

Pattern of differentiated nerve cells in hydra is determined by precursor migration

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SUMMARY

The nervous system of the fresh water polyp hydra is built up as a nerve net spread over the whole body, with higher densities in the head and the foot. In adult hydra, as a result of continuous growth, new nerve cell differentiation takes place continuously. The pattern of nerve cell differentiation and the role of nerve cell precursor migration in establishing the pattern have been observed *in vivo* by vitally labelling precursor cells with DiI. The results indicate that nerve cell precursors arise directly from stem cells, complete a final cell cycle and divide, giving rise to two daughter cells, which differentiate into nerve cells. A subpopulation of the nerve cell precursors are migratory for a brief interval at the onset of the terminal cell cycle, then complete the cell cycle and divide at the site of differen-

tiation. Labelling small patches of tissue in the head, body column and peduncle/foot with DiI indicated that formation of nerve cell precursors was nearly constant at all three positions. However, at least half of the labelled precursors in the body column migrated to the head or foot before differentiating; by contrast, precursors in head and foot differentiated *in situ* without significant migration. This redistribution leads to a net increase of nerve cell precursors in head and foot compared to body column and thus to the higher density of nerve cells in these regions.

Key words: hydra, cell migration, nerve cell differentiation, head activator, DiI labelling

INTRODUCTION

The freshwater polyp hydra has one of the simplest nervous systems in metazoan animals. It consists of a net of nerve cells (Hadzi, 1909) that extends throughout the animal with higher densities in the head and the foot (Bode et al., 1973). Individual nerve cells can be characterized as sensory or ganglionic based on cell morphology (David, 1973; Davis et al., 1968; Epp and Tardent, 1978; Tardent and Weber, 1976). Subpopulations of nerve cells containing specific neurotransmitters or recognized by specific antibodies are localized in different regions of hydra tissue (Grimmelikhuijzen et al., 1982; Hobmayer et al., 1990; Koizumi and Bode, 1986). However, little is known about the physiological function of these subpopulations.

The nerve net in adult hydra tissue is constantly undergoing renewal as a result of continuous growth and differentiation. [³H]thymidine-labelling experiments have shown that nerve cells are stable cells surviving over more than one tissue doubling while the nerve net expands continuously with ongoing tissue growth (David and Gierer, 1974). For subpopulations of nerve cells in the tentacles and the peduncle/basal disk, this expansion has been shown to involve new differentiation from interstitial cell precursors (Bode, 1992; Hobmayer et al., 1990; Technau and Holstein, 1996; Yaross et al., 1986).

Nerve cells in hydra arise by differentiation from a population of multipotent interstitial cell precursors (David and Murphy, 1977). Several experiments indicate that commitment

to the nerve cell pathway occurs in S phase and is followed by completion of the cell cycle and differentiation (Berking, 1979; Venugopal and David, 1981b; Yaross et al., 1982). Interstitial cells in hydra are abundant in the gastric region but less concentrated in head and foot tissue (Bode et al., 1973; David and Plotnick, 1980) where extensive nerve cell differentiation occurs. This has suggested that nerve precursors may migrate from the gastric region into head and foot tissue to support the high differentiation rates in these tissues (Bode et al., 1990; David and Hager, 1994; Fujisawa, 1989; Heimfeld and Bode, 1984; Teragawa and Bode, 1990, 1995).

Although the population dynamics of interstitial cell precursors and nerve cells have been extensively investigated, little is known about the nerve cell differentiation pathway *in vivo*. In particular, it is not known whether both daughter cells of a stem cell precursor differentiate to nerve cells or whether only one of the daughter cells is determined to the nerve pathway while the other daughter cell has a different fate. It is also not known whether all nerve cell precursors migrate or when migration occurs in the differentiation pathway.

To answer these questions, we have used the fluorescent carbocyanine dye DiI to follow the behaviour of individual precursor cells *in vivo*. The method was originally developed by Honig and Hume (1986) to label nerve cells *in vivo* and has recently been applied to hydra by Teragawa and Bode (1990, 1995). The method is minimally invasive and hence eliminates stimulatory effects on cell migration that are caused by tissue grafting (Fujisawa et al., 1990). We used *in vivo* labelling with

the DNA-specific fluochrome DAPI to define the time of precursor migration in the nerve cell differentiation pathway.

Our results indicate that formation of nerve cell precursors is evenly distributed along the body column and that migration of nerve cell precursors from the body column to the head and the foot is essential to establish the pattern of differentiated nerve cells along the body axis of hydra. The results show also that treatment with the neuropeptide 'head activator' (Schaller and Bodenmüller, 1981) increases the number of migrating precursors.

MATERIALS AND METHODS

Animal culture

All experiments were performed with *Hydra vulgaris*. The animals were originally isolated from Lake Zurich by Dr Pierre Tardent and have been maintained in laboratory culture since 1962. Animals were cultured at 18°C and fed daily with freshly hatched *Artemia* nauplii.

DiI labelling

The fluorescent carbocyanine dye DiI (1,1-dioctadecyl-3,3,3-tetramethylindocarbocyanine perchlorate; Molecular Probes) was dissolved in 100% ethanol. DiI was diluted to 50 µM in 0.03 M sucrose. To label hydra cells, about 10 nl of the DiI-sucrose solution was pressure-injected into the ectoderm of individual animals. The resulting small patches of labelled tissue contained an average of 1-10 interstitial cells, 5-20 nerve cells, 2-5 nests of nematoblasts and nematocytes and 3-5 epithelial cells. Since the distribution of labelled cell types does not reflect the relative numbers of cells at the site of labelling, it appears that some cell types are labelled more efficiently than others. In particular, epithelial cells are poorly labelled while nerve cells are well labelled, perhaps due to the large and ramified processes.

DiI-labelled animals were fed 1-2 *Artemia* daily and kept in the dark, in normal culture dishes, to prevent bleaching of the dye.

To examine the differentiation of the labelled cells, individual animals were placed on a microscope slide under a cover slip supported by vaseline plugs to prevent squashing of the animal. Observations were made daily and animals were then returned to the culture dishes. Animals were observed for only 3-10 minutes to prevent anoxia and bleaching of the dye. Under these conditions it was possible to follow the lineage of individual cells for up to one week.

