

enzymes, only a few appear to interact with the proteasome. Also, a number of deubiquitinating enzymes interact with the proteasome. It is possible that E3 ubiquitin ligases and deubiquitinating enzymes associated with the proteasome can edit the ubiquitin chain as the substrate approaches the proteasome. In this way, these enzymes can modulate substrate specificity or alter the overall rate of protein degradation. If UBE3A turns out to be a component of neuronal or synaptic proteasomes, certain Angelman syndrome symptoms may also be related to altered proteasome composition in the affected neurons. This may represent an alternative model for understanding UBE3A function in the brain that counters the current notion that UBE3A is a freely diffusing E3 ubiquitin ligase with perhaps a dozen specific substrates.

Although recent advances in genomics have allowed us to pinpoint the cause of hereditary neurological disorders like

Angelman syndrome to a single gene like *UBE3A*, it has proven difficult to understand the functional consequences of the gene mutation or deletion. The same can be said for parkin, an E3 ubiquitin ligase linked to autosomal recessive juvenile Parkinsonism. This reflects our general lack of understanding about how the ubiquitin-proteasome system contributes to the maintenance and regulation of the neuronal proteome. A broad-scale approach, like that taken by Greer et al., can elucidate elements both upstream and downstream of the gene of interest, positioning the mutation in a functional synaptic context where the link to the disease may be clarified.

REFERENCES

Besche, H., Haas, W., Gygi, S., and Goldberg, A. (2009). *Biochemistry* 48, 2538–2549.

Bramham, C.R., Worley, P.F., Moore, M.J., and Guzowski, J.F. (2008). *J. Neurosci.* 28,

11760–11767.

Cooper, E.M., Hudson, A.W., Amos, J., Wagstaff, J., and Howley, P.M. (2004). *J. Biol. Chem.* 279, 41208–41217.

Dan, B. (2009). *Epilepsia* 50, 2331–2339.

Dindot, S.V., Antalffy, B.A., Bhattacharjee, M.B., and Beaudet, A.L. (2008). *Hum. Mol. Genet.* 17, 111–118.

Greer, P.L., Hanayama, R., Bloodgood, B.L., Mardinly, A.R., Lipton, D.M., Flavell, S.W., Kim, T.-K., Griffith, E.C., Waldon, Z., Maehr, R., et al. (2010). *Cell*, this issue.

Jiang, Y.H., Armstrong, D., Albrecht, U., Atkins, C.M., Noebels, J.L., Eichele, G., Sweatt, J.D., and Beaudet, A.L. (1998). *Neuron* 21, 799–811.

Takiyama, Y. (2007). *Cerebellum* 6, 353–359.

Wang, X., Chen, C.F., Baker, P.R., Chen, P.L., Kaiser, P., and Huang, L. (2007). *Biochemistry* 46, 3553–3565.

Yashiro, K., Riday, T.T., Condon, K.H., Roberts, A.C., Bernardo, D.R., Prakash, R., Weinberg, R.J., Ehlers, M.D., and Philpot, B.D. (2009). *Nat. Neurosci.* 12, 777–783.

A Penetrating Look at Stochasticity in Development

Robert J. Johnston, Jr.¹ and Claude Desplan^{1,*}

¹Department of Biology, New York University, 1009 Silver Center, 100 Washington Square East, New York, NY 10003, USA

*Correspondence: cd38@nyu.edu

DOI 10.1016/j.cell.2010.02.018

In recent work published in *Nature*, Raj et al. (2010) use single mRNA molecule quantification to show that variation in gene expression in *Caenorhabditis elegans* increases in mutants displaying incomplete penetrance. They find that a bimodal response is triggered when noisy expression of an upstream regulator crosses a critical threshold.

Robustness to genetic and environmental variation is an essential feature of all biological systems. In recent years, it has become possible to address the molecular mechanisms that ensure the reproducible outcomes of biological processes. These mechanisms often break down in mutant conditions, leading to variable outcomes. In genetic terms, penetrance refers to the proportion of individuals of a particular mutant genotype displaying a mutant phenotype. In a paper that recently appeared

in *Nature*, Raj and colleagues examine mutant backgrounds of the nematode *Caenorhabditis elegans* to explore the role of variable gene expression in incomplete penetrance (Raj et al., 2010). Their analysis provides evidence that mutant conditions increase noise in gene expression. They further show that the downstream genes in affected pathways respond to these variable inputs at certain thresholds and that chromatin has a role in modulating variability of gene expression.

