J. Embryol. exp. Morph. **89**, 57–70 (1985)
Printed in Great Britain © *The Company of Biologists Limited 1985*

Cell movement in intact and regenerating planarians. Quantitation using chromosomal, nuclear and cytoplasmic markers

EMILI SALÓ AND JAUME BAGUÑÀ

Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain

SUMMARY

One of the tenets of Wolff and Dubois' 'neoblast theory' of planarian regeneration (Wolff & Dubois, 1948) is that blastema is mainly formed by the accumulation of undifferentiated parenchymal cells (neoblasts) that can migrate, if needed, over long distances to the wound. That neoblasts migrate was claimed by these authors after partial X-irradiation, and total X-irradiation and grafting using planarian strains of different pigmentation. From this they suggested that migration of neoblasts is stimulated by the wound and directed towards it.

To study the nature and extent of such 'migration' in intact and regenerating organisms, and in order to avoid the flaws of using pigmentation as a marker, we made grafts between sexual and asexual races of *Dugesia(S)mediterranea* that differ in a chromosomal marker, and between diploid and tetraploid biotypes of *Dugesia(S)polychroa* that differ in nuclear size. Also, fluorescent latex beads were used as cytoplasmic markers to follow 'migration' of differentiated cells. The hosts were irradiated or non-irradiated intact and regenerating organisms.

The results show that: 1) movement of graft cells into host tissues occurs in intact organisms at a rate of $\simeq 40\,\mu$m/day, and that this increases up to $\simeq 75\,\mu$m/day in irradiated hosts; 2) movement of cells occurs evenly in all directions; 3) regeneration does not speed up rate of movement nor drives cells preferentially to the wound; 4) spreading of cells is mainly due to the movement of undifferentiated cells (neoblasts); and 5) higher rates of movement are correlated with higher mitotic indexes. From this, it is concluded that the so-called 'migration' of neoblasts is not a true cell migration but the result of the slow, even and progressive spreading of these cells mainly caused by random movements linked to cell proliferation. The implications of these results for blastema formation and the origin of blastema cells are discussed.

INTRODUCTION

The most widely held theory of blastema formation during planarian regeneration is Wolff and Dubois' theory of neoblast migration and proliferation (Wolff & Dubois, 1948). According to it, blastema cells and undifferentiated parenchymal neoblasts are alike, and these cells, if needed, can migrate over long distances to the wound. Basically, this follows after experiments of partial X-ray irradiation and amputation (Dubois, 1949), and experiments of total X-ray irradiation and grafting (Lender & Gabriel, 1965) where a delay in blastema formation was observed, delay proportional to the distance from the unirradiated region (or graft) to the wound. That cells making the blastema came from

Key words: cell movement, cell markers, regeneration, chimaeras, planarian, nuclear, chromosomal, cytoplasmic, *Dugesia* spp.

unirradiated tissues and not from revitalized irradiated tissues was shown using planarian strains of different pigmentation.

From these experiments, two main conclusions arose: 1) blastema cells are migratory neoblasts coming from unirradiated regions; therefore, neoblasts are the source of blastema cells and most probably totipotent; and 2) the migration of neoblasts is *stimulated* by the wound and *directed* towards it.

While the first conclusion could be questioned on the grounds that migratory neoblasts could be the result of cell dedifferentiation within the unirradiated region (or graft), the second conclusion poses two main questions: 1) if neoblast migration is stimulated by the wound, we should expect little or no migration of graft cells within intact unirradiated or irradiated organisms; 2) if neoblasts migration is directed (oriented) towards the wound, we should expect that anteriorly regenerating organisms (irradiated or not) bearing a graft will show a preferential migration of graft cells towards the wound area rather than towards posterior or lateral regions.

To follow graft cells within a host tissue, strains of different pigmentation have been widely used. This method, though useful, has several flaws, the main ones being the long time needed to see a clear result, the difficulties in quantitating it, and the uncertainties of pigment cells as faithful markers of phenomena happening in internal tissues. No wonder that in a recent review on cell movements within organisms, Trinkaus (1984) says rather ironically 'these cells, called *neoblasts*, have been assigned full pluripotency and legendary migratory capacities. They have even been thought to move from one end of a creature to another, . . . there is certainly no evidence that such cells engage in their postulated peregrinations'. (p. 38, *op. cit.*).

To overcome these difficulties and criticisms, we have made use of a chromosomal marker (a heteromorphosis) that characterizes the asexual race of *Dugesia(S)mediterranea* as compared to the sexual race of the same species, as well as the difference in nuclear size between neoblasts of the diploid and tetraploid biotypes of *Dugesia(S)polychroa*. Also, fluorescent latex beads, taken up by specific differentiated cell types, have been used as cytoplasmic markers to track cell movements.

MATERIALS AND METHODS

Planarians used in this study were as follows: 1) the asexual race of *Dugesia(S)mediterranea* (Benazzi, Ballester, Baguñà & Puccinelli, 1972) from Barcelona (Spain); 2) the sexual race of *Dugesia(S)mediterranea* (Benazzi & Benazzi-Lentati, 1976) from Sardinia (Italy), kindly sent by Prof. N. G. Lepori; and 3) the biotypes A ($2n = 8$) and D ($4n = 16$) of *Dugesia(S)polychroa* (Benazzi & Benazzi-Lentati, 1976) from Sardinia (Italy), also sent by Prof. N. G. Lepori. The organisms were reared in Petri dishes with spring water in the dark at 17 ± 1°C and fed with *Tubifex* sp. In all experiments, one-week starved organisms of large size ($\geq$15 mm in length) were used, and the temperature kept at 17 ± 1°C.

