Roux's Arch Dev Biol (1992) 202:36–48

Roux's Archives of
Developmental
Biology

Cell lineage studies in the crayfish *Cherax destructor* (Crustacea, Decapoda): germ band formation, segmentation, and early neurogenesis

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Received March 6 / Accepted in revised form June 9, 1992

Summary. The cell division pattern of the germ band of *Cherax destructor* is described from gastrulation to segmentation, limb bud formation, and early neurogenesis. The naupliar segments are formed almost simultaneously from scattered ectoderm cells arranged in a V-shaped germ disc, anterior to the blastopore. No specific cell division pattern is recognisable. The post-naupliar segments are formed successively from front to rear. Most post-naupliar material is budded by a ring of about 39 to 46 ectoteloblasts, which are differentiated successively and in situ in front of the telson ectoderm. The ectoteloblasts give rise to 15 descendant cell rows by unequal divisions in an anterior direction, following a mediolateral mitotic wave. Scattered blastoderm cells of non-ectoteloblastic origin in front of the ectoteloblast descendants and behind the mandibular region are also arranged in rows. Despite their different origins, teloblastic and non-teloblastic rows cleave twice by mediolateral mitotic waves to form 4 regular descendant rows each. Thereafter, the resulting grid-like pattern is dissolved by stereotyped differential cleavages. Neuroblasts are formed during these differential cleavages and segmentation becomes visible. Each ectoderm row represents a parasegmental unit. Therefore, the segmental boundary lies within the area covered by the descendants of 1 row. Segmental structures (limbs, ganglia) are composed of derivatives of 2 ectoderm rows. The results are compared with the early development of other crustaceans and insects in relation to mechanisms of germ band formation, segmentation, neurogenesis, and evolution.

Key words: Cell lineage – Segmentation – Neuroblasts – Crayfish – Crustacea

Introduction

Detailed cell lineage studies in crustacean embryos of the peracaridan orders Cumacea, Tanaidacea, Isopoda, Mysidacea, and Amphipoda have revealed a homologous cell differentiation pattern during segmentation and neuronal development (Dohle 1970, 1972, 1976a; Hahnenkamp 1974; Scholtz 1984, 1990). A comparison of these patterns has produced insights into questions concerning homology (Dohle 1976b, 1989) and mechanisms of developmental processes such as the independent determination of single developmental steps (Dohle and Scholtz 1988). Since the specific differential cleavage pattern of ectodermal cells during segment formation has not been described in any other malacostracan group outside the Peracarida, this pattern has been discussed as an evolutionary new acquisition (apomorphy) of the Peracarida (Dohle 1976b; Sieg 1984).

On the basis of the *engrailed*-gene expression pattern in different species of insects and crustaceans, Patel et al. (1989) have suggested that the decapod crayfish *Procambarus clarki* shows the same differential cell division pattern as the peracarids. Furthermore, they consider the pattern of early neurogenesis in higher Crustacea and insects as homologous and support the idea of a common plan for neuronal development in arthropods advanced by Thomas et al. (1984). However, Patel et al. (1989) did not analyse cell lineage in *P. clarki* in detail. Also, since none of the numerous investigations on the embryonic development of decapod crayfish (e.g. Reichenbach 1888; Fulinski 1908; Zehnder 1934; Fioroni 1969; Celada et al. 1991; Sandeman and Sandeman 1991) deals with the topic of cell lineage, it seemed sensible to investigate a decapod species to find out whether decapods share the same pattern development and cell lineages during segmentation and early neurogenesis with the peracarids.

The present investigation describes the cellular differentiation pattern in the Australian crayfish *Cherax destructor* from gastrulation to segment formation, anlagen of limb buds, and early differentiation of ganglia. It was

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found that the pattern in *Cherax* resembles, in principle, the pattern found in peracarids. A comparison with developmental events in other arthropods reveals some insights into the mechanisms and evolution of segmentation and neurogenesis in arthropods.

Material and methods

Specimens of the Australian freshwater crayfish *C. destructor* were maintained in tanks filled with dechlorinated water (for details see Sandeman and Sandeman 1991). Embryos were obtained by removing eggs with forceps from the pleopods of brood-carrying females. For wholemount preparation and sectioning, all developmental stages were fixed in Duboscq-Brasil for 2 h, then washed and kept in 70% ethanol. The chorion and yolk were removed from the embryo with insect pins under a dissecting microscope before fixation. Wholemounts of the dissected and fixed germ bands were taken down to water through an ethanol series and stained in Ehrlich's haematoxylin for 30 min to 1 h. They were then differentiated in 30% hydrochloric ethanol, rinsed overnight in tap water, dehydrated in an ethanol series, cleared in methylbenzoate, and mounted in Euparal with small cover slips.

For sectioning, fixed, dehydrated embryos were cleared in xylene and embedded in paraffin. Sections of 5 µm were stained with Ehrlich's haematoxylin and mounted in DePeX.

Wholemounts for epifluorescent microscopy were fixed after dissection for 30 min in 5% formaldehyde, washed twice in distilled water for 5 min, stained in Bisbenzimid H 33258 (0.2% solution, 15 min), washed again, and mounted in glycerol.

The morphological criteria used to judge the developmental stage as a percentage of development were those defined by Sandeman and Sandeman (1991). For nomenclature of cells and cell lineages see Figs. 2, 3, and 5.

Results

Segmentation of the nauplius region and formation of the caudal papilla

The naupliar segments become visible during late gastrulation (15–20% of development). The blastopore starts to close and, in the V-shaped germinal disc in front of it, the anlagen of first and second antennae and of the mandibles start to differentiate almost synchronously (Fig. 1). Sometimes, the appearance of the anlage of the second antenna is slightly delayed. Intersegmental furrows are not recognizable. Neither ectoderm nor mesoderm cells of the germinal disc show a definable division pattern correlated with segment and limb bud formation.

The early anlage of the caudal papilla develops in parallel with the above events. Three protrusions are formed directly anterior to the blastopore (Fig. 1 A). The medial protrusion, which lies slightly in front of the two lateral bulges, merges with them at a later stage (20% development when the blastopore is almost closed), forming a horseshoe-shaped structure with the opening, the primordial proctodaeum, directed posteriorly (Fig. 1 B).

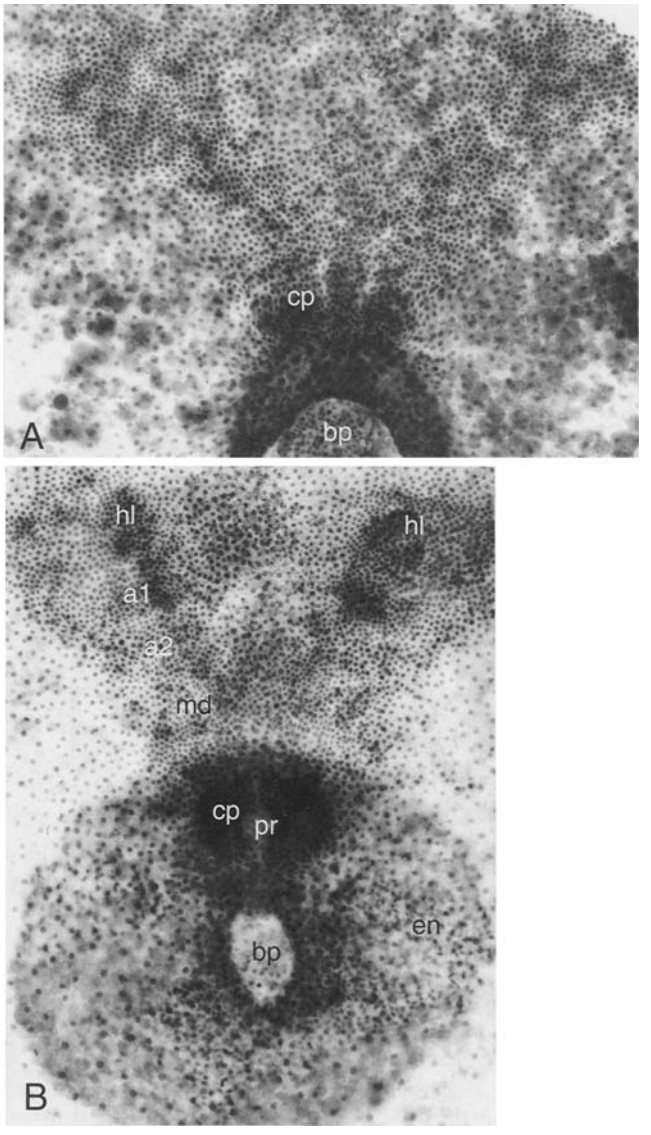

Fig. 1 A, B. Early development of naupliar segments and caudal papilla. **A** Germ band at 15% development. The V-shaped naupliar region shows no regular division patterns. Three protrusions in front of the blastopore (*bp*) form the early caudal papilla (*cp*). **B** Germ band at 20% development. The naupliar segments become visible (*a1*, antenna 1, *a2* antenna 2, *md* mandible). The proctodaeum (*pr*) lies in the centre of the caudal papilla (*cp*). *en*, entoderm; *hl*, headlobes

