1. Project background, starting hypotheses

In recent years algal biotechnology has become a rapidly developing field. Algae are currently exploited for the production of secondary metabolites as high value products. Furthermore, they are also envisaged as the ideal producers of next generation biofuels. At this point the use of algae for biofuels is hindered by high costs of the biomass production. Novel approaches such as usage of wastewater instead of fertilisers, and most importantly usage of waste CO_2 for supporting algal growth bring the double benefits of decreasing the price of algal biomass and bioremediating waste water and CO_2.

Despite the high economic potential of algal biotechnology one serious difficulty remains, the physiology of algae and the stress response in particular, need to be better understood for sustainable biomass production. This is further highlighted by the fact that adaptation to stress also means significant changes in metabolism. In fact application of stress treatments is a common practice in industry to induce the production of valuable products (Skjånes et al., 2013). This project aimed to bring novel knowledge into this so far under-studied field.

In order to sense and to appropriately regulate responses to environmental stimuli plants have evolved complex cellular signalling networks. The mitogen-activated protein kinase (MAPK) phosphorylation cascades are conserved signalling modules in all eukaryotes, they are known to have pivotal roles in regulating stress responses, cell division and growth (Avruch, 2007). A typical MAPK cascade consists of three classes of enzymes: a MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MKK), and the MAP kinase (MAPK), which activate each other in a sequential phosphorylation fashion. MAPK pathways are key regulators of adaptive processes in flowering plants (Pitzschke et al., 2009; Rodriguez et al., 2010).
At present very little is known on environmental signalling in algae, and our knowledge of algal MAPK functions is almost nothing. Our previous comparative genomics analysis identified the gene sets encoding MAPKs and MKKs in lower plant genomes, including algal species (Dóczi et al., 2012). As a logical continuation of these results we initiated collaboration with Katerina Bisova, an expert *Chlamydomonas* researcher (Department of Phototrophic Microorganisms, Institute of Microbiology, Czech Academy of Sciences, Trebon, Czech Republic). Supported by an OECD fellowship one of the project leaders, Róbert Dóczi spent five months in 2013 in the Bisova lab. During this period, he got familiar with laboratory practices of working with *Chlamydomonas*.
Of the green algae *Chlamydomonas reinhardtii* has become a well-established model species, with characterised laboratory strains, standard protocols to maintain and study cultures and genomics tools (sequenced and annotated genome, purpose vectors and efficient transformation) (Blaby et al., 2014; Merchant et al., 2007). With this toolkit it is now highly timely and feasible to carry out functional studies of key *Chlamydomonas* signalling components.

We have chosen two key components for the initial study: CrMPK8 (Cre01.g010000), a MAPK most related to the best studied group of higher plant MAPKs (e.g. AtMPK3/6); and CrMKK3 (Cre06.g249150) a MKK, belonging to a plant-specific MKK type, whose orthologue

participates in oxidative stress and pathogen signalling (Dóczi et al., 2007). Both genes are the only members of their corresponding phylogenetic clades in algae, thus minimising redundancy problems during functional work.
Application for the project was submitted in order to be able to continue this international collaboration. The project aimed to carry out a functional analysis of MAPK signalling in *Chlamydomonas* using genetic and molecular approaches, with special emphasis on its role in environmental adaptation.

2. Results

2.1. Functional genetic analyses

As a first step, we set out for optimising culturing conditions for *Chlamydomonas* in our laboratory. The wild-type laboratory strain 21gr was obtained from the foreign partner. Liquid cultures are grown on a shaker platform in TAP media, under continuous light. We produced growth curves that are regular and reproducible, with a linear correlation between cell count and optical density (measured at 750 nm). Thus, we became able to compare growth of various lines and to measure the effect of stress treatments on growth and photosynthesis parameters (e.g. by PAM fluorometry).

Due to a scientific development a major unforeseen change in the course of our project was undertaken. In the original work plan we intended to generate transgenic overexpression Chlamydomonas lines for functional analysis, due to the lack of mutant lines, which are mutated in the studied genes. The genes were cloned, and the planned expression vectors were generated, as described in the interim reports. However, during the course of the project an insertional *Chlamydomonas* mutant collection became available (Li et al., 2016). Production of such a mutant collection requires intense efforts, therefore it clearly indicates the emerging importance of *Chlamydomonas* in plant biotechnology research. Although we have generated the planned overexpression lines (these results were presented in the yearly reports) using loss-of-function (insertion) mutants for functional characterisation of the regulatory genes we study is much more efficient and precise than the originally proposed approach. Accordingly, we obtained the corresponding novel insertion lines and their background strain (for WT control) from the stock centre (https://www.chlamylibrary.org/), genotyped them, confirming the presence of the inserts at their predicted positions. As a next step, we adapted a plant RNA isolation protocol to isolate RNA from cultured *Chlamydomonas* with the help of our collaborator and demonstrated the loss of gene expression for both *CrMPK8* and *CrMKK3* in the corresponding mutants by quantitative RT-PCR.

Because MAPK pathways play an important role in the signalling of oxidative stress both in flowering plants and in mammals, we first set out to test the sensitivity of *crmpk8* and *crmkk3* mutant lines to the oxidative agent, paraquat (PQT). Surprisingly, both lines turned out to be more resistant to oxidative stress than WT control (Figure 1), suggesting that MAPK signalling is a negative regulator of oxidative stress in a unicellular green alga. This is in striking contrast

to all other eukaryotic groups, where MAPK pathways act as positive regulators of oxidative stress. These results are highly reproducible, the experiments were carried out six times with similar results. Moreover, in all experiments four different PQT concentrations were applied, triggering proportional responses.