Terminology

Since the classic paper of Wolff & Dubois (1948), authors dealing with movements of cells within intact and regenerating planarians have employed the term *cell migration* to refer to the

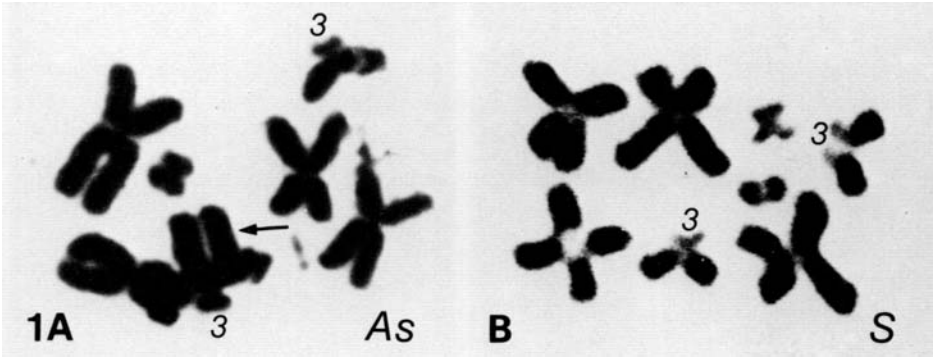


Fig. 1. Karyotypes of *Dugesia(S)mediterranea*. (A) Asexual race (*As*), having a clear heteromorphosis (arrow) in the third pair (*3*) of chromosomes; (B) Sexual race (*S*), having chromosomes of equal length.

displacement of cells from a graft to distant sites in the host. However, it is still not clear if, in planarians, this phenomenon results from an active migration of individual cells (true cell migration) or from the slow spreading of graft cells within a host due to cell proliferation (cell repopulation).

Therefore, when referring to the gradual and mutual spreading of cells between graft and host, we would avoid in this paper the use of the term cell migration speaking instead of *cell spreading* or *cell movement*.

Chromosomal marker

The asexual race (As) of *Dugesia(S)mediterranea* has an heteromorphosis in the third pair of chromosomes (Fig. 1A), the long arm of one homologue being much longer than the other (Baguñà, 1973; De Vries, Baguñà & Ball, 1984). In contrast, the sexual race (S) of the same species has chromosomes of equal length (Fig. 1B). This chromosomal marker has proved to be highly stable being present in all asexual cells (De Vries & Baguñà, in preparation).

Nuclear size marker

Planarian tissues can be dissociated by maceration into single cells, the different cell types characterized by phase-contrast microscopy, and their nuclear area measured (Baguñà & Romero, 1981). Neoblasts from biotypes A (diploid) and D (tetraploid) of *Dugesia(S)polychroa* have mean nuclear areas of $25{\cdot}76 \pm 4{\cdot}72\,\mu m^2$ ($n = 100$) and $41{\cdot}17 \pm 6{\cdot}70\,\mu m^2$ ($n = 100$) respectively, being thus easily distinguishable by size (Fig. 2A,B). This has proved to be a useful marker, making possible to follow cell movement without studying karyotypes.

Cytoplasmic marker (fluorescent latex beads)

Fluorescent latex beads (Fluoresbrite carboxylate, Polysciences; green fluorescence, $1{\cdot}0\,\mu m$ in diameter) were used to label the cytoplasm of some differentiated cell types (mainly gastrodermal and fixed parenchyma cells). To do that, planarians were fed an artificial food mixture made of beef liver, low-melting-point agarose (Sea Prep), and fluorescent latex beads (Romero, unpublished data). Once fed, the beads appear in gastrodermal cells, and 3–6 h later they appear within some parenchymal cell types (fixed parenchyma, cianophilic cells, acidophilic cells; Baguñà & Romero, 1981). The beads remain there for at least 15–20 days, disappearing slowly later on. No beads were seen to be taken up by undifferentiated cells (neoblasts) probably due to their scanty cytoplasm. Labelled cells were detected by epifluorescence (Leitz Dialux-20 microscope with a Ploemopak 2.4. equipment) (Fig. 3).

Grafting techniques

Grafting between As and S tissues of the same body level of *Dugesia(S)mediterranea*, between diploid and tetraploid biotypes of *Dugesia(S)polychroa*, and between fluorescent-labelled graft and unlabelled hosts of either species, were performed according to the classical procedures of Dubois (1949) and Schilt (1974). To avoid bacterial contamination, all the experiments were done under careful sterile conditions, and all the solutions employed had kanamycin sulphate (Sigma) at 10 $\mu g\,ml^{-1}$.

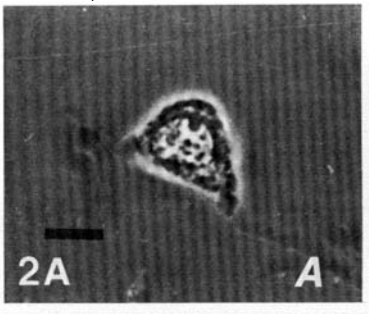
Irradiation

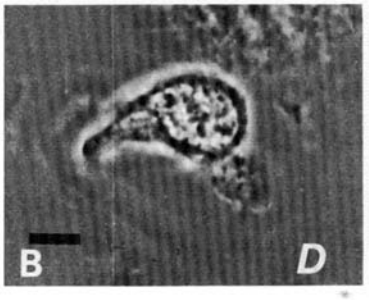
Organisms used to provide host irradiate tissues were exposed to 8000 rads (1000 rads min^{-1}) using a HT-100 Philips X-ray machine (1·7 mm A1 Filter; 100 kV, 8 mA).

