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Cell lineage and cell fate in crustacean embryos – a comparative approach

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ABSTRACT Malacostracan crustaceans undergo a complex and stereotyped cleavage pattern during formation of and segmentation in their post-naupliar germ band. This pattern has been studied in several malacostracan representatives with respect to morphogenesis and expression of the *engrailed* gene. Although this cell lineage pattern is specific and invariant in each species, comparative analyses reveal subtle differences between different parts of individual germ bands and between germ bands of different species. We conclude that despite the elaborate cleavage pattern, cell fate specification is not closely linked to cell lineage. Furthermore, some aspects of the evolutionary alterations of germ band formation and segmentation in annelids and arthropods are discussed.

KEY WORDS: *engrailed, Malacostraca, clonal analysis, segmentation, germ band*

Introduction

Invariant cell division patterns during animal development have always been fascinating for biologists. The regularity of developmental processes repeated exactly in each individual ontogeny possesses an aesthetic attraction of its own. Furthermore, the patterns of some cell lineages can be used as complex characters to reconstruct phylogenetic relationships as in the case of the spiralians which are unified by their specific cleavage type. For the developmental biologist, however, the study of invariant cell lineages might play an important role for the solution of one of the central problems in developmental biology. What mechanisms lead to the commitment of cell fate during ontogeny? The occurrence of invariant cell lineages provides an attractive hypothesis as a solution to this problem. It has almost been taken for granted that in animals where fixed and stereotyped cell lineages occur, these lineages are linked to cell fate commitment (e.g. Sternberg and Horvitz, 1981; Zackson, 1984). According to this view, the individual cells gain their information as to "what to do" with their origin and then act autonomously unaffected from influences of their surroundings. However, there is increasing evidence that this view might oversimplify the course of events. Recent experimental (e.g. Schierenberg, 1987; Schnabel, 1994) and comparative (e.g. Dohle, 1989b; Skiba and Schierenberg, 1992) studies show that even in the development of nematodes, one of the classical examples for lineage specific cell fate commitment, cell-cell interactions play a much more important role than previously thought.

The present review deals with the comparative analysis of the cell lineage during germ band formation and segmentation of malacostracan crustaceans. We present evidence for the morphological and the molecular levels that despite the elaborate cell division patterns in the malacostracan germ band (for review see Dohle and Scholtz, 1988), cell lineage and differentiation are not closely linked.

The method

There are several approaches to the problem of cell fate commitment. Experimental studies use mutants or microsurgical manipulations such as cell ablation, cell transplantation or deletion to study the causal relationship between developmental processes. We agree with Wilhelm Roux (1907), one of the pioneers of experimental developmental biology, that the description of the normogenesis in one species does normally not allow conclusions about developmental mechanisms. The comparative descriptive approach, however, takes advantage of experiments carried out by the evolution. The "mutants" produced by the evolutionary process are sometimes very subtle and they have the advantage that they are not lethal in any stage of development. Furthermore, artifactual side effects caused by experimental manipulations are avoided. The comparison of related species reveals mosaics of similarities and differences. This mosaic pattern allows conclusions about the independence of single developmental events on all levels such as gene expression, specific mitotic patterns, the arrangement of cells, the origin of germ layers, and morphogenetic events. It can be shown which steps are not necessarily coupled with preceding or subsequent steps. In addition to conclusions about developmental

Abbreviations used in this paper: en, engrailed; ET, ectoteloblasts.

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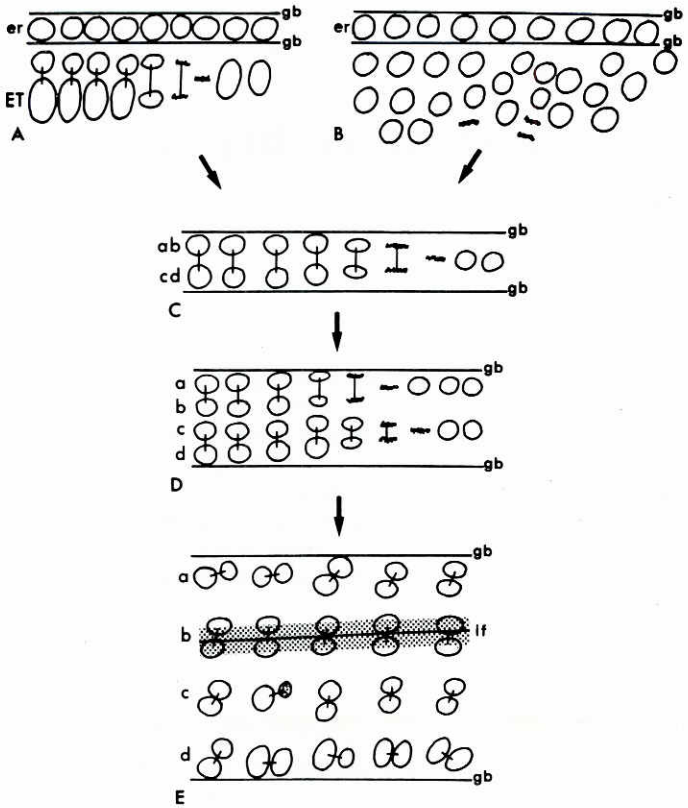