Vital labelling with the DNA-specific fluorochrome DAPI

Hydra polyps were incubated for 30 minutes in the dark in a solution of 50 µg/ml DAPI (4,6-diamidino-2-phenylindole-1-HCl, Sigma) in hydra medium and then washed 3-4 times with hydra medium. After labelling, animals were kept in the dark to prevent bleaching.

Tissue grafting

Animals starved for 24 hours were used for grafting experiments. Half of the animals were DAPI labelled. Axial grafts were prepared from DAPI-labelled lower half and unlabelled upper half animals by threading the two halves in the correct orientation onto fishing line. The two halves were gently pressed together with pieces of polyethylene tubing (PE20, Intramedic, Clay Adams). Transplants were left undisturbed for 2 hours before removing the fishing line.

Microscopy and photography

Animals were observed using a Leitz Dialux 20 microscope equipped for epifluorescence illumination with appropriate filter combinations for DiI and for DAPI and a water immersion fluorescence objective (25×; 0.75). Microscopic images were recorded with a videocamera (SANYO VC-2512; HAMAMATSU C 2400) and processed using a

frame grabber (µTech, Image/VGA Plus) attached to a IBM-compatible PC. Micrographs were taken on T-Max 400 ASA or Tungsten 320 ASA film from Kodak.

Quantitative determination of DAPI fluorescence in single cells in vivo

To determine the nuclear DNA content of individual cells in vivo, animals were vitally labelled with the DNA-specific fluorochrome DAPI. Stained animals or grafts were gently flattened under a coverslip and examined by epifluorescence illumination. The fluorescence of individual labelled nuclei was measured in arbitrary units with a photomultiplier (Leitz, MPV compact). Nematocytes, which had 2n nuclear DNA content (David and Gierer, 1974), were used as a convenient in situ standard. Since the intensity of DAPI staining varied between animals, all measurements in each animal were normalized to the average nematocyte value in that animal.

Head activator treatment

Hydra polyps were treated with the neuropeptide 'head activator' (pGlu-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe) (Schaller and Bodenmüller, 1981). Synthetic head activator was obtained from Bachem. 40 animals starved for 24 hours were labelled with DiI and then incubated in a 10⁻¹⁰ M solution of head activator in hydra medium for 24 hours (Holstein et al., 1986). Labelled animals were examined after 48 and 72 hours by epifluorescence microscopy and scored for the number of DiI-labelled nerve cells and interstitial cells that had migrated out of the labelled patch (more than 10 epithelial diameters away from the border of labelled cells).

RESULTS

Lineage of nerve cell precursors

Small patches of cells in the ectoderm of hydra polyps were labelled with the fluorescent dye DiI by injecting the dye in a sucrose solution directly into the tissue (Honig and Hume, 1986; Teragawa and Bode, 1990, 1995). The dye was taken up by cells and spread rapidly throughout the cell membranes. Labelled cells were thus clearly outlined and could be identified by their morphology. Fig. 1 shows representative examples of DiI-labelled interstitial cells, a nest of differentiating nematocytes and a nerve cell. The dye was not toxic in our experiments and individual cells and their progeny could be followed over the course of several days by repeatedly examining the same labelled patch of cells. It has thus been possible for the first time to establish cell lineages in hydra.

Newly differentiated DiI-labelled nerve cells first began to appear in large numbers on day 1-2 after labelling. New nerve cells differentiated both in the labelled patch and after migration of the interstitial cell precursor out of the patch (see below). The lineages of 183 labelled nerve cell precursors that migrated away from the site of the DiI injection are summarized in Fig. 2. In most cases (91%), the migrating precursor cell divided once and both daughter cells gave rise to nerve cells. In a few cases (8%), the labelled precursor divided a second time and all four daughter cells gave rise to nerve cells; in two cases (1%), three divisions occurred after the migration of the precursor cell and all 8 daughter cells gave rise to nerve cells. No cases were observed in which a single migrating precursor cell differentiated directly to a nerve cell.

Nerve cell differentiation that occurred inside a labelled patch without migration of the labelled precursor cell gave rise to lineages similar to those presented in Fig. 2A.

Migration of nerve cell precursors

Our experiments demonstrate that a subpopulation of nerve cell precursors migrate extensively before differentiating into nerve cells (see also Teragawa and Bode, 1995). Fig. 3 presents a typical result schematically: a precursor cell labelled with DiI in the body column migrated from the site of labelling toward the head before dividing and differentiating into two nerve cells. Migrating cells were always single and moved parallel to the vertical axis of the animal; no cells were observed to migrate circumferentially around the body column. Migration was rapid, requiring less than 6 hours from the midgastric region to either head or foot. Cells from the midgastric region migrated for variable distances: some stopped in the head or foot while others stopped, after a shorter distance, in the body column. The rate of precursor cell migration from the midgastric region towards the head was roughly 2.5-fold higher than towards the foot.

Micrographs of a migratory interstitial cell, a pair of post-mitotic interstitial cells after migration and a pair of differentiated nerve cells are shown in Fig. 4. During migration the shape of interstitial cells changed compared to non-migrating cells. They extended a process which was 1-3 cell diameters in length (Fig. 4A). This process appears to be the 'tail' of interstitial cells observed by Campbell in electron micrographs (Campbell, 1967). After migration, cells retracted the process and stayed as single cells for up to a day before dividing. Both daughter cells remained close to the site of division and differentiated to form a pair of nerve cells.

Migration occurs in S-phase

To investigate onset and duration of migration during the cell cycle of the nerve cell precursor, hydra polyps were vitally labelled with the DNA-specific fluorochrome DAPI. Lower halves of DAPI-stained animals were then grafted to unstained upper halves. Within a few hours DAPI-labelled nematocytes and interstitial cells had migrated from the lower to the upper half of the grafted animals. To determine the nuclear DNA content of migrating cells, the DAPI fluorescence of individual cells was measured in situ in whole animals. Nematocytes, which migrate in large numbers from the lower to upper halves of the grafts, have 2n nuclear DNA content (David and Gierer, 1974) and provided a convenient in situ standard for G1 cells. Comparing the fluorescence values of

migrating interstitial cells to those of migrating nematocytes in the same animal, we could identify the cell cycle position of the migrating interstitial cells.