Several groups have evaluated well-defined genetic circuits with new and highly quantitative methodologies to derive fundamental principles about the nature of these circuits and of gene regulation in general (for instance, Cağatay et al., 2009; Gregor et al., 2007; Maamar et al., 2007; Mangan et al., 2003; Süel et al., 2006, 2007). Working in a similar vein, Raj and colleagues have now sought to address the mechanisms controlling incomplete penetrance using a tech-

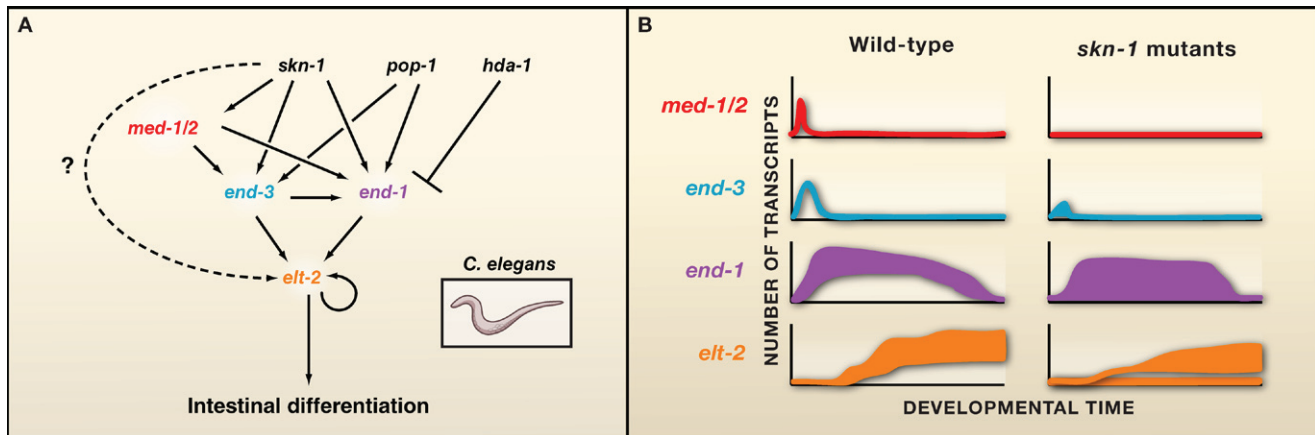


Figure 1. Gene Expression Variability and Incomplete Penetrance

(A) The circuit of transcription factors that controls intestinal differentiation. The dotted arrow indicates a putative regulatory interaction between *skn-1* and *elt-2* based on the altered threshold response observed for one *skn-1* allele.

(B) In wild-type animals, *med-1/2* and *end-3* levels peak early in development with little variation. *end-1* is expressed at high levels with low variation for a prolonged period. *elt-2* is upregulated and sustained. In *skn-1* mutants, *med-1/2* are absent, whereas *end-3* expression is minimal. *end-1* expression is induced but highly variable, and this variability is resolved by a bimodal response in *elt-2* expression. The thickness of each line indicates the degree of expression variation.

nique that allows the counting of single messenger RNA (mRNA) molecules in vivo. They chose to evaluate the genetic network that determines intestinal identity in *C. elegans*. As illustrated in Figure 1A, this circuit is a meshwork of coherent feedforward loops among transcription factors (for a review, see McGhee, 2007). Coherent feedforward loops delay the response of the most downstream element (Mangan et al., 2003) and thus may contribute to the temporal control of the circuit (Figure 1B). Specifically, *skn-1* activates expression of *med-1/med-2* (considered together as a single node), *end-3*, and *end-1*. *med-1/med-2* activate *end-3* and *end-1*, whereas *end-3* activates *end-1*. Additionally, *pop-1* mediates a WNT signal to upregulate expression of *end-3* and *end-1*. Together, *end-3* and *end-1* function in an OR gate (that is, the two genes are redundant) to activate *elt-2*. *elt-2* reinforces this input through a positive feedback loop. As a convergent node of this network motif, *elt-2* acts to upregulate hundreds of genes required for intestine fate (Figure 1A).

