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Myriad Molecules in Motion: Simulated Diffusion as a New Tool To Study Molecular Movement and Interaction in a Living Cell

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The molecular study of life is in the midst of a challenging transformation. Information and insights gained from characterization of individual cellular components have established a platform from which to take the next step, i.e., to address molecular activity in a living cell. In a cell, spatial localization, limited numbers of molecules, and molecular crowding define an environment unlike that of a test tube. To delve successfully into this new world, we need new tools, some of which will certainly be computational. A fascinating first application of such a tool is described in this issue of the *Journal of Bacteriology* by Lipkow et al. (9).

THE CHALLENGE

Many cellular metabolic and signaling pathways involve multiple steps, multiple inputs, multiple outputs, and multiple secondary connections, as well as feedback loops and other sophisticated control mechanisms. Considering these pathways in their natural complexity and designing experimental perturbations that will reveal their workings are formidable challenges. Computer-based computational models can help by simulating complex reaction and signaling networks (2). Simulations can be used to predict the consequences of experimental perturbations and assess alternative concepts of circuitry. Yet many in the molecular biosciences have not embraced mathematical models and simulations. In part, this is because the transformation described above is in progress, but more fundamentally there are at least two important concerns: (i) there is not yet a compelling set of examples in which mathematical modeling of cellular biochemistry and physiology has revealed something experimentalists care about and did not already know, and (ii) most modeling exercises have not incorporated important features of living cells, which makes experimentalists uneasy about trusting unexpected conclusions from such models.

Lipkow et al. (9) describe the situation as follows: "Many aspects of the biochemistry and physiology of living cells have in the past been simulated by networks of reactions as though they were electronic circuits. In such studies, components such as receptors, enzymes, or metabolites are portrayed as being wired together in a spatially defined manner through enzymatic and other reactions. But it is clear that living circuitry is not like this; it has unique features such as a highly malleable internal architecture and the existence of a multitude of molecular states that differ in fundamental respects from those of silicon devices. Moreover, the wiring of the cell depends on the diffusive movement of myriad different molecules large and small through the watery interstices of the cytoplasm." These elegant phrases identify some major reasons experimentalists relegate mathematical modeling to the periphery of their efforts to understand biological phenomena. Lipkow et al. (9) use a modeling tool that promises to make an important contribution toward changing this.

THE APPROACH

The modeling tool is a computer simulation program, Smoldyn, recently developed by Steven S. Andrews and Dennis Bray (1) for the study of intracellular reactions. Its application is described for the first time in the present study. The program incorporates both spatial locations of protein components and diffusion of the components within a cell. Smoldyn uses Brownian dynamics, which treats molecules as individuals rather than as concentrations and thus inherently includes stochastic behavior. Smoldyn stands for Smoluchowski dynamics, a theory for diffusive encounter of molecules in solution developed by the physicist Marjan Smoluchowski almost 90 years ago (13). Note that an important equation for diffusion is the Einstein-Smoluchowski relationship. The code for Smoldyn is publicly available, so interested researchers can adapt it for their own purposes. The authors ran it on an Apple Power Mac G5 or a PowerBook G4, either of which is within most laboratory budgets.

Lipkow et al. (9) applied Smoldyn to chemotactic signaling in *Escherichia coli* (see references 4, 6, and 7 for reviews). They constructed a three-dimensional model cell, a rectangular solid having approximately the same linear dimensions and volume as a typical *E. coli* cell. In this virtual cell they placed crucial chemotaxis components as they are thought to be distributed naturally (Fig. 1 is a cartoon of these components), assigning diffusion constants for soluble proteins derived from studies of intact cells, using binding affinities and rate constants from published in vitro studies, and stocking the cell with specific numbers of each molecule (values in the thousands) corresponding to recently determined actual contents of a standard chemotactically "wild-type" strain of *E. coli* (8). In the model, binding and chemical reactions occur as the result of diffusive encounters of binding partners or enzyme and substrate, with appropriate probabilities because a "binding radius" parameter is adjusted to yield experimentally observed reaction rates.