Fig. 1. Schematic summary of row formation and segmentation in the post-naupliar germ band of malacostracan crustaceans *(comp. Fig. 2). Only the animal's left side is shown. The midline of the germ band is on the left side. The transverse lines indicate the genealogical boundaries (gb) between the ectoderm rows. The transverse ectoderm rows are formed either by ectoteloblasts (ET), a condition found in the posterior part of most peracarids and the decapod examined* **(A)** *(these rows are designated by Roman numerals) or by scattered blastoderm cells* **(B)** *(Arabic numerals) a condition found in the anterior rows (E(0) to E(3)) of most peracarids and the decapod and in the entire post-naupliar germ band of amphipods. After formation, each row (from E(2) on - the rows in front of E(2) show a somewhat different pattern) undergoes two mediolateral mitotic waves with only longitudinal-oriented and equal mitoses, resulting in four transverse descendant rows named a, b, c, d* **(C,D)**. *Thereafter, the differential cleavages begin. They show a stereotyped pattern of mitoses with regard to size and position of the division products.* **(E)** *A simplified schematic pattern of the first differential cleavage up to the fifth cells from the midline. Some characteristics of the individual mitoses differ among the investigated species, a phenomenon not shown here (for comparison see Dohle, 1989b; Scholtz, 1984). With the differential cleavages, segmentation begins. The segment boundary (shaded area) marked by the intersegmental furrow (if) does not match the genealogical border (transverse lines). The intersegmental furrow runs transversely and slightly obliquely through the descendants of one ectoderm row in the area of descendant rows a and b. Thus, the descendants of each ectoderm row contribute to two segments.*

mechanisms, the comparative approach allows one to trace the relative sequence of evolutionary alterations in developmental steps. Moreover, comparative developmental biology provides complex characters which can be used for the reconstruction of phylogenetic relationships between organisms. Finally, it contributes to the theoretical problems of the homology concept.

The limitations of the comparative approach lie in the fact that only negative statements can be made. We can only say which processes are not linked. We cannot say one developmental event is the cause of the next following event. Nevertheless, we believe that the comparative approach is an important and necessary tool for the analysis of developmental mechanisms and that it provides hypotheses which can be tested experimentally.

Cell lineage during germ band formation and segmentation of malacostracans

Malacostracan crustaceans are unique amongst arthropods in exhibiting a complex stereotyped and iterated cell division pattern in the ectoderm and mesoderm of the posterior (post-naupliar) part of the germ band (Figs. 1, 2). This pattern allows one to trace the fate of individual cells during germ band proliferation and segmentation up to the formation of neuroblasts in the ganglion primordia and of limb buds. Representatives of the Peracarida (Cumacea: Dohle, 1970, 1976; Isopoda: Hahnenkamp, 1974; Vehling, 1994; Tanaidacea: Dohle, 1972; Mysidacea: Scholtz, 1984; Amphipoda: Scholtz, 1990) and Decapoda (Scholtz, 1992) have been investigated with respect to their cell lineage. In the ectoderm, the events during germ band formation and segmentation are characterized by three major steps which are common to all species examined (Figs. 1, 2, 3B): 1) The successive formation of regular transverse cell rows. 2) The two mediolateral mitotic waves in each of these rows resulting in four aligned descendant rows of cells. 3) The differential cleavages of the row derivatives during segment formation. These differential cleavages are characterized by a stereotyped pattern of mitoses with regard to size and position of the daughter cells.

One striking feature of the cell division pattern in malacostracans is the fact that the segment borders do not match the genealogical borders. The intersegmental furrow runs transversely and slightly obliquely through the derivatives of one ectoderm row (Figs. 1E, 2D). Thus, the ectoderm rows and their descendants form units which can be compared to the parasegments found in *Drosophila* (Dohle and Scholtz, 1988; Patel *et al.*, 1989a).

The comparison between different parts of individual germ bands and between the germ bands of the various species examined has revealed subtle differences in the patterns of all three steps which have consequently led to the following conclusions: 1) Ectodermal row formation is independent of teloblast activity. Whereas most malacostracans possess ectoteloblasts (Figs. 2B, 3B) which give rise to the ectoderm rows by unequal mitoses, amphipods lack ectoteloblasts entirely (Fig. 2E). In amphipods, the ectoderm rows are formed by scattered ectoderm cells which become arranged into regular rows homologous to the rows formed by ectoteloblasts in other species (Figs. 1, 2E). A similar phenomenon occurs in the anterior region of the post-naupliar germ band of species provided with ectoteloblasts. In these species, several rows are formed by cells not originating from the ectoteloblasts (Fig. 2B,C). 2) The further fate of the ectoderm rows is independent of their origin. All rows, regardless of their generation by ectoteloblasts or scattered blastoderm cells, undergo a very similar set of divisions. The two mitotic waves and subsequently the differential cleavages reveal an

