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SHORT COMMUNICATION

Lateral transfer of eukaryotic ribosomal RNA genes: an emerging concern for molecular ecology of microbial eukaryotes

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Ribosomal RNA (rRNA) genes are widely utilized in depicting organismal diversity and distribution in a wide range of environments. Although a few cases of lateral transfer of rRNA genes between closely related prokaryotes have been reported, it remains to be reported from eukaryotes. Here, we report the first case of lateral transfer of eukaryotic rRNA genes. Two distinct sequences of the 18S rRNA gene were detected from a clonal culture of the stramenopile, *Ciliophrys infusionum*. One was clearly derived from *Ciliophrys,* but the other gene originated from a perkinsid alveolate. Genome-walking analyses revealed that this alveolate-type rRNA gene is immediately adjacent to two protein-coding genes (*ubc12* and *usp39*), and the origin of both genes was shown to be a stramenopile (that is, *Ciliophrys*) in our phylogenetic analyses. These findings indicate that the alveolate-type rRNA gene is encoded on the *Ciliophrys* genome and that eukaryotic rRNA genes can be transferred laterally.
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Genes that are involved in transcription, translation and related processes, and interacted with many partner molecules are believed to be less transferable between different organisms (Rivera *et al.*, 1998; Jain *et al.*, 1999). Hence, lateral transfer of ribosomal RNA (rRNA) genes is generally considered not to occur. Indeed, the lateral transfer of eukaryotic rRNA genes has never been reported, whereas just a few cases of possible lateral transfer of rRNA genes between two closely related prokaryotes have been shown (for example, Mylvaganam and Dennis, 1992; van Berkum *et al.*, 2003). Thus, rRNA gene sequences have been considered as very useful phylogenetic markers and have been utilized in numerous biological studies. Especially, sequence analyses of rRNA genes amplified from environmental DNA have become the gold standard for assessing the diversity and distribution of microbial eukaryotes in a wide range of environments (for example, López-García *et al.*, 2001; Moon-van der Staay *et al.*, 2001).

Recently, we established a clonal culture of *Ciliophrys infusionum* (deposited as NIES-3355) from the sediment of the lagoon ‘Kai-ike’ (Satsuma-sendai, Kagoshima, Japan). *Ciliophrys infusionum* is a heterotrophic microbial eukaryote belonging to Dictyocophyceae (Stramenopiles) and is widely distributed in marine environments. However, as we amplified 18S rRNA gene sequences from this microbial eukaryote to confirm its morphological identification, two distinct 18S rRNA gene sequences (that is, alveolate-type (AB846664: 4418-6616) and stramenopile-type (AB846665)) were unexpectedly obtained. Note that the alveolate-type sequence was obtained first by the PCR with the primer set ‘18S forward and reverse (Yabuki *et al.*, 2010)’ and then the stramenopile-type sequence was obtained by the PCR with the primer set ‘Euk1A (Sogin and Gunderson, 1987) and EukB (Medlin *et al.*, 1988)’. The stramenopile-type sequence reasonably branched with the known sequence of *C. infusionum*, whereas the alveolate-type sequence branched with perkinsids in Alveolata, microbial eukaryotes parasitic on fishes, bivalves and algae (Figure 1). As neither contaminated nor parasitic/endosymbiotic perkinsids were observed in light and transmission electron microscopy (Supplementary Figure SI 1), the alveolate-type 18S rRNA gene sequence also seemed to be derived from a part of the *Ciliophrys* genome.

