Advance Online Publication|doi:10.1038/nature05407|Published online 19 November 2006

NEWS & VIEWS

SYSTEMS BIOLOGY

Many things from one

John R. S. Newman and Jonathan S. Weissman

Cells of the same type can generate diverse sets of physiological traits from a single set of genes. Part of this diversity could stem from 'noise' that arises from variations in the way proteins are expressed.

The census bureau will tell you that the typical American family has 2.1 children, but there are no families (we hope) that precisely match this mean. Similarly, biologists have come to realize that population-based measurements can obscure critical information about cell-to-cell diversity. The use of fluorescent proteins as tags to look at the abundances of proteins in single cells has revealed that, at least for microorganisms, individual protein levels can vary considerably, even for genetically identical populations grown under uniform conditions.

In a paper published on *Nature*'s website today, Sigal and colleagues[1] present a view of what protein variation may look like in human cells. They reveal that this variation (or 'noise') can persist over several cell generations. Additionally, for proteins belonging to the same biochemical pathway, variation seems to be larger between cells than within cells. To the extent that protein abundance and activity are correlated, this variation might contribute to the ability of cells to generate a diversity of behaviours from a single set of genes (genotype).

Variation has attracted considerable interest, particularly because it can affect cells differentially. It can be an obstacle to the precise functioning of cells, by causing levels of biological molecules to deviate from their optima, and can degrade biological signals. But noise can also be exploited by allowing cells to switch between expression states, or, more speculatively, by generating diversity in physiological characteristics (phenotypes).

Studies using microorganisms have identified at least two types of noise. Extrinsic noise results from intercellular variations in the levels of components of the pathways that regulate gene expression. Intrinsic noise, however, arises from the random production and/or destruction of messenger RNAs and proteins that is due to chance interactions occurring in cells. These interactions would persist even if every cell were otherwise identical. Extrinsic noise tends to dominate for proteins present in high amounts in a cell, whereas intrinsic noise dominates when proteins, and their corresponding mRNAs, are present in low copy number. In contrast to our understanding

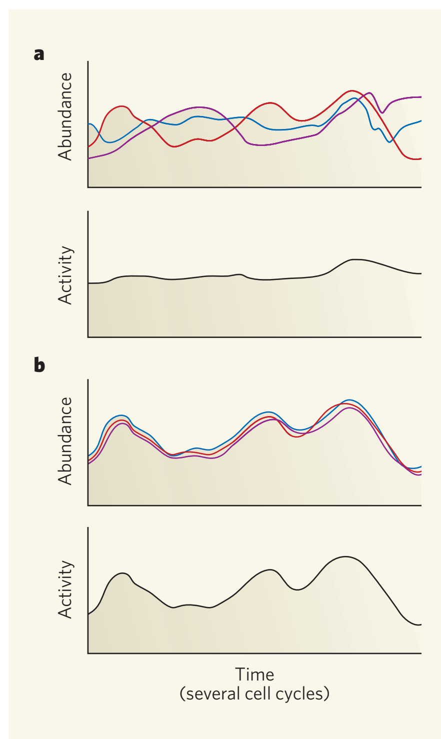

Figure 1 | Potential link between coherent protein variation and phenotype. **a**, In a three-component pathway, when the variation in the levels of each component is uncorrelated (top), the total pathway activity (bottom) is unrelated to the variation shown by any single component. This would mean that the phenotypic outcome of this pathway would be similar among a population of cells. **b**, When variation is correlated (top), the total pathway activity (bottom) varies in direct proportion to the variation shown by any single component. Sigal and colleagues[1] show that the abundances of ribosomal proteins tend to vary in concert. Extension of this finding to other proteins, particularly those for which high (or low) levels are maintained over several cell cycles, suggests that extrinsic noise may generate phenotypic diversity across a population of cells.

of variation in microorganisms, much less is known about the origins and consequences of variation in the cells of multicellular organisms. A priori one might speculate that, in these cells, the cost of noise might be greater because of the interdependence of cells within tissues or organs. This, together with the fact that there are higher numbers of mRNA molecules per cell, which tends to average out the randomness of the production and destruction of individual messages, suggests that total noise might be lower than that observed in microorganisms.