Measure of cell movement

Depending on the marker three methods were used:

1) *Chromosomal marker* (Fig. 4). Cell migration between As and S tissues of *Dugesia (S)mediterranea* was measured by placing ten grafted organisms in 0·05 % colchicine (Sigma) for 6 h at different periods after grafting (10, 21, 31 and 47 days). After, they were fixed in 1 N-HCl, stained 'in toto' with a modified Gomori's method (Saló & Baguñà, 1984), mounted according to Baguñà (1974), and all metaphase figures counted (according to Karyotypes of As and S cells) along the anteroposterior (cephalocaudal) and mediolateral axis. From them, the mitotic index of each kind of cell in 1 mm intervals along both axis was obtained. The rate





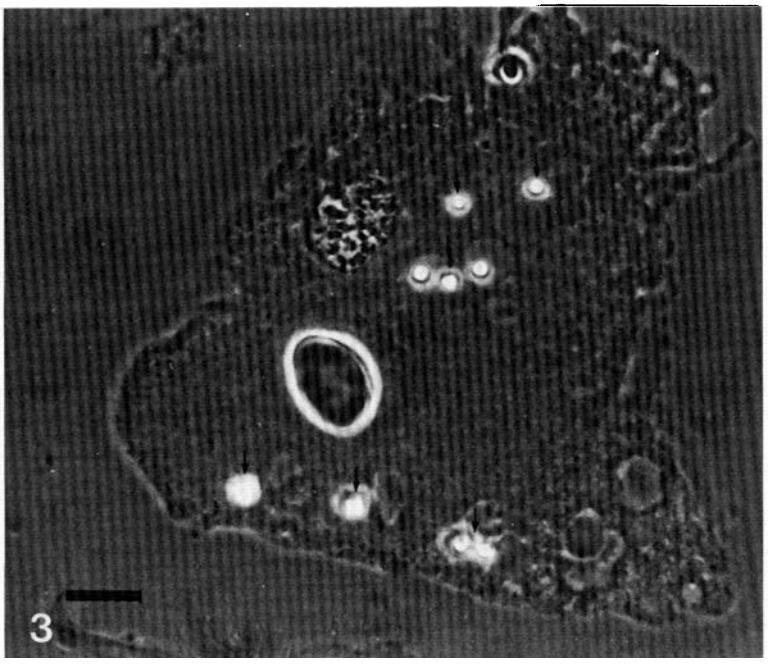


Fig. 2. (A) Neoblast of biotype A (diploid) of *Dugesia(S)polychroa*; (B) neoblast of biotype D (tetraploid) of *Dugesia(S)polychroa*. Bar equals 5 μm.

Fig. 3. Planarian fixed parenchyma cell with fluorescent latex beads (arrows) inside the cytoplasm. Bar equals 5 μm.

of movement (in average μm/day) was calculated from the distance covered by the leading edge of graft cells at different time intervals.

2) *Nuclear marker* (Fig. 5). To measure cell migration between diploid and tetraploid biotypes of *Dugesia(S)polychroa*, ten grafted organisms were fixed at different times after grafting (10, 21, 31 and 47 days), cut in 0·5 mm pieces along the anteroposterior axis, and macerated according to the procedure of Baguñà & Romero (1981). Neoblasts of both biotypes are easily distinguishable in phase-contrast microscopy according to nuclear size. In each 0·5 mm

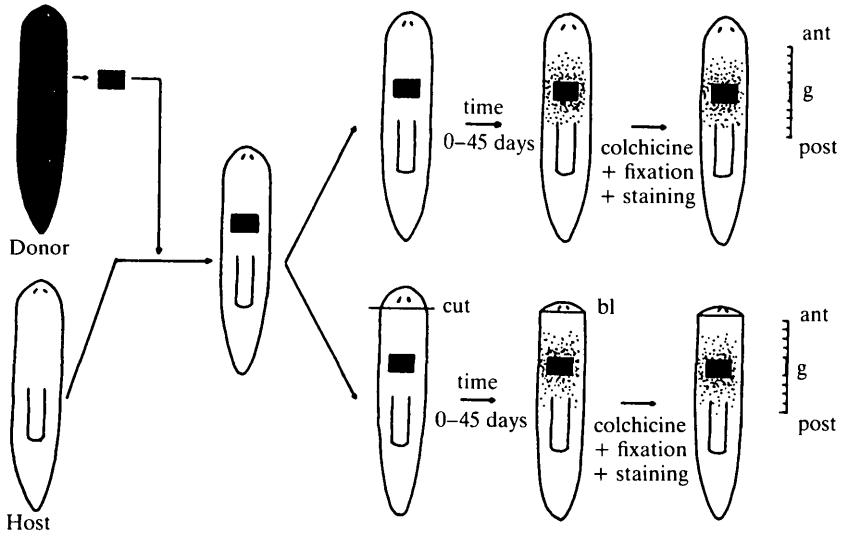


Fig. 4. Method used to measure the movement of graft cells within intact (upper right) and regenerating (lower right) host of *Dugesia(S)mediterranea* using the chromosomal marker. bl, blastema; g, graft; ant, anterior (cephalic); post, posterior (caudal). For details, see text.

