Five identical intron positions in ancient duplicated genes of eubacterial origin

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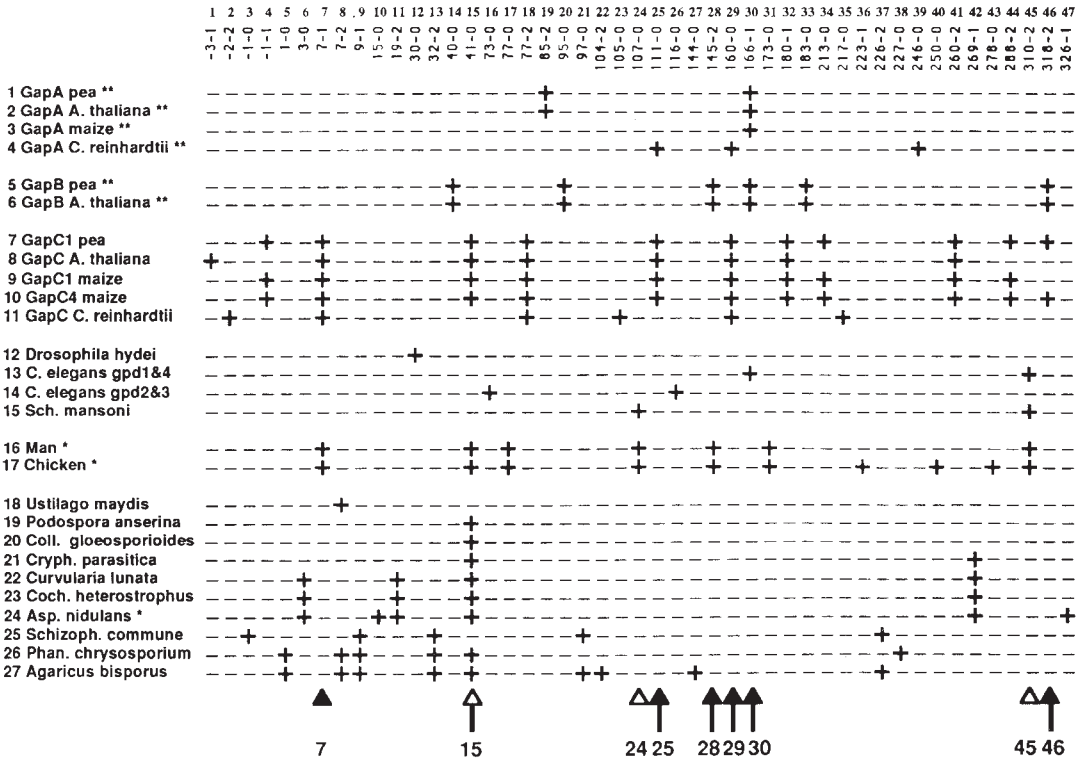
In 1985 Cornish-Bowden wrote "although there is now much to suggest that introns are an ancient relic of primordial genes, convincing proof must await the discovery of clearly corresponding intron arrangements in genes that arose by duplication before the separation of prokaryotes and eukaryotes"[1]. Genes for chloroplast and cytosolic glyceraldehyde-3-phosphate dehydrogenases of eukaryotes are descendants of an ancient gene family that existed in the common ancestor of extant eubacteria. During eukaryotic evolution, both genes were transferred to the nucleus from the antecedents of present-day chloroplasts and mitochondria, respectively[2–5]. Here we report the discovery of five spliceosomal introns at positions that are precisely conserved between nuclear genes for this chloroplast/cytosol enzyme pair. These data provide strong evidence in favour of the 'introns early' hypothesis, which proposes that introns were present in the earliest cells, consistent with the idea that introns facilitated the assembly of primordial genes by accelerating the rate of exon shuffling[6–13].

