Poster #5

An Antigen in Plant Vascular Tissue Cross-Reacts with Antibodies Towards KLH, Keyhole Limpet Hemocyanin

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The use of antisera generated against synthetic peptides bear much promise as a potent tool, not least in trying to assign functions to raw DNA sequences suspected to encode proteins. It may also serve as a complement when learning more about already known proteins—particularly with scarce proteins or ones that are hard to purify. For the purpose of provoking a strong immune response towards a peptide, it has to be coupled to a larger carrier. Keyhole limpet hemocyanin (KLH) is such a carrier that has several distinct advantages and has been utilised extensively. However, we have discovered an antigen present in plant vascular tissue that shows immunological cross-reactivity with antibodies towards KLH. Our findings prompt for caution if one intends to study plants with peptide antisera that have been raised with KLH as a carrier. We discovered the antigen while raising peptide antisera towards two distinct peptides. In both these cases KLH was used as the immunogenic carrier for coupling of the peptides. We obtained very similar staining patterns with both types of sera, in immunocytochemical staining of sections from plant tissues. This was an unexpected result, considering the different proteins from where our peptide sequences were derived. It led us to suspect that what we were seeing did not correspond to reactions with peptide epitopes but rather with a potential antigen cross-reacting with antibodies directed towards the KLH moiety of the peptide-KLH immunogen complex. Further studies using affinity-purified sera that lacked the anti-KLH specificity, in conjunction with immunodiffusion and further immunocytochemical staining, confirmed our suspicion. As a final confirmation, affinity-purified anti-KLH antibodies raised without any peptide coupled to the KLH complex also yielded similar results. Thus, our results are clearly dependent on a cross-reactive antigen, which is localised in plant vascular tissue. With various means we are presently trying to establish the identity of this cross-reacting antigen.

Poster #6

Immunolocalisation of a G Protein–Coupled Receptor in Plant Tissue Sections by Use of a Peptide Antiserum

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We have raised a rabbit antiserum against a 16-amino acid peptide, the sequence of which occurs in the amino terminal extracellular portion of a G protein–coupled receptor (GPCR) in *Arabidopsis thaliana.* The serum was obtained by coupling of the peptide to keyhole limpet hemocyanin (KLH), an immunological carrier that has often been used to provoke a strong immune response towards coupled haptens and peptides. In addition to the peptide specific antibodies, the whole sera that were obtained turned out to contain antibodies towards KLH that cross-react with an antigen present in plants (please refer also to the abstract by Höglund and Josefsson). Consequently, the specific antibodies had to be purified prior to being used for immunocytochemical staining- either by removing the anti-KLH antibodies from whole serum or by positive affinity purification on affinity resins that contained the specific peptide. The purified antibodies were then used to study the expression pattern of the protein with cellular resolution in immunocytochemical staining of tissue sections. Others have suggested a role for the receptor in cytokinin sensing. Thus our staining results are discussed largely in relation to known physiological effects of cytokinin.

Studies regarding phylogenetic relationships among the vast category of GPCR proteins were also performed. By using the PSI-BLAST search algorithm, which is very potent in detecting weak similarities, we were able to establish much more extensive kinship among these proteins than was previously known. Our data confirm evolutionary relationships between many GPCR families that were previously largely hypothetical.