The simulation program tracks every one of thousands of protein molecules as they bounce on convoluted paths through the cell. Output options allow the resulting diffusion to be shown as the distribution of all molecules at a given time after an event like stimulation (see the cover of this issue) or as the path of one molecule (or any number of individual molecules)

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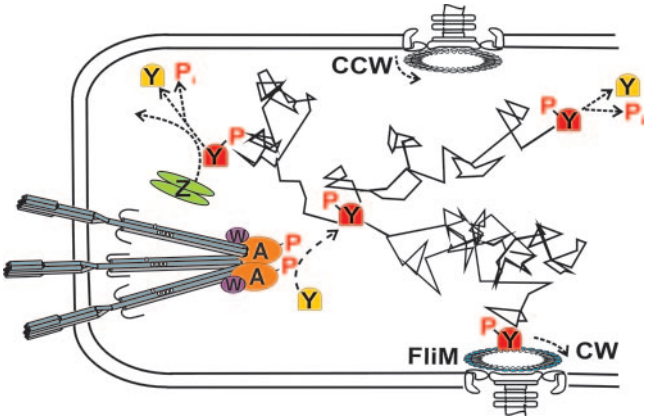

FIG. 1. Core signaling pathway in *E. coli* chemotaxis and the possible fates of phospho-CheY. In the cartoon the double line indicates the cytoplasmic membrane and the structures at the top and bottom are flagellar motors and switch complexes. Chemotaxis proteins are labeled with their single-letter names. Phosphates are shown as red "P's." On the left, a trimer of chemoreceptor dimers is in complex with a dimer of histidine kinase CheA and two copies of coupling protein CheW. CheA uses ATP to autophosphorylate; the resulting histidinyl phosphate is donated to CheY to form phospho-CheY. The cartoon show three possible fates of phospho-CheY: autodephosphorylation (upper right), CheZ-assisted dephosphorylation (upper left), and binding to the ring of FliM proteins in the flagellar switch (bottom right). The latter binding switches the direction of the flagellar rotary motor from default counterclockwise (CCW) to clockwise (CW).

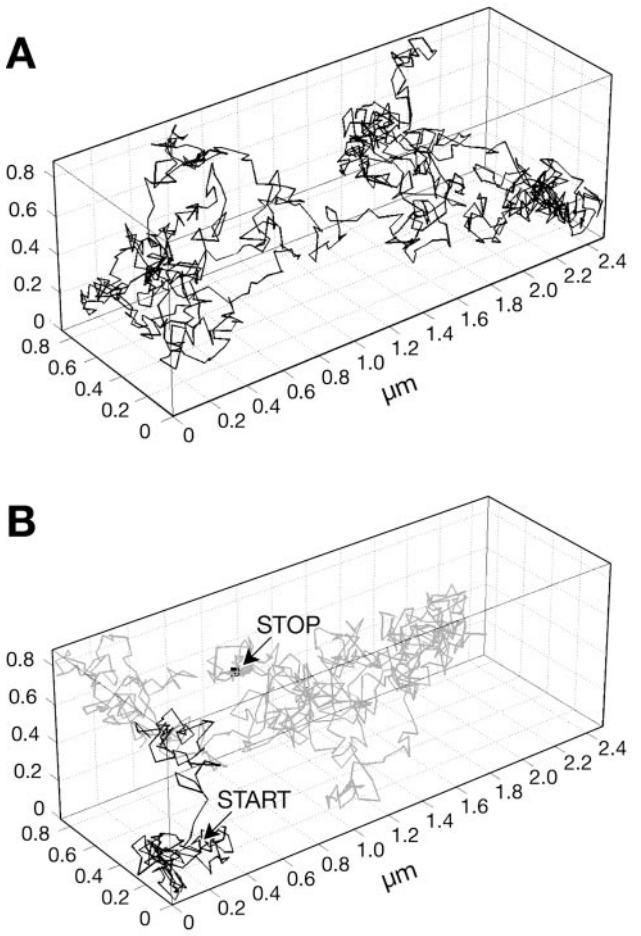

FIG. 2. Three-dimensional traces of individual CheY molecules in a Smoldyn simulation of molecular diffusion in the schematic representation of an *E. coli* cell. Traces are at a 0.1-ms resolution for 100 ms and are black for CheY and grey for phospho-CheY. The two traces are for a molecule that is not phosphorylated (A) or is phosphorylated (B) during the observation time.