Two introns strictly conserved in nuclear genes encoding chloroplast and cytosolic glyceraldehyde-3-phosphate dehydrogenases (GAPDH; *GapA/GapB* and *GapC*, respectively) have been generally regarded as strong evidence in favour of the 'introns early' hypothesis[10], although the identity of intron positions has also been dismissed as 'parallel insertion of different introns'[14]. To obtain more information on intron conservation in GAPDH genes, we have determined gene structures for *GapA* and *GapC* from the unicellular green alga *Chlamydomonas reinhardtii* and for two new *GapC* genes from higher plants, *GapC1* of pea and *GapC4* of maize. We found three new intron positions that are precisely conserved between chloroplast and cytosolic GAPDH genes. Figure 1 shows that there are 47 known intron positions in 27 different GAPDH genes. Of the ten intron positions found in chloroplast GAPDH genes (*GapA* and *GapB*;



* One intron in leader, ** two introns in N-terminal transit peptide

Introns conserved between GapA(B)/GapC, plants/animals/fungi, plants/animals and vertebrates/non-vertebrates, respectively.

FIG. 1 The distribution of introns in present-day GAPDH genes. The presence of an intron is indicated by a plus sign, its absence by a minus sign. There are 47 known intron positions, defined by codon number and phase, in 27 different genes. For introns separating triplets after the first or second base codon numbers are followed by −1 and −2, respectively. For introns falling between triplets the number of the codon located 3′ of the intron is given followed by −0. Codon positions are numbered with reference to the standard sequence alignment of GAPDH genes[2,3,12,13]. The 27 genes are shown from top to bottom as six separate groups comprising genes encoding: lines 1–4, chloroplast *GapA*; lines 5 and 6, chloroplast *GapB*; lines 7–11, glycolytic *GapC* genes from plants; lines 12–15, non-vertebrates; lines 16 and 17, vertebrates; and lines 18–27, fungi. Arrows and arrowheads at the bottom of the figure indicate intron positions precisely conserved across *GapA(B)/GapC* genes (introns 25, 28, 29, 30, 46) and across *GapC* genes of more or less distant eukaryotic organisms (introns 7, 15, 24, 45), as indicated at the bottom of the figure.

METHODS. Cloning and sequencing of cDNAs and genomic clones encoding *GapA* and *GapC* of *Chlamydomonas reinhardtii*, *GapC4* of maize and *GapC1* of pea has been described[3,13,15,22,23]. cDNA clones of *Chlamydomonas* encoding *GapA* and *GapC* were identified using heterologous probes of maize[3]. The genomic clone encoding *GapC4* of maize was identified with a cDNA probe encoding maize *GapC3* (ref. 24). The genomic clone encoding pea *GapC1* was isolated using the authentic cDNA probe[22]. Intron/exon arrangements were established by comparing the genomic sequences with their corresponding cDNA sequences, except for *Chlamydomonas GapC*, for which intron positions were deduced by homology comparisons with genes from angiosperms. Sources of sequence information: *Chlamydomonas GapA* and *GapC*, pea *GapC1* and maize *GapC4* (lines 4, 7, 10, 11), this work; maize *GapA* (line 3)[12]; pea *GapA* and *GapB* (lines 1, 5)[13]; maize *GapC1* (line 9)[23]; *GapC* genes from *Schizophyllum commune*, *Phanerochaete chrysosporium* and *Agaricus bisporus* (lines 25 to 27)[25]; all other sequences can be found in release 35 of GenBank. *A. thaliana*, *Arabidopsis thaliana*; *C. reinhardtii*, *Chlamydomonas reinhardtii*; *C. elegans*, *Caenorhabditis elegans*; *Sch. mansoni*, *Schistosoma mansoni*; *Coll. gloeosporioides*, *Colletotrichum gloeosporioides*; *Cryph. parasitica*, *Cryphonectria parasitica*; *Coch. heterostrophus*, *Cochliobolus heterostrophus*; *A. nidulans*, *Aspergillus nidulans*; *Schizoph. commune*, *Schizophyllum commune*; *Phan. chrysosporium*, *Phanerochaete chrysosporium*.

