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Regeneration and pattern formation in planarians

II. Local origin and role of cell movements in blastema formation

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Summary

In planarians, blastema cells do not divide, and growth of blastema is thought to result from the steady accumulation, beneath the wound epithelium, of undifferentiated cells produced by cell division in the stump. However, whether these cells come only from local stump sources or whether cells placed far from the wound can also participate, after long-range migrations, in the growth of blastema, is still uncertain.

To study this problem, we have analysed three basic parameters of the process of regeneration: cell kinetics of blastema growth; number of cells produced by mitosis in the stump areas near the wound (postblastema); and rates of movement ('migration') of undifferentiated cells using grafting procedures with nuclear and chromosomal markers.

The results show that: (1) cells near the wound area spread (move) at higher rates than cells placed far from it (90–140 μm day^{-1} *versus* 40–50 μm day^{-1}); (2) cells originally placed farther than 500 μm from the wound boundary are hardly represented within 3- and 5-day-old blastemata; and (3) the number of cells produced by mitosis within a 200–300 μm postblastema area around the wound seem sufficient to explain, provided their rates of movement are taken into account, the increasing number of blastema cells. From this, it is concluded that blastema cells in planarians originate from local stump areas, and that mitotic activity jointly with local cell movement within a 200–300 μm postblastema area around the wound match the increasing number of blastema cells during regeneration. The implications of these results for blastema growth and pattern formation mechanisms are discussed.

Key words: regeneration, planarians, pattern formation, blastema, cell movement, cell proliferation, cell markers.

Introduction

When a planarian is cut transversally a blastema made of small undifferentiated cells forms above the wound. Although the blastema grows in size and cell number, several studies have shown that blastema cells in planarians do not divide (Spiegelman & Dudley, 1973; Morita & Best, 1984; Saló & Baguñà, 1984). From this, it has been suggested that blastema grows by the continuous entrance of undifferentiated cells, produced by cell division in the old stump (postblastema), to the base of blastema (Saló & Baguñà, 1984, 1985). However, it is still uncertain if these cells come from local stump sources or if cells placed far from the wound can participate in blastemata growth.

To answer this question, the growth kinetics of blastemata, the mitotic kinetics of stump cells near the wound, and estimates of the rates of cell movement and the extent of the stump area where cells are recruited should be known. Unfortunately, these data are, at present, not available. Blastema growth rates have not been studied and, though data on mitotic indices of several planarian species are known (*Dugesia(S)mediterranea*, Baguñà, 1976; *Dugesia(G)tigrina*, Saló & Baguñà, 1984; *Dugesia(S)lugubris*, Brugal *et al.* 1985), the cumulative number of undifferentiated cells produced by mitosis in the stump at given time periods is still unknown. Moreover, although undifferentiated cells (neoblasts) are known to move in regions far from the wound at rates of 40 μm day^{-1} (Saló & Baguñà, 1985) their actual rate in regions near the wound has not been assessed.

If the number of cells produced by mitosis in the postblastema and the rate of migration of neoblasts near the wound were known, the number of cells crossing the wound boundary to make the blastema and, hence, the area where cells are recruited could be estimated. If the number of cells produced turned out to match the increasing number of cells making the blastema, a local origin of blastema cells could be suggested. Conversely, if this number is found to be lower than the number of blastema cells, other mechanisms like cell dedifferentiation or long-range cell migration should be invoked.

The main aim of this paper was, therefore, to obtain data on: (1) the number of cells present in anterior and posterior blastemata during regeneration; (2) the number of cells produced by mitosis within a 500–600 μm

deep stump region from the wound (postblastema); and (3) the rates of migration of neoblasts in regions near the wound during the first week of regeneration. From these data, the local (short-range) *versus* general (long-range) hypotheses of the origin of blastema can be checked, and the implications of it on old questions like the cellular origin of blastema cells in planarians (neoblasts *vs* dedifferentiation; Slack, 1980; Baguñà, 1981) and the mechanics of blastema formation properly assessed.

