SYSTEMS BIOLOGY

Mutations that rock the boat

The phenomenon of incomplete penetrance — whereby organisms with genetically identical alleles can develop distinct phenotypes — has been known for 80 years, and several mechanisms have been proposed to explain it. A paper now provides a quantitative description of the effect of an incompletely penetrant mutation on its gene-network properties. By using a single-mRNA counting method during *Caenorhabditis elegans* development, the authors show that such mutations can compromise the buffering mechanisms that normally maintain stability in gene-expression levels, variability and timing.

The 20 cells that make up the intestine of *C. elegans* derive from an invariant cell lineage as a result of successive interactions among the members of a small transcriptional network. The authors looked for transcriptional variation in the genes in this pathway as a consequence of mutations in its components. They did this by using a FISH-based technique that allows each mRNA molecule to be counted as a fluorescent spot, permitting gene expression to be measured accurately in each cell at successive stages of development (see image).

Mutations in *skn-1*, which encodes a protein at the top of the gene-expression cascade, cause the gene *elt-2,* which lies at the bottom of the cascade, to be bimodal: *elt-2* expression is either 'on' or 'off'. The mRNA-counting analysis of hundreds of wild-type and mutant embryos across developmental stages showed that *skn-1* mutations eliminated nodes at the top of the cascade entirely, and that the bimodality of *elt-2* expression was due to a thresholding effect imposed by the penultimate member of the cascade, *end-1* — *elt-2* would be on only if *end-1* expression was above a certain level between the 65- and 120-cell stages. The decision to switch on *elt-2* was made as early as the two-intestinal-cell stage.

SKN-1 affects *end-1* expression both directly and indirectly through the gene regulatory network. Activation of *end-1* is in fact controlled epigenetically by SKN-1, which relieves the repressive state imposed by the histone deacetyltransferase HDA-1 on the *end-1* promoter; the authors hypothesized that in *skn-1* mutants, *end-1* would not be activated as efficiently, resulting in variable *end-1* expression levels. This idea was supported by the effects of downregulating *hda-1* by RNAi in *skn-1* mutants — the expression levels of *end*-1 were stabilized, causing almost all cells to express *elt-2*.

An analysis of the effects of mutations in other members of the cascade on *elt-2* expression variation were less striking but followed the general rule that genes with higher numbers of connections (such as *skn-1* and a lower pathway member, *end-3*) tend to be more disruptive to stability then less well connected components, such as *end-1*.

The study has shown that the incomplete penetrance of *skn-1* mutations is caused by stochastic fluctuations in gene expression that are normally buffered. Stochasticity can therefore be induced even by mutations in genes with specific functions — and not necessarily globally acting ones, such as heat-shock protein 90 — and might itself drive the evolution of robustness mechanisms.

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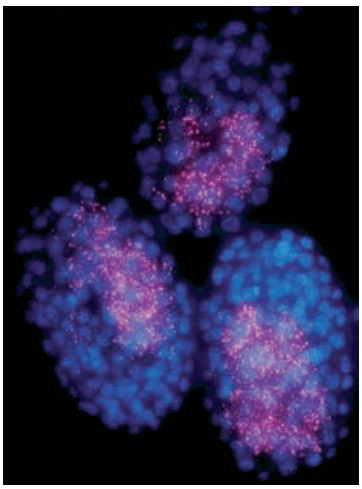
ORIGINAL RESEARCH PAPER Raj, A., Rifkin, S. A., Andersen, E. & van Oudenaarden, A. Variability in gene expression underlies incomplete penetrance. *Nature* 18 Feb 2010 (doi:10.1038/nature08781)



Wild-type *C. elegans* embryos stained to show nuclei (blue) and *elt-2* RNA (pink). Image is reproduced, with permission, from Raj, A. & Rifkin, S. A. *et al.* © (2010) Macmillan Publishers Ltd. All rights reserved.