Poster Number

15 **Gene Expression Noise in Transcriptional Negative Auto-regulation in Mammalian Cell**

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Negative auto-regulation (N.A.R) is the simplest and most widely spread type of genetic network motif. Although previous work has demonstrated that N.A.R can reduce gene expression noise, the dynamic relationship between repression strength and its noise reduction effect has not been well documented in Mammalian cell. Here we construct N.A.R cascades using repressor tetR to suppress its own promoter, which contains different number of tetR binding site, in HEK 293 cell. To measure protein production, a red fluorescent protein mCherry has been fused to C-terminal of tetR. Moreover, to increase nuclear repressor concentration, a nuclear localization sequence has been fused to tetR::mCherry fusion protein C-terminal. Consistent with previous work, our data indicate that comparing to non-auto-regulation, N.A.R. dramatically decrease the steepness of the dose-response curve and reduce gene expression noise. Interesting, our data suggest that repressor binding site number in promoter region could significantly affect N.A.R noise reduction effect. For instance, when there are only two repressor binding sites inserted in the promoter region, noise of the regulated gene expression is relatively high, in non-induced state, while in induced state the noise is low and stay at a low level in a wide input dynamic range. Increasing the binding site number in the promoter region decrease the gene expression noise in non-induced state. However, in induced state, the noise of the regulated gene increase as the inducer concentration increase. These results is useful for synthetic biologist to design genetic circuits to achieve precisely gene expression and tightly noise repression.

16 **Activation of Gq without subunits dissociation**

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Heterotrimeric G proteins are one of the most well studied signaling proteins. With hundreds of different coupled receptors and multiple downstream effectors, G proteins constitute a major hub in signal transduction, sorting extracellular signals into proper second messengers. According to extensive in vitro evidence, the activation of G proteins requires the dissociation of GTP-bound Gα subunit and Gβγ dimer. However, the question of whether all G proteins follow such a model in vivo remains difficult to confirm due to technical limitations. We have observed a subtype-dependent G protein dissociation by using flag-tagged Gβ1 subunit to pull down different Gα subunits upon GTPγS or AlF4- activation. Most members of the Gi family showed obvious dissociation. On the contrary, Gαq and Gα16, both from the Gq family, remain associated with Gβ even in the activation state. We further confirmed this tight association in Gq activation by constructing artificial Gβ-Gα fusion proteins. The Gα subunit was linked to the Gβ1 subunit by a Leu linker or a Phe-(Gly4 Ser)2- Gly4-Phe linker, providing a tightly stuck condition or a more flexible connection. Our functional results showed that Gβ-Gαq fusion protein activated PLCβ as efficiently as the wild type non-fusion proteins. Gβ-Gαs fusion proteins are also functional. But Gαi3 protein lost its function when tightly linked with Gβ. These results indicate the subtype-dependent variance of activation of G proteins and that Gq proteins may not dissociate upon activation. (Supported by 2013CB530900 and T13-607/12R).