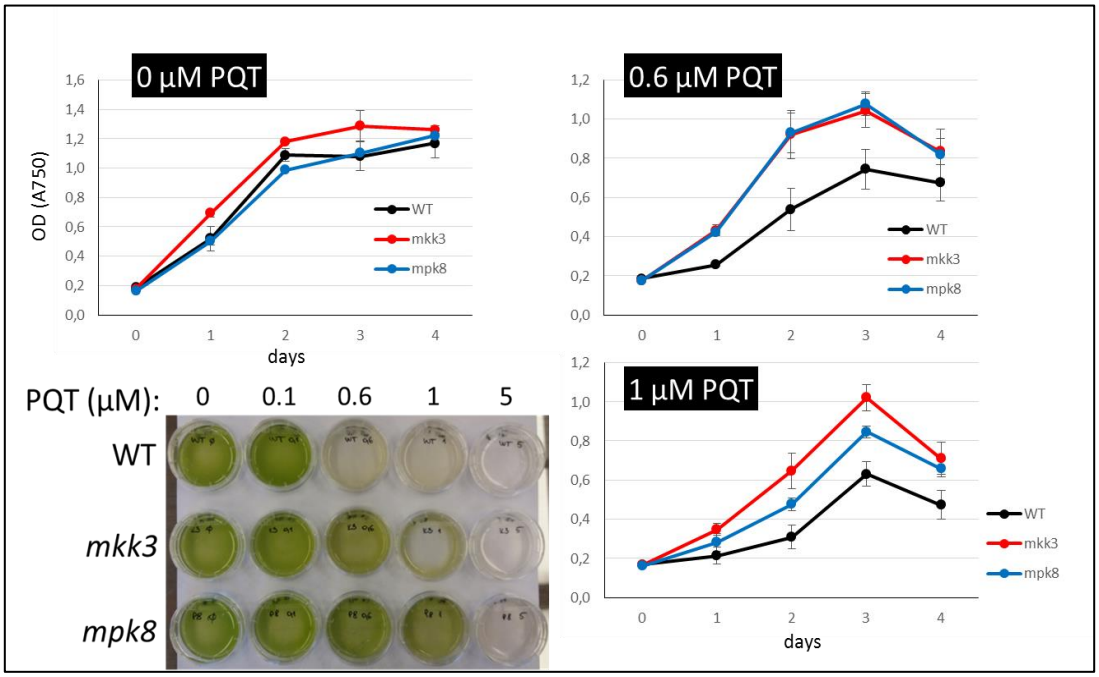

Figure 1. MAPK signalling mutants are more tolerant to PQT-induced oxidative stress
WT and MAPK signalling mutant *Chlamydomonas* lines were cultured in flasks under continuous rotation and light in liquid TAP media in the presence of PQT at the indicated concentrations. Culture density was assayed by photometry (OD) at 750nm. Cultures grown in microtiter plate are shown in the lower left panel.

Because PQT treatment led to a culture bleaching, we also measured the effect of PQT stress on photosynthesis in collaboration with É. Darkó (Dep. of Plant Physiology, MTA ATK). Fv/Fm tests demonstrated sensitivity of photosystem II of cultured *Chlamydomonas* to PQT and that these values in both mutants were higher than WT under all PQT concentrations, confirming their increased tolerance to PQT-triggered ROS (Figure 2).

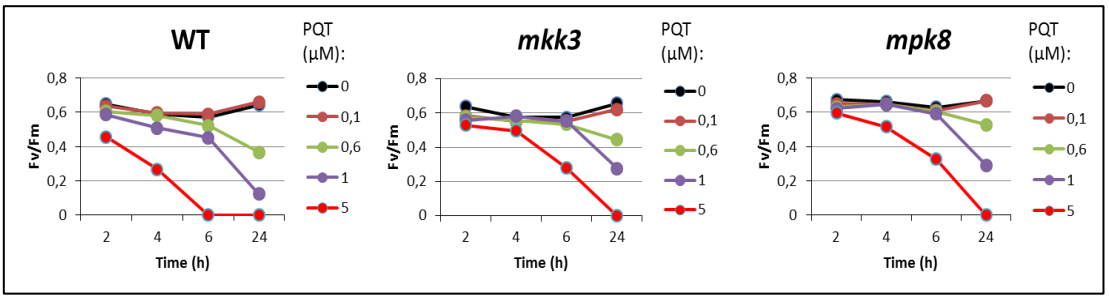

Figure 2. Lower degree of photosystem II damage in MAPK signalling mutants under PQT-induced oxidative stress
Fv/Fm values of WT and MAPK signalling mutant *Chlamydomonas* lines grown in petri dishes under continuous light and rotation to exponential phase in TAP media than subjected to the PQT treatment in the indicated concentrations and time periods.

To study the regulatory relationship between oxidative stress and MAPK signalling further, a set of genes known to be involved in ROS metabolism in flowering plants was selected for comparative gene expression analysis. qRT-PCR assays show that CrAPX1, CrSOD1, CrDHAR1, CrFDX4 are induced by PQT in a negatively MPK-dependent manner, i.e. their basal expression is already higher and their induction is stronger in the KOs than WT. CAT was interestingly repressed by PQT and in the mutants it had a lower baseline expression and a more pronounced repression in response to PQT treatment. CrGPX and CrFER2 had no significant change, while we had technical issues with detecting CrGSHR1 (Figure 3). These results suggest that the Foyer-Halliwell-Asada pathway is a primary MAPK regulatory target and further confirm the negative regulatory role of MAPK signalling in oxidative stress.

