SYSTEMS BIOLOGY

Strength in numbers

By bundling together receptor domains, researchers identify important extracellular protein-protein interactions that are otherwise too weak to detect.

Membrane-bound proteins are essentially the sensory organs of the cell, responding to interactions with neighboring cells or changes in the external environment. Unfortunately, they are also challenging to study, and many experimental techniques that work well with soluble proteins are ill-suited for membrane proteins. "We could not biochemically reproduce many of the receptor-ligand interactions reported in the literature," says K. Christopher Garcia, of the Stanford University School of Medicine.

Garcia and colleagues recently devised a screening strategy that allowed them to catalogue such cell-surface binding events in the *Drosophila* proteome with greatly improved accuracy. Many intracellular binding partners grip each other tightly, but interactions between extracellular proteins tend to be weaker and more fleeting. To amplify these events, the researchers used an oligomerization-based approach, generating receptor-derived 'prey' constructs modified with a helical domain that enables them to pentamerize. They then performed assays in multiwell plates, with each well coated with a different 'bait' protein. The resulting multivalent interaction between bait and prey is more robust, making it possible to confidently identify binding events that are otherwise too transient to capture.

As an initial demonstration, Garcia and colleagues performed their screen with recombinant extracellular domains from 202 *Drosophila* proteins, representing three major families of transmembrane proteins. Their screen revealed 106 reproducible bait-prey interactions, 83 of which were previously unreported. In addition to clarifying some critical cellular signaling cascades, this surprisingly dense network of interactions also yielded useful insights into *Drosophila* evolutionary history, such as likely gene-duplication events.

The technique proved highly robust with minimal false positives, and many of the bait-prey interactions were subsequently validated by other assays. Indeed, the researchers could even use their pentameric prey constructs to accurately label expression of interacting partners in live *Drosophila* embryos. Moving forward, Garcia is working on multiplexing strategies that could transform this 'extracellular interactome assay' into a true high-throughput screen. "We'd like to go 'all against all' for an entire genome," he says.

Michael Eisenstein

RESEARCH PAPERS

Özkan, E. *et al.* An extracellular interactome of immunoglobulin and LRR proteins reveals receptor-ligand networks. *Cell* **154**, 228–239 (2013).