

Cloning of segment polarity gene homologues from the unsegmented brachiopod *Terebratulina retusa* (Linnaeus)

Peter W.H. Holland, Nicola A. Williams and Jeremy Lanfear*

Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

Received 8 August 1991

We have used the polymerase chain reaction (PCR) to amplify, clone and sequence homologues of the *Drosophila* segment polarity genes *engrailed* (*en*), *cubitus interruptus* *Dominant* (*ci^D*) and *wingless* (*wg*) from the genome of the brachiopod, *Terebratulina retusa* (Linnaeus). The deduced translation products of brachiopod *en* and *ci^D* share high levels of sequence identity with their *Drosophila* homologues. The brachiopod *wg*-related clone is divergent from *Drosophila* *wg*, although clearly a member of the *wg/Wnt* gene family. These results indicate that structural diversity of *Drosophila* segment polarity genes has been evolutionarily conserved in a divergent, ancient and unsegmented animal phylum.

Engrailed, Homeobox, Zinc finger gene, *Wingless*, Brachiopod, Molecular evolution

1 INTRODUCTION

The establishment of the segmental body pattern during embryogenesis in *Drosophila melanogaster* involves the sequential activation of several groups of genes, each group leading to the division of the embryo into progressively smaller units [1,2]. The 'segment polarity' genes represent the final tier of this genetic cascade, being responsible for establishing and maintaining the spatial limits and polarity of the metameric units. The *Drosophila* segment polarity gene group may be subdivided both functionally, on the basis of mutant phenotypes, and structurally, since the genes encode a wide diversity of protein products [2].

Putative homologues of several segment polarity genes have been reported from other organisms, including vertebrates, but in most cases the phylogenetic distribution of the genes is poorly known [2]. In particular, it is not known whether the structural diversity of segment polarity genes seen in *Drosophila* has been evolutionarily conserved in a diversity of animals, including in the many groups of unsegmented animals which may be employing these genes for distinct roles.

In an attempt to address this question, we have investigated whether the genome of a representative species of the Brachiopoda, a phylogenetically ancient and divergent phylum of unsegmented animals, contains ho-

mologues of three *Drosophila* segment polarity genes. The strategy we adopted is based on the use of degenerate oligonucleotide primers in the Polymerase Chain Reaction (PCR) to amplify related sequences, prior to recombinant DNA cloning. We report the isolation and sequence determination of brachiopod genomic DNA clones derived from genes homologous to three functionally and structurally divergent *Drosophila* segment polarity genes, a homeobox gene, *engrailed* (*en*), a zinc-finger gene, *cubitus interruptus* *Dominant* (*ci^D*); and a gene coding for a secreted protein, *wingless* (*wg*).

2 MATERIALS AND METHODS

Genomic DNA extraction, purification, PCR, cloning and sequencing were as previously described [3,4]. The PCR primer sequences used were *en*, primers A and C of Holland and Williams [5], *wg* primers of Gavin et al. [6], *ci^D*, 5'-GAGAGGATCCNITTYAARGCN-CARTAYATG-3' and 5'-GAGAAGCTTRTGNACNGTYTFN-ACRTGYTT-3', designed by Drs P.W. Ingham and G. Paterno (ICRF/DBU Oxford, UK) to complement conserved regions between the *Drosophila* *ci^D* and human *GLI* genes.

3 RESULTS AND DISCUSSION

3.1. Cloning of a brachiopod *en* gene homologue

PCR-mediated amplification of brachiopod DNA was performed using primers complementary to conserved regions within, and downstream of, the *en* homeobox. Following isolation and cloning of the major band, 11 recombinants were sequenced, and found to derive from the same homeobox gene (Fig. 1A). The deduced translation product of the cloned region shares 77% sequence identity with *Drosophila en* (Fig. 1B), and comparable identity with vertebrate *en* related genes [5].

*Present address: Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, UK

Correspondence address: P.W.H. Holland, Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK. Fax (44) (865) 310 447

A
 Brachiopod engrailed homologue
 A AAC GAA CAG CTC GCC AGA CTG AAA AAA GAA TTC GAA ATA AAC AGA TAC TTG
 ACT GAA CAG AGA AGA CAA GAA CTC TCA CCG GAG TTG ATG CTA AAC GAG AGT CAA
 ATT AAA ATT TGG TTC CAG AAC AAG AGA GCA AAG TTG AAG AAA TCA ACT GGG ACA
 AAG AGT GGT CTG GCA TTG CAC

B
 Brachiopod
 1 NEQLARLKKPEINRYLTFRROELSRELMHLESQIKINFONKRAKLNKSTGKSGALAH
 20 R-----R-NE-----R--Q--S--G---A-----I-----S-NP----Q
 40
 60