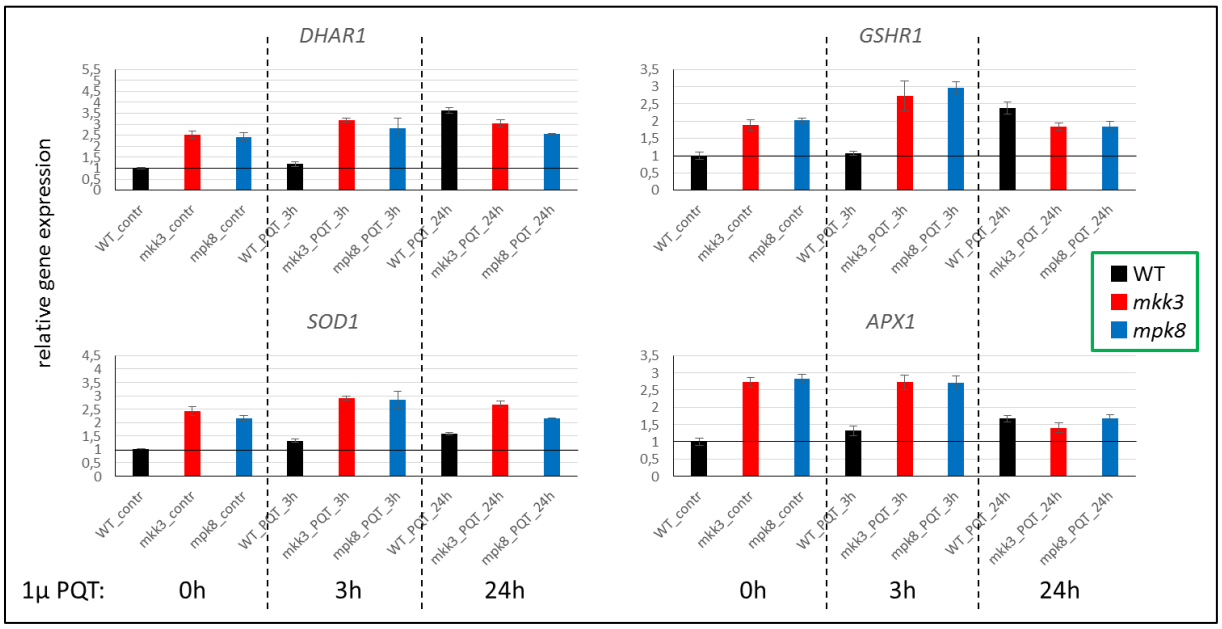

Figure 3. Genes encoding ROS detoxifying enzymes are negative targets of CrMPK8 and CrMKK3

Relative expression values of the Glutathione-dependent dehydroascorbate reductase 1 (DHAR1), Glutathione reductase (GSHR1), Superoxide dismutase (SOD1) and Ascorbate peroxidase (APX1) were assayed by quantitative real-time PCR. *Chlamydomonas* liquid cultures were treated with 1µM PQT, samples were taken at the indicated time points.

Moreover, as initially a 2µM PQT concentration was applied for the gene expression experiments, we also observed degradation of RNA in samples collected from WT, but not in the two mutant lines, after 12h treatment, further indicating their enhanced antioxidant defence (Figure 4). Therefore, gene expression experiments were carried out using 1µM PQT treatment.

Besides oxidative stress we also tested salt tolerance of the mutants. Interestingly, similarly to other species, the MAPK mutants are less tolerant to salt than WT control. Unlike PQT-induced oxidative stress, salt stress is a complex stress, comprising of osmotic stress, ion stress and (indirect) oxidative stress. The differential functions MAPKs play in the signalling of oxidative and other stress factors in *Chlamydomonas* are enigmatic at this point and we can only assume that redox responses and other stress components may have differential roles in algal life style.

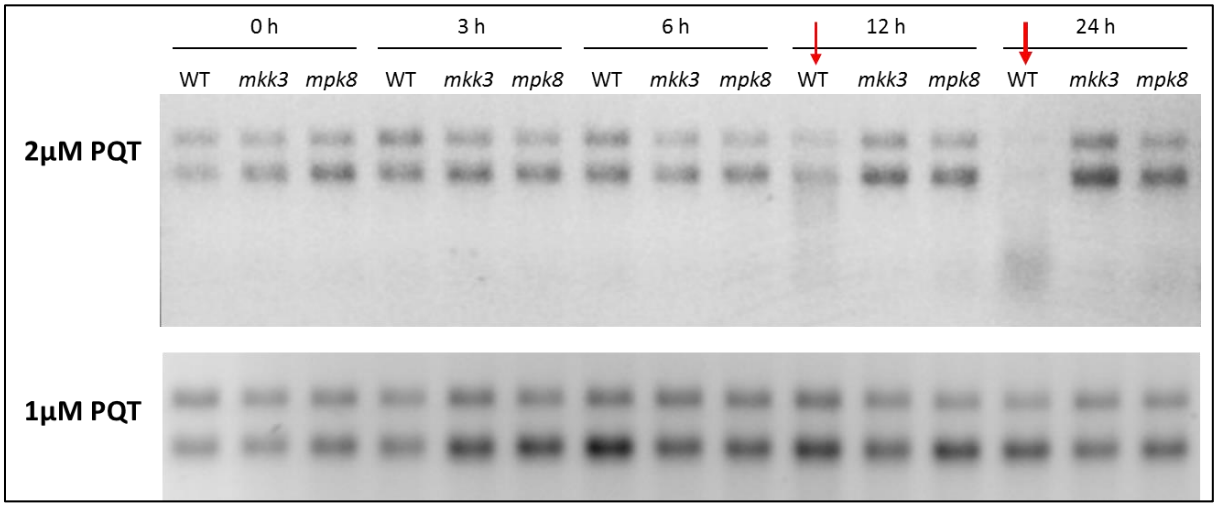

Figure 4. RNA is less prone to oxidative damage in MAPK signalling mutants.
RNA samples collected from the indicated genotypes at the indicated time points of 2 or 1 µM PQT-treated cultures were separated by agarose gel electrophoresis. RNA degradation is visualised by smearing (arrows).

