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NOTE

DISTRIBUTION OF THE MITOCHONDRIAL DEVIANT GENETIC CODE AUA FOR METHIONINE IN HETEROKONT ALGAE[1]

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ABSTRACT

*The DNA sequence of the cytochrome oxidase subunit I (*COX*I) gene (1059 bp), was determined in a number of heterokont algae, including five species of the Phaeophyceae [*Chorda filum *(Linnaeus) Stackhouse,* Colpomenia bullosa *(Saunders) Yamada,* Ectocarpus *sp.,* Pseudochorda nagaii *(Tokida) Inagaki,* Undaria pinnatifida *(Harvey) Suringar], and a member of the Raphidophyceae [*Chattonella antiqua *(Hada) Ono]. The distribution of a deviant mitochondrial code, the AUA codon for methionine (AUA/Met), which was previously reported in the Xanthophyceae, was inferred from these* COX*I sequences. Comparative analyses of these sequences revealed that all the algae described above bear the universal genetic code, including the assignment for the AUA codon. A phylogenetic tree was constructed using the obtained sequences along with already-published* COX*I sequences of various heterokont algae. The clusters of the Xanthophyceae and the Phaeophyceae were resolved as sister groups with high bootstrap support, excluding a bacillariophycean species, a raphidophycean species, and three species of the Eustigmatophyceae. Taking the distribution of the deviant code and the* COX*I phylogenetic tree together, the genetic code change most probably occurred in an ancestor of the Xanthophyceae after it had branched off from the Phaeophyceae.*

Key index words: cytochrome oxidase subunit I; deviant genetic code; heterokont algae; Phaeophyceae; Xanthophyceae

Recently, several types of mitochondrial genetic code changes in algae have been reported. UGA codes tryptophan (UGA/Trp) in a member of the Rhodophyceae (Boyen et al. 1994) and in all examined species of the Prymnesiophyceae (Hayashi-Ishimaru et al. 1997), excluding those in the Pavlovales (Ehara, unpubl.). UAG codes for leucine or alanine in several colony-forming green algae (Hayashi-Ishimaru et al. 1996). In heterokont algae, it has been reported that AUA codes for methionine (Met) in the Xanthophyceae, whereas this codon is used for isoleucine (Ile) in the Eustigmatophyceae (Ehara et al. 1997) and *Melosira ambigua* (Bacillariophyceae; Inagaki et al. 1998). In this study we investigated whether there are any deviant genetic codes in other heterokont algae and inferred when the genetic code change (AUA/Met) occurred in the course of heterokont evolution.

The sources of organisms and availability of the DNA sequences of the cytochrome oxidase subunit I (*COX*I) gene used in this study are summarized in Table 1. Total DNA was extracted from the whole organism or zoospores as described previously (Kawai et al. 1995, Hayashi-Ishimura et al. 1996) and purified using a gel matrix (Geneclean Spin Kit, Bio 101, Vista, California). A set of degenerate primers (p1B: 5′-GCNACNACRTARTTANGTRTCRTG-3′ and P1C: 5′-TGGTTNTTYTCNACNAAYCAYAARGAYAT-3′) was used for the PCR amplification of a 1059 bp region of the *COX*I gene. Amplification, isolation of the PCR generated fragments, cloning, and sequencing were performed according to Ehara et al. (1997).

The deduced amino acid sequences were aligned (alignment is available on request) with the published or registered sequences of the heterokont algae using the programs in SINCA version 3.0 (Fujitsu System Engineering, Japan). The neighbor-joining (NJ) tree (Saitou and Nei 1987) also was inferred using the programs in SINCA with a Kimura two-parameter model (Kimura 1980). The unweighted most parsimonious (MP) trees were found using PAUP version 3.1.1 (Swofford 1993). Bootstrap resampling (Felsenstein 1985) was conducted 500 times, both in the NJ and MP analyses. Three species of the Prymnesiophyceae (*Isochrysis galbana, Phaeocystis pouchetii,* and *Pavlova lutheri*) were used as the outgroup in these analyses (Table 1). Maximum likelihood (ML) analysis was conducted by the star decomposition search using the JTT transition probability matrix in the PROTML program from the MOLPHY version 2.2 package (Adachi and Hasegawa 1992). In this analysis, the number of species were reduced to 10 (Table 1), and bootstrap resampling was not performed.

