

Minireview

Unexpected intron location in non-vertebrate globin genes

Luc Moens^a, Jacques Vanfleteren^b, Ivo De Baere^a, Anna M. Jellie^c, Warren Tate^c and Clive N.A. Trotman^c

^aDepartment of Biochemistry, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium, ^bLaboratory of Animal Morphology and Systematics, University of Ghent, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium and ^cDepartment of Biochemistry, University of Otago, Dunedin, New Zealand

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The *Caenorhabditis elegans* and *Artemia* T4 globin sequences are highly homologous with other invertebrate globins. The intron/exon patterns of their genes display a single intron in the E and G helices respectively. Precoding introns in multirepeat globins are inserted in homologous positions. Comparison of the intron/exon patterns in the known globin gene sequences demonstrates that they are more diverse than first expected but nevertheless can be derived from an ancestral pattern having 3 introns and 4 exons.

Globin gene; Intron location; Evolution

1. INTRODUCTION

Vertebrate globin genes invariably have two introns resulting in three exons with boundaries between amino acid residues B12 and B13 and between G6 and G7 (helix notation). Plant globin genes have an extra intron between E14 and E15, making four exons, whereas most insect globin genes contain no intervening sequences [1,2]. It is currently thought that the 4 exons, 3 introns arrangement is ancestral and that evolution has sometimes led to the elimination of introns [1-3].

Recently the gene sequence of the internally duplicated globin from the nematode *Pseudoterranova decipiens* was described [4]. This sequence contains six introns and seven exons altogether. The first intron separates a secretory peptide leader sequence from the functional protein coding sequence (precoding intron). The next three introns are in the B, E and G helix coding sequences similar to plants. The fifth intron separates the two globin repeats (bridge intron) and the final one is found in the second repeat which has retained only this single intron in the B helix.

The globin of the clam *Barbatia reeveana* (Mollusca) also has a duplicated globin structure but has the vertebrate intron-exon pattern, together with a precoding intron before the first repeat and a bridge intron between the repeats [5].

This contrast suggests that the globin gene structure

of invertebrates is more diverse than at first expected.

Here we examine the protein and gene structures of the monomeric haemoglobin of the nematode *Caenorhabditis elegans* and repeat T4 of the multi-repeat globin of the crustacean *Artemia* and we evaluate their globin gene structures from an evolutionary perspective.

2. ALIGNMENT OF GLOBIN AMINO ACID SEQUENCES

The proper alignment of the translated globin sequences is a prerequisite for the assessment of equivalent intron positions. The invertebrate globin sequences from which the genomic structures are known are aligned in Fig. 1.

Only the alignment of the *C. elegans* globin is discussed in detail below. The stereochemical interpretation of the other sequences has been published previously [11-17].

The proposed alignment recognizes the obligatory CD1 Phe and F8 His, as well as the conservation of the Gly at B6 and E8 associated with the crossing of the B and E helices [18].

Although E7 in *C. elegans* cannot be occupied by the usual His, the alignment is notably strong from E2 though E18. Moreover, the observed Gln at E7 is, in invertebrates, the most frequently seen alternative at this position [19,20].

The G helix is unusual in having two Trp residues at G5 and G9. However, alignment in the G helix is strengthened by the confidence with which we can lo-

Correspondence address: L. Moens, Department of Biochemistry, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium. Fax: (32) (3) 8202248.

