

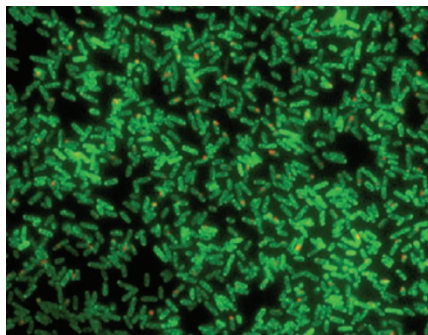
SINGLE MOLECULE

Single molecules meet systems biology

Application of single-molecule methods to assay genome-wide mRNA and protein levels in single bacteria provides a systems-level view of their relationship.

In biology, working with large samples of cells helps ensure a good signal, but this comes with a drawback: the measurement is an average that will mask differences in individual cells. A prominent example of this is single persistent bacteria that resist antibiotic killing of a larger genetically identical population. This antibiotic resistance must come from differences in the repertoire of proteins expressed by these cells but population-based methods cannot be used to examine expression differences between single bacteria in a population.

Sunney Xie at Harvard University has spent years working with methods to understand biological phenomena in single molecules and cells, from measuring the activity of single enzymes to visualizing the activity of a single gene in a single bacterium. “The process of transcription and translation is a single-molecule problem,” says Xie. “There is only one copy of



Simultaneous fluorescence image of YFP and the corresponding mRNA from the same *yfp*-tagged gene recorded and quantified in a microfluidic device. Image courtesy of Yuichi Taniguchi (Harvard University).

the gene, and the processes of gene regulation and transcription initiation are much slower than subsequent steps, so they are rate-limiting, and ‘stochasticity’ comes into play.” This stochastic nature can result in phenotype switching, a possible explanation for the existence of persisters.

Xie previously visualized the expression of individual proteins produced from

a single gene tagged with *yfp* in a growing bacterium in real time, but he wanted to apply these techniques across the genome. This requires a library of bacterial strains in which each gene is tagged at its 3’ end with *yfp*. Xie says, “my graduate student Paul Choi came up with a great idea of swapping *yfp* into an affinity-tagged library, which was previously constructed by Andrew Emili’s group at University of Toronto, with only one primer using λ -Red-mediated recombination. Huiyi Chen worked with him to do this cheaply and quickly.”

Once Xie and his group had their library, they needed a high-throughput way of quantifying YFP expression in each strain. For this they turned to microfluidics. Postdoc Yuichi Taniguchi designed and built a microfluidic device that could image 96 strains at a rate of 4,000 cells in 25 s per strain. By careful calibration, normalization for cell size and deconvolution to remove autofluorescence, they obtained accurate measurements of protein abundance. Xie says, “we have five decades of dynamic range from 0.1 to 10⁴ copies per cell.”

SYSTEMS BIOLOGY

LEARNING FROM HISTORY

The strength of selection that acts on a growing population of cells and the presence of a stochastic switching mechanism may be inferred from lineage data.

There are several ways in which one might study how cells behave in a fluctuating environment. At one extreme, one might look at average properties of the population, for instance, the extent to which a certain protein is phosphorylated. At the other extreme, one might look at single cells, for instance, at the signal of a fluorescent reporter expressed by the cells. Studying populations of dividing cells at the single-cell level, however, remains a challenge, although there is growing interest in cellular heterogeneity and its biological consequences.

In recent theoretical work, Edo Kussell at New York University and Stan Leibler at the Rockefeller University describe a framework for measuring the effective strength of selection acting on populations of cells. The makeup of a cellular population may change in response to environmental fluctuation both via individual cells sensing and responding to the changing conditions and via stochastic fluctuations upon which selection can act. Could they disentangle these two effects, Kussell and Leibler wondered, and thus measure the effective strength of selection in a growing population of cells?

The researchers describe a thought experiment that should allow one to do this. “Imagine that you grow cells in the condition you’re interested in, and in parallel you grow them in the same conditions except you now increase the fitness differences between cells by some constant multiple,” explains Kussell. This should now accentuate the fitness differences between the cells so that selection can act on them. “At the end of the day, if the population structure is different between these two experiments, then you know selection is in play under those conditions.” In other words, you disentangle the effect of selection from that of cells sensing and responding to environmental change.

The problem is, it is difficult if not impossible to actually translate this experiment into reality. How would one practically increase the fitness of all individuals in a population by a small constant factor? What Kussell and Leibler found, however, is that by measuring a property that they call the variance of the historical fitness of cells, the results of the above thought experiment can be inferred. This property can be measured by examining independent individual lineage histories of cells in a growing population and by simply recording the number of times a cell divides over time under different conditions of interest.

Because Xie wanted to investigate the relationship between transcription and translation system-wide in single molecules, he also needed a way to count the mRNA molecules encoding the YFP-tagged protein in each strain. Using fluorescence *in situ* hybridization, graduate student Gene-Wei Li could detect an individual *yfp* mRNA labeled with a single red fluorophore in a fixed cell.

While Taniguchi collected protein expression data in live cells for all 1,018 strains, Li used the library and RNA counting to simultaneously measure mRNA and protein levels in single fixed cells for 137 of the more highly expressing strains.

