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Tentacle morphogenesis in hydra

II. Formation of a complex between a sensory nerve cell and a battery cell

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Summary

Differentiation of sensory nerve cells in tentacles of *Hydra magnipapillata* was investigated using the monoclonal antibody NV1. $NV1^+$ sensory nerve cells form specific complexes with battery cells in tentacles. $NV1^+$ cells can only be formed by differentiation from interstitial cell precursors. These precursors complete a terminal cell cycle in the distal gastric region at the base of tentacles; differentiation from the S/G_2 boundary to expression of the NV1 antigen requires 30 h. During this time, precursors move from the distal gastric region into the tentacles, differentiate to morphologically fully formed nerve cells and then begin expressing NV1 antigen. The neuropeptide head activator stimulates $NV1^+$ differentiation in S-phase of the precursor's cell cycle.

Key words: hydra, nerve cell differentiation, battery cell.

Introduction

In the accompanying paper, we characterized a monoclonal antibody, NV1, which identifies nerve cells embedded within battery cells in the tentacles of *Hydra magnipapillata* (Hobmayer *et al.* 1990). Similar nerve cells have been identified in *Hydra oligactis* using another monoclonal antibody, JD1 (Dunne *et al.* 1985). Based on cell morphology both $NV1^+$ and $JD1^+$ cells appear to be sensory: they have a sensory cilium extending apically toward the surface of the battery cell and several processes that extend laterally from the base of the cell to make contact with 3–5 neighboring battery cells (Yu *et al.* 1985). Consistent with their close morphological association with battery cells, differentiation of $NV1^+$ cells is also tightly coupled to formation of battery cells (Hobmayer *et al.* 1990).

In addition to this close association with battery cells, two other aspects of $NV1^+$ and $JD1^+$ cells in tentacles set them apart from other nerve cells in hydra. First, these cells do not exhibit 'phenotypic plasticity', i.e. they cannot arise by redifferentiation from preexisting nerve cells. Instead, they can only be formed by new differentiation from interstitial cell precursors. Yaross *et al.* (1986) have shown this to be true for $JD1^+$ cells and the experiments reported here show this also to be true for $NV1^+$ cells.

Second, differentiation of $NV1^+$ cells could not be stimulated in head tissue by head activator treatment although differentiation of other nerve cell types could be stimulated (Hobmayer *et al.* 1990). This result suggested that all potential sites for $NV1^+$ differentiation in head tissue were occupied and led to the idea that NV1 precursors and battery cell precursors form a 'pretentacle' complex. Formation of this complex occurs in the distal gastric region and the complex is then transported into tentacles by tissue movement (Campbell, 1967).

In the present report, we have investigated the requirements for formation of this 'pretentacle' complex. We show that $NV1^+$ cells in tentacles arise only by differentiation from interstitial cell precursors. We also show that NV1 precursors are normally incorporated into differentiating battery cells and not into mature battery cells. Finally, we have examined the differentiation kinetics of $NV1^+$ cells and localized their formation to tissue in the distal gastric region adjacent to the base of tentacles.

Materials and methods

Experimental animals and culture conditions as well as methods for maceration of hydra tissue, production of monoclonal antibodies, determination of cell numbers and treatment with head activator are described in the accompanying report (Hobmayer *et al.* 1990).

Double staining with nerve cell- and nematocyte-specific antibodies

Animals were anesthetized in 2 % urethane in hydra medium for 1 min. The relaxed hydra were fixed with Lavdowsky's fixative (ethanol: formalin: acetic acid: water – 50: 10: 4: 40) for more than 12 h and then washed in PBS/BSA 0.1 %/azide 0.02 % for 30 min. After transferring them into wells of a 96-well microtitre plate (Costar), animals were incubated in 50 μl of the nerve cell-specific monoclonal antibody NV1 for 12 h

and then for 6 h in 50 μl of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin IgG/IgM (TAGO, California) diluted 1:20 in PBS/BSA/azide. After washing briefly in PBS/BSA/azide, animals were incubated in unconjugated goat anti-mouse immunoglobulin (TAGO, California) diluted 1:20 in PBS/BSA/azide to saturate free NV1-binding sites. They were then washed again briefly and incubated in 50 μl of a nematocyte-specific monoclonal antibody, H22, for 2 h. Binding of this antibody was visualized using tetramethylrhodamine-B-isothiocyanate (TRITC)-conjugated goat anti-mouse immunoglobulin IgG (Sigma Chemical Co.) diluted 1:50 in PBS/BSA/azide for 2 h. Staining was terminated by washing in PBS for 30 min. All steps were done at room temperature.

