self-shielding (*9*–*11*). The principle is similar to that of laser isotopic separation of gases, but uses either interstellar ultraviolet (UV) radiation or the light of the growing Sun. In either case, UV radiation dissociates CO molecules. However, owing to the abundance of $C^{16}O$ molecules, the radiation penetrating the system runs out of photons capable of breaking up this isotopic combination before it runs out of photons that dissociate $C^{17}O$ or $C^{18}O$. The result is that in such regions, ^{17}O and ^{18}O are free to react, either directly with dust grains or with H atoms to form H_2O that eventually reacts with the dust. Meanwhile, the extremely stable $C^{16}O$ sequesters the ^{16}O in the gas phase and prevents it from reacting with silicates. As long as CO remains stable, the self-shielding mechanism remains a viable explanation for the mass-independent fractionation of oxygen isotopes throughout the solar system.

Unfortunately, Ciesla and Sandford demonstrate that CO is not a stable sink for ^{16}O, and that such molecules will be converted into complex organics even in the cold outer reaches of the solar nebula, just as they are in cold molecular cloud cores. If CO is converted into organic molecules by radiation processing in icy grains in the outer nebula, just as it is via Fischer-Tropsch–type reactions (*12*–*14*) on the surfaces of warmer grains in the inner nebula, and if such organics are free to react with silicate dust or to react with the bulk silicate in planetesimals, then the effects of photochemical self-shielding will be minimized or even completely erased. A new mechanism may be needed to explain the oxygen isotopic fractionation observed in the solid bodies of the solar system.

Our understanding of the chemistry in protostellar systems has made enormous progress over the last few decades, fueled by an increased awareness of the complex dynamics of these evolving energetic nebulae. We can no longer consider just the simple local environment (*15*) to explain the composition of a planet, asteroid, or comet as was done in the past, but must now consider chemical processes that might take place within the nebula as a whole as well as the probability of transport and mixing the products of such reactions throughout the system. Just as we now find it impossible to explain the complex chemistry of the terrestrial atmosphere without reference to detailed transport models that interconnect highly dissimilar chemical environments, so chemical models of protostars and of the solar nebula must eventually treat these environments as tightly coupled, interactive systems. The demonstration that the chemistry on the surfaces of outward-flowing, dynamically mixing icy grain surfaces both mimics the chemistry in cold cloud cores and strikes at the central assumption of the photochemical self-shielding model for oxygen isotopes in solar system solids only adds emphasis to this conclusion.

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CELL BIOLOGY

Using Cell-to-Cell Variability—A New Era in Molecular Biology

Studying the phenotypic differences between genetically identical cells rather than their general features can reveal novel regulatory mechanisms for diverse cellular processes.

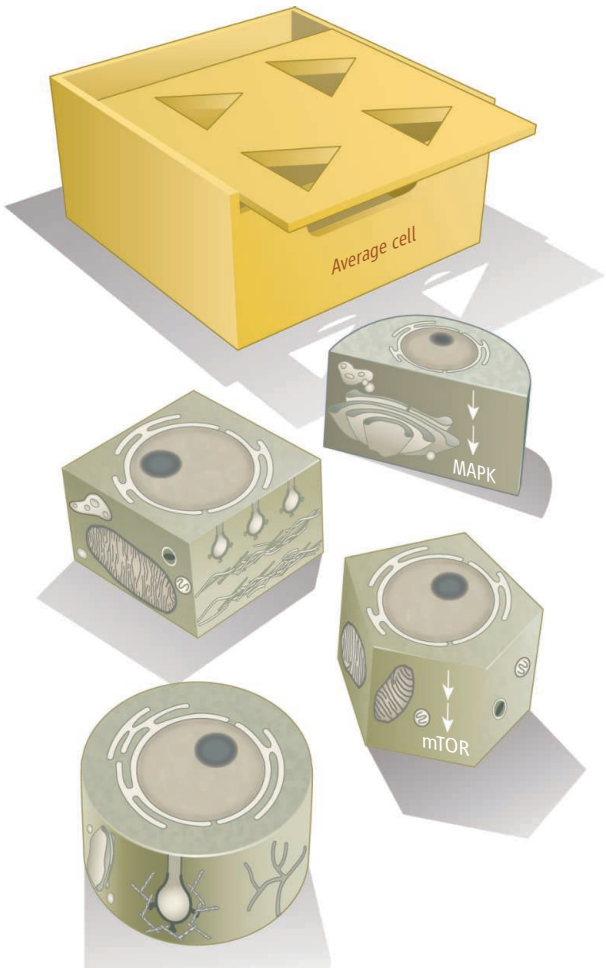
Lucas Pelkmans

Every cell biologist who has used a microscope knows that single cells in a population display variable behavior (*1*). Although heterogeneity between single cells is obvious in tissues and organisms, it can also be observed in populations of monoclonal cells that have been cultured under identical conditions. Besides having stochastic sources (*2*, *3*), phenotypic cell-to-cell variability among genetically identical cells can be deterministic and regulated, in both prokaryotic and mammalian cells (*4*, *5*). Molecular and cell biologists have traditionally ignored this phenomenon, in part because of technical limitations, but also because, historically, research has focused on mechanisms and processes that are common between cells. However, the mechanisms that make them different are likely to be equally important. Embracing this cell-to-cell variability as a fact in our scientific understanding requires a paradigm shift, but it will be necessary. In fact, it may be a strong boost for the field by uncovering novel and previously overlooked mechanisms of regulation at the cell population level.