	H/LEADER SEQUENCE	HC/LINKER/PRE-A	A	AB	B	C	CD	D	
	1	1	1	1	1	1	1	1	
	123456789012345678901	1234 5678901234567	1234567890123456	1234567	123456789012 3456	1234567	1234567890	1234567	
MbW			VL	SEGSHQLVLHVWAKVE	-----A	DVAGHGQDILIR LFES	HPETLEK	FD--RFXHLK	TEAEMKA
Gm			VAF	TEKQDALVSSSFBAFK	-----A	NIPQXSVVFYTS*ILEK	APAAKDL	FS---FLANG	-VDFT--
Ct4	#(KLLILALCFARASA)		L	TADQISTVQSSFAQVK	-----C	----DAVGILYA VEKA	DPSIQAK	FT---F-AGK	DLDSIKG
Ct2			APL	SADSEASLVRSWAGVK	-----H	----SEVDILY*IFKA	NPDIMAK	FF--QF-AGK	DLETLKG
Lt			DEHEHCC	SEEDHRIVQKQWDILW	-----H	IKIGFGRLLLT*LAKE	IPDVNDL	FK--RV----	DIEHAE-
At			VADAVAKVCG	SEAIKGNLRSSWGLM	-----SA	DIEATGLTYLAN*LFEL	RPLTKTY	FT--RLGQVQ	KGA---
At4	[DEFTVKSFNHVFEVIFLNE]GLRQ*ADI---VDPVTHL		TGRQKEMIKASWSKAR	-----T	DLRSLGQELFMR MFKA	HPEYQTL	FVNKGADV-	PLVSLRE	
At5	[DAASVEAWKFFDVSIGVIAQ]GL-K*VATSEEDPVTGL		YGKEIVALRQAFAAVT	-----P	RNVEIGKRVFAK LFAA	HPEYKTL	FK--KFEQV-	SVEELPS	
Br1		#*MSVSAKLDEVT--	QFANKNLIRSTWNMMV	-----	GDRGNGVELMGL*LFQR	APDSKID	FK--RLGDVS	-AENIPY	
Br2	[-DDAIQAWHKLIDVICAVLKE]--SK*KGVLETIEEVT--		KPANKGLIRSTWNMMIA	-----	GDRKNGVELMAL*LFEM	APDSKID	FR--RLGDVS	-PSNIPN	
Pd1	#(MHSSIVLAIVLFVAIASA)	SK*-----	---TRELCKMSLEHAK	--VGTSK	EAKQDCIDLYKH*MFEL	YFAMKKY	FKHRENYT--	-PADVQK	
Pd2	[DEPTKHAHQEIGKEFSHEISH]	G-RH*-----	---VRDHCMNSLEYIA	--IGDKE	HQKQNGIDLYKH*MFEL	YFAMKKY	FKHRENYT--	-PADVQK	
Ce		# SM N-----	RQESIDLCVKSLEGRM	--VGTEA	QNIENGNAFFRY FFTN	FPDLRVY	FKGAKEYT--	-ADDVKK	

	E	EF	F	FG	G	GH	H
	1	1	1	1	1	1	2
	123 4567 89 01234 567890	1234567890123	1234567890	12345	123456 7890123456789	123456789	123456789012345678901
MbW	SED LKKH GV TVLTA LGAILK	---KKGHHEAE--	LKPLAQSHAT	KH-KI	PIKYLE*FISEAIIHVLHGR	HPQDF----	GADAQCGAMNKALELFRKDIAA
Gm	NPK LTGM AE KLFAL*VRDSAG	---ASGTVVAD--	-AALGSVHAQ	KA--V	TDPQFV*VVKALLKTIKAA	VQLK-GDKW	SDELSRAWEVAYDELAATAIKK
Ct4	SAD FSAH AN KIVGF FSKIIQ	---LPNIDGD--	VTFVASHTP	RG--V	THDQLN NFRAGFVSYMKAH	---DTD-	FAGAEAAWGATLDAFFGMVFA
Ct2	TQO FATH AG*RIYGF VSEIVA	MGNANHPAMETL	IKDMAANHKA	RG--I	PKAQFN EFRASLVSYLQSK	---L-VSW	NDSLGAANTQGLDNVFNMMFS
Lt	GPK FSAH AL RILNG LQLAIN	LLDDPPALDAA--	LDHLAHQHEV	REG-V	QKAHFK*KFGEILATGLPQV	---L----	DDYDALAHKSCLEKILTKISS
At	NSK LRGH AI TLTYA LDWFDV	SLDDPSRLKCV--	VEKFAVNHN	AK--I	SGDAFG*SIIPEMKETLKAR	-MGSY----	SDDVGAHVQAILGMQNAVLIS
At4	DER FISH MA NVLGG FDTLLQ	-LDSSYFIYS--	LRNLGDAHIQ	AK--A	GTQHFR*SFEEILIPILQES	-NQGL----	DAASVEAWKFFDVSTIGVIAQ
At5	TDA PHYH IS LVHNR FSSIOK	-IDNVSEVYL--	LKKLGRHRIK	RG--L	SRKQFD QFVELYIABISSE	---VL----	SDTGRNGLEKVLTFATGVIEQ
Br1	NKK LNCH GI TLHYA LMMFVD	DLDSSKDLLEDV--	CRKFAVNHVI	RG--V	LDVKFG*HIKEPMAELLRRK	---CGNDC-	-DDAIQAWHKLIDVICAVLKE
Br2	NKK LNCH GI TLHYA LMMFVD	GLONKIDLEDV--	CRKFAVNHVN	RG--V	LDVKFG*HIKEPMAELLRRK	---CGQTC-	TDQHIQAWHKLIDVICAVLEE
Pd1	DPF FIKQ*GQ NILLA CHVLCA	TYDDRETFDAY--	VGELMARHER	DHVKI	PNDVHN*HFWHEHFIETFLGSK	---TTL----	DEPTKHAHQEIGKEFSHEISH
Pd2	DAF FVKQ GH KILLA LRMICS	SYDDEPTFDYF--	VDALMDRHIC	DDIHL	PQEQWH EFWKLFAYELNEK	SHQHL----	TEAEKHAHSTIGEDFAHEADK
Ce	SER*FDKQ GQ RILLA CHLLAN	VYTNEEVFKGY--	-RETINRHRI	--YKM	DPALWM AFFTVFTGYLESV	---CGL----	NDQQAAMMALGKEFNABESQT