Whole mounts and macerated cells were observed with a Leitz Dialux 20 microscope equipped with epifluorescence attachment and filterblocks I2 (excitation wavelength 450–490 nm, barrier filter 515 nm) and N2 (excitation wavelength 530–560 nm, barrier filter 580 nm). $NV1^+$ nerve cells and $H22^+$ nematocytes in double-stained whole mounts could easily be observed by switching from filterblock I2 to N2.

Elimination of interstitial cells by nitrogen mustard-treatment

Selective elimination of interstitial cells was achieved by treating hydra with nitrogen mustard (2.2 dichloro-*N*-methyldiethylamin–HCl; Sigma Chemical Co.) according to the procedure of Diehl and Burnett (1964). 50–100 experimental animals were stirred carefully in 100 ml of a 0.01 % solution of nitrogen mustard in hydra medium for 10 min. Then they were washed five times in hydra medium and cultured further without feeding. Interstitial cells disappeared within 6–8 days after treatment at which time animals had ≤0.001 interstitial cells per epithelial cell.

Carbon marking of epithelial cells

Epithelial cells were marked with carbon particles by injecting India ink (Faber-Castell Corp.) into the interstitial spaces of the ectoderm with a fine glass needle (Campbell, 1973). The ink particles are phagocytized by ectodermal epithelial cells and incorporated into an intracellular vacuole, where they stably mark the cell for periods of several days.

Tissue grafting

Axial grafting of heads onto body column pieces was done by bisecting hydra directly below the tentacle ring (see also Fig. 2) and threading head and body column pieces with correct alignment onto nylon fishing line (diameter 0.20 mm; D.A.M.). Pieces were gently pressed together by sleeves of PE10 polyethylene tubing (INTRAMEDIC, Clay Adams). Grafts were left undisturbed for 2 h to allow them to heal, removed from the fishing line and cultured further without feeding.

Results

Immunocytochemical characterization of the NV1–battery cell complex

Battery cells in tentacles of hydra constitute a functional complex between an ectodermal epithelial cell, 10–20 nematocytes and a sensory nerve cell (Hufnagel *et al.* 1985; Campbell, 1987). A detailed description of the morphology of this sensory nerve cell has been given by Westfall and Kinnamon (1978). Its exact localization, however, has only recently been revealed as a result of staining with highly specific monoclonal antibodies. In *H. magnipapillata*, these nerve cells are recognized by the monoclonal antibody NV1 (Hobmayer *et al.* 1990); in *H. oligactis*, they are recognized by the monoclonal antibody JD1 (Yu *et al.* 1985).

$NV1^+$ sensory nerve cells are completely embedded within battery cells. They have an apical cilium, which extends to the surface of the surrounding battery cell, and two or more processes, which extend laterally from the basal part of the cell. In whole-mount preparations, these processes can be seen to innervate nematocysts in several neighboring battery cells (Fig. 1A,B). Processes from individual $NV1^+$ cells do not make contact with each other. The precise relationship between a $NV1^+$ cell and the surrounding battery cell is best observed in macerations (Fig. 1C,D). In good preparations, it is possible to see the processes of the $NV1^+$ cell extending laterally along the base of the battery cell adjacent to the mesoglea. Short side branches extend from these main processes to innervate individual nematocytes both in the host battery cell and in neighboring battery cells. Fig. 1E outlines schematically the location of the different cells that combine to form a battery cell.

Formation of $NV1^+$ nerve cells requires interstitial cell differentiation

Tentacle tissue is continuously formed at the base of tentacles and lost at the tentacle tips (Campbell, 1967; Dübel *et al.* 1987). During this process the entire cellular constituents of a tentacle – epithelial cells, nerve cells and nematocytes – are replaced.

Nerve cells in tentacles are derived from two sources: new nerve cell differentiation associated with tentacle formation and transfer of preexisting nerve cells from the body column into tentacles by tissue movement. These two sources can be distinguished by analyzing the nerve cell populations in tentacles in interstitial cell-depleted animals (Yaross *et al.* 1986). In such animals, differentiation of new nerve cells is excluded. Thus, nerve cells that appear in the regenerating head of interstitial cell-depleted animals arise from preexisting nerve cells of the body column; nerve cells that fail to form under these conditions require differentiation of interstitial cell precursors.

Interstitial cell-free animals were prepared by incubation of polyps in nitrogen mustard as described in Materials and methods. Then the treated animals were decapitated directly below the ring of tentacles and allowed to regenerate. All preexisting tentacle-specific $NV1^+$ nerve cells were eliminated by decapitation. When head regeneration was complete six days after head removal, the polyps were fixed and assayed for the presence of nerve cells in the tentacles.

