Dispatches

Gene Regulation: Enhancers Stepping Out of the Shadow

The expression of many animal genes has been shown to be controlled by two — rather than one — enhancers with similar regulatory content. Such enhancer redundancy ensures robustness of gene expression under adverse environmental or genetic conditions.

Oliver Hobert

In spite of much genetic variation and exposure to a wide and diverse range of environmental conditions, phenotypic variation between animals within a species is generally low. This insight led Conrad Waddington to coin the term 'canalization': "*Developmental reactions* […] *are in general canalized. That is to say, they are adjusted so as to bring about one definite end-result regardless of minor variations in conditions during the course of the reaction*" [1]. Waddington was not the first to notice. Back in the even earlier days of experimental embryology, Hans Spemann had observed the robustness of developmental systems against perturbation and called it a "*double assurance*" [2]. In the meantime countless studies have confirmed the robustness of various systems to experimental manipulation. However, it has been much less forthcoming to interpret canalization in molecular genetic terms [3,4].

One potential mechanism that may provide canalizing function is through the regulation of gene expression, in particular transcription. The story began in 2008 when the lab of Mike Levine discovered that enhancers of individual developmental control genes in *Drosophila*, responsible for driving expression in specific spatio-temporal domains, do not just come in one, but two copies [5]. Each enhancer copy is occupied by a similar set of cognate *trans*-acting factors, is phylogenetically conserved and is able to produce similar expression patterns in reporter gene assays [5]. The more proximal enhancer was termed the 'primary enhancer', while the second, most distal copy was called the 'shadow enhancer'. Two recent reports, one from the Levine lab in this issue of *Current Biology* [6], the other from the lab of David Stern [7], have undertaken mutational analysis to probe the functional relevance of such a regulatory architecture.

Perry *et al.* [6] use the *Drosophila snail* locus as a paradigm. The authors show that two distinct enhancers — the shadow enhancer located more distally to the *snail* coding region and the primary enhancer located more proximally — are each sufficient to recapitulate the mesodermal expression pattern of *snail* when hooked to a reporter gene. The authors then use BAC-based genomic reporter constructs in which either the primary or shadow enhancer is deleted and find that each BAC construct can reproduce authentic mesodermal expression. However, compared to the wild type, either deletion construct (Figure 1A) results in less faithful reporter gene expression under more extreme environmental conditions (increased temperature). This manifests itself essentially in a binary manner, i.e. the fraction of cells expressing the reporter gene becomes more variable (Figure 1A). The authors also examine the effect of deleting one of the two enhancers on the ability of a genomic BAC clone to rescue the *snail* mutant phenotype. The picture is the same as with the reporter gene: lacking the shadow enhancer does result in rescue, but compared to a wild-type genomic clone, the rescue is less robust under adverse environmental conditions.

The study by the Stern lab [7] draws a similar picture for another developmental control gene, the *Drosophila* transcription factor *shavenbaby* (*svb*). Here again, separate enhancers that drive similar expression patterns were identified. In this case, the authors engineered a mutation in the genome that deletes the shadow enhancer. The authors find that such mutant animals display subtle phenotypes under optimal conditions. Much stronger phenotypes are seen either upon adverse environmental conditions or in a genetically sensitized background in which the system was partially de-stabilized by removal of one copy of the *wingless* gene, which under normal conditions has no detrimental effect. Showing that duplicated *cis*-regulatory control elements provide a buffer against two completely different types of 'Waddingtonian' perturbations — intrinsic (genetic) *and* extrinsic (environmental) — is a testament to the importance of the *cis*-regulatory robustness mechanism and suggests that buffering systems may be more versatile than previously thought [8].

Duplicated regulatory signatures similar to the two recent *Drosophila* cases were also described in the nematode *C. elegans* [9]. Several genes expressed in the gustatory neuron ASE contain not one but two (or more) binding sites for the Zn-finger transcription factor CHE-1, a terminal selector gene. Genomic regions containing either binding site alone are entirely sufficient to drive *che-1*-dependent expression in the ASE gustatory neurons. Yet in their normal genomic context, mutation of either binding site alone only results in an incompletely penetrant effect on the expression of *che-1* target genes in ASE, while loss of both sites results in complete loss of expression. Moreover, a mutation in one of the two CHE-1 binding sites controlling the homeobox gene *cog-1* results in a partially penetrant loss of *cog-1* expression and therefore ASE differentiation defects [9].