	HC
	1
	12345678901234567890
MbW	KYKELGYQG
Gm	A
Ct4	KM
Ct2	YL
Lt	RLNA
At	AL
At4	GLKV
At5	GL
Br1	SK
Br2	KK
Pd1	H
Pd2	HAKAEKDHIIEGEGHKEEHH
Ce	HLKNSNLPVH

Fig. 1. Alignment of non-vertebrate globin sequences showing the intron locations. Sequences were aligned using FASTA [9] and the template of Bashford et al. [10]. ▼ = intron position; # = precedes coding sequence; {} = leader sequence; [] = H helix of preceeding repeat Mb = Sperm whale myoglobin [1]. Gm = *Glycine max* globin [14,33]. Ct4 = *Chironomus thummi* globin IV [11,32]. Ct2 = *Chironomus thummi* globin IIb [11,27]. Lt = *Lumbricus terrestris* globin [12]. At = *Anadara trapezia* minor globin [13,31]. AT4 = *Artemia* globin repeat T4 [17,23]. AT5 = *Artemia* globin repeat T5 [17,23]. Br1 = *Barbatia reeveana* globin repeat 1 [5]. Br2 = *Barbatia reeveana* globin repeat 2 (5). Pd1 = *Pseudoterranova decipiens* globin repeat 1 [4,16]. Pd2 = *Pseudoterranova decipiens* globin repeat 2 [4,16]. Ce = *Caenorhabditis elegans* globin [6,7]. (Deduced from genomic DNA sequence data obtained as a partial result of the *C. elegans* genome sequencing project (cosmid zk 637; PIR/NBRF Accession number I39344) [6,7]).

cate F8 His and its flanking sequence to one side, and the H helix with its characteristic H8 Trp to the other side. The steric compensation of the bulky side chain of G9 Trp by the single hydrogen side chain of H12 Gly has been discussed previously [17,21]. The alignment in Fig. 1 is in accordance with Dixon et al. [4] but the unconventional nature of the 5-residue AB turn is further discussed below with the interpretation of the intron structure.

3. ORGANIZATION OF GLOBIN GENES

Cosmid zk 637 has an insert sequence that can be identified as globin like [6,7]. Analysis of this putative globin gene confirms the presence of a TATAA, a CAATA and a polyadenylation signal sequence as well as start and stop codons; therefore it contains the neces-

sary elements of a functional gene. A single intron of 298 bases is present, having the characteristic nematode splicing consensus sequences [22] (Figs. 1 and 2).

