

Analyses of the E3 ubiquitin-ligase target adapter-encoding *BTB* gene families in algal species

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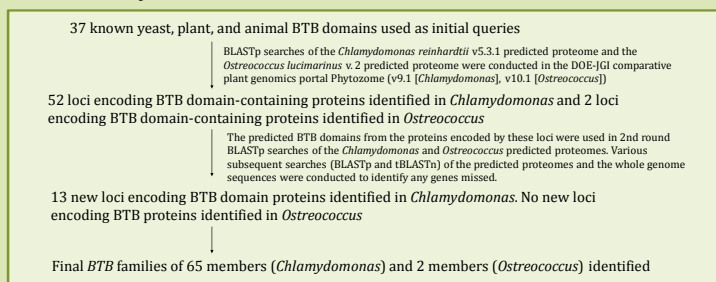
Introduction

Ubiquitylation, the attachment of ubiquitin to proteins to mark for degradation by proteasomes, is crucial for proper organism function. One family of complexes that play a role in this process is the BTB/Cullin 3/RBX E3 ubiquitin-protein ligases. Three proteins; a BTB (Bric-a-Brac, Tramtrack, Broad Complex) domain-containing protein, CUL3, and RBX, form a complex to catalyze attachment of ubiquitin to target proteins (Fig. 1). The BTB proteins are the target-adapters, binding to the proteins to be ubiquitylated via motifs appended to the BTB domain¹.

Genes encoding BTB proteins have been identified in a wide range of eukaryotic organisms (including fungi, protists, animals, and plants) but the *BTB* gene families in different groups show great variability in size, complexity, and composition². In land plant genomes thus far studied, *BTB* gene families are large (~75-150 members) and complicated (with multiple subtypes based on the presence of a diverse set of encoded target-binding motifs)². We are interested in when the particular *BTB* family composition seen in the higher plants may have arisen in evolution.

To help answer this question, we have characterized the *BTB* gene families in the fully-sequenced genomes of the green algal species *Ostreococcus lucimarinus* and *Chlamydomonas reinhardtii*. Aquatic green algae are thought to be the ancestors of the land plants, so comparison of algal and land plant *BTB* families may give us clues as to how the *BTB* family evolved as the transition to land occurred.

Identification of the *BTB* Gene Families in Genomes of *Chlamydomonas reinhardtii* and *Ostreococcus lucimarinus*



Phylogenetic Analysis of the *BTB* Families of *Chlamydomonas reinhardtii* and *Ostreococcus lucimarinus*

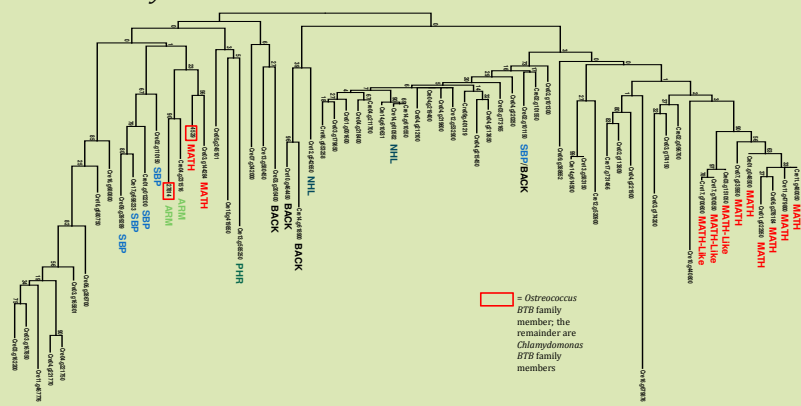


Figure 2. Phylogenetic tree of BTB family members from *Chlamydomonas reinhardtii* and *Ostreococcus lucimarinus*. The tree was generated in MEGA 6.06 by maximum-likelihood analysis of a Gblocks-edited alignment of the amino acid sequences of BTB domains from the family members. The tree with the highest log likelihood is shown. Bootstrapping with 500 replicates was performed; the numbers at each node indicate bootstrap values. Bootstrap values >75% indicate moderate to strong support for that node. Branch lengths are scaled to number of substitutions per site. Other domains or motifs identified by SMART and/or Pfam in the predicted proteins are indicated for some family members.