Fig. 5A,B shows the DNA contents of migrating interstitial cells and nematocytes measured in vivo. The distributions are rather broad due to the thick tissue layer in vivo and thus the scattering of fluorescence light. Nevertheless the two distributions are clearly similar indicating that migrating interstitial cells have the same DNA content as nematocytes, i.e. 2n. Comparison of the in vivo measurements on migrating cells with measurements on single cells in macerates (Fig. 5C,D) confirmed the 2n DNA determination and also demonstrated clearly the narrower distribution of single cell measurements due to the improved optical conditions in macerated tissue.

The results demonstrate that migrating interstitial cells have 2n DNA content. Thus they were in G1 at the time of labelling or they must have passed through mitosis after labelling.

Stem cells and nematoblast precursors do not migrate

The DiI-labelling experiments revealed extensive cell

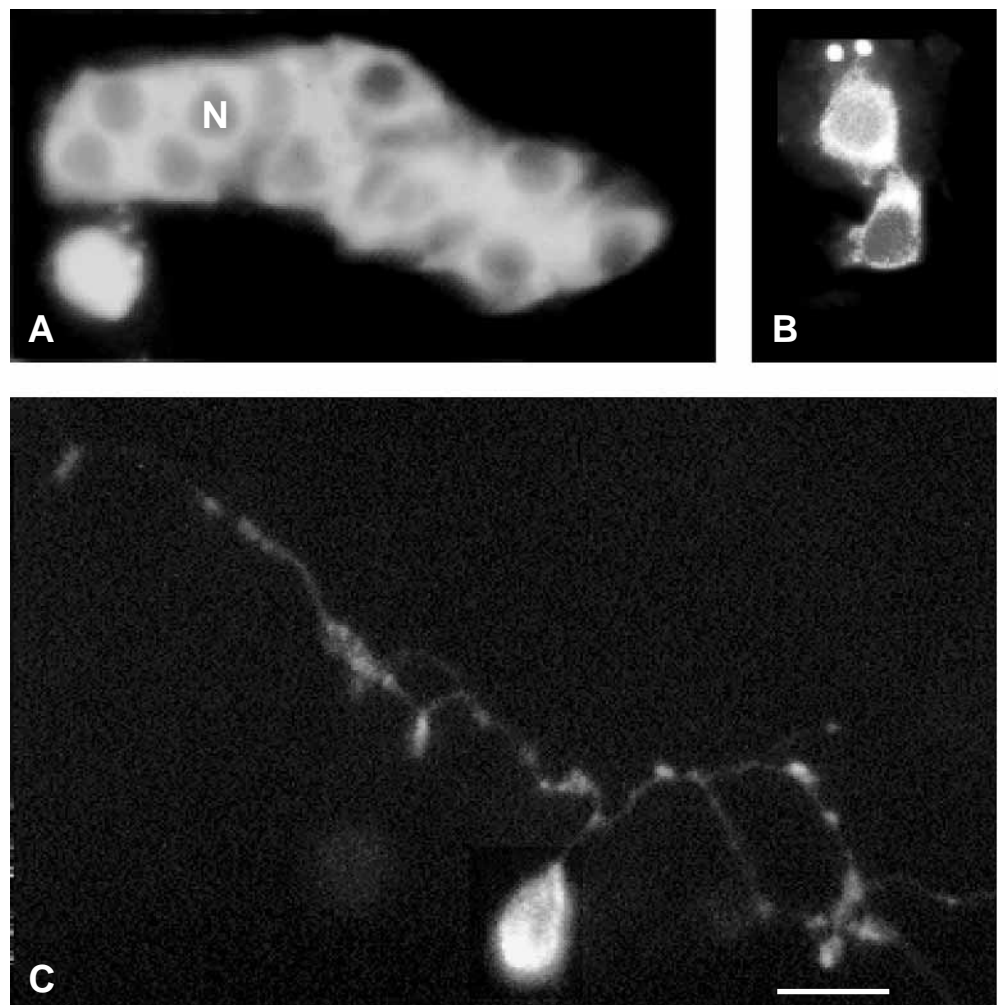


Fig. 1. Micrographs of DiI-labelled cells in the ectoderm of hydra. DiI labels the cell membranes and is also distributed throughout the cytoplasm. (A) nest of differentiating nematocytes, (B) pair of interstitial cells and (C) nerve cell. N, cell nucleus. Scale bar represents 10 μ m.

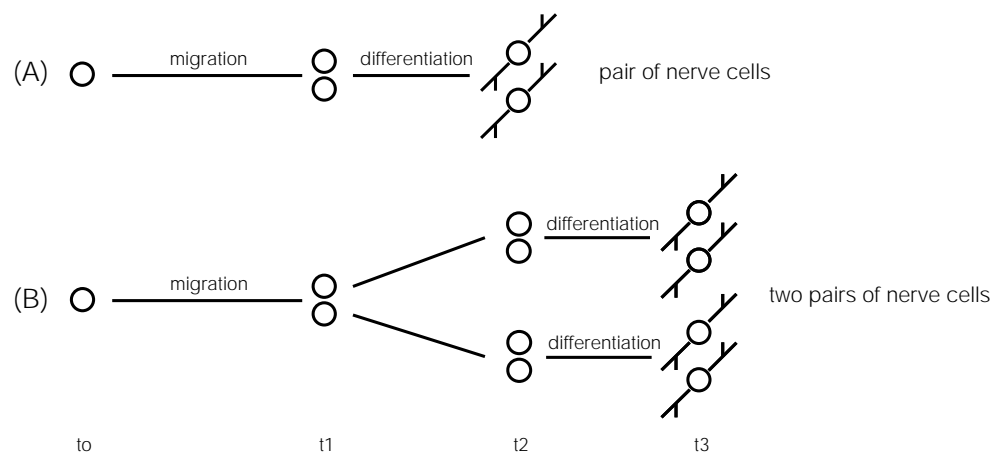


Fig. 2. Summary of 183 nerve cell lineages from migrating precursor cells. (A) 91% of observed lineages; (B) 8% of observed lineages.

migration from patches of labelled tissue in the body column. The majority of these cells could be identified as migrating nematocytes based on the nematocyte capsule. The remaining cells were single interstitial cells, many of which differentiated to nerve cells (see above).

