Switches, Switches, Every Where, In Any Drop We Drink

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In this issue, Broussard et al. (2013) report genetic switches that regulate cell fate selection; a recombinase attachment site is embedded within a repressor coding sequence, such that integration truncates a proteolysis domain, stabilizing the repressor and setting the switch.

The capacity to switch between states underlies the control of all systems, living or otherwise. For example, microprocessors are now approaching 10 billion transistors per system with the electronics industry selling upward of 1 billion units annually. Stated differently and rounding up, humans are making almost 10^{19} electrical switches per year. Meanwhile, existing natural switches have been found throughout biology and are being repurposed to support biotechnology applications. The most abundant switches in nature are likely those controlling cell fate selection within bacteriophage. For example, if only 10% of the nearly 10^{31} phage in the Earth's oceans are capable of lysogeny, then phage represent $\sim 10^{30}$ switches that collectively execute $\sim 10^{24}$ decisions per second (Brüssow et al., 2004). Incredibly, we still do not fully understand even the best-studied phage-based genetic switch, and almost all the natural diversity among phage-based switches remains unexplored. In this issue, Broussard et al. (2013) uncover and detail many distinctive aspects of a new category of genetic switches controlling cell fate selection during bacteriophage infection. Most wonderful is their finding that switch setting is controlled by integrase-mediated editing of DNA encoding a switch-specific transcription repressor (Figure 1A). Another tantalizing observation is the authors' isolation of a single amino acid substitution within the repressor's proteolysis domain that, independent of integration, both stabilizes the repressor and enables activation of transcription; such a mutation could seemingly support evolution toward positive feedback control found in other genetic switches.

Bacteriophage lambda is the best understood natural genetic switch wherein cross-regulating transcription factors (CI and Cro) create a bistable system leading to either host cell lysis or stable integration and maintenance of a prophage within the host chromosome (lysogeny) (Figure 1B). Switch setting during lambda infection is controlled by a third transcription factor (CII), whose expressed level and activity are sensitive to the number of infecting phage particles and host cell protease activity (Ptashne, 2004). However, conditions exist wherein genetically identical cells grown in a common environment and infected with exactly one phage particle per cell realize divergent fates. Cell cycle position correlates with a 16-fold biasing of infection outcomes; larger cells just prior to division are much more likely to undergo lysis (St-Pierre and Endy, 2008). It is still not perfectly understood to what extent and by what molecular mechanism(s) an infecting lambda phage samples spontaneous physical variation among individual cells in order to determine infection outcomes (Zeng et al., 2010). It could be that a combination of physical variables entirely determines individual cell fates (e.g., differences in cell volume within an asynchronous population plus spontaneous variation in protease activity combined with the availability of specific tRNA, etc.). Alternatively, random processes during infection may contribute spontaneous variation to developmental outcomes (Arkin et al., 1998). Such possibilities are not idle curiosities to those working to engineer synthetic genetic systems, as the approaches used to realize switching reliability and precision are heavily biased by perceptions of how natural switches have been evolved to work within seemingly noisy intracellular milieux (Schrödinger, 1944).

To this fray, Broussard et al. (2013) introduce a new family of phage-based switches that are distinct from lambda in both mechanism and system-level architecture. Such differences are exciting in considering how switches are formed from molecular components and also how switches might have evolved. To engineers, the results of Broussard et al. (2013) further emphasize the layered sophistication to be found within naturally evolved genetic systems and suggest new ways for improving synthetic systems. For example, Broussard et al. (2013) show that phage immunity regions embed an integrase attachment site within the coding sequence of a transcription repressor. In one example, site-specific recombination between the phage genome and host chromosome changes an in-frame TGT (cysteine) to a TGA (stop) that shortens the repressor coding sequence by 33 amino acids; other presented examples result in similar coding sequence changes. Functionally, the abridged repressor proteins are more stable and steady switches within a lysogenic state. Engineers working with integrases to implement synthetic genetic logic and data storage have yet to realize such sophistications (Friedland et al., 2009; Bonnet et al., 2012). However, the natural examples from Broussard et al. (2013) suggest that integration-mediated editing of transcription factor coding sequences can be used to realize feedback control of integrase expression, which should enable the engineering of formally bistable integrase-based switches (Bonnet et al., 2012).

Not surprisingly, detailing a new natural genetic switch leads to many unanswered questions about the system itself and also regarding evolutionary comparisons to be made among switches. As examples of the former, it remains unclear how the Cro protein inhibits lysogeny in Broussard et al. (2013) newly uncovered switches. Also, it is striking that no recombination directionality factor (e.g., an excisionase) has been identified, suggesting that these integrases are bidirectional. Could multiple integration and excision events occur prior to cell fate determination? Next, given that ClpXP proteolysis is thought to degrade switch proteins, could queue saturation of ClpXP by unrelated phage or host proteins (Cookson et al., 2011) bias infected cells toward lysogeny? Additionally, what directs expression of integrase during induction of a prophage, and what is the significance, if any, of these phage integrating into tRNA loci (and so on)?

