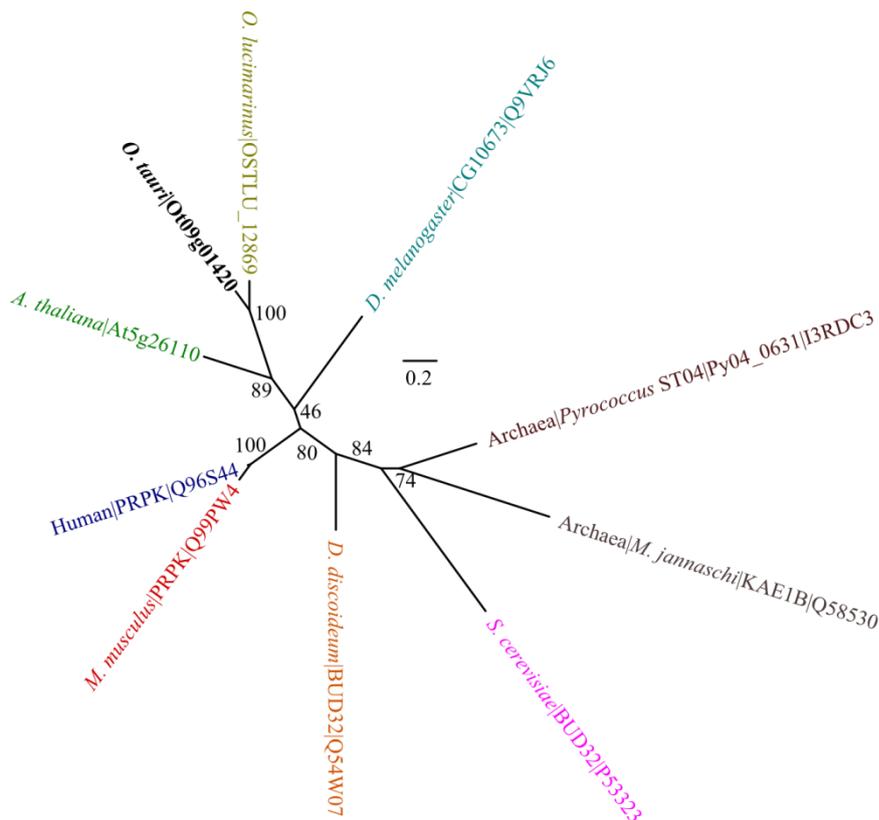




## Atypical kinases

### B) BUD32

The BUD32 protein kinase (Ot09g01420) in *O. tauri* is related to Bud site selection protein (BUD32) in yeast and human p53-related protein kinase (PRPK). BUD32 is found in almost all eukaryotes, in archaea, and some bacteria. Universally, it forms a complex or gene-fusion with kinase-associated endopeptidase 1 (Kae1) (Hecker *et al.*, 2009). Kae1 is also conserved in *O. tauri* (Ot13g00210). This complex is involved in telomere maintenance and transcriptional control in yeast (Hecker *et al.*, 2008; Kisseleva-Romanova *et al.*, 2006).