2.2. Transcriptome analysis

To address this question further we decided to use the more amenable mutant genetic system for the transcriptome analysis originally planned by using the overexpression lines. To gain further insight into this regulatory mechanism we carried out a transcriptome analysis of MAPK mutant lines under control and stressed conditions. The experiment was designed around a parallel culturing of control and *crmpk8* and *crmkk3* mutant lines under control (mock) and paraquat-treated conditions. Samples were collected at 0, 3 and 24 hours, thereby generating a total of 16 samples. Following RNA extraction, RNA quality was assessed by spectrophotometry, agarose gel electrophoresis and qPCR, where previously established oxidative stress marker gene expression patterns were assayed. Expression of the marker genes reflected the previous results, indicating a successful treatment course.

Samples were arranged pairwise (mock vs. treated) on eight arrays on two slides that were custom-made by Agilent Technologies, according to Toepel et al. (Toepel et al., 2011). cDNA synthesis, labelling and array hybridisation was carried out by our collaborator, Z. Doleschall at the National Institutte of Oncology, Budapest, Hungary. Following initial quality check of the raw data, the entire transcriptome experiment was repeated to obtain an independent biological reproduction.

The microarray probesets were re-annotated based on the recent NCBI RefSeq genome assembly GCF_000002595.1. Approximately 44.000 nucleotide probes were mapped on 14.202 individual gene (mRNA) sequences which were identified also at protein level. 3072 of the 14.202 genes were expressed with a minimum of +/-1.5 fold change, at least in one of the eight samples (Figure 5). Public databases were used for functional annotations of the 3072 differentially expressed genes (DEGs), including KEGG (Kyoto Encyclopedia of Genes and Genomes), GO (Gene Ontology) terms and Pfam (Protein Families) protein domain collections. Based on the KEGG database we have mapped 575 individual DEGs in 104 different metabolic

pathways. This analysis revealed that the investigated gene mutations were more relevant on the gene expression patterns compared to the paraquat treatment alone. We have found that the MPK8 and MKK3 mutations have quite similar effects on many metabolic pathways. The genes, involved in the 'Lipid metabolism', 'Carbohydrate metabolism', 'Energy metabolism' and 'Glycan biosynthesis and metabolism', furthermore many genes from the 'Folding, sorting and degradation' and the 'Replication and repair' related pathways were basically up regulated. In parallel, most of the genes in the 'Amino acid metabolism' and in the 'Metabolism of cofactors and vitamins' were downregulated. These results were confirmed by GO term analysis as well, which describes our knowledge of the biological domain with respect to three aspects. Genes from the over-represented categories, including the members of 'Photosynthesis', 'Proteasomal ubiquitin-independent protein catabolic process' and 'Response to abiotic stimulus' groups were upregulated, while the elements of 'Branched-chain amino acid biosynthetic process' and interestingly the 'Cilium organization and motility' related genes were downregulated.

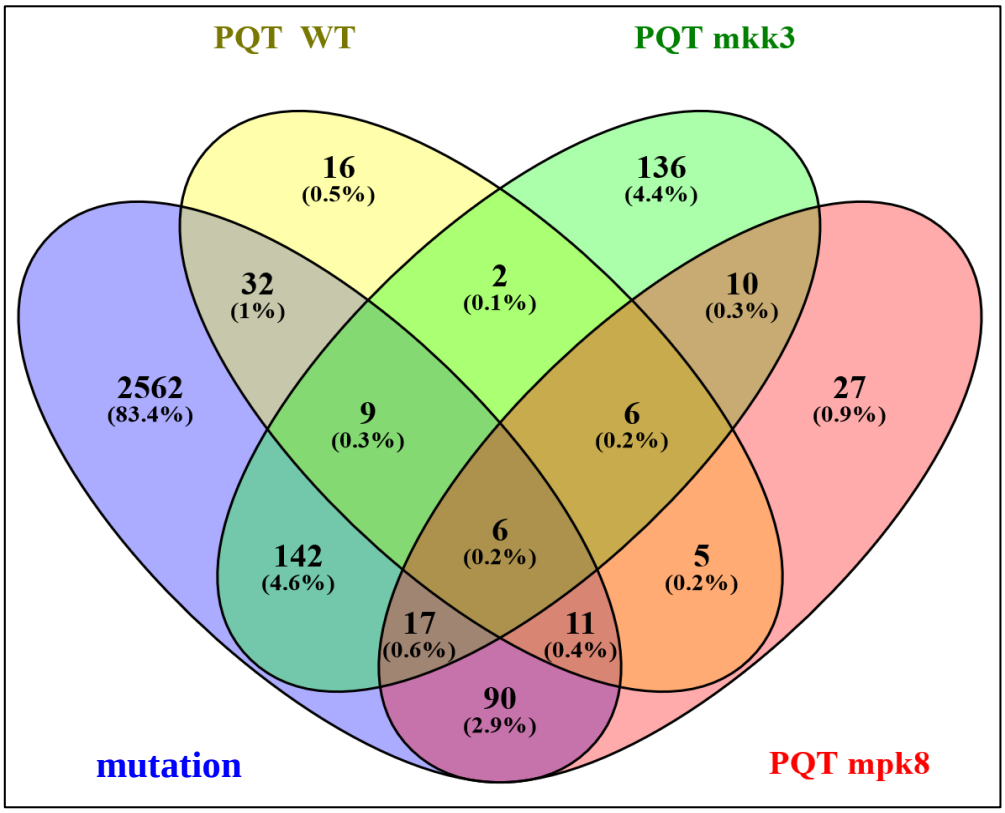

Figure 5. Distribution of the differentially expressed genes (DEGs) on the microarray with at least +/-1.5 fold change.

The Venn-diagram represents the number of genes changed due to the mutation or the paraquat (PQT) treatment in all genotypes and all combinations of these two effects. The different colours indicates the percentage distribution of the total 3072 genes expressed differentially with at least +/- 1.5 fold change.

Overview of the experiments revealed transcriptional regulatory networks controlled by CrMKK3 and CrMPK8. These data verify their negative regulatory roles in oxidative response, but also highlight their involvement in various biological processes, photosynthesis perhaps being the most remarkable finding (Figure 6).

