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Fate-mapping in wild-type *Drosophila melanogaster*

III. A fate map of the blastoderm

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Summary. Here we propose a fate map of the *Drosophila* blastoderm based on reconstructions of increasingly aged embryos and on results of horseradish peroxidase (HRP) injections in early gastrula cells. Boundaries of blastoderm anlagen have been extrapolated from size, form and location of the corresponding larval primordia, once these primordia become distinguishable at later embryonic stages.

Key words: Blastoderm fate map – Embryogenesis – *Drosophila*

Introduction

In the following we present a fate map of the blastoderm of *Drosophila* that includes the location and the number of progenitor cells of most larval organs. This fate map has been obtained from two different sets of data. One was derived from normal histological studies on the spatio-temporal pattern of embryonic cell proliferation. The pattern of embryonic cell divisions in *Drosophila* was found to be regular; therefore by counting the number of cells in several larval organs, once they become recognizable in embryogenesis, we were able to estimate the number of cells in the corresponding blastoderm anlagen (Hartenstein and Campos-Ortega 1985). The other data were collected by injecting a marker substance, horseradish peroxidase (HRP), in cells of the early gastrula stage and studying the distribution of the label in embryos at, or shortly after, germ band shortening. This approach permitted us to follow blastoderm cells through subsequent morphogenetic movements and elucidate their fate (Technau and Campos-Ortega 1985). Thus both approaches gave complementary results. Most of the evidence for the conclusions presented below can be found in these two accounts.

Materials and methods

The bulk of material used to construct the blastoderm fate map is described in Hartenstein and Campos-Ortega (1985) and Technau and Campos-Ortega (1985). The fate map is based upon a computed planimetric reconstruction of the blastoderm. For this reconstruction we calculated (i) the average surface of a lateral projection of the blastoderm and (ii) the average number of cells in this stage. First of all, the lateral profile of each of ten living embryos at the cellular blastoderm stage was drawn with a camera lucida at 440× magnification. These drawings were digitalized with a semi-automatic image analyser (MOP 2, Kontron-Meßgeräte, GmbH) and averaged by a computer, which calculated the mean area of the blastoderm and processed the data to elaborate a planimetric reconstruction. Since blastoderm cells are of very similar shape and size, with an approximately hexagonal arrangement, cells on this planimetric reconstruction were represented by evenly spaced dots. Second, the total number of cells to be included in the reconstruction was obtained from directly counting blastoderm cells in four different fuchsin-stained whole mounts. Dividing the computed blastoderm area by this figure gave 51 μm^2 as the surface occupied by a single cell, corresponding to a diameter of 7.7 μm per cell. Additionally the average distance of two cell nuclei on the blastoderm was measured directly in fuchsin-stained whole mounts using a semi-automatic image analyser, and found to be 7.5 μm; this value was then taken as diameter of the hexagonal arrangement of blastoderm cells. The area occupied by a single cell as determined by this method amounts to 48.3 μm^2.

In order to enable direct comparison of the results of HRP injections with those of the histological analysis, the positions at which injections were performed in the early gastrula (see Technau and Campos-Ortega 1985) were translated onto the planimetric reconstruction of an embryo of similar age, and from there to the blastoderm, as indicated in Fig. 1.

Results and discussion

The fate map

Figure 1C shows the fate map. The fate map is presented as a lateral prospect of the blastoderm and includes the location and the number of progenitor cells of several larval organs and of the imaginal optic lobes. The anlagen of other larval organs and imaginal discs could not be precisely located and were therefore omitted.

The rationale of fate map construction

The rationale followed to establish the fate map consisted of the following steps. Counting (i) how many times embryonic cells have divided until the various larval organs be-

