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Eukaryotic evolution

Early origin of canonical introns

Spliceosomal introns, one of the hallmarks of eukaryotic genomes, were thought to have originated late in evolution[1,2] and were assumed not to exist in eukaryotes that diverged early — until the discovery of a single intron with an aberrant splice boundary in the primitive 'protozoan' *Giardia*[3]. Here we describe introns from a close relative of *Giardia*, *Carpediemonas membranifera*, that have boundary sequences of the normal eukaryotic type, indicating that canonical introns are likely to have arisen very early in eukaryotic evolution.

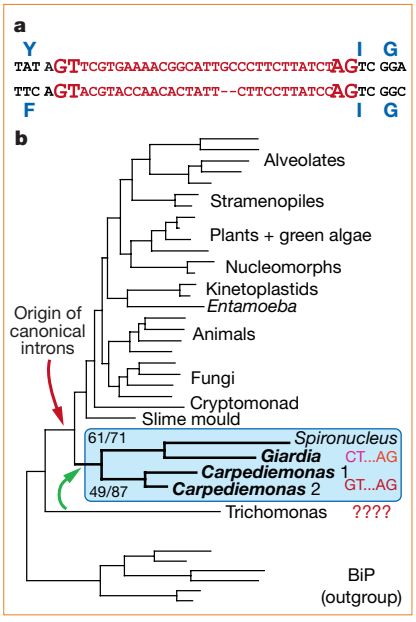

Figure 1 Introns and evolutionary affinities of *Carpediemonas*. **a,** Portions of two *Carpediemonas* carbamate kinase genes, showing intron sequences (red) interrupting the protein-coding sequence (in blue). The introns have canonical splice boundaries (GT…AG; large red type). **b,** Maximum-likelihood evolutionary tree of eukaryotic cytosolic Hsp70 proteins ('Γ + invariable sites' model). Endoplasmic-reticulum Hsp70 ('BiP') is used as an outgroup. The grouping of *Carpediemonas* with *Giardia* and *Spironucleus* is shown in the blue box; statistical support (bootstrap percentages) for this grouping is assessed using likelihood (upper left of box), and likelihood distance (lower left). The higher percentages (right in each pair) apply when the outgroup is omitted. The basal placement of *Trichomonas* is weakly supported with likelihood (21%); green arrow shows a more plausible position on the basis of other evidence[7,13]. The intron splice boundaries for the relevant groups and the origin of canonical introns are shown in red. New sequences have been deposited at GenBank under accession numbers AY131204–AY131209.

Carpediemonas membranifera is a poorly studied, free-living microbial eukaryote that is considered to be a relative of *Giardia* on the basis of its morphology[4]. Using the polymerase chain reaction (PCR) with *Carpediemonas* genomic DNA as template, we determined the partial sequences of two distinct carbamate kinase genes from this organism. In both genes, an insertion of 33 or 31 nucleotides interrupts the similar protein-coding sequence shared with carbamate kinase genes from other organisms (Fig. 1a). These insertions are bounded by guanine and thymine (GT) nucleotides at the 5′ end and adenine and guanine (AG) nucleotides at the 3′ end, which is a characteristic of most of the spliceosomal introns that interrupt protein-coding genes in other eukaryotes.

We used PCR with reverse transcription to recover the messenger RNA sequence of one of the two *Carpediemonas* carbamate kinase genes. This sequence lacks the insertion, which is presumably removed (spliced) from the messenger RNA before translation. We conclude that the insertions in the *Carpediemonas* carbamate kinase genes are canonical 'GT…AG' spliceosomal introns, albeit comparatively small ones.

To determine the evolutionary affinities of *Carpediemonas*, we used PCR to amplify near-complete sequences for two genes that encode cytosolic heat-shock protein 70 (Hsp70). We also sequenced a cloned Hsp70 gene from *Spironucleus barkhanus*, a very close relative of *Giardia*. Maximum likelihood analysis of Hsp70 proteins reveals a specific evolutionary relation between *Carpediemonas*, *Giardia* and *Spironucleus* (Fig. 1b); three other molecular markers also support this relationship[5].

The single intron found in a *Giardia* gene has a non-canonical CT dinucleotide at its 5′ splicing boundary[3], which could be interpreted as a 'frozen' primitive eukaryotic condition: canonical 'GT…AG' spliceosomal introns might then be a later innovation in more modern cells. Our results indicate that this is not the case, however, as canonical introns seem to be an ancestral feature of the larger evolutionary grouping that includes *Giardia* and *Carpediemonas*. The aberrant *Giardia* intron probably represents a lineage-specific (or intron-specific) secondary alteration of the 5′ splice boundary.

The extremely early divergence attributed to *Giardia* is based on the absence or aberration of many typical eukaryotic features, such as mitochondria and introns, and on its arguably deep-branching position in many phylogenetic trees[6–9]. The grouping of *Giardia* with *Carpediemonas* (which, as well as canonical introns, has organelles that are probably derived from mitochondria[4]) weakens this argument for early divergence.

Irrespective of the true evolutionary position of *Giardia*, the only potentially 'early' eukaryotic group in which introns have not been found are the parabasalids, such as *Trichomonas*[10,11]. *Trichomonas* is already known to possess some of the cellular machinery for intron splicing[12], however, and there is evidence to indicate that it is evolutionarily affiliated with *Giardia* and its relatives[7,13] (and is slightly misplaced in many phylogenies, including that shown in Fig. 1b). An affiliation with *Giardia* implies a similar closeness to *Carpediemonas*, and it is likely that parabasalids have, or had, canonical introns. There is now every reason to assume that canonical introns were present in the most recent common ancestor of living eukaryotes.

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