

[Note]

Revisiting the β -Tubulin Genes in the Red Alga Genus *Chondrus*: Ancient Lineage Sorting or Faster Evolution

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ABSTRACT

To test whether β -tubulin genes in *Chondrus crispus* species vary as a result of ancient lineage or accelerated evolution, we added *Porphyra Tub* β 1 and *Tub* β 2 genes into a neighbor-joining analysis of the genes. The length difference between *Chondrus* and *Physarum* branches was non-significant. The β -tubulin gene phylogeny, rooted at α -tubulins, revealed paralogy of β -tubulin genes in red algae as well as in slime molds. The *Chondrus crispus* β 1 and *Physarum polycephalum* β 2 represented basal and independent lineages, indicating the effects of lineage sorting, as an ancient β -tubulin allele was passed down to *Chondrus* exclusively. The long branch of *Chondrus crispus* β 1 is the result of accumulated genetic variation, which should be ascribed to its ancient history.

Key words: *Chondrus crispus*, β -tubulin, lineage sorting, phylogeny, slime molds

Introduction

Tubulin is a heterodimer of two distinct polypeptides α and β . In most eukaryotes α and β tubulin are encoded by multiple genes, which are mostly unlinked to each other and dispersed in the genome (Goddard *et al.*, 1994). Genes of the tubulin family have been isolated and characterized from a wide range of organisms. Liaud *et al.* (1995) cloned a β -tubulin gene from the marine red alga *Chondrus crispus*. The deduced *Tub* β 1 protein was divergent from other β -tubulin of lower and higher eukaryotes, with only 64% to 69% amino acid identity. Their neighbor-joining tree indicated that the *Chondrus* β -tubulin gene was most related to a slime mold *Physarum polycephalum* β 2, and that the branch leading to *Chondrus* was much longer than for the β -tubulin gene of other genera. The topology of the tree showed taxonomic affinity between animals and fungi with the robustness at most major nodes supported by bootstrap analysis. Liaud *et al.* (1995) concluded that *Tub* β 1 had an accelerated evolutionary rate due to the release of

functional constraints in connection with the specialization of microtubular structures in rhodophytes.

Long branches on a given phylogenetic tree, however, may be simply an illusion. Branch-length can change when the tree is orientated at different roots. Liaud *et al.* (1995) rooted their neighbor-joining tree with the β -tubulin genes of *Euglena* and *Trypanosoma*, but did not describe their rationale. Some bias is unavoidable in determining the root for a group of taxa at the same hierarchical level (in this study, β -tubulins). In addition, the great dissimilarity between *Tub* β and β -tubulins implies that the former can possibly be recognized as a new lineage. Therefore, we re-analyzed the phylogeny of β -tubulins rooting the neighbor-joining tree with two α -tubulin genes, *Zea mays* and *Eimeria acervulina*.

Materials and Methods

The amino acid sequences used in Liaud *et al.* (1995) were obtained from the GenBank (Table 1).

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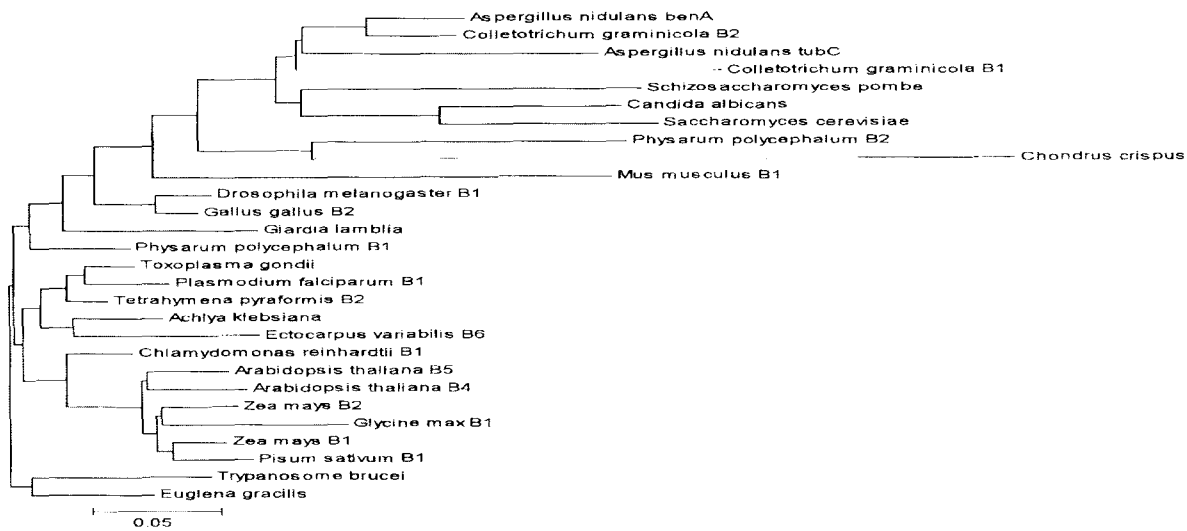