The authors focus their analysis on the variation in gene expression that occurs in mutants in the most upstream member of this pathway, *skn-1*, which exhibit intestinal differentiation defects with incomplete penetrance. In wild-type animals, levels of *med-1/med-2*, *end-3*, and *end-1* mRNA peak with distinct and robust temporal dynamics, culminating

in the expression of *elt-2* at a sustained maximum (Figure 1B). In *skn-1* mutants, expression of *med-1/med-2* is completely absent, whereas *end-3* levels are minimal. The single mRNA molecule detection technique allows the authors to observe noisiness in *end-1* expression that appears to be the only variable input into *elt-2* in this mutant condition. This variation is resolved at the level of *elt-2* expression, which displays a bimodal ON/OFF distribution of mRNA levels (Figure 1B).

The bimodal response in *elt-2* expression suggests that a thresholding mechanism controls this phenomenon. In other words, when *end-1* reaches a specific level, the *elt-2* positive feedback loop is triggered, resulting in an ON/OFF response. To test this, Raj and coworkers evaluated the relative mRNA levels of *end-1* and *elt-2*. They find that at low *end-1* levels, *elt-2* expression is never observed. Nevertheless, *skn-1* mutant worms with high *end-1* mRNA levels similar to wild-type are capable of triggering the *elt-2* positive feedback loop. However, some worms exhibiting high levels of *end-1* do not trigger *elt-2* expression, arguing against a simple thresholding model. The investigators provide an explanation for this apparent contradiction. In null *end-3* mutants, *end-1* levels remain similar to wild-type levels, but expression of *elt-2* is often not induced. Given that *end-3* also feeds into *elt-2* regulation, this observation suggests

that *end-3* allows for high *end-1* levels to efficiently induce *elt-2* expression. In *skn-1* mutants, *end-3* is nearly absent, which explains why high levels of *end-1* are not always capable of *elt-2* induction. The frequency of high *end-1* levels with low *elt-2* levels within embryos is greater than the actual penetrance of intestinal defects. On the basis of this observation, the authors conclude that high levels of *end-1* are likely activating *elt-2* in most cases, but there is a temporal delay in induction. Given that the single mRNA counting technique only allows for static observations, new methodologies will be needed to further test this hypothesis.

Strangely, for one *skn-1* mutant allele, *elt-2* expression is activated even at low levels of *end-1*. This is peculiar, given that *skn-1* is the most upstream regulator in this network and is not known to directly regulate *elt-2*. Therefore, it is surprising that a different type of mutation in this upstream gene would modulate a gene that it only indirectly regulates. This observation suggests that *skn-1* probably regulates *elt-2* either directly on its promoter or via an unknown mediating factor (Figure 1A). Alternatively, the worms with this particular *skn-1* allele may carry a background mutation that affects the threshold response.

Raj and colleagues also examine the role of *hda-1*, a chromatin regulator that is a repressor of *end-1* expression (Calvo et al., 2001) (Figure 1A). The authors con-

clude that knockdown of *hda-1* levels with RNA interference (RNAi) in *skn-1* mutants leads to upregulation of *end-1* transcripts and a decrease in its variability. Indeed, the coefficient of variation for *end-1* levels does decrease with RNAi knockdown of *hda-1* in *skn-1* mutants as compared to the variation in *skn-1* mutants alone. However, this is still markedly higher than the variation observed in the wild-type. It appears that the double-mutant phenotype is complex. Not only is *end-1* derepressed, but the variation is also partially limited. Is the partial decrease in variation simply a byproduct of derepression of this gene? Or do multiple mechanisms feed into the control of variation? Or both? These questions are complex, but this paper lays the groundwork for addressing them.

The work of Raj and colleagues begins to address the mechanisms that cause incomplete penetrance. Their highly quantitative single molecule approach is new to developmental biology, which typically makes use of reporter transgenes, antibody staining, and in situ hybridization to assess gene expression. With this methodology, Raj et al. clearly show that

variation in expression occurs in specific mutant conditions and that the architecture of a developmental network is able to compensate for noisy expression. However, one of the most challenging problems in the field of gene expression (in wild-type or mutant conditions) is to identify the source of transcriptional stochasticity. Although a role for chromatin state is proposed in this paper, this is hardly surprising given that its regulation is so fundamental to gene expression in general. The next challenge will be to show how these alterations affect variability in gene expression at individual loci.