Fig 1 (A) Consensus nucleotide sequence (internal to primers) from brachiopod *en* homologue (B) Deduced amino acid sequence from (A), aligned with *Drosophila en*. Dashes indicate identity with *en*

3.2. Cloning of a brachiopod *ci^D* gene homologue

Following amplification of brachiopod DNA with primers within the zinc-finger region of *ci^D*, a single band was produced, found to hybridize to a putative *ci^D* homologue from *Xenopus laevis* (not shown), and cloned. The DNA sequence of a single recombinant is shown in Fig 2A. Alignment with *Drosophila ci^D* [7] and 3 putative human homologues (*GLI*, *GLI2*, and *GLI3* [8]), suggests that the brachiopod clone derives from a true *ci^D* homologue and contains 2 introns. Interestingly, both intron positions have been highly conserved, being identical in brachiopod, beetle, human and nemertean ([8] and J.L., unpublished data).

The deduced translation product of the cloned region shares 91% sequence identity with *Drosophila ci^D*, revealing exceptional evolutionary conservation of *ci^D* compared to other zinc-finger gene families ([9] and J.L., unpublished data)

3.3. Cloning of a brachiopod *wg* gene homologue

PCR-mediated amplification of brachiopod DNA was performed using primers complementary to a conserved region shared by *Drosophila wg* and two vertebrate *Wnt* genes (putative *wg* homologues [6,10]). The major product was cloned, the DNA sequence of a single recombinant is shown in Fig 3A. Alignment of the deduced translation product of this clone with *Drosophila wg* (Fig. 3B) reveals identity of only 46% (excluding an additional stretch of 85 amino acids in *wg*). We

A
 Brachiopod cubitus-interruptus Dominant homologue
 CTG GTT GTA CAT ATG AGA COT CAT ACT GGA GAA AAA CCT CAC AAA TGC ACG gta
 agggootttgtgattatcaggggocctgtttgtggtatcttttgcctccctgctcagctatgaaatgccc
 atcaaatcgactgactgaaatgcttgaatttatctttttgtag TTT GAG GGT TOT CAG AAG GCC TAC
 AGT CGA CTG GAG AAC CTA AAA ACC CAC CTG AGO TCA CAT ACT GGG GAA AAA CTA
 TAT ATG TGT GAA TTC CCA GGC TOT GCO AAG GCC TTC AGC AAT GCT TCA GAC CGA
 GCC AAG CAC CAG AAC ACG ACA CAT TCT AAT GCT ctgagctatgtaccggaggatgata
 totattaccatagagtagagggattttatgtatctatctacccaattgcatgcaactttggaatacta
 catctatgggtgatcaatgtttttgtttatataatattgtatcttcaatgattatcaatggtgataacatg
 tttccactatataatcaatgaatgacttgcttatataatctggatataatgataatgataataataatata
 atataatag AAA CCC TAT GTG TGC AAA GCA GCC GGC TOT ACC AAG AGA TAC ACT
 GAC CCC AGC TCA CTG AAG

B
 Brachiopod
 1 LVVIGRRRHTGSEKHKCTFECCQKAYSRLNLNHLRSHGTGKLYNCSPPQAKAFSHASD
 20 -----P-T--Y--S-----
 40
 60
 81 RAKHQWRTHSHAKPYVCKAAGCTKRYTDPSSLR
 80
 Drosophila -----E--I--P-----

Fig 2 (A) Nucleotide sequence (internal to primers) from clone of brachiopod *ci^D* homologue. Lower case letters are putative intron sequence (B) Deduced amino acid sequence from exon regions of (A), aligned with *Drosophila ci^D*. Dashes indicate identity with *ci^D*.

A
 Brachiopod wingless homologue
 GGT GTG AGT GGG TCG TCT ACA ATG AAG ACA TGC TGG TCT AAA CTG TCG CCT TTC
 AGA GTG GPT GGA ACT CAC CTG ATG AAG CGC TAC CTG AAG GCC ABA CAC GTG GCC
 ACT ATA AAG GGC CCG AGG AGG CCG GTC TTC CTA AAA CTC AAA CGT TCC CGG AGG
 CCA AAT AAA AAA CCG AGA AAA AGA GAC TTC GTT TAC TTA CAA AAA TCC CCC AAC
 TAC TGT GAC AGG GAC GTT AAG AAG GGC TGA CTG GGC ACC CAC GGA CGA CTA TGC
 AAT AAG ACG TGT CCA GGC ACA GAT GGC TGT AAC CTG CTC TGT TGT GGC AGG GGA
 TAC AAC ACA CAC CTC AAA CCA AAG ACG TGG CAA TCC TAC TGC AAG

