

# POGs/PlantRBP: a resource for comparative genomics in plants

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## ABSTRACT

**POGs/PlantRBP (<http://plantrbp.uoregon.edu/>) is a relational database that integrates data from rice, Arabidopsis, and maize by placing the complete Arabidopsis and rice proteomes and available maize sequences into 'putative orthologous groups' (POGs). Annotation efforts will focus on predicted RNA binding proteins (RBPs): i.e. those with known RNA binding domains or otherwise implicated in RNA function. POGs form the heart of the database, and were assigned using a mutual-best-hit-strategy after performing BLAST comparisons of the predicted Arabidopsis and rice proteomes. Each POG entry includes orthologs in Arabidopsis and rice, annotated with domain organization, gene models, phylogenetic trees, and multiple intracellular targeting predictions. A graphical display maps maize sequences on to their most similar rice gene model. The database can be queried using any combination of gene name, accession, domain, and predicted intracellular location, or using BLAST. Useful features of the database include the ability to search for proteins with both a specified domain content and intracellular location, the concurrent display of mutual best hits and phylogenetic trees which facilitates evaluation of POG assignments, the association of maize sequences with POGs, and the display of targeting predictions and domain organization for all POG members, which reveals consistency, or lack thereof, of those predictions.**

## INTRODUCTION

Comparative analysis of orthologous genes is a powerful method for elucidating gene structure, function and evolution. Identification of orthologs on a gene-by-gene basis can be labor-intensive, and accessing the data available for orthologous genes typically requires visits to multiple species-specific databases. Thus, there is a need for resources that predict orthologous groups and bring together information

about the orthologs in a manner that simplifies comparative analyses.

To facilitate cross-species comparisons among the major model plant species *Arabidopsis thaliana* (Arabidopsis), *Oryza sativa* (rice) and *Zea mays* (maize), we developed the database POGs/PlantRBP (<http://plantrbp.uoregon.edu/>). POGs/PlantRBP clusters proteins in the rice and Arabidopsis proteomes into putative orthologous groups (POGs) based on a mutual-best-hits strategy, with POG assignments subsequently evaluated by phylogenetic analysis. Each POG display page includes a graphical representation of the domain organization of POG members, the results of two targeting predictors for each of the nucleus, chloroplast, mitochondrion, and secretory system, and a phylogenetic tree from which users can navigate to related POGs. Maize genomic and cDNA sequences are associated with the POG containing the rice gene with which they are most similar. The design of the database is illustrated in Supplementary Figure 1.

A web interface allows searches that combine search terms for different feature types (e.g. predicted intracellular location and predicted domain content), and displays data in a form that aids comparisons among orthologs. Annotation efforts are focusing on 'RNA binding proteins' (RBPs), i.e. those proteins predicted to interact with RNA and/or influence RNA function. This protein class is more complex in plants than in metazoa (1–4) and includes several protein families that are largely specific to plants [e.g. the PPR (5) and CRM (6) families]. A prerequisite for a comprehensive understanding of the network of RNA–protein interactions in plants is a catalog of plant RBPs. POGs/PlantRBP provides a new tool for this purpose.

## DATABASE CONSTRUCTION

### POG assignment

Predicted rice and Arabidopsis proteins were assigned to POGs in the following manner. The proteins predicted by all gene models in both species were compared to one another using WU-Blast 2.0 (<http://blast.wustl.edu/>) (7). For each of the ~90 000 gene models, the top 20 blast hits with an *E*-value <1e–5 were re-aligned using NEEDLE, an implementation of the Needleman–Wunsch algorithm (8) distributed with the EMBOSS package (9). These global

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alignments were used to cluster the gene-models into POGs via the mutual-best-hits strategy summarized in Supplementary Figure 2. This strategy yields both one-to-one and many-to-one orthology relationships. It also yields clusters in which no gene is in more than one cluster. All gene-models for a particular locus were placed in the same POG when at least one of the gene models met the mutual-best-hits criterion.

A phylogenetic approach was used to complement the mutual-best-hit method and aid evaluation of POG assignments (see Supplementary Figure 3). The top blast hits for each protein in a POG that met an *E*-value cutoff of  $<1e-5$  (up to 20 such proteins) were examined, and those with  $>50\%$  coverage (either hit/query or query/hit) were designated 'closely related'. The 'closely related' protein sets for all members of a POG were combined, and this combined set of proteins was used as input to MUSCLE version 3.6 (10) to produce a multiple alignment and corresponding guide tree. Where the tree topology supports the POG assignments, the POG is marked as 'well-supported'. POGs containing only proteins that are not 'closely-related' to any other protein (*E*-value  $<1e-5$ ) in either species are also marked as 'well-supported'. Trees and multiple alignments were stored for later display.

