Characterizing the effect of shared transcriptional resource in mammalian cells

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Competition for a variety of cellular resources such as transcription factors, splicing factors, and protein degradation machinery have been reported in eukaryotic cells. A phenomenon known as "squelching" occurs in eukaryotes expressing strong transcriptional activators; most notably the popular Gal4-VP16. Squelching is thought to result when *trans*-activation domains bind and sequester transcriptional cofactors, thereby effectively repressing transcription of genes with no regulatory link to the activator. We have experimentally tested the effects of squelching by transfecting HEK 293, HEK 293FT, HeLa, Vero 2.2, and CHO K1 cells with plasmids encoding Gal4 fused to different activation domains (Gal4-AD), a Gal4-responsive promoter driving EYFP, and a constitutive test promoter driving mKate2. Our results provide a reference for understanding the "hidden" biological interactions between simple circuit elements that arise due to resource sharing effects. Many constitutive promoters are significantly up- or down-regulated by the activation domains, obviating problems with normalization to constitutive transfection controls and potential circuit-breaking behavior. Importantly, we demonstrate that highly expressed activators can negatively regulate their target promoters via squelching and can feedback to repress their own transcription, complicating our view of the system. With a model of cofactor resource sharing, we aim to elucidate circuit topologies to relieve squelching for any given activator, and establish experimental design principles to reduce the effect of resource sharing on experimental outcomes. Our model and experiments to date indicate that there is a fundamental trade-off between on-target activation and off-target repression by strong activators.