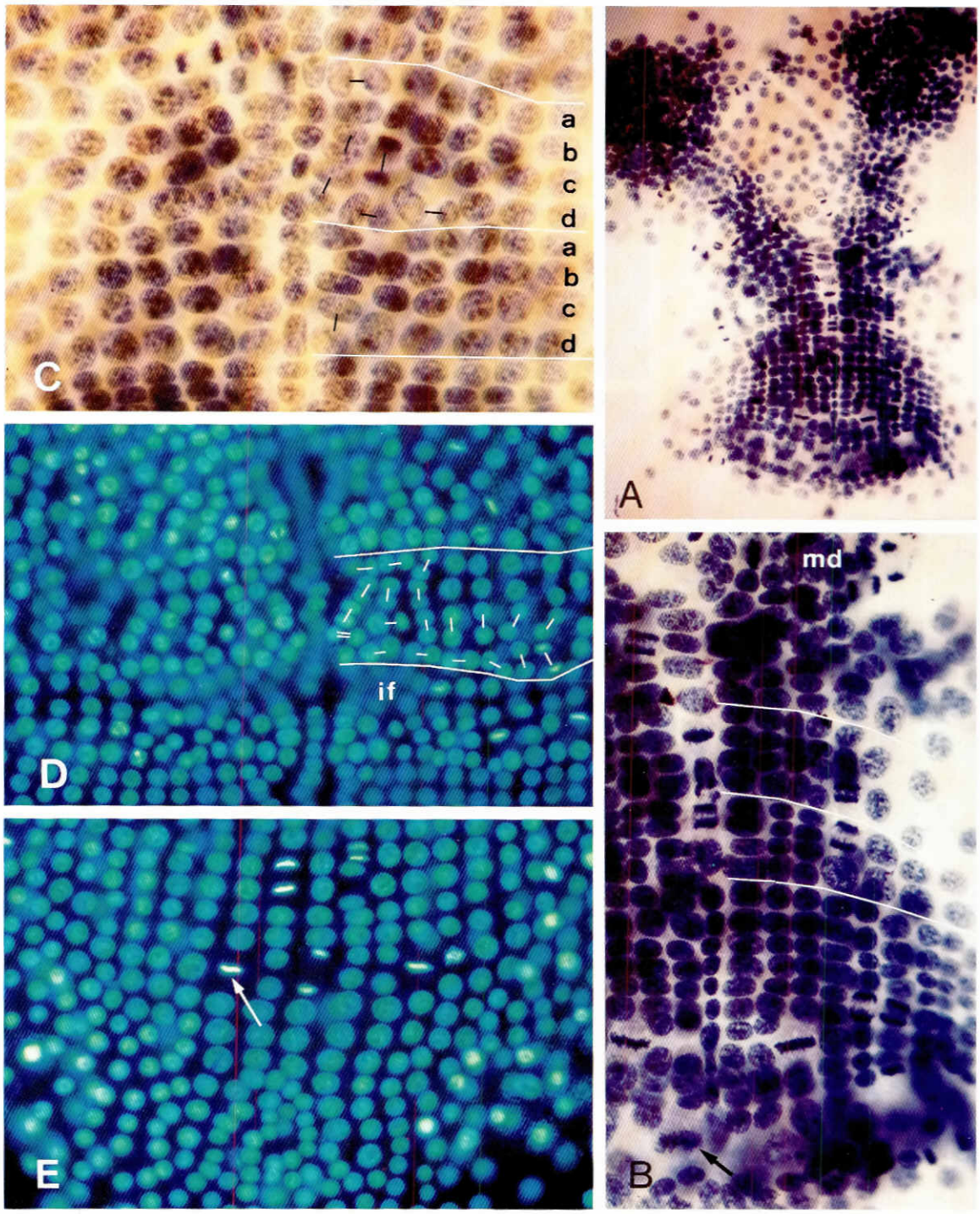

Fig. 2. Germ bands of the cumacean *Diastylis* (A,B,C) (stained with Delafield's Hematoxylin) and the amphipod *Orchestia* (D,E) (fluorescent dye: Bisbenzimid) to show formation of rows and early differential cleavages. (A) *Total germ band in a stage when ectodermal cell row VII is formed by ectoteloblasts.* **(B)** *Magnified part of same preparation as* **(A)**. *White lines are drawn in front of and behind the derivatives of the non-ectoteloblastic rows E(2) and E(3). The rows behind E(3) are formed by ectoteloblasts. Ectoteloblast ET_1 is in metaphase of its seventh division (arrow) on the animal's right side. md rudiment of mandible.* **(C)** *Slightly later stage than (B) showing the begin of differential cleavages in rows E(2) and E(3). White lines as in (B). a, b, c, d are the descendant cell rows after the second mitotic wave. Sister cells after the first differential cleavage are connected by a dark line.* **(D)** *Part of the germ band of Orchestia. White lines in front of and behind derivatives of ectodermal cell row E(3). Sister cells after the first differential cleavage on the animal's left side of E(3) are connected by a white line. Note the position of the intersegmental furrow (if) with respect to the genealogical border (comp. Fig. 1E).* **(E)** *Hind end of same germ band as in* **(D)**. *The first mitotic wave begins in E(15), the second mitotic wave begins in E(13). No ectoteloblasts are formed. $E(15)_2$ is in metaphase and marked by an arrow.*