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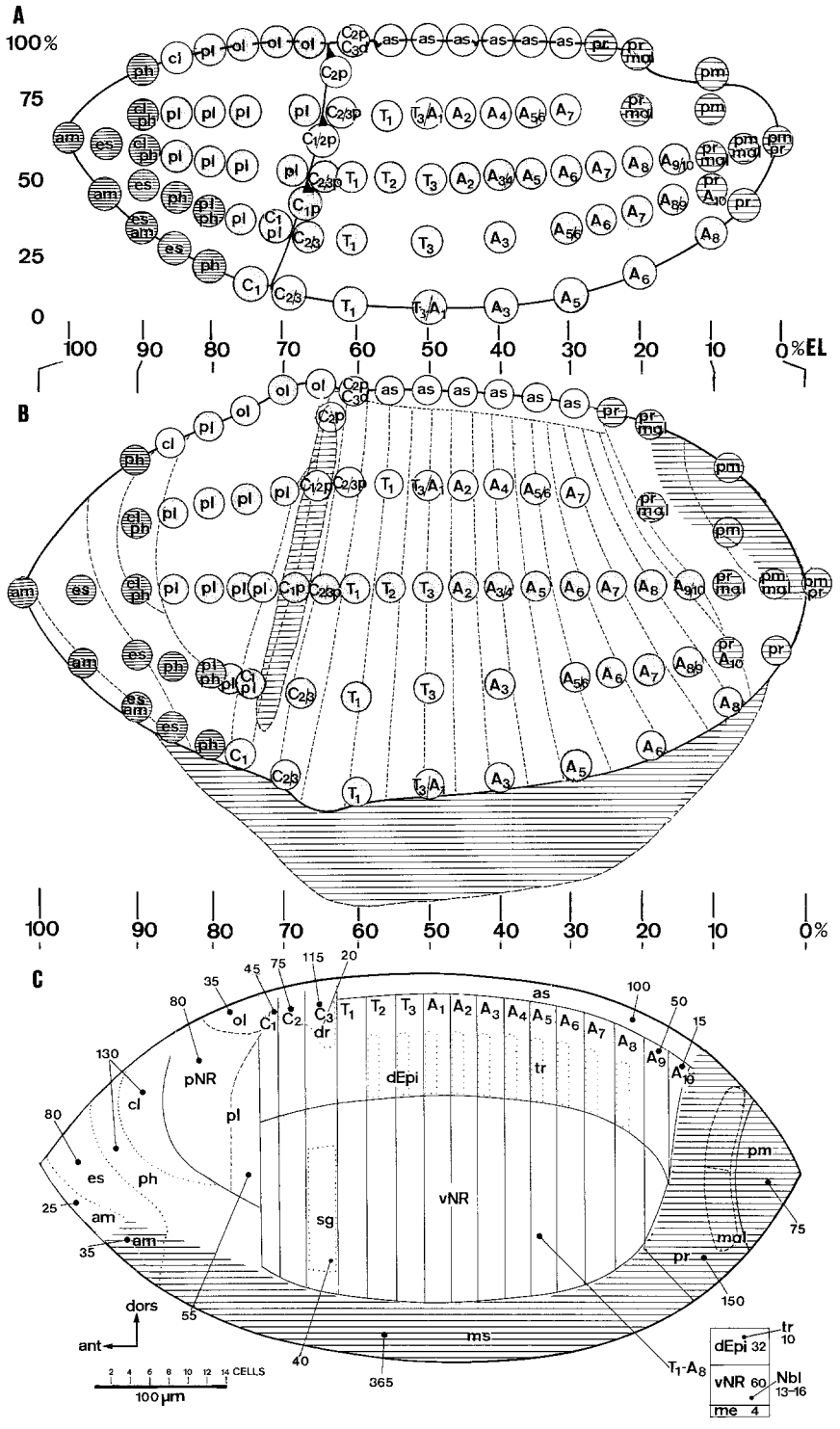

Fig. 1 A–C. Synopsis of the results of HRP injections in cells of the early gastrula (**A, B**), referred to the blastoderm fate map (**C**). Same magnification for all drawings

A Lateral view of the gastrulating embryo. The *circles* indicate injection sites, and the *symbols in the circles* location of the dye in the late embryo (see Technau and Campos-Ortega 1985). *Light shading* indicates injection sites resulting in labelling of neural tissue, hatched circles in gut regions

B Planimetric reconstruction of left half of the early gastrula shown in **A**. Injection sites are indicated as in **A**. Boundaries between anlagen are tentatively drawn as *dashed lines*. Note that germ band elongation has started, leading to an increase in length of the ventral part of the abdominal segments. *Hatching* shows either already invaginated anlagen (major part of anterior midgut and mesoderm; posterior midgut and part of the proctodeum form by this stage a conspicuous concave plate whose outlines are indicated by *hatching* as well) or transiently folded (cephalic furrow)

C Fate map of the *Drosophila* blastoderm. A planimetric reconstruction of the left half of the blastoderm is shown. *Hatched* areas will invaginate at gastrulation. *Numbers* indicate the size of the different anlagen referred to one side of the blastoderm. Notice that the size of the anlagen of the salivary glands (40) and of the dorsal ridge (20) is included in the size of C3. Scales indicate EL% (0–10% and 90%–100% values are distorted due to the reconstruction procedure). *am*: anterior midgut; *as*: amnioserosa; *C3d*: dorsal ridge; *cl*: clypeolabrum; *dEpi*: dorsal epidermis; *dr*: dorsal ridge; *es*: oesophagus; *mp*: Malpighian tubes; *ms*: mesoderm; *ol*: optic lobes; *p*: gnathal protuberances; *ph*: pharynx; *pl*: procephalic lobe; *pm*: posterior midgut; *pNR*: procephalic neurogenic region; *pr*: proctodeum; *sg*: salivary gland; *tr*: tracheae; *vNR*: ventral neurogenic region; *C1–C3*: gnathal segments; *C1p*: mandible; *C2p*: maxilla; *C3p*: labium; *T1–T3*: thoracic segments; *A1–A10*: abdominal segments. See text for further details

come distinguishable as primordia, and (ii) the number of cells of each larval primordium, permitted us to calculate the size of the corresponding blastoderm anlagen. Then (iii) analysing the morphogenetic movements performed by cells of the blastoderm, and (iv) using some assumptions, to be discussed at each particular case, we translated those data on the surface of the blastoderm.

