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

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Introduction
Ubiquitination, 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/CCIN3/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 domain.

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 composition. 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). 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 families may give 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
37 known yeast, plant, and animal BTB domains used as initial queries
52 loci encoding BTB-domain-containing proteins identified in Chlamydomonas and 2 loci encoding BTB-domain-containing proteins identified in Ostreococcus
13 new loci encoding BTB-domain proteins identified in Chlamydomonas. No new loci encoding BTB proteins identified in Ostreococcus
Final BTB families of 65 members (Chlamydomonas) and 2 members (Ostreococcus) identified

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.

Architecture of the Two BTB Protein Families in Ostreococcus lucimarinus

<table>
<thead>
<tr>
<th>Gene #</th>
<th>MATH</th>
<th>ARM</th>
</tr>
</thead>
<tbody>
<tr>
<td>4529</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27814</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of BTB Family Members</th>
<th>Genome Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oryza sativa (rice)</td>
<td>149</td>
<td>372Mb</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>81</td>
<td>135Mb</td>
</tr>
<tr>
<td>Physcomitrella patens</td>
<td>65 (unpublished data)</td>
<td>473Mb</td>
</tr>
<tr>
<td>Chlamydomonas reinhardtii</td>
<td>65</td>
<td>27 chromosomes</td>
</tr>
<tr>
<td>Ostreococcus lucimarinus</td>
<td>2</td>
<td>13.2Mb</td>
</tr>
</tbody>
</table>

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.

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.g21170) and there is evidence that 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.

• 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-SPB, NBL-BTB) are not seen in land plants and vice-versa.

• There is significant sequence diversity within the collection of Ostreococcus BTB domains. This is similar to the BTB domains found in animals and plants, which are quite variable mostly 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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