### POG annotation

Each predicted protein was analyzed for predicted intracellular localization using Predotar (11), TargetP (12), NucPred (<http://www.sbc.su.se/~maccallr/nucpred/>), and PredictNLS (13). This set of algorithms provides two independent predictions for targeting to each of the chloroplast (TargetP and Predotar), mitochondrion (TargetP and Predotar), nucleus (PredictNLS and NucPred) and secretory system (TargetP and Predotar). Searches can be performed for proteins predicted by either one or both of the 'redundant' algorithms to localize to a specific compartment. Each predicted protein was analyzed for domain content using InterproScan (14) version 3.3 (<http://www.ebi.ac.uk/interpro/index.html>) with Pfam (15) version 19 and SuperFamily (16) version 1.69 models. POGs were annotated as 'putative RNA binding' based on a hand-curated list of Interpro domains related to RNA metabolism (Supplementary Table 1), and on entries in the Arabidopsis Splicing Related Genes (ASRG) Database (1) (<http://www.plantgdb.org/SRGD/ASRG/>). Available maize genomic and expressed sequence tag (EST) assemblies and ESTs from the maize full-length cDNA project (see Source Sequences below) were associated with their closest rice counterpart using BLASTn against rice genomic DNA with an *E*-value cutoff of  $<1e-10$ . BLASTn was chosen for this purpose because nucleotide similarity provides greater resolution than amino acid similarity for species as closely-related as maize and rice.

### Source Sequences

Sequences incorporated into PlantRBP were derived from the following sources. Arabidopsis sequences are from version 6 of the Arabidopsis genome annotation and were downloaded from TAIR (<ftp://ftp.arabidopsis.org/>). Rice sequences (version 4) were downloaded from TIGR (<ftp://ftp.tigr.org/>). Maize genomic assemblies (MAGI version 4) were downloaded from (<http://magi.plantgenomics.iastate.edu/>), maize EST assemblies from PlantGDB (<http://plantgdb.org/>) and

ESTs associated with the Arizona full-length cDNA project from (<http://www.maizecDNA.org/download>).

## USER INTERFACE

### Searches

A search page includes fields for searching by gene (gene name, gene description, or gene identifier), domain, and predicted intracellular location. Multiple search criteria entered in the same or different field are linked with 'and', serving to narrow the search. For example, entering 'RRM PPR' under domain, and 'chloroplast' under targeting prediction returns POGs containing proteins with both an RRM and PPR domain that are predicted by either Predotar or TargetP to be targeted to chloroplasts. Searches can be further limited to return proteins predicted by two different algorithms to be targeted to either the chloroplast, mitochondrion, nucleus, or secretory system. A search can be broadened by adding a wild card symbol (\*) to a truncated term. For example, typing 'At\*' in the Gene field will limit the search to Arabidopsis proteins. A BLAST (7) interface is also available. The BLAST results are parsed and displayed using Bioperl (17). Each entry within the BLAST results is linked to the POG with which the gene is associated.

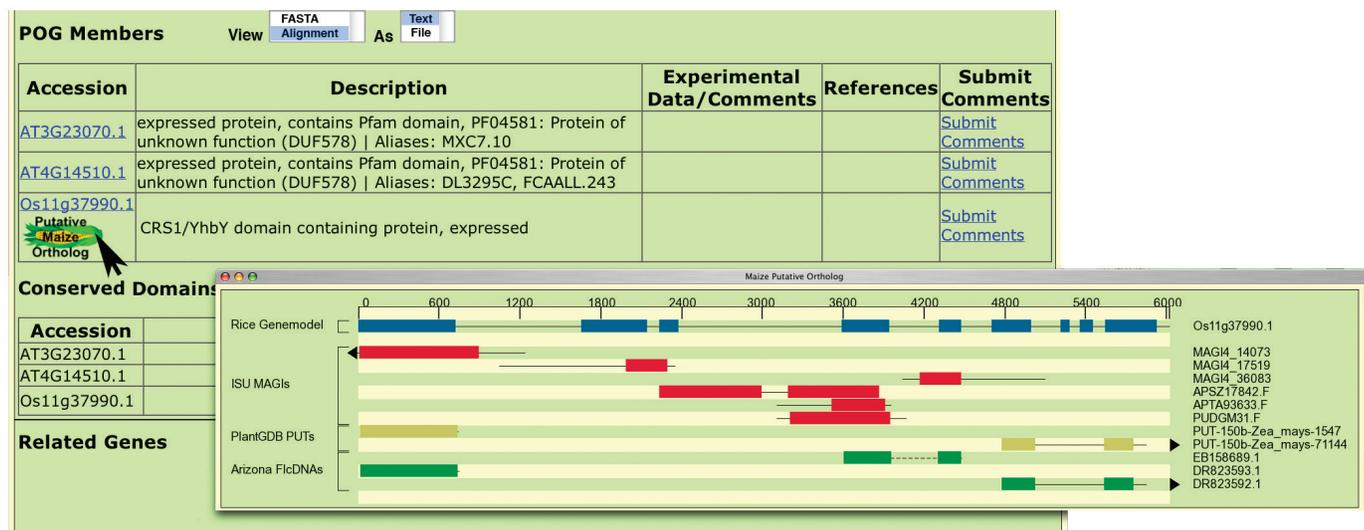
Search results are returned as a list of POGs that include any proteins that meet the search criteria. POGs in the list are annotated with domains shared by all POG members, protein name (where assigned), and an indication of whether the POG is 'well-supported' according to the criteria described above. Each POG on the list is linked to a more detailed 'POG View' annotation page.

