Cloning, knockout and physiology of the phytochrome family in *Physcomitrella patens*

Jon Hughes[1], Gerhard Brücker, Elmar Hartmann, Franz Mittmann[1]
Plant Physiology, Free University of Berlin, Germany.
[1] Current address: Plant Physiology, Justus-Liebig University, Giessen, Germany.
Contact e-mail: jon.Hughes@bot3.bio.uni-giessen.de

The red / far-red photochromic photoreceptor phytochrome plays a critical role in steering development throughout the plant life cycle. It is, however, still unclear how phytochrome acts. Both classical signal-transduction intermediates as well as a more direct involvement in transcription control have been implicated in Pfr action. The phytochrome-dependent polaro- and phototropic behaviour of moss protonemal tip cells is rather interesting in this context. This response requires not only that local Pfr levels within the cytoplasm be sensitive to the direction of irradiation, but also that this vectorial information be retained by the signal transduction system which coordinates the direction of cell growth. Clearly, this is incompatible with gene activation as a sole mechanism of phytochrome action. With Didier Schaefer's discovery of efficient homologous recombination in the moss *Physcomitrella patens*, the targeted knockout of specific genes has become available as an effective tool in studying plant gene function. We have developed this technique for studying phytochrome function in *Physcomitrella* protonemata.

In addition to *Phypa*;PHY1 (PP1) discovered earlier, we have now cloned three phytochrome genes from this species (PP2-PP4). Phylogenetically, these are sister genes forming two distinct clades with two classical phytochromes from the moss *Ceratodon* (CP2 & CP3), consistent with the notion that the *Physcomitrella* genome has been duplicated. We have successfully targeted each of the four *Physcomitrella* phytochrome genes using kanamycin- and hygromycin- resistance cassettes in single- and/or double cross-over strategies. According to extensive gPCR, Southern blots, RTPCR and Northern blots the single targeted phytochrome gene has been genetically and functionally destroyed in each case. We have documented the phototropic behavior of the mutants alongside that of the wild type in exhaustive fluence-response studies. Unlike in *Ceratodon*, wild type *Physcomitrella* caulonemata show a complex fluence-response curve with both positive and negative (avoidance) regions. The response is strictly red-light-dependent and is far-red reversible - indicating the involvement of phytochrome. Each knockout mutant is impaired in one aspect of the phototropic response.