Differentiation of ectoteloblasts and ectoderm row formation

The ectoteloblasts differentiate in situ from blastoderm cells which change their appearance and function at a specific time. The first ectoteloblasts can be detected between 20 and 25% development (Fig. 2 A). Three or four cells appear on either side at the anterior edge of the anlage of the caudal papilla that are larger than their neighbours and possess relatively large nuclei. These large cells form a crescent with the opening facing posteriorly. Once differentiated, the ectoteloblasts divide unequally giving rise to smaller cells in an exclusively ante-

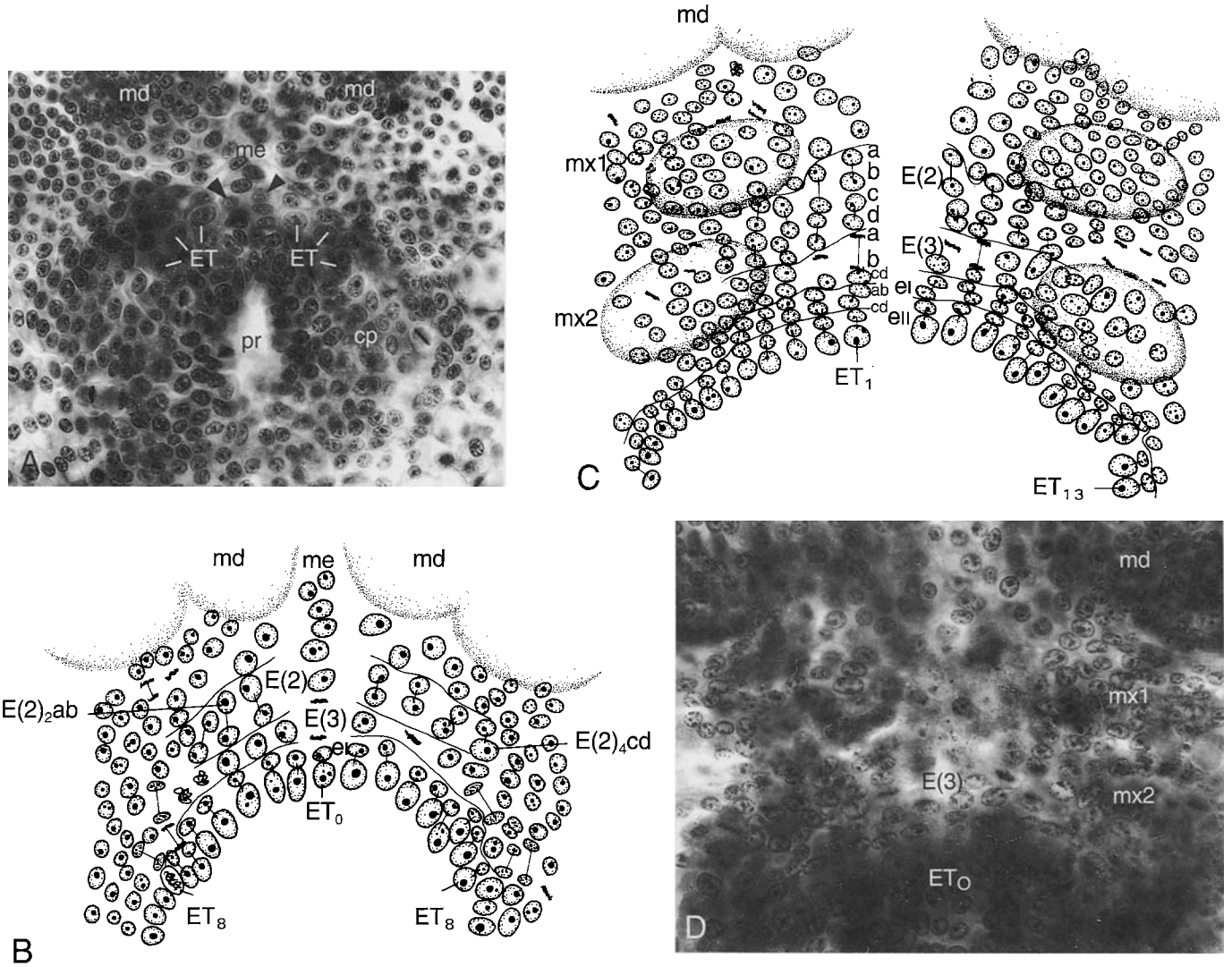

Fig. 2A–D. Differentiation of ectoteloblasts and early ectodermal row formation of teloblastic descendants and non-teloblastic rows, mitotic waves in the anterior rows, and anlage of the two maxillary segments. The distance of the ectoteloblasts (*ET*) from the midline (left or right) is shown by *index numbers*. The ectoteloblast nearest to the midline is named ET_1, the next ET_2, etc. The median ectoteloblast is labelled ET_0. Ectodermal transverse cell rows produced by ectoteloblasts are labelled with an *e*. The succession of these rows is indicated by *roman numerals* (e.g. *eI, eVI*). The distance of the cells from the midline is designated by *index numbers* corresponding to the ET (e.g. eVI_{11}). Ectodermal transverse cell rows of non-ectoteloblastic origin are labelled with an *E* and *arabic numerals in brackets* (e.g. *E(2)*). Indices are used as above (e.g. $E(2)_3$). After the first cleavage of a row (of ectoteloblastic and non-ectoteloblastic origin) the two descendant rows are named ab and cd (e.g. *eIab* and *eIcd*; **C**). After the second mediolateral mitotic wave the four descendant rows of each ectoderm row are designated a, b, c, d (e.g. *E(2)a, E(2)b, E(2)c, E(2)d*; **C**). **A** The first ectoteloblasts (*ET*) become visible at the anterior margin of the caudal papilla (*cp*) at 20–25% development. They are characterised by deeper lying large nuclei. *Arrowheads* mark the two median cells with uncertain fate, one of them will later become ET_0. The median longitudinal row (*me*) can be recognised. *pr*, Proctodaeum. **B** Formation of row *eI*. Row *E(3)* begins the first mitotic wave, row *E(2)* is divided once (25% development). *md*, Mandible; *me*, median row. **C** Post-naupliar region of an embryo at 28% development. Second mitotic wave in row *E(2)* finished; it begins in row *E(3)*. First mitotic wave in row *eI*. Row *eII* is formed. About 12 or 13 ectoteloblasts (*ET*) are differentiated. The buds of first (*mx1*) and second maxillae (*mx2*) are visible. Note their early appearance in relation to the cellular degree of differentiation compared to posterior segments (Fig. 8A). The median row is omitted. **D** Micrograph of the same preparation

rior direction (i.e. the longitudinal direction of the germ band). At this early stage, there are two cells lying between the innermost ectoteloblasts of the left and right side (ET_1), which have not increased in size (Fig. 2A). One of them differentiates into the median ectoteloblasts (ET_0) somewhat later. The fate of the supernumerary cell is not clear. It may become part of the median cell row in front of ET_0.

Blastoderm cells lateral to the first ectoteloblasts increase in size and differentiate into ectoteloblasts. At 30% development, the crescent of ectoteloblasts becomes a complete ring consisting of 39–46 cells (Fig. 3). The final number varies between individuals. No transverse division of differentiated ectoteloblasts was ever seen that could have produced the additional ectoteloblasts. The recruitment of new ectoteloblasts thus propa-

