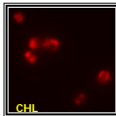
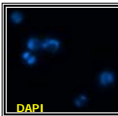
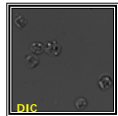
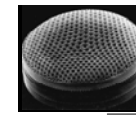
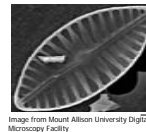


# Genetics of Programmed Cell Death in the Model Diatom *Thalassiosira pseudonana*

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## Objectives

- Use microarray and qRT-PCR analysis to relate gene expression to programmed cell death markers
- Elucidate the potential role of two novel death specific genes (DSP), TpDSP1 and TpDSP2 in aging and iron starved diatom cultures

## Abstract

Understanding the evolutionary lineage of autocatalyzed programmed cell death (PCD) could clear our understanding of the essential factors and executioners that proved important throughout time. PCD has been investigated in metazoans, but remains a mystery in many unicellular species. PCD was recently shown in diatoms under Fe stress with a putative role for metacaspases, caspase orthologues. In our study, aging and iron starved cultures of the model diatom, *Thalassiosira pseudonana*, were analyzed for the expression of genes specifically involved in PCD. Specifically, changes in expression of six metacaspase genes and two death specific protein genes was quantified in aging and iron starved conditions. Physiological characteristics were monitored through cell abundance and photosynthetic health as measured by Fv/Fm; caspase activity was measured by the levels of hydrolysis of the fluorogenic caspase substrate, IETD-AFC. The presence of active metacaspases was observed using Western blot analysis. Caspase activity was revealed by IETD-AFC cleavage and was taken as an indication of PCD. Cell samples were harvested for microarray analysis and quantitative PCR (qRT-PCR). Microarray data revealed 4.2 and 25.5 fold increases in the expression of two proteins (designated TpDSP1 and TpDSP2, respectively) in iron starved cells compared to the replete control. These proteins have closest homology to a death specific protein another diatom, *Skeletonema costatum*. Subsequent analyses with qRT-PCR revealed a 2.6 fold increase and 1.4 fold decrease in gene expression of TpDSP1 and TpDSP2, respectively, between stationary and exponentially growing cells. Our results suggest that these putative death specific proteins may play a key role in triggering PCD under Fe stress.

## Introduction

Phytoplankton, a microscopic class of organisms, accounts for less than 1% of the Earth's biomass, but contributes almost 50% of the world's carbon-based primary production.<sup>1</sup> Recent data suggests that a certain organized mode of self-destruction, known as programmed cell death (PCD) is involved in governing the life cycles of phytoplankton.<sup>2</sup> Research revealed a correlation between the expression of a so called death-specific gene, ScDSP, and cell death in a marine diatom, *Skeletonema costatum*.<sup>3</sup> In that study, ScDSP mRNA levels in cells in the stationary phase were upregulated compared to exponentially growing cells.<sup>3</sup> Caspases (cysteine-containing aspartate-specific proteases), enzymes that initiate the pathway to autocatalytic death by cleaving essential proteins, are linked to essential PCD machinery in metazoans. Metacaspases, caspase orthologues, are suspected to have a role in PCD in plants, fungi and protozoa. In a study<sup>2</sup> on the model diatom, *T. pseudonana*, six metacaspases (TpMCs) were identified whose distinct patterns of expression revealed a potential role in PCD. The study also indicated that *T. pseudonana* possesses a homologous protein (Protein ID 11118; BLASTp E value of 1 10 55) to *S. costatum* ScDSP (GenBank accession no. AAY27742) with conserved EF-hand calcium binding motifs (cd00051 and COG5126)<sup>2</sup>. It is hypothesized that this protein serves to couple stress signals to the PCD execution machinery. However, we have very little understanding of the molecular machinery that is involved in regulation of PCD. The main goal of the microarray study is to expand our knowledge of potential genes involved when PCD markers (like metacaspases, caspase activity) are triggered. Microarrays allow us to examine global gene expression under a specific condition and identify interesting candidate genes. We aim to analyze expression patterns of TpMCs and two homologues to ScDSP, TpDSP1 and TpDSP2, under aging and iron starved conditions to elucidate their potential roles in PCD.

