Taking the inventory inside single cells

G Balázsi & J J Collins

Despite having an increasingly accurate parts list for biological cells, much is left to discover about how these parts act together to create functional cells, and how distinct individual cells interact to create functional tissues and organs. Biologists are increasingly aware of the cell-to-cell variability in molecule copy numbers—a trend that is revealed by several new techniques, including one that permits counting molecules in single cells.

Methods developed to measure cell-to-cell differences have typically been based on microscopic imaging of fluorescent proteins[1] or RNA[2,3]. These techniques make it possible to monitor RNA or protein fluctuations both in single cells over time and across the cell population, and they are even capable of visualizing single molecules in single cells[4,5]. However, they can only be used to monitor a few types of molecules (for example, ones selectively labeled) in relatively stable conditions. A technique recently introduced by Huang *et al.*[6] enables one to measure a larger number of molecular species in changing environmental conditions.

This new approach involves all the steps typical to a gel assay, but the 'laboratory bench' is squeezed down to the impressive size of a square inch (**Fig. 1**). Cells are pumped into a microfluidic channel and captured one by one in micrometer-sized reaction chambers. The addition of a buffer solution causes cell lysis, which can be followed by fluorescent labeling of the resulting protein mass, if necessary. The various protein species are separated by capillary electrophoresis and quantified with the clever use of cylindrical optics and fluorescence microscopy. Various molecular species can be measured individually, molecule by molecule, and therefore it is possible to capture proteins that are low in copy number within cells. Importantly, capillary electrophoresis separates proteins based on their charge and molecular weight (**Fig. 1**). This eliminates the need of tagging different molecular species with different fluorescent labels, thereby avoiding the potential adverse effects that tags can have on protein expression. For similar reasons, this approach can also be readily applied to organisms for which genetic manipulation is difficult, if not impossible.

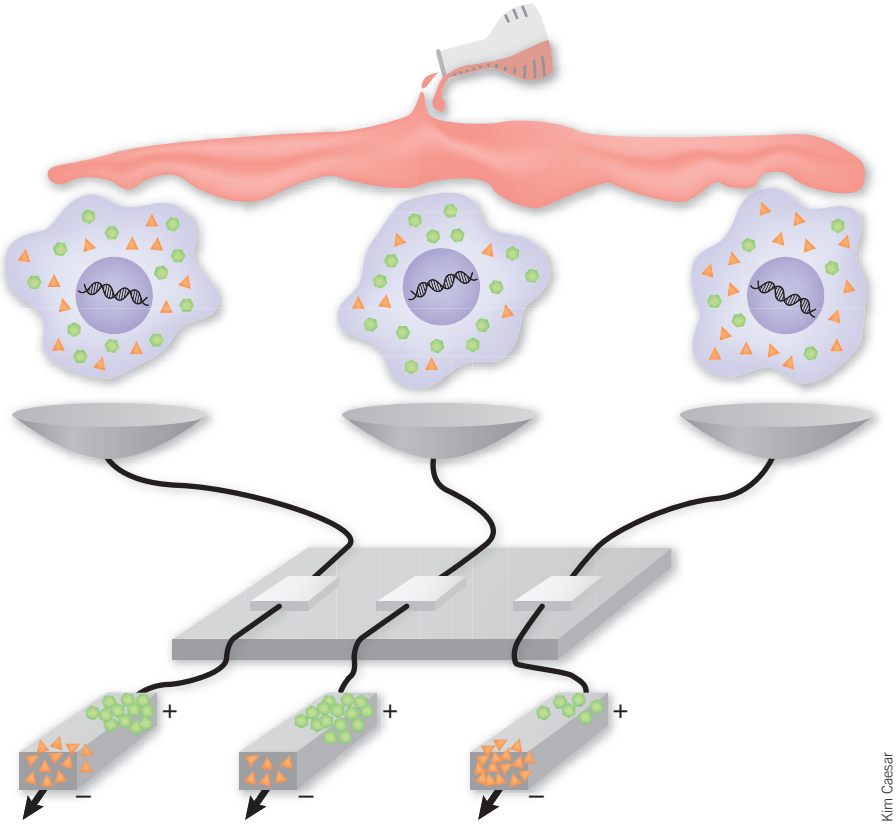

Figure 1 Taking the inventory inside single cells. Single cells are manipulated and lysed in micrometer-sized reaction chambers, and then various protein species are separated via capillary electrophoresis and counted molecule by molecule.