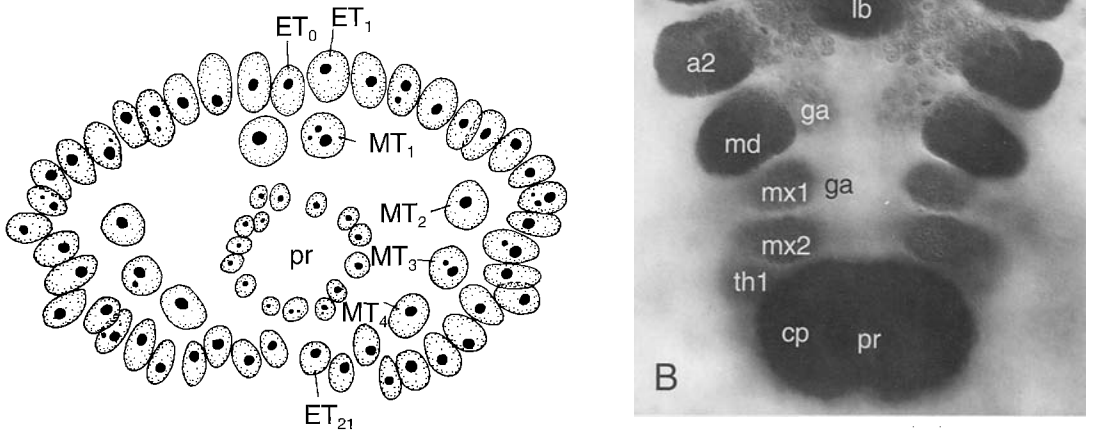

Fig. 3. A Rings of teloblasts of an embryo at 35% development. The full complement of 43 ectoteloblasts (*ET*) and 8 mesoteloblasts (*MT*), four MT per side (MT_1 to MT_4), is formed. The teloblast rings surround the proctodaeum (*pr*). **B** The complete germ band of the same stage. The limb buds up to the first thoracic segment (*th1*) are visible. *a1*, First antenna; *a2*, second antenna; *cp*, caudal papilla; *ga*, ganglion anlage; *hl*, head lobes; *lb*, labrum covering stomodaeum; *md*, mandible; *mx1*, first maxilla; *mx2*, second maxilla; *pr*, proctodaeum

gates in a lateral direction (Fig. 2), only the unpaired median ectoteloblast ET_0 being differentiated later than its lateral neighbours. However, differentiation does not propagate cell by cell, instead 4–6 cells per side become ectoteloblasts with each round of divisions in the teloblast area. The caudal papilla becomes a tube-like structure surrounding the lumen of the proctodaeum as ectoteloblasts are differentiated.

A ring of mesoteloblasts differentiates and gives rise to the mesodermal structures in each segment. It consists of 8 mesoteloblast cells in a specific arrangement (Fig. 3A). The differentiation pattern of these cells and their progeny during embryonic development has not been analysed.

Since the first ectoteloblasts form a slightly curved row, their descendant rows are arranged similarly. The first row, consisting mainly of ectoteloblast derivatives (6–8 cells per side), is row eI (Fig. 2B). Some scattered blastoderm cells are added laterally resulting in a row of about 10–12 cells on each side of the midline. Row eII consists of about 13 ectoteloblast descendants per side (Fig. 2C). The ring of ectoteloblasts is closed with the formation of row eV. Subsequent descendant rows are arranged in rings from this stage on. Thus, in contrast to the rows anterior to row eV which give rise to only ventral structures, these posterior rows form ventral and dorsal parts of the embryo. The ventrally folded caudal papilla elongates by the proliferation of ectoteloblast descendant rows.

Each descendant row is formed by a wave of division of the ectoteloblasts which runs from close to the midline in a lateral direction (Fig. 4). The sequential gradient is not very steep and six or more ectoteloblasts in a row frequently exhibit the same mitotic figure simultaneously. The divisions producing rows eXIV and eXV are equal, and the ectoteloblasts are no longer recognisable (Fig. 7). Row eXV is thus the last ectoteloblast descendant row.

A number of irregularly scattered blastoderm cells separates the first ectoteloblasts from the anlage of the mandibular segment (Fig. 2A). During the formation of ectoteloblast descendant rows, these scattered cells also become arranged in rows (Fig. 2B). Row E(2) is formed exclusively by these blastoderm cells of non-ectoteloblastic origin. The origin of row E(3), is partly unclear. It cannot be excluded that some early ectoteloblast descendants become part of row E(3), as there are few cytological differences between the blastoderm cells and the ectoteloblast derivatives at this developmental stage. Despite their different origins, rows E(2) and E(3) resemble the rows formed by ectoteloblasts in their appearance and further development. The differentiation of the anteriormost part of the post-naupliar germ band differs from the posterior region and was not analysed in detail, although some of these cells were apparently organized in rows (corresponding to row E(1) in peracarids?) and showed distinct mitotic differentiation patterns, particularly in advanced stages (Figs. 2C, 5A).

Waves of division in ectoderm rows

Despite their different origin and arrangement, rows E(2) to eXV initially undergo similar patterns of divisions. Each row undergoes two rounds of mediolateral mitotic waves resulting in four descendant rows a, b, c, and d (Figs. 2C, 6, 7). All the divisions of these mitotic waves are equal and the spindle axes lie exclusively in a longitudinal direction.

Row E(2), which initiates this phase on the germ

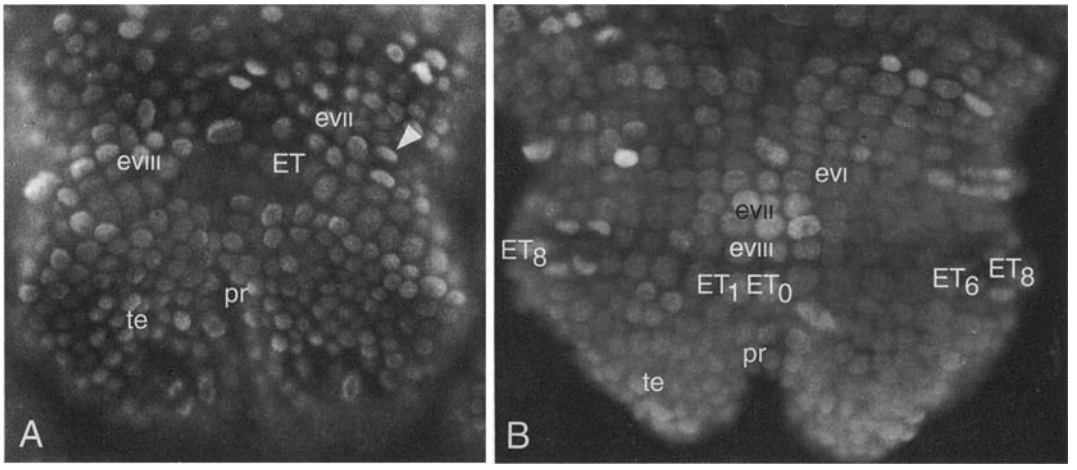

Fig. 4A, B. Caudal papilla (folded backward) of an embryo at 35–40% development during formation of row *eVIII*. **A** Dorsal view showing the less regular arrangement of the rows compared to the ventral side. The propagation of the first mitotic wave in row eVII is indicated (*arrow*). **B** Ventral view. The mediolateral gradient of the mitoses of the ectoteloblasts (*ET*) (seven ET have divided and ET_8 is in metaphase on the animal's left and right sides) and during the second mitotic wave (row *eVI*) is shown. *pr*, Proctodaeum; *te*, telson ectoderm

band, undergoes its first wave of division during formation of rows E(3) and eI at 25% development (Fig. 2B). This can be seen only in the inner four to five cells per side. More laterally, in the area of limb bud formation, in this case maxilla 2, mitotic activity is very high and neither row structures nor definable division patterns can be detected at this stage. The first mitotic wave in row E(3) starts during formation of row eI (about eight ectoteloblasts per side are differentiated; Fig. 2B). As in row E(2), only the four or five innermost cells per side are involved. The lateral cells of non-teloblastic origin show no regular divisions. Due to the small number of cells arranged in rows in E(3) and eI, all divisions of the first mitotic wave are more or less simultaneous. The growing caudal papilla obscures the events in the first few ectoteloblast descendant rows, so the following developmental sequence is based on the posterior parts of the germ band: Each row starts its first wave of division when, in the adjacent anterior row, almost all cells have divided once, and the second mitotic wave includes five to eight cells in descendant row ab. The lateral propagation of the mitotic waves in the rows is relatively fast, like that seen during ectoteloblast differentiation. Up to six adjacent cells can be in a similar mitotic phase at the same time in the more posterior ring-like rows. In these rows (eV–eXV), however, the mitoses of the ventral part are always in advance of those in the dorsal region (Fig. 4).

The second round of mitotic waves in each row (E(2) to eXV) follows the same mode as the first. Each of the two descendant rows, ab and cd (products of the first mitotic wave), undergoes a second mediolateral wave of division. At least in the circularly arranged rows, the second mitotic wave starts when the adjacent anterior row has almost finished its second wave of division (except for three to four cells on the dorsal side of descendant row cd). Descendant row ab is always in advance of the corresponding descendant row cd (Figs. 4, 6) and has almost completed its second mitotic wave (except for three to four cells per side) when cd begins to divide. The temporal gradient of lateral propagation of the second mitotic wave is, like that of the first, relatively flat. Row E(2) initiates the second round of divisions on the germ band at 25–30% development. Rows E(2)ab and E(2)cd begin their cleavage, closely followed by rows E(3)ab and cd (Fig. 2C).

