

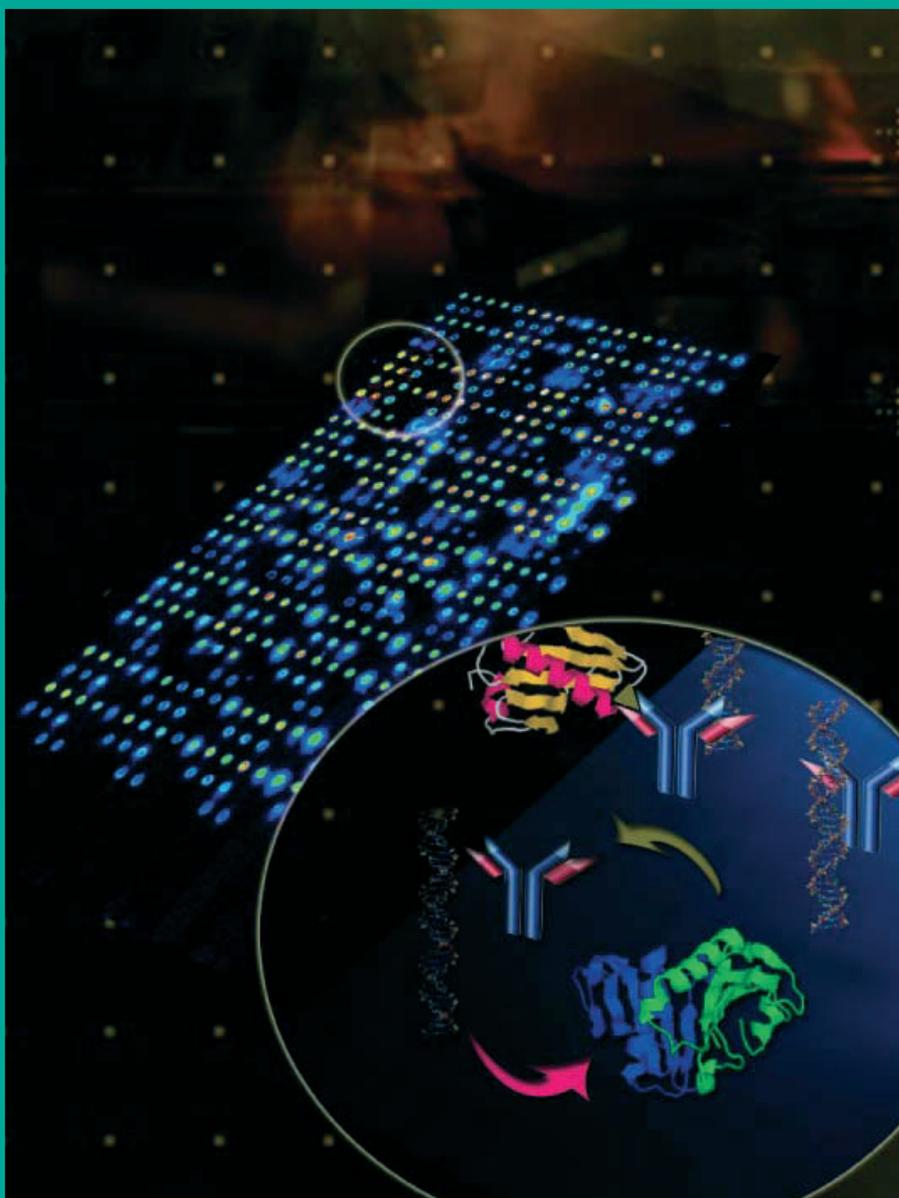
ISSN 1742-464X



www.febsjournal.org

# the FEBS Journal

Volume 272 Number 21 November 2005



## Minireview Series

Identifying protein interactions: experimental approaches

## Review Article

Initiator caspases



Blackwell  
Publishing

FEBS Journal

Volume 272

Number 21

November 2005

pp.5389-xxxx

Blackwell

## Identifying protein interactions

### Experimental approaches

Ettore Appella and Carl W. Anderson

Protein–protein interactions are crucial for most biological processes, and hundreds of thousands of physical protein interactions may occur among the tens of thousands of proteins in a cell at any one instant. In a previous issue of *FEBS Journal*, computational approaches for identifying and characterizing protein networks were explored in four minireviews [1–4]. In this issue, we present four complementary minireviews that explore experimental approaches to identifying, characterizing and validating protein–protein interactions. Each minireview is derived from a presentation given at the *15th Methods in Protein Structure Analysis Conference (MPSA2004)*, which took place in Seattle, WA, in early September, 2004. MPSA meetings are biannual, international meetings that promote the discovery and exchange of new methods and techniques for the analysis of protein structure that facilitate the pursuit of solutions to biological problems. The next MPSA conference, MPSA2006, will be held in Lille, France, August 29–September 2, 2006 (<http://www.iapsap.bnl.gov>).

