A glimpse of a putative pre-intron phase of eukaryotic evolution

Alexander V. Sverdlov¹, Miklos Csuros², Igor B. Rogozin¹ and Eugene V. Koonin¹

¹National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, USA
²Department of Computer Science and Operations Research, Université de Montréal, Montréal, Québec QC H3C 3J7, Canada

Comparison of the exon–intron structures of ancient eukaryotic paralogs reveals the absence of conserved intron positions in these genes. This is in contrast to the conservation of intron positions in orthologous genes from even the most evolutionarily distant eukaryotes and in more recent paralogs. The lack of conserved intron positions in ancient paralogs probably reflects the origination of these genes during the earliest phase of eukaryotic evolution, which was characterized by concomitant invasion of genes by group II self-splicing elements (which were to become introns in the future) and extensive duplication of genes.

The mystery of the intron invasion

The hallmark of eukaryotic gene structure is the interruption of protein-coding sequences by noncoding sequences known as introns [1–4]. Introns are present in all of the eukaryotic genomes that have been sequenced, including the most compact ones, those of parasitic, unicellular eukaryotes [5–7]. Accordingly, the key components of the spliceosome – the elaborate RNA- and protein-containing molecular machine that mediates the removal of introns and the joining of exons – are conserved throughout the eukaryotic domain of life [8,9]. Moreover, the positions of numerous introns are conserved between orthologous genes from the most evolutionarily distant eukaryotes, in particular between animals and plants [10–12]. Thus, introns seem to have been present in eukaryotic genes since the earliest stages of eukaryogenesis and might have had an important role in the emergence of the nucleus and other features of eukaryotic cell organization [13,14]. By contrast, and counter to the introns-early hypothesis [15,16] (see Glossary), there is no evidence that these elements were ever present in prokaryotes, although spliceosomal introns are thought to be derived from group II self-splicing elements, which are found in many bacteria, in eukaryotic organelles and in certain archaea. Some of these group II self-splicing elements are bona fide introns, whereas others insert themselves in intergenic regions [2,17–19]. The lack of information on the transitional phase between the almost intronless genomes of prokaryotes (the presence of the occasional self-splicing introns notwithstanding) and the genomes of eukaryotes, which are riddled with introns in protein-coding genes, leaves a large gulf in our understanding of the origins of eukaryotic cell organization.

Lack of conservation of intron positions in ancient eukaryotic paralogs

We sought insight into the events that occurred during the early, formative, phase of eukaryogenesis by comparing the intron positions in paralogous genes that are duplicated in all sequenced eukaryotic genomes but not in any prokaryotic genomes. More than 2000 sets of such ancient eukaryotic paralogs have been identified and inferred to have evolved by duplications during the early phase of eukaryotic evolution (i.e. between the emergence of the eukaryotic cell and the radiation of the extant eukaryotic lineages) [20]. Many of the ancient paralogs show limited sequence conservation, which complicates the accurate alignment of these sequences and the identification of conserved introns. We generated amino acid sequence alignments for the 157 most-conserved sets of ancient paralogs, and we mapped the intron positions onto the alignments, essentially as previously described [11]. We then counted the intron positions that were shared between the ancient paralogs in unambiguously aligned regions (see the supplementary material online). This analysis was carried out either for paralogs within a genome or for multiple alignments of paralogs from a set of genomes (Figure 1). Unexpectedly, we observed very low conservation of intron positions between ancient paralogs in each of the comparisons, <3% of the intron positions were shared (Figure 1; Table S2 in the supplementary material online), which is similar to the level of independent intron insertion that is expected in the same positions.

Glossary

**Group II self-splicing elements**: a distinct class of mobile element that combines the ability to undergo ribozyme-catalyzed self-splicing with transposition mediated by the reverse transcriptase that is encoded by the element. These elements insert themselves in protein-coding genes (mainly in the organelles of plants, fungi and protozoa) or in intergenic regions (mainly in bacteria and certain archaea). They are thought to be ancestors of eukaryotic spliceosomal introns.