As shown in Table 1, no $NV1^+$ nerve cells appeared in the tentacles of nitrogen-mustard (NM)-treated regenerates. By comparison, untreated control regenerates had about 240 $NV1^+$ cells per tentacle set. Thus, tentacle-specific $NV1^+$ nerve cells can only arise by differentiation from interstitial precursors.

Table 1 also shows that nerve cells do indeed appear

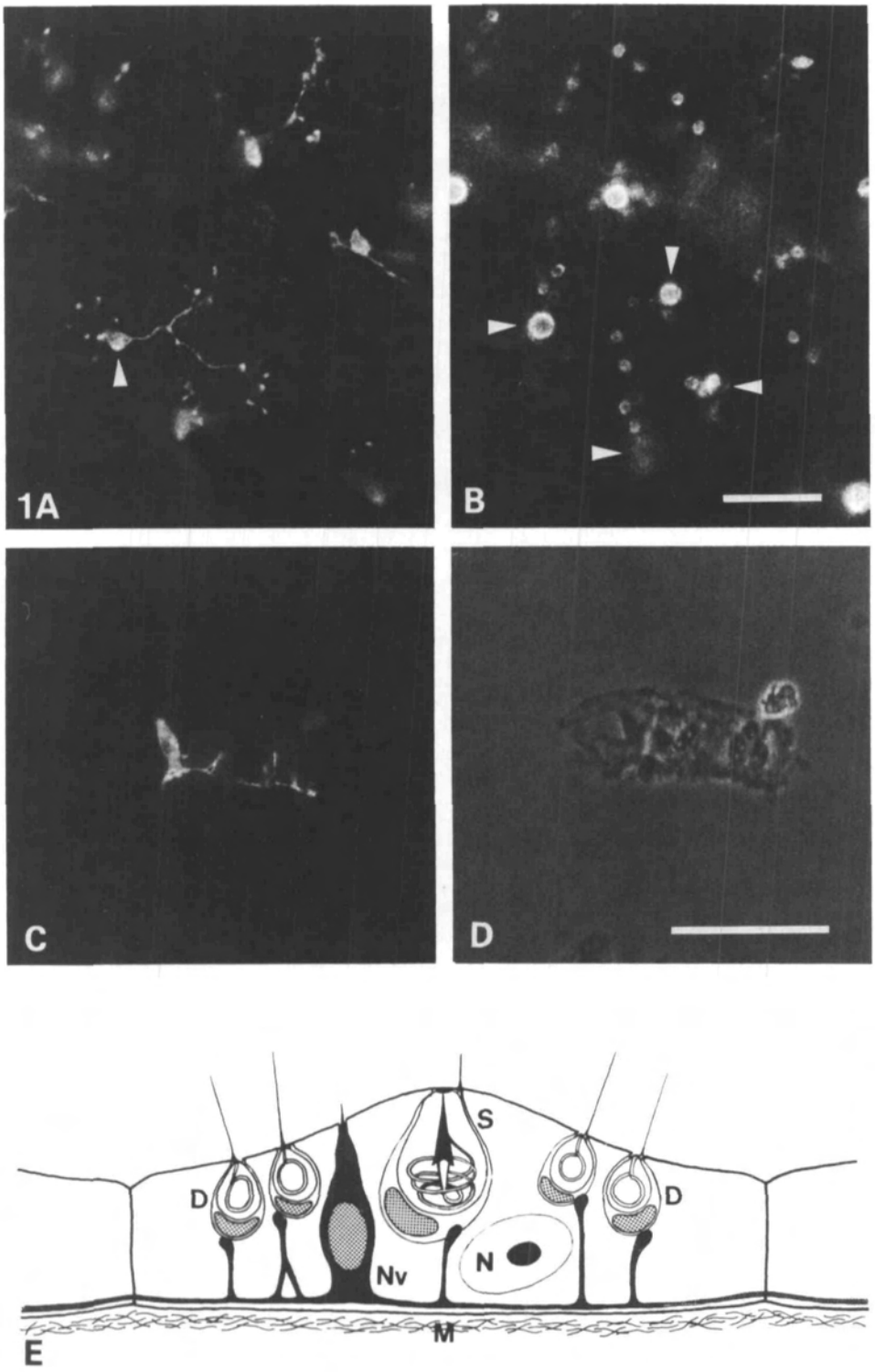

Fig. 1. $NV1^+$ nerve cells in tentacle tissue of *Hydra magnipapillata* visualized by indirect immunofluorescence. (A,B) Double staining of whole mounts with the monoclonal antibodies NV1 and H22 (nematocyte-specific) shows innervation of nematocysts of several neighboring battery cells by one sensory $NV1^+$ cell. Arrows indicate the $NV1^+$ nerve cell body (A) and the stenoteles of 4 battery cells (B). (C,D) Sideview of a $NV1^+$ nerve cell and the surrounding battery cell (phase-contrast optics) in NV1-stained maceration preparation. (E) Schematic representation showing the location of the different cell types in a tentacle-specific battery cell; Nv, $NV1^+$ nerve cell; N, battery cell nucleus; S, stenotele; D, desmonemes; M, mesoglea. Bars: 25 μm.