Materials and methods

Species

Planarians used in this study were as follows: (1) the asexual race or *Dugesia(S)mediterranea* (Benazzi *et al.* 1972) from Barcelona, Spain; (2) the sexual race of *Dugesia(S)mediterranea* (Benazzi & Benazzi-Lentati, 1976), from Sardinia, Italy; and (3) the diploid (A, 2n = 8) and tetraploid (D, 4n = 16) biotypes of *Dugesia(S)polychroa* (Benazzi & Benazzi-Lentati, 1976), both from Sardinia, Italy. The organisms were reared in Petri dishes in the dark at 17 ± 1 °C in planarian saline (PS, Saló, 1984) and fed with *Tubifex* sp. In all experiments, one-week-starved organisms were used and the temperature kept at 17 ± 1 °C.

Regenerating organisms: terminology

Organisms, 9–10 mm long, were cut prepharyngeally and both pieces, anterior (cephalic) and posterior (caudal), were left regenerating for two weeks. Anterior and posterior blastemata refer to the small unpigmented mound of tissue made of small undifferentiated cells (neoblasts) that forms and grows above the wound in caudal and cephalic pieces, respectively (Fig. 1). Stump refers to the pigmented old body, or regenerant, below the wound, and operationally it is convenient to consider it as made of a small region (aprox. 500–600 μm in length) near the wound (postblastema, pb) where most mitotic activity occurs, and a larger region that covers the rest of the regenerant that can be taken as a control region (see Fig. 1).

Number of blastema cells

The number of cells making the blastema was measured using a maceration technique that dissociates tissues into single cells (Baguñà & Romero, 1981). Regenerating organisms (at 1, 2, 3, 4, 6 and 9 days of regeneration) were killed in methanol: acetic acid: glycerol: distilled water (2:1:1:13), and blastemata carefully cut from the stump with a microscalpel, macerated in the same solution, and the number of cells counted under phase-contrast microscopy with a hemocytometer (Neubauer cell counter, 0·1 mm depth; Saló & Baguñà, 1986). For each measurement, five blastemata were used, the results being the mean of three different experiments.

Recovery of cells following this technique is very high (>99 %) as assessed comparing the DNA content and the cell titer of a maceration preparation using the average nuclear DNA content of *Dugesia(S)mediterranea* (1·45 pg DNA cell^{-1}) and *Dugesia(S)polychroa* biotypes A and D (1·12 and 2·37 pg DNA cell^{-1}, respectively) as references (Romero, 1987; Prats, Romero & Baguñà, unpublished data).

Cumulative number of cells produced by mitosis

To estimate the number of cells produced by mitosis in a given area during a given period of time, two basic methods have been traditionally used: autoradiography (mainly the fraction of labelled mitosis method, or FLM), and metaphase arrest (stathmokinetic) techniques (Aherne *et al.* 1977). For reasons still unknown, planarians do not take thymidine into their cells (Coward *et al.* 1970); this precludes the use of autoradiographic techniques and compels, instead, the use of stathmokinetic methods.

To have a stathmokinetic daily estimate of the number of cells produced by mitosis and their spatial distribution, the number and position of arrested metaphases after colchicine blockage was studied in successive 8 h intervals during the first 9 days of regeneration. Briefly, organisms of different regenerative ages were incubated for 10 h in 0·05 % colchicine (Sigma, London) in planarian saline (PS, Saló, 1984), and at 2 and 10 h of incubation some individuals were set apart, fixed in Carnoy, stained in acetic orcein, and mounted *in toto* (Saló & Baguñà, 1984). Arrested metaphases within the postblastema area (approx. 600 μm around the wound, see Fig. 1) were plotted within four successive transverse 150 μm length strips along its anteroposterior axis (for more details, see Saló & Baguñà, 1984; Fig. 1). The number of metaphases produced within each transverse strip in each 8 h interval was determined from the difference in number between organisms incubated in colchicine for 2 and 10 h, and an average value calculated from equivalent strips of five different organisms of the same regenerative age. From the data obtained in the three 8 h intervals, the daily number of metaphases produced during the first 9 days of regeneration was estimated.