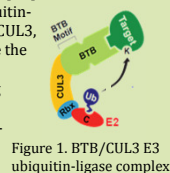


Figure 1. BTB/CUL3 E3 ubiquitin-ligase complex

Sequence Alignments of *Ostreococcus lucimarinus* and Representative *Chlamydomonas reinhardtii* BTB Domains

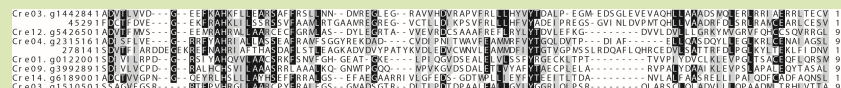


Figure 3. Alignment of representative BTB domains from *Chlamydomonas reinhardtii* and *Ostreococcus lucimarinus*. Predicted BTB domains from 7 *Chlamydomonas* BTB proteins and the 2 *Ostreococcus* BTB proteins (4529 and 27814) were aligned with CLUSTALW2 using the default settings. The alignment was displayed with BOXSHADE 3.21 using a 55% sequence identity threshold. Conserved and similar amino acids are shown in black and gray boxes, respectively. Dashes denote gaps.

Architecture of the Two BTB Proteins in *Ostreococcus lucimarinus*

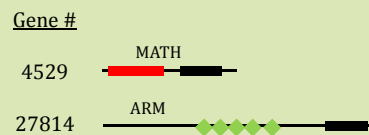


Figure 4. Only two *BTB* genes were identified in *Ostreococcus*. One is predicted to encode a protein with a C-terminal MATH domain and the second is predicted to encode a protein with Armadillo (ARM) repeats. MATH-BTB and ARM-BTB proteins are also seen in *Chlamydomonas* and land plants. MATH-BTB proteins are also found in yeasts and in animals and are one of a very few BTB types shared generally among the eukaryotes.

BTB Gene Family Size and Genome Size in Various Algal and Plant Species

Species	Number of BTB Family Members	Genome Size
<i>Oryza sativa</i> (rice)	149 ⁽²⁾	372Mb 12 chromosomes
<i>Arabidopsis thaliana</i>	81 ^(1,2,3)	135Mb 5 chromosomes
<i>Physcomitrella patens</i>	65 ^(unpublished data)	473Mb 27 chromosomes
<i>Chlamydomonas reinhardtii</i>	65	111Mb 17 chromosomes
<i>Ostreococcus lucimarinus</i>	2	13.2Mb 21 chromosomes

Conclusions

- The *BTB* family in *Chlamydomonas reinhardtii* is large (65 members) and complex, but the family in *Ostreococcus lucimarinus* is very small (2 members).
- The two *Ostreococcus* *BTB* genes appear to each have a homolog in *Chlamydomonas* (27814 and Cre04.g231516; 4529 and Cre03.g144284).
- While the architectures found in the two *Ostreococcus* *BTB* proteins are also found in *BTB* proteins from land plants and *Chlamydomonas* (MATH-BTB and ARM-BTB), most of the architectures found in *Chlamydomonas* (e.g. BTB-SBP, NHL-BTB) are not seen in land plants and vice-versa.
- There is significant sequence diversity within the collection of *Chlamydomonas* *BTB* domains. This is similar to the *BTB* domains found in animals and plants, which are quite variable mostly yet share a common function (Cullin 3 protein interaction).
- Increasing genome size is generally associated with increasing *BTB* family size.
- These results suggest there have been drastic changes in the size and composition of this E3 ubiquitin-ligase gene family during Viridiplantae evolution.

References

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