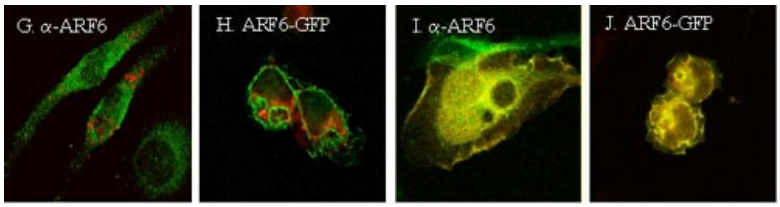
Study of protein interactions

Individual cells from living organisms can be specifically stimulated by their extracellular environment to perform a defined task, for example a hormone from the blood supply may stimulate cell growth. This requires the transduction of the extracellular stimulant into an intracellular signal. Intracellularly, the transduction of such a signal can be mediated through consecutive modular interaction between two or more proteins. It is probable that the pathology of many diseases results from complete loss or aberrant interactions between such proteins. Although the homogeneous purification of specific proteins facilitates the in vitro characterisation of an interaction, these studies provide no information with regards to the spatial and temporal regulation of interactions within a living cell; information that is likely to be essential to enable future therapeutic interventions.

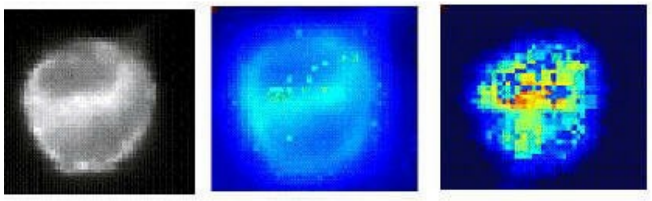
With the aid of standard optical microscopy it is possible to see whether two proteins co-localise (i.e. they are close one to another in the cell). To achieve this, the proteins of interest are each tagged with a different coloured fluorescent molecule (fluorophore), for example red and green. Each of the fluorophores is individually stimulated and images of the resulting fluorescence are taken through the matched filters (e.g. red and green). In the registered images yellow hues indicate the locations where both the red and the green fluorophores co-localise.

Antigen-stimulated co-localisation



The protein interactions can be detected more reliably using Fluorescence Resonance Energy Transfer imaging (FRET). Using this techniuqe the proteins of interest are tagged with specially matched fluorophores. One of them will fluoresce only if it is at a 1-10 nm distance from the other, which is likely to occur only if the two tagged proteins interact. Novel semi-quantitative methods of the analysis of fluorescence microscopy images are being developed in collaboration between the Institute for Cancer Studies and the School of Computer Science.

Multispectral imaging and analysis shows evidence of FRET between ARF6 and PLD1b proteins labelled with CFP and YFP respectively. Excitation light stimulates CFP only. Shown are: a brightness image (left), CFP levels (centre) and YFP levels (right)



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