Results are expressed as absolute numbers (±S.D.). As a general control, similar data in strips of the same length of similar body regions of intact organisms and of regions far from the wound of regenerating organisms were measured.

Measure of cell movement in regions near the wound

Movement of cells has been studied by grafting procedures using chromosomal (a heteromorphosis between the sexual and asexual races of *Dugesia(S)mediterranea*) and nuclear (difference in nuclear size between neoblasts of diploid and tetraploid biotypes of *Dugesia(S)polychroa*) markers. The markers, the grafting procedures and some terminological

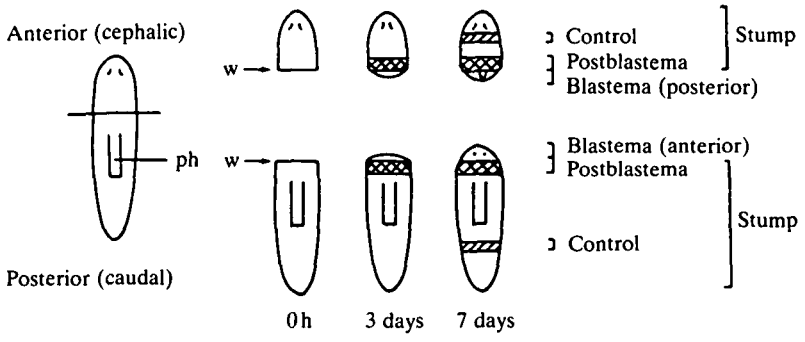

Fig. 1. Diagrammatic dorsal view of intact and regenerating *Dugesia(S)mediterranea* displaying the main regions referred to in this work. ph: pharynx; w: wound.

remarks as regards 'cell migration' *vs* 'cell spreading/cell movement' concepts have already been described and discussed (Saló & Baguñà, 1985).

To estimate the rate of movement of graft cells in regions near the wound, groups of grafted animals were carefully cut at different distances (1·5, 1·0 and 0·5 mm) anterior to the graft and left regenerating. At different periods of regeneration (1, 2, 3, 4, 5, 7 and 9 days) the presence of graft cells in the host stump region between graft and blastema or within the blastema itself was checked by fixing some organisms ($n = 5$) and studying the spatial distribution of graft metaphases (chromosomal marker) or nuclei (ploidy marker) as described (Saló & Baguñà, 1985). The rate of movement (in $\mu m\,day^{-1}$) was calculated from the distance covered by the leading edge of the graft cells within the stump at different time intervals. As controls, the rate of movement of graft cells towards the lateral and posterior sides of the host was studied.

Since cutting the host to such predetermined tiny distances from the graft appeared rather difficult, the actual distance between the anterior edge of the graft and the wound level (blastema/postblastema boundary) was carefully measured in fixed organisms before staining.

Density of undifferentiated cells (neoblasts) in the postblastema

Changes in the density of undifferentiated cells (neoblasts) in the postblastema during regeneration were assessed by isolating this area, with a microscalpel, from blastema and stump and macerating it into single cells as described (Baguñà & Romero, 1981). The percentage of neoblasts was estimated, according to morphological criteria (Baguñà & Romero, 1981) under phase-contrast microscopy in samples of at least 1000 cells. As controls, equivalent areas of intact organisms were similarly processed and their neoblasts counted.

Results are expressed as the ratio between the number of neoblasts in the postblastema as compared to control regions, the latter value being set to one (this being equivalent to $12\,000 \pm 14\,000$ neoblasts for a 600 μm region in length in a 9 mm long intact organism; Baguñà & Romero, 1981; Romero, 1987).