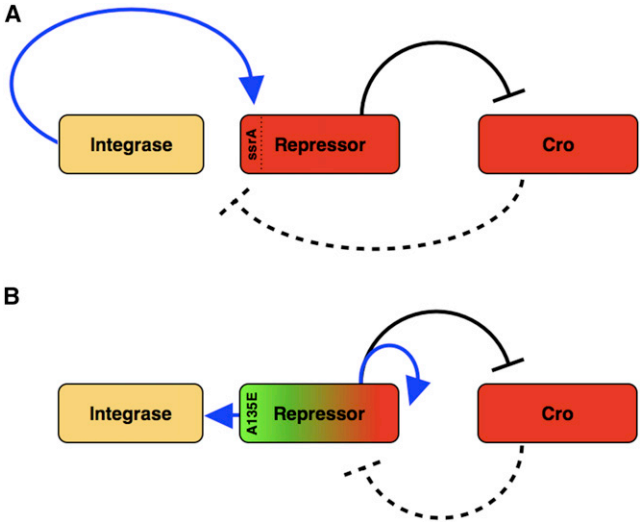

Figure 1. Architectural Adjacencies within Natural Genetic Switches
(A) Abstraction of control relationships as revealed by Broussard et al. (2013). Integration stabilizes the repressor protein by removing a site-specific ssrA proteolysis epitope (solid blue, positive regulation). Repressor inhibits lytic functions (solid black, negative regulation). Cro inhibits lysogenic functions, albeit via a currently unknown mechanism (dashed black, negative regulation). (B) As in (A) but via different molecular couplings more akin to the bacteriophage lambda-switch architecture. Repressor (CI) again inhibits lytic functions (solid black, negative regulation, red ORF shading) but also autoregulates repressor and integrase levels via a point substitution (A135E) that enables transcription activation (solid blue, positive regulation, green ORF shading). Cro still inhibits lysogenic functions via transcription repression (dashed black, negative regulation).

While many questions can be asked regarding switch architecture and evolution, in closing we highlight four observations that alone seem of modest significance but taken together suggest how switches might evolve between different forms. First, Broussard et al. (2013) observe that both the full-length (unstable) and abridged (stable) forms of the repressor do not increase expression from the promoter upstream of the repressor coding sequence (P_{Rep}). Second, a single point substitution that stabilizes the full-length repressor (A135E) also increases expression from P_{Rep} by ~2.5-fold. Third, a point mutation just upstream of P_{Rep} inhibits lysogeny in an A135E background. Fourth, Bushman and Ptashne (1988) turned the Cro repressor from phage lambda into a transcription activator by creating a small acidic patch including a T17E substitution. Thus, perhaps one form of genetic switch that relies on relief from repressor degradation to realize or stabilize lysogeny could evolve to another form that uses positive autoregulation of repressor synthesis; both architectures would boost repressor levels (Figure 1). Regardless of what might be found true, as scientists set sail on the high seas of phage-based genetic switch research, please do not shoot the albatross.

REFERENCES

Arkin, A., Ross, J., and McAdams, H.H. (1998). Genetics *149*, 1633–1648.

Bonnet, J., Subsoontorn, P., and Endy, D. (2012). Proc. Natl. Acad. Sci. USA *109*, 8884–8889.

Broussard, G.W., Oldfield, L.M., Villanueva, V.M., Lunt, B.L., Shine, E.E., and Hatfull, G.F. (2013). Mol. Cell *49*, this issue, 237–248.

Brüssow, H., Canchaya, C., and Hardt, W.-D. (2004). Microbiol. Mol. Biol. Rev. *68*, 560–602.

Bushman, F.D., and Ptashne, M. (1988). Cell *54*, 191–197.

Cookson, N.A., Mather, W.H., Danino, T., Mondragón-Palomino, O., Williams, R.J., Tsimring, L.S., and Hasty, J. (2011). Mol. Syst. Biol. *7*, 561.

Friedland, A.E., Lu, T.K., Wang, X., Shi, D., Church, G., and Collins, J.J. (2009). Science *324*, 1199–1202.

Ptashne, M. (2004). A Genetic Switch, Third Edition (Cold Spring Harbor, NY: CSHL Press).

Schrödinger, E. (1944). What is life? (Dublin: Trinity College).

St-Pierre, F., and Endy, D. (2008). Proc. Natl. Acad. Sci. USA *105*, 20705–20710.

Zeng, L., Skinner, S.O., Zong, C., Sippy, J., Feiss, M., and Golding, I. (2010). Cell *141*, 682–691.