Robustness compensates for variation caused by the stochastic low level expression of key regulators. However, mechanisms that ensure robustness also provide a buffer in the wild-type, which allows for the evolution of new regulatory interactions (for a review, see Masek and Siegal, 2009). It will be exciting to determine not only how variation occurs due to the break down of wild-type biological programs but also how novel cryptic modes of regulation are revealed when robustness mechanisms are impaired.

ACKNOWLEDGMENTS

C.D. is supported by NIH R01 EY013010. R.J.J. was supported by a Jane Coffin Childs Memorial Fund fellowship.

REFERENCES

- Cağatay, T., Turcotte, M., Elowitz, M.B., Garcia-Ojalvo, J., and Süel, G.M. (2009). *Cell* 139, 512–522.
- Calvo, D., Victor, M., Gay, F., Sui, G., Luke, M.P., Dufourcq, P., Wen, G., Maduro, M., Rothman, J., and Shi, Y. (2001). *EMBO J.* 20, 7197–7208.
- Gregor, T., Tank, D.W., Wieschaus, E.F., and Bialek, W. (2007). *Cell* 130, 153–164.
- Maamar, H., Raj, A., and Dubnau, D. (2007). *Science* 317, 526–529.
- Mangan, S., Zaslaver, A., and Alon, U. (2003). *J. Mol. Biol.* 334, 197–204.
- Masek, J., and Siegal, M.L. (2009). *Trends Genet.* 25, 395–403.
- McGhee, J.D. (2007). The *C. elegans* intestine. In *WormBook, The C. elegans Research Community*, ed. 10.1895/wormbook.1.133.1. <http://www.wormbook.org>.
- Raj, A., Rifkin, S.A., Andersen, E., and van Oudenaarden, A. (2010). *Nature*. Published online February 18, 2010. 10.1038/nature08781.
- Süel, G.M., Garcia-Ojalvo, J., Liberman, L.M., and Elowitz, M.B. (2006). *Nature* 440, 545–550.
- Süel, G.M., Kulkarni, R.P., Dworkin, J., Garcia-Ojalvo, J., and Elowitz, M.B. (2007). *Science* 315, 1716–1719.

MicroRNAs: From Decay to Decoy

Michaela Beitzinger¹ and Gunter Meister^{1,2,*}

¹Universität Regensburg, Universitätsstrasse 31, 93053 Regensburg, Germany

²Center for Integrated Protein Science Munich, Max-Planck-Institute of Biochemistry, 82152 Martinsried, Germany

*Correspondence: gunter.meister@vkl.uni-regensburg.de

DOI 10.1016/j.cell.2010.02.020

MicroRNAs interact with Argonaute proteins to guide posttranscriptional gene silencing. Eiring et al. (2010) now show that *miR-328* has a second function, acting as a decoy by binding to hnRNP E2 and lifting its translational repression of an mRNA involved in myeloid cell differentiation.

Although microRNAs (miRNAs) were discovered a decade ago, a detailed understanding of these tiny gene regulators is still in its infancy. Genes encoding miRNAs are transcribed as primary miRNA transcripts (pri-miRNAs) by RNA polymerase II. Pri-miRNAs are processed by Drosha to produce stem-loop-structured miRNA precursors (pre-miRNAs). Pre-miRNAs are exported to

the cytoplasm, where Dicer generates ~21 nucleotide double-stranded RNA intermediates. Such double-stranded RNAs are processed further, and one strand, the mature miRNA, interacts with Argonaute (Ago) proteins to form miRNA-protein complexes (miRNPs) (Bartel, 2009; Carthew and Sontheimer, 2009). miRNAs are able to silence gene expression posttranscriptionally

by binding to partially complementary target sites in the 3' untranslated region (UTR) of target messenger RNAs (mRNAs), leading to repression of translation or destabilization of the mRNA by deadenylation. Almost perfectly complementary target sites in the mRNA can be cleaved by the miRNA through an RNA interference-like mechanism (Filipowicz et al., 2008). In contrast to these