Figure 1. Neighbor-joining tree of of β -tubulin genes based on amino acid sequences arbitrarily rooted at *Euglena* and *Trypanosome*.

These sequences were aligned with the aid of Clustal V (Higgins *et al.*, 1992). Phylogenetic analyses were based on neighbor-joining methodology using both PHYLIP (Version 3.2, Felsenstein, 1989) and MEGA (Kumar *et al.*, 1993).

Results and Discussion

We recovered a neighbor-joining tree identical to that of Liaud *et al.* (1995), which was rooted at *Euglena* and *Trpanosome* arbitrarily. The branch of *Chondrus crispus* was two to three times longer than that of *Physarum polycephalum* (Figure 1), as shown in Figure 3 of Liaud *et al.* (1995). However, in the unrooted tree that we reconstructed based on amino acid sequences, the branch (0.2145 ± 0.0223) leading to *Chondrus* remained longer compared to that (0.1873 ± 0.0160) of *Physarum polycephalum* β 2. The length difference between the two branches was non-significant ($p < 0.10$).

To reveal the effect of lineage sorting in β -tubulins, we incorporated two β -tubulin sequences from *Porphyra purpurea*, both β 1 (Z67991) and β 2 (Z67992), into the analysis and used two α -tubulin genes, *Zea mays* (X63177) and *Eimeria acervulina* (X88776), as outgroups. The rooted neighbor-joining tree identified by MEGA suggested that the *Tub* β 1 gene was a basal taxon, representing an independent lineage (Figure 2). This gene may have duplicated and

Table 1. Tubulin sequences obtained from GenBank and used for phylogenetic analysis to clovify the divergence of β -tubulin genes in *Chondrus* species.

Gene	Organism	GenBank Accession No.	
Tubulin β	<i>Achlya klebsiana</i>	J05597	
	<i>Toxoplasma gondii</i>	M20025	
	<i>Euglena gracilis</i>	X15797	
	<i>Giardia lamblia</i>	X06748	
	<i>Candida albicans</i>	M19398	
	<i>Saccharomyces cerevisiae</i>	V01296	
	<i>Schizosaccharomyces pombe</i>	M10348	
	<i>Chondrus crispus</i>	X71748	
	<i>Trypanosome brucei</i>	K02836	
	Tubulin β 1	<i>Zea mays</i>	X52878
<i>Porphyra purpurea</i>		Z67991	
<i>Pisum sativum</i>		X54844	
<i>Glycine max</i>		M21296	
<i>Chlamydomonas reinhardtii</i>		M10064	
<i>Plasmodium falciparum</i>		M28398	
<i>Physarum polycephalum</i>		M58521	
<i>Drosophila melanogaster</i>		M20419	
<i>Mus musculus</i>		A25437	
<i>Colletotrichum graminicola</i>		M34491	
Tubulin β 2		<i>Zea mays</i>	X52879
		<i>Porphyra purpurea</i>	Z67992
		<i>Tetrahymena pyraformis</i>	L01415
	<i>Gallus gallus</i>	V00389	
	<i>Physarum polycephalum</i>	M20191	
Tubulin β 4	<i>Arabidopsis thaliana</i>	M21415	
	<i>Arabidopsis thaliana</i>	M84702	
Tubulin β 6	<i>Ectocarpus variabilis</i>	M32875	
	<i>Zea mays</i>	X63177	
Tubulin α	<i>Eimeria acervulina</i>	X88776	
	<i>Aspergillus nidulans</i>	M17520	
Tubulin C	<i>Aspergillus nidulans</i>	M17519	
	<i>Ben A</i>	M17519	