The results of the phylogenetic analysis of the *COX*I sequences conducted by the NJ, MP, and ML methods were essentially the same. The resulting

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TABLE 1. *List of species, whose* COXI *gene sequences were analyzed, and availability of sequences used for phylogenetic analyses.*

Algal species	GenBank accession no.	Culture code or sampling locality
Bacillariophyceae		
Melosira ambigua[a] (Grunow) O. Müller	AB009418	NIES[b] 20
Eustigmatophyceae		
Eustigmatos magnus (Petersen) Hibberd	AB000205	CCAP[c] 860/2
Nannochloropsis sp.	AB000207	CCMP[d] 505
Nannochloropsis oculata[a] (Droop) Hibberd	AB000209	CCAP 849/1
Phaeophyceae		
Chorda filum[a,e] (Linnaeus) Stackhouse	AF037991	Oshoro, Hokkaido, Japan
Colpomenia bullosa[a,e] (Saunders) Yamada	AF037995	Muroran, Hokkaido, Japan
Ectocarpus sp.[a,e]	AF037994	Hiroshima, Japan
Pseudochorda nagaii[e] (Tokida) Inagaki	AF037992	Nemuro, Hokkaido, Japan
Pylaiella littoralis (L.) Kjellman	Z72500	
Undaria pinnatifida[e] (Harvey) Suringar	AF037993	Muroran, Hokkaido, Japan
Prymnesiophyceae		
Isochrysis galbana[f] Parke	AB000119	CCMP 1323
Pavlova lutheri[f] (Droop) Green	AB009420	CCMP 1325
Phaeocystis pouchetii[a,f] (Hariot) Lagerheim	AB000120	NIES 388
Raphidophyceae		
Chattonella antiqua[a,e] (Hada) Ono	AF037990	NIES 1
Xanthophyceae		
Botrydiopsis alpina[a] Vischer	AB000203	UTEX[g] 295
Botrydium granulatum var. *kolkwitzianum* Vischer	AB000204	CCAP 805/4
Heterococcus caespitosus[a] Vischer	AB000206	CCAP 835/2A
Mischococcus sphaerocephalus Vischer	AB000208	CCAP 847/1
Tribonema aequale Pascher	AB000211	CCAP 880/1
Vaucheria sessilis[a] (Vaucher) De Candolle *ex* Collins	AB000212	CCAP 745/1C

[a] Species used for maximum likelihood analyses (Adachi and Hasegawa 1992).
[b] Microbial Culture Collection at the National Institute for Environmental Studies (Watanabe and Nozaki 1994).
[c] Culture Collection of Algae and Protozoa (Tompkins et al. 1995).
[d] Provasoli-Guillard National Center for Culture of Marine Phytoplankton (Andersen et al. 1997).
[e] *COX*I sequence determined in this study.
[f] *COX*I sequence used as outgroup.
[g] The Culture Collection of Algae at the University of Texas at Austin (Starr and Zeikus 1993).

MP tree is shown in Figure 1 with bootstrap values for the NJ and MP analyses. The cluster of the phaeophytes (Ph cluster) is resolved as a sister clade of the xanthophytes (Xa cluster) with high bootstrap support [89% (NJ)/82% (MP)], excluding *M. ambigua, Chatonella antiqua,* and the three eustigmatophyte clades (Fig. 1). Both the Xa and Ph clusters are supported by high bootstrap values, 99% (NJ)/98% (MP) and 99% (NJ)/77% (MP), respectively. In support of our *COX*I tree, the robust clustering of the phaeophytes and the xanthophytes has been shown previously by the phylogenetic analysis of nuclear-encoded small subunit of rRNA sequences (Ariztia et al. 1991, Saunders et al. 1995, Potter et al. 1997) or large subunit of rRNA sequences (Van der Auwera and De Wachter 1997). The lineage of the Xa cluster, which bears a deviant genetic code, AUA, for methionine, is shown with a dotted line in Figure 1. The phylogenetic positions of *M. ambigua* and *C. antiqua* are less evident because of their low bootstrap support [47% (NJ)/44% (MP) and 30% (NJ)/31% (MP), respectively]. The evolutionary distances separating the phaeophytes (*Chorda filum, Pseudochorda nagaii, Undaria pinnatifida, Ectocarpus* sp., *Pylaiella littoralis,* and *Colpomenia bullosa*), which have been classified into three orders (Lim et al. 1986), are relatively short compared to the distances separating the xanthophytes or the eustigmatophytes (Fig. 1). This suggests the relatively recent divergence of the Phaeophyceae compared with the Eustigmatophyceae and the Xanthophyceae.