in regenerated tentacles of nitrogen-mustard-treated animals. Some of these cells could be identified with the monoclonal antibody NV4, which recognizes 60 % of all nerve cells in hydra (Hobmayer *et al.* 1990). Table 1 shows that about 40 $NV4^+$ cells appeared in the tentacles of interstitial cell-free regenerates. A similar number of $NV4^+$ cells was present in the distal fifth of the gastric region – from which head tissue formed during regeneration – at the time of head removal (Table 1). The simplest explanation for these observations is that preexisting $NV4^+$ cells in the distal gastric region were displaced into the regenerated tentacles. These cells did not acquire the NV1 antigen when they moved into tentacles, confirming that $NV1^+$ cells can not be formed by redifferentiation from preexisting nerve cells.

Table 1. *Number of nerve cells in interstitial cell-free head regenerates*

Time after head removal	Treatment	NV1$^+$	NV4$^+$
0 days*	NM-treated	0	52
6 days†	NM-treated	0±0.5	43±11
	Control	237±63	261±45

* Results are average of 20 macerated explants of the distal 1/5 gastric region.
† Mean (±S.D.) of three independent experiments; 5–7 regenerates were analyzed in each experiment.

NV1$^+$ nerve cells differentiate in association with newly formed battery cells

To investigate whether differentiation of NV1$^+$ nerve cells requires coincident differentiation of new battery cells or whether NV1 precursors can differentiate in association with mature battery cells in tentacles, we grafted NV1-free heads onto body columns of normal animals and followed the appearance of NV1$^+$ cells in the grafted heads (Fig. 2). Prior to transplantation, the donor animals were injected with India ink at the site of transplantation to mark the ectodermal epithelial cells and thus permit tracking of epithelial cell movement from the body column into tentacles following transplantation (Campbell, 1973). In some animals, the tentacles were excised following transplantation; the difference in tentacle length between such animals and intact animals defines the 'old' tentacle tissue, present at the time of transplantation. 4 days after transplantation, animals were fixed, stained with NV1 antibody and analyzed for NV1$^+$ cells in tentacles.

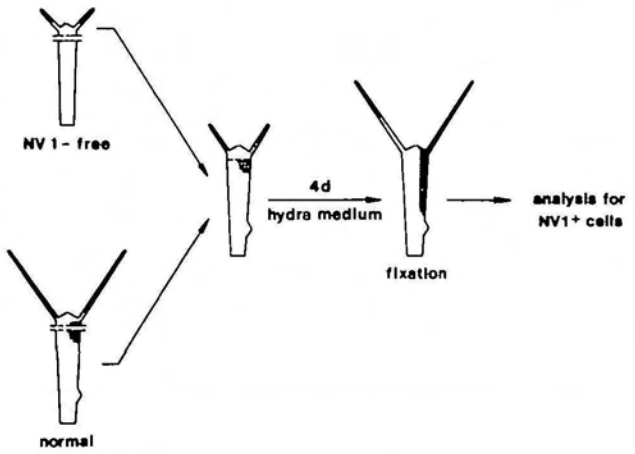

Fig. 2. Axial grafting procedure to repopulate NV1-free heads with NV1$^+$ nerve cells. NV1-free heads were transplanted onto body columns of normal animals. Prior to transplantation, the normal animals were injected with India Ink (stippled area) to mark ectodermal epithelial cells. 4 days after transplantation, the positions of newly differentiated NV1$^+$ nerve cells and ink-marked epithelial cells were analyzed. NV1-free heads were prepared by inducing head regeneration in nitrogen-mustard-treated hydra (Table 1).