In the first of these minireviews, Stan Fields discusses the evolution of the yeast two-hybrid technique, a powerful genetic approach for identifying protein–protein interactions. Critical for the high-throughput usage of this technique has been the development of computational approaches for dealing with the great number of false-positive identifications, which may represent 50% or more of the interactions that can be recovered. Fields provides guidance for addressing this issue, illustrated with two examples from his own laboratory: high-throughput analyses of the protein interactions of *Plasmodium falciparum*, the malaria parasite, and an analysis of yeast membrane protein interactions. To evaluate this information, an approach was developed in collaboration with William Noble that uses sets of positive and negative examples to learn features that discriminate between sets to provide a likelihood classification of uncharacterized examples. Bertone & Snyder

then review recent advances in functional protein microarray technology. Protein arrays have typically been limited to purified antibodies that are mechanically printed on supports and peptides that can be synthesized *in situ* using photolithography or the SPOT protocol, which is based on conventional solid-phase chemistry. The authors review progress that has been made in protein array formats and contact-printed functional protein microarrays, such as those that include the whole yeast proteome. This review leads naturally to our third review by Niro Ramachandran and colleagues, which describes emerging methods for the real-time, label-free detection of protein–ligand interactions on functional protein microarrays. Ramachandran in LeBaer's group at Harvard pioneered a self-assembling protein microarray technique – a nucleic acid programmable protein array (NAPPA) – that utilizes expression plasmids spotted on the array surface together with a capture agent, followed by cell-free expression to make 'just-in-time' protein arrays. In this review they describe several techniques in development, including grating-coupled surface plasmon resonance, colorimetric resonant reflection, nanohole array sensors and the use of carbon nanowires and microelectromechanical cantilever systems (MEMS) for high-throughput detection of a wide array of ligands with proteins. The final review in this series, by Houtman, Barda-Saad & Samelson, describes how complementary techniques have been used to characterize membrane-associated signaling complexes formed by the hematopoietic-specific adapter protein, linker for activation of T cells (LAT). LAT is a transmembrane domain-containing protein, without apparent enzyme activity, that serves as a scaffold for the assembly of signaling complexes. The role of LAT and its binding partners has been elucidated through genetic studies using mouse knock-in technology, imaging with both light and electron microscopy, and a host of biophysical techniques, including isothermal titration calorimetry and analytical ultracentrifugation. The

doi:10.1111/j.1742-4658.2005.04969.x

review illustrates how the combined use of these complementary techniques can build a comprehensive understanding of complex formation and function for such multiprotein complexes.

## References

- 1 Altschul SF, Wootton JC, Gertz ME, Agarwala R, Morgulis A, Schäffer AA & Yu YK (2005) Protein database searches using compositionally adjusted substitution matrices. *FEBS J* **272**, 5101–5109.
- 2 Bowers PM, O'Connor BD, Cokus SJ, Sprinzak E, Yeates TO & Eisenberg D (2005) Utilizing logical relationships in genomic data to decipher cellular processes. *FEBS J* **272**, 5110–5118.
- 3 Noble WS, Kuang R, Leslie C & Weston J (2005) Identifying remote protein homologs by network propagation. *FEBS J* **272**, 5119–5128.
- 4 Dunker AK, Cortese MS, Romero P, Iakoucheva LM & Uversky VN (2005) Flexible nets: the roles of intrinsic disorder in protein interaction networks. *FEBS J* **272**, 5129–5148.



Ettore Appella is Chief of the Chemical Immunology Section, Laboratory of Cell Biology, National Cancer Institute, NIH. Raised in Italy, he received his MD from the University Medical School, Rome, Italy, in 1959 and then undertook postdoctoral studies in protein chemistry and immunology at Johns Hopkins University with Clement Markert and subsequently at the National Institutes of Health with Bruce Ames, Gordon Tomkins and Michael Potter. In 1966, he started his group at the National Cancer Institute. His research interests encompass protein chemistry, immunology, tumor suppressors, and the synthesis of small organic molecules that target the HIV nucleocapsid and the p53 tumor suppressor.



Carl W. Anderson is a Senior Geneticist and Chair of the Biology Department at the Brookhaven National Laboratory (BNL) on Long Island, NY. He received his BA in chemistry and physics from Harvard College in 1966 and his PhD in Microbiology from Washington University, St Louis, in 1970. After postdoctoral work in the tumor virus group at the Cold Spring Harbor Laboratory, he joined the Biology Department at BNL in 1975 and became department chair in 1999. His research interests encompass the roles of post-translational modifications in regulating cellular stress responses, including activation of the tumor suppressor p53, and the mechanism of DNA repair by nonhomologous end-joining.