“This is the first time the proteome has been characterized at single-molecule sensitivity in any organism,” remarks Xie. They found that half of all the proteins are present at fewer than ten molecules per cell and 20% of proteins are expressed at under one copy per cell. Xie admits that some were leakages, but others were functioning proteins. They found that the protein expression is represented by a gamma distribution and, perhaps surprisingly, there was no correlation between the mRNA copy number and protein copy number in the same cell. This is explained by the stochastic nature of transcription and the fact that most of the proteins had a lifetime longer than the cell cycle, whereas the mRNAs had a much shorter lifetime.

These results promise to be the first of many biological insights to come. Xie is making the library available for other people to use and is looking forward to applying single-molecule systems biology to questions such as bacterial persistence.

Daniel Evanko

RESEARCH PAPERS

Taniguchi, Y. *et al.* Quantifying *E. coli* proteome and transcriptome with single-molecule sensitivity in single cells. *Science* **329**, 533–538 (2010).

Not only does the formulation of Kussell and Leibler provide a tool to study the effective strength of selection in a particular system, it also allows one to infer whether a cell or unicellular organism is using a stochastic switch to adapt to a particular environmental fluctuation. “For stochastic switches, there is a very pronounced peak in the historical fitness variance, which occurs at fluctuation periods that are longer than the generation time,” says Kussell. “So you can infer the existence and the type of an internal mechanism when you essentially don’t know what the organism is doing, and you don’t know what genes are important.” Undoubtedly, it will be of interest to begin to dissect the contribution of particular genes to such predicted stochastic switches.

In this work, Kussell and Leibler used lineage data obtained from simulations of growing *Escherichia coli*. However, experimental data in which growing cells, either microorganisms or mammalian cells, are imaged over time by live-cell video microscopy are increasingly becoming available. The theory developed in this work may well prove useful for studying the mechanisms by which populations of dynamic cells, stem cells or tumor cells, for instance, change in response to the environment, in contexts in which there is a selective advantage for the cells to be in one state or another.

Natalie de Souza

RESEARCH PAPERS

Leibler, S. & Kussell, E. Individual histories and selection in heterogeneous populations. *Proc. Natl. Acad. Sci. USA* **107**, 13183–13188 (2010).

BIOCHEMISTRY

iCLIP at nucleotide resolution

Most, if not all, human mRNAs are spliced into several isoforms by heterogeneous nuclear ribonucleoprotein (hnRNP) particles that associate with the nascent transcript. Koenig *et al.* now precisely map binding of an hnRNP by a twist on UV-light cross-linking and immunoprecipitation (CLIP). In individual-nucleotide-resolution CLIP (iCLIP), each RNA reveals the exact site of protein binding. These data will yield insight into the effect of hnRNP binding on splicing.

Koenig, J. *et al.* *Nat. Struct. Mol. Biol.* **7**, 909–915 (2010).

PROTEOMICS

O-glycosylation stoichiometry

Sugar-based post-translational modifications remain a challenge to characterize, owing to their low abundance and diverse chemistries. Rexach *et al.* now describe a straightforward approach to characterize the stoichiometry and dynamics of O-GlcNAc glycosylation *in vivo*, by selectively labeling terminal O-GlcNAc sugars with a polyethylene glycol-based mass tag that can be easily detected by denaturing polyacrylamide electrophoresis and immunoblotting. This allowed them to follow the interplay of phosphorylation and glycosylation in mammalian cells.

Rexach, J.E. *et al.* *Nat. Chem. Biol.* advance online publication (25 July 2010).

STEM CELLS

Changing substrate rigidity to grow stem cells

There is increasing interest in understanding how mechanical properties of the growth substrate influence cellular biology. Gilbert *et al.* now show that muscle stem cells can be cultured for one week on hydrogel surfaces with a rigidity that matches that of muscle. In contrast to rigid surfaces, the soft surface promotes stem cell self-renewal and maintains the ability of the cells to regenerate muscle when transplanted *in vivo*. Whether the effect persists for culture over longer periods awaits further study.

Gilbert, P.M. *Science* advance online publication (15 July 2010).

MICROSCOPY

tRNA movement through the ribosome

Time-resolved, single-particle electron cryomicroscopy allows biological processes to be followed by taking multiple snapshots of dynamic systems, but a very large computational effort is required to sort images of heterogeneous complexes. By taking and analyzing two million images of tRNA movement through the ribosome at various time points, Fischer *et al.* used this technique to observe 50 distinct ribosome sub-states, gaining insights into the mechanism of the translocation step of protein synthesis.

Fischer, N. *et al.* *Nature* **466**, 329–333 (2010).

PROTEOMICS

Characterizing protein ubiquitination

The low abundance of ubiquitination sites make them difficult to detect. Xu *et al.* now describe a method to enrich these previously ubiquitinated peptides with a monoclonal antibody reagent that recognizes a unique diglycine adduct left on modified lysine residues after tryptic digestion. Using mass spectrometry, they identified 374 ubiquitination sites on 236 human proteins, adding substantially to the number of known ubiquitination sites.

Xu, G. *et al.* *Nat. Biotechnol.* advance online publication (18 July 2010).