To determine if other cell types – e.g. stem cells or nematoblast precursors – also migrate, we analysed DiI-labelled animals one week after labelling in order to permit more extensive cell division by migrating cells. DiI was stable and mitotic progeny were still detectable after one week when labelled animals were kept in the dark and fed daily.

The results are shown in Table 1. All animals (25/25) contained DiI-labelled migrating nematocytes. Most animals (21/25) also contained migrating interstitial cells and nerve cells that had differentiated from migrating interstitial cell precursors. Clones of proliferating interstitial cells and nests of proliferating nematoblasts were not observed outside of the DiI-labelled patch in any animal (0/25). Labelled interstitial cells (23/25) and nests of proliferating nematoblasts (12/25), however, were still present in the patch indicating that cell division had not diluted the DiI below the limit of detection. Since 7 days is more than sufficient time for clone formation (David and Murphy, 1977) or nest formation (David and Gierer, 1974), we conclude that stem cells and nematoblast precursors do not migrate.

Table 1. Cell types migrating from patches of DiI-labelled tissue

Cell type	Cells/nests per animal	Observed in
<i>cells outside the patch</i>		
<i>(= migrating cells)</i>		
nematocytes	86.2±39.5	25/25
interstitial cells	3.4±2.5	21/25
nerve cells	6.1±4.5	21/25
nests of nematoblasts/nematocytes	0	0/25
clones of interstitial cells	0	0/25
<i>cells inside the patch</i>		
nests of nematoblasts/nematocytes	1.7±1.2	12/25
interstitial cells	4.8±2.9	23/25

25 animals were DiI labelled, fed normally and kept in the dark. After 7 days, all cells observed outside the patch (more than 5-10 epithelial cell diameters away from the border of labelled cells) were counted. Cells inside the patch were counted separately.

Proportion of in situ differentiation and precursor migration at different axial positions

The DiI-labelling technique has demonstrated that some nerve cell precursors migrate before differentiating while others differentiate in situ at the site of labelling. To analyse the relative contributions of migration and in situ differentiation to the pattern of nerve cell differentiation, the DiI-labelling experiment was carried out at three different positions along the body column: head/upper gastric, midgastric and lower gastric region/peduncle (Fig. 6). Individual animals were injected at one of the three positions and nerve cell differentiation from DiI-labelled precursors was followed for the next 3 or 4 days.

The results indicate a striking difference in the behaviour of labelled nerve cell precursors at the three positions. In the midgastric region, about half of the new nerve cells differentiated in situ while the remaining half differentiated either in the head or foot after migration of a precursor cell from the labelled midgastric patch. By comparison, most DiI-labelled precursors in head and foot patches differentiated in situ or migrated only a very short distance beyond the periphery of the labelled patch. Migration never occurred from a head or foot patch into the gastric region.

The results are shown quantitatively in Fig. 6 as the number of labelled nerve cells that newly differentiated on each day either in situ or after precursor migration out of the labelled patch. A total of 100 animals were analysed at each position. Every day, the number of newly differentiated (DiI-labelled) nerve cells inside and outside the patch was determined. This number was obtained by counting the total number of labelled nerve cells per animal on each day and subtracting the number of nerve cells counted on the previous day. Thus, fully differentiated (old) nerve cells labelled at the time of injection (see Materials and Methods) are not included in the numbers of newly differentiated nerve cells shown in Fig. 6.

The total number of newly differentiated nerve cells varied between 100 and 200 per day per 100 animals at each position and was roughly the same at all three positions on all days. Since the size of the labelled patches was approximately the same at all three positions, the results indicate that the rate of precursor commitment to nerve cell differentiation is nearly constant along the body column.

The results in Fig. 6 indicate a striking difference in the

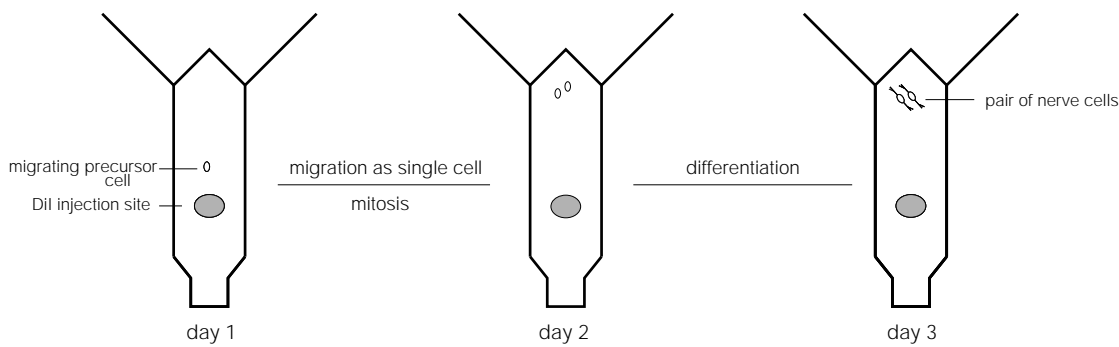


Fig. 3. Schematic representation of nerve cell differentiation from a migrating precursor cell labelled with DiI in the midgastric region (see text for details).

migration behaviour of the nerve cell precursors. On day 1 essentially all DiI-labelled nerve cells differentiate in situ. By comparison, on days 2, 3 and 4, essentially half of the nerve cell precursors that arose in the body column migrated out of the labelled patch toward the head or foot before differentiating into nerve cells. The simplest explanation for this difference is that precursor migration is limited to a well-defined time point along the differentiation pathway from interstitial cell to nerve cell. Precursors that were labelled late in the pathway, shortly before differentiation, have passed the migration phase and differentiate in situ. The majority of newly differentiated DiI-labelled nerve cells on day 1 are such cells. By contrast, precursors that complete differentiation on days 2, 3 and 4 were labelled earlier in the differentiation pathway (or even labelled as stem cells before birth of the nerve cell precursor) prior to the migration phase.

In a few cases, single labelled cells differentiated directly to nerve cells. Essentially all such cases occurred on day 1 after labelling and differentiation took place, in situ, at the site of labelling. This suggests that, in these cases, DiI labelling occurred after mitosis of the nerve cell precursor and that only one daughter cell picked up the dye.