The number of cells in each larval organ could be determined once boundaries between developing organs became distinguishable. Since most boundaries are well defined, no ambiguities were generally found while counting cells, nor when counting the number of divisions. Thus we are confident that the values obtained for the sizes of blastoderm anlagen are quite good approximations. In fact adding the numbers of cells calculated for each anlage (given in Fig. 1 C) results in a total of 4972 cells, which closely corresponds to the average figure of 5010 (SD 88) cells directly obtained from four different blastoderms (Hartenstein and Campos-Ortega 1985). However, it is a different matter to translate the course of larval organ boundaries to the blastoderm, for this procedure relies on indirect assumptions. In several cases boundaries become distinguishable at rela-

tively late stages, for example, the tracheal pits, the salivary glands or the Malpighian tubules. In other cases the prospective location of boundaries already becomes evident at early stages, either because of regional differentiations of the proliferation pattern, e.g. intersegmental boundaries, or because of morphogenetic movements, e.g. boundaries between germ layers. In any case, however, it is quite unfortunate that when boundaries become visible the original topological relationships of blastoderm cells have suffered considerable distortion because of intervening morphogenetic movements. Although morphogenetic movements can be analysed fairly well, both by reconstructing embryos of slightly different ages with methods of normal anatomy (see Hartenstein and Campos-Ortega 1985), and by following the fate of embryonic cells injected with HRP (see Technau and Campos-Ortega 1985), the *Drosophila* embryo is fairly large and those movements rather complex. Thus, there always remains a certain degree of uncertainty while tracing the course of larval organ boundaries back to the blastoderm, an operation that is each time somwhat arbitrary.

The first boundary distinguishable in development is the boundary between germ layers, i.e. between the primordia invaginated at gastrulation (mesoderm and endoderm) and the ectoderm; boundaries between the primordia of dorsal and ventral epidermis, of epidermis and hindgut, of epidermis and foregut, of hindgut and posterior midgut, of central nervous system and epidermis, and of consecutive segments, appear later on. Finally, particular organs, e.g. salivary glands, tracheal tree, Malpighian tubules etc. become evident. The total extent of the blastoderm region giving rise to mesoderm and endoderm, which invaginates at gastrulation, was easily determined from countings of transverse sections and of fuchsin-stained whole mounts, all remaining cells corresponding to the ectoderm and the amnioserosa. After counting the cells invaginated at gastrulation, boundaries between mesoderm and endoderm anlagen (posterior midgut primordium – mesoderm – anterior midgut primordium), as well as the boundary between proctodeum and posterior midgut, were determined, chiefly based on both quantitative considerations and the results of HRP injections. We assumed that the pole cells retain their original position relative to the underlying cells during germ band elongation. Under this assumption the bottom of the amnioproctodeal invagination, which carries the pole cells and is going to form the posterior midgut, must derive from the posterior pole of the blastoderm. The walls of the amnioproctodeal invagination can then be brought in relation to the posterior pole, and the number of cells can be counted. The number of cells of the posterior midgut anlage was found to be 150; on the basis of the shape of the amnioproctodeal invagination we assumed that the anlage of the posterior midgut has a regular shape around the posterior egg pole and, thus, we fixed the border to the proctodeum anlage at the position corresponding to a blastoderm area containing 150 cells. All these assumptions are strongly supported by the results of HRP injections. The anterior midgut-mesodermal border and the proctodeal-mesodermal border were determined in a similar way.

HRP injections have shown the proventriculus and the region of the gastric caeca to derive from cells invaginating with the stomodeum (Technau and Campos-Ortega 1985). Extrapolating from the HRP injections, it seems that stomodeal cells contributing to proventriculus and anterior midgut are located as a ventral blastoderm strip that extends up to the anterior tip and contains about 50 cells. On the other hand cells invaginating at the tip of the ventral furrow will form the remaining parts of the anterior midgut. From countings performed once these cells become distinguishable from the mesodermal primordium we estimate the size of the anlage to be about 70 cells. Thus the entire blastoderm anlage of proventriculus and anterior midgut consists of 120 cells.

The proctodeum develops from the anterior, lateral and posterior walls of the amnioproctodeal invagination, its anlage invaginating during gastrulation and germ band elongation. Therefore the proctodeum forms in fact part of a sac, the ground of which is the primordium of the posterior midgut. This means that the anlage of the proctodeum must be organized like a ring around the anlage of the posterior midgut. The results of HRP injections fully support this hypothesis.