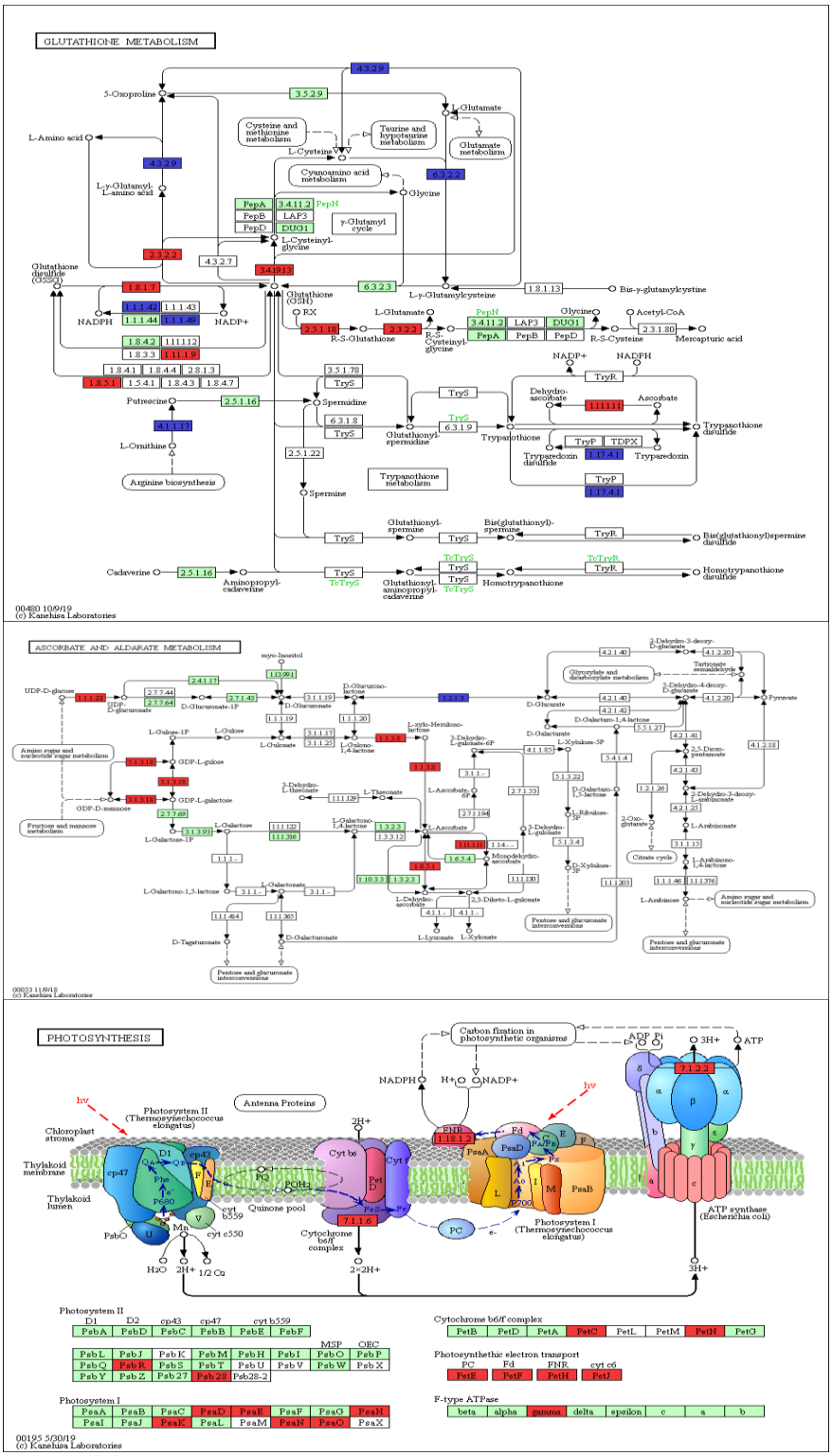

Figure 6. Mapping of the differentially expressed genes involved in the 'Glutathione metabolism', 'Ascorbate and aldarate metabolism' and 'Photosynthesis' KEGG pathways.

The figures represent the schematic structure of three metabolic pathways. The rectangles represent genes and enzymes, the overexpressed ones are filled with red and the repressed ones with blue, respectively. Green means that the expression of those genes/enzymes did not reach the +/- 0.5 fold change on the arrays, while there are no information about the white ones in the KEGG database.

2.3. Pigment biosynthesis

As suggested by the transcriptome analysis we also measured pigment content in the MAPK mutant *Chlamydomonas* lines. The total chlorophyll and carotenoid contents were determined spectrophotometrically and their amount was related to the starting cell number (10^7 cell/measurement).
We found that under standard culturing conditions both the carotenoid and the chlorophyll content of *mkk3* mutants is higher than that of the wild type control. However, we could not detect any changes in the pigment content of the *mpk8* mutant compared to the WT (Figure 7). This is the only experiment where differential results were obtained by the two mutant lines, which is rather unexpected, but suggest possible redundancy at the MAPK level in this process. Nevertheless, these results reveal that MAPK signalling plays a role in the regulation of biosynthesis of pigments, a finding with important implications in biotechnology, as in recent years carotenoids from algae gained commercial recognition in the global market for food and cosmeceutical applications (Ambati et al., 2019).