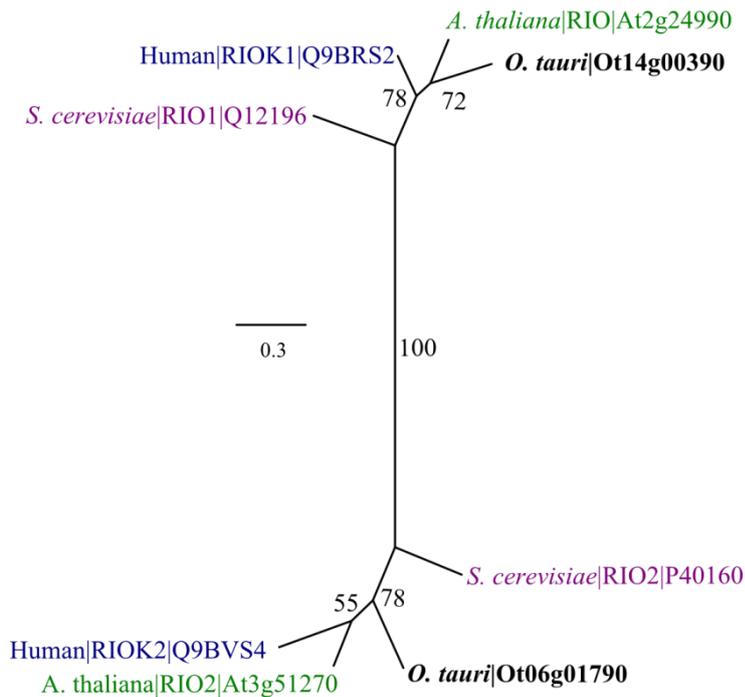


**A phylogeny of the BUD32/PRPK protein kinases.** Kinases are included from *O. tauri* (**bold**), *O. lucimarinus* (teal), *A. thaliana*. (green), *H. sapiens* (blue), *S. cerevisiae* (magenta), *M. musculus* (red), *D. melanogaster* (cyan), slime mould (orange), and two Archaea protein sequences (brown). KAE1B is a fusion protein. The numbers attached to edges/branches are bootstrap values.

### C) RIO

The atypical Right Open reading frame (RIO) family of kinases were named after RIO1, which in yeast regulates checkpoints at two stages of the cell-cycle (Angermayr *et al.*, 2002). Both RIO1 and RIO2 are involved in ribosome biogenesis in yeast, and probably perform distinct roles as knock-out of either is lethal (LaRonde-LeBlanc & Wlodawer, 2005). Both RIO1 (Ot14g00390) and RIO2 (Ot06g01790) are present in *O. tauri*, supporting functional divergence of these two kinases.

## Supplemental Information S6



**A phylogeny of the RIO protein kinases.** Kinases are included from *O. tauri* (**bold**), *H. sapiens* (blue), *S. cerevisiae* (magenta), and *A. thaliana*. (green). The numbers attached to edges/branches are bootstrap values.

### D) STN

The State Transition (STN) family of kinases in *O. tauri* are related to STN7 and STN8 in *A. thaliana*. STN7 is required in higher-plants and algae for adaptation of the photosystem to different qualities of light (Bellafiore *et al.*, 2005; Depège *et al.*, 2003; Tikkanen *et al.*, 2010; Tikkanen & Aro, 2012). STN7 phosphorylates light harvesting proteins (LHC2), and STN8 phosphorylates core photosystem 2 (PS2) proteins (Bonardi *et al.*, 2005). The expanded STN family of proteins in *O. tauri* may be a function of the aquatic environment (Six *et al.*, 2009), and could provide an interesting insight into kinase control of photoadaptation.

### E) SCYL

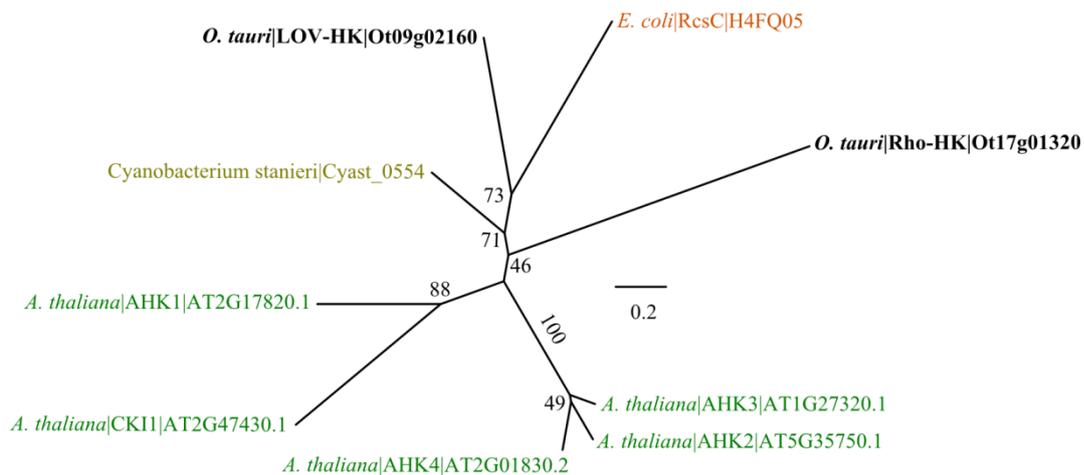
Protein kinase-like SCY1-like (SCYL) proteins are highly conserved throughout eukaryotes, and may be pseudo-kinases (Scheeff *et al.*, 2009). Although their function is poorly understood, the high degree of conservation across eukaryotes and presence in the *O. tauri* minimal kinome potentially reflects essential conserved cellular roles that remain to be identified.

