Minireview

Protein synthesis molecule by molecule

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Abstract

Since the earliest days of molecular biology it has been known that even a seemingly uniform culture of bacteria is made up of cells very different from each other in terms of their levels of a given protein. This individuality has now finally been quantified at single-molecule resolution, as reported in two recent papers.

In 1953 Seymour Benzer asked the following question [1]: when we induce β-galactosidase in a culture of *Escherichia coli*, do all the cells make the enzyme, or is there considerable heterogeneity in the population? He could not measure the amount of enzyme in each cell directly, so he infected cultures with a phage whose replication depended on β-galactosidase activity. The production of new phage can be detected on a cell-by-cell basis and became the measure of β-galactosidase concentration in each cell. Benzer found that there was considerable individuality in an induced population, although how much he could not say, nor could he identify its origins. This classic experiment has served to keep the question of cell individuality alive [2,3] and is often quoted, but only recently have the tools become available to study transcription and translation in single living cells.

It has recently become possible to follow individual RNA molecules as they are made [4-6]. The method depends, however, on an amplification scheme in which a single mRNA molecule binds around 50-100 molecules of green fluorescent protein (GFP). The detection of single protein molecules in living cells seemed beyond the reach of current technology. Although a single GFP molecule can be imaged when it is constrained to a surface or pinned down in space [7-10], a single molecule diffusing rapidly through a cell, and in and out of the focal volume, could not be reliably imaged. This technical problem has now been overcome by Xie and colleagues and, in a recent paper in *Science* [11], they provide some beautiful results bearing on the kinetics of single-molecule synthesis in growing *E. coli* cells.

Detecting single protein molecules

First, their experimental system. The authors used a GFP variant called Venus [12] that is known to fold rapidly *in vitro* (it fluoresces bright yellow, like the planet in the night sky). Venus was fused to a membrane protein, the transmembrane serine receptor Tsr, which allowed Yu *et al.* [11] to image individual Venus-Tsr molecules as they appeared in the membrane, where diffusion is restricted and single-molecule imaging is possible (although not easy). Synthesis of β-galactosidase was kept repressed in these cells, so that just a few molecules were made per generation. They also used a very sensitive CCD camera and photon-counting statistics to quantify the number of Tsr molecules appearing as a function of time in dividing cells. To keep the counting manageable, and to preserve the distinction between new and old events, they photobleached each new molecule shortly after it was made. With this combination of techniques, they found they could image each protein molecule as it was made, follow single molecules in the membrane as they moved about the cell, follow the segregation of the new molecules as the cells divided, and ask if the newly synthesized proteins are preferentially associated with one or other region of the cell.

Second, the results. As well as being a technical *tour de force*, the work did indeed demonstrate a high degree of individuality in the population, as Benzer foretold [1]. That the number of molecules per cell varies widely is not surprising, given the small average number per cell - it would be remarkable if there were precisely four per cell, for example, and probably impossible to design a system with this kind of accuracy. The interesting and significant result comes from measurements of the kinetics of protein production. Yu *et al.* [11] found that synthesis occurred in bursts, with a geometrical distribution of burst sizes that could be modeled after the theoretical work of Berg [13]. Berg supposed that the simplest model for protein synthesis involved competition between mRNA degradation on the one hand, and successful initiation of protein synthesis on the other. Under this model, the probability of producing n protein molecules from one mRNA follows a geometric distribution:

$$P(n)=\rho^n(1-\rho)$$

where ρ is the probability that the ribosome will bind to the mRNA and get started and $(1-\rho)$ is the probability that the RNA will get degraded. The data by Yu *et al.* [11] show a good fit for the small values of n observed in these experiments, and the authors use this result to argue that Berg's model accurately describes protein synthesis on a cell-by-cell basis.

Although Yu *et al.* [11] mention the localization of Tsr only in passing, noting that it first appears at random and then moves to the poles of the cell, the ability to observe single molecules as they appear in the membrane shows that it is possible to follow the dynamics of assembly of higher-order membrane complexes in real time. This will now allow us to study, for example, the order in which signaling complexes are assembled at the pole of the bacterial cell, which members of the complex arrive first, who recruits whom, and perhaps even to discover if localized mRNA can account for some of the spatial dynamics, as it can in many eukaryotic systems [14]. Although protein localization has previously been addressed using single-molecule immunochemistry in fixed specimens [15], the signal-to-noise limitations of these methods in prokaryotes made it impossible to answer these questions.

Single molecular assays in picoliter chambers

In a second paper from the same laboratory, Cai *et al.* [16] report experiments to assay single living cells for β-galactosidase activity. They designed 100-picoliter microfluidic chambers for single-cell assay and used a substrate that yields a fluorescent product upon cleavage by β-galactosidase. Because β-galactosidase assays are linear for long periods of time, it was possible to determine the number of molecules of enzyme in each cell, distinguishing one from two from three, and so on, by inspecting the slope of product accumulation versus time. Broadly speaking, these experiments also demonstrated a burst phenomenon. Under conditions of repression, under which many cells have no β-galactosidase activity (see above), they found around 0.1 bursts per cell cycle, and around 20 monomers per burst, in good agreement with earlier measurements on similar cell populations. The authors also compared their results with living cells to measurements on chloroform-permeabilized cells using the same picoliter chambers. Here they could examine the numbers of active molecules in a population of cells without the complications of cell growth and division. These data were in good agreement with the live-cell results, and the data were best fitted by a gamma distribution. The significance of this particular fit lies in the fact that this distribution results from the convolution of two independent random processes: a Poisson process, which represents the random occurrences of some event in time - here presumably the uncorrelated initiations of protein translation from mRNA - and a geometrically distributed protein production event, presumably due to the finite lifetime of the mRNA molecules. The fact that the active form of β-galactosidase is a tetramer, and thus the appearance of activity is once removed from the synthesis of individual protein chains, is not taken into account in this simplified description.

It is instructive to compare the results discussed above with recent experiments in *E. coli* [5] and in *Dictyostelium discoideum* [17] in which mRNA synthesis was followed in individual living cells. In both organisms it was found that transcription occurred in bursts, with a geometrical distribution of burst sizes - a very similar behavior to that observed by Yu *et al.* [11] for protein production. This similarity immediately leads to a possible alternative interpretation of the results by Yu *et al.* [11]: that the observed characteristics of protein bursts are not a reflection of the exponential lifetime of cellular mRNAs ('Berg's picture'), whereby each mRNA molecule gives rise to a random burst of proteins, with a geometrical distribution of burst sizes, but rather, they are a direct result of the randomness of mRNA production, with each random burst of RNA production reflected in a one-to-one manner in the protein kinetics, thus appearing to us as a protein burst. The existing data may not be sufficient to resolve this issue, and the experimental systems are quite different in detail. It is, however, now possible to combine the two measures in the same experiment, given the advances described by Yu *et al.* [11] and the availability of a wide range of fluorescent protein colors [18]. This type of system, in which mRNA and protein production from the same gene could be followed simultaneously, at single molecule resolution, is a natural next step and promises exciting new results.

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