Differential cleavages

After the two mediolateral mitotic waves, the cells of the resulting four descendant rows a, b, c, d of each ectoderm row undergo a set of differential cleavages. Mitoses are no longer all equal and not all spindle axes are longitudinally orientated. However, all individual divisions of the differential cleavages are defined and invariant with regard to their equality and spindle direction. Only the sequence of the individual divisions varies between different embryos and between both sides of an embryo. The mediolateral propagation of divisions ceases and morphogenesis of segments and appendage buds begins. Early neurogenesis takes place by differentiation of specific cells to neuroblasts which in turn give rise to ganglion mother cells. An analysis of the division pattern beyond the fifth lateral cells of the descendant rows was made difficult by the evagination of appendage buds and the corresponding cell divisions in this area with short mitotic cycles.

The differential cleavages on the germ band begin in row E(2) at 30% development (Fig. 5). Table 1 displays the characteristics of the individual mitoses of the first differential cleavage in row E(2). The mitotic activity during the first differential cleavage propagates from two centres on either side of the midline. One is the region of the evaginating appendage bud and the adjacent part of the invaginating segmental border (cells b_4,

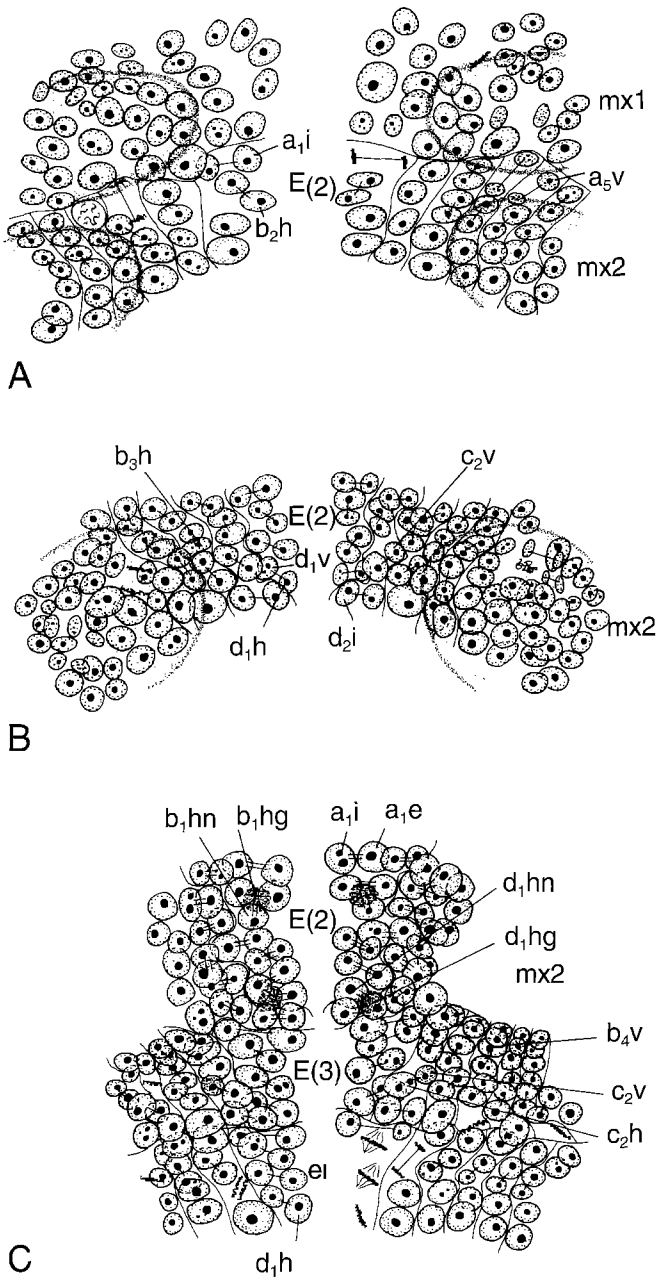

Fig. 5A–C. Differential cleavages in row *E(2)*. Compare Table 1. The median row is omitted. The daughter cells resulting from differential cleavages in the ectoderm rows are labelled *e, i, v, h*. This indicates the relative position of cells. For instance, the cell d_1 in row E(2) divides into an anterior cell d_1v and a posterior cell d_1h, cell a_1 divides into an inner cell a_1i and an outer cell a_1e (Figs. 2B, C). **A** Beginning of first differential cleavage (*one connecting line*). Several cells in descendant rows *E(2)a* and *b* are divided. Note the pattern in the area in front of row *E(2)*. **B** Advanced differential cleavage. Most cells have undergone their mitoses. The interpretation of the cellular differentiation of the limb buds of second maxilla (*mx2*) is difficult due to high mitotic activity and short cell cycles. **C** Early second differential cleavage in row E(2) (*two connecting lines*). The first neuroblasts (*n*) and ganglion mother cells (*g*) are differentiated in row *E(2)*, the cells b_1h and d_1h divide into b_1hn, d_1hn, b_1hg, d_1hg. Row *E(3)* shows an advanced first differential cleavage. The mitosis of cell $E(3)c_2$ differs from the corresponding mitosis in row *E(2)* (see Table 1 and Fig. 5B). It is highly unequal and the anterior daughter cell $E(3)c_2v$ is small with a darkly staining nucleus. The differential cleavage in row *eI* begins. *mx1*, first maxilla; *mx2*, second maxilla

Table 1. Characteristics of the individual mitoses of first differential cleavage in row E(2)

Cell	Direction[a]	Size[b]
a_1	–	a_1 i > a_1 e
a_2	/	a_2 v = a_2 h
a_3	|	a_3 v = a_3 h
a_4	|	a_4 v = a_4 h
a_5	|	a_5 v = a_5 h
b_1	|	b_1 v = b_1 h
b_2	|	b_2 v = b_2 h
b_3	|	b_3 v = b_3 h
b_4	|	b_4 v = b_4 h
b_5	|	b_5 v = b_5 h
c_1	/	c_1 v = c_1 h
c_2	/	c_2 v ≦ c_2 h
c_3	|	c_3 v = c_3 h
c_4	|	c_4 v = c_4 h
c_5	|	c_5 v = c_5 h
d_1	/	d_1 v = d_1 h
d_2	–	d_2 i = d_2 e
d_3	–	d_3 i > d_3 e
d_4	\	d_4 i = d_4 e
d_5	\	d_5 v = d_5 h

[a] Shows the spindle orientation in relation to the midline (on the left)
[b] Indicates the relative size of the daughter cells

b_5). From there division activity propagates in all directions. The other centre lies close to the midline (cells a_1 to d_1) with division activity proceeding laterally. Both events are overlayed by an anteroposterior decline of differentiation. The divisions in descendant row E(2)b are always in advance of those in row E(2)a and followed by those in E(2)c. Divisions in E(2)d are delayed. As in the earlier stages, lateral halves of the germ band show a certain independence with regard to the timing of their divisions (Fig. 5).

As mentioned above, the areas forming the first and second maxillae are characterised by a relatively early mitotic activity. Row structures were never recognised in this area although the arrangement of ectoderm cells gives the appearance of a defined pattern of division.

In principle, the first differential cleavage of all subsequent rows (except for the last row eXV, Fig. 7) follows the procedure described for row E(2). Slight differences concerning spindle directions and equality of single mitoses were observed between the cell rows (Figs. 5, 6, 7, 8). Rows E(3) to eIV contain more lateral cells than E(2) and all rows from eV on are arranged in rings. Events in the dorsal region of the blastoderm during the differential cleavage have not been studied in detail, but lateral and dorsal to the appendage bud anlagen all mitoses are more or less equal and longitudinal spindle axes are the same as those of the preceding two waves of division (Fig. 8B).

The first differential cleavage in a given row begins when first differential cleavages in the next anterior row are advanced and the posterior adjacent row is undergoing the second mitotic wave. An example of the degree of differentiation in several neighbouring rows is shown in Fig. 6.