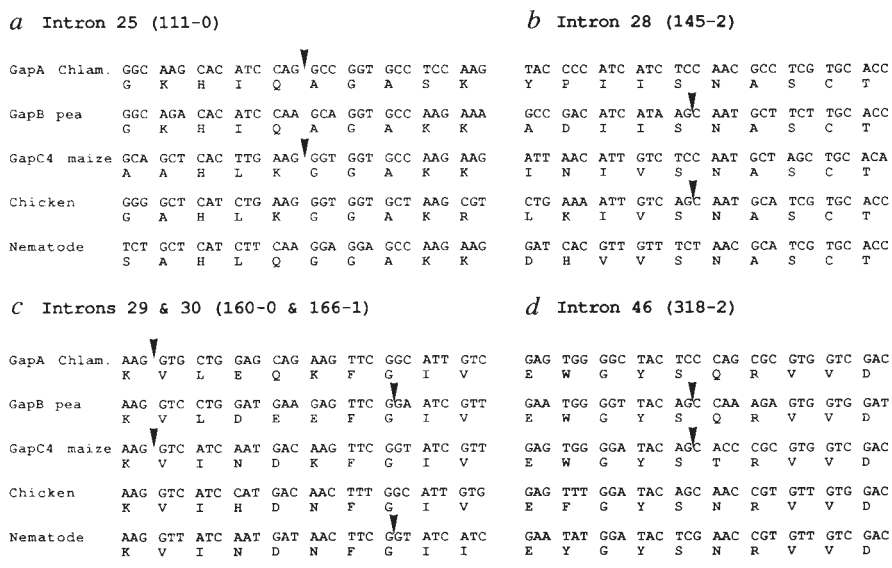


FIG. 2 Conservation of intron-exon junctions between chloroplast and glycolytic GAPDH genes, *GapA(B)* and *GapC*, respectively. The alignments show that the five introns interrupt homologous coding sequences at identical positions. Intron designations and sources of sequence information are as in Fig. 1. For the estimation of the probability that the five intron positions coincide by chance, we considered *GapA/GapB* (999 bp, 6 genes, 10 different introns; see Fig. 1) versus *GapC* (999 bp, 21 genes, 42 different introns; see Fig. 1) as a pairwise comparison and determined a probability of 2.2×10^{-5} according to ref. 26. If fungal genes with their highly non-random intron distributions (Fig. 1, genes 18 to 27) are excluded from this calculation, the probability becomes 2.2×10^{-6}.

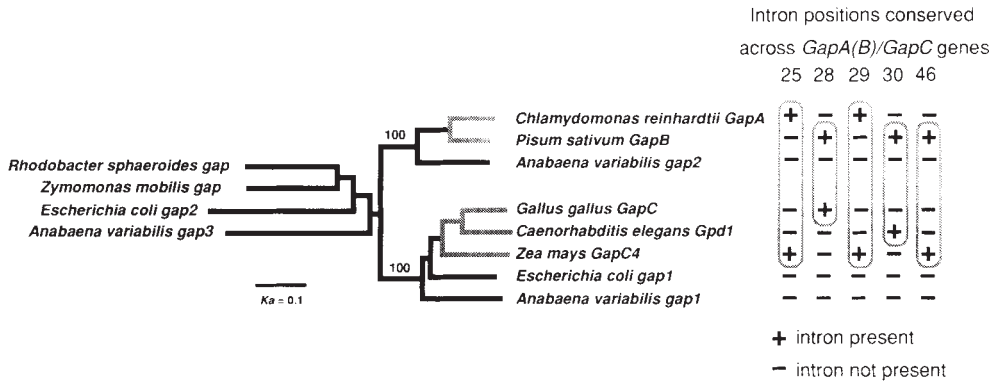


FIG. 3 Phylogenetic tree for GAPDH sequences showing intron conservation patterns. The tree was inferred by the neighbour-joining method[27] from a divergence matrix of non-synonymous substitutions per non-synonymous site, Ka[28]. Branches bearing genes found in eubacterial genomes are shown as solid lines, those bearing genes found in eukaryotic genomes are shown as grey lines. The scale bar indicates 0.1 substitutions per site. The numbers above the *GapA* and *GapC* branches indicate that these were found in 100/100 bootstrap parsimony replicates (PAUP, version 3.0) for the amino-acid alignment; these are the only branches in the figure that are essential to arguments concerning the age of introns conserved across *GapA(B)/GapC* genes (see text). Designations of intron positions correspond to those indicated in Figs 1 and 2. The *E. coli* GAPDH genes were designated in the figure as *gap1* and *gap2* instead of *gapA* and *gapB*, respectively, as in the original literature[29], in order to avoid confusion with the plant *GapA* and *GapB* genes[4]. Sources of bacterial sequences are given in[4], with the exception of Rhodobacter sphaeroides[30]. For all other sources, see Fig. 1 legend.