A deviant genetic code (AUA/Met) has been reported in the Xanthophyceae (Ehara et al. 1997), although no sign of modified genetic codes was detected in the analyzed *COX*I genes of the five phaeophytes and one raphidophyte. Codon reassignment requires two subsequent steps, temporary disappearance of a specific codon from the whole genome and appearance of a mutated tRNA that is able to recognize the codon when it emerges again (Osawa et al. 1992). So far, there is no exception to the rule that there is a preference to use the AUA codon over the AUG codon for Met in mitochondrial genes if AUA is available as another Met codon (Nakamura et al. 1997). This is also the case for the reported *COX*I sequences within the Xanthophyceae. An analysis of codon usage showed that AUA/

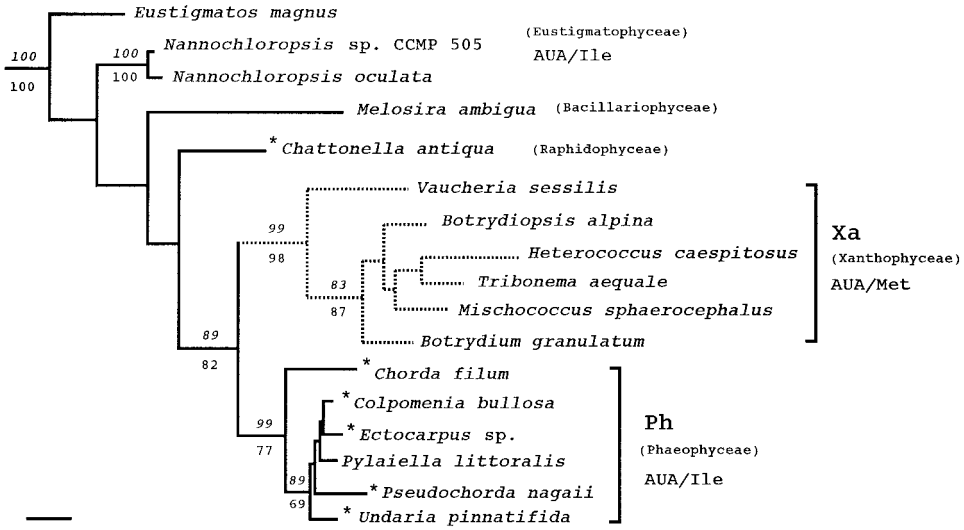

FIG. 1. One of the two most parsimonious (MP) trees derived from the deduced amino acid sequences (353 residues) of mitochondrial *COX*I genes. Number of steps: 456; CI = 0.682. Bootstrap values (500 replicates) are in roman under the internodes for the MP analysis and in italic above the internodes for the NJ analysis (values >60% are shown). An asterisk (*) denotes the species for which the *COX*I sequences was determined in this study. Scale bar equals 10 changes. The other MP tree differs from Figure 1 in local rearrangements in the Ph clade.

Met is much preferred over AUG/Met with AUA/AUG ratio's of 14/4, 20/2, 16/3, 17/1, 16/4, and 16/1, for *Botrydiopsis alpina, Botrydium granulatum, Heterococcus caespitosus, Mischococcus sphaerocephalus, Tribonema aequale,* and *Vaucheria sessilis,* respectively. We could detect no peculiar driving force that might remove the AUA codon in the examined Phaeophyceae through their codon usage analyses. The codon used frequently in the genome is unable to change the amino acid assignment without disadvantageous effects on the species (Osawa et al. 1992). Therefore, it seems highly unlikely that a common ancestor of the Phaeophyceae and the Xanthophyceae already utilized the AUA codon for Met, and then, in the lineage of the Phaeophyceae, the AUA codon threw back to code Ile atavistically. Considering the above, the distribution of the deviant genetic code and the phylogenetic tree obtained from the mitochondrial *COX*I sequences (Fig. 1), the most parsimonious scenario is that this genetic code change occurred in an ancestor of the Xanthophyceae after this group branched off from the Phaeophyceae.

Horizontal transfer of the deviant genetic code system is apparently impossible, because of the required evolutionary steps described above (Osawa et al. 1992). Moreover, it is known that once a modified genetic code system is established, such a system is able to maintain itself in its lineage (Osawa et al. 1992, Inagaki et al. 1998). Therefore, superimposition of the genetic code appears to be potentially very useful to check the appropriateness of a molecular phylogenetic tree.

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