Inner space exploration: the chemical biologist's guide to the cell

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As scientists, we live in the throes of technology — pushed forward by it, prodding it on, prompted by it to explore new landscapes. New technologies reveal to us the intricacies of the natural world. Our environment becomes more vivid and real than it was before. For example, Archaea, the oddball phylogenetic domain that straddles ground between bacteria and eukaryotes, has long been considered a denizen only of extreme environments and specialized ecological niches. Yet reports in the past several years that use polymerase chain reaction (PCR) amplification of ribosomal RNA have identified robust archaeal lineages in environs as normal as Wisconsin dirt [1]. That this intriguing phylogenetic cousin of ours, an analysis of which might yield hints to our own evolutionary history, lives not just at a sulfurous vent at the bottom of the ocean but also, simply, underfoot is rather wonderful. One is reminded that the mundane world visible to us now is just one face of a more complex, more elegant universe that we have no means to sense.

The eukaryotic cell itself might turn out to be just this kind of complex universe. A recent meeting on new technologies in cell biology and genomics reminded us of a classic movie from the 1960s called 'The Fantastic Voyage' (a comic remake in 1987 was entitled 'Inner Space'). In this movie, a team of scientists and their spacecraft were miniaturized for a journey through the human body, an 'inner space' no less cosmic or mystical than the galaxies and nebulae of outer space. These lucky scientists experienced a real-time image of the cells, tissues and systems that sustain us; they navigated, first hand, vital processes such as breathing and digestion that we take for granted every day. What would those scientists have seen if they could have been shrunk further to the size of a single molecule, traveling along the surface or through the innards of a living cell?

For years scientists have prodded cells with electrodes, freeze-fractured them for microscopy, immunoprecipitated their contents in twos and threes, and reconstituted their elements *in vitro*. Yet the obvious questions — what does it actually look like in there, are cytoplasmic enzymes organized or free floating, how do the membranes of subcellular organelles orchestrate themselves — have remained recalcitrant to study. The astounding advances in optical microscopy and miniaturization techniques presented at this recent meeting have made it possible to analyze the physiology of single cells in the environment of complex tissues, and single molecules within isolated living cells. In concert with these techniques, chemical biology might now offer a tenable route to the intracellular frontier.

Consider the problem of detecting transient interactions within a cell, which has long frustrated biochemists. Fleeting contacts between enzymes as one passes a substrate to the next, low affinity stickiness that might cluster proteins about a scaffold, such subtle contacts — if they occur — might be invisible or indistinguishable from artifact using many *in vitro* tests of affinity. Intracellular assays such as the yeast two-hybrid system and elegant derivatives thereof allow cellular constituents to remain in their native milieu but tether them to sensors designed to monitor intracellular associations. Clearly, the more subtle the interaction, the more sensitive it will be to such baggage. Other approaches that outfit a protein with a unique chemical handle might serve as the basis of new detection techniques. Work in our lab [2] has demonstrated that installation of a ketone — a functional group unique to the extracellular landscape — on cell surface carbohydrates can serve as a novel means to fluorescently tag or otherwise label them. Others have accomplished the analogous feat on a cytoplasmic protein, by installing in it a hexapeptide containing four cysteine residues that is recognized *in situ* by a fluorescein derivative carrying two dithioarsenicals ([3]; reviewed in this issue). Administered extracellularly, the dye becomes highly fluorescent upon binding. An alternative, the *in vivo* nonsense suppression technique [4], has been adapted to incorporate unnatural amino acids into the nicotinic acetylcholine receptor within a eukaryotic cell [5]. One is left with the intriguing suggestion that such methods for specifically labeling proteins within living cells could generate the means to detect protein interactions using fluorescence resonance energy transfer (FRET) or direct covalent trapping.