## References

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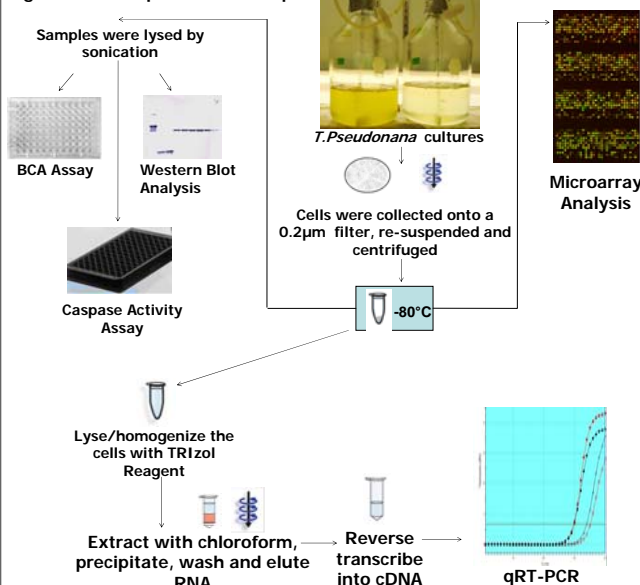
## Acknowledgements

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## Materials and Methods

Figure 1. The experimental setup

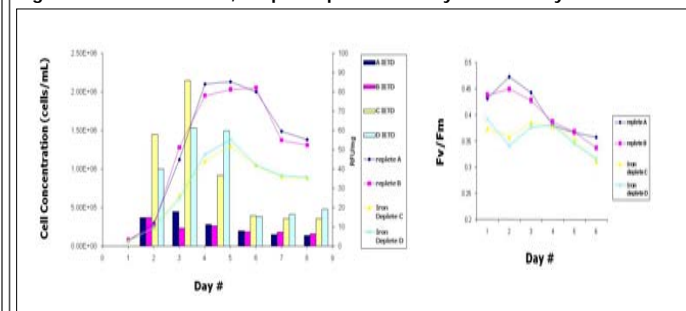


## Results

Figure 2. Analysis of Death Specific Genes in *T. pseudonana*

Protein ID <sup>1</sup>	Abbreviation (this study)	Best BLAST hit <sup>2</sup> (accession no.); E value	Conserved Domain (E value)	Stationary-Iron vs. Stationary Replete		Stationary vs. Exponential Replete
				Fold Change in Gene Expression	p value	Fold Change in Gene Expression
11117	TpDSP1	Death specific protein, <i>Skeletonema costatum</i> (AAY27742); 1e-44	pfam00051, EFh, calcium binding motif (2e-08)	4.28 up	0.015938	2.62 up
11118	TpDSP2	Death specific protein, <i>Skeletonema costatum</i> (AAY27742); 3E-54	pfam00051, EFh, calcium binding motif (5e-11)	25.51 up	0.002245	1.62 down

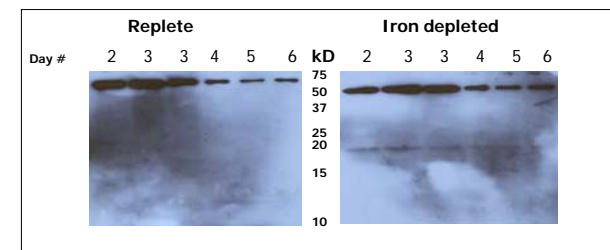
Figure 3. Cell Abundance, Caspase Specific Activity and Photosynthetic Health



Caspase specific activity (shown as bars) was determined through the measurement of IETD-AFC substrate cleavage normalized to protein concentration. Photosynthetic health is indicated by Fv/Fm, which is a measure of the photosynthetic efficiency of photosystem II. We observed:

- Caspase activity was highest just prior to the stationary growth phase
- Caspase activity was notably higher for iron starved vs. replete cultures
- Iron starved cultures had low Fv/Fm indicative of photosynthetic stress

Figure 4. Western Blot Analysis



Membranes were first probed with a polyclonal antibody raised against a metacaspase from *Emiliania huxleyi* (EhMC; titer 1:500). A secondary antibody, anti-rabbit IgG HRP; titer 1:10,000 was used for detection. We observed:

- immunohybridization to a ~52 kD protein (indicative of TpMC3) was constitutively expressed in both treatments
- Immunohybridization to a ~18 kD protein (indicative of TpMC6) was observed only in iron starved samples and correlated with elevated caspase specific activity

## Conclusions

• Our data suggests the involvement of *T. pseudonana*'s death specific genes in PCD. They may play a role in PCD regulation given their conserved EF-hand, calcium binding domains

• Further characterization of the function and regulation of these genes is necessary

<sup>1</sup> <http://genome.jgi-psf.org/Thaps3.home.html>  
<sup>2</sup> <http://www.ncbi.nlm.nih.gov/>