

Analyses of E3 ubiquitin-ligase target adapter-encoding *BTB* gene families in Viridiplantae



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Introduction

Ubiquitylation, the covalent attachment of the small protein ubiquitin (Ub), can modify the degradation, localization, or activity of target proteins and is crucial for proper organism function. This attachment is achieved by the sequential action of E1 (Ub-activating), E2 (Ub-conjugating), and E3 (Ub-ligase) enzymes. The E3 complexes bind the target protein and facilitate attachment of the Ub moiety. There are multiple families of E3's encoded in eukaryotic genomes; one of these are the CRL3 type. CRL3 E3 complexes consist of three proteins; a BTB (Bric-a-Brac, Tramtrack, Broad Complex) domain-containing protein, Cullin 3, and RBX1 (Fig. 1). The BTB proteins are the target-adapters, binding to the proteins to be ubiquitylated via motifs appended to the ~100 amino-acid 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 composition^{2,3}. 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 target-binding motifs)². We are interested in when the particular CRL3 family composition seen in the higher plants (as defined by the BTB target-adaptor repertoire available) may have arisen in evolution.

To help answer this question we searched the publically-available whole genome sequences of a variety of land plant and green algal species (Table 1), looking for all of the genes within each genome that encode proteins that contain BTB domains. Land plants evolved from green algal ancestors, so comparing the BTB families in these species should provide clues as to how and when the land plant BTB family composition arose. Previous work in the Gingerich lab had suggested that the BTB families of some green alga (within a group known as the chlorophytes) are very different than the BTB families seen in the land plants. However, it is another group of green algae (the charophytes) that are more closely related to the land plants. Until recently, no charophyte sequences have been publically available. However, the first whole-genome sequence of a charophyte (*Klebsormidium flaccidum*) was recently released. This now allows us to compare the BTB families from chlorophytes, charophytes, and land plants, which should give a more detail view as to when certain land-plant BTB types arose in evolution.

Procedure

Representative species chosen for this study are show in Table 1. BLASTP (BLASTP 2.2.26+) searches of predicted proteomes were done with an 0.1 E-value cut-off value, using a list of known plant and algal BTB domains as queries. The target predicted proteomes were provided by Phytozome 11.0 (<https://phytozome.jgi.doe.gov/pz/portal.html>), with the exception of the *Klebsormidium flaccidum* predicted proteome, which was retrieved from the *Klebsormidium* genome portal website (http://www.plantmorphogenesis.bio.titech.ac.jp/~algae_genome_project/klebsormidium/). The full-length predicted protein sequences from these searches were then submitted to the Simple Modular Architecture Research Tool (SMART v.7) (<http://smart.embl-heidelberg.de/>) to identify the BTB domains and to determine if any other domains were present. Due to the high volume of information needing to be collected from the SMART website to create this database, a web crawler was designed to gather and format the predicted BTB domain sequences along with the search E-value and other possible domains. (<http://smart.embl-heidelberg.de/>). Predicted BTB domains from all of the proteins were aligned using MAFFT version 7 (<http://mafft.cbrc.jp/alignment/software/>). Ambiguous regions of the alignment were removed using Gblocks version 0.91b (<http://molevol.cmima.csic.es/castresana/Gblocks.html>). This modified alignment was then used to generate a Neighbor-Joining phylogenetic tree in MEGA 7 (<http://www.megasoftware.net/>) using the following parameters: evolutionary distance between sequences was calculated using the p-distance method, pairwise deletion of gaps, and bootstrapping with 1000 replications was performed to determine support for the nodes. The tree after cosmetic editing is provided in Figure 2 .

Species	Representing
<i>Arabidopsis thaliana</i>	Land Plant Eudicots
<i>Chlamydomonas reinhardtii</i>	Green Algae Chlorophytes
<i>Coccomyxa subellipsoidea</i>	Green Algae Chlorophytes
<i>Klebsormidium flaccidum</i>	Green Algae Charophytes
<i>Micromonas pusilla</i>	Green Algae Chlorophytes
<i>Oryza sativa</i>	Land Plant Monocots
<i>Ostereococcus lucimarinus</i>	Green Algae Chlorophytes
<i>Physcomitrella patens</i>	Land Plant Moss
<i>Selaginella moellendorffii</i>	Land Plant Lycophytes
<i>Volvox carteri</i>	Green Algae Chlorophytes

Table 1. Land plant and algal species analyzed in this study.