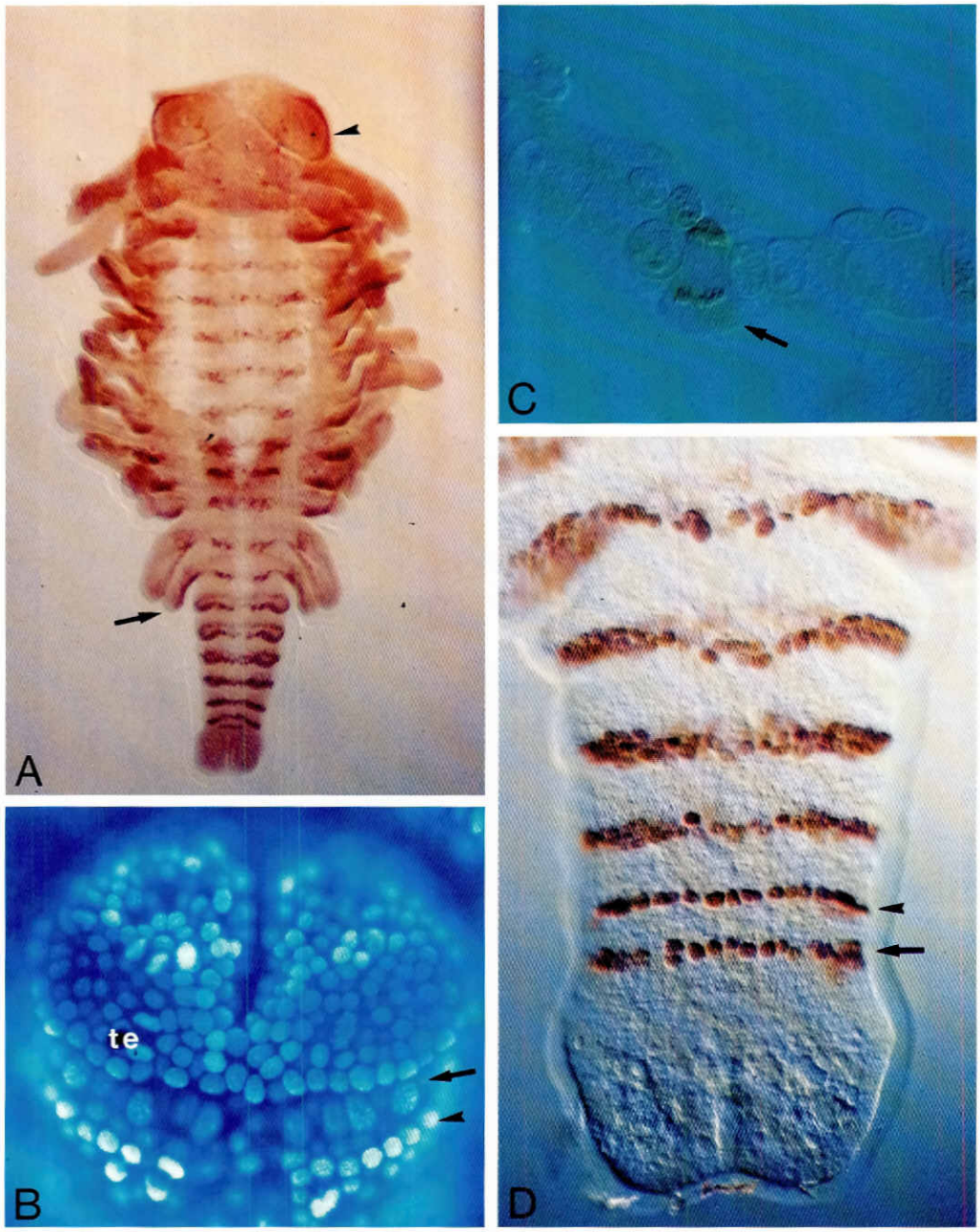

Fig. 3. Aspects of germ band development and *en* stripe formation in *Cherax destructor*. **(A)** *en expression in the germ band at about 55% development (see Sandeman and Sandeman, 1991) (Nomarski optics). The number of en stripes is almost complete. The anteriormost stripe (arrowhead) marks the ocular-protocerebral region (comp. Fig. 4A,B) (see Scholtz, 1995a). In the abdomen, 7 stripes are formed (two more stripes will be added somewhat later (Scholtz, 1995b)). The arrow points to the last (8th) thoracic segment.* **(B)** *Posterior end of the caudal papilla of an embryo at 35%-40% development (Bisbenzimid). The arrow points to the large ectoteloblasts which surround the caudal papilla. The arrowhead indicates an ectoderm row formed by ectoteloblast activity. te telson ectoderm.* **(C)** *Unequal division of an en-positive neuroblast (arrow) in the thoracic region (about 40% development) (Nomarski optics). Dorsal is up. The yolk has been removed.* **(D)** *Abdomen (about 50% development) showing the regulation of early en expression (Nomarski optics) (comp. Figs. 4, 5, 6). The en positive cells in abdominal stripe 5 (arrow) undergo the second mitotic wave. The median cells of descendant row b have already ceased their en expression whereas it is maintained in the cells of descendant row a. More lateral, cells of a and b express en. The process of the decay of en expression in descendant row b is completed in abdominal segment 4 (arrowhead) leading to a narrowed en stripe which is restricted to descendant row a.*

almost identical pattern when comparisons are drawn between different rows in the germ band or between different species (Figs. 1, 2). 3) The individual mitoses of the differential cleavages are independent of each other. The interspecific comparison shows that the spindle orientations or the equality of the division products of single mitoses can be altered without affecting the surrounding mitotic pattern. This indicates the independent determination of the individual mitosis and shows that a complex information is required to produce the overall pattern of the differential cleavages (see Dohle, 1989b, Scholtz, 1984).

In summary, the comparative analysis shows that the cell lineage cannot be the clue to the understanding of cell fate specification. Homologous cell division patterns, segmental borders, limb buds, and neuroblasts are differentiated independent of the preceding cell lineage and of the origin of cells.

Cell lineage and *engrailed* expression

In *Drosophila*, the segment polarity gene *engrailed* (*en*) is expressed in the posterior portion of each segment from the embryo to the adult (Kornberg *et al.* 1985; DiNardo *et al.* 1985). A corresponding expression pattern of *en* homologues has been found in annelids (Wedeen and Weisblat, 1991), various insects (e.g. Patel *et al.*, 1989a; Fleig, 1990; Sommer and Tautz, 1991; Brown *et al.*, 1994; Patel, 1994), and some crustacean species (Patel *et al.*, 1989a; Manzanares *et al.* 1993; Scholtz *et al.*, 1993,