### F) ABC1

The ABC1 kinase family in algae is considerably larger than in other eukaryotes. Across the green lineage, the few ABC1 kinases that have been characterised are thought to be involved in stress responses (Yang, 2012), regulation of photosynthesis (Yang *et al.*, 2012b), and light sensing (Boyd *et al.*, 2011). We speculate that they may fulfil similar but expanded signalling requirements in algae.

## G) Histidine Kinases

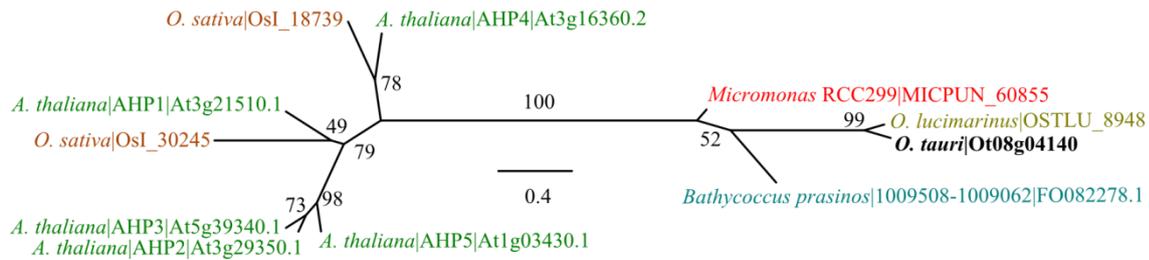
Histidine Kinases (HKs) are an ancient family of proteins, pervasive across the tree of life, which typically mediate extracellular signalling through multi-component pathways, characterised by phosphorylation of a histidine residue. In *O. tauri* the HK sequences of components of a light-signalling pathway sit at structural midpoints in the phylogeny between cyanobacteria and land plants. The relative scarcity of components of the HK-mediated pathway in *O. tauri* makes it an attractive target for study. The *O. tauri* kinome contains two membrane-bound HK light receptors, LOV-HK and Rho-HK, and a single histidine-containing phosphotransfer (Hpt) factor. Hpts are typically cytoplasmic messengers downstream of membrane-bound HKs. LOV-HK has already been shown to mediate light input into the circadian clock in *O. tauri* (Djouani-Tahri *et al.*, 2011). LOV-HK contains a LOV receptor domain, which is responsive to red light. Rho-HK contains a blue light responsive bacterial-like rhodopsin domain. A phylogeny of the HK domain of LOV-HK and Rho-HK demonstrates a close relationship to cytokinin-responsive HKs in *A. thaliana*. Both LOV-HK and Rho-HK contain C-terminal intracellular response domains, which typically interact with Hpt and facilitate the phosphorylation of a histidine in Hpt.



**Supplemental Figure 1 A phylogeny of the HK protein kinases.** Kinases are included from *O. tauri* (bold), and *A. thaliana*. (green), *Escherichia coli* (orange), and *Cyanobacterium stanieri* (yellow). The numbers attached to edges/branches are bootstrap values.

Histidine-containing phosphotransfer (Hpt) factors are related to histidine kinases, but contain large deletions within the kinase domain. *A. thaliana* contains six Hpt proteins, which are phosphorylated by cytokinin-responsive HKs. In *O. tauri*, there is only a single Hpt. This leads to the hypothesis, recently proposed by Pfeuty (2012), that both *O. tauri* light receptors share a single Hpt component. The high conservation of the HK domains within the HK receptor and Hpt phylogenies of *O. tauri* and the HK domains of cytokinin signalling in *A. thaliana*, likely reflect that *O. tauri* components are prototypical for this signalling pathway.