Sisters to transient protein interactions within the cytoplasm are temporary associations among cell membrane constituents, or among components of membranous organelles such as the endoplasmic reticulum and Golgi complex. Such clustering is implicit in the current image of the cell membrane, in which transient 'rafts' of certain

lipids and proteins form within the larger membrane pool. Their dynamic nature has made these structures intangible *in vivo*. Yet recent work in which GPI-anchored proteins in living cells were chemically cross-linked identified oligomers of up to 15 molecules [6]. A complementary approach monitored the extent to which FRET between GPI-anchored proteins depended on that protein's concentration in the membrane; the unwavering FRET signal, even with dilute levels of protein, suggested that the assembly of the proteins into subdomains, and not mere concentration, effected the energy transfer between molecules [7]. These techniques and others, including FRET-based indicators of cell membrane potential [8], suggest that the membrane is not a simple barrier but rather a complex, intricately organized organ of the cell. Already it is possible to fluorescently label subcellular structures such as mitochondria and nuclei. If intracellular targeting methods could be applied to sensors of membrane organization, we might be able to achieve a similarly multi-dimensional view of intracellular membrane architecture, thus elucidating such sagas as the multi-step process of protein glycosylation in the secretory pathway.

Amidst the collisions of proteins and the slosh of lipid membranes lie the fluxes of small molecules that comprise many intracellular signaling pathways. Calcium, cAMP, inositol triphosphate and nitric oxide are known to propagate externally derived information within the cell, but the precise localization and timing of their delivery is more elusive. For example, to identify whether signals pass in waves or rivulets from their origins and how precisely the proteins responsive to them are clustered could yield new insights into how crosstalk between signaling pathways is established or minimized. Startling progress has been achieved in the field of calcium study, with the commercial availability of membrane-permeable calcium-sensitive dyes that are captured in the cell by enzymatic deprotection of acetate esters. The reciprocal approach, introduction of caged calcium for the spatially and temporally controlled release of intracellular calcium, recapitulates signaling processes at will [9]. Analogous methods for monitoring other small molecule messengers are less plentiful but avidly sought. A novel fluorosensor of cAMP is currently available, and advances in electron paramagnetic resonance have enabled the quantification of intracellular nitric oxide in real time [10,11]. Further refined, such techniques might illuminate the interplay of different intracellular communication processes occurring in concert. If comparable chemical tags can be directly appended to intermediates in metabolism, a myriad of interconnected biosynthetic pathways could be analyzed in real time as well.

Ultimately, it might be that to describe a cell in its true complexity requires that one monitor its feints and enthusiasms a single molecule at a time. After all, this is the level at which biological work is actually accomplished. Those who engage in single-molecule measurements of DNA and proteins have observed that a population presents only an idealized, most probable version of reality [12]. The behavior of single molecules may seem stochastic and perversely unlikely, but such improbable contortions could be essential for the molecule's activity. To capture these moments within a living cell could revise our scientific understanding in radical ways. Crafting the means to temporarily immobilize, tag or otherwise manipulate individual molecules will require subtle and sophisticated chemistry.

Our ability to visualize the world has telescoped from the level of whole animals and their viscera to the specialized tissues that comprise each organ, further to individual cells and their contents, and finally to molecular assemblies and even solitary molecules. As chemists attempt to breach the cellular frontier, they need to adopt a similarly layered strategy. Reactions of the past assumed an inert or easily manipulated solvent, reagents draped in protecting groups, and the precise steering of one functional group into the next. More recent transformations accommodate a new level of complexity, using highly selective chemistry on unprotected, richly functionalized molecules such as proteins and oligosaccharides within an aqueous milieu. The next era of reactions — transplanted from the round bottom flask to the cell — will have to accept a complete loss of control of the ambient environment, surrendering to a solvent densely populated with proteins and carbohydrates, specialized electron donors, and highly activated biological energy sources. Reagents will need to be targeted and finely tuned to enact the desired transformations. Technology steeped in this cellular context is challenging, alluring and potentially significant. It is unpredictable what the consequences will be of visualizing life from the inside out, one molecule at a time.

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