



Figure 1 Shh protein secreted by the notochord signals cells in the adjacent somite of the zebrafish embryo. The response to this signal is mediated by the Gli transcription factors that activate expression of slow-twitch muscle lineage-specific genes. Gli activity is attenuated by maternally supplied *Su(fu)*, which has now been shown to be negatively regulated by *miR-142*.

defect in morphogenesis but rather reflects a loss of particular types of muscle fibers that are specified by distinct levels of Hedgehog activity⁸. Prompted by this finding, these authors searched for transcripts encoding components of the Hedgehog signaling pathway among the predicted targets of *miR-214* and came up with one mRNA, encoded by the *Suppressor of fused* gene, that represents a plausible candidate for a *bona fide miR-214* target.

Su(fu) and Hedgehog signaling

So what exactly is *Suppressor of fused* or *Su(fu)*?

Originally identified in *Drosophila melanogaster* through suppression of the hedgehog-like phenotype caused by *fused* mutations, *Su(fu)* encodes a new PEST domain protein that is one of the more enigmatic components of the Hedgehog signaling pathway. Various lines of evidence have implicated *Su(fu)* in regulating the nuclear import and activity of the Gli family of transcription factors that mediate Hedgehog signaling activity⁹, yet at least in flies, the gene turns out to be completely dispensable. In the zebrafish, however, morpholino-mediated knockdown of *Su(fu)* causes a subtle phenotype, increasing the number of slow-twitch muscle fibers in each somite⁸, implying that it acts to modulate the response of progenitor cells to graded Shh activity. So the effects of *miR-214* knockdown could be explained by an increase in *Su(fu)* activity in the muscle progenitors, inhibiting their response to the Shh signal (Fig. 1). Consistent with this interpretation, Patton and colleagues show that the effects of *miR-214* knockdown can be mitigated by simultaneous knockdown of *Su(fu)*.

Modulating the activity of *Su(fu)* in this way provides a means of fine-tuning the response of cells to Hedgehog signaling. But whether *Su(fu)* is under similar control in other vertebrates remains unclear. In contrast to its subtle roles in flies and fish, mammalian *Su(fu)* has a key role as a major negative regulator of the Hedgehog pathway; in its absence, the pathway is constitutively activated throughout the embryo, causing catastrophic changes in cell fate that result in embryonic lethality^{10,11}. Modulation of mam-

malian *Su(fu)* is thus not the most obvious way to fine-tune the response of cells to Shh or other Hedgehog family proteins, and it may well be that its miRNA-mediated regulation is restricted to certain species. In this regard, it is notable that zebrafish embryos homozygous for a *Su(fu)* mutation do not show the muscle phenotype caused by the morpholino-mediated knockdown of the gene¹². This implies that the *Su(fu)* activity required in muscle progenitors is furnished principally by maternally expressed mRNA. The key requirement for *miR-214* may thus be to reduce the levels or translation of this maternally deposited transcript, a function not dissimilar to that of *miR-430* in clearing maternal mRNAs⁷.

COMPETING INTERESTS STATEMENT

The author declares that he has no competing financial interests.

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np A DNA methylation–based switch generates bistable gene expression

Matthew R Bennett & Jeff Hasty

A new study examines the *agn43* epigenetic switch of *Escherichia coli*, providing an alternative to feedback regulation as a model for gene expression regulation. Through a combination of synthetic network construction and computational modeling, the authors show that the generated bistable gene expression involves transitions between several rarely occupied states between ‘on’ and ‘off’.

Phenotypic diversity is not solely the consequence of differences in the genetic makeup of individual organisms but is also a result of variation in gene expression. Epigenetic differ-

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ences, in the form of methylation of *cis*-regulatory regions of genes, contribute to regulation of gene expression. Epigenetic switches can often be characterized by multiple, concurrently stable gene expression states in which the same genes may be expressed in two (‘on’ or ‘off’) or more ways.

On page 265 of this issue¹, Han Lim and Alexander van Oudenaarden explore the dynamics of one such epigenetic switch within

the *agn43* system of *Escherichia coli*, modified with a synthetic construct. This system is of particular interest because it is known not to involve feedback regulation, which has previously been shown to generate stable ‘on’ and ‘off’ gene expression states. The *agn43* epigenetic switch in *Escherichia coli* is controlled by the methylation of three GATC sequences downstream of the promoter. When these sites are fully methylated, *agn43* is expressed at highest levels. However, in



Mendel's headache

the absence of methylation, the transcription factor OxyR can bind to these sites, blocking both transcription and methylation by DNA adenine methylase.

Through disruption of the *agn43* system, the authors found that this switch was not just bistable but that transitions between the two extremes consisted of several intermediate states. While each intermediate state was rarely occupied, cells that leave either the 'on' or 'off' state enter one of these intermediate states and then experience pressure to revert to their original state. As explored in model simulations, the authors show how this can occur, as the intermediate states between full methylation and repression are strongly biased to resist occupation and quickly distribute cells toward the two extremes. Furthermore, the authors' simulations suggest how, in theory, the intermediate states enable the expression state of the gene to be inherited.

Mendel's noisy puzzle

Epigenetic traits provide cells with a unique strategy for surviving in fluctuating environ-

ments. As the environment changes, a cell might switch gene expression states in order to survive. However, due to the noisy nature of gene expression², cells may also switch states even when the environment is stable³. For instance, a positive-feedback gene circuit may be in either a low (no-transcription) or high (fully induced) state. If the promoter is leaky, accidental transcription might cause positive feedback to force the gene into the high state. This sudden change in a gene's expression level may have wide-ranging effects, leading to a phenotypic change in the cell. Imagine Mendel's shock if he were to study the heritability of flower color (see illustration), only to witness a flower's color changing before his eyes! Although such an occurrence would be unlikely in multicellular organisms, it is analogous to what can happen in single cells that exhibit bistable gene expression. In large populations, random switching between two states creates a bimodal population representing the two phenotypes, even though one state might be more fit than the other in the current envi-

ronment. The unfit subpopulation enables the entire population to hedge its bets in case of an environmental change in the future.

Multistability in gene expression

The characterization of multistability in gene expression is important to many fields, ranging from immunology to synthetic and systems biology. From an immunological and evolutionary standpoint, cells that show bistability can have a distinct advantage over those that are monostable, especially in highly variable environments^{4,5}. Multistability not based on feedback could also be a useful tool for synthetic biologists. Previous studies have shown that one source of bistability in gene networks can be created by direct or indirect feedback loops^{6–8}. These networks are controlled by the very proteins they create⁹, which can bind to regulatory sites of the promoters to either repress or activate transcription. However, this type of regulation can be metabolically costly, as it requires the production of large amounts of regulatory proteins. The study by Lim and van Oudenaarden, however, shows that non-feedback based epigenetic switches can also create bistability, although it remains to be seen how common a mechanism this may be.

As the authors point out, a synthetic switch based on methylation has the additional advantage that it can be combined with other forms of regulation. For example, consider a gene whose basal transcription level is very low but that can be activated by the presence of a transcriptional activator bound to an upstream regulatory site. If the methylation sequence were placed downstream of the promoter, then the gene would be fully expressed only when activator concentrations were high and the promoter region was fully methylated. The creation of such a modified version of the *agn43* switch is an intriguing possibility and warrants further study.

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