Head activator treatment stimulates formation of migrating nerve cell precursors

Previous studies have shown that the neuropeptide 'head activator' stimulates nerve cell differentiation in hydra (David and Holstein, 1985; Holstein et al., 1986; Schaller, 1976). To investigate whether head activator treatment stimulates formation of migrating nerve cell precursors, we labelled hydra with DiI in the midgastric region, treated them with head activator for 24

hours and counted the number of labelled (migrating) interstitial cells outside the labelled patch on day 1 and 2 after treatment. The results are shown in Table 2. Head-activator-treated animals contained 1.5 times more interstitial cells migrating toward the head on day one than untreated control animals.

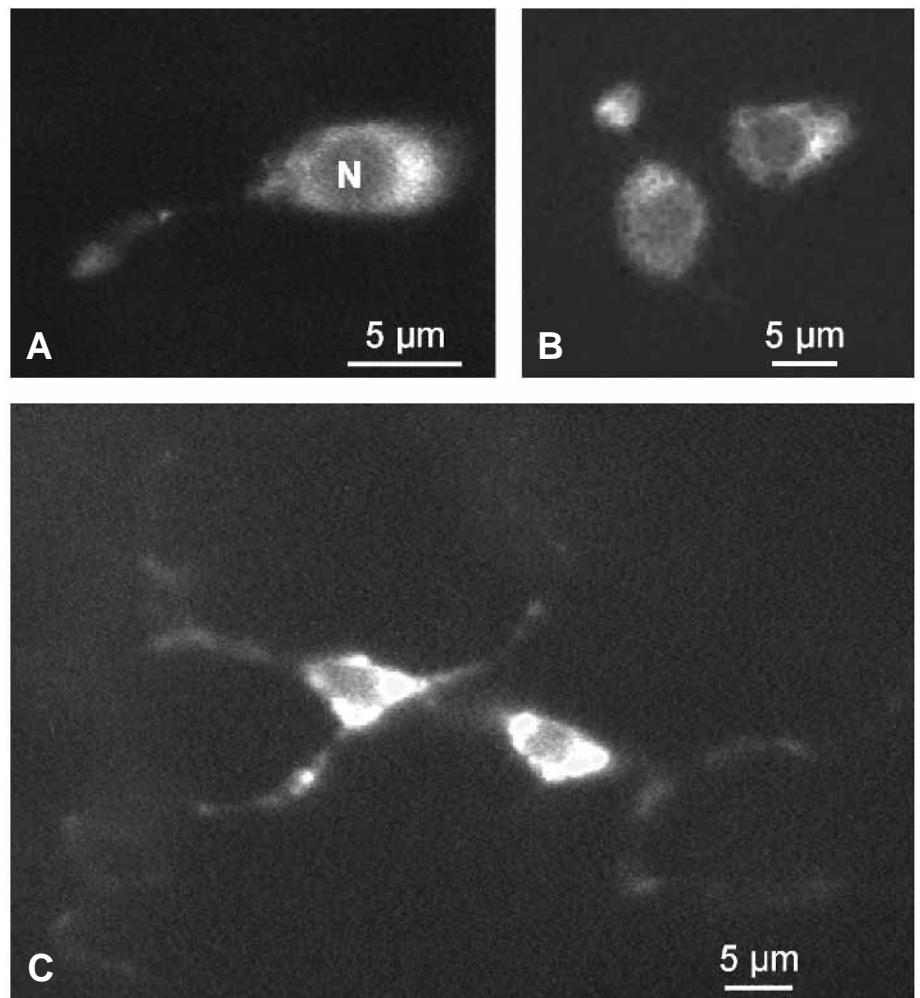


Fig. 4. Micrographs of DiI-labelled cells in the nerve cell lineage. (A) Migrating nerve cell precursor in the body column with their process, (B) pair of nerve precursors after mitosis and (C) pair of newly differentiated nerve cells. N, cell nucleus. Scale bar represents 5 µm.

Table 2. Interstitial cells migrating from DiI-labelled patches in head-activator-treated hydra

	Day 1		Day 2	
	-HA	+HA	-HA	+HA
foot	1.5±1.9	1.8±1.9	1.5±1.9	1.9±2.4
head	4.2±3.5	6.7±4.3*	3.8±3.7	5.6±4.7

Hydra polyps were DiI labelled in the midgastric region and incubated for 24 hours with 10^{-10} M head activator (+HA). Control animals were DiI-labelled and incubated in hydra medium (-HA). The number of interstitial cells that migrated out of the labelled patch toward the head or foot were scored on day 1 and 2 after treatment in 40 animals. The numbers indicate the means \pm standard deviation of migrating cells calculated per animal. Asterisks indicate a statistically significant difference (* $P=0.05$).

DISCUSSION

Commitment and migration of nerve cell precursors

The results of the present experiments enrich our understanding of nerve cell differentiation in hydra. By labelling precursor cells with a vital dye, it has been possible to follow in detail the differentiation process *in vivo*. The results show clearly that individual nerve cell precursors arise from stem cells, complete a final cell cycle and after mitosis both daughter cells differentiate as nerve cells. The involvement of a terminal cell cycle in the differentiation pathway had been suggested previously based on kinetic studies of nerve cell differentiation stimulated by head regeneration and by the inhibitory effects of hydroxyurea on this process (David and Gierer, 1974; Venugopal and David, 1981a,b,c; Yaross et al., 1982). It was not known, however, whether one or both daughter cells give rise to nerve cells.

The second striking result of the present observations is the fact that a subpopulation of nerve cell precursors migrate. Migration can be extensive, carrying a precursor from the midgastric region to the head or foot in a short time. Migration also has a significant effect on the pattern of nerve cell differentiation along the body axis (see below).

Our results show that migration is a characteristic property of nerve cell precursors and not simply a general feature of interstitial cells. Since migrating cells never gave rise to nests of differentiating nematocytes or to clones of interstitial cells (Table 1), nematoblast precursors and stem cells are not migratory in normal animals. This agrees well with previous observations of Bosch and David (1990) showing that clones of genetically marked interstitial cells grow as contiguous patches of cells and never give rise to satellite clones. It should be noted, however, that extensive interstitial cell migration occurs in grafts between normal and interstitial cell-free tissue (Minobe et

al., 1995) or in graft combinations between tissue of different sexes or different strains (David et al., 1991; Nishimija-Fujisawa and Sugiyama, 1995).