lines 1 to 6 in Fig. 1), five are precisely conserved in glycolytic GAPDH (*GapC*) genes of plants and animals: introns 28 and 30 (corresponding to positions 145-2 and 166-1) in vertebrates (lines 16 and 17 in Fig. 1) and nematodes (line 13), respectively[12,13], and the three new introns 25, 29 and 46 (positions 111-0, 160-0 and 318-2) in *GapC* genes of higher plants (Fig. 1, lines 7 to 10). In addition to these precisely conserved introns across the *GapA(B)/GapC* boundary, there are four cases of conserved introns between *GapC* genes of different major eukaryotic groups (introns 7, 15, 24, 45) and numerous cases of quasi-conservation or 'slippage' of introns[15,23], in which positions differ by only one to eight bases both across gene classes and major taxonomic groups, such as introns B40-0/C41-0, B95-0/C97-0, C144-0/B145-2, C180-1/B183-0, C-3-1/C-2-2/C-1-0/C-1-1/C1-0 (plants/fungi), C7-1/C7-2 (plants/fungi), C30-0/C32-2 (*Drosophila*/fungi), C77-0/C77-2 (vertebrates/plants), C104-2/C105-0/C107-0 (fungi/*Chlamydomonas*/animals) C226-2/C227-0 (fungi/fungi), where B and C denote *GapB* and *GapC*, respectively.

Figure 2 shows a detailed alignment of the exon sequences flanking the five introns precisely conserved between chloroplast and cytosolic GAPDH genes. A representative set of five genes has been selected from Fig. 1, two genes encoding chloroplast GAPDH (*GapA* of *Chlamydomonas* and *GapB* of pea) and three encoding the cytosolic enzyme (chicken *GapC*, nematode *Gpd1* and maize *GapC4*). Clearly, in all five pairwise comparisons, introns interrupt the *GapA(B)* and *GapC* coding sequences in identical positions. This is also true for intron 25 (position 111-0), where the two flanking codons 110 and 111 have changed from Gln|Ala in *Chlamydomonas GapA* to Lys|Gly in maize *GapC4*. The probability of finding these five identical intron positions across the *GapA(B)/GapC* boundary as a result of independent insertion is extremely low and is estimated to be roughly 2×10^{-5} (Fig. 2 legend).

In Fig. 3, the same five sequences have been incorporated into a phylogenetic tree together with the GAPDH genes of the cyanobacterium *Anabaena variabilis* (genes *gap1, gap2* and *gap3*), the γ-purple bacterium *Escherichia coli* (genes *gap1* and *gap2*) and the α-purple bacteria *Rhodobacter sphaeroides* and *Zymomonas mobilis*. The tree topology and the corresponding bootstrap values clearly show that the duplication event that gave rise to chloroplast and cytosolic GAPDH genes of eukaryotes occurred long before the separation of distinct organismal lineages leading to present day eukaryotes and eubacteria. The subtrees for *GapA(B)* and *GapC* both bear genes found in eukaryotic and eubacterial chromosomes. Thus, either the duplication

event that gave rise to *GapA(B)* and *GapC* genes occurred in ancient eubacteria and the eukaryotic genes are of endosymbiotic origin, as we believe[2–5,12,13], or the gene duplication took place in the common ancestor of eubacteria and eukaryotes. In the former case, the five indisputably identical spliceosome intron positions in *GapA(B)/GapC* (Fig. 2) were occupied in eubacterial GAPDH genes long before the divergence of purple and cyanobacteria (*E. coli* and *A. variabilis*). In the latter case, they were occupied in the ancestral GAPDH gene of the progenote.

Although the five introns conserved across present-day *GapA(B)* and *GapC* genes are removed with the aid of spliceosomes, the data do not indicate how their predecessors in the ancestral GAPDH gene were spliced. The discovery of group II introns in eubacteria[16] has given support to the notion that these elements may have been the precursors from which both spliceosomal introns and small nuclear RNAs in eukaryotic nuclei arose[17,18–21]. Introns in *GapA(B)*- and *GapC*-ancestors within eubacterial chromosomes may have been mechanistically group II, and if so, may have evolved into contemporary spliceosomal introns *in situ*. The conservation of five introns at identical positions in GAPDH genes which were duplicated in ancient eubacteria and perhaps in progenotic DNA, lends strong support to the exon theory of genes[9]. □

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