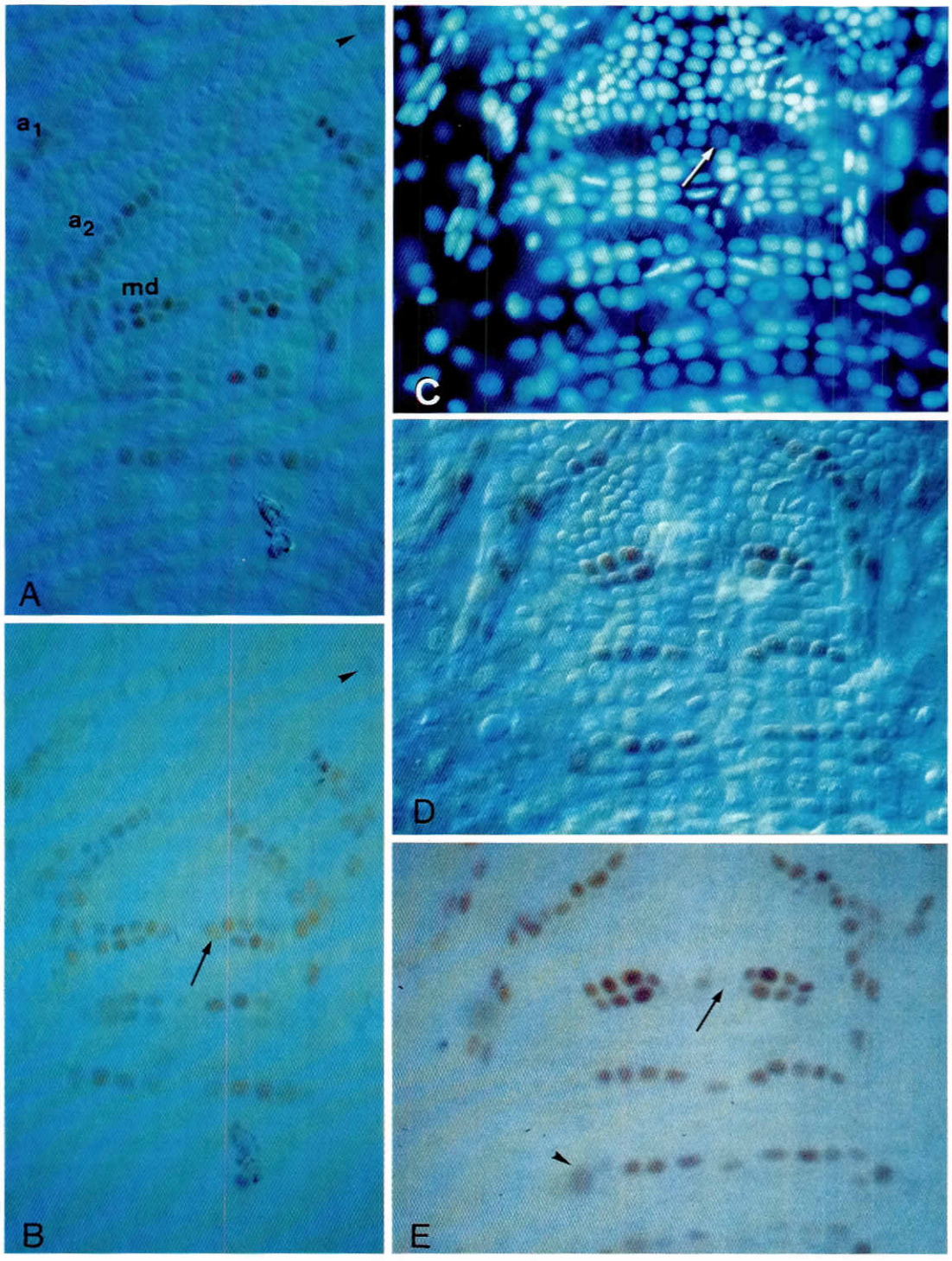

Fig. 4. Regulation of early *en* expression in the germ band of *Neomysis*. **(A-B)** *Earlier stage (7 cell rows have been formed by ectoteloblasts).* **(C-E)** *Slightly later stage (9 cell rows have been formed by ectoteloblasts).* **(A)** *Nomarski optics;* **(B,E)** *bright field;* **(C)** *fluorescent dye (Bisbenzimid);* **(D)** *Nomarski combined with epifluorescence.* **(A-B)** *First antennal (a1) and second antennal rudiments (a2) are formed. Arrowhead points to preantennular en expressing cells marking the ocular-protocerebral region (comp. Fig. 3A). md mandibular stripe. Four en-positive cells of the mandibular stripe have divided on either side; all 8 daughter cells are stained, the median cell is not yet stained. E(2)ab divides into descendant rows a and b; the anterior descendant row a maintains en expression, descendant row b loses it (especially evident in the animal's left side); row E(3)ab still undivided, four cells express en on either side and the median cell is not yet stained.* **(C-E)** *The cell marked in* **(B)** *by an arrow has divided once more and its daughters have nearly completely lost en expression. The arrow points to the inner larger daughter cell. E(2)a expresses en, the cells of E(2)b are en-negative. In E(3) the cells a_0-a_4 are stained, also b_4 and ab_5 (arrowhead). The cells b_0-b_3 have already lost en expression (comp. Figs. 3D, 5, 6). Descendant row elab is dividing.*