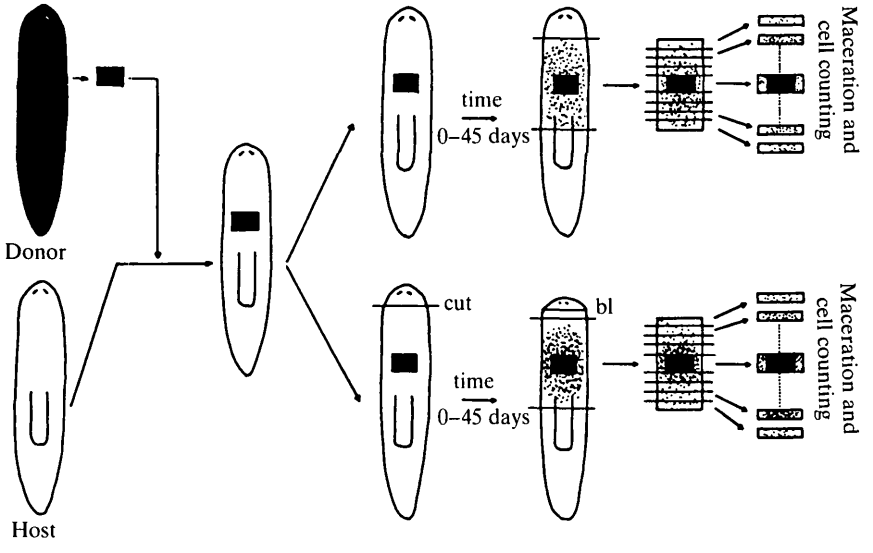


Fig. 5. Method used to measure the movement of graft cells within intact (upper right) and regenerating (lower right) hosts of *Dugesia(S)polychroa* using the nuclear marker. bl, blastema. For details, see text.

interval, 10% of neoblasts (~1000 cells) were counted and classified according to their biotype (A or D). The rate of graft cell movement (in average μm/day) was calculated from the distance covered by the graft cells between different time intervals.

3) *Cytoplasmic marker*. Movement of fluorescent (green) labelled cells was measured by fixing ten organisms at different times after grafting (5, 10, 15 and 20 days), mounting them 'in toto', and observing by epifluorescence the edge of fluorescent cells. The rate of cell movement (in average μm/day) was calculated from the distance covered by the leading edge of fluorescent cells at different time intervals.

All measurements were made for unirradiated and irradiated intact and regenerating hosts.

RESULTS

Histology

a) *Unirradiated hosts*

Grafts of As or S tissue into S or As hosts of *Dugesia(S)mediterranea* take very quickly. Two days after grafting, epidermal and mesodermal continuity is complete and no scar or connective tissue formation at the graft–host boundary is seen. Similar results were obtained with biotypes A and D of *Dugesia(S)polychroa*, and with either species using the fluorescent marker.

b) *Irradiated hosts*

Grafts of unirradiated tissue into irradiated hosts in both species also take very easily, epidermal and mesodermal continuity appearing quickly after grafting. As expected, grafts of unirradiated tissue lead to survival and regeneration of the irradiated host.

Cell movement in unirradiated intact organisms

The spreading of grafted As cells within S host tissues, and *vice versa*, in unirradiated *Dugesia(S)mediterranea* is shown in Fig. 6A. Estimates of the average rate of movement give values of $39{\cdot}7 \pm 11{\cdot}0$ μm/day ($n = 40$) in both directions (graft to host and host to graft). Since the only mitotic cells in planarians are neoblasts, and assuming a mean cell diameter of 8–10 μm for these cells, this rate of movement is equivalent to 4 cell diameters. No differences are detected in the direction of movement (spreading) along the A–P axis and mediolateral axis.

The rate of movement obtained using the difference in nuclear area as marker between biotypes A and D of *Dugesia(S)polychroa* amounts to $42{\cdot}2 \pm 10{\cdot}5$ μm/day (~4 cell diameters) ($n = 40$). On the other hand, fluorescent cells (all of them differentiated) move (or spread) at a rate far slower than the one for undifferentiated cells: $\leqslant 15$ μm/day ($n = 36$). Since the mean cell diameter of differentiated cells is ~30 μm (Baguñà & Romero, 1981), this is equivalent to less than half a cell diameter.

Cell movement in unirradiated regenerating organisms

Mutual cell spreading between graft and host tissues in regenerating *Dugesia(S)mediterranea* is shown in Fig. 6B. The rate of cell movement is very close

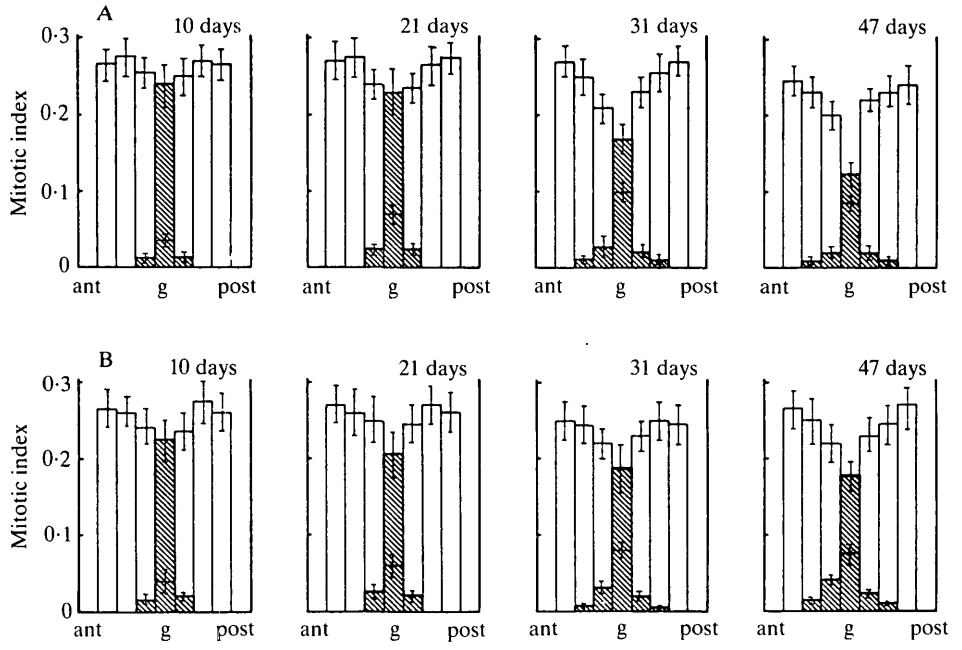


Fig. 6. Mitotic index ±S.D. of graft cells (hatched bars) and host cells (plain bars) by interval of length (1 mm) along the anteroposterior (cephalocaudal) axis of intact (A), and anteriorly regenerating (B) unirradiated hosts of *Dugesia(S)mediterranea* at different times (in days) after grafting and cutting. Movement (spreading) of cells occurs evenly in both directions. g; graft; ant, anterior (cephalic); post, posterior (caudal).