Orientation of mitotic axis in the postblastema area

To assess if stretching and contraction of tissues during wound healing constrain mitotic cells to orient their axis in particular directions, namely parallel to the anteroposterior axis, the orientation of mitotic axis in the postblastema area was studied. Whole-mount regenerating organisms, stained in acetic orcein (Saló & Baguñà, 1984) were studied at 0, 4 and 8 h and 1, 2, 3 and 5 days of regeneration. Mitotic axes of dividing cells were plotted within a 1 mm deep area from the stump/blastema boundary and the angle between it and the anteroposterior body axis measured. Angular values were grouped in three 30 sectors (a, 0–30°; b, 30–60°; c, 60–90°) as related to anteroposterior body axis, and the number of mitoses within each sector estimated. At each stage of regeneration no less than 500 mitoses were counted.

Results

(1) *Blastema growth rates*

The first visible accumulation of blastema cells is seen between 1 and 2 days of regeneration as a very thin strand of unpigmented cells above the wound. At this stage, neat separation of blastema from the underlying postblastema and stump appears difficult; therefore, estimates of cell number in blastemata at 1 and 2 days of regeneration were obtained through several independent methods: direct cell counting of cut blastemata after maceration; extrapolation to 1 and 2 days of regeneration of the curve of increase in blastemal cell number (see Fig. 2); and measuring the volume of blastemata, in whole fixed organisms, from their height, width and length and estimating the number of cells using the mean volume of blastema cells (approx. $520 \pm 42\,\mu m^3$; Saló, 1984). All these methods gave rather similar values, the first one being represented here.

On day 3, and from then on, blastemata are clearly visible and easily cut, macerated and their cells counted. The increase in the number of cells of anterior and posterior blastemata is plotted in Fig. 2, each data point representing the average of fifteen blastemata from three different experiments. It can be concluded that from the very early stages of regeneration anterior blastemata are always bigger than posterior blastemata. Moreover, from the slope of the curve in Fig. 2, it becomes evident that fewer cells are entering the blastema from the stump as regeneration progresses.

(2) *The pattern of mitotic accumulation*

An 8 h exposure to colchicine resulted in a high proportion of arrested division in each anteroposterior 150 μm interval in length, enabling their distribution to be seen clearly.

As could be expected from previous observations on the mitotic indices of this and related species (Baguñà, 1976; Saló & Baguñà, 1984; Brugal *et al.* 1985; Saló & Baguñà, unpublished data), metaphases accumulate steadily from the very beginning of regeneration, their values being well above those of control nonregenerating organisms and those of regions far from the wound of regenerating organisms (Table 1). The spatial pat-

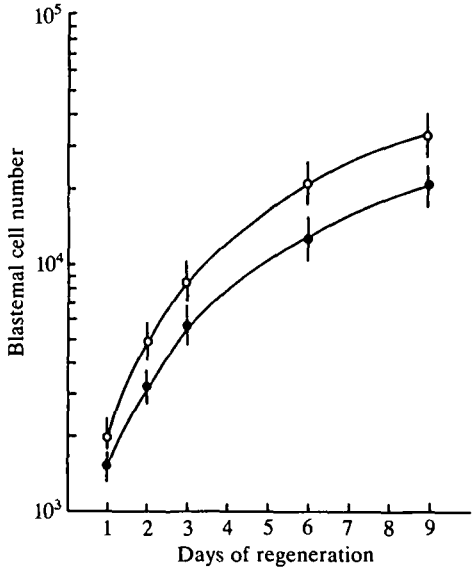

Fig. 2. Semilog plot of the increase in cell number in anterior (○) and posterior (●) blastemas of 9–10 mm long *Dugesia(S)mediterranea* during the first 9 days of regeneration. Each point represents the average of 15 values.