**Introns-early hypothesis**: a hypothesis that states that introns in protein-coding genes are an ancestral feature of gene organization that was present in the last universal common ancestor of cellular life but was eliminated in prokaryotes during ‘genome streamlining’. The opposing hypothesis – the introns-late hypothesis – states that introns were inserted in protein-coding genes during eukaryotic evolution.

**Paralogs**: genes that evolved by duplication of an ancestral gene.
The few shared intron positions seemed to be randomly scattered over the analyzed set of ancient paralogous genes; therefore, there is no indication that the exon–intron structure is conserved in any subset of ancient paralogs (data not shown). As a control, we examined the conservation of intron positions in more-recent, lineage-specific paralogs of the same genes (e.g. those resulting from duplications only in animals or only in plants) and observed a much greater level of conservation (Figure 1), in agreement with previous findings [22].

Concomitant intron invasion and extensive gene duplication in the early phase of eukaryotic evolution

Our analysis shows the absence of appreciable conservation of intron positions between ancient paralogs, in contrast to more-recent paralogs of the same genes. This could be explained either by the rapid loss of introns after gene duplication during the early stages of eukaryotic evolution or by the absence of shared intron positions in ancient paralogs immediately after gene duplication. However, rapid intron loss is unlikely to be the main factor underlying the absence of conserved intron positions in ancient paralogs because, within orthologous sets, the same genes have many intron positions that are conserved between the most evolutionarily distant eukaryotic genomes available [11]. Specifically, in the genes comprising the ancient paralogous sets analyzed in *Homo sapiens* and *Arabidopsis thaliana*, the positions of 193 of the 6624 introns present (2.9%) are conserved between these genomes, compared with 1230 introns (18.6%) between orthologs in the same gene set.

Thus, it seems most probable that the ancient paralogs lacked introns at the time of duplication and/or duplicated genes by a mechanism, such as reverse transcription, that involved loss of introns in one of the copies. Conceivably, both of these factors made important contributions to the observed pattern (Figure 2). If the duplications that generated ancient paralogs occurred concomitantly with the initial process of intron insertion in the genes of the emerging eukaryote [13,14], then many of these duplications would involve intronless or intron-poor genes. Presumably, this early phase of eukaryogenesis was also characterized by a high level of reverse transcription.

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**Figure 1.** Conservation of intron positions in ancient and recent eukaryotic paralogs. Conservation of introns was assessed by the following: (i) multiple alignments of paralogous sequences from six species (i.e. 12 sequences); (ii) alignments of paralogs from two of these species, *Arabidopsis thaliana* and *Homo sapiens* (two sequences against two sequences); and (iii) alignments of paralogs from each of the six species separately (six separate twofold alignments). In the first two comparisons, an intron position was considered to be conserved if it was shared by any pair of paralogs. The results are given for the alignment stringency ±5 (see the supplementary material online).
conferred by proliferating group II self-splicing elements, the progenitors of introns. This would drive extensive reverse-transcription-mediated gene duplication, with any previously inserted introns lost from the new copies of the duplicated genes. Therefore, the lack of conservation of intron position between ancient paralogs is likely to reflect their origination by gene duplication during the earliest, formative, phase of eukaryotic evolution, which pre-dates the relatively intron-rich state that has been inferred\[3,11,12\] for the genes of the last common ancestor of modern eukaryotes.

**Concluding remarks**

We found that, in contrast to orthologs from even the most distant eukaryotic species or to relatively recent paralogs, ancient paralogs that evolved by duplication before the divergence of the main lineages of eukaryotes have almost no shared intron positions. It seems most probable that the duplications that generated the ancient eukaryotic paralogs occurred concomitantly with the massive invasion of group II self-splicing elements (the ancestors of spliceosomal introns) into protein-coding genes. Therefore, the ancient duplications might often have involved intronless or intron-poor genes. In addition, many of these duplications might have occurred by the reverse-transcription pathway, mediated by the reverse transcriptase encoded by group II self-splicing elements. This pathway also would have yielded intronless gene duplicates, which, subsequently, would have accumulated introns independently of their paralogs.

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**Supplementary data**

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tig.2007.01.001.

**References**

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