first migrate to the wound and then deposit new collagen and other ECM components to heal it. Because this process requires coordinated action by multiple cells, fibroblasts are a useful model system in which to investigate collective response to chemical signals. Fibroblasts stimulated by ATP release calcium, a second-messenger signal for processes ranging from proliferation to collagen

synthesis, and the intracellular calcium concentration can be measured using fluorescent calcium indicators.

Analogies to unicellular organisms suggest potential factors for collective chemosensing in mammalian cells. Because collective behavior emerges in bacteria and amoebae when the density of cells is sufficiently high, Sun et al. (1) first create adjacent 2D communities of fibroblasts of different cell densities in a microfluidic chamber. The fluorescence intensity of individual cells oscillated over time when exposed to an external flow of ATP, showing that ATP stimulation induced release of calcium. On average, the activation time required for a cell to reach a threshold intensity was shorter for cells in the high-density colony than for cells in the low-density colony, independent of flow direction. Differences in distributions of activation times for colonies of low and high densities showed that the response process was not random. Together, these observations indicated that cells must communicate to generate responses that are internally correlated.

Fibroblasts communicate with each other using two main mechanisms. Adjoining fibroblasts can form gap junctions, channels that connect the cytoplasm of the cells and allow direct diffusion of small molecules; in addition, diffusing molecular messengers can transmit information through the extracellular space. Because

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both mechanisms of communication rely on diffusion, the signals that most strongly influence the response of a cell should come from its closest neighbors. Likewise, the greatest synchronization should arise between neighboring cells, as suggested by earlier experiments that correlated the periods of calcium oscillations for neighboring cells (8). Sun et al. (1) sensitively measure synchronization by calculating the cross-correlation between the gradient of the fluorescence response curves of adjacent cells. Even if cells do not oscillate in perfect synchronization, the width and magnitude of the cross-correlation nonetheless yield information on the extent of temporal correlations. With this measure, Sun et al. (1) confirm that the response of fibroblasts was correlated only when the cell density exceeded a critical threshold.

In unicellular systems, dense colonies of cells produce a large concentration of signaling molecules that initiate collective behavior; by analogy, the concentration of ATP should also affect the collective spatiotemporal dynamics of calcium response in fibroblasts. Indeed, in dense colonies, the shape of the fluorescence response curves varied with ATP concentration, affecting the width of the distribution of oscillation frequencies but not the peak frequency. Comparison with earlier work on calcium oscillations (9) suggests that the signaling pathway used by the cells did not vary significantly with ATP concentration. Fibroblasts also exhibited a nonuniform spatial response to ATP stimulation, with “pacemakers” that consistently exhibited the fastest activation times. This result suggests that the threshold level for activation of calcium response varies between fibroblasts, simi-

lar to variations in threshold observed in social amoebae (7). Surprisingly, the neighbors of pacemakers also responded in a reproducible order (Fig. 1A), as previously seen in smooth muscle cells (10).

Sun et al. quantify the spatiotemporal evolution of calcium response to ATP stimulation in fibroblasts.

Inhibiting the permeability of gap junctions disrupted the established ordering of activation times (Fig. 1B), indicating that the signals for this response propagated through gap junctions.

Do these spatiotemporal features of collective response persist in the absence of gap junctions? To prevent the formation of gap junctions, Sun et al. (1) physically separate the cells by encasing them in a hydrogel matrix. However, cells could still communicate via messenger molecules that diffuse through the extracellular space. The absence of gap junctions eliminated pacemakers and destroyed the correlations between activation times of neighboring cells (Fig. 1C). Nonetheless, certain spatiotemporal features persisted at high cell densities: Calcium oscillations induced via signals transmitted through gap junctions or through diffusing molecules shared a characteristic frequency. This observation again suggests that different short-ranged mechanisms for cellular

communications excite at least one similar signaling pathway within the cells.

The elegant experiments of Sun et al. (1) demonstrate that fibroblasts exhibit collective chemosensing in which the spatial and temporal characteristics depend on cell density and connectivity. These similarities to social behavior in unicellular organisms suggest other factors, such as population size (4), that may influence collective chemosensing in fibroblasts. The quantitative techniques developed by Sun et al. (1) offer a previously undescribed route to characterize the collective response of fibroblasts to mechanical stress, and thereby gain unique insight into collective behaviors initiated by calcium signaling during wound healing (11) or cell migration (12). In addition, combining the careful measurements of oscillation frequency with genetic analyses (9) may illuminate specific pathways associated with collective behavior. Finally, analogies to systems that exhibit emergent behavior inspire questions that are testable within this framework: Does the topology of connections between neighbors (13) affect collective response? Do multipoint correlations (14) in the calcium response identify heterogeneous regions of dynamics? Does the correlation length of the response (15) scale with the number of cells? Answers to these and related questions will yield unique insight into the processes by which many cells generate a unified response.

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