Table 1. *Number of blastema cells and daily number of arrested metaphases in postblastema and stump regions during anterior and posterior regeneration of* Dugesia(S)mediterranea*

Body region†	a–p strips†† (in μm)	Days of regeneration 0	1	2	3	4	5	6	7	8	9	Σ 0–3‡	Σ 0–6‡
Stump	–	340	400	450	430	410	460	490	440	410	460	–	–
	450–600	400	450	600	720	850	900	720	520	500	440	1770	4240
Postblastema	300–450	400	900	1320	1440	1270	990	1040	1020	950	780	3660	6960
	150–300	400	1450	1470	1550	1730	1290	1340	1130	1000	950	4470	8830
	0–150	400	1890	2080	2760	3280	2700	2330	2150	2040	1850	6730	15040
Blastema (posterior)	–	–	1500	3200	5800	7700	10000	12800	15500	18200	21300	–	–
Blastema (anterior)	–	–	2000	4800	8650	11400	15000	20820	25000	30000	33850		
	0–150	400	1560	2280	3560	5560	3530	2650	1900	1950	1550	7400	19140
Postblastema	150–300	400	1360	1540	1890	2820	2230	1800	1630	1240	970	4790	11640
	300–450	400	880	1070	1770	2660	2160	1335	1140	1190	1060	3330	9485
	450–600	400	550	600	1100	1540	1450	910	720	540	480	2250	6150
Stump	–	450	400	440	410	450	440	450	370	410	440	–	–

*For the sake of clarity all the values have been rounded and standard deviations (with ranges not higher than 15% of the average value) omitted. For further details, see Methods.
†For more details, see Fig. 1.
††For more details, see Methods.
‡Cumulative number of metaphases during the first three (Σ 0–3) and six (Σ 0–6) days of regeneration.

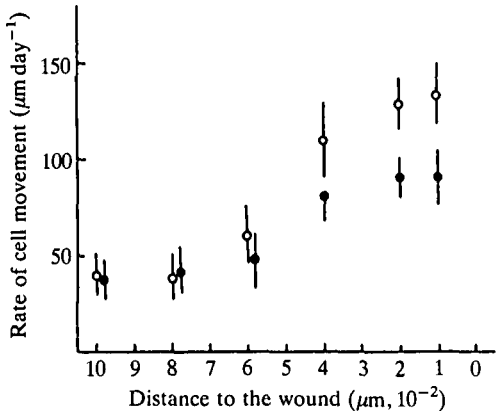

Fig. 3. Changes in the rate of cell movement (in $\mu m\,day^{-1}$) ± S.D. of graft cells within host tissues as they move closer to the wound/stump boundary. (○) anterior regeneration; (●) posterior regeneration. Each point represents the average of 15 values.

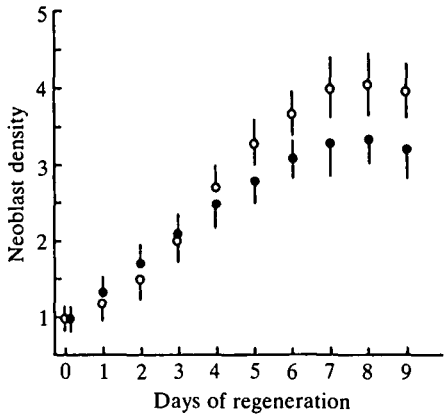

Fig. 4. Changes in neoblast density (±S.D.) within anterior (○) and posterior (●) postblastemas during the first 9 days of regeneration in *Dugesia(S)mediterranea*. Density values are ratios between number of neoblasts at different periods of regeneration and number of neoblasts at 0 days, the latter value being set to one. 10–12 organisms were analysed for each point. For more details, see text.

tern of mitoses is uneven, being always higher in regions near the wound, and shows a temporal maximum between 3 and 5 days of regeneration both in anterior and posterior stumps. Moreover, the number of mitoses produced in the 600 μm around the wound seems well above the number required to explain the increasing number of blastema cells, provided that extensive movements of stump cells to the wound do occur (see below). Indeed, the cumulative number of metaphases produced within the first distal strip (0–150 μm) of tissue in both anterior and posterior stumps during the first 3 (Σ 1–3) or 6 (Σ 1–6) days of regeneration match the number of blastema cells at 3 and 6 days of regeneration.