To explore this issue directly, Sigal and colleagues[1] created a series of strains of a human cancer cell line in which DNA encoding yellow fluorescent protein (YFP) was inserted into the genome in the middle of genes encoding various proteins (one insertion per strain). The genes therefore maintained their natural regulation, but encoded fluorescent fusion proteins. As the cells grew, time-lapse microscopy was used to monitor their overall fluorescence (and thus the abundance of the respective protein). The images were 'synchronized' with respect to cell divisions, eliminating a major source of variation that was not pertinent to these studies. The authors found that the coefficient of variation (standard deviation/mean) is 10–30%, similar to measurements in microorganisms[2].

To explore the level of intrinsic and extrinsic noise, the authors succeeded in obtaining a strain in which both copies of the gene that encodes the RPL5 protein were tagged, one with the DNA encoding YFP and one with DNA encoding a red fluorescent protein (RPL5 is found in a multi-subunit complex called the ribosome, which is involved in protein synthesis). Such a strain is useful because it makes it possible to distinguish between extrinsic and intrinsic noise (observed as intercellular and intracellular differences in fluorescence, respectively). In double-tagged cells, the levels of yellow and red fluorescence tended to fluctuate in concert, showing that extrinsic noise is

© 2006 Nature Publishing Group

a major component of total noise. This is in line with what one would expect given the greater numbers of mRNA molecules in human cells and the study's bias towards abundant proteins.

An extension of this double-tagging approach shows that, at least for ribosomal subunits, proteins that are involved in similar processes show similar variation, whereas those participating in different biological pathways do not always do so. Additionally, Sigal *et al.* looked at the length of time proteins spend above or below a population mean. For the 20 fusion proteins used in their study, they find that proteins require 0.8 to 2.5 cell cycles to 'relax' towards the average.

The picture that emerges from their observations is one in which the coherent induction or repression of a pathway occurs such that high or low protein levels can be maintained for several days. This phenomenon, particularly if it proves to be general, is provocative because it hints that proteins that must act together will be induced or repressed in a coordinated manner in individual cells, possibly even generating phenotypic diversity among cells (Fig. 1). So how might this long-term action occur, and what are the probable physiological consequences? One proposed model is that random fluctuations in regulatory components are amplified as a signal cascades down a pathway. Alternatively, long-lived responses may result from self-reinforcing mechanisms, such as positive feedback, that lead to slow rates of conversion between distinct activation states of gene networks.

Harder to gauge is the phenotypic impact of this extrinsic variation. A possible benefit of coherent variation could be to ensure that processes that are energetically expensive, such as protein secretion, are restricted to a few cells[3], rather than having many cells incur a high fixed cost to produce a set of proteins that are underused. Also, by analogy to microorganisms, where variation is postulated to generate phenotypic heterogeneity[4], one might speculate that noise could allow a subpopulation of cancer cells to evade endogenous or exogenous attempts to inhibit unrestrained cell growth.

At least three issues await further investigation. First, to what extent do the tissue-culture conditions reflect what occurs *in vivo*? Although tissue culture is an artificial environment that might generate noise that doesn't occur *in vivo*, cells typically divide much more slowly *in vivo*, which could cause deviations to persist for even longer timescales than Sigal *et al*. report. Second, to what extent will the observations reported here prove general? As the authors point out, the set of proteins they examine are biased (for example, by their abundance and accessibility for YFP integration). Third, do variations in protein concentrations lead to proportional changes in their activities? After all, cells have many mechanisms for controlling activities. These include modifying a protein after it has been made, and regulating the protein's cellular localization and the availability of key partners, all of which might act to suppress variation. More broadly, can we link heterogeneity in protein concentrations or activities to phenotypic differences? Although these questions show that much remains to be done, it is clear that these and related topics can now be explored in the cells of multicellular organisms. ■

John R. S. Newman and Jonathan S. Weissman are in the Department of Cellular and Molecular Pharmacology, and at the Howard Hughes Medical Institute, University of California, San Francisco, Byers Hall 403B, 1700 4th Street, San Francisco, California 94158-2330, USA.
e-mail: jrsnewman@alum.mit.edu

1. Sigal, A. *et al. Nature* doi:10.1038/nature05316 (2006).
2. Newman, J. R. S. *et al. Nature* **441,** 841–846 (2006).
3. Michaelson, J. *Lab. Invest.* **69,** 136–150 (1993).
4. Balaban, N. Q. *et al. Science* **350,** 1622–1625 (2004).

© 2006 Nature Publishing Group