The T4 repeat gene of *Artemia* globin contains a single intron of about 1,000 bases, in addition to inter-domain introns located between domains T3 and T4 and between T4 and T5 (Figs. 1 and 2). All introns show the consensus splicing sequences [23].

Having correlated the gene sequence data with structural features of the protein, we can now evaluate the intron locations. Two classes of intron location are recognized, namely (A) intra-repeat and (B) inter-repeat.

3.1. Intra-repeat introns

The intron positions in invertebrate globin B and G helices, determined so far, are precisely conserved at the B12/B13 and the G6/G7 location (Fig. 1). In contrast

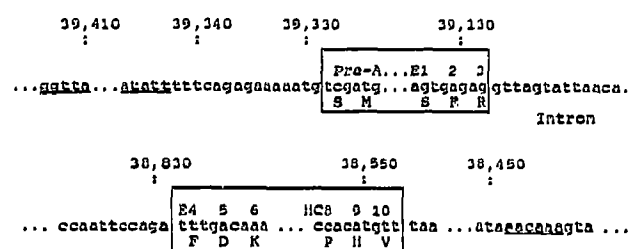
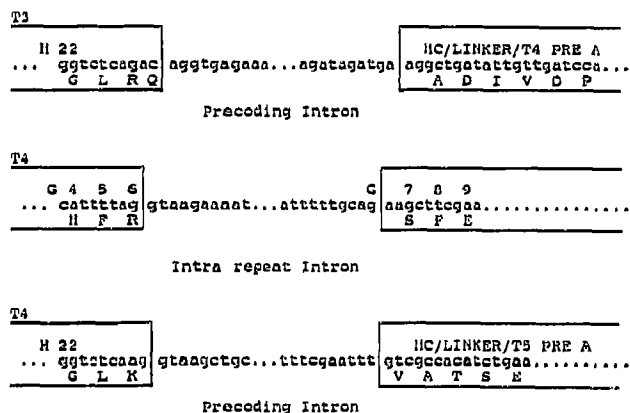
A: *CAENORHABDITIS ELEGANS* GLOBIN GENE.B: *ARTEMIA* T3/T4/T5 GLOBIN GENE REPEATS.

Fig. 2. Gene organization of the *C. elegans* globin and of repeat T4 of *Artemia*. (A) Partial nucleotide sequence of the *C. elegans* globin gene showing regulatory sites (underlined) and splice junctions. Exons are boxed. Numbers refer to the sequence of the template strand insert in cosmid zk 637 [6,7]. Deduced coding strand sequence is shown for convenience. Helix notification as in Fig. 1. Amino acids are given by the single letter code underneath the second base of each codon. (B) Partial nucleotide sequence of *Artemia* T3/T4/T5 globin repeats showing splice junctions. Exons are boxed. Helix notification as in Fig. 1.

the location of the intron within the E helix seems to be variable. The central intron in plants is inserted at E14–15, which is 4 residues after the position predicted by G6 [24,25]. This intron separates the haem binding region into two structural units, F2 and F3 that make contact with haem from opposite sides [24]. In *P. decipiens* the equivalent central intron is inserted at E7–E8 (CAA/Q G[intron]GT/G CAA/Q) [24]. The equivalent *C. elegans* intron is inserted at a still more anterior position E3–E4. It is tempting to speculate that it has migrated secondarily from its ancestral more distal position, which from E7 now reads: (CAA/Q GGC/G CAA/Q) and is coincident with the splice site in the *P. decipiens* gene. Similar intron sliding is observed in other genes such as triose phosphate isomerase [26]. In these two globins the nucleotide sequences differ by only one base in this region.

Intron insertion may differ by as much as six codons [2], however the conservation of the insertion positions in the B and G helices contrasts with the variation in the E helix.

In contrast to all other *Chironomus* globin genes sequenced so far, a single intron is present within the E helix (E9–E10) of the gene coding for globin IIB [1,2,27]

Single introns are also present in the B helix of *P. decipiens* repeat 2 and in the G-helix of *Artemia* repeat T4 [23].

