SUMMARY

During the work for this thesis cellular and molecular approaches were used to study photomorphogenesis in the mosses *Ceratodon purpureus* and *Physcomitrella patens*.

Protonemata of *Ceratodon* were used as a model for these analyses. Growth is restricted to the apex of the tip cell whereas unilateral irradiation with red light induces a positive phototropism. This response is red/far-red reversible implying that it is mediated by phytochrome. Phytochrome also modulates chlorophyll biosynthesis in the moss, as in higher plants. In earlier work with *Ceratodon*, various phototropism mutants were isolated and characterized. In Class 1 mutants these phytochrome effects are lost because of a deficiency in the phytochrome chromophore resulting from lesions in the bilin biosynthetic pathway. Microinjection methods were established in *Ceratodon* and used to complement Class 1 mutants in an effort to identify the genetic lesion. Phycocyanobilin, which substitutes for the phytochrome chromophore phytochromobilin (PÖB), was injected into protonema tip cells. This led in ~70 % of the filaments to a clear phototropic response and elevated chlorophyll levels in unilateral red light. The studies implied the existence of an extreme stable subfraction of holophytochrom, localized apparently to the apex of the tip cell. In a series of experiments expression plasmids carrying for heme oxygenase (HO) genes from rat, *Arabidopsis* (*AtHO1*) and *Ceratodon* were microinjected. HO catalyzes the conversion from heme to biliverdin, a precursor of PÖB. The *Ceratodon* HO wild type (wt) gene *CpHO1* was cloned and sequenced in preceding work of Franz Mittmann. In ~40 % injected filaments the wt phenotype could be restored. These results indicate that Class 1 mutants lack the enzymic activity of this gene product. A GFP-CpHO1 fusion protein expressed from an appropriate plasmid showed that the enzyme is localized to the plastids. However, both plastidic (full-length CpHO1 and AtHO1) and cytosolic (rat HO and CpHO1 without chloroplast transit peptide) HOs were able to suppress the mutant phenotype.

The work presented here was intended to shed light on light-mediated responses in *Physcomitrella*. Because dark-grown material provide clearer results in studying photomorphogenesis, it was first necessary to establish conditions under which *Physcomitrella* protonemal filaments grow in darkness. It seems that the adaptation to a gravitropic stimulus is a prerequisite for dark growth of *Physcomitrella* protonema. Thorough physiological studies charcterised phytochrome-mediated phototropism in protonemata. A blue light receptor seems not to be involved in this response. Fluence response curves of caulonemal phototropism in red light are rather complex. Depending on the fluence rate, filaments grew positively and/or negatively phototropically. At low light levels, the majority grow away from the light. Intermediate light irradiances induce a positive response in most filaments, whereas under strong light, again negative phototropism dominates (avoidance response). Experiments with polarized light indicate that active phytochrome molecules are dichroically orientated in close vicinity to the plasma membrane.

Physcomitrella is able to integrate DNA efficiently by homologous recombination. Franz Mittmann identified and disrupted by targeted knockout the four phytochrome genes *PhypaPhy1 – 4*. A quantitative comparison of the phototropic behavior of the knockouts with that of the wildtype provided a detailed picture of the function of each of the four genes. It appears that each phytochrome has a specific role in the phototropic response. In *PhypaPhy4*-knockouts positive phototropism is lost completely. With these results the first photoreceptor in mosses responsible for this response was identified. PhypaPhy3 seems to be responsible for the avoidance response, in which the filaments bend away from the light under high fluence rates. *PhypaPhy1* and *2* knockouts showed a more subtle phenotype. Their function could be a modulation of the effects of PhypaPhy3 and 4.

The Class 1 phototropic mutations described above were the basis for gene targeting experiments in *Ceratodon*. HO knockouts should be easily identifiable on the basis of their aphototropic phenotype. Transformations with a knockout construct directed against the HO gene in the wildtype resulted in ~40% of the regenerating protoplasts showing the aphototropic phenotype. Surprisingly however, this was not the result of the expected integration of the construct at the homologous genomic locus, but probably of the homologous DNA acting via RNAi or DNA methylation. Franz Mittmann sequenced also *CpHO1*-Loci of several Class 1 mutants. In one line the cause was a mutation resulting in a stop codon just downstream of the start codon. A plasmid carrying a modified *CpHO1* gene fragment was transfected into mutant protoplasts in an attempt to repair the lesion via homologous recombination (gene replacement). PCR- und Southern-analysis's supplied evidence for the integration of a single fragment at the homologous region in the *Ceratodon* genome providing unambiguous evidence of successful gene replacement. This is the first report of a nuclear gene replacement in plants. The high efficiency of 0,9 % regenerating protoplasts with wildtype phenotype demonstrate the potential of this method. Site-directed mutagenesis of chromosomal genes *in vivo* raises interesting new possibilities for functional analysis of plant gene products using moss systems. Moreover this experiment has shown in an elegant manner that efficient gene targeting by homologous recombination is possible in *Ceratodon purpureus*.