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Tuning In to Noise: Epigenetics and Intangible Variation

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In this special issue of *Developmental Cell*, we discuss the role of chromatin in phenotypic variation as a counterpoint to the reviews on chromatin dynamics in development and cancer. We highlight some recent work on the role of chromatin in transcriptional noise in yeast and *Caenorhabditis elegans* and consider the implications in understanding intangible variation or developmental noise in mammals.

The myriad processes that make us unique as individuals lie at the heart of biology. Molecular biologists and geneticists have used classic approaches to tease out the heritable component of phenotype, but we have made less progress in unraveling the nonheritable component. The idea that probabilistic or chance events in early development play a role in the latter is rarely considered, despite long-standing evidence from embryologists and naturalists. In undergraduate textbooks this is called "developmental noise" or 'intangible variation." In essence, highly inbred animals, ostensibly isogenic, reared in tightly controlled environments, show a surprisingly broad range of phenotypes for many measurable traits.

Interestingly, Waddington's classic papers on epigenetics were stimulated in part by his curiosity about developmental accidents, or "noise," during the growth of a complex organism—hence his interest in asymmetric development or fluctuating asymmetry (Waddington, 1957). Even before the structure of DNA was elucidated, Waddington foresaw that the bridge between genotype and phenotype must be complex. Over 50 years later, evidence for a role for stochastic events in development is accumulating and our understanding of the molecular events underlying developmental noise is becoming clearer (Losick and Desplan, 2008). Recent studies on transcription in yeast and *Caenorhabditis elegans* have revealed that "transcriptional noise" is regulated by proteins involved in establishing and maintaining the epigenome. Other systems of buffering cellular noise such as microRNAs or the molecular chaperone Hsp90 are also likely to be involved, but here we are focusing on the role of chromatin.

Modern molecular techniques, in particular the development of single-cell transcription assays, have given us greater insight into these processes. The first single-cell study of transcriptional noise in eukaryotes used a two-reporter-gene strategy in diploid yeast. Both cyan and yellow fluorescent proteins (CFP and YFP) were inserted into the same site on homologous chromosomes and each was driven by the same *PHO5* promoter sequence (Raser and O'Shea, 2004). This system allowed the researchers to measure extrinsic noise (affecting the expression of both reporters) and intrinsic noise (affecting only one of the reporters). The ability to measure intrinsic noise was vital; they were able to resolve intrinsic fluctuations in expression due to inefficient promoter activation that was not evident when transcripts were studied from pooled cells, in which the levels averaged out. They found that reduction in the levels of chromatin remodeling factors (i.e., components of SWI/SNF, INO80 or SAGA) that were known to act at the *PHO5* promoter resulted in increased intrinsic noise, indicating that faithful epigenetic gene regulation buffers against noise arising from sluggish promoter transitions.

A role for chromatin in suppressing phenotypic variation has also emerged from studies in *C. elegans*. RNA interference was used to analyze the phenotypic consequences of knocking down paired combinations of genes annotated as having "signaling," "chromatin," or "transcription factor" function (Lehner et al., 2006). The phenotypes of 37 mutant strains that carry mutations in different signaling components were compared to the phenotypes when RNAi was used to knockdown each of 1860 genes encoding proteins with signaling, chromatin or transcription factor functions, i.e., they compared ∼65,000 combinations. The results showed that knockdown of certain chromatin genes (e.g., orthologs of the NuA4/Tip60 histone acetyltransferase complex or the nucleosome remodeling and histone deacetylase [NuRD] complex) clearly had the greatest effect on enhancing the phenotypes of the mutant strains. They described these chromatin genes as network "hubs" and showed that knockdown of these hubs uncovered a broad range of mutant phenotypes. These hub genes interacted with over one-quarter of the signaling mutant genes from multiple different signal transduction pathways, suggesting that they can modulate many different processes. The authors propose that chromatin hub genes modulate the phenotypic consequences of mutation to a large number of genes. So, in this system, chromatin modifiers appear to be functioning as general buffers of genetic variation.

Raj and colleagues, also working with *C. elegans*, carefully measured the transcriptional changes underlying incomplete penetrance of mutations affecting intestinal specification (Raj et al., 2010). The strength of their study lies in the simplicity of the system; the *C. elegans* intestine is made up of 20 cells and a well-defined network of only six genes determines intestinal cell specification. Mutation of one of the genes in the pathway, *skn-1*, is embryonic lethal but with incomplete penetrance; in some cases there is no intestinal cell specification and in some

cases there are intestinal cells present in embryos. By counting transcripts (using a version of FISH that makes each mRNA molecule visible as a single fluorescent spot) of various downstream genes in mutant and wild-type embryos, they showed that the variation in mutant phenotypes is caused by large variations in the transcript levels of an intermediary gene (*end-1*), which must reach a threshold level before it can activate a third gene that acts as a switch to intestinal cell fate. *skn-1* activates *end-1* by chromatin remodelling and the authors hypothesize that inefficient recruitment of the chromatin remodelling machinery underlies the variable expression levels. In summary, this study shows that wild-type offspring have epigenetic mechanisms that control large fluctuations in gene expression and that if these mechanisms are disrupted, stochastic transcription in the cell can lead to altered cell fates.

