

LARGE SCALE APPROACHES TO THE DETERMINATION OF PROTEIN FUNCTION

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The cell can be viewed as a collection of dynamic multiprotein complexes, each of which has a specific function. We have suggested the large-scale mapping of such multi-protein complexes in the following way: The complex is biochemically purified using an antibody or a tagged member of the complex. Modern mass spectrometric methods are then used to identify gel-separated interaction partners of the targeted protein. We will present advances in the technology used to purify and mass spectrometrically analyse the protein complexes. The human spliceosome was the first large mammalian complex analysed in this way (collaboration with A. Lamond, Dundee). More than 70 proteins were excised and measured by mass spectrometry in this study, leading to the identification of many known and novel splicing factors. Essentially all the novel proteins were identified in expressed sequence tag databases. Several of these were cloned and functionally investigated. Colocalisation studies were used as further evidence of in vivo involvement in splicing. The above strategy was also used in an iterative fashion to detect additional interaction partners, resulting in the identification of further novel proteins.

We have now applied the technology to a variety of situations. For example, we have investigated various signalling complexes, such as those involved in the EGF receptor pathway. In this case, proteins that were tyrosine phosphorylated in response to receptor stimulation were precipitated with phosphotyrosine antibodies. Difference bands between proteins in stimulated cells and a control were excised and the proteins sequenced by mass spectrometry. Several known and novel proteins in the EGRF pathway were found in this way and are currently being characterised in our laboratory.
