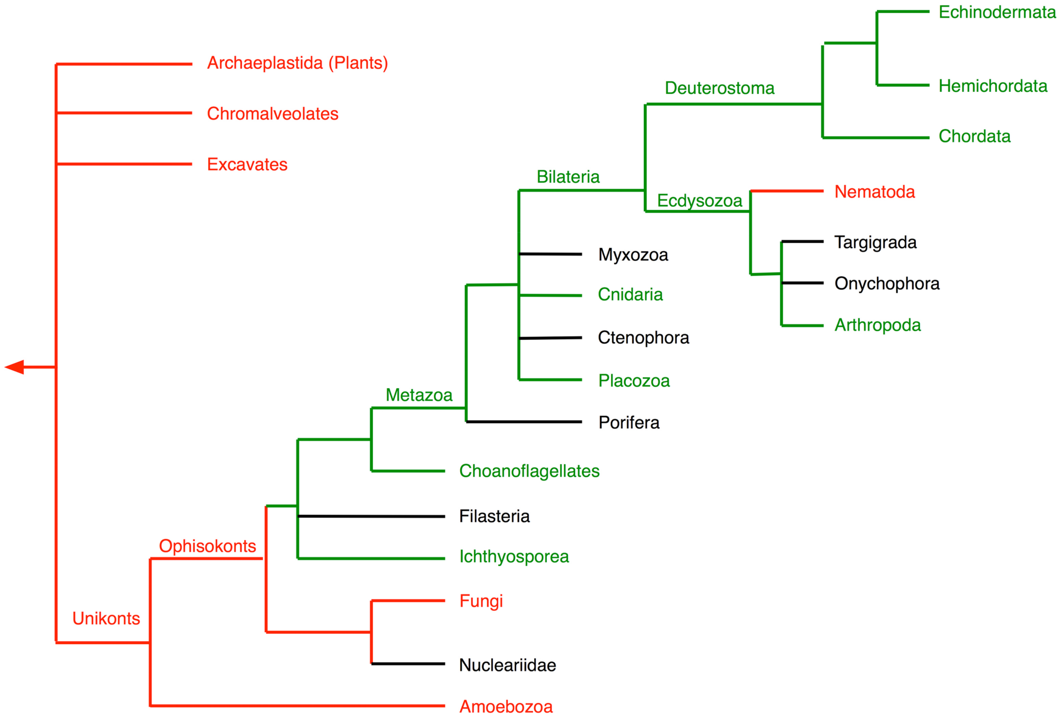
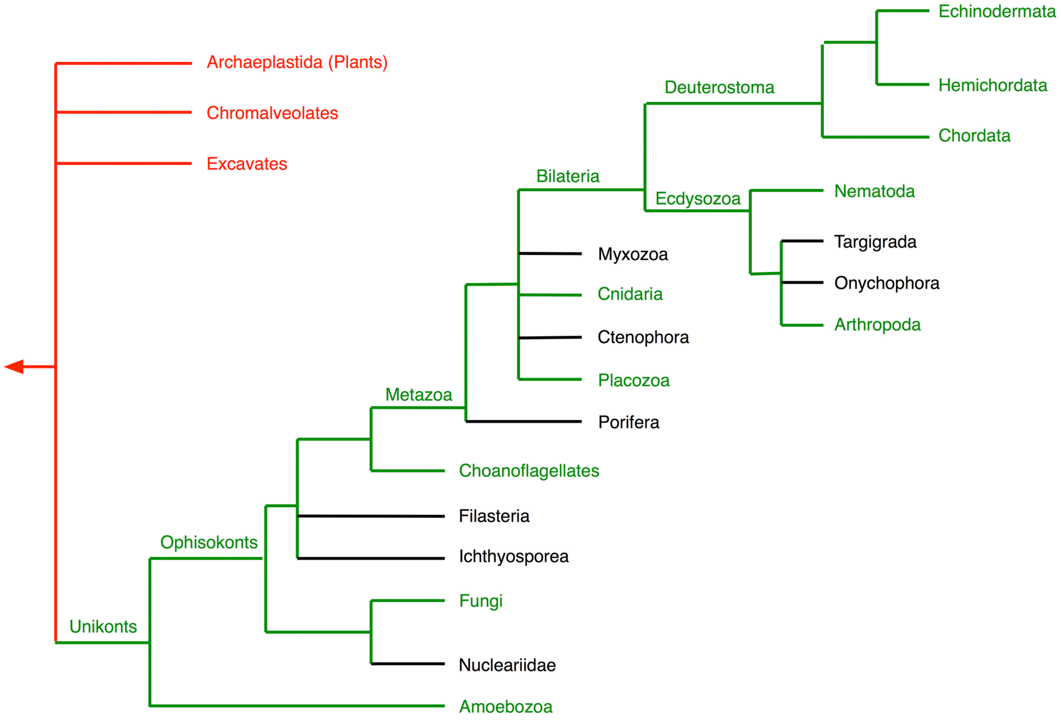
A