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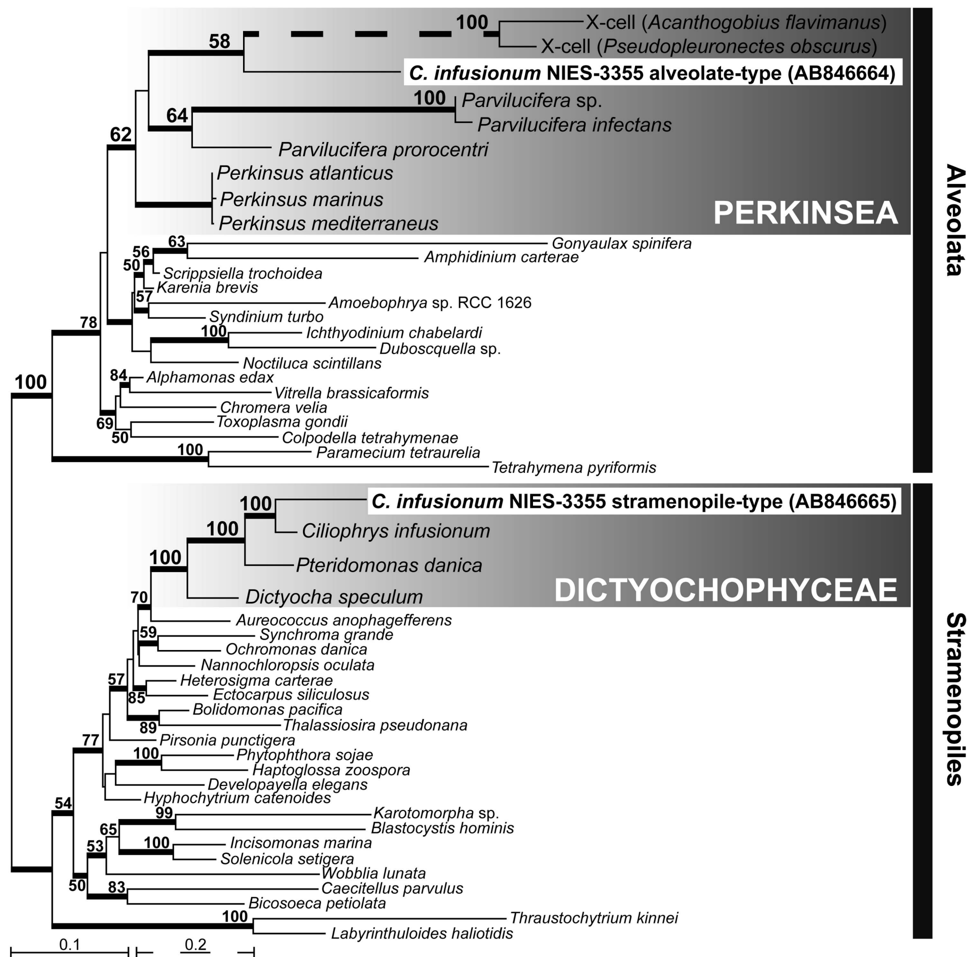
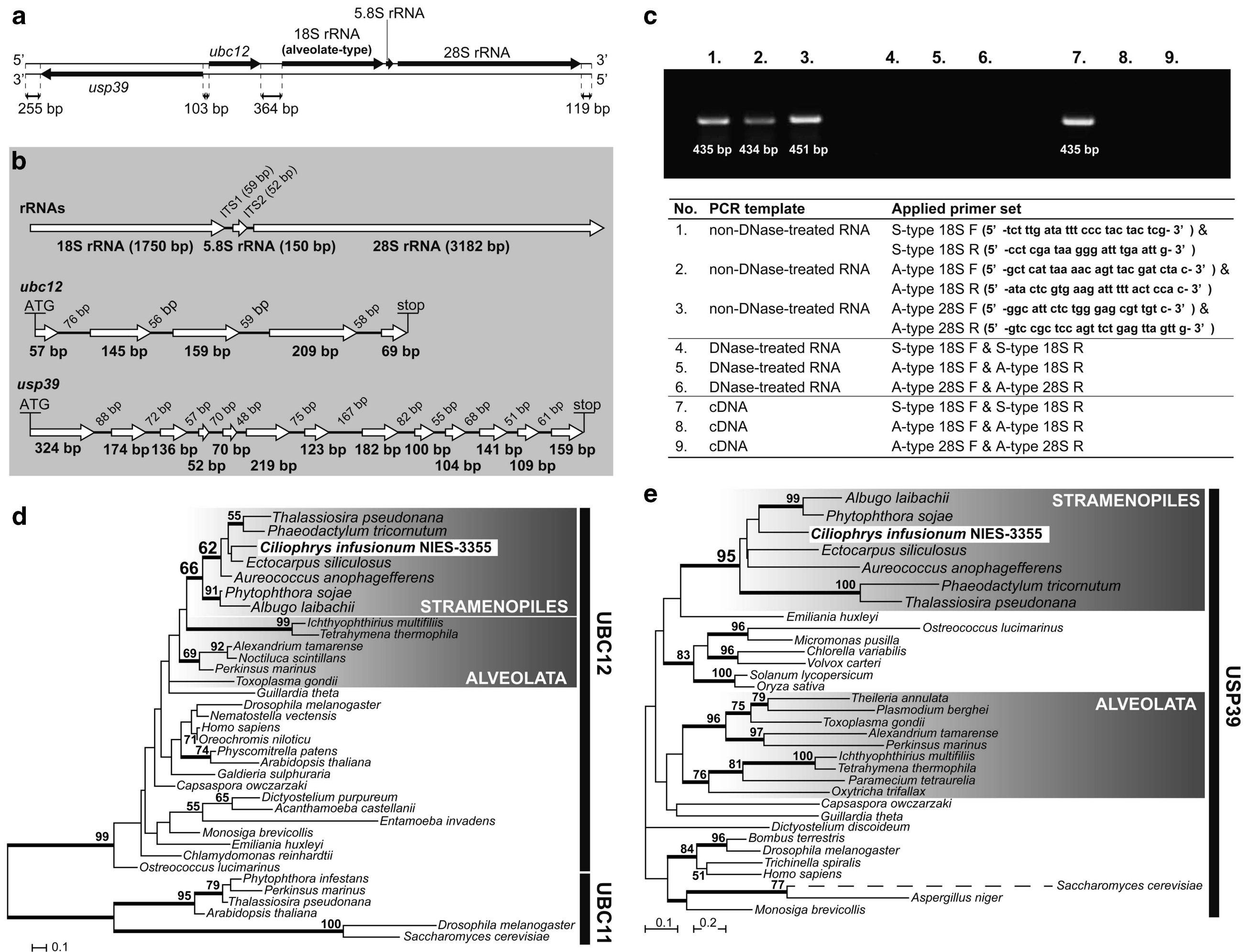


