Fold-change detection of signaling molecules in social amoeba

In the social amoeba *Dictyostelium*, the reponse to cell-cell communication molecule cAMP is fold-change dependent. The mathematical model proposed corresponds well to the quantitative live-cell imaging evidence, and it may have implications on robustness of oscillatory signaling observed in other developmental systems.

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THERE are quite a lot of "model" organism in biological researches, for example *Escherichia coli*, fruitly fly *Drosophila* and the nematode worm *Caenorhabditis*. But beginning back into the nineteenth century there have been many others that have played a similar role. For example, there was Mendel with his garden peas, followed by other organism such as maize, amphibian, chick and sea urchin embryos, yeast, myxobacteria, zebra fish, the small plant *Aribidopsis* and the cellular slime molds. Since the establishment of *Dictyostelium discoideum* as "model" species by Kenneth Raper[1], it has been used in the majority of the experimental work today. One of the most interesting behavior of the slime molds is their feeding. Individually, each amoeba surrounds a bacterium with its pseudopods, encases it in a food vacuole, and extracts the needed nutrients. Once the energy of the bacterium is depleted, they form collectively as a multicellular organism. In starvation, a few cells, start emitting an attractant, and the amoeba around those hot spots turn and orient towards the source. Interestingly, before it was known what was the chemical nature of the attractant, it was called *acrasin*, inspired by a literary allusion to Edmund Spenser's *The Faerie Queene*. These slime molds are members of the *Acrasiales*, and there is a witch in *The Faerie Queene* who, like Circe, attracts men and turns them in beasts. Today we now know that the chemical nature of the acrasin for this species is cyclic adenosine mono phosphate (cyclic AMP, cAMP), a substrate found everywhere and important in animals for passing on the signals from hormones into cells.

Writing in *PNAS*, Kamino *et al.*[2] has exploited the simplicity of this model system, to study the well established question of fold-change detection (FCD)(Fig.1), i.e. how cells adapts to the variation of extracellular stimuli. In *Dictyostelium*'s case, it's the secretion and sensing of cAMP. Before the study, it was previously unclear how a wide range of cell densities will respond collectively to extracellular stimuli, and to what extend will the robustness exhibit. With the aid of fluorescence microscopy and microfluidic devices, they found that the fold-change dependence is a single-cell level property. They also mathematically proved that the secret-and-sense systems with FCD elements can naturally derive the characteristic of invariance of the oscillations to density transformation.

In order to study the magnitude of oscillations, they first quantify a index called FRET, the ratio of fluorescence intensities at 485 and 540 nm (CFP and YFP), which are the reflective wavelength of cAMP molecules under the illumination of 434-nm light, detected by the cAMP sensor Epaclcamps. They calculated FRET ratio at different cell density and different exogenous cAMP concentration conditions, which could be manipulated in the profusion chamber. To do so, they first purfused the developmental buffer (DB) to remove the effects from past stimuli, and then cAMP at different concentrations was injected at constant flow rate. Their result showed that the oscillations were more easily suppressed at low cell densities and by high exogenous cAMP concentrations.

They next wanted to the find the fine boundary at which extracellular cAMP concentration and magnitude change can sustain oscillations. Thus they quantified the initial peak amplitude, and increase cAMP concentration in fold-change step manner. They found: 1) the time to reach peak amplitude showed no dependency on background extracellular cAMP concentration, 2) fold change of stimuli correlated linearly with response amplitude 3) response amplitude plateaued at around 0.1 nM background concentration and maintained the rescaling property for two orders of magnitude. When compared the response amplitude at low and high background concentration of individual cells, they found the linear relationship between the two, suggesting that fold-change dependence is a single-cell level property.

To reconfirm the conclusion, they employed a novel microfluidic device called lighthouse to find cAMP relay response to traveling-wave stimuli, and the result showed that peak FRET index is almost constant in different background concentrations, thus reinforcing the conclusion.