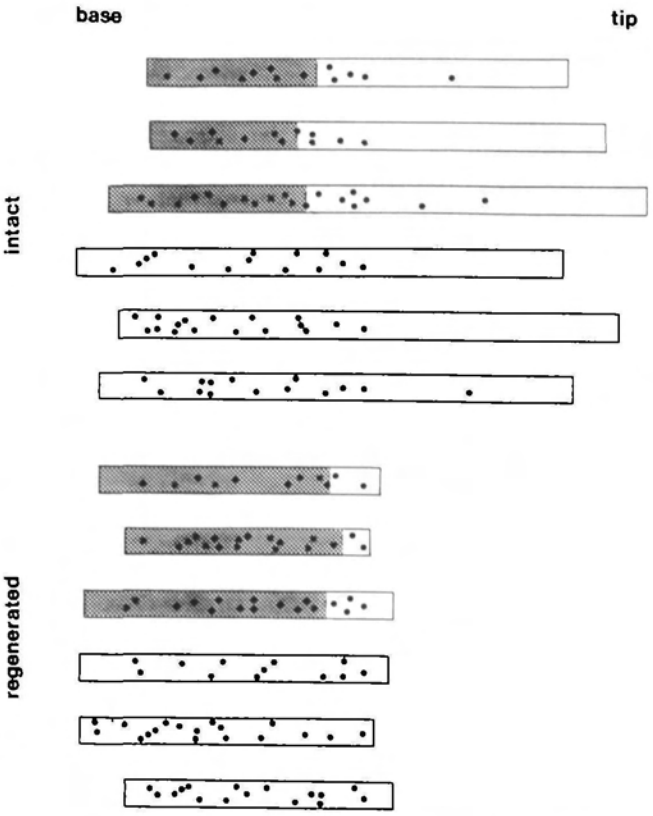

Fig. 3. Localization of newly differentiated NV1$^+$ nerve cells in the tentacles of grafted NV1-free heads. The experimental protocol is shown in Fig. 2. Individual tentacles from representative animals were traced in *camera lucida* and are presented schematically as block diagrams scaled to the length of each tentacle. NV1$^+$ nerve cells are represented by dots. The tentacles are positioned such that the distal boundaries of the NV1$^+$ regions are vertically aligned. The shaded areas indicate carbon-marked tissue. Top: 6 tentacles from intact animals; bottom: 6 regenerated tentacles.

Fig. 3 shows schematically the position of NV1$^+$ nerve cells, based on *camera lucida* sketches of individual tentacles from representative animals on day 4. Regenerated tentacles were uniformly filled with NV1$^+$ cells, while tentacles from intact animals exhibited a proximal region filled with NV1$^+$ cells and a distal region nearly free of NV1$^+$ cells. The tentacles in Fig. 3 are lined up such that the distal boundaries of the NV1$^+$ regions are above each other. The size of the NV1$^+$ regions in both regenerated and intact tentacles was roughly similar, indicating that the rate of NV1$^+$ differentiation is essentially the same in both tissues. The larger size of the intact tentacles is due primarily to the distal NV1-free region. This region consists of mature tentacle tissue present at the time of grafting. The fact that it is nearly free of NV1$^+$ cells indicates that these cells normally do not differentiate in association with mature battery cells.

The absence of NV1$^+$ cells in mature tentacle tissue could be due to the failure of NV1 precursors to migrate into the NV1-free head following grafting. This was clearly not the case. Fig. 3 shows that in all 6 carbon-

marked tentacles $NV1^+$ cells differentiated distal to the carbon boundaries. Thus, NV1 precursors must have migrated from the normal donor tissue into the NV1-free head. The position of these $NV1^+$ nerve cells in close proximity to cells from the graft boundary suggests that they differentiated together with battery cell precursors near the base of the grafted head and not with mature battery cells in the tentacles.

Kinetics of $NV1^+$ differentiation during head and tentacle regeneration

To localize the precursors to $NV1^+$ nerve cells, we determined the kinetics of $NV1^+$ differentiation following removal of the head (cut just below the tentacle ring) and removal of the tentacles (cut just above the tentacle base). The results in Fig. 4 show that $NV1^+$ cells begin to reappear 20 h after tentacle removal and 42 h after head removal. Assuming that tentacle formation is not markedly delayed as a result of cutting, we conclude that NV1 precursors at the body column–tentacle boundary require 20 h to move into tentacles and differentiate while those at the gastric region–head boundary require 42 h to complete this process (see also Fig. 7).

Determination of the S/G_2 boundary of NV1 precursor cells

To determine the cell cycle position of NV1 precursors in the distal gastric region, we used the 'hydroxyurea-escape' technique of Fujisawa and David (1982). Heads were excised from a set of experimental animals at time 0 and at various times thereafter groups of regenerating animals were placed in 10 mM hydroxyurea and incubated further. After 4 days, all experimental animals were fixed and scored for $NV1^+$ cells.