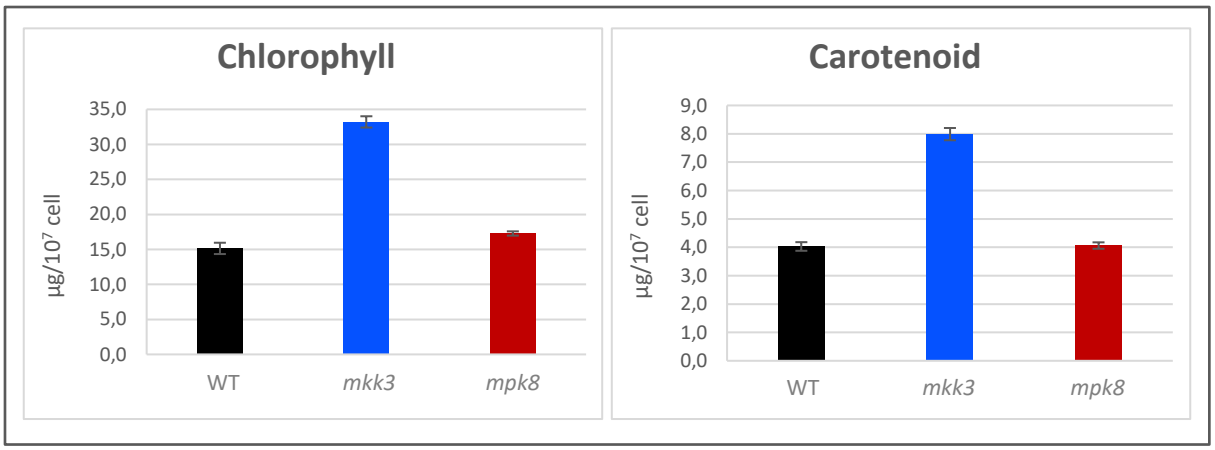

Figure 7. Pigment content of the *mkk3* mutant is twice that of the wide type.
WT and MAPK signalling mutant *Chlamydomonas* lines were cultured in flasks under continuous rotation and light in liquid TAP media until the cell number reached 10^7 cell/ml. Total chlorophyll and carotenoid content were measured from the cell culture spectrophotometrically. For the measurement we used three biological replicates.

2.4. Interaction of CrMKK3-CrMPK8

Two genes were selected for initial functional analysis in *Chlamydomonas*: CrMPK8, a MAP kinase and CrMKK3, a MAP kinase kinase, representing two hierarchy levels of the MAPK phosphorylation cascade. As detailed above, the two mutant lines displayed highly similar responses under all experimental conditions. Moreover, there is a high degree of overlap in their transcriptomes. This phenocopying raises the possibility that the two kinases are part of the same pathway. To test this hypothesis we assayed protein interaction between CrMPK8 and CrMKK3 by yeast two-hybrid and BiFC (split YFP) methods in collaboration with V. Soós at the Department of Applied Genomics (MTA ATK). No interaction was detected in the yeast system, while BiFC in transfected *N. benthamiana* cells was positive (Figure 8). These

conflicting results imply that the MPK8-MKK3 interaction requires a plant-specific third partner. Accordingly, their orthologues in Arabidopsis, MPK6 and MKK3, also fail to interact in the Y2H setting (Dóczi et al., 2007; Jin et al., 2008). These results confirm that CrMKK3-CrMPK8 constitute a MAPK pathway in *Chlamydomonas*. To further confirm their functional connection activation of CrMPK8 by CrMKK3 will have to be demonstrated.

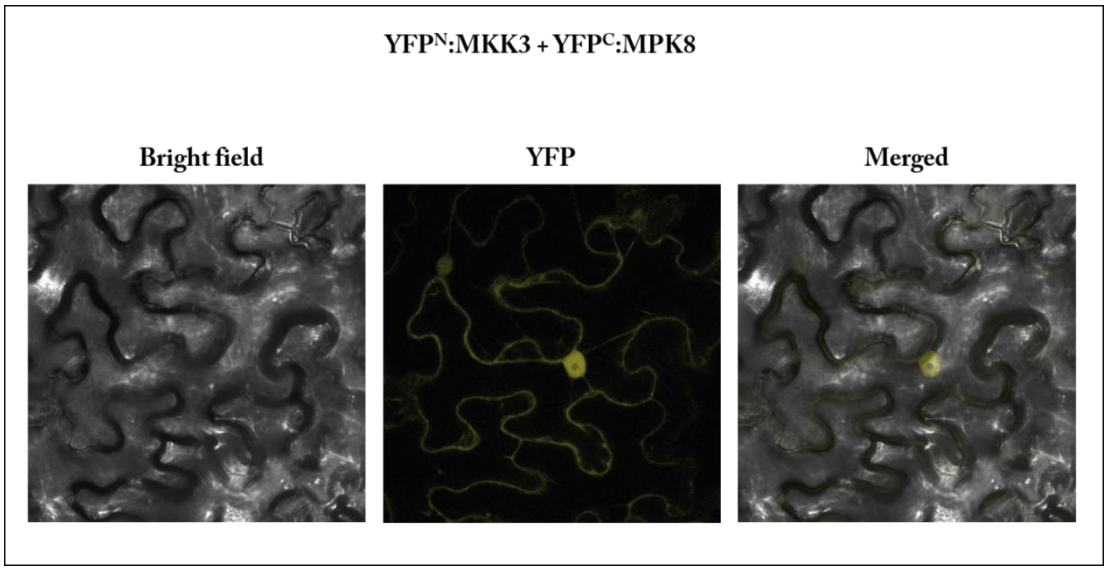

Figure 8. Interaction of CrMKK3 and CrMPK8

Interaction between MKK3 and MPK8 was assayed by bimolecular fluorescence complementation (BiFC – split YFP) in agroinfiltrated *N. benthamiana* leaves. N-terminal YFP was fused to MKK3 and C-terminal YFP was fused to MPK8 in N-terminal orientation. Similar results were obtained in three biological replicates in all other possible combinations, with the exception of MKK3:YFP^N + YFP^C:MPK8, where no interaction was detected (not shown).