A somewhat different approach has been taken by Choi and Kim. They have used a single-cell proteomic analysis in yeast to study cellular noise and also found that it is largely controlled by chromatin regulation (Choi and Kim, 2009). They found that genes with the most variable expression (among cells) have a common sequence in their promoters that enhances the flexibility of the DNA and attracts nucleosome occupancy. Others had shown previously that nucleosome occupancy in these regulatory regions prohibits transcription and so nucleosome removal is required for expression. The authors found that chromatin modifier levels (e.g., chromatin remodelers, histone acetyltransferases, deacetyltransferases, and methyltransferases) had a substantial effect on variability, whereas transcription factor levels did not (Choi and Kim, 2009). Thus, the variable expression at these promoters is initially encoded in the promoter sequence, but is ultimately directed by epigenetic processes.

Numerous genome-wide studies on nucleosome positioning in eukaryotes have found that most promoters have nucleosome depleted regions that lie upstream of the transcriptional start site. A recent study in yeast shows that nucleosome occupancy can govern the probability that a gene will be expressed. The authors embedded a transcription factor binding site within a nucleosome-bound region of the promoter; normally, the site is located in a nucleosome-depleted region and gives very efficient gene activation. When the site was placed within a nucleosome, expression became bimodal, i.e., an on/off pattern was observed in single-cell assays, indicating stochastic binding of the transcription factor (Bai et al., 2010). Bimodal expression was also observed at an endogenous gene in which the transcription factor binding site is naturally embedded in a nucleosome. Furthermore, the probability of a cell being "on" was changed by altering the dosage of a SWI/SNF remodeler or a histone deacetylase. This study suggests that the preservation of nucleosome-depleted regions in promoter elements is a widely conserved epigenetic mechanism that suppresses transcriptional noise. It is interesting that some functional promoters lack a nucleosome-depleted region. Could these lie adjacent to genes for which bimodal or variable expression is advantageous?

Intangible variation (developmental noise) has been studied mainly in *Drosophila* and mammals. In these organisms, in which developmental processes are complex, it will be difficult to design methods of counting transcripts in individual cells at critical stages of a developmental pathway, as has been done in worms. Nevertheless, there is every reason to believe that the same underlying principles occur. Consistent with this, we have observed increased phenotypic noise among inbred littermates, in mice haploinsufficient for chromatin regulators (N.C.W., unpublished data). Similarly, a screen for modifiers of epigenetic reprogramming, also carried out in an inbred background, identified a number of mutant strains that displayed stochastic death; some mutants died and others did not (Ashe et al., 2008). This can be considered an extreme form of phenotypic noise. Although reports of incomplete penetrance in mouse colonies are not uncommon, mixed genetic backgrounds often make it difficult to rule out underlying genetic explanations.

We should not forget that there are situations in which transcriptional noise will be advantageous; the development of mammalian olfactory neurons, each with a specific receptor, is one stunning example. Each olfactory sensory neuron expresses only one allele of one type of olfactory receptor (Serizawa et al., 2000). There are over 1000 olfactory receptor genes spread throughout the genome, so to achieve mutually exclusive expression is challenging. One can view this process as a cell fate decision; each neuron is making a different decision. The choice to express a single olfactory receptor is stochastic and, as such, can be considered transcriptional noise. *Cis*-acting sequences and feedback mechanisms ensure that only one receptor is expressed (Lomvardas et al., 2006; Serizawa et al., 2003).

At the root of any change in cell fate is a single event that triggers a cascade of subsequent changes. It may well turn out that the capacity for some gene promoters to act in a bimodal fashion is a fundamental requirement of multicellularity. The study by Raser and O'Shea on intrinsic transcriptional noise in yeast identified both *cis*- and *trans*-acting factors that alter the level of noise, suggesting that noise is an evolvable trait that can be optimized to balance fidelity and diversity at each gene. It is difficult in outbred populations, such as humans, to measure the contribution of such processes to what makes us each unique, but the idea that transcriptional noise is involved is worthy of consideration.

REFERENCES

Ashe, A., Morgan, D.K., Whitelaw, N.C., Bruxner, T.J., Vickaryous, N.K., Cox, L.L., Butterfield, N.C., Wicking, C., Blewitt, M.E., Wilkins, S.J., et al. (2008). Genome Biol. *9*, R182.

Bai, L., Charvin, G., Siggia, E.D., and Cross, F.R. (2010). Dev. Cell *18*, 544–555.

Choi, J.K., and Kim, Y.J. (2009). Nat. Genet. *41*, 498–503.

Lehner, B., Crombie, C., Tischler, J., Fortunato, A., and Fraser, A.G. (2006). Nat. Genet. *38*, 896–903.

Lomvardas, S., Barnea, G., Pisapia, D.J., Mendelsohn, M., Kirkland, J., and Axel, R. (2006). Cell *126*, 403–413.

Losick, R., and Desplan, C. (2008). Science *320*, 65–68.

Raj, A., Rifkin, S.A., Andersen, E., and van Oudenaarden, A. (2010). Nature *463*, 913–918.

Raser, J.M., and O'Shea, E.K. (2004). Science *304*, 1811–1814.

Serizawa, S., Ishii, T., Nakatani, H., Tsuboi, A., Nagawa, F., Asano, M., Sudo, K., Sakagami, J., Sakano, H., Ijiri, T., et al. (2000). Nat. Neurosci. *3*, 687–693.

Serizawa, S., Miyamichi, K., Nakatani, H., Suzuki, M., Saito, M., Yoshihara, Y., and Sakano, H. (2003). Science *302*, 2088–2094.

Waddington, C.H., ed. (1957). The Strategy of the Genes: A Discussion of Some Aspects of Theoretical Biology (London: Allen & Unwin).