From direct observation of the behaviour of migrating nerve cell precursors, it is clear that such cells wait a considerable time, in some cases as much as a day, after migration before undergoing division (Fig. 3). This suggests that migration occurs relatively early in the cell cycle of the precursor cell. To localize this time point more precisely, we determined the nuclear DNA content of migrating interstitial cells by DAPI labelling. The results showed that migrating interstitial cells had G1 (2n) nuclear DNA content (Fig. 5A). Since G1 cells are quite rare in a population of interstitial cells (Fig. 5C), it seems likely that the 'G1' cells were actually in G2 at the time of DAPI labelling and that they divided prior to migration distributing the DAPI label to both daughter cells. Since the DAPI labelling was carried out 3-4 hours prior to measurement, the migrating cells must have been at the beginning of the cell cycle at the time of measurement in agreement with the conclusion reached above.

The localization of the migration phase to the beginning of the terminal cell cycle of nerve cell precursors neatly resolves a controversy about the role of migration in nerve cell differentiation. Venugopal and David (1981c) showed that nerve precursors did not change their position in tissue between the end of S phase and nerve cell differentiation and concluded that nerve cell precursors did not migrate. By comparison, Heimfeld and Bode (1984) demonstrated that migrating interstitial cells could be labelled with [3 H]thymidine and that at

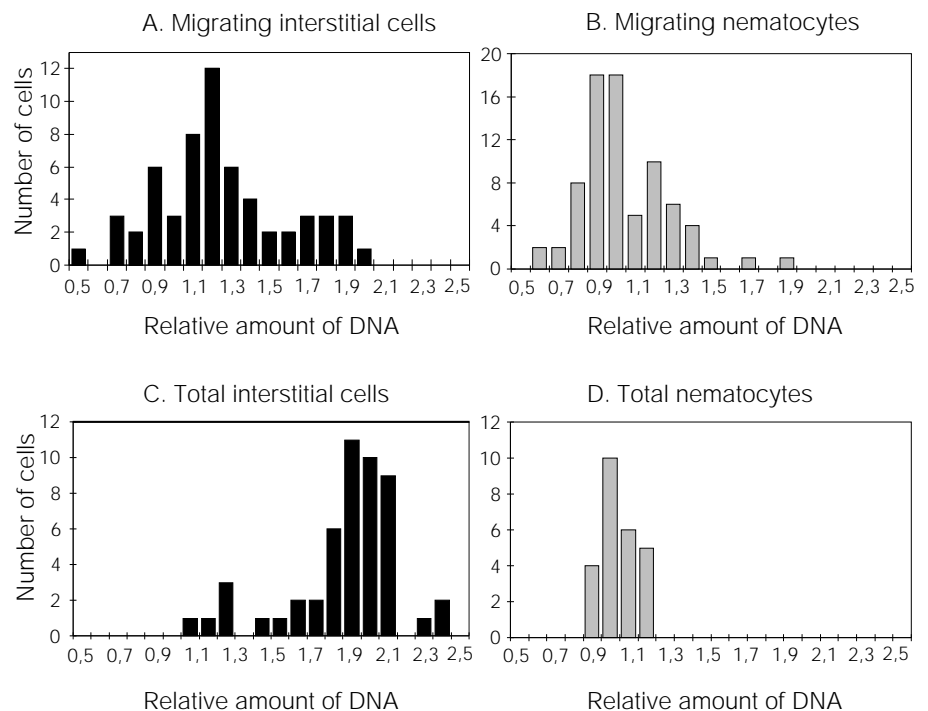


Fig. 5. Nuclear DNA content of migrating interstitial cells (A) and migrating nematocytes (B) determined *in vivo* 3-4 hours after transplantation of DAPI-labelled tissue onto unlabelled host tissue. Nuclear DNA content in total interstitial cells (C) and total nematocytes (D) determined in macerates of tissue from the gastric region. Abscissa shows the DNA content expressed as relative DAPI fluorescence (arbitrary units).

least some of these cells differentiated to nerve cells. This apparent difference can now be simply resolved: nerve cell precursors migrate in hydra but they do so in the S phase at the time when Heimfeld and Bode assayed them and prior to the time at which Venugopal and David found their position to be fixed in tissue.

Formation of migrating nerve precursor cells is stimulated by treatment with ‘head activator’

Little is known about the signals inducing the formation of nerve cell precursors from stem cells. Treating hydra polyps with the neuropeptide ‘head activator’ at pM concentration almost doubles the number of nerve cell precursors throughout the body column (David and Holstein, 1985; Holstein et al., 1986a,b). The kinetics of this increase indicate that head activator affects precursors in early S-phase at the beginning of the final cell cycle. Head activator also stimulates formation of migrating precursors (Table 2). The results in Fig. 5 indicate that migrating cells are in S-phase (see above). Hence, the simplest model of head activator action is that peptide treatment increases the number of precursors and these cells subsequently begin migration. However our experiments do not rule out the alternative hypothesis that head activator stimulates migration directly and thus increases the number of migrating precursors.

Migration of nerve cell precursors and the pattern of nerve cell differentiation

There is a striking pattern of differentiated nerve cells in hydra: nerve cell densities are higher in head and foot tissue (0.5 nerve cells/epithelial cell) compared to tissue of the gastric region (0.1 nerve cells/epithelial cell) (Bode et al., 1973). Previous attempts to explain this pattern of differentiation have involved either increased levels of nerve cell commitment in head and foot tissue (Yaross and Bode, 1978; Venugopal and David, 1981a,b,c) or migration and hence redistribution of precursors from the gastric region toward the extremities (Heimfeld and Bode, 1984).

The results in Fig. 6 indicate that roughly the same number of nerve cell precursors arise in head/upper gastric, midgastric and lower gastric/peduncle tissue. Although the results are not strictly quantitative because we could not control the exact size of the DiI-labelled patch, they show quite clearly that the rate of precursor formation in these three regions is comparable. Thus, the pattern of nerve cell differentiation in hydra can not be explained by differences in the rate of precursor formation along the body axis.