(Fig. 2 and Lipkow et al., Fig. 3). The paths are arresting and memorable representations of diffusion, showing 100 ms in the life of individual CheY molecules. Many in the chemotaxis field were made aware of the essence of diffusion 20 years ago in Howard Berg's wonderful treatise (3): the diffusion coefficient of a small protein like CheY (~14 kDa) means it traverses the length of an *E. coli* cell in less than 0.1 s, exploring much of the volume along the way. What I retained from that book were not mathematical expressions but tracings of diffusing particles or swimming bacteria that resemble those in Fig. 2. For many biologists, the visual representation means the mathematical insight is digested and the essence is incorporated into an active body of knowledge. This is not an esthetic issue but a crucial scientific one for all biologists who aim to study and understand biochemical and cellular phenomena. We may know that movement of molecules in the cell is the random walk of diffusion, but we are all guilty of saying (and thinking) that "the signal is sent" or that the protein is "targeted to the membrane," allusions that evoke images of an arrow on its way to the bull's eye. If graphics of molecular paths created from programs like Smoldyn were ubiquitous in textbooks and reviews and as research tools, these common misstatements and the resulting conceptual errors would largely disappear.

THE APPLICATION

Lipkow et al. focused on the soluble signaling molecule of bacterial chemotaxis, response regulator CheY, and components with which it interacts (Fig. 1). CheY becomes phosphorylated by interaction with the autophosphorylated histidine kinase CheA, which is part of signaling clusters of chemoreceptors located at cell poles. Phospho-CheY interacts with the switch complex of flagellar rotary motors to alter the direction of rotation, thereby affecting swimming patterns. There are four to six motors distributed along the cytoplasmic membrane. Phospho-CheY is short-lived because of an inherent phosphatase activity. In *E. coli* and related species cellular half-life is further reduced by a phosphatase-like protein, CheZ. The authors represented the signaling cluster as a square array of kinases placed at one end of the virtual cell, near the membrane, and four flagella as four rings of switch protein FliM distributed at regular intervals along the membrane. Placements and dimensions were reasonable approximations of the current understanding of cellular locations and sizes of signal-generating and signal-receiving complexes. A separate program developed previously by the Bray laboratory (5) was used to generate a continuous input of the amount of phosphorylated CheA in resting and stimulated states. Smoldyn produced a continuous record of each CheY molecule in the cell, including phosphorylation upon interaction with phospho-CheA, diffusion through the cytoplasm, interaction with

motors, and spontaneous, as well as CheZ-induced, dephosphorylation. In addition the program continuously determined the occupancy of each flagellar switch with phospho-CheY. These records are gratifyingly similar to experimental data.

Smoldyn does more than mimic known features of the chemotaxis signaling system; it can investigate features difficult to probe with available experimental approaches, for instance, the distribution of a protein across a cell dimension, distributions of lifetimes of an unstable molecule like phospho-CheY, and effects on motors at different distances from the signaling complex. Particularly interesting to this reader was the assessment of effects of molecular crowding (10) on diffusion of signaling molecules. The literature is full of warnings about molecular crowding (11), but it is not always clear what an experimentalist should do except worry. To investigate the effects of crowding on diffusion of CheY, the authors introduced into their virtual cell cubic obstacles with functional dimensions and numbers (12,544) approximating the cellular complement of ribosomes and the level of crowding in a typical bacterial cell. The authors observe subtle effects but, reassuringly for the in vitro experimentalist, no drastic alterations in the features examined.

THE FUTURE

Lipkow et al. describe steps they will take to make Smoldyn simulations even more realistic, including linkage to an input program (12) that provides positions and dynamics of activated and phosphorylated kinase molecules in the cluster. As subjects of additional investigations, they identify gradients and waves of phospho-CheY, stochasticity and noise in chemotaxis signaling, and several other issues that the field is only starting to consider. My hope is that this program and its derivatives become tools for those studying the myriad cellular phenomena in which diffusion and spatial localization play important roles.

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