Variability dynamics of protein levels in human cells

Alex Sigal[1*], Ron Milo[2*], Ariel Cohen[3*], Tamar Danon[3], Natalie Perzov[3], and Uri Alon[3]

We investigated variability and its temporal dynamics in proteins in human cells. We measured fluctuations in endogenous proteins which were fluorescently labeled by introducing *YFP* as an additional exon into the coding sequence of one genomic allele. Variability (STD/Mean) ranged between about 0.15 and 0.3 for the proteins studied. Protein level differences between cells were transient: cells higher than average could become lower and vice versa. The timescale for the auto-correlation to decay to half was typically several cell generations. The relatively long auto-correlation time observed in protein levels may account for some of the individuality in cell behavior.

I. Purpose

Clonal cell populations in a homogeneous environment can show different behaviors[1]. In bacteria and yeast, there is significant non-genetic variability in gene expression[2-4], which may determine important phenotypic behaviors such as whether a bacterium survives when challenged with antibiotics[5]. While variability in microorganisms has been characterized, this study[6] quantified protein level variability in human cells, and the time-scale on which protein levels fluctuate. We further asked whether fluctuations in protein levels are due to global factors (for example, the number of ribosomes), local factors in the network (such as shared regulation), or whether there was no correlation between different proteins.

II. Results

A. *Variability between human cells*

We quantified the variability between cells in the expression of 20 different proteins. To quantify protein behavior, we retrovirally inserted the gene coding for yellow fluorescent protein (*YFP*) into the genome of human lung carcinoma cells. The *YFP* contained splicing sequences which enabled it to be spliced into the mRNA of a gene as a new exon, resulting in the expression of a fusion protein. The tagged gene was therefore expressed under its native promoter on the chromosome. We used time-lapse microscopy to image the fluorescently labeled proteins over time in individual cells. We found variability with a standard deviation that ranged, for different proteins, between about 15% and 30% of the mean. In the proteins examined, the level of variability was independent of the cell cycle.

B. *Mixing timescale*

We asked whether and on what timescale do protein levels mix in individual human cells. Mixing, in this context, occurs when a cell lineage, given enough time, reaches the different states found in a snapshot of a cell population, and can be characterized by a timescale τ_m for the average auto-correlation of individual cells to decay to half. All proteins showed mixing, with τ_m longer than 2 generations (over 40 hours) for many proteins.

C. *Cross-correlations between different proteins*

To view cross-correlations, cells were double labeled with YFP and mCherry tags, and specific protein pairs selected based on localization. Pairs of unrelated proteins, and proteins in the ribosome pathway, were thus tagged in the same cells. We found that the levels of proteins in the same biological pathway (the ribosome) were strongly correlated, while proteins in different pathways were not, indicating that fluctuations in protein levels are due to factors acting on the local network.

III. Conclusion

This type of long-memory noise, concerted between proteins in the same network, may act to generate variability in cellular responses and phenotypes.

Acknowledgements: We thank the Kahn family foundation and the Israel Science Foundation for support.

*These authors have contributed equally to this work

[1]Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA. E-mail: sigal@caltech.edu

[2]Department of Systems Biology, Harvard Medical School, Boston, MA, 02115 USA. E-mail: ron_milo@hms.harvard.edu

[3]Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, 76100 Israel. E-mail: uri.alon@weizmann.ac.il

References

[1] Spudich, J. L., and D. E. Koshland, Jr. (1976) Non-genetic individuality: chance in the single cell. Nature **262**, 467-471.

[2] Elowitz, M. B., et al. (2002) Stochastic gene expression in a single cell. Science **297**, 1183-1186.

[3] Ozbudak, E. M., et al. (2002) Regulation of noise in the expression of a single gene. Nat Genet **31**, 69-73.

[4] Raser, J. M., and E. K. O'Shea **(**2004) Control of stochasticity in eukaryotic gene expression. Science **304**, 1811-1814.

[5] Balaban, N. Q., et al. (2004) Bacterial persistence as a phenotypic switch. Science **305**, 1622-1625.

[6] Sigal, A., et al. (2006) Variability and memory of protein levels in human cells. Nature **444**, 643-6.