Dissecting multi-protein signalling complexes by Bimolecular Complementation Affinity Purification (BiCAP).

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Background

The dynamic assembly of multi-protein complexes is a key regulatory mechanism for modulating the spatio-temporal specificity required for an accurate, yet adaptive response to rapidly changing cellular conditions.

Despite significant advances in interactomics (Figure 1), there is still a lack of experimental techniques to facilitate the specific and sensitive deconvolution of these multi-molecular signalling complexes.

Here we describe a novel approach that overcomes many of the existing limitations and allows the specific isolation and proteomic characterisation of any two interacting proteins, to the exclusion of their individual moieties and competing binding partners (Croucher et al., Science Signaling, 2016).

BiCAP Method

This novel technique is achieved through the combination of a protein-fragment complementation assay (Figure 2) and affinity purification with a conformation-specific nanobody, termed Bimolecular Complementation Affinity Purification (BiCAP) (Figure 3).

Figure 1: The interaction of adaptor protein Shc1 following EGF stimulation (Zheng et al., Nature 2013).

Figure 2: Bimolecular fluorescence complementation can be used to visualise protein interactions.

Split domains of the Venus fluorescent protein were fused to ErbB2 (Figure 2). Upon co-transfection of these two constructs a prominent membranous fluorescent signal could be detected.

Figure 3: GFP-TRAP allows the selective isolation of interacting proteins.

While bimolecular fluorescence complementation allows the visualisation of protein interactions, immunoprecipitation with a GFP antibody does not isolate these interacting proteins (Figure 3). However, the GFP-TRAP nanobody recognises a three-dimensional epitope on Venus and therefore can specifically isolate only the interacting protein complex.

Figure 4: Adapting the BiCAP technique to investigate the interaction of ErbB2 dimers.

The co-transfection of ErbB2-V1 with either EGFR-V2, ErbB2-V2 and ErbB3-V2 results in a strong membranous fluorescence (Figure 4). Through affinity purification with GFP-TRAP, tryptic digestion and analysis by mass spectrometry, the interaction of each receptor dimer can be determined.

Figure 5: The interaction of ErbB2 as either a homodimer, or a heterodimer with EGFR or ErbB3.

A core group of proteins can be seen to interact with all three receptor dimers (Figure 5). Although a select group of proteins interact exclusively with either one or two dimers. This is especially apparent for the ErbB2/ErbB3 heterodimer.

Figure 6: Validation of novel protein interactions.

A number of these novel protein interactions were validated within the BiCAP method by western blotting (Figure 6). The endogenous interactions could also be observed by Proximity Mediated Ligation assays in ErbB2+ breast cancer cell lines.

Figure 7: Differing mechanisms of MAPK activation.

ErbB2 containing heterodimers are known to have an increased signalling capacity (Kennedy et al., 2016). Accordingly, the ErbB2:EGFR and ErbB2:ErbB3 heterodimers activate higher levels of ERK than the ErbB2 homodimer (Figure 7).

Despite this strong activation of ERK by the ErbB2:ErbB3 heterodimer, there is less Grb2 and no SOS2 interacting with this heterodimer. However, an alternative interacting protein, FAM95A, may account for the activation of ERK by this heterodimer.

Results

Here we have adapted the BiCAP technique to specifically interrogate the interaction of ErbB2 as either a homodimer, or a heterodimer with EGFR or ErbB3.

Results cont’d

Both FAM95A and ErbB3 are expressed in ErbB2+ breast cancer cell lines (Figure 8). While the interaction between ErbB2 and FAM95A cannot be detected by immunoprecipitation, it can be detected by PLA. Additionally, ErbB3 knockdown significantly decreased this interaction.

Figure 8: Endogenous FAM95A interacts with ErbB2:ErbB3 heterodimers.

The knockdown of either ErbB3 or FAM95A in ErbB2+ breast cancer cell lines significantly reduces ERK activation. FAM95A knockdown also significantly reduces growth in colony formation assays.

Conclusions

• The BiCAP technique allows the specific isolation and characterisation of protein-protein interactions.
• ErbB2 containing dimers display a diverse array of interacting proteins.
• The differential recruitment of signalling proteins by these receptor dimers underlies their differing signalling capacity.
• The ErbB2:ErbB3 heterodimer activates ERK through a non-canonical pathway involving FAM95A.

References


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