Echinodermata
Archaeplastida (Plants)
Hemichordata
Deuterostoma
Chromalveolates
Chordata
Excavates
Bilateria
Nematoda
Ecdysozoa
Targigrada
Myxozoa
Onychophora
Cnidaria
Arthropoda
Ctenophora
Placozoa
Metazoa
Porifera
Choanoflagellates
Filasteria
Ophisokonts
Ichthyosporea
Fungi
Unikonts
Nucleariidae
Amoebozoa

B


Echinodermata
Archaeplastida (Plants)
Hemichordata
Deuterostoma
Chromalveolates
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Nematoda
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Amoebozoa

Figure S7 Phylogenetic analysis of Gwl and Endos. Phylogenies are adapted from The Tree of Life Web Project (http://tolweb.org/tree/). For simplicity and also because of the lack of fully sequenced genomes, several clades among the Bilateria are not shown (including Ecdysozoa such as Priapulida, Loricefera, Kinorhyncha, and Nematomorpha; as well as Bilateria such as Rotifera and Platyhelminthes that are not categorized as Ecdysozoa). **(A)** Gwl. Green indicates lineages whose genomes include authentic Gwl [that is, proteins not only containing strong homologies with the Gwl kinase domain, but also containing the regions that ensure Gwl activation at M phase (particularly amino acids 180-222, 708-739, and 864-878 in the *H. sapiens* enzyme)]. Red indicates lineages without authentic Gwl genes, although some include genes for kinases closely related to Gwl such as Rim15 in *S. cerevisiae,* Cek1 in *S. pombe*, and IRE in the plant *Arabdipopsis thaliana.* Note particularly the absence of authentic Gwl in the Nematoda. Black indicates lineages for which the presence or absence of authentic Gwl genes cannot be determined based on available sequence information. Beyond the well-characterized authentic Gwl proteins in Chordata (e.g., human and frogs) and Arthropoda (e.g., flies), other presumed Gwl orthologs used to assemble this figure include: XP_001180570.1 in the Echinoderm *Strongylocentrotus purpuratus*, XP_002737566.1 in the Hemichordate *Saccoglossus kowalevskii*, XP_002167997.1 in the Cnidarian *Hydra magnipapillata*, XP_002108611.1 in the Placozoan *Trichoplax adhaerens*, XP_001745592.1 in the Choanoflagellate *Monosiga brevicollis*, and EFW39780.1 in the Ichthyosporean *Capsaspora owczarzaki*. **(B)** Endos. Green indicates lineages whose genomes encode at least one Endosulfine-like protein containing a motif homologous to that phosphorylated on human ENSA by Gwl or on yeast Igo1/2 by Rim15. Red indicates lineages in which fully sequenced genomes cannot be demonstrated to include Endosulfine-like genes. Black indicates lineages for which the presence or absence of Endosulfine-like genes cannot be determined based on available sequence information. The figure indicates the presence of an Endosulfine family gene precursor in a common ancestor to all the Unikonts. However, this conclusion is somewhat uncertain, as it is based on the results of tblastn searches of the genome of the Amoebazoan *D. discoideum* using the Igo1 amino acid sequence from *S. cerevisiae*. The hypothetical protein XM_639611.1 uncovered by this analysis has only low overall homology with Igo1, but the Dictyostelium protein includes the motif KYFSADWA, which is similar to the target site for Rim15/Gwl in Igo1/Endosulfine (KYFDSGDYA/N, with the phosphorylated site underlined; see Fig. S2).

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