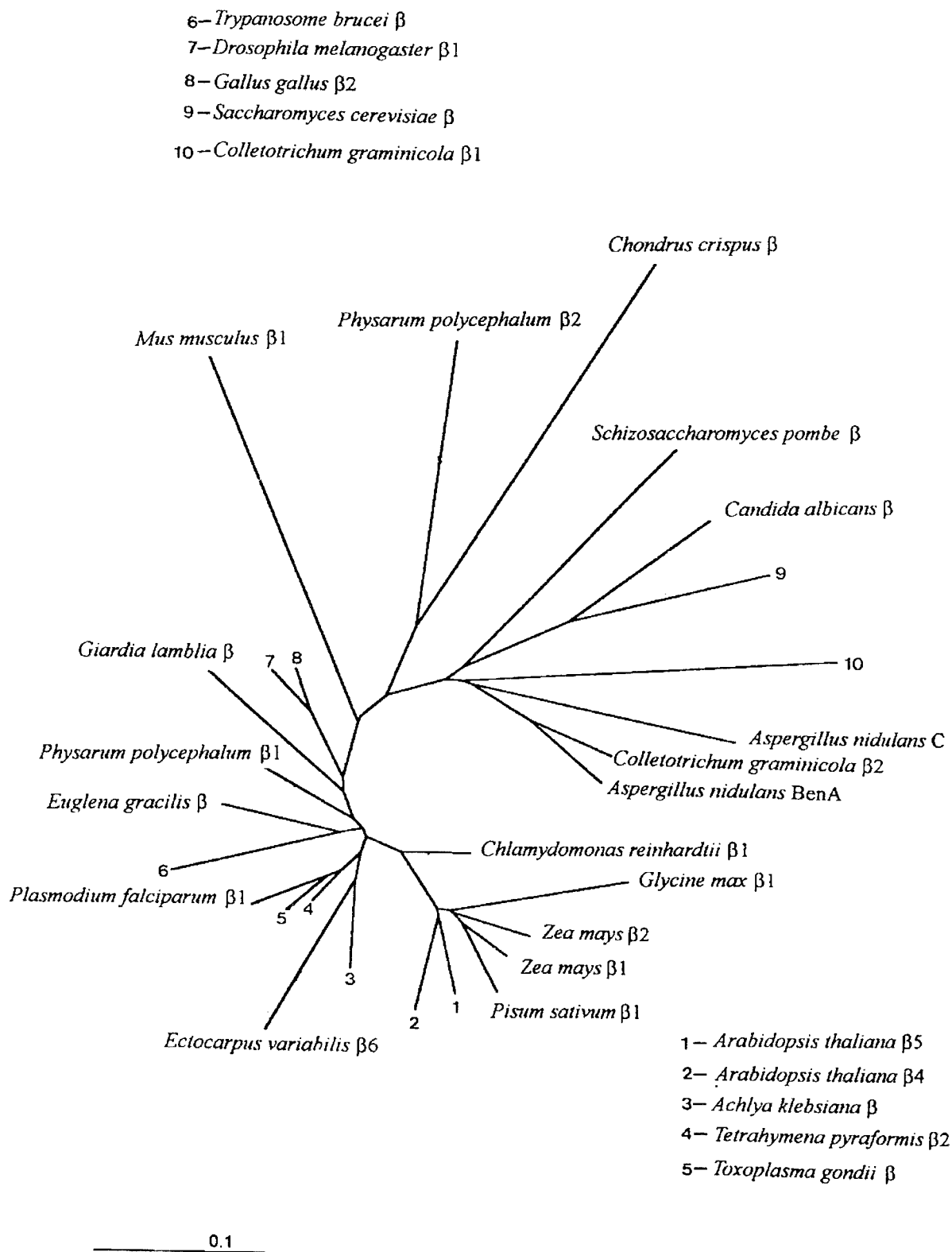


Figure 2. Unrooted neighbor-joining tree of β -tubulin genes based on amino acid sequences. 1. *Arabidopsis thaliana* β 5, 2. *Arabidopsis thaliana* β 4, 3. *Achlya klebsiana* β , 4. *Tetrahymena pyriformis* β 2, 5. *Toxoplasma gondii* β , 6. *Trypanosome brucei* β , 7. *Drosophila melanogaster* β 1, 8. *Gallus gallus* β 2, 9. *Saccharomyces cerevisiae* β , 10. *Colletotrichum graminicola* β 1. *Chondrus crispus* is the basal taxon.