3.2. Inter-repeat introns

In addition to the intra-repeat introns described above, the multi-repeat globin genes of *Artemia*, *Barbaltia* and *Pseudoterranova* have inter-repeat introns separating the individual globin units (Fig. 1). From an evolutionary point of view, intra- and inter-repeat introns are not equivalent. Intra-repeat introns represent functional regions of the protein in the sense of the mini-gene hypothesis of Gilbert [28] and thus refer to the primordial gene organization. Inter-repeat introns have accompanied gene duplication events and can thus be considered as secondarily acquired and much more recent.

The presence of inter-repeat introns may help us to assign structure to the N-terminal region of the nematode sequences, which align well with each other but are difficult to reconcile with other globins in the A helix and AB turn. A 5-residue AB turn is not known structurally in globins and the resulting alignment places an intron illogically in the A-helix [4]. The emerging precedent is for introns to be located between functional repeats, e.g. in the *Artemia* globin gene at both ends of domain T4 where the clear alignment of the H and A helices and the length of the linkers leave no doubt that this is the case. An attractive alignment is obtained by modelling the nematode sequences on *Chironomus* globin IIB in which the start of the B helix is shortened by 4 residues (Table I). This places favourable AsX residues at the start of the B helix and logically positions the inter-repeat intron before an inter-repeat linker of about 6 residues

4. GLOBIN GENE EVOLUTION

The globin gene organization found in invertebrates is compatible with the idea of an ancestral gene containing 4 exons and 3 introns, with a tendency for introns to have been lost during evolution (Fig. 3) [1,2]. This arrangement appears to have been retained in known plant globins and in the first repeat of the *P. decipiens* gene whereas all introns except one have been secondarily deleted from the second repeat. Only a single intron has been retained in the E helix of *C. elegans* and in the *Chironomus* IIB gene. This intron is equivalent to the ancestral and plant central introns.

It might be argued that these introns are a more recent acquisition and that the ancestral globin gene contains two introns only (in the B and G helices). However novel intron insertion would be opposed to the general evolutionary tendency of intron reduction.