Hydroxyurea blocks cells in S-phase; nerve cell precursors that have proceeded past the S/G_2 boundary at the time of hydroxyurea addition complete differentiation (Holstein and David, 1986). Fig. 5 shows that hydroxyurea completely inhibited $NV1^+$ differentiation during the first 15 h after head removal. Later addition of hydroxyurea allowed progressively more NV1 precursors to differentiate. In other words, more NV1 precursors progressed from S into G_2 and became hydroxyurea-resistant. The increase in hydroxyurea-resistant precursors, describing their S/G_2 boundary, parallels the increase in $NV1^+$ cells during normal head regeneration (dotted line) but precedes it by about 30 h. Thus, the S/G_2 boundary of NV1 precursor cells lies 30 h prior to expression of the NV1 antigen.

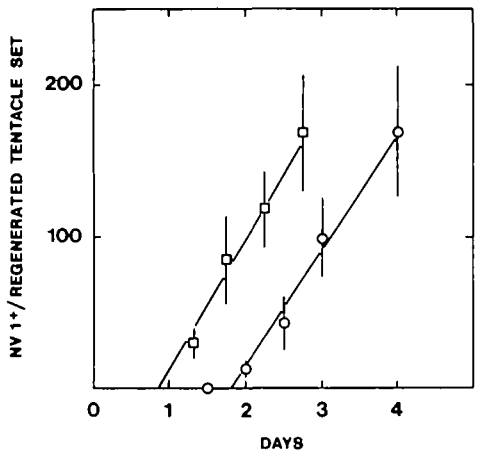

Fig. 4. Appearance of $NV1^+$ nerve cells during tentacle (□) and head (○) regeneration. Tentacles or whole heads were removed at t_0. $NV1^+$ cells were scored in whole-mount preparations at different times during regeneration. Each point represents mean (±one standard deviation) of 10 animals.

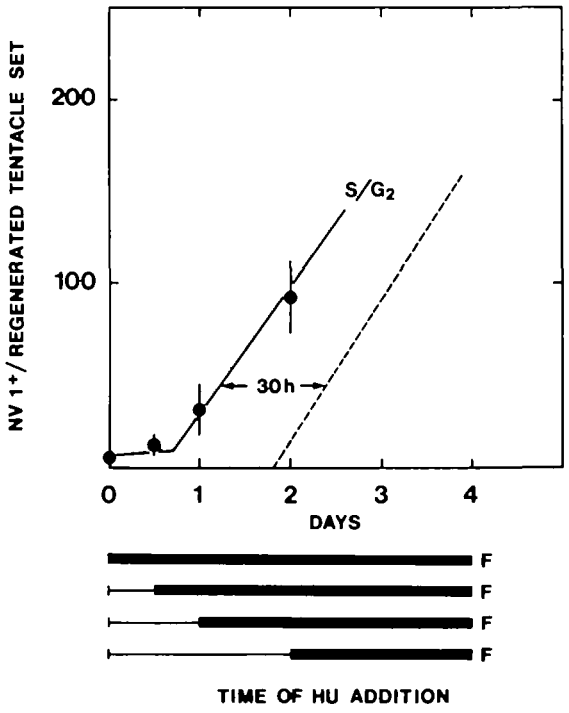

Fig. 5. Localization of the S/G_2 boundary of NV1-precursor cells by the 'hydroxyurea-escape' procedure (see text). Head regeneration was induced at t_0. At various times during regeneration, 0.01 M hydroxyurea (HU) was added to the regenerates (black bars). 4 days after head removal all regenerates were fixed and scored for $NV1^+$ cells. The results are plotted at the time of hydroxyurea addition. The dotted line shows the kinetics of $NV1^+$ differentiation during normal head regeneration (from Fig. 4). Each point represents mean (±one standard deviation) of 10 animals.

Localization of head-activator-sensitive NV1 precursor cells

To determine when NV1 precursors become sensitive to the neuropeptide head activator, we removed tentacles at their base and incubated animals in 1 pM head activator for 18 h. Thereafter the animals were incubated in hydra medium and scored for the appearance of $NV1^+$ cells in tentacles.