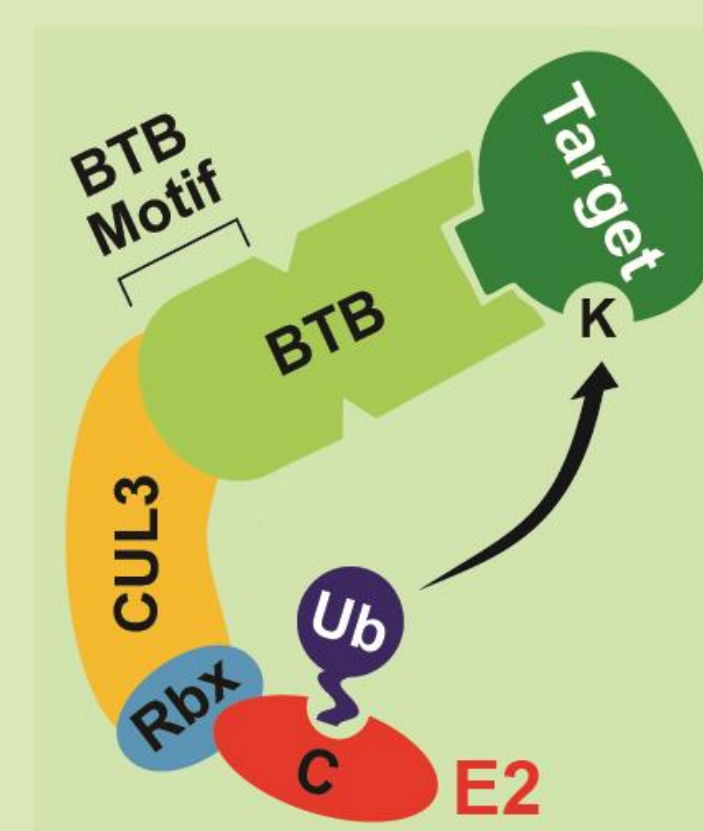


Figure 1. CRL3 BTB/CUL3 E3 ubiquitin-ligase complex structure

Conclusions

- The number of genes encoding BTB proteins varied greatly between the genomes studied, ranging from only 2 in *Ostereococcus lucimarinus* up to 146 in rice (*Oryza sativa*). The size and complexity of the charophyte *Klebsormidium* BTB family (86 members) is similar to that seen in the land plants.

- The land plant and chlorophyte algae BTB families are very different in composition from each other, with only a few BTB types in common.

- The MATH-BTB configuration is seen in all chlorophytes, charophytes, and land plants studied. It is also seen in animals and yeast, suggesting this is an ancient BTB type. There has been a very large expansion of genes encoding this type in the monocots, including *Oryza sativa* (rice).

- The BTB family of the charophyte *Klebsormidium* is more similar to the BTB families of the land plants than the chlorophyte families are. Several BTB types (including BTB-NPH3, BTB-TPR, and BTB-pentapeptide), previously thought to only exist in land plants, were found to be encoded in the *Klebsormidium* genome. This suggests that BTB family composition seen in the land plants had started to develop prior to emergence of the land plants from their algal ancestors.

- The *Klebsormidium* genome has two genes encoding BTB-NPH3 proteins. In all of the land plant genomes this family has greatly expanded (dozens of members), suggesting a proliferation of BTB-NPH3 proteins may have correlated with land colonization.

- The Gingerich lab studies the LRB (Light-Response BTB) proteins, which regulate the levels of the phytochrome red light photoreceptors. Previously we had believed that this type was only present in land plants. However, this analysis has identified a likely homolog in *Klebsormidium*.

- Interestingly the *Klebsormidium* genome encodes BTB types not seen in either the chlorophyte algae or the land plants, suggesting that the BTB family was very dynamic in evolution. As the BTB proteins act as target adapters in E3 ubiquitin-ligases, this suggests different lineages altered their BTB family composition to regulate the levels of different groups of target proteins.

Future Work

- The whole genome sequences of many additional land plants are available. It is planned to incorporate these in our analysis. This will require development of more streamlined/automated analysis methods, as this will require working with thousands of sequences.

References

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