If one considers these case studies, one should perhaps start wondering whether it actually makes sense to designate one of the enhancers a 'shadow enhancer'. In all cases, both enhancers appear to behave similarly in terms of both sufficiency (i.e. each individual enhancer is sufficient to drive expression in specific tissues) and necessity (i.e. each individual enhancer is not absolutely necessary). Naturally,

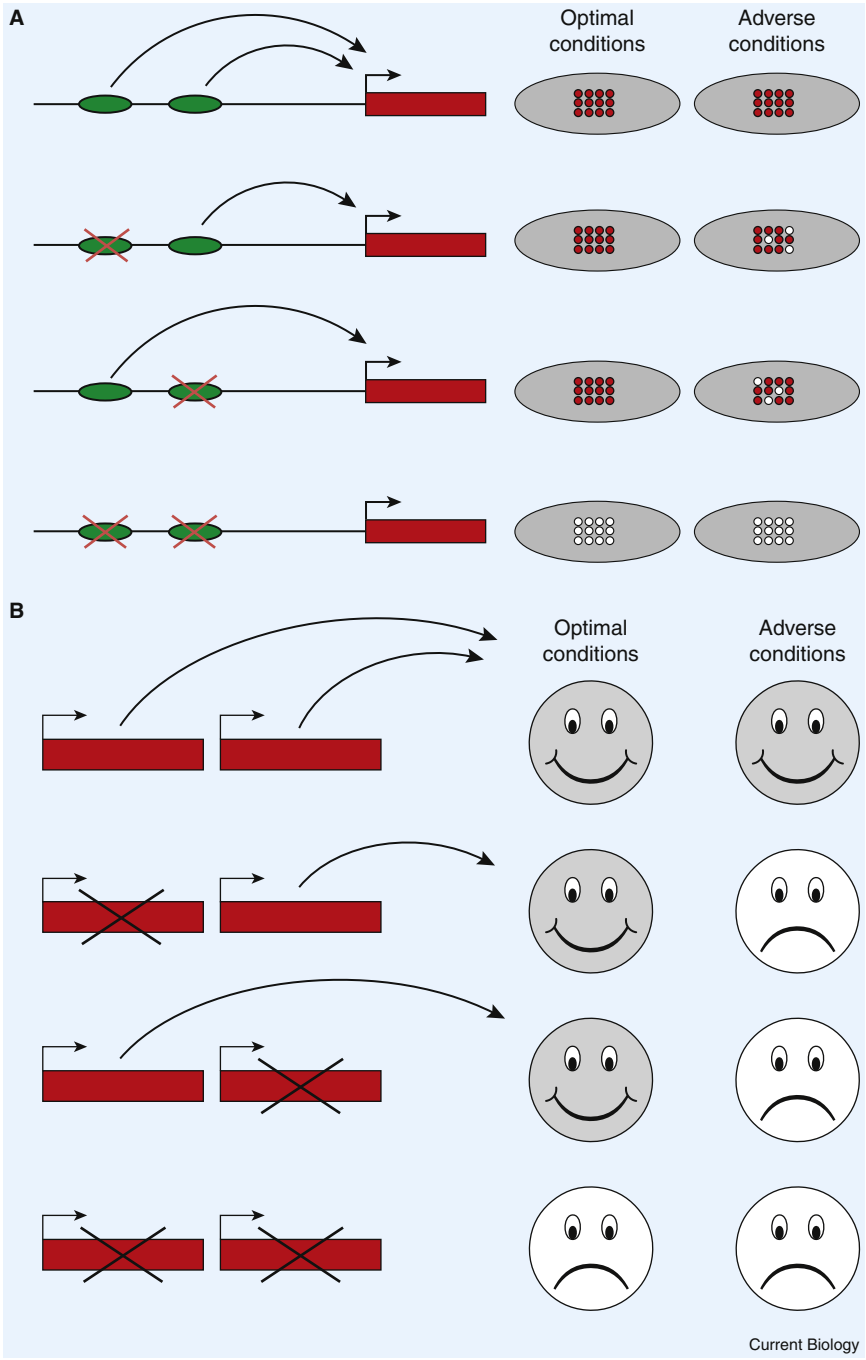

Figure 1. Robustness and redundancy.