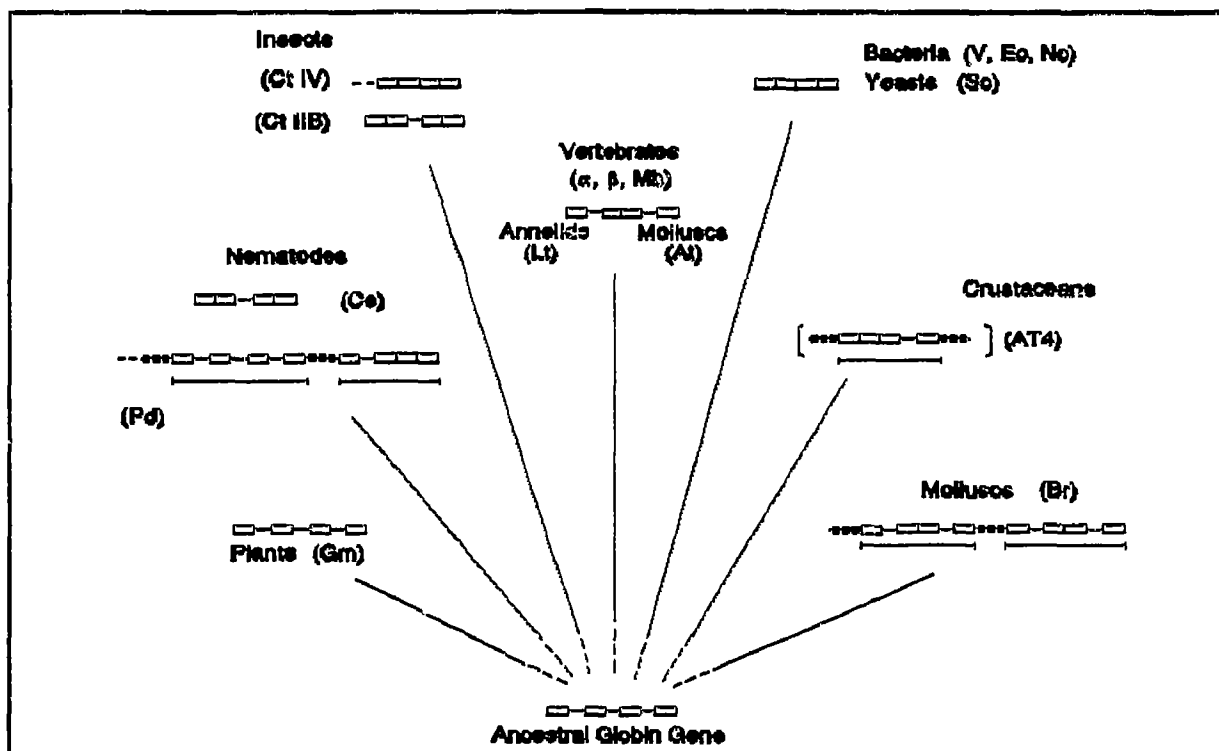


Fig. 3. Evolution of globin gene arrangement. This figure illustrates how the array of globin gene structures found in extant species can all be derived from a single ancestral 4 exon/3 intron configuration. A general tendency of rejecting introns is obvious. Note that the diagram depicted is not a phylogenetic representation. Signal sequences = ---. Exon = closed box. Intron: intra-repeat intron = ---, Inter-repeat intron = -.-.-. In multi-repeat structures the repeats are underlined. Vertebrates (α, β, Mb) [2]. Bacteria (V = *Vitreoscilla*) [34]. (Ec = *Escherichia coli*) [30]. (Nc = *Nostoc commune*) [35]. Yeast (Sc = *Saccharomyces cerevisiae*) [29]. Other abbreviations as in Fig. 1.

Moreover, the occurrence of the central intron conceivably hinders the concerted evolution of the haem binding region. Therefore, it is more likely that it would be eliminated in the course of evolution instead of being acquired.

The conservation of intra-repeat introns in the B and G helices of *Pseudoterranova* (repeat 2) and *Artemia* (T4) genes at the conserved positions supports their

derivation from the ancestral pattern (Fig. 3). Intron deletion in the globins of bacteria, yeasts and *Chironomus* (except *IIB*) has reached the stage where there are none left [2,29,30,35].

The ancestral Hb molecule was presumably an intracellular molecule, since it is present in yeasts, bacteria and protozoa. Derivation of extracellular globins would have required particular adaptations: e.g., the recruit-

Table I
Alternative alignment of nematode A and B helices based on the location of inter-repeat introns

	HC/Linker/Pre-A 1 123456789012	A 1 1234567890123456	AB	B 1 1234567890123456
Mbw	V-L	SEGEWQLVLHVWAKVE	A	DVAGHGQDILIRLFKS
Ct3	L	SADQISTVQASFDKVK	G	----DPVGILYAVFKA
As1	NKT-RELCM	KSLEHAKVDTSNEARQ	-	----DGIDLYKHMFEFEN
As2	NKHGRHQCM	RSLQHIDIGHSETAKQ	-	----NGIDLYKHMFEFEN
Pd1	SKT-RELCM	KSLEHAKVGTSKEAKQ	-	----DGIDLYKHMFEFEN
Pd2	RHSVRDHCM	NSLEYIAIGDKEHQKQ	-	----NGIDLYKHMFEFEN
Ce	SMNRQEISDLVCV	KSLEGRMVGTEAQNIE	-	----NGNAFFRYFFFTN
Tc	AKSDEEIRKDAL	SALDVVPLOSTPEKLE	-	----NGREFYKYFFFTN

Ct3, *Chironomus III*; As1 and 2, *Ascaris suum* repeat 1 and 2; Tc, *Trichostrongylus colubriformis* globin (36); rest of abbreviations as in Fig. 1.

ment of a leader sequence and an increase in M_r to minimise excretion.

The equivalence of the inter-repeat introns suggests that they are derived from an original intron separating the leader sequence from the globin coding sequence. A potential mechanism has been described for the *Barbatia* globin gene [5].

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