

# Gene-Ontology-based analysis of gene expression changes in early development of *Ceratopteris* spores

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## Abstract

Following exposure to light, single-celled spores of the aquatic fern *Ceratopteris richardii* undergo a series of rapid developmental changes similar to germination in higher plants. To investigate this process, Salmi, et al. tracked gene expression changes over 48 hours post-light exposure using a microarray printed with partially-sequenced *Ceratopteris* clones [1]. Here, we extend this work by analyzing over-representation of Gene Ontology terms among differentially up-regulated genes from each time point.

## 1. Introduction

Early development of the single-celled spores of the aquatic fern *Ceratopteris richardii* serves as an experimental model system for studying germination in plants, in which previously dormant cells, such as in seeds or pollen, undergo rapid developmental changes in response to an activating signal. For *Ceratopteris* spores, this signal is exposure to light, which triggers a series of well-characterized events, including: a polar calcium current at 6 hours, nuclear migration at 24 hours, a polar cell division at 48 hours, and rhizoid (root analog) emergence at 72 hours in a position determined by the prior nuclear migration.

To investigate this process in germinating *Ceratopteris* spores, Salmi and coworkers tracked gene expression changes over 48 hours post-light exposure using a custom microarray printed with partially-sequenced clones from a *Ceratopteris* spore cDNA library [1]. They interrogated several time points (0 h, 6 h, 12 h, 24 h, and 48 h post-germination) and identified *Ceratopteris* genes that showed evidence for differential gene up-regulation relative to at least one other time point. Using functional annotations associated with most closely-related *Arabidopsis* homologs (best hits in a blastx search), they proposed theories explaining the role of individual genes in

germination. Here, we extend this work by looking at the behavior of many genes at once, using Gene Ontology classifications transferred from *Arabidopsis*. Specifically, we attempt here to create a broad strokes picture of *Ceratopteris* development by analyzing over-representation of Gene Ontology terms among differentially up-regulated genes from each time point.

## 2. Methods

Over-representation analysis was performed using version 2.04 of the ErmineJ software [2], which uses the binomial approximation to the hypergeometric distribution to assess significance and compute p-values. In this study, terms with p-values equal to or less than 0.0001 were counted as significantly over-represented. ErmineJ requires a microarray annotations file that relates array identifiers (Genbank ids) to Gene Ontology codes. To create this file, we performed a provisional GO annotation of the *Ceratopteris* cDNAs using results from a prior blast analysis in which the *Ceratopteris* sequences were searched against an *Arabidopsis* protein sequence database [1,3]. GO terms associated with the putative *Arabidopsis* homologs were transferred to the *Ceratopteris* clones. GO annotations were obtained from the Gene Ontology Web site in March, 2005 and included annotations from TAIR and TIGR [4]. The microarray annotation and other relevant files are available at [http://www.transvar.org/roux\\_collab](http://www.transvar.org/roux_collab).

## 3. Results

We find that at 0 hours, terms related to seed storage (GO:0045735, nutrient reservoir activity), metal transport (GO:0005506, iron binding protein), and disease resistance (GO:0006915, apoptosis) are over-represented among the up-regulated clones. The term “rhodopsin-like receptor activity” (GO:0001584) is also over-represented. The clones annotated with this

term appear to encode homologs of signal transduction molecules that might act downstream of a rhodopsin-like GPCR. Taken together, these GO terms create a picture of an organism well-stocked with mRNAs encoding nutrient storage proteins, transporters for the import of metal ions, and signal transduction proteins.

At 6 hours following the light signal, one term: GO:0005744, "mitochondrial inner membrane presequence translocase enzyme," was over-represented. At 12 hours, no categories were over-represented at p-values below 0.0001; the most significant categories included genes encoding putative peroxidases (GO:0016209, antioxidant activity, p-value 0.000321), proteases (GO:0006510, ATP-dependent proteolysis, p-value 0.000957), and enzymes involved in starch and sugar mobilization (GO:0005975, carbohydrate metabolism, p-value 0.00099.)

At 24 hours, 13 GO categories were over-represented. These categories included genes involved in lipid-related functions, proteolysis, and cellular differentiation. Taken together, these terms suggest that by 24 hours after germination, the spore is engaged in the breakdown and recycling of proteins as well as lipid biosynthesis.

At 48 hours, 7 categories were over-represented at p-values below 0.0001. These included GO terms related to protein synthesis, response to hormones and external signals, and glyoxylate metabolism. The next most significant terms (p-value < 0.001) related to cell wall biosynthesis and carbohydrate metabolism.

#### 4. Conclusions and Discussion

Using Gene Ontology annotations transferred from *Arabidopsis*, we identified functional categories that are over-represented among the differentially-expressed genes at five time points during *Ceratopteris* spore germination and therefore are most likely to be relevant to underlying biological processes. The relevant terms include: terms for seed storage proteins, signal transduction, and metal-binding at 0 hours; terms for mitochondrial functions at 6 and 12 hours; fatty acid biosynthesis, lipid transport, and protein degradation at 24 hours; and terms for protein synthesis, glyoxylate metabolism, fatty acid oxidation, cell wall biosynthesis, and response to hormones at 48 hours. Taken together, these results evoke a developmental time course in which the newly-activated gametophyte establishes signal transduction pathways (0-6 hours), activates mitochondrial

functions (6 and 12 hours), breaks down proteins and synthesizes fatty acids (24 hours), and then undergo a burst of protein and cell wall biosynthesis (48 hours) that apparently coincides with setting up a new sensitivity to hormone-related signal transduction pathways.

**Table 1. GO categories over-represented at 24 hours following germination**

<b>Lipid-related</b>
GO:0004315, 3-oxoacyl-[acyl-carrier protein] synthase activity
GO:0000038, very-long-chain-fatty acid metabolism
GO:0004312, fatty-acid synthase activity
GO:000828, lipid binding
GO:0006869, lipid transport
GO:0044255, cellular lipid metabolism
GO:0006631, fatty acid metabolism
<b>Proteolysis</b>
GO:0004175, endopeptidase activity
GO:0004194, pepsin A activity
<b>Misc</b>
GO:0031225, anchored to membrane
GO:0044274, organismal biosynthesis
GO:0016747, transferase activity, transferring groups other than amino-acyl groups
GO:0030154, cell differentiation

**Table 2. GO categories over-represented at 48 hours following germination**

<b>Protein synthesis</b>
GO:0015935, small ribosomal subunit
GO:0005830, cytosolic ribosome (sensu Eukaryota)
GO:00162283, eukaryotic 48S initiation complex
GO:00162282, eukaryotic 43S preinitiation complex
<b>Signal transduction</b>
GO:0009733, response to auxin stimulus
GO:0009605, response to external stimulus
<b>Misc</b>
GO:0006097, glyoxylate cycle

#### References

- [1] M.L. Salmi, T.J. Bushart, S.C. Stout, and S.J. Roux, "Profile and analysis of gene expression changes during early development in germinating spores of *Ceratopteris richardii*, in press.
- [2] <http://microarray.genomecenter.columbia.edu/ermineJ/>
- [3] [http://www.sbs.utexas.edu/roux/Ceratopteris%20Page/ceratopteris\\_research.htm](http://www.sbs.utexas.edu/roux/Ceratopteris%20Page/ceratopteris_research.htm)
- [4] <http://www.geneontology.org>