FUSING ENZYMES TO TRANSCRIPTION ACTIVATOR LUXR FOR THE RAPID CREATION OF METABOLITE SENSORS

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Metabolite sensors have been applied for high-throughput screening for improved biosynthetic pathways, as well as for dynamic control of the metabolic networks. Obviously, however, current repertoire of natural sensors covers only a small fraction of the known metabolite.

We have been developing the new robust workflow for the rapid creation of metabolite sensors where biosynthetic enzymes can be adopted as the sensory (recognizing) components. Most of the known metabolites act as the direct substrates of some enzymes, and they are recognized and converted by these enzymes in physiologically relevant concentrations. Thus, ever-increasing repertoire of available enzymes is a rich and reliable source of sensory units. We found that the transcription activator LuxR can be fused with various biosynthetic enzymes without losing its function. By adding moderately de-stabilizing mutations, typically by random mutagenesis of the resultant fusion proteins followed by screening a small number (~100) of variants, we could have quickly isolated variants that can activate LuxR-dependent promoter in response to the substrates of the enzymes fused to LuxR. In this presentation, we demonstrate various metabolites can be detected by this manner.

Detailed analysis of the thus-obtained fusion proteins indicated that function of LuxR is dependent on the substrate binding-induced stabilization of the enzymes. The biosensors with this mode of action exhibited various unique features. For instance, we found that the sensitivity (EC50) and dynamic range of these sensors to the target metabolites can be flexibly altered by the concentration of homoserine lactones, the cognate ligand of LuxR, in the media. Also, this provides unique opportunity to indirectly visualizing the substrate-binding to the enzyme in high-throughput manner. Indeed, multi-round mutagenesis and screening of the fusion protein of isopentenyl diphosphate isomerase (IDI) with LuxR variant (IDI-LuxR) revealed that many of the mutations that improved sensory performance of IDI-LuxR also elevated the catalytic performance of IDI. Some of such mutations turned out to elevate IDI activity even without fusion partner LuxR. Altogether, by fusing to LuxR, random mutagenesis, and traditional reporter (fluorescence)-based screening, one can not only adopt a variety of biosynthetic enzymes as sensor components but also laboratory evolve their catalytic functions.