### POG View

The POG View page (Figure 1) summarizes information about members of each POG in a format that facilitates comparative analyses. The page starts with a list of the component gene models linked to either TIGR (rice) or TAIR (Arabidopsis), and annotated with gene descriptions derived from TIGR (rice) or TAIR (Arabidopsis). Experimental data and literature citations for the RBP subset will be displayed on this page, after manual curation by POGs curators and with community input (see Future Work). The sequences and multiple alignments of POG members can be viewed or downloaded. The alignments are displayed using Mview (18). For each rice gene model, a pop-up window presents the putative maize ortholog(s) in a graphical alignment (Figure 2).

Below the table of gene models is a graphical representation of the conserved domains in each predicted protein. This display highlights inconsistent gene models and inconsistent domain predictions among orthologs. Each domain is linked to its Interpro page. A phylogenetic tree of rice and Arabidopsis proteins related to POG members (see criteria for 'related' proteins above) is displayed beneath the domain maps. Each gene model in the tree is linked to its respective POG, simplifying navigation to related POGs. The sequences, multiple alignment and domain maps of the related genes can be viewed or downloaded. Finally, a summary of the predictions from the four targeting prediction algorithms is shown in a table at the bottom of the page, in a manner that highlights the consistency, or lack thereof, of those predictions.





**Figure 2.** Pop-up window showing putative maize orthologs. Rice loci in each POG are linked to a graphical representation of putative maize orthologs. Maize genomic assemblies (MAGI's at <http://magi.plantgenomics.iastate.edu/>), EST assemblies (PlantGDB PUTs at <http://plantgdb.org/>), and ESTs from the maize full-length cDNA project ([www.maizecDNA.org](http://www.maizecDNA.org)) are displayed below the TIGR gene model for their best rice hit in BLASTn searches against TIGR's rice pseudochromosomes, version 4.

## CONCLUSIONS AND FUTURE WORK

The efficient exploitation of information gleaned with different model organisms, each with unique experimental attributes, requires the identification of orthologous gene sets, representations of data that facilitate comparisons among orthologs, and user-friendly interfaces that aid searches for orthologous groups with specified properties. Previously, two databases have been described that aim to achieve the first of these purposes for model plant species: OrthologID (19) (<http://nypg.bio.nyu.edu/orthologid/>) uses a rigorous phylogenetic method to predict orthologs in the fully-sequenced genomes of Arabidopsis, rice, and poplar, and displays phylogenetic data for each orthologous group; the Genome Cluster Database (GCD) (<http://bioinfo.ucr.edu/projects/GCD>) (20) clusters proteins in rice and Arabidopsis based on BLAST similarity and domain organization but does not aim to distinguish orthologs from paralogs. Genes in GCD are linked to protein alignments and expression profiling data. POGs/PlantRBP is distinct from these and other available resources in its use of both mutual-best-hit and phylogenetic analysis to assign orthologous groups, in the types of data that are displayed concurrently for orthologous proteins (domain organization, targeting predictions, literature citations, phylogenetic trees, links to putative maize orthologs), and in the types of searches that are easily accomplished (e.g. searches for orthologous groups containing proteins with both a specified domain content and predicted intracellular location). In addition, this database has a unique emphasis on the annotation of 'RNA binding' proteins, a protein class that is particularly complex in plants (1–5).

Future work will focus on three areas:

- (i) An immediate priority is to enhance the annotations of POGs for predicted RBPs. In addition to the annotations available for all POGs (e.g. domains and targeting

predictions), annotations for RBPs will include literature citations, mutant phenotypes, and established intracellular locations. Where appropriate, POGs will be linked to subcellular proteome databases [e.g. mitochondria (21) and plastids (22)] and to smaller databases that focus on RBP subsets [e.g. the ASRG Database (1)]. In addition, gene models will be displayed simultaneously for all POG members, to highlight differences in gene prediction. An interface will be provided for community input.

- (ii) High quality POG assignments are essential for comparative analyses. Currently, the mutual-best-hit and phylogenetic approaches agree for ~65% of the POGs; these POGs are designated as 'well-supported'. A priority for the future is to increase the number of well-supported POGs by modifying the strategies used for defining mutual-best-hits and by using more rigorous methods for building phylogenetic trees. Those POGs encoding RBPs and for which the POG is not supported by the tree will be manually curated to resolve the discrepancy. Users with an interest in a POG that is not supported by the tree can assess orthology by downloading the sequences of closely-related proteins from the POG View page, generating and editing global protein alignments and using the edited alignments to calculate trees that include bootstrap values. Users who take this step will be encouraged to share their results through the community annotation interface.
- (iii) New maize genome and cDNA sequence data are deposited in public repositories on a frequent basis. To maintain an up-to-date representation of putative maize orthologs, we will develop a pipeline to automate the incorporation of new maize genome and EST sequence data. In addition, the database will be updated with recalculated POGs, protein domains, and targeting predictions when new versions of the rice and Arabidopsis gene sets are released.

## SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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