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

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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 evolutionary 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 homologues 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)*.

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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'-GAGAGGATCCNTTYAARGCN-CARTAYATG-3' and 5'-GAGAAGCTTRTGNACNGTYTTN-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].

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 CGG 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

```
           1                  20                  40                  60
Brachiopod NEQLARLKKEFEINRYLTEQRRQELSRELMLNESQIKIWFQNKRAKLKKSTGrKSGLALH
Drosophila S-------R--NE------R---Q--S--G---A------------I-----S-NP---Q
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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 CGT CAT ACT GGA GAA AAA CCT CAC AAA TGC ACG gta
agggccttttgctgattcagggccactgtatggtgatatcttgtgatatcaccatgtcagatatgaaatgcc
atcaacaatcgactgaatgttaaattatattttttgtag TTT GAG GGT TGT CAG AAG GCC TAC
AGT CGA CTG GAG AAC CTA AAA ACC CAC CTG AGG TCA CAT ACT GGG GAA AAA CTA
TAT ATG TGT GAA TTC CCA GGC TGT GCG AAG GCC TTC AGC AAT GCT TCA GAC CGA
GCC AAG CAC CAG AAC AGG ACA CAT TCT AAT GCT gtgagttatgtacccgtgggtatgata
tctattaccataggcgtagtagggtatttattgcatatactacccaatggcatgtgacttttggaatccta
cctctatgggtagtaatgttttgttatataatattgatatttaataagtgtatcacttgtgtgataacatg
ttgtcaccctcatcattcatgaaatgacttgcctctataatctgctatacatgtagctaatatatatcaatcta
atatatctag  AAA CCC TAT GTG TGC AAA GCA GCC GGC TGT ACC AAG AGA TAC ACT
GAC CCC AGC TCA CTG AGG
```

B

```
           1                  20                  40                  60
Brachiopod LVVHGRRHTGEKPHKCTFEGCQKAYSRLENLKTHLRSHTGEKLYMCEFPGCAKAFSNASD
Drosophila -----------------------Y-----------------------F-T--Y---S--------

          61                  80
Brachiopod RAKHQNRTHSNAKPYVCKAAGCTKRYTDPSSLR
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 TGT ACA ATG AAG ACA TGC TGG TCT AAA CTG TCG CCT TTC
AGA GTG GTT GGA ACT CAC CTG ATG AAG CGC TAC CTG AAG GCC AAA CAC GTG GCC
ACT ATA AAG GGC CGC AGG AGG CCC GTC TTC CTA AAA CTC AAA CGT TCC CGG AGG
CCA AAT AAA AAA CCG AGA AAA AGA GAC TTG GTT TAC TTA CAA AAA TCC CCC AAC
TAC TGT GAC AGG GAC GTT AAG AAG GGC TCA 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

```
           1                                     38
Brachiopod GVSGSCTMKTCWSKLSPFRVVGTHLMKRYLKAKHV ATI
Drosophila -M-----V----MR-AN---I-DN-KA-FDG-TR-QV-NSLRATNALAPVSPNAAGSNSV
           1                                                          60

Brachiopod
Drosophila GSNGLIIPQSGLVYGEEEERMLNDHMPDILLENSHPISKIHHPNMPSPNSLPQAGQRGGR
          61                                                         120

             39                                                      93
Brachiopod    KGRRRPVF LKLKRSRRPNKKPRKRDLVYLQKSPNYCDRDVKKGSLGTHGRLCNKT
Drosophila NGRRQ--KHNRYHFQ-NPHNPEH-P-GSK-----EP--SF-EKNLRQ-I------Q--C-
         121                                                          180

          94                         123
Brachiopod CPGTDGCNLLCCGRGYNTHLKPKTWQCYCK
Drosophila SL-V---G-M------RRDEVVVCR-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*.

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