The results in Fig. 6 show that the first $NV1^+$ cells appeared roughly 20 h after removal of tentacles in both head-activator-treated and untreated control regenerates. However, beginning about 35 h after the onset of

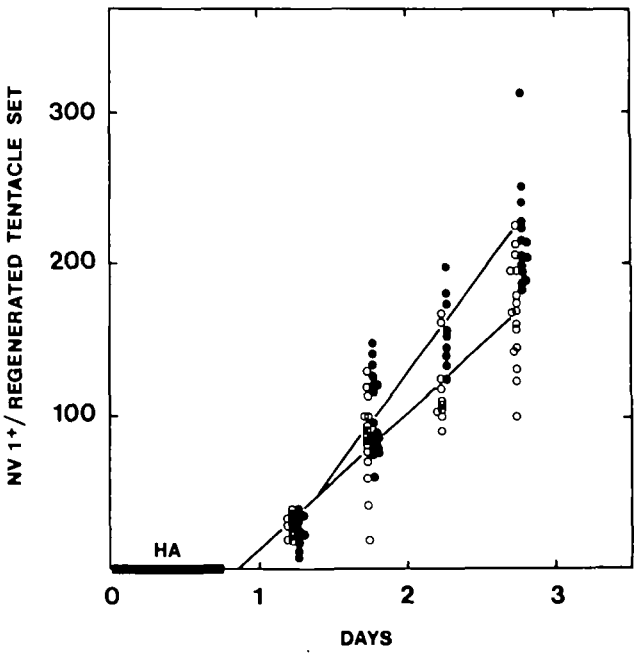

Fig. 6. Kinetics of $NV1^+$ differentiation in head activator (HA)-treated (●) and untreated (○) regenerates. Tentacles were removed at t_0. Then experimental animals were incubated in 1 pM head activator or hydra medium for 18 h, injured with three transverse cuts to stimulate nerve cell differentiation (Holstein *et al.* 1986) and transferred to hydra medium to continue tentacle regeneration. $NV1^+$ cells were analyzed in whole mounts. Each point represents a single polyp. Lines are derived by linear regression analysis; lines are significantly different at a 95 % confidence limit (*t*-test analysis of covariance; Weber, 1964).

head activator treatment, more $NV1^+$ cells appeared in head-activator-treated animals than in untreated control animals. Thus, precursors that respond to head activator require 35 h to complete differentiation and express the NV1 antigen. These precursors are in S-phase since the S/G_2 boundary of precursors lies 30 h prior to NV1 antigen formation (Fig. 5).

Discussion

Origin of nerve cells in tentacle tissue

Yaross *et al.* (1986) showed that nerve cells in the head of hydra are derived from two sources: (1) mature nerve cells embedded in body column tissue, which are passively carried into newly formed head tissue, and (2) new nerve cells, which differentiate during head formation. Our results on head regeneration in interstitial cell-depleted animals confirm this conclusion (Table 1). Because we counted the number of nerve cells directly in such animals, we could estimate quantitatively the relative contribution of the two sources. The results in Table 1 indicate that tentacle formation is accompanied by massive nerve cell differentiation; about 85 % of $NV4^+$ cells in the tentacles are derived from new differentiation while only 15 % are derived from preexisting nerve cells of the body column.

Plasticity of the nerve cell phenotype in hydra

A variety of experiments indicate that nerve cell phenotype in hydra is not rigidly fixed but can vary as nerve cells are transferred by tissue movement from one region of the animal to another. Koizumi and Bode (1986) showed that FMRFamide-negative cells of the gastric region express FMRFamide as they move into head tissue. Furthermore, Koizumi *et al.* (1988) have shown that mature ganglion cells in the body column can transform into sensory cells of the hypostome during head regeneration.

In view of these dramatic examples of 'phenotypic plasticity' in hydra nerve cells, it was surprising to discover that $JD1^+$ nerve cells (Yaross *et al.* 1986) and $NV1^+$ cells (Table 1) can only be formed by new differentiation from interstitial cell precursors. Based on the results here, it appears that the requirement for new differentiation is associated with formation of a complex between NV1 precursors and battery cell precursors. This differentiation complex apparently cannot be formed from already differentiated parts: mature nerve cells from the gastric region cannot form complexes with differentiating battery cells (Table 1) nor can nerve cell precursors form complexes with mature battery cells (Fig. 3).

Formation of a NV1–battery cell complex

By comparing the results of several experiments in the present report, it is possible to outline the formation of the NV1–battery cell complex as shown schematically in Fig. 7. It takes 20 h to reform $NV1^+$ cells when tentacles are excised at their base (Fig. 4). This is the maximal time required for NV1 precursors to move from the tentacle base out to a position at 1/10 tentacle length where the NV1 antigen first appears. The results in Fig. 5 indicate that the end of the S-phase of NV1 precursors (S/G_2 boundary) lies roughly 30 h before appearance of the NV1 antigen. Thus, precursors in