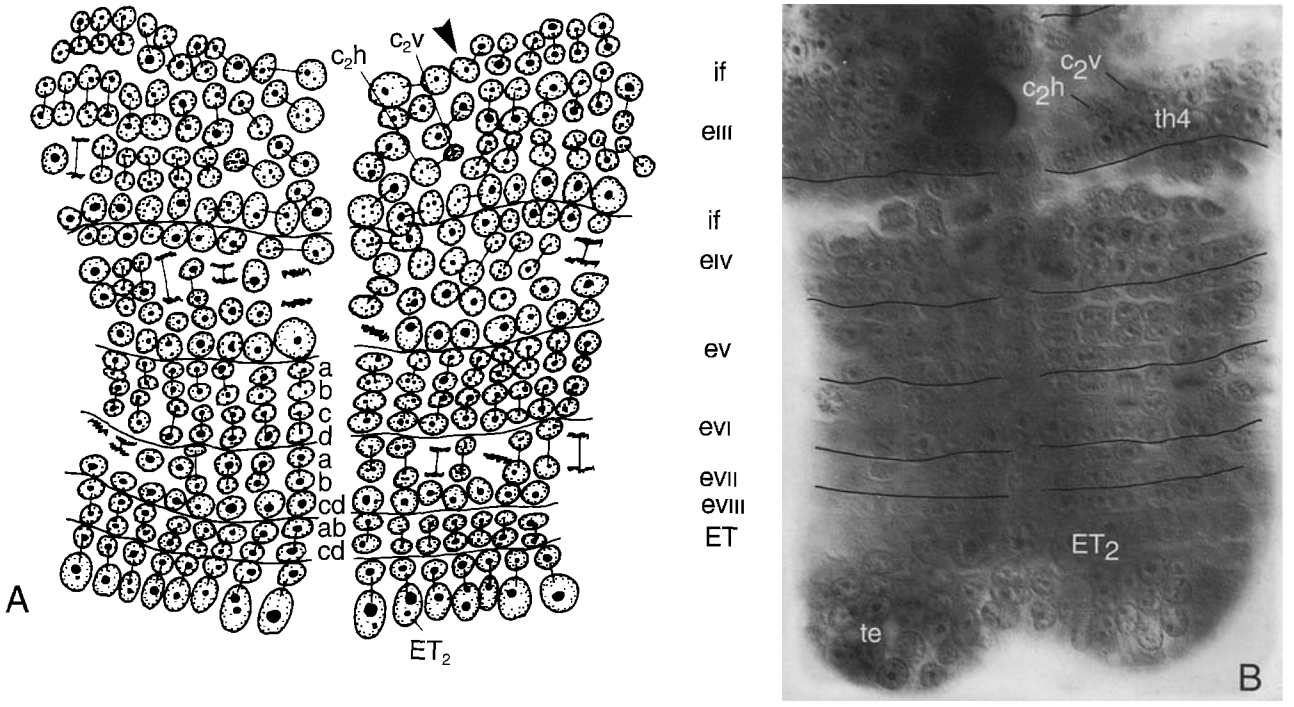

Fig. 6A, B. Survey of the ventral side of the posterior region (caudal papilla folded backward) of an embryo at 42% development. The anteroposterior decrease of the degree of differentiation becomes evident. Row *eIII* shows an advanced first differential cleavage and segmentation begins. The mitosis of cell $eIIIc_2$ ($eIIIc_2h >$ $eIIIc_2v$, the latter with a darkly staining nucleus) shows the same characteristics as in row E(3). The spindle direction of the mitosis of cell $cIIIa_2$ (*arrowhead*) differs from those of rows E(2) and E(3) (compare Fig. 5) and corresponds to those in all other ectoteloblast descendant rows (compare Fig. 7). Row *eIV* begins its first differential cleavage. Row *eV* is in the stage of an advanced second mitotic wave. In row *eVI*, some cells of descendant row *ab* have devided beginning the second mitotic wave. Row *eVIII* shows an advanced first mitotic wave. Row *eVIII* is formed. **A** Camera lucida drawing. **B** Micrograph of the same preparation. *if*, Intersegmental furrow; *te*, telson ectoderm; *th4*, fourth thoracic segment

Analysis of the second differential cleavage could not be taken very far because invagination of the intersegmental furrows and evagination of limb buds obscure the fate of single cells and their progeny. Fig. 10A shows the beginning of the second differential cleavage in row eII. Several neuroblasts and ganglion mother cells are differentiated during the second and following differential cleavages. These events are described later under neurogenesis.

Segmentation

In contrast to the nauplius region where the segments of the first and second antennae and the mandible are formed without a corresponding recognisable temporal and spatial division pattern, segmentation in the post-naupliar germ band is related to a clearly definable differential division pattern of ectoderm rows. This correlation is not so strict in the maxillary and first thoracic segments where intersegmental furrows and limb buds appear relatively early – even before differential cleavages have started (Fig. 2). Segments of the post-naupliar germ band are formed successively from front to rear corresponding to the stage of differentiation in the ectoderm rows and their progeny (Figs. 6, 9). Segmentation begins during the early first differential cleavage or immediately before it. The intersegmental furrow is formed first followed by the evagination of the limb buds (Figs. 6, 8). Morphogenetic events on the ventral side are in advance of those on the dorsal side.

Each ectoderm row represents a parasegmental unit. Thus, the segmental borders do not match the clonal or genealogical boundaries which are marked by the four descendant rows a, b, c, d of each ectoderm row. Instead, the intersegmental furrow runs transversely and slightly obliquely through the area of the descendants of row b after the first differential cleavage, so that one segment is composed of cells which derive partly from descendants of an anterior cell row, namely from rows c and d, and partly from descendants of row a of the adjacent posterior cell row (Figs. 6, 8).

The tips of appendage buds are built of cells from the area $c_{4/5}$, $d_{4/5}$, and $a_{4/5}$ of the next posterior row (Figs. 6, 8A). The last abdominal appendages, the uropods, are formed from derivatives of rows eXIII and eXIV (Fig. 7). No major differences were found between the thoracic and abdominal segment anlagen at the level of the first differential cleavage. Only the maxillae show slight deviations with regard to some mitotic characteristics in row E(2) (Figs. 5, 6, 7, Table 1).

As mentioned above, the area in front of row E(2) undergoes a different set of cleavages which could not be analysed. The derivatives of this region are the material for the anterior part of the first maxilla and the posterior border of the mandibular segment. The caudal

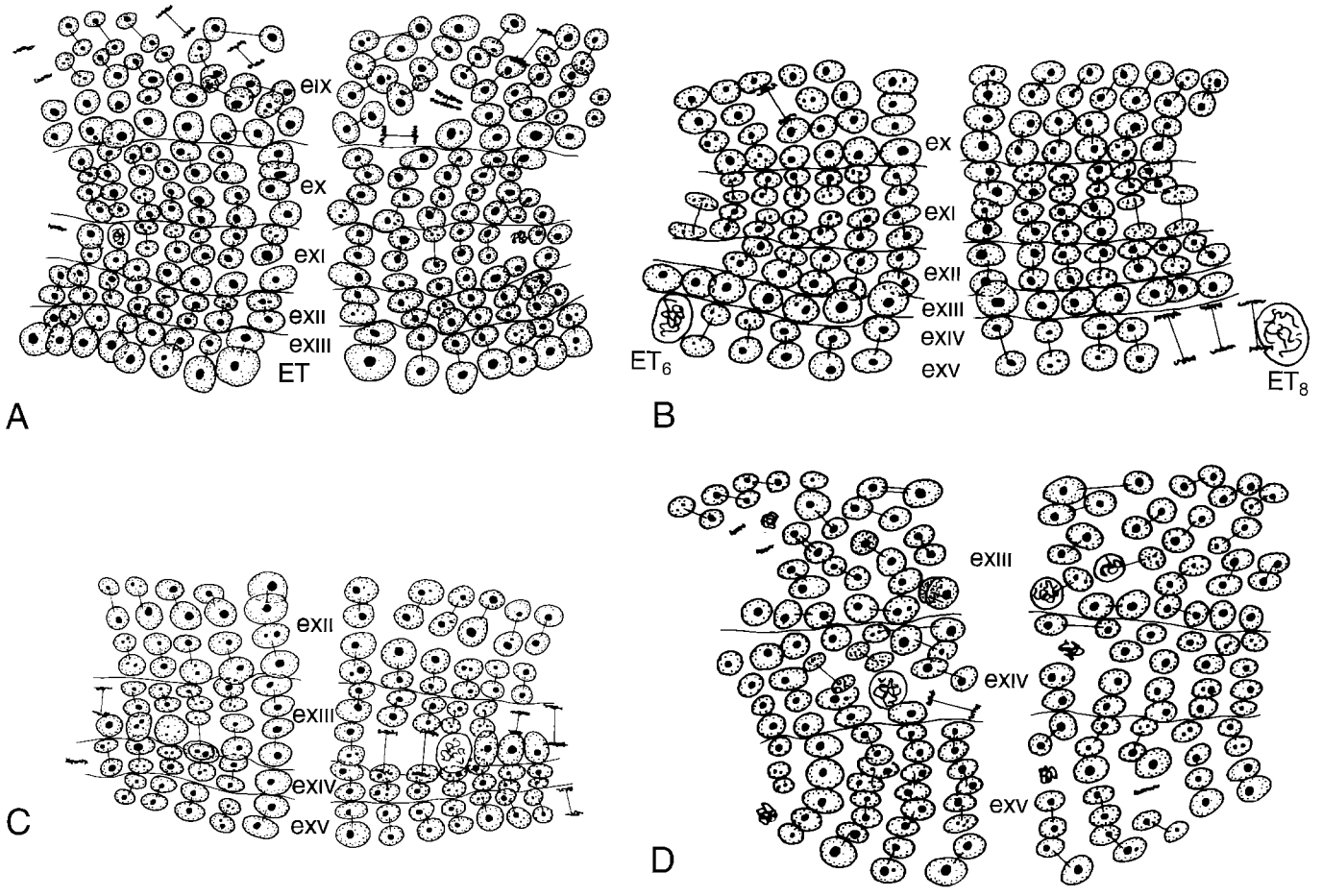

Fig. 7A–D. The last divisions of the ectoteloblasts (*ET*)-formation and cleavage of the posteriormost rows at 55–60% development. **A** Posterior end of an embryo. Row *eXIII* is formed by the last unequal division of the ectoteloblasts. **B** The ectoteloblasts divide equally forming rows *eXIV* and *eXV*. **C** The first mitotic waves in rows *eXIV* and *eXV* occur simultaneously. **D** Row *eXIV* undergoes a normal first differential cleavage, whereas the cells of row *eXV* divide with high frequency only in longitudinal direction