By comparison, the observation of migrating nerve cell precursors made here and independently by Teragawa and Bode

(1990, 1995) and by Technau and Holstein (1966) provide a simple explanation for the observed pattern of nerve cell differentiation in hydra. The results in Fig. 6 indicate that about half of the nerve cell precursors in midgastric tissue migrate toward the head or foot and about half differentiate in situ. By comparison, almost all nerve cell precursors that arise in head and foot tissue differentiate in situ. Adding the migrating precursors to the in situ nerve cell differentiation occurring in head and foot tissue leads to a nearly 3-fold increase in the rate of differentiation in head and foot tissue compared to gastric tissue. The exact difference depends on the relative size of gastric tissue, which is exporting nerve cell precursors, compared to the size of the head and foot tissues, which are importing precursors. Although the data are not sufficiently detailed to allow a quantitative description, the general solution to the problem is clear: the increased rates of differentiation at the extremities are due to import of precursors from the body column.

Recent observations of nerve cell differentiation in the

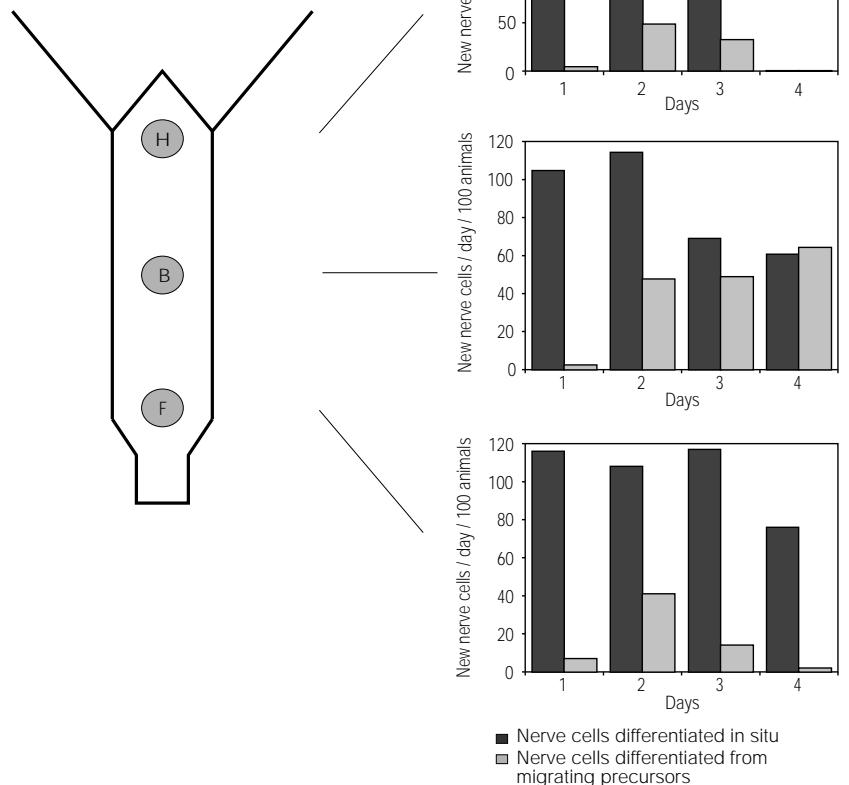


Fig. 6. Pattern of nerve cell differentiation along the body column. Patches of cells at different positions along the body column were labelled with DiI (H, head/upper gastric region, B, body/midgastric region, F, foot/lower gastric region). Individual animals contained a single labelled patch. New nerve cells differentiating in the patch (dark bars) and differentiating outside the patch (grey bars) were scored every day. The ordinate indicates the total number of newly differentiated nerve cells per day per 100 labelled animals.

peduncle and basal disk have provided the most complete description of the role of migration in nerve cell formation (Technau and Holstein, 1996). Using grafts between genetically marked tissue, these authors have shown that essentially all newly differentiated nerve cells in the lower peduncle arise from interstitial cell precursors that migrate from the gastric region. Similar grafting experiments have also shown that the precursors for new nerve cell differentiation in regenerating head tissue and in intact animals are derived from the body column (David et al., 1987; Fujisawa, 1989). Although all these experiments clearly demonstrated migration of nerve cell precursors, the experiments required grafting between marked and unmarked tissue to demonstrate the effects. Since it has been shown that grafting stimulates interstitial cell migration (Fujisawa et al., 1990), it could be argued that the observed migration might not occur in normal animals. This caveat has now been eliminated by the results obtained with the *in vivo* DiI-labelling procedure presented here, which shows that nerve cell precursors also migrate in undisturbed intact animals.