Figure 1 Maximum-likelihood tree of 18S rRNA gene sequences (25 alveolates and 26 stramenopiles, 1411 positions) reconstructed by RAxML 7.2.8 (Stamatakis, 2006) with a GTRGAMMAI model selected as the best-fit model by the modeltest 3.7 (Posada and Crandall, 1998). Bootstrap supports were calculated from the analyses of 100 replicates and more than 50% values are shown. Nodes supported by Bayesian posterior probabilities ⩾0.95 are highlighted with bold lines.

Polymorphism of the 18S rRNA gene has been reported for several eukaryotes such as early-branching fish and diatoms (Krieger and Fuerst, 2002; Alverson and Kolnick, 2005). However, the degree of their polymorphism was very low, suggesting that such polymorphism was generated during species diversification. Therefore, the polymorphism found in this study is apparently different from previous cases, and the possibility that the alveolate-type 18S rRNA gene was laterally transferred to the *Ciliophrys* genome was raised. To confirm the existence of the alveolate-type 18S rRNA gene in the *Ciliophrys* genome, we obtained both upstream- and downstream-flanking regions of this unexpectedly detected gene from a genome library prepared by restriction enzyme treatment followed by adapter ligation with a Straight Walk Kit (BEX co Ltd, Itabashi, Tokyo, Japan).

Consequently, a genome fragment (AB846664) containing the alveolate-type 18S rRNA gene (9709 bp in length) was obtained. BLAST searches indicated that two protein-coding genes (that is, *ubc12* encoding ubiquitin-conjugating enzyme 12 (UBC12) and *usp39* encoding ubiquitin specific peptidase 39 (USP39)) were located in the upstream-flanking region of the alveolate-type 18S rRNA gene. Four and 12 introns were identified in *ubc12* and *usp39*, respectively, and the transcription of both genes was confirmed by RT–PCR with exactly matching primers (data not shown, see experimental procedures in supplement). The physical map of this genome fragment and the structures of *ubc12*/*usp39* are shown in Figures 2a and b, respectively. In the downstream-flanking region, 5.8S rRNA and 28S rRNA genes were identified (Figure 2b). The transcription of the stramenopile-type 18S rRNA gene was confirmed by RT–PCR, whereas the transcription of neither the alveolate-type 18S rRNA nor its adjacent 28S rRNA genes was confirmed (Figure 2c and supplement: note that the primers used were verified to work by positive controls). These findings suggest that the alveolate-type 18S rRNA gene and its adjacent 28S rRNA gene are pseudogenes, although the possibility that these



No.	PCR template	Applied primer set
1.	non-DNase-treated RNA	S-type 18S F (5' -tct ttg ata ttt ccc tac tac tcg- 3') & S-type 18S R (5' -cct cga taa ggg att tga att g- 3')
2.	non-DNase-treated RNA	A-type 18S F (5' -gct cat taa aac agt tac gat cta c- 3') & A-type 18S R (5' -ata ctc gtg aag att ttt act cca c- 3')
3.	non-DNase-treated RNA	A-type 28S F (5' -ggc att ctc tgg gag cgt tgt c- 3') & A-type 28S R (5' -gtc cgc tcc agt tct gag tta gtt g- 3')
4.	DNase-treated RNA	S-type 18S F & S-type 18S R
5.	DNase-treated RNA	A-type 18S F & A-type 18S R
6.	DNase-treated RNA	A-type 28S F & A-type 28S R
7.	cDNA	S-type 18S F & S-type 18S R
8.	cDNA	A-type 18S F & A-type 18S R
9.	cDNA	A-type 28S F & A-type 28S R