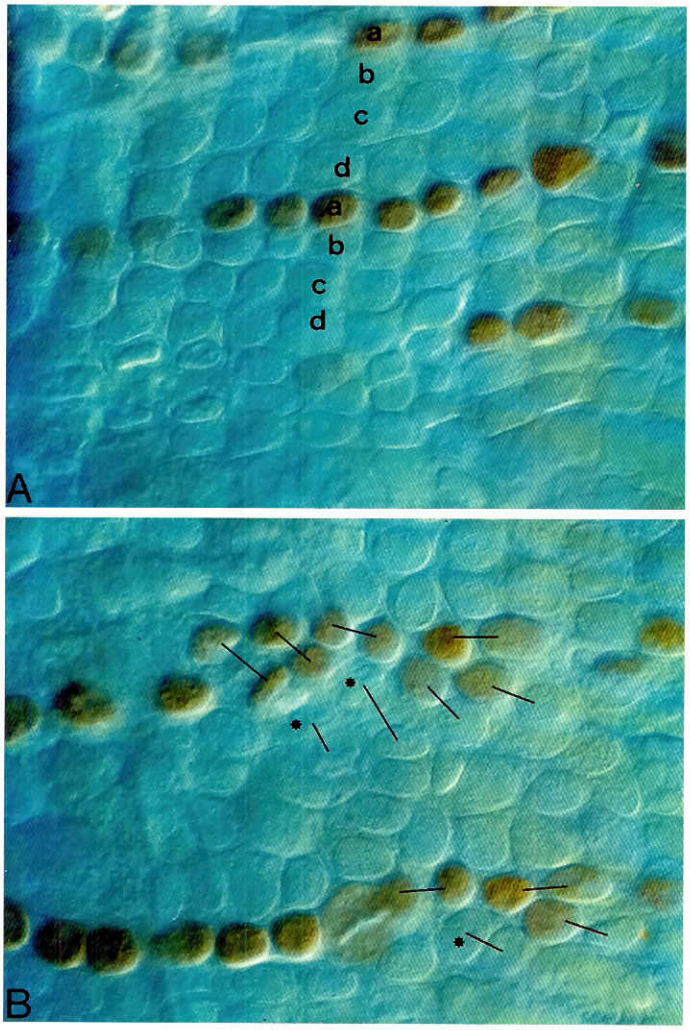

Fig. 5. *en* expression in the amphipod *Orchestia cavimana* (Nomarski optics). (A) *Initial en expression during the second mitotic wave of three subsequent ectoderm rows. The cells of descendant rows a express en whereas the cells of descendant rows b, c, and d are en-negative (comp. Figs. 3D, 4).* **(B)** *The pattern of en expression during the first differential cleavage of the derivatives of two adjacent thoracic ectoderm rows. The stripes widen by a combination of clonal transmission and recruitment. Cells of descendant row a transmit en expression to their progeny. In addition, anterior cells of descendant row b begin to express en whereas their posterior sisters remain en-negative. Nuclei of sister cells involved in en expression are connected by lines. The asterisks mark nuclei of anterior derivatives of b which are about to express en. In both pictures the midline of the germ band is to the right.*

1994; Patel, 1994). This widespread occurrence indicates a conserved role of *en* in connection with segmentation throughout the Articulata (annelids and arthropods). It has been suggested for *Drosophila* that *en* is a selector gene and plays a crucial role in establishing lineage restrictions in the posterior compartment from early embryonic stages up to the adult fly (for review see Lawrence, 1992). According to this view, *en* defines and maintains the anterior border of the embryonic parasegments (the boundary between the anterior and posterior compartments of segments) from the onset of its expression (e.g. Kornberg *et al.*, 1985; Lawrence, 1992; Lawrence and Morata, 1992).

In malacostracans, the regular pattern of *en* expression which can be related cell-by-cell to the complex cell division pattern in the post-naupliar germ band in individual species (Scholtz *et al.*, 1994) suggests that initial *en* expression might be controlled by cell lineage. However, several findings from our comparative studies in several malacostracans using the monoclonal anti *en* antibody mAb 4D9 (Patel *et al.*, 1989b) lead to a different view (see Scholtz *et al.*, 1993, 1994; Dohle and Scholtz, 1995; Dohle, unpublished):

1) Similar *en* stripes are correlated with different cell division patterns in different regions of malacostracan germ bands. Transverse *en* stripes form in the naupliar region where no regular cell division patterns occur as in the post-naupliar germ band with its stereotyped cell lineage (Figs. 2A, 3A, 4).

2) The onset of *en* expression is related to different stages in the cell lineage in different species. We have found that in the post-naupliar germ bands of *Neomysis* and of *Cherax, en* expression starts one cell cycle earlier (first mitotic wave of the ectodermal rows) than in those of amphipods (second mitotic wave of the ectodermal rows) (Figs. 3, 4, 5, 6).

3) The widening of *en* stripes is a combination of clonal transmission and recruitment. This can be clearly shown for the post-naupliar germ band and some data indicate that this is also true for the naupliar *en* stripes. In the post-naupliar region, *en* expression is passed on to the derivatives of each descendant row a and, in addition, anterior derivatives of descendant row b express *en de novo* after the first differential cleavage (Figs. 5, 6).

4) *en* expression is not stable from the onset and not simply clonally inherited. In *Cherax* and *Neomysis* cells at the posterior border of the early *en* stripes (cells of descendant row b after the 2nd mitotic wave of each row) cease to express *en* (Figs. 3, 4, 6). A similar regulation has been described in *Drosophila* (Vincent and O'Farrell, 1992). In addition, we have found that in the mandibular stripe of *Neomysis* some cells at the anterior margin lose *en* expression two mitotic cycles after the initial *en* expression (Fig. 4). Whether a corresponding phenomenon occurs in *Drosophila* is not known.

5) The resulting *en* pattern is similar throughout the malacostracan species examined – independent of the origin of cells that make up the germ band. Fig. 6D shows a scheme of the *en* patterns after the first differential cleavage in amphipods, mysids and decapods (compare Fig. 5). The patterns are very similar despite the fact that the cells originate from ectoteloblasts in *Neomysis* and *Cherax* and from scattered blastoderm cells in amphipods. The same can be said when the patterns in non-teloblastic and teloblastic rows of the germ band of *Neomysis* and *Cherax* are compared.