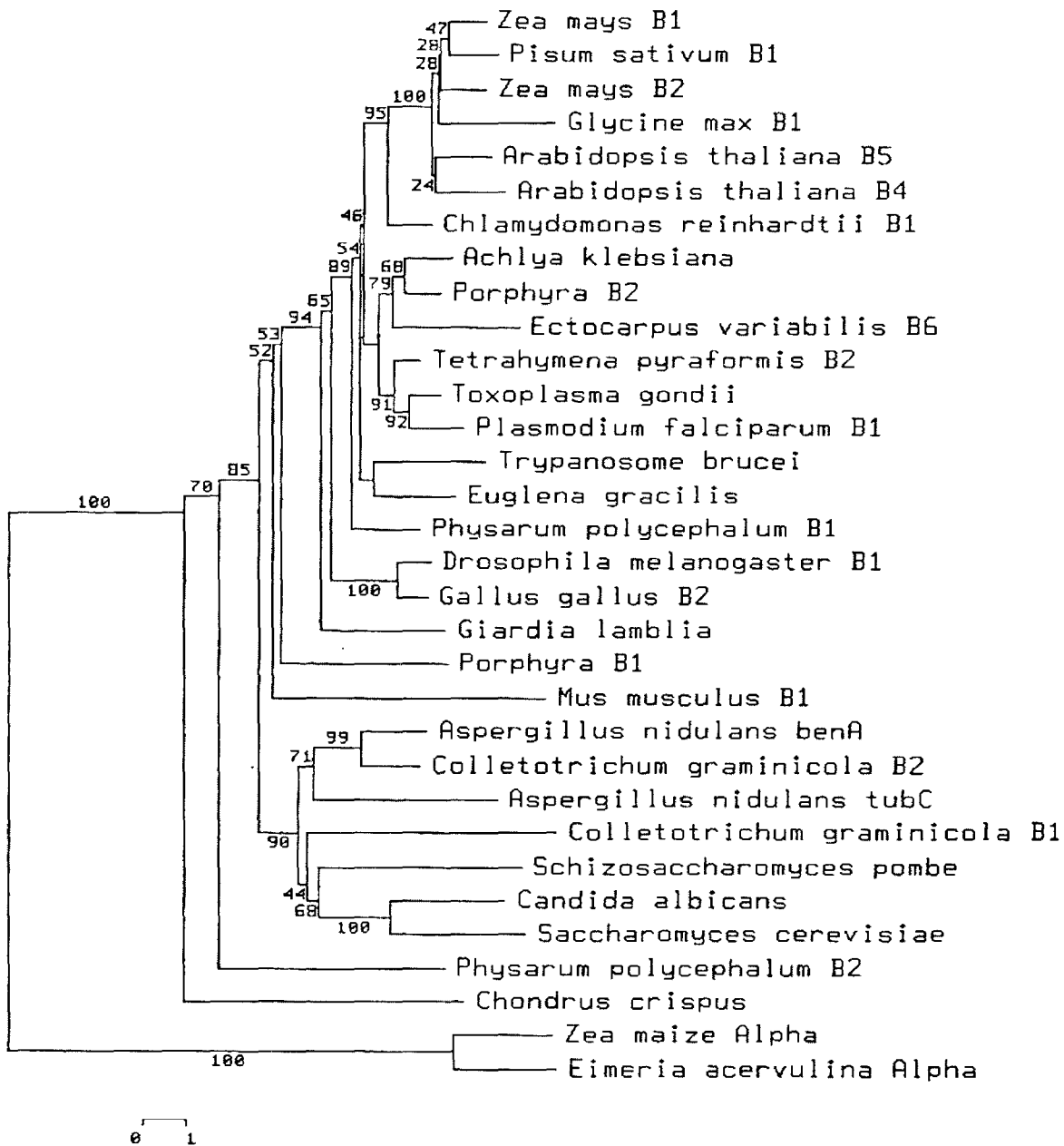


Figure 3. Neighbor-joining tree of β -tubulin genes rooted at *Zea mays* α and *Eimeria acervulina* α . Numbers at nodes indicate bootstrap values with 400 replicates. *Chondrus crispus* is basal to *physarum* and all other Tub β genes.

separated from others before the β -tubulin genes diverged. Like *Chondrus*, the divergence of the β 1 lineage in *Porphyra purpurea* may also be ancient. The β 2 tubulin in *Porphyra purpurea* was nested in a clade of *Achlya klebsiana* (flagellate fungus) and *Ectocarpus variabilis* (brown algae). No exclusive clade containing all the β -tubulins of both *Chondrus* and *Porphyra* was recognized.

The paralogy in the β -tubulin genes of red algae indicated an effect of lineage sorting, suggesting that the polymorphic loci in the ancestral population "entered" each descendant lineage unequally (Lyons-Wiler and Milinkovitch, 1997). Lineage sorting can distort phylogenies when genes coalesce deeper in time than the taxa (Maddison, 1997). The effects of lineage sorting, which have been discovered in many

organelle (Tiedmann and Noer, 1998; Young, 1998; Chiang, 2000) and nuclear genes (Harris and Disotell, 1998), may be more a rule than an exception in the molecular evolution of β -tubulin genes. According to the reconstructed phylogeny (Figure 3), the *Tub* $\beta 1$ may have been duplicated prior to divergence of most β -tubulin genes in eukaryotes. Likewise, the divergence of the $\beta 1$ lineage in *Porphyra purpurea*, as well as of *Physarum* $\beta 2$, may also be ancient.

