Class 14: Engineering orthogonal regulated promoters for synthetic biology

Reading assignment

- Blount et al. Rational diversification of a promoter providing fine-tuned expression and orthogonal regulation for synthetic biology. PLoS One. 2012. 7:e33279

Classroom activity (limit 45 minutes)

Pokholok et al.

1. The authors of this paper planned to create orthogonal promoters that could be used in synthetic biology applications. What is the advantage in a synthetic biology application to having these promoters? How do they imagine that a set of promoters of the type they planned to design could be used to accomplish synthetic biology purposes?

   Because the promoters have different sequences and do not use any normal cellular control scheme, it would be possible to use multiple promoters to control the expression of a set of proteins without having unwanted consequences (like homologous recombination between promoters that would destroy the control scheme). The purpose is to construct control schemes that allow the cells to perform previously unknown behaviors that would be useful to biotechnologists.

2. What process(es) did the authors use to find the candidate gene that would be the basis of their experiments? What features were they looking for in the candidate gene? How did they show that the chosen gene (*PFY1*) had those features?

   Their plan was to alter an existing yeast promoter to impose an artificial regulatory scheme on it. To make that easiest, they wanted to identify promoters with no observable regulation, meaning that the promoter was constitutively active in all physiological conditions. They used databases of gene expression data to identify a small group of promoters that showed as little variation as possible. Because they planned to insert these promoters into multiple genes, it was important that the promoter be as small as possible. The PFY1 *promoter satisfied these conditions best.*

3. What are Reb1 and the poly-dT elements? What effect did the authors propose they have on the function of the *PFY1* promoter? Why were those features important to their synthetic biology goals? How did the authors change the wild type *PFY1* promoter to create their set of orthogonal promoters?

   Reb1 is a transcription activator protein that binds in the region of the PFY1 *promoter and poly-dT is a stretch thymidine in the promoter DNA that cause a bend in the DNA molecule. The authors state that they have been shown to "maintain stable and constitutive expression", which was one of the features of the promoter they were looking for as the basis for this experiment. The authors altered blocks of nucleotides in two regions of the promoter in order to create previously unknown promoters (not present elsewhere in yeast) that would be different enough to not be seen by the cell as similar enough to allow homologous recombination and to support different rates of transcription.*

4. The mutant promoters the authors created have varied activities (that is, rates of transcription). How do the authors explain the variation in activity for these promoters? Why do you think the authors needed to construct promoters with such varied activities?

   The authors suggest that the various sequences introduced with interact with the transcription initiation machinery differently enough that they would produce different rates of occupancy of the promoter, and therefore different rates of transcription. The differences might include differences in melting temperature, which would affect the ability of the polymerase to unwind the DNA to begin transcription. They wanted different activities because they wanted to control the amounts of proteins produced in the circuits created for synthetic biology purposes.

5. What is the TetR protein and how does it affect transcription? Why do you think the authors decided to insert multiple tandem copies of the TetR binding site into their modified *PFY1* promoters? What type of regulatory scheme did they plan to make and what effect did they expect to see in the presence of active TetR? How did they measure transcription from the *PFY1* promoter and what result did they see?

   The TetR protein is a non-native (bacterial) repressor protein that can bind a Tet operator to repress transcription. They inserted multiple copies because more TetR proteins bound are more effective at repressing transcription (the authors don't state this explicitly). The regulatory scheme they designed was an "inverter" in which TetR represses the promoter in the absence of anhydrotetracycline (aTC) but in its presence the repression is relieved. In effect aTC "induces" rather than represses the promoter. This is the behavior they saw, with a rough dose response to aTC.

6. What are the TALOR proteins, what is the advantage of the TAL proteins from which they are derived and how do they regulate transcription? How do they show that the TALOR proteins can be engineered to control the targeted promoters for which they were designed but not other, similar promoters?

   TALOR proteins are derived from TAL proteins, which can be rationally designed to bind any DNA sequence (we can discuss how this works later if the class desires). By binding the DNA they block transcription of an overlapping promoter. They designed TALOR proteins to bind two of their orthogonal promoters that have different DNA sequences, showing that the TALOR only represses the designed target promoter and not others.

7. How do the authors believe they have improved on previous generations of promoters designed for synthetic biology purposes? They showed that TALOR proteins have a relatively modest effect (this background activity might be too high for many purposes); how do they suggest their effect could be amplified? Why is the alternative of making TALOR proteins that have an intrinsically higher effect a less attractive solution?

   The authors claim that their promoters are more useful because smaller, that they are dissimilar enough not to undergo recombination (which would scramble the genome, interfering with the regulatory scheme) and that they lack any endogenous control elements, so regulating them will not disturb cellular physiology by changing expression of cellular genes. They suggest that repression can be increased by combining multiple TALORS and say that increasing the affinity of such a protein would result in increased off-target interactions, which could create problems.