to the one found for intact organisms ($36{\cdot}8 \pm 6{\cdot}9\,\mu$m/day) (n = 42). Similar experiments done with *Dugesia(S)polychroa* gave values ($38{\cdot}2 \pm 8{\cdot}2\,\mu$m/day) (n = 46) very close to those found for intact organisms. Moreover, the amount of spreading is similar in all directions along the A–P axis and the mediolateral axis. Fluorescent cells also move at a similar rate as in intact organisms ($\leqslant 15\,\mu$m/day) (0·5 cell diameter) (n = 40).

Cell movement in irradiated intact organisms

After irradiation at 8000 rads, planarian cells do not proliferate, cell renewal is halted, tissues necrose, and organisms die within a 3–5 weeks period depending on temperature, age, and nutritional conditions (Lange, 1968; Chandebois, 1976). Moreover, the number of neoblasts decreases steadily approaching zero values at 15–20 days postirradiation (Saló, 1984).

Grafting unirradiated tissues into irradiated hosts leads to survival of the host through repopulation from graft cells. That graft cells and not revitalized host cells are responsible for this behaviour appear evident because *all* mitotic figures belong to graft cells. The rate of movement of As-cells into irradiated S-hosts and of S-cells into irradiated intact As-hosts in *Dugesia(S)mediterranea* gave values of $75{\cdot}3 \pm 12{\cdot}4\,\mu$m/day (~7–8 cell diameters) (n = 38) (Fig. 7A). This value is

significantly higher than the one found in similar experiments using irradiated intact hosts (Fig. 6A). Moreover, the spreading occurs evenly in all directions along the A–P and mediolateral axis. Similar data were found using the nuclear size marker of biotypes A and D of *Dugesia(S)polychroa* (70·23 ± 10·80 μm/day) (7–8 cell diameters) (n = 40). As a control experiment, the spreading of As-cells grafted

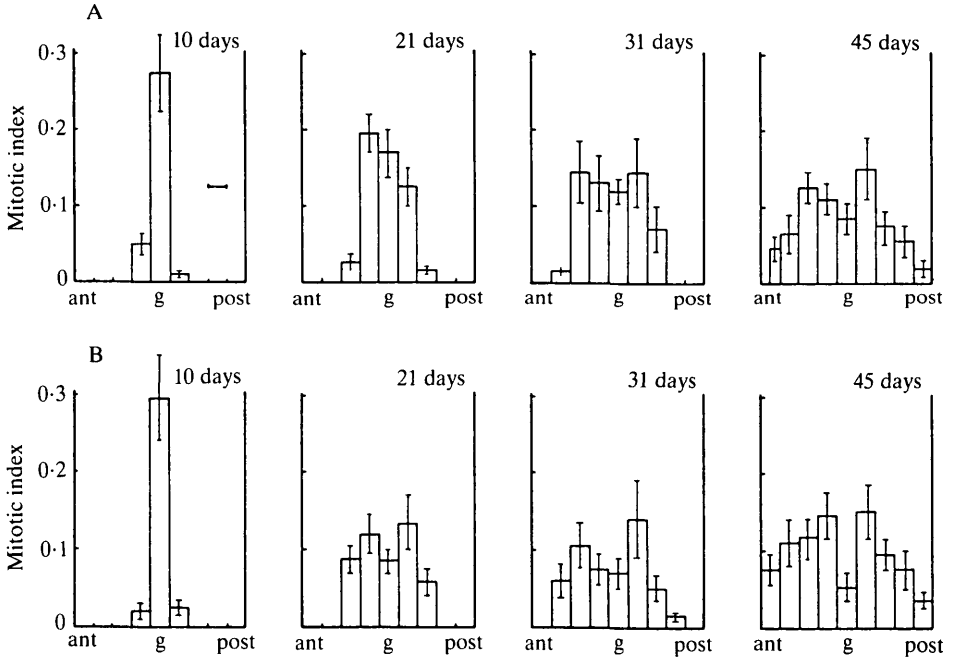


Fig. 7. Mitotic index ± S.D. of graft cells by interval of length (1 mm) along the anteroposterior (cephalocaudal) axis of intact (A) and anteriorly regenerating (B) irradiated hosts of *Dugesia(S)mediterranea* at different times (in days) after grafting and cutting. Movement (spreading) of cells occurs evenly in both directions. g, graft; ant, anterior (cephalic); post, posterior (caudal).

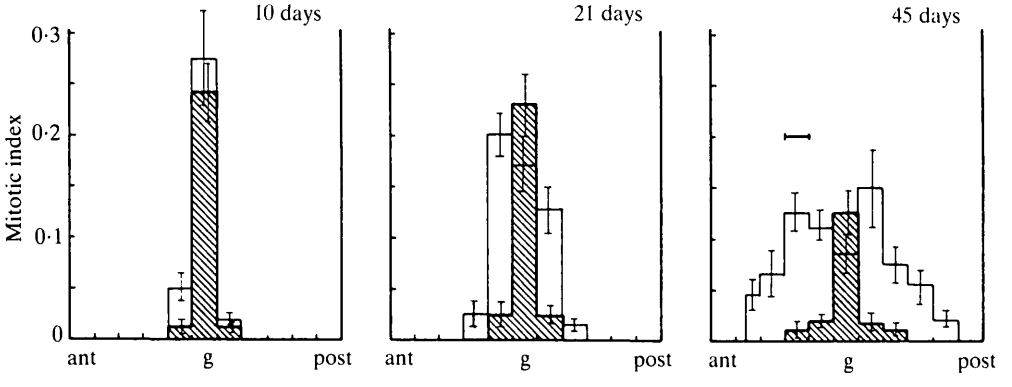


Fig. 8. Mitotic index ± S.D. and movement of graft cells along the anteroposterior (cephalocaudal) axis of intact unirradiated (thick line and hatched bars) and irradiated (thin line and plain bars) hosts at different times (in days) after grafting. g, graft; ant, anterior (cephalic); post, posterior (caudal).