(3) *Cell movement in regions near the wound*

Movement of cells in areas more than 500–600 μm from the wound were found to be similar to those found for nonregenerating organisms or for regions far from the wound (approx. 4–5 mm) in regenerating organisms (approx. $40\,\mu m\,day^{-1}$; Saló & Baguñà, 1985) (Fig. 3). Moreover, movement of cells from lateral and posterior areas of the graft towards the tissues of anteriorly and posteriorly regenerating hosts gave values only slightly higher than nonregenerating controls (~50–55 *vs* $38–42\,\mu m\,day^{-1}$).

When the 500–600 μm stump area around the wound is considered, both methods (chromosomal markers or ploidy markers, see Methods) have higher estimates of cell movement with average values around $134 \pm 18\,\mu m\,day^{-1}$ and $90 \pm 15\,\mu m\,day^{-1}$ for anterior and posterior regenerants, respectively. These results clearly suggest that stump cells near the wound move towards it at higher rates than those found in control nonregenerating organisms ($\sim 40\,\mu m\,day^{-1}$; Saló & Baguñà, 1985) and in regions far from the wound of regenerating organisms ($50–55\,\mu m\,day^{-1}$; this work).

(4) *Changes in neoblast density in the postblastema area*

Figure 4 shows the changes in density of neoblasts within a 500–600 μm area of anterior and posterior postblastemata from the blastema/stump boundary. As expected from the increases seen in mitotic indices and rates of cell movement near the wound, a steady accumulation of these cells follows after cutting, already apparent from the second day on of regeneration and increasing at a slower pace as regeneration goes on.

(5) *Can cumulative mitoses and local cell movement explain blastema growth?*

From data of Table 1, the cumulative number of cells crossing the blastema/stump boundary in anterior (a) and posterior (b) regenerants, under two different hypothetical situations, can be estimated (Fig. 5). In the first (filled circles), each average division produces one cell which is left behind in the postblastema and another cell moving to the wound at an average daily rate of $140\,\mu m\,day^{-1}$ (anterior regeneration, Fig. 5A) or $90\,\mu m\,day^{-1}$ (posterior regeneration, Fig. 5B). In this case, the number of cells crossing the boundary daily (filled circles) matches, being actually slightly above it in posterior regenerants, the daily increase in the number of blastema cells (open circles).

A second situation results if within the nearest strip to the wound both daughter cells produced after cell division move together to the boundary. If this was so, the number of cells crossing the boundary daily (open triangles) is well above the number needed to explain the increase in the number of blastema cells (open circles).

(6) *Changes in the orientation of mitotic axes during regeneration*

Figure 6 shows the changes in direction of mitotic axes of dividing cells during regeneration from a random distribution in control regions (0 h) to a preferential alignment with the anteroposterior axis from 4 h of regeneration on. This situation holds during the first 3

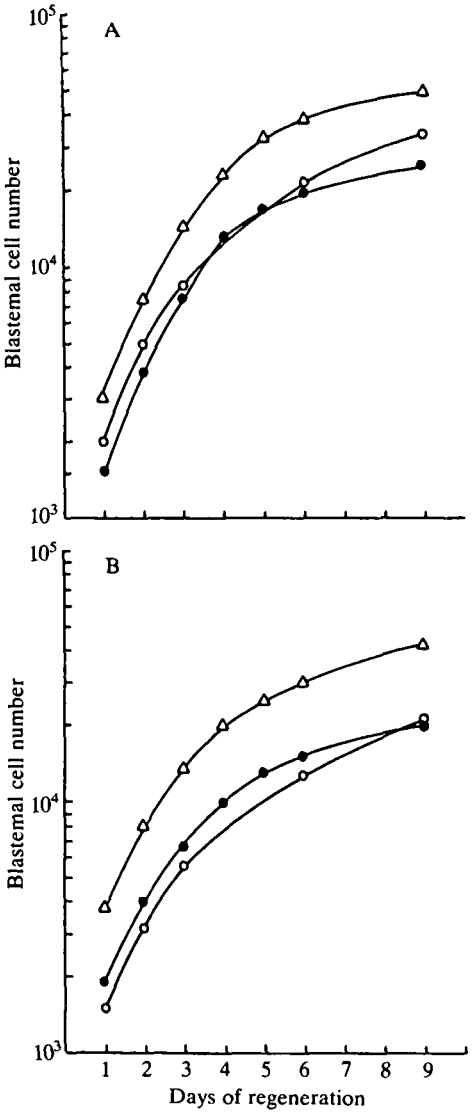

Fig. 5. Semilog plots of the actual number of blastema cells (open circles) and the predicted cumulative number of postblastema cells crossing the blastema/stump boundary to make the blastema under two different hypothetical situations (closed circles and open triangles) in anterior (A) and posterior (B) regeneration. For more details, see text.

