Regulatory Role of Tandem Repeats

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Tandem repeats of DNA are prevalent and hypervariable in higher eukaryotic genomes. If these repeats contain transcription factor (TF) binding sites, they could act as 'decoys' and influence gene expression of target genes. Using a synthetic system in budding yeast, we find decoy sites for an activator can competitively inhibit expression of a target gene. Importantly, the dose-response of target gene to the activator becomes more ultrasensitive in the presence of decoys. Using a simple model, we suggest the effective affinity of the activator to the repeats is >10-fold higher versus the promoter.

***Keywords* — gene regulation, tandem repeats**

I. Background

Tandem repeats (TR's) of DNA represent a section of the genomic that may be highly evolvable, as repeat numbers can change with frequencies 100-10000 higher than point mutation [1]. The numbers of TR's present in coding regions and promoters, and introns have demonstrated functional roles in modulating protein activity and gene expression and are correlated with disease [2-4]. Less is known about the functional role of intergenic TR's that are found far away from coding regions. Because these TR's can often include TF binding sites [5], they may have an indirect regulatory role by competitively binding the TF, functioning as a 'decoy' site.

If the TF is an activator, addition of decoy sites would be expected to decrease target gene expression. However, the nature of the TF interaction with decoy sites could qualitatively change the target gene's response. For example, if decoy sites have a much higher affinity for the activator, the target gene's response will become more ultrasensitive [6]. Indeed, the sequestration of an activator by a competitive protein inhibitor has been shown to result in a highly ultrasensitive response in downstream target gene expression [7]. Thus, decoy sites might play a similar functional role, although one might expect their affinity for the activator to be similar to the promoter affinity.

II. Results

To test these ideas, we constructed a synthetic gene circuit based on the tet-OFF system where the tetracycline controlled transcriptional activator (tTA) binds to a tetO operator site and induces downstream gene expression. We then introduced various numbers of decoy tetO binding sites on both plasmids and integrated in various genomic locations and measure the effect on target gene expression.

We analyzed our results in the context of a simple thermodynamic-based model for gene expression. Decoy tetO sites both decreased expression levels and converted the normal linear activator/promoter response to an ultrasensitive one. The degree depended on the number and location of the tetO repeats. The results suggested that the effective affinity of tTA to decoy sites is 10-100 fold higher than the promoter.

To determine how sequestration of tTA depends on decoy number, we constructed tTA-YFP fusions and quantified the intensity of the YFP dot that appears in cells due to tTA localization. Sequestration appears linearly dependent on repeat number, suggesting there is no cooperative binding to the decoys.

To verify the ultrasensitive response could have a phenotypic consequence, we constructed a positive feedback gene circuit that responded in a graded manner to changes in feedback strength (via addition of doxycycline, an inhibitor of tTA binding to tetO). Upon adding decoy sites, we observed a conversion of the graded response to a bimodal, all-or-none response. We also verified that the decoys were not causing this conversion by changing noise in gene expression [8].

The "effective" affinity of tTA binding to tetO may be decreased by active processes at the promoter. We eliminated two active processes – tTA ubiquitination and RSC chromatin remodeling activity – and found neither to affect the the ultrasensitive response.

III. Conclusion

Our work suggests that small changes in the numbers of tandem repeats containing TF binding sites can have a qualitative effect on downstream gene expression, through molecular titration. This may be one plausible mechanism to explain why repeat variation in intergenic regions is correlated with disease phenotypes. In addition, the effective TF affinity may be much weaker at active promoters compared to native binding sites.

Acknowledgements: This work was funded by HFSP RGY79/2007.
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