Table 1. *Rates of cell movement (in μm/day) of graft cells within host tissues (irradiated and non-irradiated; intact and regenerating) using chromosomal, nuclear, and cytoplasmic markers*

	HOST			
	Unirradiated		Irradiated	
Marker	Intact	Regenerating	Intact	Regenerating
Chromosomal	39·7 ± 11·0	36·8 ± 6·9	75·3 ± 12·4	73·9 ± 8·9
Nuclear	42·2 ± 10·5	38·2 ± 8·2	70·2 ± 10·8	77·1 ± 10·4
Cytoplasmic	≤15	≤15	≤15	≤15

into irradiated As-hosts was measured. The results obtained also gave similar values (72·3 ± 11·85 μm/day) (~7–8 cell diameters) (n = 46). However, using fluorescent-labelled grafts, the spreading rate found (≤15 μm/day; ~0·5 cell diameter) (n = 40) is similar to the one found for unirradiated organisms, regenerating or not. This means that, unlike undifferentiated cells, differentiated cells do not change their rate of spreading after irradiation.

Cell movement in irradiated regenerating organisms

The spreading of graft cells within irradiated regenerating hosts gave values very close to the ones found for irradiated intact hosts: 73·9 ± 8·9 μm/day (~7–8 cell diameters) (n = 38) for *Dugesia(S)mediterranea* (Fig. 7B), and 77·06 ± 10·40 μm/day (~7–8 cell diameters) (n = 42) for *Dugesia(S)polychroa*. The amount of spreading was seen to be very similar in all directions along the A–P and mediolateral axis. In addition, fluorescent cells migrate at a similar rate to that in irradiated intact hosts (≤15 μm/day; ~0·5 cell diameter) (n = 32).

To study why undifferentiated grafted cells move faster in irradiated than in unirradiated hosts, the mitotic indices of grafted cells in both kinds of hosts were compared. The results (Fig. 8, for *Dugesia(S)mediterranea*) show clearly a higher mitotic index for graft cells within irradiated hosts than within unirradiated hosts. Moreover, in irradiated hosts, the number of graft cells in mitosis at sites distant from the graft site is higher than at the graft site itself, whereas in non-irradiated hosts it is the opposite.

Table 1 summarizes for comparison the rates of cell movement found for all experiments done and groups tested, using chromosomal, nuclear and cytoplasmic markers.

DISCUSSION

The use of chromosomal, nuclear, and cytoplasmic markers to track cell movements in planarians

Previous attempts to track cell movements within planarian tissues made use of radioactively labelled cells and differences in pigmentation between graft and host

strains. However, since planarian cells do not take up thymidine (Coward, Hirsh & Taylor, 1970), the most commonly used labels were [^{3}H]cytidine (Cecere, Grasso, Urbani & Vannini, 1964), [^{3}H]uridine (Lender & Gabriel, 1965), and $^{14}CO_2$ (Flickinger, 1964), all of them labelling unspecifically RNAs and proteins of most cell types. Once labelled, movement of cells was followed by autoradiography. The results found were diverse, a fact not surprising if we take into account the differences in markers and techniques employed (see Brønsted, 1969, for general references). One of the main flaws of these experiments is that labelled RNAs and proteins can, through cell lysis and diffusion into the intercellular space, be made available to non-labelled cells and blur the results. Moreover, these markers are non-specific, and non-permanent, being diluted with time up to the point of no detection. On the other hand, the use of pigmentation differences between planarian strains (or species) also has several disadvantages (see Introduction).

The chromosomal and ploidy markers used in this work have several advantages over RNA/protein labelling and pigmentation markers: 1) they are permanent; 2) they are present in all the cells of either graft or host; and 3) are of easy identification. Moreover, the fluorescent marker, though non-permanent, has proved very useful to follow specifically the movement of cells other than neoblasts.

Cell movement within intact planarians

The ease with which graft and host cells mix suggest at first that cell movement within planarian tissues is rather unrestrained, a fact not surprising if we consider that parenchymal tissue in planarians is in a rather loose state of organization (Baguñà & Ballester, 1978). But, what kind of cell moves?

That neoblasts move is shown clearly in data on cell spreading based on mitotic and non-mitotic cells (Table 1). Moreover, using fluorescent beads, it has been shown that cells other than neoblasts (e.g. fixed parenchymal cells, acidophilic and basophilic secretory cells, gastrodermal cells,..) move too, though their movements are much more restrained. Why this is so is at present difficult to assess, though it could be linked to their lack of cell proliferation. From this, we can conclude, tentatively, that cell movement between graft and host is mainly due to neoblasts.

Active cell movement or passive cell repopulation?

One of the uncertainties of the phenomenon of neoblast movement between graft and host tissues is to know if this is due either to an active process of individual cell movement (true cell migration) or to a passive process of 'cell spreading' due to cell proliferation. The first alternative was suggested by Dubois (1949) on the grounds that mitosis was not found within the irradiated host until unirradiated cells or cells from the graft reached the wound.