(A) Robustness through multiple enhancers. Summary of the results of three studies discussed in the text [6,7,9]. Blue ovals indicate either complex enhancers that bind multiple proteins [6,7] or single transcription factor binding sites [9]. Red circles indicate expression domains of a gene. (B) Robustness applied to paralogous genes. Note the conceptual similarity to the mechanism described in (A). See [3] for further discussion of paralogous genes.

one of the two will always be located closer to the coding region, but since distance imposes no restriction on enhancer function, it is not clear whether distance is a truly functionally meaningful criterion. As for now, the perhaps most conservative interpretation of the data mentioned above is that robustness is conferred by two more or less equivalent regulatory mechanisms.

There is no reason to believe that the logic of buffering through seemingly redundant enhancers should be restricted to invertebrates. Multiple regulatory elements that drive similar expression patterns have also been identified in mice [10,11]. Moreover, deletions of well-characterized and highly-conserved vertebrate enhancers do not always lead to recognizable phenotypes under standard lab conditions [12], but, as the *Drosophila* studies described above imply, phenotypes may become apparent under more adverse conditions.

Besides providing a molecular framework for how robustness is conferred, these studies [6,7] should perhaps also be viewed in the context of two classic genetic phenomena — redundancy and partial penetrance. Traditionally, co-expressed paralogous genes generated through duplication have been thought to provide redundant gene functions, such that one copy of the gene becomes free to acquire new functions [3]. Indeed, the existence of a co-expressed paralog is the most frequently used explanation for why the knockout of one paralog produces no obvious phenotype. Yet, as pointed out by Wilkins [3], co-expressed paralogous genes may actually also serve the specific purpose of canalizing gene function. Thus, much in analogy to the apparently redundant enhancer elements described above, the function of individual paralogous genes may only manifest itself upon exposure to adverse environmental conditions, or in genetically sensitized backgrounds (Figure 1B).

Likewise the robustness concept as described by the Levine and Stern labs [6,7] bears on the phenomenon of partial penetrance that has been observed countless times in genetic knockout studies, e.g. [13]. Partial penetrance can be easily explained by postulating that the activity of a gene (or one or multiple pathways) is somewhat fluctuating under normal and/or adverse conditions, but is usually so much higher than a critical threshold that these fluctuations do not result in any phenotypic consequence. Disruption of a component of the system may lower gene activity towards the threshold value, such that random fluctuations now matter significantly and some animals (or cells) will display a mutant phenotype — as their gene activity happens to be below the threshold — while others won't

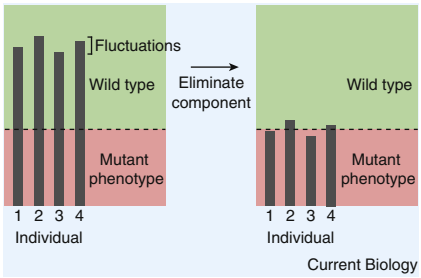

Figure 2. A conceptual framework to explain partial penetrance.

Removal of a component in a buffered system results in a partially penetrant phenotype. In the example shown, 50% (2/4) of the animals will display a mutant phenotype. See [14] for an intriguing recent report on the phenomenon of partially penetrant phenotypes.

(Figure 2). The enhancer studies fit nicely into this picture. Two enhancers ensure firing probability and/or sufficient mRNA output above a threshold even in the presence of perturbations, while removal of one enhancer decreases the firing probability and/or level of mRNA output to a threshold where fluctuations result in a significant impact in some but not all animals or cells (Figure 2). The bottom line of all this is quite simple and surely would have pleased Spemann and Waddington: having two copies of the same thing is good, but the adaptive advantage of such duplication may only be apparent under specific, perturbing conditions.