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REFERENCES

- Berking, S.** (1979). Control of nerve cell formation from multipotent stem cells in hydra. *J. Cell Sci.* **40**, 193-205.
- Bode, H. R., Berking, S., David, C. N., Gierer, A., Schaller, H. C. and Trenkner, E.** (1973). Quantitative analysis of cell types during growth and morphogenesis in hydra. *Wilhelm Roux's Arch. Dev. Biol.* **171**, 269-285.
- Bode, H. R., Gee, L. W. and Chow, M. A.** (1990). Neuron differentiation in hydra involves dividing intermediates. *Dev. Biol.* **139**, 231-243.
- Bode, H.** (1992). Continuous conversion of neuron phenotype in hydra. *Trends Genetics* **8**, 279-284.
- Bosch, T. C. and David, C. N.** (1990). Cloned interstitial stem cells grow as contiguous patches in hydra. *Dev. Biol.* **138**, 513-515.
- Campbell, R. D.** (1967). Tissue dynamics of steady state growth in hydra littoralis III. Behaviour of specific cell types during tissue movements. *J. Exp. Zool.* **164**, 379-392.
- David, C. N.** (1973). A quantitative method for maceration of hydra tissue. *Roux Arch. Dev. Biol.* **171**, 259-268.
- David, C. N. and Gierer, A.** (1974). Cell cycle kinetics and development of Hydra attenuata III. Nerve and nematocyte differentiation. *J. Cell Sci.* **16**, 359-375.
- David, C. N. and Murphy, S.** (1977). Characterization of interstitial stem cells in hydra by cloning. *Dev. Biol.* **58**, 372-383.
- David, C. N. and Plotnick, I.** (1980). Distribution of interstitial stem cells in hydra. *Dev. Biol.* **76**, 175-184.
- David, C. N. and Holstein, T.** (1985). The head activator, a neuropeptide, controls nerve cell differentiation in hydra. *Arch. Sci. Geneve* **38**, 461-471.
- David, C. N., Bosch, T. C. G., Hobmayer, B., Holstein, T. and Schmidt, T.** (1987). Interstitial stem cells in hydra. In *Genetic Regulation of Development* (ed. Loomis, W. F.) p. 389-408. New York: A. R. Liss.
- David, C. N., Fujisawa, T. and Bosch, T. C.** (1991). Interstitial stem cell proliferation in hydra: evidence for strain-specific regulatory signals. *Dev. Biol.* **148**, 501-507.
- David, C. N. and Hager, G.** (1994). Formation of a primitive nervous system: nerve cell differentiation in the polyp hydra. *Perspectives on Developmental Neurobiology* **2**, 135-140.
- Davis, L. E., Burnett, A. L. and Haynes, J. F.** (1968). Histological and ultrastructural study of the muscular and nervous systems in hydra. II. Nervous system. *J. Exp. Zool.* **167**, 295-331.
- Epp, L. and Tardent, P.** (1978). The distribution of nerve cells in Hydra attenuata Pall. *Wilhelm Roux's Arch. Dev. Biol.* **185**, 185-193.
- Fujisawa, T.** (1989). Role of interstitial cell migration in generating position-dependent patterns of nerve cell differentiation in hydra. *Dev. Biol.* **133**, 77-82.
- Fujisawa, T., David, C. N. and Bosch, T. C.** (1990). Transplantation stimulates interstitial cell migration in hydra. *Dev. Biol.* **138**, 509-512.
- Grimmelikhuijzen, C. J., Dockray, G. J. and Schot, L. P.** (1982). FMRFamide-like immunoreactivity in the nervous system of hydra. *Histochemistry* **73**, 499-508.
- Hadzi, H.** (1909). Über das Nervensystem von Hydra. *Arb. Zool. Inst. Univ. Wien* **17**, 225-268.
- Heimfeld, S. and Bode, H. R.** (1984). Interstitial cell migration in Hydra attenuata. II. Selective migration of nerve cell precursors as the basis for position-dependent nerve cell differentiation. *Dev. Biol.* **105**, 10-17.
- Hobmayer, B., Holstein, T. W., and David, C. N.** (1990). Tentacle morphogenesis in hydra II. Formation of a complex between a sensory nerve cell and a battery cell. *Development* **109**, 897-904.
- Holstein, T. W., Schaller, H. C., and David, C. N.** (1986a). Nerve cell differentiation in hydra requires two signals. *Dev. Biol.* **115**, 9-17.
- Holstein, T. and David, C. N.** (1986b). The properties of nerve cell precursors in hydra. *Dev. Biol.* **115**, 18-26.
- Honig, M. G. and Hume, R. I.** (1986). Fluorescent carbocyanine dyes allow living neurons of identified origin to be studied in long-term cultures. *J. Cell Biol.* **103**, 171-187.
- Koizumi, O. and Bode, H. R.** (1986). Plasticity in the nervous system of adult hydra. I. The position-dependent expression of FMRFamide-like immunoreactivity. *Dev. Biol.* **116**, 407-421.
- Minobe, S., Koizumi, O. and Sugiyama, T.** (1995). Nerve cell differentiation in epithelial hydra from precursor cells introduced by grafting. I. Tentacles and hypostome. *Dev. Biol.* (submitted).
- Nishimiya-Fujisawa, C. and Sugiyama, T.** (1995). Genetic analysis of developmental mechanisms in hydra. XXII. Two types of female germ stem cells are present in a male strain of Hydra magnipapillata. *Dev. Biol.* (in press).
- Schaller, H. C.** (1976). Action of the head activator on the determination of interstitial cells in hydra. *Cell Differ.* **5**, 13-25.
- Schaller, H. C. and Bodenmüller, H.** (1981). Isolation and amino acid sequence of a morphogenetic peptide from hydra. *Proc. Natl. Acad. Sci. USA* **78**, 7000-7004.
- Tardent, P. and Weber, C.** (1976). A qualitative and quantitative inventory of nervous cells in Hydra attenuata Pall. In *Coelenterate Ecology and Behaviour* (ed. G.O. Mackie), pp. 501-512. New York: Plenum Publishing Corp.
- Technau, U. and Holstein, T. W.** (1996). Phenotypic maturation of neurons and continuous precursor migration in the formation of the peduncle nerve net in hydra. *Dev. Biol.* **177**, 599-618.
- Teragawa, C. K. and Bode, H. R.** (1990). Spatial and temporal patterns of interstitial cell migration in Hydra vulgaris. *Dev. Biol.* **138**, 63-81.
- Teragawa, C. K. and Bode, H. R.** (1995). Migrating interstitial cells differentiate into neurons in hydra. *Dev. Biol.* **171**, 286-293.
- Venugopal, G. and David, C. N.** (1981a). Nerve commitment in hydra. I. Role of morphogenetic signals. *Dev. Biol.* **83**, 353-360.
- Venugopal, G. and David, C. N.** (1981b). Nerve commitment in hydra. II. Localization of commitment in S phase. *Dev. Biol.* **83**, 361-365.
- Venugopal, G. and David, C. N.** (1981c). Spatial pattern of nerve differentiation in hydra is due to a pattern of nerve commitment. *Dev. Biol.* **83**, 366-369.
- Yaross, M. S. and Bode, H. R.** (1978). Regulation of interstitial cell differentiation in Hydra attenuata. IV. Nerve cell commitment in head regeneration is position-dependent. *J. Cell Sci.* **34**, 27-38.
- Yaross, M. S., Baca, B. A., Chow, M. H. and Bode, H. R.** (1982). Commitment of hydra interstitial cells to nerve cell differentiation occurs by late S-phase. *Dev. Biol.* **89**, 425-436.
- Yaross, M. S., Westerfield, J., Javois, L. C. and Bode, H. R.** (1986). Nerve cells in hydra: monoclonal antibodies identify two lineages with distinct mechanisms for their incorporation into head tissue. *Dev. Biol.* **114**, 225-237.