The results found by us clearly contradict Dubois' proposal. First of all, many graft cells in mitosis are found within unirradiated and irradiated hosts soon after

grafting. Secondly, the mean rates of movement found (35–75 μm/day) are rather low for a cell often qualified as a 'migratory cell', values that can be easily accounted by random movements of the daughter cells during and after telophase. Finally, similar rates of movement are found using the chromosomal marker (seen in mitotic neoblasts) and the nuclear size marker (seen in non-mitotic neoblasts), indicating that non-mitotic neoblasts do not move at a faster rate than mitotic neoblasts. The last two points clearly suggest that the spreading of neoblasts should be mainly linked to cell proliferation, and more specifically to random movements occurring during and/or after cell division.

This leads us to conclude that, as already suggested by Sugino, Okuno & Yoshinobu (1970), movement of cells (neoblasts) from graft to host and *vice versa* is not the result of active cell movements of individual cells but the consequence of a passive process of cell spreading (repopulation) mainly linked to cell division.

Cell movement from distant sites is neither stimulated by the wound nor directed towards it

That wounding does not stimulate the spreading of cells far from the wound appears very clear when comparing the similar rate of movement of neoblasts in regenerating and intact organisms (Table 1). Moreover, that cells other than mitotic cells do not spread faster after wounding is also clear when looking at the data obtained using the nuclear and the cytoplasmic markers (Table 1).

These data contradict one of the tenets of Wolff and Dubois' theory of blastema formation: that wounding stimulates the 'migration' of cells even in regions far from the wound, stimulation mediated by some kind of diffusable 'necrohormone' released at or near the wound. Besides the results found in this work, we think their conclusions are unproved for two reasons: 1) in their experiments they did not have rates of movement in intact organisms to compare with the values found for regenerating organisms and infer from the latter only that stimulation had occurred; and 2) the existence of the postulated 'necrohormones', or any other stimulatory mechanism, have never been substantiated.

If spreading of cells is not stimulated in regenerating organisms, it follows that anteriorly regenerating organisms should have similar spreading rates of cells towards the wound (anterior) than towards posterior intact areas. That this is so appears very clear from all the data gathered using both markers (chromosomal and nuclear), where an even rate of spreading was seen along the A–P axis (Figs 6A,B, 7A,B) and the mediolateral axis (results not shown) in all groups studied. From this we can conclude that, contrary to another of the tenets of Wolff and Dubois' theory, cell movement (spreading) is not directed (oriented) preferentially to the wound.

Why cells move faster in irradiated than in unirradiated hosts?

The rate of movement of grafted cells in irradiated hosts is twice the value found for unirradiated hosts (Table 1). This could be due either to an easier movement of graft cells going through the spaces left by lysed or dying cells, or to a higher

mitotic rate of graft cells in irradiated hosts due to the decreasing number of neoblasts and to the absence of mitosis in the latter.

The mitotic index of graft cells in irradiated hosts is two to four times higher than in unirradiated hosts (Fig. 8). Moreover, the rate of cell movement measured using the chromosomal marker (seen in mitotic neoblasts) and the nuclear size marker (seen in non-mitotic neoblasts) are similar (Table 1). Both results favour the second alternative; that is, that absence of mitotic cells in the irradiated host and the ever decreasing number of host neoblasts allow graft cells to engage in mitosis at a rate higher than in unirradiated host. From this it follows that cell proliferation in planarians could be controlled by a density-dependent inhibitory mechanism mediated by factors produced and released by neoblasts themselves. This, jointly with the action of some still poorly characterized factors that trigger neoblast proliferation after wounding (Baguñà, 1976; Friedel & Webb, 1979; Saló, 1984) and feeding (Baguñà, 1974), may control the final balance of neoblast density and proliferation.

Some implications for mechanisms of blastema formation and the origin of blastema cells

In a previous paper (Saló & Baguñà, 1984) it has been shown that regenerative blastemata in planarians do not have mitotic activity, and that their growth could be explained through the continuous entrance of undifferentiated cells from the stump to the base of blastemata. However, it is still uncertain if these cells come only from local stump sources or if cells placed far from the wound can participate in blastemata growth.

The low spreading rates of planarian cells found in this work (see Table 1) suggest at first a local origin of blastema cells. Thus, taking into account that mitotic activity in the stump region near the wound is three to four times higher than in control intact organisms (Saló & Baguñà, 1984), and assuming that cell spreading is mainly due to movements related to mitotic activity, it is sound to suggest that spreading rates in regions near the wound may attain values around 100 μm/day (recent unpublished data obtained on cell spreading near the wound support this suggestion; Saló & Baguñà, in preparation). If this is so, a 4- to 5-day blastema must be the result of the proliferation and spreading of cells within a region of, at the most, 300–500 μm around the wound. This means that, in normal (non-irradiated) regenerating organisms, blastema cells have a local origin.

However, as first shown by Wolff & Dubois (1948), partially irradiated regenerating organisms form a blastema after a delay proportional to the distance between wound and healthy unirradiated tissue. From this they suggested that blastema cells were neoblasts that had migrated throughout the irradiated tissue from the unirradiated regions, and, therefore, that neoblasts were totipotent or pluripotent migratory cells.

The results obtained in this work suggest an alternative interpretation of Wolff and Dubois experiments and conclusions. First of all, the so-called 'migration' of neoblasts is not a true cell migration but the result of the slow and progressive

spreading of undifferentiated cells (neoblasts) through the irradiated region by cell proliferation. Secondly, although neoblasts from the unirradiated region make the blastema in partially irradiated organisms, this does not necessarily mean that neoblasts are totipotent or pluripotent since these cells (neoblasts) may have resulted from the dedifferentiation of differentiated cells in the unirradiated region before their 'migration' (spreading) to the wound. Therefore, until more precise experiments using cell-specific markers are performed (Saló & Baguñà, work in progress), the idea of neoblasts as totipotent or pluripotent cells will remain unproved.

