7.61 Discussion #D3: Single Cell Assays: G Proteins and Ca^{++} Detection

Assigned Reading for Discussion #3, Wed.. October 4, 2006

This set of readings addresses several different ways of analysing specific signalling pathways in individu cells and single cell assays to monitor intracellular events.
We have assigned three papers (marked by *) but we include for your information some other reference addressing the same issues.

G PROTEINS

***1.** Kleuss, C., Hescheler, J., Ewel, C., Rosenthal, W., Schultz, G. and Wittig, B. (1991). Assignment G-protein subtypes to specific receptors inducing inhibition of calcium currents. Nature 353:43-48.

***2**. Kleuss, C., Scherübl, H., Hescheler, J., Schultz, G. and Wittig, B. (1992). Different β-subunits determine G-protein interaction with transmembrane receptors. Nature 358:424-426.

These two papers describe and use methods for microinjection of antisense oligonucleotides to ablate specific Gα or Gβ subunits and then test whether ion channel function alterations in response to specific hormones are affected. They should be discussed together

A third follow up paper extends this analysis to Gγ subunits.
Kleuss, C., Scherübl, H., Hescheler, J., Schultz, G. and Wittig, B. (1993). Selectivity in signal transduction determined by γ subunits of heterotrimeric G proteins. Science 259:832-834.

Gollasch, M., Kleuss, C., Hescheler, J., Wittig, B . and Schultz, G. (1993). G_{i2} and protein kinase are required for thyrotropin-releasing hormone-induced stimulation of voltage-dependent Ca^{2+} channels in rat pituitary GH3 cells. Proc. Natl. Acad. Sci. USA 90:6265-6269.
This paper uses the same approach plus others to dissect a branched signal transduction pathway involving several different Gα subunits, implicating different G proteins in the different branches.

Ca^{++} DETECTION and GREEN FLUORESCENT PROTEIN

***3.** Miyawaki, A., Llopis, J., Heim, R., McCaffery, J.M., Adams, J.A., Ikura, M. and Tsien, RY. (1997). Fluorescent indicators for Ca^{2+}based on green fluorescent proteins and calmodulin. Nature 388: 88 887.

This paper is one of a series of papers developing modified forms of GFP to assay various parameters at the single cell level.

Rizzuto, R., Brini, M., DeGiorgi, F., Rosssi, R., Heim, R., Tsien, R.Y. and Pozzan, T. (1996). Doub labelling of subcellular structures with organelle-targeted GFP mutants *in vivo.* Curr. Biol. 6:183-188.
An earlier paper in the sequence.
Scales, S.J., Pepperkok, R. and Kreis, T.E. (1997) Visualization of ER-to-Golgi Transport in living cells reveals a sequential mode of action for COPI and COPII. Cell90: 1137- 111148.
Use of similar methods to address the role of COPs I and II in intracellular transport.
Kao, J.P.Y., Harootunian, A.T. and Tsien, R.Y. (1989). Photochemically generated cytosolic calcium pulse and their detection by Fluo-3*. J. Biol. Chem. 264:8179-8184.
An even earlier development of one of the most widely used intracellular Ca^{++} sensors
John F. Presley, Carolyn Smith, Koty Hirschberg, Chad Miller, Nelson B. Cole, Kristien J. M. Zaal, and Jennifer Lippincott-Schwartz Golgi Membrane Dynamics Mol. Biol. Cell 1998 9: 1617-1626.
Photoactivatable GFP – This is likely to be used extensively in the future:
Patterson GH, Lippincott-Schwartz J. A Photoactivatable GFP for Selective Photolabeling of Proteins and Cells. Scien 2002 Sep 13;297(5588):1873-7.