To test their method, Huang *et al.*[6] measured the expression of a human transmembrane protein expressed in an insect cell line. They also examined the degradation of phycobilisome protein complex components that takes place when the cyanobacterium *Synecococcus* sp. PCC 7942 is transferred from a nitrogen-rich environment to a nitrogen-depleted environment (in the absence of external nutrients such as nitrogen, *Synecococcus* spp. degrade their phycobilisomes in an ordered manner and use them as nutrients). The advantage of the strategy of electrophoretically separating the components before analysis is particularly clear in the second case: Huang *et al.* quantified phycobilisome components with highly overlapping fluorescence spectra in single cells, which could not have been measured by microscopy. They observed a high variability of protein copy numbers across the cell population in both cases. Interestingly, when cyanobacteria are transferred to a nitrogen-free medium, they sequentially degrade the components of their phycobilisome complex—but there are exceptions. Approximately 5% of the population maintains the stoichiometry of the

G. Balázsi is at the Department of Systems Biology, University of Texas M.D. Anderson Cancer Center, 7435 Fannin Street, Houston, Texas 77054, USA, and J.J. Collins is at the Center for BioDynamics and the Department of Biomedical Engineering, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA.
e-mail: gbalazsi@mdanderson.org or jcollins@bu.edu

phycobilisome components as if the environment never changed. This subpopulation would have been missed by traditional techniques such as gel assays or mass spectrometry, which measure only the mean protein concentrations.

Many recent studies have reported on the heterogeneity of protein copy numbers across cell populations[7–9]. But what is the importance of quantifying cell-to-cell differences, rather than only measuring average concentrations, as in standard gel assays and microarray experiments? If cells were to live in a constant, stable environment, it would be detrimental to deviate from the protein concentrations that are optimal for survival in that specific niche. However, recent theoretical work suggests that cell population heterogeneity can be advantageous in changing environments[10,11]. Accordingly, a recent experimental-computational study showed that cell population heterogeneity is of critical importance in acute stress conditions, in which the more heterogeneous population has a better chance of survival[12]. The new technique developed by Huang *et al.* is suitable for studying heterogeneously expressed proteins that confer better chances of survival to a cell population after a drastic change in the environment. Such approaches could provide important insights into the networks, pathways and stochastic dynamics involved in cellular defense mechanisms.

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

1. Elowitz, M.B., Levine, A.J., Siggia, E.D. & Swain, P.S. *Science* **297**, 1183–1186 (2002).
2. Chubb, J.R., Trcek, T., Shenoy, S.M. & Singer, R.H. *Curr. Biol.* **16**, 1018–1025 (2006).
3. Golding, I., Paulsson, J., Zawilski, S.M. & Cox, E.C. *Cell* **123**, 1025–1036 (2005).
4. Raj, A., Peskin, C.S., Tranchina, D., Vargas, D.Y. & Tyagi, S. *PLoS Biol.* **4**, e309 (2006).
5. Cai, L., Friedman, N. & Xie, X.S. *Nature* **440**, 358–362 (2006).
6. Huang, B. *et al. Science* **315**, 81–84 (2007).
7. Blake, W.J. *Nature* **422**, 633–637 (2003).
8. Ozbudak, E.M., Thattai, M., Kurtser, I., Grossman, A.D. & van Oudenaarden, A. *Nat. Genet.* **31**, 69–73 (2002).
9. Raser, J.M. & O'Shea, E.K. *Science* **304**, 1811–1814 (2004).
10. Thattai, M. & van Oudenaarden, A. *Genetics* **167**, 523–530 (2004).
11. Wolf, D.M., Vazirani, V.V. & Arkin, A.P. *J. Theor. Biol.* **234**, 227–253 (2005).
12. Blake, W.J. *et al. Mol. Cell* **24**, 853–865 (2006).