2.5. Comparative genomics of algal MAPKs

Photosynthetic green microalgae (Chlorophyta) are a large and diverse, early diverging group within the plant kingdom (Viridiplantae), consisting of uni- and multi-cellular species, which are distributed across a wide range of physiological and ecological conditions. Comparative analysis of gene families across microalgae and more complex land plants can facilitate reconstruction of the gene family's evolutionary history prior to separation from the last common ancestor. In order to gain a comprehensive inventory of algal MAPKs we screened 13 algal species with sequenced genomes to identify MAPK sequences. The 13 species represent a wide range of green algae in terms of phylogeny, life style, geographical distribution, ecological or economic importance. For example, they represent the three major classes, including widely distributed cosmopolitan marine planktonic or fresh water species as well as highly specialised species adapted to extreme conditions or parasitic life style. The species also represent the spectrum of cell sizes, genome sizes and gene numbers across green algae.

Analysis of the identified sequences led to a number of interesting findings. Low number of MAPKs is characteristic across algae, the few losses or duplications are associated with genome complexity rather than habitat ecology, despite the importance of MAPKs in environmental

signalling in flowering plants. Our results reveal that the plant MAPK gene family emerged from three types of progenitor kinases, which are ubiquitously present in algae, implying their formation in an early ancestor. Most importantly, based on phylogenetic analysis the main variants of the plant-type MAPKs must have formed in a very early common photosynthetic ancestor and the MAPK groups found in flowering plants emerged through serial duplications of three types of ancestral MAPK types, designated A/B, C and D (Figure 9), based on the plant MAPK phylogeny nomenclature convention of groups A-D (Ichimura et al., 2002). Interestingly, kinases related to ERK8, a non-canonical MAPK type, which is missing in higher plants are present throughout Chlorophyta.

In opisthokonta species ERK-type MAPKs are involved in cell cycle regulation, and thus mutations affecting the ERK pathway are highly associated with cancers. To investigate transcriptional regulation of MAPKs during the cell cycle, our collaborating partner carried out a systematic transcriptional analysis of these early MAPK forms throughout the cell cycle, using synchronised *Chlamydomonas* cultures. Their qRT-PCR results reveal that most *Chlamydomonas* MAPKs are transcriptionally upregulated around transition from G1 to S/M phase, suggesting their involvement in cell cycle regulation. This is in line with the notion that the eukaryotic common ancestral MAPK was an ERK-like MAPK, because ERK1/2 are key cell cycle regulators in mammals and all plant MAPKs are most related to ERK. These results were presented in a recently published article (Kalapos et al., 2019).

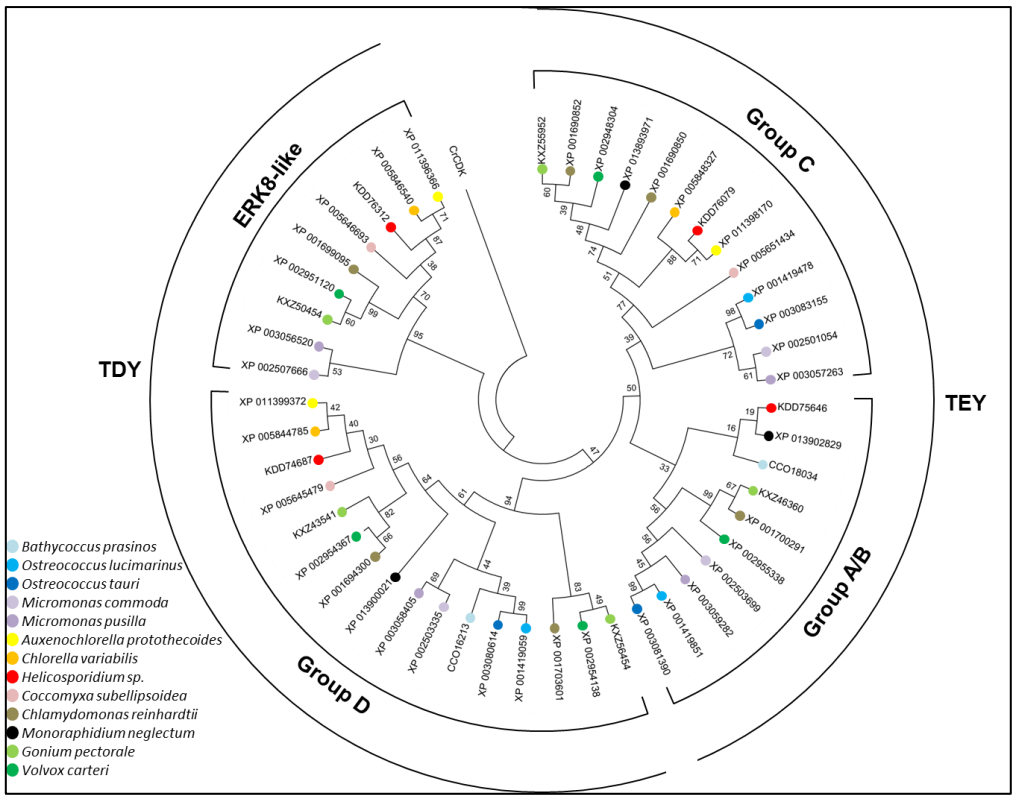

Figure 9. Phylogenetic relationships of MAPK sequences in 13 algal species.
Amino acid sequences of the kinase domains of 48 identified MAPKs were aligned by the MUSCLE alignment method and the phylogenetic tree was constructed by the Maximum-likelihood method using the MEGA6 software package. A cyclin-dependent kinase (CDKG1) of *Chlamydomonas reinhardtii* was used as outgroup. Species are indicated by each protein ID in the tree with coloured dots according to the key on the left. Phylogenetic grouping and the type of the conserved phosphorylation site (TxY) are indicated at the inner and outer perimeters, respectively.

3. Conclusions

Despite the ubiquitous importance of microalgae (ecology, biotechnology), the knowledge on their physiological adaptation mechanisms and in particular on the signal transduction mechanisms that regulate the underlying molecular responses is very limited. Our work contributes to this knowledge gap and has implications for biotechnology.