days of regeneration to return to the former state at 5 days of regeneration.

Discussion

Earlier attempts to follow cell movements within regions near the wound in regenerating planarians made use of autoradiography of labelled graft cells within unlabelled regenerating hosts. Since planarian cells do not take up thymidine, precursors of RNA and protein were commonly used (see Brønsted, 1969; and Saló & Baguñà, 1985, for general references). The results found were highly diverse and unreliable as all cells, and not only neoblasts as claimed by some authors (Lender & Gabriel, 1965; Gabriel, 1970), were highly labelled.

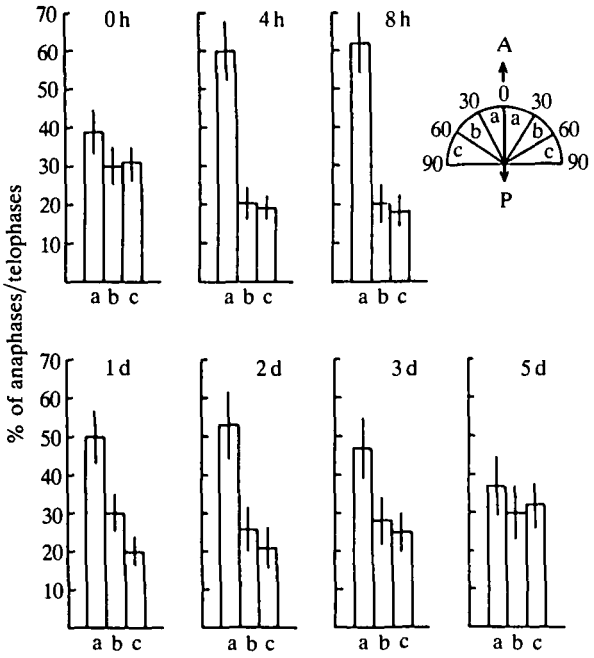

Fig. 6. Angular distribution of anaphase/telophase mitotic figures within the three consecutive 30 sectors (a, 0°–30°; b, 30°–60°; c, 60°–90°) between anteroposterior (A–P) and transversal body axes, during the first five days of regeneration in *Dugesia(S)mediterranea*. Assignment of mitotic figures to sectors followed from the angle formed between mitotic axis and A–P body axis. Ordinate: percentages of anaphase/telophase in each sector (a,b,c). For more details, see text.

The chromosomal and ploidy markers used here, already employed to estimate the spreading of cells in intact planarians and in regions far from the wound of regenerating organisms (Saló & Baguñà, 1985), have several advantages over RNA/protein labelling and other markers such as pigmentation; mainly, their permanence, easy scorability and, for chromosomal markers, their specificity for undifferentiated (mitotic) cells (neoblasts).

Using these markers, we have analysed the local (short-range) *versus* general (long-range) origin of blastema cells in planarians comparing the increasing number of cells in anterior and posterior blastemata with the cumulative number of cells produced by mitosis within a 500–600 μm stump region (postblastema) around the wound and taking into account their rate of migration to the stump/blastema boundary. Two main conclusions stem from these results. First, cells near the wound spread (move) at a substantially higher rate than cells placed far from it. Second, the number of cells produced by mitosis within the postblastema area seem sufficient to explain, provided we take into account their rates of migration, the growth of blastemata. From this it follows that blastema cells in planarians have a local origin and that other mechanisms invoked to provide undifferentiated blastema cells, like cell dedifferentiation or long-range cell migration, would not need to be involved.