Uncovering a novel mechanism, structure, or organelle that is generally observed in all cells is still regarded as the ultimate goal for most scientists in the field. Although clearly important, this fixation on results that can be generalized while dismissing other, conflicting findings has hindered progress. Fortunately, the field is slowly accepting that multiple mechanisms often exist for a particular cellular behavior or activity and that these mechanisms can vary between individual cells, even within the same monoclonal population (*6*–*8*).

The great value of cell-to-cell variability for uncovering novel cellular processes comes from its being a natural source of "perturbation." A similar approach is applied in the social sciences and economics, where relationships between individual factors are inferred through the analysis of variability in these factors in dynamically changing social and economic systems (*9*). However, these sciences can generally only make use of naturally occurring perturbations to test hypotheses. In contrast, the tools available to the molecular cell biologist provide some unique advantages, such as combining the analysis of cell-to-cell variability with intentional, targeted molecular perturbations. In addition, with the advent of high-throughput image-based technologies and computational image analysis (*10*, *11*), large data sets can be constructed comprising measurements from multiple molecular and phenotypic states in large numbers of single cells within their

Institute of Molecular Life Sciences, University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland. E-mail: lucas.pelkmans@imls.uzh.ch



Above average. Single, genetically identical (monoclonal) cells (circle, half circle, pentagon, and square) are not the same as the average cell (triangle). Individual cells may display many cell-to-cell variabilities, such as in the signaling kinases mitogen-activated protein kinase (MAPK: half circle) and mammalian target of rapamycin (mTOR: pentagon), and in clathrin-mediated (circle) or caveola-mediated (square) mechanisms of endocytosis, making each individual cell clearly different.

microenvironment over long periods of time. These data sets are particularly well suited for statistical approaches to find physiologically relevant connections, such as causality and feedback (*12*). Once such approaches become mainstream, we may find that cell-to-cell variability is fundamental to most, if not all, molecular cellular processes, from the number of ribosomes in a cell or its metabolic capacity to cytoskeletal properties and the activity of signaling pathways. One consequence of this will be the realization that the concept of a single "textbook-model" cell does not factually exist (see the figure).

We all struggle with the amount and the complexity of molecular information available on the regulation of cellular processes, as well as the layers of cross talk between them. Most of this knowledge has been obtained with cell population-averaging techniques or from a relatively few single cells and then generalized to all cells. However, it is likely that when these processes are studied at the single-cell level, it becomes clear that not all parts of a complex pathway (e.g., of signal transduction) or all existing alternative routes (e.g., of endocytosis) act together in an individual cell, but rather in different subsets of cells within one population. Thus, we may find that this molecular complexity manifests itself in part at the cell population level and can be explained by studying patterns of cell-to-cell variability.

A rich hunting ground for molecular cell biology is to find out the molecular mechanisms by which regulated cell-to-cell variability emerges in a population. Quantitative measurements of how cells spatially organize themselves in a growing population will reveal how heterogeneity in the single-cell microenvironment is created (*5*). Integrating this with molecular measurements of important signal transducers will explain how cell-to-cell variability patterns of cellular signaling emerge. This, in turn, may affect a wide range of cellular processes. For instance, it may cause heterogeneity in transcription factor activity and subsequently in gene transcription (*13*, *14*), and it may affect signaling pathways that control protein translation (*15*) or metabolic rate (*16*). At a higher level, this may cause variation in the state of the cytoskeleton, lipid composition of membranes, and membrane trafficking (*5*), as well as in intrinsically cycling cellular states, such as the cell cycle (*17*, *18*). On top of this, we may expect different cellular states to affect the type and level of soluble factors secreted by individual cells (*19*) and how other individual cells respond to them (*20*, *21*). Because the activity and state of these diverse cellular processes influence each other and are determined by—but also define how—cells spatially organize themselves, there exists extensive feedback at both the single-cell and the cell population level.

Remarkably, although a large amount of knowledge exists for individual cellular processes, very little is known about how they act together to determine cell-to-cell variability. Specifically, quantitative assessment of how information flows from one cellular property to the next is almost completely absent. I expect that a particularly intriguing class of molecular regulators, namely those that control and establish cell-to-cell variability, will emerge and become important for studying this. When such regulators are perturbed, there may be no change in overall cellular activity, but the pattern of cell-to-cell variability of the activity will be different (*22*). These regulators could represent an entirely missed concept in molecular cell biology. Another fascinating topic will be the molecular mechanisms by which cells control their intrinsic variability. For clathrin-mediated endocytosis, we have shown that cells that are sparsely growing display more cell-to-cell variability than cells growing in more crowded regions (*5*). This suggests that local cell density can regulate intrinsic variability.

Finally, hypothesis-driven statistics and causal inference from large sets of accurate measurements may allow us to overcome one of the greatest problems in molecular cell biology: the fact that genetic and pharmacological perturbations have a variety of indirect effects, obscuring the fine detail of the molecular causality underlying cellular activities. Ironically, it is exactly the biologically determined variability in such measurements, whether of single cells in a population or subcellular structures within a cell (*23*), that will enable us to reveal this.

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