6) *en* expression is regained in cells whose precursors have lost its expression. In *Cherax* and *Neomysis* one cell cycle after the loss of *en* expression in descendant row b, the anterior derivatives of b regain the competence to express *en* again (Fig. 6). Corresponding events have been reported for another mysid species (Patel, 1994).

7) Initial *en* expression in non-malacostracan crustaceans and insects is not related to a certain lineage. As mentioned above, the stereotyped cleavage pattern of malacostracans can neither be found in non-malacostracan crustaceans (e.g. Freeman,

1989; Manzanares *et al.*, 1993; Gerberding, 1994) nor in insects. Nevertheless, *en* is expressed in a homologous manner (e.g. Patel *et al.*, 1989a; Manzanares *et al.*, 1993).

All these comparative data show the independence of early *en* expression from the underlying cell division pattern. In other words, the competence of certain cells to express *en* is not the result of a specific cell lineage. This does not necessarily mean that the two processes of cell genealogy and *en* expression are not closely and perhaps inextricably linked in the normogenesis of an individual species. Ablation experiments in crayfish embryos, however, have indicated that even this is not the case in malacostracan crustaceans – *en* stripes are formed despite the perturbation of the cell division pattern (Scholtz and Sandeman, in preparation).

The findings of a loss of *en* expression in cells at the posterior and anterior margins of *en* stripes have an additional implication. They challenge the view that *en* expression, once switched on, is clonally transmitted throughout development (Lawrence and Morata, 1992). In particular, the decay of *en* expression at the anterior border indicates that the parasegmental boundary might not be as stable during development as suggested – at least in crustaceans.

Phylogenetic considerations and homology

Comparative analyses are not only capable of demonstrating the independence of single developmental steps. They can also reveal the relative sequence of alterations in development in the course of evolution. Based on the principles of phylogenetic systematics (Hennig, 1966; Ax, 1987), the ancestral conditions can be reconstructed, and conserved versus evolutionarily altered elements in the developmental process can be distinguished.

The existence of 19 ectoteloblasts arranged in a ring is part of the ground plan of the Malacostraca (Dohle, 1972). These ectoteloblasts are convergent to those of clitellates (Dohle, 1972) since ectoteloblasts neither occur in polychaetes nor in onychophorans, myriapods, insects, nor in most of the non-malacostracan crustaceans (only in Cirripedia, a pattern of teloblasts, different from that of malacostracans, has been reported; Anderson, 1973). This original malacostracan pattern has been altered in the ancestral lineages leading to freshwater crayfish within the decapods and to the large group of the Peracarida. In freshwater crayfish, the circular arrangement of the ectoteloblasts has been maintained but the number has increased to about 40 (Fig. 3B) (Scholtz, 1992, 1993). The Peracarida have lost the ectoteloblast ring. A ventral transverse row of ectoteloblasts with a variable number has formed (Fig. 2A,B). In a second step, the amphipods within the peracarids have lost ectoteloblasts entirely (Fig. 2E). The complex and stereotyped cleavage pattern in the post-naupliar germ band, however, has been conserved throughout. This pattern must have evolved in the ancestral lineage of malacostracans or within this group as it has not been found either in non-malacostracan crustaceans (e.g. Freeman, 1989; Gerberding, 1994) or in other arthropods.

Expression of *en* in iterated transverse stripes at the posterior border of developing segments has been shown to occur in annelids (Wedeen and Weisblat, 1991; Dorresteijn *et al.*, 1993), crustaceans (Patel *et al.*, 1989a; Manzanares *et al.*, 1993; Scholtz

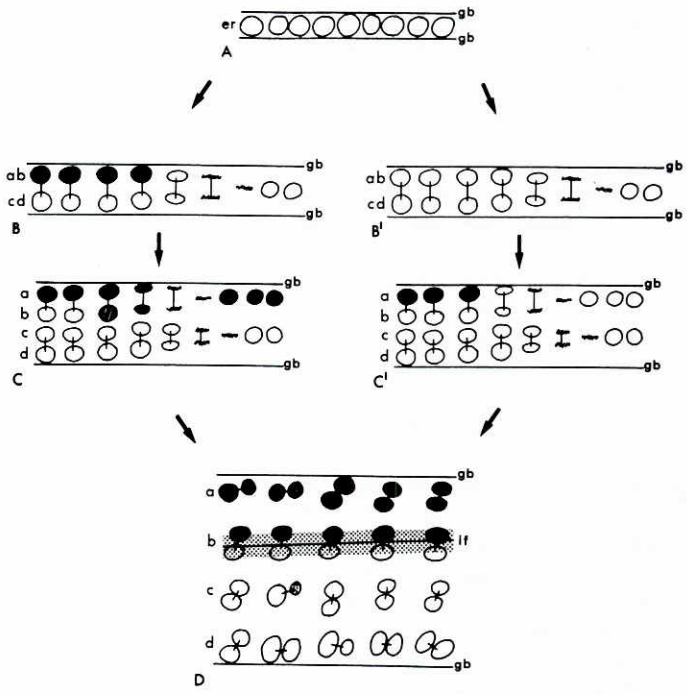

Fig. 6. Schematic representation of the differences and similarities in early *en* expression between *Cherax* and *Neomysis* on the one hand and amphipods on the other hand *(comp. Fig. 1). In Cherax and Neomysis, en expression starts in descendant row ab (black nuclei) during the first mitotic wave of each transverse ectoderm row (er)* **(A,B)**. *In amphipods no en expression takes place at this stage* **(B')**. *During the second mitotic wave in the rows of Cherax and Neomysis, en expression is lost in a mediolateral sequence in descendant row b (represented by the shaded nucleus)* **(C)**. *In the corresponding stage of amphipods, en expression (black nuclei) begins in descendant row a and propagates mediolaterally* **(C')**. *No loss of en expression occurs. During the first differential cleavage, de novo en expression occurs in anterior derivatives of descendant row b in front of the intersegmental furrow (if)* **(D)** *(comp. Fig. 5B). This pattern is very similar in all investigated species independent of the preceding differences. gb genealogical border.*