To look into the rescaling prop-

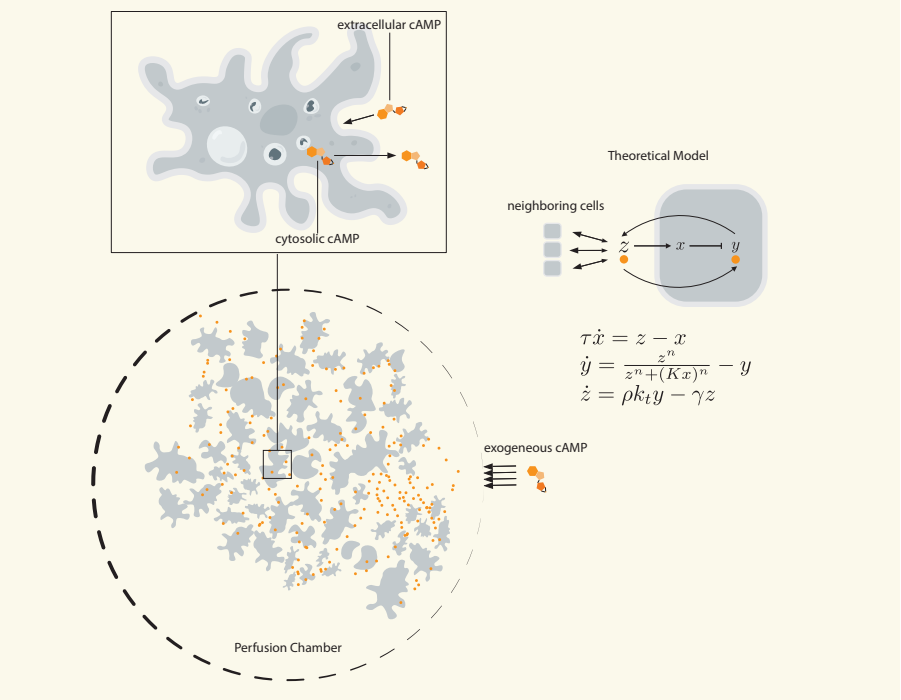

Fig. 1. Social amoeba is capable of detecting fold-change variation of their signaling molecules, cAMP. Kamino *et al.*[2] combined experimental and theoretical approaches to reveal the robustness of fold-change detection. Their theoretical models have predicted on the find boundary of oscillation of cAMP relay, depended on cell density and influx rate of exogenous cAMP. Model A″ shown at the right incorporated one incoherent feedforward loop and one negative feedback loop to exhibit the property of scaling invariance of extracellular cAMP. It also matched with their experimentally obtained phase diagram very well. (In the ODE system, ρ, k_t, and γ represent the cell density, the secretion rate, and the dilution rate, respectively.)

erty, they constructed RH_{CRAC}-RFP fluorescence report system. By the observed synchronous oscillations between the two factors, they concluded that the information encoding and transduction of fold change is related to the degree of membrane translocation of CRAC.

The observations in experiments turned out to have well conjugated explanation in theory, as they have shown next. They extended the proposed FCD theoretical framework to incorporate secret-and-sense variable in the system of ordinary differential equations (ODE), of order 1 and dimension 3 (Fig.1). Out of 3, 2 parameters x, y characterize intracellular state, x relates to adaptation and y relates to cytosolic cAMP concentration. z characterizes extracellular cAMP concentration. With adequate derivations, they found that the ODE system maintains its form under the given transformation: $x \to \phi(p, X), y \to y, z \to pZ, \rho \to p\rho$ where p is the scaling factor imbued with experimental meaning and ρ the cell density. They also proposed the explicit form of functions affecting x and y time derivative, along with the metabolic pathway representations, and thus two kinds of phase diagram were obtained: ρ versus dilution rate γ ; ρ versus the influx of exogenous cAMP concentration z_0. The diagrams showed the demarcation of oscillatory and nonoscillatory domain. Noticeably, these models had almost uniform $\rho - z_0$ diagrams, corresponding well with the experimentally plotted phase diagram. They also revisited the $\rho - \gamma$ diagram of M&G model[3], and the model is also capable of predicting excitability and oscillations in cell suspension, perfused cells and propagating waves in cells on a solid support. The deficiency that it fails to satisfy the rescaling property may suggest further investigations of explicit forms of functions. Despite this, they found that by incorporating FCD into a secrete-and-sense circuitry, the resulting cell–cell communication is robust to cell density change due to the scale-invariant property of the system equations, one of the major insights of their modeling work.

These findings bear the implication that given the ubiquity of adaptation and secrete-and-sense circuits[4], FCD-based robustness may have a wide connotation in other multicellular phenomena, and the research itself has exemplified the approach of finding such invariance, experimentally and theoretically. ■

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