Interestingly, in the β -tubulin genes tree of eukaryotes (Figures 2, 3), several branches, e.g. soybean, mouse, *Ectocarpus* and *Colletotrichum graminicola* $\beta 1$, were longer compared to their related taxa. Nested in the clade of angiosperms, soybeans should have shared a common evolutionary history with other dicots. Long branch of the *Glycine* $\beta 1$ lineage indicates an accelerated evolution and may be correlated with the short life span of soybeans (an annual plant; Li, 1997). Faster evolution also occurred in the mouse. In contrast, the basal position of *Chondrus crispus* β -tubulin in the phylogenetic tree as indicated by longer branch suggests that the accumulated genetic variation within lineage is due to the long evolutionary history.

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〔札記〕

校對紅藻之 β -tubulin 基因：ancient sorting 或快速演化

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摘 要

Liaud 等人(1995)從海洋性紅藻 *Chondrus crispus* 基因組中選殖出一 β -tubulin 基因，其氨基酸序列與其他 *Chondrus crispus* 的 β -tubulin 基因之氨基酸序列不十分相似，而與黏菌 *Physarum polycephalum* β 2 較相似。依據親緣樹形圖分析之結果顯示，此 *Chondrus crispus* 之 β -tubulin 分枝長度較其他的 β -tubulin 分枝長度為長，Liaud 等人認為此結果是因，此 β -tubulin 基因在 *Chondrus crispus* 具有特化之功能，使得其演化速度較快。但是，根據我們的重新分析發現，*Chondrus crispus* 與 *Physarum polycephalum* 分枝之長度差異並不顯著。當分析加入以 α -tubulin 基因為外群時，結果顯示此 *Chondrus crispus* 之 β -tubulin 基因為一獨立分枝，因此，造成此 β -tubulin 基因分枝長度較長之因素，與其說是快速演化造成，不如說是因共同祖先具多型性，在演化的過程中由親系分配(lineage sorting)所造成。

關鍵詞：紅藻，快速演化，親系分配，外群

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