et al., 1993, 1994; Patel, 1994), insects (e.g. Patel *et al.*, 1989a; Fleig, 1990; Sommer and Tautz, 1991; Patel, 1994), and probably in myriapods (Whitington *et al.*, 1991). This suggests that the stem species of the Articulata (annelids and arthropods) has already had this *en* expression pattern. There is good evidence that *en* expression is originally not correlated with an invariant cell division pattern on the germ band. This can be inferred from the fact that an invariant cell lineage during germ band formation and segmentation has neither been described for polychaetes, onychophorans, chelicerates, non-malacostracan crustaceans, myriapods, nor for insects. Only in the lineages leading to the clitellates and the malacostracans, complex invariant cell lineage patterns during segmentation have evolved. These phylogenetically novel patterns are very different (for review and comparison of the clitellate pattern see Weisblat and Shankland, 1985; Shankland, 1991, 1994; Weisblat *et al.*, 1994; Ramírez *et al.*, 1995) and have evolved convergently and they have independently superimposed the older *en* expression pattern.

The observation that in the insect *Drosophila* (Vincent and O'Farrell, 1992) and in the malacostracan species *Cherax*

destructor (Decapoda) (Scholtz *et al.*, 1993), *Neomysis integer* and *Mysidium columbiae* (Peracarida, Mysidacea) (Scholtz *et al.*, 1993; Patel, 1994) *en* expression is lost in posterior cells of clones originating from *en* positive cells suggests that this regulation is an ancestral character within arthropods. The lack of regulation of this kind in amphipod crustaceans (Peracarida) would therefore represent the derived condition.

It is a widespread view that homology of differentiated cells such as neuroblasts or neurons can only be proven when it can be shown that these cells share an identical lineage (e.g. Pearson *et al.*, 1985; Larimer and Pease, 1990; Doe, 1992; Boyan and Williams, 1995). Of course, similar development corroborates the supposed homology of corresponding structures. However, the examples given above show that homologous developmental pathways cannot be a mandatory prerequisite for the homology of structures (this is true for interspecific homology as well as for serial homology (homonomy); for further discussion see e.g. Dohle, 1976, 1989a; Sander, 1983, 1989; Scholtz, 1993; Scholtz *et al.*, 1994; Wagner and Misof, 1993). For instance, the early pattern of neuroblasts in the cumacean *Diastylis* (Dohle, 1976), the amphipod *Gammarus* (Scholtz, 1990), and the decapod *Cherax* (Scholtz, 1992) is identical concerning position and formation of individual neuroblasts and therefore homologous, irrespective of the different origin of these neuroblasts from cells which are either formed by teloblasts (*Diastylis, Cherax*) or scattered blastoderm cells *(Gammarus)* (see above). As mentioned before, insects do not show any cell division pattern, comparable to that of malacostracans, on their germ band. In addition, because myriapods which are believed to be the closest relatives of insects do not differentiate neuroblasts (e.g. Dohle, 1964; Whitington *et al.*, 1991) and crustacean and insect neuroblasts show some characteristic differences, it is doubtful whether neuroblasts in crustaceans are at all homologous to those in insects (Dohle, 1976; Dohle and Scholtz, 1988; Scholtz, 1992). Nevertheless, it has been shown that the arrangement of neurons is quite similar in crustaceans and insects and that individual neurons can be homologized in embryos (Thomas *et al.*, 1984; Whitington *et al.*, 1993) and adults (Mittenthal and Wine, 1978; Wiens and Wolf, 1993; for review see Kutsch and Breidbach, 1994).

Perspectives

The analysis of cell division patterns should be extended to include earlier developmental stages so as to gain a more complete picture of cell lineage in malacostracans. The eggs of amphipods are well suited for addressing this problem. In contrast to other malacostracans examined by us, amphipods seem to undergo a total cleavage with a stereotyped pattern during early development. Exact data on early cell division patterns would complement the cell lineage studies dealing with later embryonic stages in amphipods (Scholtz, 1990). Furthermore, a descriptive basis would thus be provided for comparisons with other malacostracans which undergo, in contrasts to amphipods, a larval development (Hertzler and Clark, 1993; Hertzler *et al.*, 1994).

Experimental studies such as cell ablations and perturbations in the cell division pattern of the post-naupliar germ bands of malacostracans will be useful to confirm the postulated independence of cell lineage and cell fate specification. In particular, the relationship between cell division and gene expression can be resolved with this kind of investigation. Experiments are in progress in the Australian freshwater crayfish *Cherax destructor* (Scholtz and Sandeman, in preparation).

Detailed cell by cell comparisons of neuroblast patterns between insects and crustaceans are required using molecular markers (see Fig. 3C) (Doe, 1992). These investigations will be carried out to establish whether the neuroblasts of crustaceans and insects are truly homologous as proposed by Thomas et al. (1984) and Patel et al. (1989b) or convergent as discussed by Dohle (1976), Dohle and Scholtz (1988), and Scholtz (1992).

Finally, detailed knowledge of cell lineage events in the crustacean germ band offers a good opportunity to study general aspects of the evolutionary alterations in the mechanisms of segmentation in arthropods (see also Patel, 1993, 1994). Additional molecular markers for crustaceans (e.g. Averof and Akam, 1993, 1995) will be particularly useful for such detailed comparative studies.

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