We would like to thank Rafael Romero for permission in quoting unpublished data, Professor N. G. Lepori (Università di Sassari, Sardegna, Italia) for sending stocks of the sexual race of *Dugesia(S)mediterranea* and the biotypes A and D of *Dugesia(S)polychroa*, and Dr J. Schilt (Université de Nancy I, France) for teaching one of us (ES) grafting techniques in planarians. We warmly acknowledge the comments of an anonymous referee which greatly helped to improve the manuscript. This work was supported by the grants AIUB 611/81 of the University of Barcelona and 1108/81 from Comisión Asesora de Investigación Científica y Técnica (CAICYT) to JB.

REFERENCES

BAGUÑÀ, J. (1973). Estudios citotaxonómicos, ecológicos, e histofisiología de la regulación morfogenética durante el crecimiento y regeneración de la raza asexuada de la planaria *Dugesia mediterranea* n.sp. (Turbellaria, Tricladida, Paludicola). Ph. D. Thesis. Univ. de Barcelona.

BAGUÑÀ, J. (1974). Dramatic mitotic response in planarians after feeding, and a hypothesis for the control mechanism. *J. exp. Zool.* **190**, 117–122.

BAGUÑÀ, J. (1976). Mitosis in intact and regenerating planarian *Dugesia mediterranea* n.sp. II. Mitotic studies during regeneration and a possible mechanism of blastema formation. *J. exp. Zool.* **195**, 65–80.

BAGUÑÀ, J. & BALLESTER, R. (1978). The nervous system in planarians: peripheral and gastrodermal plexuses, pharynx innervation, and the relationship between central nervous system structure and the acoelomate organization. *J. Morph.* **155**, 237–252.

BAGUÑÀ, J. & ROMERO, R. (1981). Quantitative analysis of cell types during growth, degrowth and regeneration in the planarians *Dugesia mediterranea* and *Dugesia tigrina*. *Hydrobiologia* **84**, 181–194.

BENAZZI, M., BALLESTER, R., BAGUÑÀ, J. & PUCCINELLI, I. (1972). The fissiparous race of the planarian *Dugesia lugubris* s.l. found in Barcelona (Spain) belongs to the biotype G: comparative analysis of the karyotypes. *Caryologia* **25**, 59–68.

BENAZZI, M. & BENAZZI-LENTATI, G. (1976). *Animal Cytogenetics.* Vol. I. *Platyhelminthes* (ed. B. John). Berlin: Gebrüder.

BRØNSTED, H. V. (1969). *Planarian Regeneration.* London: Pergamon Press Ltd.

CHANDEBOIS, R. (1976). *Histogenesis and Morphogenesis in Planarian Regeneration.* Basel: S. Karger.

CECERE, F., GRASSO, M., URBANI, E. & VANNINI, E. (1964). Osservazioni autoradiografiche sulla rigenerazione di *Dugesia lugubris*. *Rend. Ist. Sci. Camerino* **5**, 193–198.

COWARD, S. J., HIRSH, F. M. & TAYLOR, J. H. (1970). Thymidine kinase activity during regeneration in the planarian *Dugesia dorotocephala*. *J. exp. Zool.* **173**, 269–278.

DE VRIES, E. J., BAGUÑÀ, J. & BALL, I. R. (1984). Chromosomal polymorphism in planarians (Turbellaria, Tricladida) and the plate tectonics of the western Mediterranean. *Genetica* **62**, 187-191.

DUBOIS, F. (1949). Contribution à l'etude de la régénération chez planaires dulcicoles. *Bull. Biol.* **83**, 213–283.

FLICKINGER, R. A. (1964). Isotopic evidence for a local origin of blastema cells in regenerating planaria. *Expl Cell Res*. **34**, 403–406.

FRIEDEL, T. & WEBB, R. A. (1979). Stimulation of mitosis in *Dugesia tigrina* by a neurosecretory fraction. *Can. J. Zool*. **57**, 1818–1819.

LANGE, C. S. (1968). Studies on the cellular basis of radiation lethality. I. The pattern of mortality in the whole-body irradiated planarian (Tricladida, Paludicola). *Int. J. Radiat. Biol*. **13**, 511–530.

LENDER, TH. & GABRIEL, A. (1965). Les néoblastes marqués par l'uridine tritiée migrent et édifient le blasteme de régénération des planaires d'eau douce. *C. r. hebd. Séanc. Acad. Sci., Paris* **260**, 4095–4097.

SALÓ, E. (1984). Formació del blastema i re-especificació del patró durant la regeneració de les planàries *Dugesia(S)mediterranea* i *Dugesia(G)tigrina*: Anàlisi morfològic, cel.lular i bioquímic. Ph. D. Thesis. Univ. de Barcelona.

SALÓ, E. & BAGUÑÀ, J. (1984). Regeneration and pattern formation in planarians. I. The pattern of mitosis in anterior and posterior regeneration in *Dugesia(G)tigrina*, and a new proposal for blastema formation. *J. Embryol. exp. Morph*. **83**, 63–80.

SCHILT, J. (1974). Rôles respectifs du système nerveux et des territoires au cours de la régénération chez les planaires. Ph. D. Thesis. Univ. de Nancy I.

SUGINO, H., OKUNO, Y. & YOSHINOBU, J. (1970). Effect of transplanted pieces from non X-irradiated worms on irradiated ones in *Dugesia japonica. Mem. Osaka. Kyoiku. Univ*. **19**, 63–76.

TRINKAUS, J. P. (1984). *Cells into Organs* (2nd. ed.). Englewood Cliffs (NJ, USA): Prentice-Hall Inc.

WOLFF, E. & DUBOIS, F. (1948). Sur la migration des cellules de régénération chez les planaires. *Rev. suisse Zool*. **55**, 218–227.

(Accepted 5 March 1985)