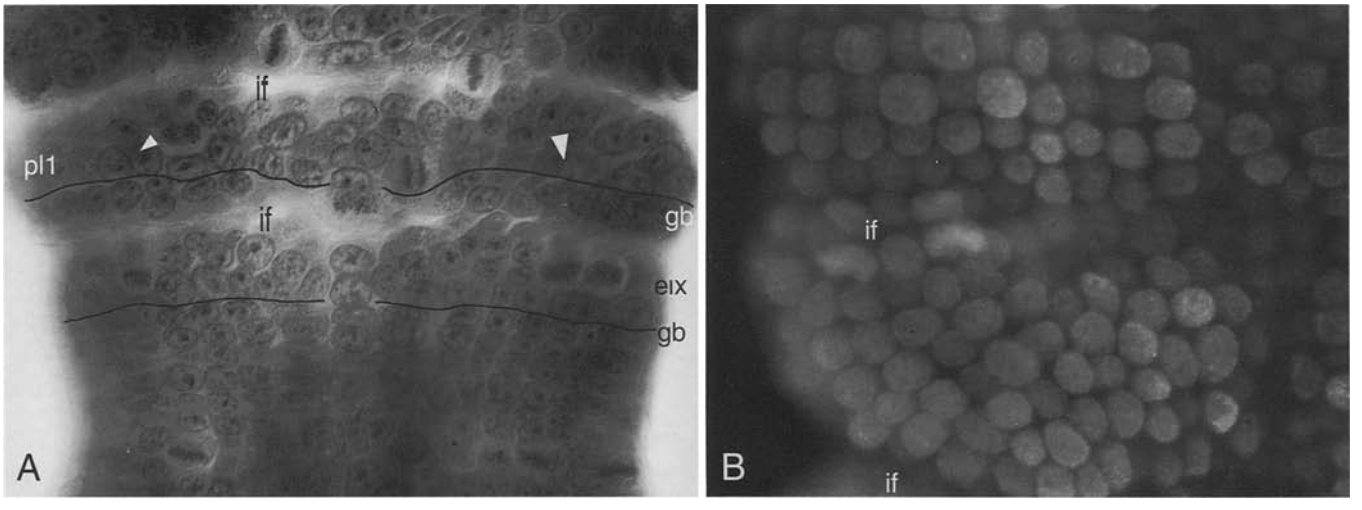

Fig. 8. A Anlage of the first abdominal segment (*pl1*) at 45% development (ventral side). Row *eXIII* is formed. Note the position of the intersegmental furrow (*if*) within the descendants of row *eIX*. *Arrows* mark the tips of the appendage buds. *gb*, Genealogical border. **B** The left dorsal side of forming abdominal segments showing the little differentiation on the cellular level compared to the ventral side. *if*, intersegmental furrow

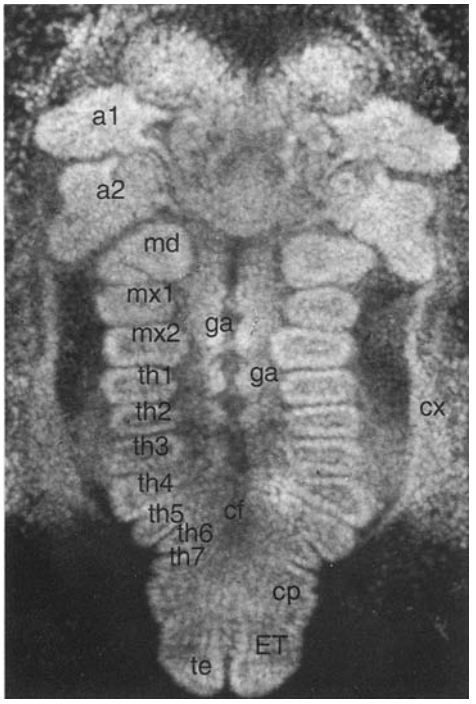

Fig. 9. Embryo at 45% development. Row *eXI* is formed. *a1, a2*, First and second antennae; *cf*, caudal furrow; *cp*, caudal papilla (folded backward); *cx*, carapace; ga, ganglion anlage; *md*, mandible; *mx1, mx2*, first and second maxillae; *th1, th5*, first and fifth thoracic appendage

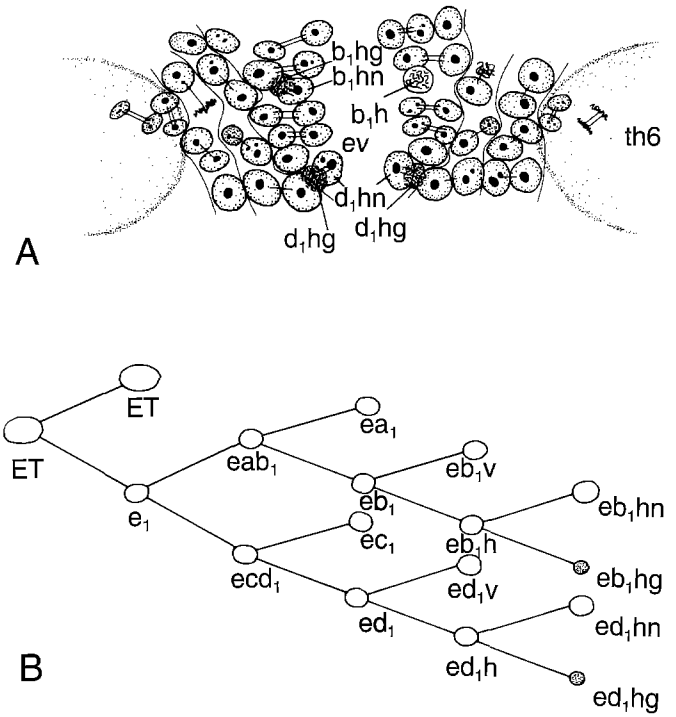

Fig. 10A, B. The differentiation of the first neuroblasts and ganglion mother cells in thoracic and abdominal segments (compare Fig. 5C). **A** Camera lucida drawing of row *eV* at 45% development. The neuroblasts, eVd_1hn and eVb_1hn, and the corresponding ganglion mother cells, eVd_1hg and eVb_1hg, are formed on the animal's right side. On the left side, cell eVb_1h is in metaphase. *th6*, sixth thoracic appendage. **B** Schematic representation of the lineage of first neuroblasts and ganglion mother cells

furrow matches the border between the fifth and sixth thoracic segment, the region of row eVb (Fig. 9). Thus, the caudal papilla consists of the last three thoracic segments and all the abdominal segments and the telson. The posteriormost intersegmental furrow invaginates in the region of row eXIVb and marks the border between the sixth abdominal segment and the telson (Fig. 7). The anlagen of all segments are formed at about 55 to 60% development.

Early neurogenesis

Neuroblasts can be identified when they start their typical mode of division. They give rise to smaller derivatives (the ganglion mother cells) toward the centre of the embryo, thus producing columns of ganglion mother cells lying beneath the outer ectoderm layer (Fig. 11). The ganglion mother cells undergo equal mitoses to produce neurons – how many rounds and whether the ganglion mother cell derivatives are exclusively neurons was not clarified.

The first two neuroblasts are differentiated during the second differential cleavage of a row (Figs. 5C, 10). Divisions of the cells b_1h and d_1h are unequal and the spindle directions point toward the centre of the embryo. The larger division products, the neuroblasts b_1hn and d_1hn, remain in the surface layer. Their smaller sister cells, the first ganglion mother cells b_1hg and d_1hg, have a darkly-staining nucleus and lie deeper in the yolk due to the spindle direction. Additional neuroblasts are differentiated during the following rounds of differential cleavage. The neuroblasts are situated in the area between the forming limb bud and the midline (Fig. 11). Analysis becomes more difficult with the progression of morphogenetic events (segmentation, limb bud formation) and the increasing number and density of cells. The neuroblast numbers increase to a total of 25–30 per hemisegment arranged in 4–5 irregular files containing 6–7 neuroblasts. One additional median neuroblast is differentiated at the posterior margin of the segment (Fig. 11). Early neuroblast differentiation is almost identical in most ectoderm rows (E(2) to eXIV). The sequence of the appearance of the first two neuroblasts varies slightly between E(2) and the other rows (Figs. 5C, 10A). The first neuroblasts in the post-naupliar region appear at 35% development in the maxillary area (Fig. 5C).