Figure 2 (**a**) Physical map of the genome fragment (AB846664) obtained by genome-walking analyses. Black arrows represent the coding regions of the predicted genes. *usp39* is encoded on the strand opposite to other genes. (**b**) Structures of the transcription unit (18S, 5.8S and 28S rRNA genes), *ubc12* and *usp39* on the obtained genome fragment are separately shown. White arrows represent coding regions. The possible coding regions of rRNA genes were predicted by comparison with the rRNA gene sequence from *Perkinsus atlanticus* (AF509333). The sizes and positions of the introns in *ubc12* and *usp39* were identified by the comparisons between the sequences of genome DNA and RT–PCR products. (**c**) Summary of PCR amplification of stramenopile-type 18S, alveolate-type 18S and alveolate-type 28S rRNA genes using non-DNase-treated RNA, DNase-treated RNA and cDNA synthesized from the DNase-treated RNA as template. PCR primers exactly matching with the stramenopile-type 18S rRNA gene (S-type 18S F and S-type 18S R), alveolate-type 18S rRNA gene (A-type 18S F and A-type 18S R) and alveolate-type 28S rRNA gene (A-type 28S F and A-type 28S R) were independently designed, and their sequences are shown in the table. (**d**) Maximum-likelihood tree of 29 UBC12 and 6 UBC11 (outgroup) sequences reconstructed by RAxML 7.2.8 with an LGGAMMAF model selected as the best-fit model by Aminosan (Tanabe, 2011). Bootstrap supports were calculated from the analyses of 100 replicates and more than 50% values are shown. Nodes supported by Bayesian posterior probabilities ⩾0.95 are highlighted with bold lines. (**e**) Maximum-likelihood tree of 33 USP39 sequences reconstructed by RAxML 7.2.8 with an LGGAMMAF model selected as the best-fit model by Aminosan. Bootstrap supports were calculated from the analyses of 100 replicates and more than 50% values are shown. Nodes supported by Bayesian posterior probabilities ⩾0.95 are highlighted with bold lines.

genes are transcribed under different situations (such as different culture conditions) cannot be completely excluded.

Phylogenetic analyses showed that both UBC12 and USP39 found in this study affiliated with stramenopile homologues (Figures 2d and e). Statistical support for the affiliation of USP39 from *C. infusionum* with the stramenopile homologues was very high (bootstrap probability (BP) of 95% and Bayesian posterior probability (BPP) of 1.00). Although the support for the affiliation of UBC12 from *C. infusionum* with the stramenopile homologues was moderate (BP of 66% and BPP of 0.98), its tight affiliation was confirmed by the approximately unbiased test (Supplementary Figure SI2). These results support the idea that the 9709 bp fragment is a part of the *Ciliophrys* genome and that the alveolate-type 18S rRNA sequence is actually encoded on the *Ciliophrys* genome. The 28S rRNA gene in the fragment was shown to form a clade with the homologue of *Perkinsus* with high support (Supplementary Figure SI3), suggesting that this

28S rRNA gene shares the same origin with the alveolate-type 18S rRNA gene. On the basis of our present findings it can be concluded that the lateral transfer of eukaryotic rRNA genes (18S, 5.8S and 28S rRNA genes) occurred from a perkinsid to *Ciliophrys* as a single evolutionary event.

In the past few years, large-scale environmental rRNA gene surveys conducted with next generation sequencing technology have become common practice (for example, Bråte *et al.*, 2010; Cheung *et al.*, 2010). These large-scale surveys may detect transferred rRNA genes and such transferred rRNA genes may confuse our understanding of the true diversity and distribution of microbial eukaryotes, even if the frequency of lateral transfers of the rRNA gene is rare and the copy numbers of the transferred rRNA gene in environments are low. We agree that environmental rRNA gene surveys with PCR are still useful and effective to estimate the diversity/distribution of microbial eukaryotes. However, the fact that recovered rRNA gene sequences do not always reflect the actual existence of microbial eukaryotes corresponding to these sequences should be kept in mind based on our findings.

Conflict of Interest

The authors declare no conflict of interest.

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