References

1. Waddington, C.H. (1942). Canalization of development and the inheritance of acquired characters. Nature *150*, 563–565.
2. Hamburger, V. (1988). The Heritage of Experimental Embryology: Hans Spemann and the Organizer (Oxford: Oxford University Press).
3. Wilkins, A.S. (1997). Canalization: a molecular genetic perspective. Bioessays *19*, 257–262.
4. Hornstein, E., and Shomron, N. (2006). Canalization of development by microRNAs. Nat. Genet. Suppl. *38*, S20–S24.
5. Hong, J.W., Hendrix, D.A., and Levine, M.S. (2008). Shadow enhancers as a source of evolutionary novelty. Science *321*, 1314.
6. Perry, M.W., Boettiger, A.N., Bothma, J.P., and Levine, M.S. (2010). Shadow enhancers foster robustness of Drosophila gastrulation. Curr. Biol. *20*, 1562–1567.
7. Frankel, N., Davis, G.K., Vargas, D., Wang, S., Payre, F., and Stern, D.L. (2010). Phenotypic robustness conferred by apparently redundant transcriptional enhancers. Nature *466*, 490–493.
8. Scharloo, W. (1991). Canalization: Genetic and developmental aspects. Annu. Rev. Ecol. Sys. *22*, 65–93.
9. O'Meara, M.M., Bigelow, H., Flibotte, S., Etchberger, J.F., Moerman, D.G., and Hobert, O. (2009). Cis-regulatory mutations in the Caenorhabditis elegans Homeobox gene locus cog-1 affect neuronal development. Genetics *181*, 1679–1686.
10. Jeong, Y., El-Jaick, K., Roessler, E., Muenke, M., and Epstein, D.J. (2006). A functional screen for sonic hedgehog regulatory elements across a 1 Mb interval identifies long-range ventral forebrain enhancers. Development *133*, 761–772.
11. Werner, T., Hammer, A., Wahlbuhl, M., Bosl, M.R., and Wegner, M. (2007). Multiple conserved regulatory elements with overlapping functions determine Sox10 expression in mouse embryogenesis. Nucleic Acids Res. *35*, 6526–6538.
12. Xiong, N., Kang, C., and Raulet, D.H. (2002). Redundant and unique roles of two enhancer elements in the TCRgamma locus in gene regulation and gammadelta T cell development. Immunity *16*, 453–463.
13. Bülow, H.E., and Hobert, O. (2004). Differential sulfations and epimerization define heparan sulfate specificity in nervous system development. Neuron *41*, 723–736.
14. Raj, A., Rifkin, S.A., Andersen, E., and van Oudenaarden, A. (2010). Variability in gene expression underlies incomplete penetrance. Nature *463*, 913–918.

Department of Biochemistry and Molecular Biophysics, Howard Hughes Medical Institute, Columbia University Medical Center, New York, NY 10032, USA.
E-mail: or38@columbia.edu

DOI: 10.1016/j.cub.2010.07.035

Female Meiosis: Coming Unglued with Age

Chromosome abnormalities in humans are strikingly associated with increasing maternal age. Studies in mice implicate loss of chromosome cohesion as an important cause of age-related meiotic errors in the oocyte.

Patricia Hunt* and Terry Hassold

In the early 1930s, the noted British geneticist Lionel Penrose realized that Down syndrome babies are far likelier to be born to older women [1]. At the time, Down syndrome was known only as a form of mental retardation with characteristic phenotypic features. The understanding that the condition results from three copies of chromosome 21 (trisomy 21) would not be made for another 25 years [2,3]. In short, the recognition that advancing maternal age affects the likelihood of producing a normal, healthy child predated our understanding that chromosome abnormalities represent not only the leading cause of birth defects in humans, but also the major cause of pregnancy loss.

The meiotic errors that result in chromosome abnormalities are common in humans, and approximately 0.2–0.3% of newborn infants are trisomic [4]. However, this represents just the tip of a large iceberg, because most aneuploid conceptions die *in utero*. Indeed, studies of preimplantation embryos suggest that a large proportion, if not a majority, of fertilized human eggs have extra or missing chromosomes [5]. Because the vast majority of errors result from the fertilization of a chromosomally abnormal egg by a normal sperm, attention has focused on why human female meiosis is so error-prone.

How does maternal age factor into this equation? Hugely. Among women in their twenties, approximately 2–3% of clinically recognized pregnancies involve trisomic fetuses but, among women in their forties, this value skyrockets to over 35% (Figure 1). Given the importance of the age effect and the research attention devoted to it, it may seem odd that we know so little about its basis. Indeed, like the enigmatic smile on the Mona Lisa, the mechanism(s) by which maternal age induces its effects on chromosome segregation have remained a tantalizing mystery. However, three papers in this issue of *Current Biology* [6–8] lend strong support to a mechanism involving the ties that bind meiotic chromosomes. Physical connections — whether between sister chromatids during mitosis or between homologs and sister centromeres during meiosis — are essential for proper chromosome segregation and depend on a class of proteins known as the cohesins [9,10]. The production of haploid