B
 Brachiopod
 1 CVSGSCTMKTCNSKLSPPFRVVGTHLHKRYLKAHV ATI
 Drosophila -H-----V----MR-AN---I-DN-KA-FDG-TR-QV-NSLRATNALAPVSPNAAGSNV
 1
 60
 Brachiopod
 Drosophila GSNGLIIPQSGLVYGEERHNLNHDHMPDILLENSHPISKIHHPNMPSPNSLPQACGRGGR
 61
 120
 Brachiopod
 Drosophila KGRRRRVF LKLKSRPRPKKPRKRDVLYLQKSPNYCDRDUVKKGLGTHGRLCNKT
 93
 AGRRG--KHNRYHFQ-NPHNPEH-P-GSK----EP--SF-EKNLRQ-I-----Q--E--
 121
 180
 Brachiopod
 Drosophila CPGTDCCNLCCGRGYNHLKPKTTWQCYCK
 123
 SL-V---G-H-----RRDEWVVCV-A-T
 181
 210

Fig 3 (A) Nucleotide sequence (internal to primers) from clone of brachiopod *wg* homologue (B) Deduced amino acid sequence from (A), aligned with *Drosophila wg*. Dashes indicate identity with *en*, blanks indicate gaps introduced to maximize alignment.

believe that this clone does derive from a true brachiopod *wg/Wnt* homologue, since the deduced translation product shares all of the 33 amino acid residues which are invariant between *wg* and 9 mouse *Wnt* genes in this region [6,10]

3.4. Diversity of brachiopod segment polarity gene homologues

Homologues of the *Drosophila* segment polarity gene *en* have been cloned from representatives of several taxa, including arthropods, annelids, nematodes, echinoderms and vertebrates [5,11-14]. However, homologues of the structurally divergent segment polarity genes *ci^D* and *wg* have been reported from fewer organisms [2,6,8,12].

The *Terebratulina en*, *ci^D* and *wg* homologues described here represent the first protein-coding genes to be cloned from a member of the Phylum Brachiopoda. Their identification demonstrates that divergent segment polarity genes are conserved in at least insects, vertebrates and brachiopods, suggesting that functional interaction between these genes, as seen in *Drosophila*, may be evolutionarily ancient. However, since the Brachiopoda are unsegmented metazoa, any developmental roles of these genes are likely to be fundamentally different from those in *Drosophila*.

Acknowledgements We thank Gary Paterno, Phil Ingham and Uwe Strähle for *ci^D* and *wg* primers. This work was supported by SERC Grant GR/I1507 6 to P.W.H. and N.A.W. and an NERC studentship to J.L.

REFERENCES

[1] Akam, M. (1987) Development 101, 1-22
 [2] Ingham, P.W. and Nakano, Y. (1990) Dev. Growth Differ. 32, 563-574

- [3] Holland, P W H and Hogan, B L M (1986) *Nature* 321, 251-253
- [4] Holland, P W H (1991) *Gene* 98, 253-257
- [5] Holland, P W H and Williams, N A (1990) *FEBS Lett* 277, 250-252
- [6] Gavin, B J, McMahon, J A and McMahon, A P (1990) *Genes Dev* 4, 2319-2332
- [7] Orenic, T V, Slusarski, D C, Kroll, K L and Holmgren, R A (1990) *Genes Dev* 4, 1053-1067
- [8] Ruppert, J M, Kinzler, K W, Wong, A J, Bignor, S H, Kao, F-T, Law, M L, Seunanez, H N, O'Brien, S J and Vogelstein, B (1988) *Mol Cell Biol* 8, 3104-3113
- [9] Lanfear, J and Holland, P W H (1991) *J Mol Evol* 32, 310-315
- [10] Rijsewijk, F, Schuermann, M, Wagenaar, E, Parren, P, Weigel, D and Nusse, R (1987) *Cell* 50, 649-657
- [11] Dolecki, G J and Humphreys, T (1988) *Gene* 64, 21-31
- [12] Kamb, A, Weir, M, Rudy, B, Varmus, H and Kenyon, C (1989) *Proc Natl Acad Sci USA* 86, 4372-4376
- [13] Patel, N H, Martin-Blanco, E, Coleman, K G, Poole, S J, Ellis, M C, Kornberg, T B and Goodman, C S (1988) *Cell* 58, 955-968
- [14] Wedeen, C J, Price, D J and Weisblat, D A (1991) *FEBS Lett* 279, 300-302