Why cells move faster near the wound than far from it?

The finding of higher rates of cell movement within the 500–600 μm postblastema area around the wound was not entirely unexpected given the higher rates of mitosis there (Table 1) and the positive correlation found in intact organisms between rates of cell movement and mitotic indices (Saló & Baguñà, 1985). It is more debatable, however, whether these higher rates of movement can be entirely explained through higher rates of mitosis or whether other mechanisms should be invoked to account for it. We think particularly of phenomena like: (a) an enhanced capacity of cell movement due to the presence of empty intercellular spaces left by dead cells around the wound during the first 2–3 days of regeneration (Bowen *et al.* 1982); (b) a more directed (towards the anteroposterior axis), as opposed to random, orientation of mitotic axes during the first days of regeneration (Fig. 6); and (c) a passive bulk spreading of postblastema cells eased by the distal expansion of the wound epithelium due to relaxation of longitudinal and circular muscle fibers and to the pressure of the underlying proliferating postblastema cells.

Overall, it is possible that the faster rates of cell movement within the wound (postblastema) area result from the individual or joint action of these three mechanisms considered. However, the relative importance of each mechanism, or of any other here not considered (e.g. active cell migration), with regard to the actual rate of cell movement, is still uncertain. Computer simulations are in progress to assess their relative weight (Solé, Ransom, Ocaña, Saló & Baguñà, unpublished observations).

Some implications for blastema growth and pattern formation mechanisms in planarians

Whatever the actual mechanism behind the faster spread of cells as they approach the wound turns out to be, the outcome is that a 3-day-old blastema must have been formed by cells placed placed less than 400 μm around the wound; that is, about 40–50 cell diameters. Indeed, new cells produced by mitosis within the distal (approx. 200 μm from the wound) range of this area (see Table 1) may explain both the increasing number of blastema cells found (Figs 2 and 5) and the increasing density of neoblast within the postblastema (Fig. 4). Similarly, a 5-day-old blastema, already having most new pattern elements (e.g. eyes and brain ganglia in anterior regeneration; pharynx in posterior regeneration), though mainly composed of local cells, presents in its proximal area cells originally placed at 500–600 μm from the wound.

Bearing in mind that blastema cells in planarians do not proliferate, this suggests that formation and growth of blastemata in planarians may be seen as an orderly phenomenon where stump cells are incorporated earlier or later into the blastema, and forming, therefore, their distalmost or proximal areas, depending on their initial distance from the wound site.

Since 3-day-old blastemata are autonomous units able to self-regulate (see Brønsted, 1969, for references), pattern must by then have been restored. Indeed, using grafting procedures it has been estimated that head and pharynx determination do occur within the postblastema area between 6 and 24 h of regeneration when blastema is either nonexistent or barely visible (Saló, 1984; Saló & Baguñà, manuscript in preparation). This suggests that pattern must be set early within a rather restricted (~500 μm) area of the stump below the wound, to be amplified and refined at later stages by proliferation and orderly spreading of postblastema cells to the stump/blastema boundary.

Why blastema cells in planarians do not proliferate?

A peculiar oddity of planarian regeneration as compared to other epimorphic (blastema-forming) systems is the lack of proliferation of blastema cells (Spiegelman & Dudley, 1973; Saló & Baguñà, 1984: Morita & Best, 1984). This phenomenon has evident parallelisms with the accumulation of undifferentiated cells during morphallactic regeneration of head structures in small Turbellarians (e.g. the rhabdocoel *Microstomum lineare*, Palmberg & Reuter, 1983) as well as with the formation of the terminal anlage in several species of Cestoda (Wikgren *et al.* 1971). In both cases, a small core of proliferating cells first forms, growing later by peripheral recruitment of new proliferating migratory cells while core cells stop dividing probably due to high cell density and/or close cell packing. Similarly, close apposition of undifferentiated cells below the wound epithelium and within the blastema in freshwater planarians may halt their proliferation.

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