Acta Bot. Neerl. **40**(2), June 1991, p. 172–173

Meeting of The Netherlands Society for Plant Cell and Tissue Culture on 27 September 1990

Signal Transduction of Phytochrome in Leaf Protoplasts of Wheat

M.E. Bossen, P.A.P.M. Verhoeven-Jaspers and R.E. Kendrick. Department of Plant Physiological Research, Wageningen Agricultural University, Generaal Foulkesweg 72, 6703 BW Wageningen, The Netherlands

Protoplasts, isolated from the primary leaves of dark-grown wheat, have been used as a model system to study signal transduction of phytochrome. Research has been focused on changes in plasma membrane properties and the role of Ca^{2+}. After exposure to red light (R), protoplasts swelled only when Ca^{2+} was present in the medium. Far-red light (FR) inhibited swelling after exposure to R. R-induced swelling was also inhibited by the Ca^{2+}-channel blockers Verapamil and nifedipine. Swelling was induced in darkness by the Ca^{2+}-channelagonist Bay K-8644. A GTP-binding protein is thought to be also involved as GDP-β-S, known as an inhibitor of G-proteins, inhibited the R-induced swelling, and the activator GTP-γ-S stimulated swelling in darkness. Li^+, neomycin and H_7, known as as antagonists of the phosphatidyl inositol cycle in animal cells, inhibited the R-induced swelling. PMAL, an agonist, induced swelling in darkness.

Using the Ca^{2+}-sensitive dye murexide, an increase in $[Ca^{2+}]_{medium}$ was found after R-irradiation, which was reversed by FR. This effect was inhibited for ≈80% by the Ca^{2+}-channel blocker Verapamil and the calmodulin antagonist W_7. After R-irradiation an increase in $[Ca^{2+}]_{cyt}$ was found in oat protoplasts as measured with the Ca^{2+}-indicator quin2 (Chae *et al.* 1990, *Biochim. Biophys. Acta* **1051:** 115–122). FR reversed this effect.

Ion channel activity in the membranes of protoplasts can be measured with the patch-clamp technique. Lew *et al.* (1990, *Plant Physiol.* **92:** 822–830) applied this technique on protoplasts of the alga *Mougeotia*. After R-irradiation they found an increased activity of K^+-channels in the plasma membrane. FR reversed the activation. Activation of these K^+-channels is probably regulated by changes in $[Ca^{2+}]_{cyt}$.

The activation of Ca^{2+}-channels via a G-protein and the phosphatidyl inositol cycle, and changes in $[Ca^{2+}]_{cyt}$, are proposed to be involved in the signal transduction of phytochrome.

Tuber Formation in Potatoes

D. Vreugdenhil. Department of Plant Physiology, Wageningen Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands

The process of tuber formation in potatoes can be divided into the following steps: stolon induction and initiation, stolon growth, tuber induction and initiation (Vreugdenhil & Struik 1989, *Physiol. Plant.* **75:** 525–531). Each step is regulated by (the interaction of) various hormones.

Each axillary bud of a potato plant has the potential to grow out as a stolon. Stolon formation is favoured by darkness and high relative humidity, and will occur when the bud is subjected to apical dominance. The diageotropic growth of stolons is due to a low level of cytokinins. Application of exogenous cytokinins to the stolon tip converts the stolon to a negative geotropic leafy shoot.

Longitudinal growth of stolons continues as long as conditions are unfavourable for tuber induction, e.g. under long day conditions. This day-length effect is probably mediated by gibberellins, their level decreasing under short day conditions. In-vitro, the elongation of stolons is reversibly inhibited by ethylene, thus indirectly enabling earlier tuber formation.

In in-vitro cultures, tubers can be induced by a high level of sucrose and by cytokinins. Ethylene and gibberellins inhibit tuber induction. An unknown stimulus might also be involved in tuber induction. Recent research indicates that a (derivative of) jasmonic acid might play an important role (Koda *et al.* 1988, *Plant Cell Physiol.* **29:** 1047–1051.

In the intact plant the various steps in tuber formation occur simultaneously. As indicated above, a certain regulator can influence more than one step, stimulating tuber formation in one step and inhibiting it in another: in the intact plant, cytokinins convert a stolon to a leafy shoot and thus prevent tuber formation early in development, but they stimulate tuber formation on isolated stolons cultured *in vitro*. Such complex regulating mechanisms make it hard, if not impossible, to extrapolate results obtained in relatively simple in-vitro system about the regulation in whole plant.

Flower Bud Formation In-Vitro: a Model System for Research on the Developmental Biology of Plants

A.F. Croes. Department of Experimental Botany, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

Explants consisting of peels from pedicels of tobacco regenerate flower buds when cultured on a suitable medium. Development is reasonably synchronous and proceeds in two stages of approximately one week each: primordium development and flower bud formation. The concentrations of auxin (IAA, NAA) and cytokinin (BA) in the medium determine the number and position of regenerating buds on the explant. The sensitivity of the tissue towards the hormones is modified by many factors so that hormone concentration is not an absolute determinant.

The presence of auxin and cytokinin in the medium is only required during the first days of culture. The physiological significance of this exposure period is different for auxin and cytokinin. After removal of NAA from the medium the accumulated dose is sufficient to maintain a physiologically active concentration in the tissue. Upon withdrawal of BA, however, the BA concentration in the tissue falls far below the concentration of the endogenously synthesized dihydrozeatin, a cytokinin that is as active as BA in flower bud induction. When the administration of BA is postponed for a few days, flower bud formation is delayed. No such delay occurs when IAA is supplied some time after the onset of culture. This indicates that cytokinin is the first-acting hormone in the system.

More insight in the mode of action of the hormones is sought by studying the regulation of genes involved in flower bud development. A number of cytokinin-inducible genes are expressed during the early phase of primordium development. Among them is an extensin gene that might function in cell division. Genes more specifically related to flower bud formation are being isolated by screening a cDNA library prepared from 7-day-old bud-forming explants.