The amnioserosa derives from a narrow cell strip at the dorsal midline of the blastoderm in register with the anlage of the metameric germ band, an arrangement that is evident from both normal anatomy (Poulson 1950) and HRP injections. Since the length of the amnioserosa anlage corresponds to that of the metameric germ band, its width can be extrapolated from the total number of amnioserosa cells, for the amnioserosa cells do not divide throughout embryogenesis.

The anterior margin of the germ band becomes evident very early, allowing the number of cells of procephalon and metameric germ band to be counted and these values to be translated onto the blastoderm map. Since intersegmental boundaries are orientated perpendicular to the midline, and no indication of intervening morphogenetic movements which could derange this orientation is observed during development, it was assumed that the boundaries between the segmental anlagen are oriented orthogonal to the ventral midline of the blastoderm as well. This assumption is strongly supported by the results of HRP injections. The entire anlage of the metameric germ band was then subdivided in the various segmental anlagen according to the number of cells in each segment. The anteroposterior extent of the anlage of each segment corresponds to less than 3 cells, and that of the entire germ band to roughly 40 cells.

Four regions can be morphologically distinguished in the embryonic procephalon from early stages on. These are (i) the procephalic neurogenic region (containing precursors of supra-oesophageal neuroblasts and of epidermal cells of the procephalic lobe), (ii) the anlagen of foregut and clypeolabrum, (iii) a narrow dorsomedian strip of "parietal cells" (to partially become integrated in the optic lobe primordium); (iv) and a narrow vertical strip of cells located between the procephalic neurogenic region and the anterior border of the metameric germ band, which we assume, chiefly on the basis on quantitative estimations, will become integrated into the epidermis of the procephalic lobe. Since boundaries between the above regions become evident before distortions due to foregut invagination and anteroventral shift of the gnathal epidermis occur, these boundaries can be directly projected onto the blastoderm after compensating for minor alterations caused by gastrulation. The boundaries drawn in our map between oesophagus, pharynx and clypeolabrum are inferred chiefly from the results

of HRP injections; the size of these anlagen is based on cell countings.

The dorsal ridge, gnathal buds and salivary glands (all arising from various parts of the gnathal segments); the optic lobe placode, tracheal placodes and Malpighian tubules become evident late in development. The size of their blastoderm anlagen has been defined based on quantitative considerations, and their location chiefly on the results of HRP injections. Finally, the results of HRP injections suggest that the dorsal territory of the mandible does not extend up to the dorsal midline, being continued by the anlage of the optic lobes.

A comparison with previous maps

Most of the pertinent comparisons have already been made in a previous account (see Technau and Campos-Ortega 1985). The most important points, which we would like to emphasize again, are the following. The fate map of the blastoderm of *Drosophila melanogaster* that we propose is very similar to that of Poulson (1950) in most respects, although it differs from Poulson's in a few points. First of all, concerning the neurogenic ectoderm, Poulson located the ventral neurogenic ectoderm separated from the ventral epidermogenic anlage. Hartenstein and Campos-Ortega (1984) showed neuroblasts and dermatoblasts to be intermingled within the ventral neurogenic ectoderm, and this was confirmed by the results of HRP injections (Technau and Campos-Ortega 1985). Poulson drew the ventral and the procephalic neurogenic ectoderm as contiguous regions, whereas our normal anatomical analysis indicates that they are separated. This point is still controversial for the results of HRP injections do not fully support this hypothesis. Since in some instances the results of HRP injections are difficult to interpret, due to our not being able at present to evaluate the extent of diffusion of the injected HRP (discussed in Technau and Campos-Ortega 1985), the issue cannot yet be solved satisfactorily. We favour a separation of both neurogenic anlagen on the basis of the histological organization. Poulson (1950) located the anlage of the proctodeum laterally, and this was modified by Underwood et al. (1980), who located the proctodeum restricted to ventral levels, caudal to the anlage of the mesoderm. However, the anlage of the proctodeum does in fact extend as a ring around the posterior egg pole, i.e. around the anlage of the posterior midgut; this is clearly indicated by normal anatomy and HRP injections. Finally, the anlage of the amnioserosa seems to be over-represented in Poulson's map. Other minor differences to Poulson's map are discussed in Technau and Campos-Ortega (1985).

Lohs-Schardin et al. (1979) mapped the larval epidermis by means of a uv-laser microbeam, chiefly concentrating on the anlage of T1-A8, but also referring to the gnathal segments and telson. Our results are in very good agreement with theirs and we shall not repeat those results here. Estimations of these authors as regards the number of cells per segment are in the range of those counted directly, as presented in this report.

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