Like the limb buds of each segment in the post-naupliar germ band, segmental ganglion anlagen are composed of derivatives of two ectoderm rows (Fig. 11A, B).

The situation in the post-naupliar area anterior to row E(2) was unclear, although neuroblasts and ganglion mother cells were identified there (Fig. 11D). Neuroblasts are also differentiated in the naupliar segments,

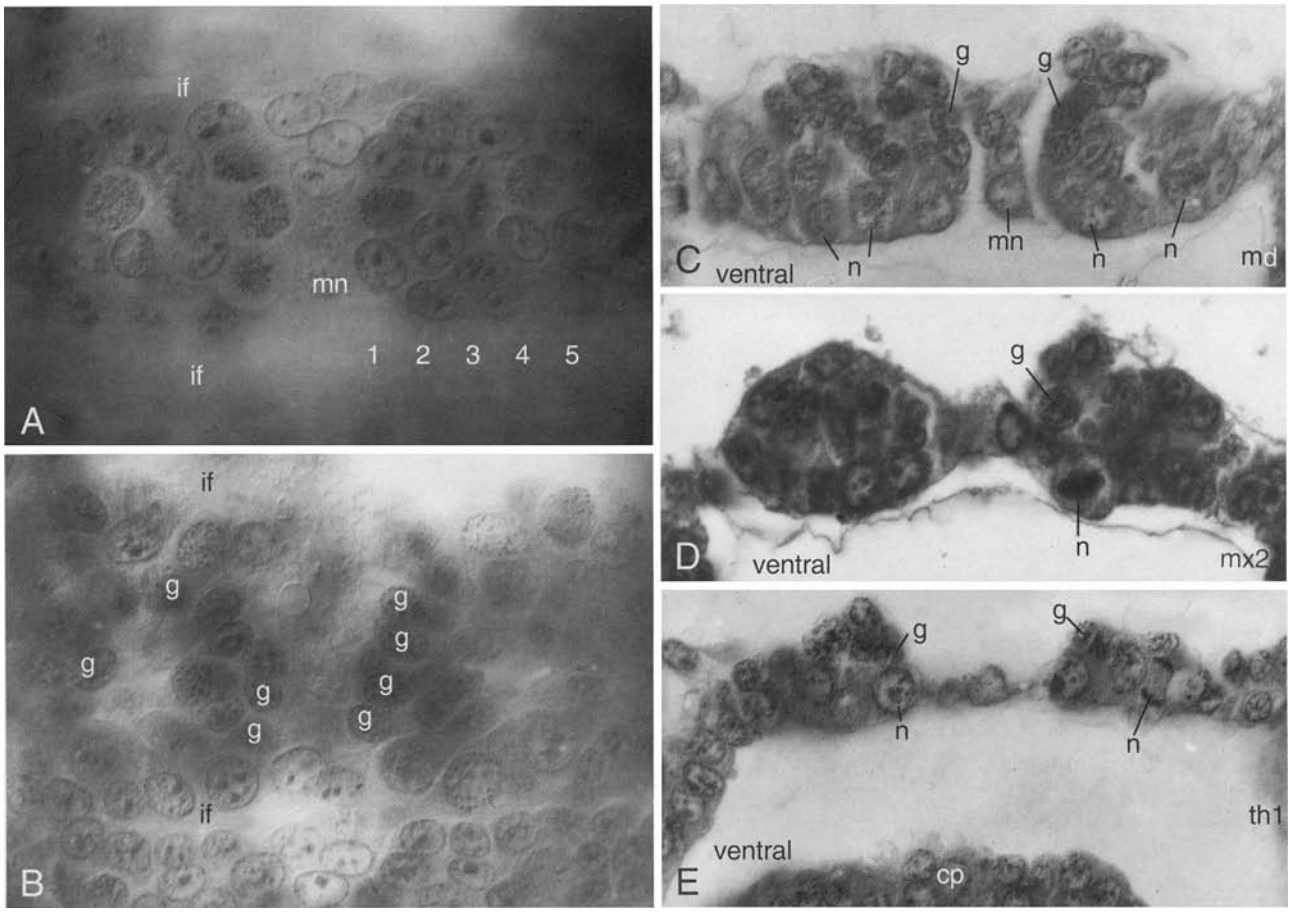

Fig. 11. **A** Ventral view of the neuroblasts of the sixth thoracic segment at 45% development (wholemount). Many neuroblasts are in metaphase. *if*, Intersegmental furrow; *mn*, median neuroblast. The numbers represent the tiles of neuroblasts. **B** Same preparation focussed somewhat deeper. The arrangement of the ganglion mother cells (*g*) is shown (not all designated). **C** Transverse section through the ganglion anlage of the mandibular segment at 40% development. The columns of ganglion mother cells (*g*) are recognisable. *md*, Mandible; *mn*, median neuroblast; *n*, neuroblast. **D** Transverse section through the ganglion anlage of the second maxillary segment at 40% development. The mitosis of a neuroblast (*n*) is seen in the right side. *mx2*, Second maxilla. **E** Transverse section through the ganglion anlage of the first thoracic segment at 40% development. The mitosis of a neuroblast is to be seen on the right side. Only a few ganglion mother cells (*g*) are formed. Note the similarity between the patterns of the mandibular, the maxillary and the thoracic ganglionic primordia. This similarity exists despite the different origin and patterning of these segments (see text). *cp*, Caudal papilla; *th1*, first thoracic appendage

where they appear at 20–25% development between the limb buds and give rise to columns of ganglion mother cells by unequal mitoses as in the post-naupliar segments (Fig. 11 C). No division pattern comparable to the differential cleavages of the post naupliar region is recognisable.

Discussion

Ectoteloblasts and evolutionary alterations of early developmental events

The ancestral condition for the higher Crustacea (Malacostraca), 19 ectoteloblasts and 8 mesoteloblasts arranged in rings (Dohle 1972; Zilch 1974), has also been described for many species of decapods (e.g. Oishi 1959, 1960). Recorded ectoteloblast numbers close to 19, e.g. 18 in *Panulirus japonicus* (Shiino 1950), are probably identification errors as discussed by Oishi (1959, 1960). Furthermore, Oishi (1959, 1960) describes for several decapods a defined invariant division pattern forming the ectoteloblasts. In contrast to this, in *Cherax*, a ring with a variable number of about 39–46 ectoteloblasts is formed without a stereotyped division pattern. The number of mesoteloblasts in *Cherax*, on the other hand, remains conservative (8 cells). Figures in the publications of Reichenbach (1888), Fulinski (1908) and Patel et al. (1989) on various freshwater crayfish species reveal a number of ectoteloblasts similar to that of *Cherax*. The higher number of ectoteloblasts apparently represents a derived character of freshwater crayfishes in general.

The crayfishes are not the only example of an alteration of the basic malacostracan pattern of teloblasts. In the Peracarida, the teloblastic rings are rearranged as transverse rows giving rise to a flat germ band that lacks a true caudal papilla (Dohle 1972; Scholtz 1984). The Peracarida have a variable number of ectoteloblasts (15–23) but still possess 8 mesoteloblasts (reviewed by

Dohle and Scholtz 1988). Within the peracarids, the amphipods have entirely lost the ectoteloblasts and the budding zone consists of scattered cells with no regular divisions (Dohle 1976b; Scholtz 1990).

The evolutionary alterations in the number, arrangement, and formation of ectoteloblasts in Malacostraca give another clear example of the possible modification of early developmental characters without a necessary effect on subsequent differentiation (e.g. Sander 1983; Dohle and Scholtz 1988; Henry and Raff 1990; Wray and Raff 1990). At least in decapods and peracarids, the cellular division pattern of the post-naupliar germ band is homologous (see below) despite its different mode of generation.

Pattern development in Cherax *and in the Peracarida*

Segment formation in the ectoderm of *Cherax* resembles in many details the events described in species of Cumacea, Tanaidacea, Isopoda, Mysidacea, and Amphipoda (Peracarida) (Dohle and Scholtz 1988) – in particular, with respect to the cell division pattern in the post-naupliar region, the differentiation of neuroblasts, the parasegmental character of the ectoderm rows, the position of the intersegmental furrow which does not match the genealogical border, the different origin of the maxillary segments, and the formation of the naupliar region without a recognisable early cell division pattern. The similarity of the differential cleavage patterns in the post-naupliar germ bands of *Cherax* and the peracarids in particular, is so great that homology is evident in this character. Therefore, this specific pattern must have appeared earlier in malacostracan phylogeny than proposed by Dohle (1976b) and Sieg (1984), who suggested it was a new acquisition (apomorphy) of the Peracarida. It is now necessary to investigate the embryos of Leptostraca, Hoplocarida, and non-malacostracan Crustacea to find out whether this pattern is shared by all Malacostraca and whether it is an apomorphy for this group.