## Supplemental Information S6



**Supplemental Figure 2 A phylogeny of the histidine-containing phosphotransfer (HPT) protein.** Proteins are included from *O. tauri* (**bold**), *O. lucimarinus* (*teal*), and *A. thaliana*. (*green*), *O. sativa* (*orange*), *Micromonas RCC299* (*red*), and *Bathycoccus prasinos* (*blue*). The *Bathycoccus* identifier refers to the genomic coordinates on the referenced scaffold, as there was no published gene model for this protein. The numbers attached to edges/branches are bootstrap values.

In *A. thaliana* 18 response regulators (ARR) translate Hpt activation into transcriptional changes. In *O. tauri*, there is only one candidate ARR protein (Ot16g01320), which contains a response regulator and Myb transcription factor domain). An APRR (TOC1) is also present in *O. tauri* and forms a central component of the transcriptional circadian clock circuitry (Troein *et al.*, 2011; Corellou *et al.*, 2009). TOC1 is therefore an obvious candidate for an input of light into the transcriptional clock, mediated by histidine kinase signalling. Similar mechanistically to HKs: Pyruvate Dehydrogenase Kinase (PDK) phosphorylates and deactivates pyruvate dehydrogenase across eukaryotes (Thelen *et al.*, 2000; Korotchkina & Patel, 2001), and works reversibly with pyruvate dehydrogenase phosphatase (PDPC) (Holness & Sugden, 2003), also known as Protein Phosphatase 2C (PP2C), to regulate glucose homeostasis. Interestingly, while PDK phosphorylates S/T residues, mechanistically it works very similar to Histidine Kinases (Mooney *et al.*, 2000).

## Other Kinases

In addition to CMGC kinases, the protein kinase families BUB1, Haspin, and Polo-like kinases (PLK) are also involved in cell-cycle control. The importance of protein kinases to regulate the cell cycle is demonstrated in the quantity and diversity of families of cell cycle protein kinases in *O. tauri*. Significant progress has already been made on the study of components and properties of the *O. tauri* cell cycle (Farinas *et al.*, 2006; Corellou *et al.*, 2005; Robbins *et al.*, 2005).

### H) BUB

The BUB1-like protein kinase (Ot12g00570) is named after the Budding Uninhibited by Benzimidazoles 1 (BUB1) protein kinase in yeast, and has been identified across the green lineage (Karpov *et al.*, 2010). BUB1 is involved in the spindle assembly checkpoint (SAC) and DNA damage response (DDR) (Yang *et al.*, 2012a). Two phosphatidylinositol 3' kinase-related kinases (PIKK) kinases are also associated with the mitotic checkpoint control and DDR (Abraham, 2001). BUB1 has been suggested to be an ATM and ATR target (Matsuoka *et al.*, 2007).

## I) Haspin

Haspin is another *O. tauri* kinase that is associated with spindle formation during mitosis (Dai *et al.*, 2005). It is conserved in many eukaryotes, including plants (At1g09450), human, yeast (ALK1), and *O. tauri* (Ot05g03870).

## J) PLK

Polo-like kinases (PLK) are involved at several stages throughout the cell cycle (Glover *et al.*, 1998). *O. tauri*, like most eukaryotes, only contains one copy of PLK (Ot04g00800). PLK is activated by Aurora (AUR) kinase, which thereby controls entry into mitosis (Seki *et al.*, 2008). *O. tauri* contains a single ortholog of AUR (Ot10g01060), and an AUR-like protein kinase (CALK) (Ot13g01960). The latter is conserved throughout algae, and has been shown to regulate flagella length in *Chlamydomonas* (Luo *et al.*, 2011; Pan *et al.*, 2004). Mammalian AurA conducts a similar role in cilium disassembly (Pugacheva *et al.*, 2007). The presence of CALK in algae devoid of flagella, like *O. tauri*, indicates an additional role for CALK.

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