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NEWS AND COMMENTARY

Gene regulation

Stochastic and deterministic effects in gene regulation

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The large majority of genes in all organisms are under deterministic control—that is, their activity can be predicted from their environment, usually the relative concentrations of positive and negative regulators. Other genes are subject to stochastic effects, as in the case of genes subject to X inactivation in female eutherians, in which one of two identical X-linked alleles in the early embryo is designated at random for life-long silencing. Once established, X inactivation is essentially irreversible in somatic tissues but is re-randomized in each generation. X inactivation is both stochastic and deterministic in that the outcome is always essentially the same: half of all cells have the X chromosome of maternal origin inactivated, while the other half inactivate the X chromosome of paternal origin.

Chromosomal rearrangements can also cause genes normally subject to strict deterministic control to show stochastic regulation; important examples are position effect variegation in *Drosophila* (Henikoff, 1990), telomere position effect in yeasts (Gottschling *et al*., 1990; Grewal and Klar, 1996) and coat color variegation in mice caused by transposition (of an IAP) into the region 5′ of the *agouti* gene (Michaud *et al*., 1994). In these cases, the artificial rearrangements cause the allele to be so finely balanced between activation and repression that adjacent cells can have different phenotypes, and phenotypic diversity arises within a population of identical cell types. Gene regulation in such cases can be almost completely stochastic and very sensitive to minor perturbations.

There are a number of cases in which genes normally subject to deterministic regulation can be converted into stochastic control. The *E. coli lac* operon is a classic example (Novick and Weiner, 1957). The *lac* operon is normally induced by the presence of lactose when glucose is absent, a clear case of deterministic control. However, if exposed briefly to a pulse of lactose just above the inducing threshold, only some of the cells in a genetically identical population will form colonies when plated on medium that contains lactose at concentrations below the inducing threshold. Those cells within the lactose-exposed population that were induced to express *lac* permease survive, and the higher internal concentrations of lactose allow them to proliferate, while cells in which *lac* permease was not induced do not grow. Hence, by experimental manipulation, a deterministic system can be caused to show stochastic regulation, and persistent phenotypic diversity arises within a genetically identical population of organisms.

Lim and van Oudenaarden (2007) developed a new defined system that shows persistent stochastic gene regulation. In their experiment they replace the coding region of the *E. coli agn43* gene with a T7 polymerase gene, which, when turned on, will drive expression of a plasmid in which a T7 promoter drives expression of a GFP reporter gene. When the reporter gene is turned on, the cells yield a strong fluorescent signal, which gives a quantitative measure of the on/off state of the gene. The methylation status of three GATC sites in the *agn43* promoter controls expression of T7 RNA polymerase. When the adenine residues in these sites are fully methylated, the gene is turned on. When they are unmethylated the gene is turned off (Figure 1). A balance between DNA adenine methyltransferase (Dam), which methylates the switch, and OxyR, which binds to the unmethylated switch and prevents methylation, controls the methylation state of this switch. If the switch is methylated, after cell division each daughter cell will contain a hemi-methylated copy (Figure 1). However, if the switch begins as unmethylated, each daughter cell will also contain an unmethylated copy of the switch. The unmethylated copy is then protected from Dam methylation by OxyR, which can bind only to the fully unmethylated state (Figure 1). Because neither methylation by Dam nor protection by OxyR is completely efficient, cells will switch expression states at easily measurable rates.

There are several key underlying points in this model that define its inheritance properties. The first is that there is no enzyme with demethylation properties that can remove the methylation signal on the switch. These methylation signals can only be passively diluted when the DNA replication of cell division creates initially unmethylated copies. OxyR can outcompete Dam for the unmethylated template to block methylation of the switch at an efficiency of less than 1. Second, there is no feedback loop present in this system.

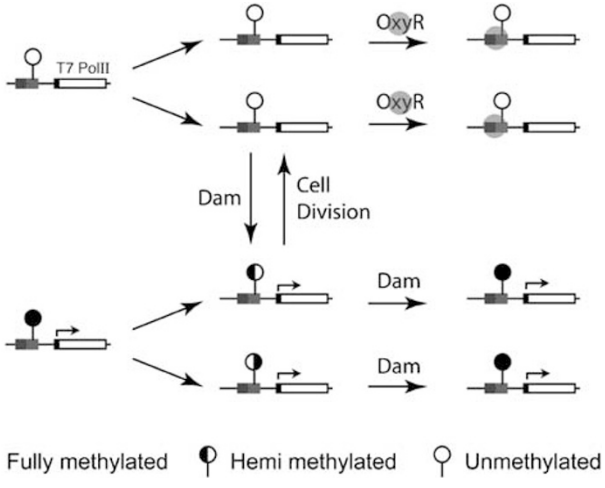

Figure 1 Competition between the OxyR repressor and Dam controls activity of the *agn43* promoter. Methylation of GATC tetranucleotides by Dam prevents repression by OxyR, which activates the reporter gene; binding of OxyR occludes the GATC sites, prevents methylation by Dam, and leads to repression. Neither OxyR or Dam is 100% efficient, with the result that there are rare switching events that interconvert methylated, active reporter constructs and unmethylated, repressed reporters. Dam, DNA adenine methyltransferase.

Agn43 neither directly nor indirectly regulates the expression of this system, by regulating Dam or OxyR expression—the authors assume that the same is true of their modified T7 expression system without direct proof. Last, there is no theoretical need for the inheritance of additional machinery such as RNA or protein between cell divisions. The state of the initial cell is sufficient to determine the outcome. The real advantage of the system developed by Lim and van Oudenaarden is its amenability to quantitative description and for examination of the effects of environmental perturbations on switching rates, which are much more difficult to analyze in animals.

How important are fully stochastic epigenetic switches in nature? Virtually all of those studied to date can be considered largely artificial. Variegating phenotypes are conspicuous and intriguing in the laboratory and are considered attractive by breeders of plants and animals. Variegating strains are therefore propagated by biologists and breeders under protected conditions. It is very much open to question whether epigenetic switches serve to expand the range of phenotypes that are produced by a single genotype, and whether such variability can provide a selective advantage in nature.

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