Apart from the general similarities, some differences in the segmentation patterns between *Cherax* and the peracarids can be seen as follows:

– During the first differential cleavage, the nuclei in *Cherax* are more similar in size and staining properties than in peracarids.

– In contrast to the peracarids, where the proliferation zone gives rise only to the ventral side of the embryo, in *Cherax* the dorsal side posterior to and including the sixth thoracic segment is also formed by ectoteloblast activity.

– Ectoteloblasts in *Cherax* bud one ectoderm cell row more than in *Diastylis rathkei* (Dohle 1976a) and *Neomysis integer* (Scholtz 1984) before they disappear, perhaps indicating the rudimentary anlage of a seventh abdominal segment.

– The velocity of mediolateral propagation of the two mitotic waves is higher in *Cherax* than in peracarids resulting in more irregularities in the sequence of divisions.

The most profound difference between *Cherax* and the peracarids is found in the temporal relation between posteroanterior germ band proliferation and subsequent anteroposterior segmentation. Compared to *Cherax*, segmentation in the peracarids occurs later in relation to the formation of new rows, resulting in a longer unsegmented germ band (see next paragraph).

Segmentation mechanisms in short-germ and long-germ types of development

The extreme cases of the insect "egg types" (Krause 1939), the short-germ and the long-germ type of development, have been considered by several authors to be fundamentally different with regard to their mechanisms of segmentation. In particular, stress is placed on the difference between segment proliferation by a caudal budding zone (short-germ) and the segmental subdivision of an extended germ band (long-germ) (Sander 1983; Mee and French 1986; Krause 1987; Tear et al. 1988; Patel et al. 1989). Dohle (1972), on the other hand, has suggested that the budding zone does not proliferate segments but only produces competent cellular material which is subsequently subdivided and that germ band formation and segmentation are two overlapping and partly independent processes. Experiments in *Artemia salina* support this concept (Freeman 1989). Larvae treated with bromodeoxyuridine, a suppressor of cell differentiation, show no further segmentation, whereas proliferative growth is not affected.

Compared to insects, *Cherax* would clearly represent the short-germ type of development. Peracarids, on the other hand, show an intermediate type; their germ bands are relatively extended before segment formation occurs. Interestingly, within the peracarids, amphipods which do not differentiate ectoteloblasts (Dohle 1976b; Scholtz 1990) resemble most the long-germ type. In a corresponding stage where 15 ectodermal cell rows are formed, in the amphipod *Gammarus* no segments can be seen (Scholtz 1990), whereas in *Cherax* segmentation has already proceeded up to the first abdominal segment. These differences between the germ bands of *Cherax* and *Gammarus* confirm that proliferation of material and segmentation are independent events and that the temporal correlation between these two processes can be altered to a great extent. This leads to the suggestion that evolution toward the long-germ type in general was initiated by a delay of the segmentation process and/or an acceleration of material formation, and that fundamental changes of the basic mechanisms of segment formation are not necessarily required – a point of view supported by theoretical models (Meinhardt 1982). This does not exclude the possibility that additional mechanisms have evolved in long-germ embryos – for instance, it seems likely that a role for pair-rule genes during segmentation is an evolutionary innovation in long-germ insects, at least in dipterans (Patel et al. 1989). According to this hypothesis, the shift from short germs to long germs would represent a case of heterochronic evolution (Gould 1977). In the nematode *Caenorhabditis elegans*, for instance, several heterochronic mutants are known (reviewed by Ambros 1988) which show advanced development of certain structures in relation to other embry-

onic events. A genetic change of this kind could have underlain the initial evolution of the long-germ type of development.

Because it seems likely that only minor evolutionary changes in the temporal pattern are necessary, the convergent tendency toward long-germ development in different insect lines (Sander 1983), in myriapods (Heymons 1901), in Crustacea (e.g. Grobben 1879; Scholtz 1986), and in chelicerates (e.g. Moritz 1957) becomes understandable.

Cell lineage and differentiation

The formation of segments, appendages, and ganglia in the post-naupliar region is correlated with the ordered cellular division pattern – despite the different origin of the cell rows (teloblastic vs. non-teloblastic). On the other hand, homologous structures are formed in the naupliar segments without a comparable early cellular division pattern. Thus, the comparative analysis of cell fate in the different regions of the germ band of *Cherax* reveals that the commitment of a certain cell to become a neuroblast, part of a limb bud, etc. is not due to a specific genealogy or division pattern. The same has been shown for germ band proliferation and segmentation in several Crustacea (Dohle and Scholtz 1988) and for blastoderm formation in dipteran insects (Sommer and Tautz 1991). Transplantation and ablation experiments with neuroblasts and their precursors, respectively, in insects, where no regular division patterns occur, point to the same conclusion (Technau et al. 1988; Doe and Goodman 1985b). Germ band formation, metamerization, and early neurogenesis in arthropods are obviously not controlled by cell lineage. The highly ordered lineage pattern found in higher Crustacea might merely be a specific way to form the material for subsequent segmentation and neurogenesis, which could be regulated by positional information or gradients (Dohle and Scholtz 1988; Patel et al. 1989).

A common plan for neuroblasts in insects and crustaceans?

In thorax and abdomen of several insect species, neuroblasts have been described which are arranged in a specific pattern and with a fixed number per segment (e.g. Bate 1976; Doe and Goodman 1985a; Tamarelle et al. 1985; Hartenstein et al. 1987). The similarity in appearance, arrangement, and number of neuroblasts and ganglion mother cells between insects and crustaceans is at first sight striking (compare Fig. 11 to Fig. 1 in Bate 1976). This has led to claims of homology and a "common plan for neuronal development", including neuroblast differentiation in arthropods (Thomas et al. 1984). This point of view was supported by similar patterns of *engrailed* gene expression in insect and crustacean neuroectoderm (Patel et al. 1989). However, a closer look at neuroblast differentiation reveals some characteristic differences which suggest that the main similarity between insect and crustacean neuroblasts is only that they both act as typical stem cells. Neuroblasts in insects separate from the undifferentiated ectoderm layer by sinking into the embryo before they begin their proliferation of ganglion mother cells (e.g. Malzacher 1968; Tamarelle et al. 1985; Doe and Goodman 1985a; Hartenstein et al. 1987), whereas crustacean neuroblasts remain in their superficial position during division (e.g. Dohle 1976a; Scholtz 1990; present investigation). The differentiation of neuroblasts in *Schistocerca americana* is accompanied by the formation of several specialised cells such as sheath cells, cap cells, and glia precursors arranged in a complex manner (Doe and Goodman 1985a). We cannot be certain whether this complicated pattern is shared by the insect ancestor or not, but at least the sheath cells are common to many different insects (e.g. *Periplaneta americana, Carausius morosus*: Malzacher 1968; *Schistocerca americana*: Doe and Goodman 1985a; *Drosophila melanogaster*: Hartenstein et al. 1987). No such corresponding specialised cells can be found in early crustacean neurogenesis. The final number of neuroblasts per segment in insects (which varies between different species) is reached by three waves of neuroblast differentiation (Doe and Goodman 1985a; Tamarelle et al. 1985; Hartenstein et al. 1987). In Crustacea there is a continuous addition of new neuroblasts with each round of differential cleavages in the post-naupliar region (Dohle 1976a; Scholtz 1990).

The current study supports the view of Dohle (1976a) and Dohle and Scholtz (1988), based on a phylogenetic comparison, that neuroblasts have evolved convergently in insects and higher crustaceans, rather than the hypothesis that these cells are homologous in the two groups (Thomas et al. 1984; Patel et al. 1989). There is no evidence for the existence of true neuroblasts (i.e. stem cells with unequal divisions) in the lower (non-malacostracan) Crustacea (e.g. Benesch 1969; Weygoldt 1960). "Myriapods" the closest relatives of insects, also lack neuroblasts. Ventral ganglia in these animals are formed by an invagination process (e.g. Heymons 1901; Tiegs 1940; Dohle 1964; Whitington et al. 1991) and the anti-*engrailed antibody* does not bind to cells in ganglionic primordia (Whitington et al. 1991).

The differences of neuroblast formation and the phylogenetic analysis lead to the conclusion that neuroblasts have evolved independently in insects and in higher Crustacea.

Acknowledgements. I am very grateful to David Sandeman and Renate Sandeman for the opportunity to work in their laboratory and the stimulating atmosphere they always create. I thank Wolfgang Dohle, John Freeman, David Sandeman, and Paul Whitington for critical reading of the manuscript and Rosa Sezak for technical assistance. This work was supported by a visiting fellowship from the University of New South Wales and an Australian Research Council grant to David Sandeman.

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