Within the framework of an international collaboration we have introduced a unicellular plant model system into the host institute to study environmental signalling. Instead of relying solely on overexpression as originally proposed, we switched to working with a versatile genetic tool of insertional mutant lines for functional genetics studies. It has turned out to be a robust experimental system, providing interesting and highly reproducible results. Most importantly, we have discovered an intriguing evolutionary flip of the highly-conserved regulatory role of MAPK signalling in oxidative stress in a unicellular photosynthetic alga. At present the biological reason underlying this phenomenon is unclear, it is presumably a consequence of algal lifestyle.
With high relevance from a biotechnological perspective, we found that MAPK signalling is also involved in the regulation of pigment biosynthesis, as underscored both by compound measurements and transcriptome results. Carotenoids are of great interest for industry, therefore modulation of MAPK signalling in cultured microalgae now seems a promising approach to enhance carotenoid production.
Besides, by using comparative genomics and gene expression analysis we have clarified the origin and relationship of algal MAP kinases and produced preliminary data suggesting their involvement in cell cycle regulation. Our results also revealed a high degree of similarity in MAPK signalling components throughout green algae, implying transferability of results obtained in *Chlamydomonas* to other species.

Taken together, we have discovered and characterised an evolutionarily unique function of a novel MAPK pathway in algae. Cellular signalling in lower plants is basically an uncharted area, thus our results can have significant contributions to the broader field of signalling and environmental adaptation of plants and to the understanding of the evolution of signalling in the plant kingdom. Moreover, considering the economic significance of green algae, our results can have important implications in biotechnology.

4. Publications

We presented our results to the scientific community at several national and international conferences, e.g. one of our abstracts was selected for an oral presentation at the International Plant Molecular Biology 2018 Conference. Most of our results presented in this report are currently unpublished as we are still in the process of complementation experiments. Once these are successful, we plan to publish the results demonstrating the first functional characterisation of a novel MAPK pathway in algae and its evolutionarily unique role as a negative regulator of oxidative stress response in a high-impact journal. Besides, we have already published a MAPK

methodology paper (Dory et al., 2016), a review on MAPK substrates in a renowned plant science journal (Dóczi & Bögre, 2018) and comparative genomics paper about the evolution of the MAPK family in algae (Kalapos et al., 2019).

Peer-reviewed publications:

Kalapos B., Hlavová M., Nádai T.V., Galiba G., Bisova K. and Dóczi R. (2019) Early Evolution of the Mitogen-Activated Protein Kinase Family in the Plant Kingdom. *Scientific Reports* 9:4094. IF: 4.011

Dóczi R.* and Bögre L. (2018) The Quest for MAP Kinase Substrates: Gaining Momentum. *Trends in Plant Science* 23:918-932. IF: 14.006
* Corresponding Author

Dory M., Doleschall Z., Nagy S.K., Ambrus H., Mészáros T., Barnabás B., Dóczi R. (2016) Kinase-Associated Phosphoisoform Assay: A Novel Candidate-Based Method to Detect Specific Kinase-Substrate Phosphorylation Interactions In Vivo. *BMC Plant Biol* 16:204. IF: 3.67

Conference presentations:

Nádai TV, Boldizsár Á, Kalapos B, Darkó É, Galiba G, Bisova K, Dóczi R (2019) The Adaptive Role of Mitogen-Activated Protein Kinase (MAPK) Signalling in *Chlamydomonas Reinhardtii* Grown Under Paraquat Induced Oxidative Stress. International Conference on Plant Science Research, 04-06 March, 2019, Baltimore, MD, USA (oral presentation)

Nádai TV, Boldizsár Á, Kalapos B, Darkó É, Bišová K, Galiba G, Dóczi R (2019) The Role of Mitogen-Activated Protein Kinase (MAPK) Signalling in Oxidative Stress in the Unicellular Model Organism, *Chlamydomonas Reinhardtii*. International Conference on Plant, Cellular and Molecular Biology (Plant2019), 18-20 February, 2019, Valencia, Spain (oral presentation)

Nádai TV, Kalapos B, Galiba G, Bisova K, Dóczi R (2018) Mitogen-Activated Protein Kinase Signalling in the Model Unicellular Microalga, *Chlamydomonas reinhardtii*. FEBS3+ Conference, From Molecules to Living Systems, 2-5 September 2018, Siófok, Hungary (poster presentation)

Dóczi R, Nádai TV, Kalapos B, Galiba G, Bisova K (2018) The role of mitogen-activated protein kinase signaling in oxidative stress in the model unicellular microalga, *Chlamydomonas reinhardtii*. IPMB 2018 - 12th International Congress of Plant Molecular Biology, 5-10 August, Montpellier, France (oral presentation)

Dóczi R, Kalapos B, Nádai TV, Galiba G (2018) Towards understanding early evolution of the plant mitogen-activated protein kinase family through comparative genomics across Chlorophyta. IPMB 2018 - 12th International Congress of Plant Molecular Biology, 5-10 August, Montpellier, France (poster presentation)

Nádai TV, Kalapos B, Galiba G, Bisova K, Dóczi R (2018) Functional analysis of mitogen-activated protein kinase (MAPK) signalling in the model unicellular microalga, *Chlamydomonas reinhardtii*. FIBOK, National Conference of Young Biotechnologists 28-29 March, Budapest, Hungary ISBN 978- 963- 315- 370-3 (oral presentation)

Nádai TV, Bisova K, Dóczi R (2017) Exploring environmental signal transduction in the model unicellular microalga, *Chlamydomonas reinhardtii*. 8th Symposium on "MICROALGAE AND SEAWEED PRODUCTS IN PLANT/SOIL-SYSTEMS" 26-27 June, Mosonmagyaróvár, Hungary (oral presentation)

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Published in *Biochemistry*, journal of the Hungarian Biochemical Society, issue XL/3 (HU ISSN 2060 8252, HU ISSN 0133 8455) p. 44.

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