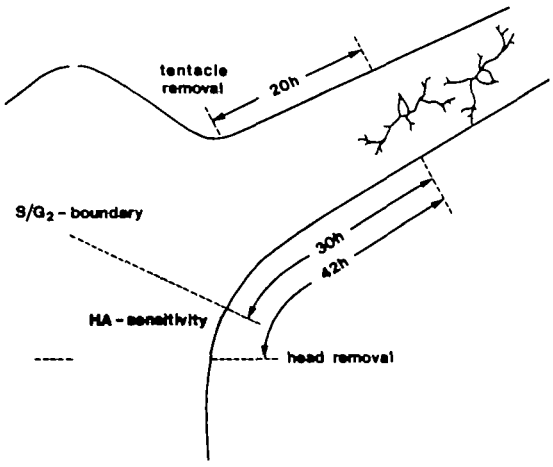

Fig. 7. Temporal sequence of events during development of a battery-cell-specific $NV1^+$ nerve cell. Times are derived from Fig. 4 and Fig. 5.

S-phase are expected to lie within the body column below the tentacle base.

Previous work (Holstein and David, 1986) has shown that nerve cell precursors are sensitive to head activator in their S-phase. Hence, we would locate the head-activator-sensitive precursors at a position proximal to the S/G_2 boundary. The results in Fig. 6 appear to confirm this scheme: beginning 30–35 h after the onset of head activator treatment, more $NV1^+$ cells appeared in treated than in untreated tissue indicating that these precursors were sensitive to head activator. These cells were presumably derived from below the S/G_2 boundary which lies roughly 30 h prior to NV1 antigen formation. By comparison, the first $NV1^+$ cells to appear during tentacle regeneration are derived from precursors above the S/G_2 boundary; these do not respond to head activator treatment, i.e. the same number appear in treated and untreated tissue.

Cell–cell interactions in the development of sensory nerve cells

Results reported here and in the previous paper (Hobmayer *et al.* 1990) indicate that differentiation of $NV1^+$ sensory nerve cells is dependent on coincident differentiation of surrounding battery cells. The structure of the NV1–battery cell complex is similar to sensory organs in nematodes, insects and vertebrates where sensory neurons also occur in multicellular complexes with surrounding support cells (Hüllzellen; McLaren, 1976; Thurm, 1965; Steinbrecht, 1969). In some cases, differentiation of such sensory complexes, e.g. external sense organs (bristles) and internal sense organs (chordotonal receptors) in insects, occurs from a sensory mother cell *via* a series of asymmetric divisions (for review see Ghysen and Dambly-Chaudiere, 1989). In other cases, e.g. hypodermal chemoreceptors in the nematode *C. elegans*, sensory organs are formed by interaction between cells of different lineages as is also the case for the NV1–battery cell complex.

One of the best understood examples of cell–cell interactions in sensory cell differentiation occurs during the formation of ommatidia in insect eyes (for review see Ready, 1989). The ommatidia consist of modified sensory neurons (the photoreceptors) and a set of support cells (cone cells, pigment cells). The set of cells constituting an ommatidium are not clonally related. Rather they arise by inductive cell–cell interactions starting with a founder cell (Tomlinson and Ready, 1987). Recent studies of the *Drosophila* mutant *sevenless*, in which the R7 photoreceptor cell in each ommatidium is missing, have shown that the *sevenless* gene encodes a typical membrane receptor with a cytoplasmic domain homologous to tyrosine kinases (Basler and Hafen, 1988). The fact that defects in such a membrane receptor perturb cell commitment in ommatidia provides strong support for a cell–cell interaction model.

Is the NV1–battery cell complex a sensory organ?

Based on their morphology, $NV1^+$ nerve cells appear to be sensory. In addition, they are completely surrounded by a battery cell. Together these two facts suggest that the NV1–battery cell complex may be a sensory organ in which the battery cell fulfills the function of the support cell(s), which typically surround sensory neurons in chemo- and mechano-receptors. The fact that formation of the NV1–battery cell complex requires interaction between nerve and epithelial cell precursors similar to the interactions involved in sensory organ formation is also consistent with this hypothesis.

If battery cells are sensory organs, what are they sensing? The most likely possibility appears to be prey. The sensitivity of nematocytes to mechanical stimulation is known to be enhanced if the stimulating glass needle has been coated with food extract (Ewer, 1947). Since the processes of $NV1^+$ nerve cells extend to nematocytes in several neighboring battery cells, such a chemosensory function could enhance the sensitivity of nematocytes in neighboring battery cells to mechanical stimulation and thus amplify the killing response.

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References

Basler, K. and Hafen, E. (1988). Control of photoreceptor cell fate by the *sevenless* protein requires a functional tyrosine kinase domain. *Cell* **54**, 299–311.

Campbell, R. D. (1967). Tissue dynamics of steady state growth in *Hydra littoralis*. II. Patterns of tissue movement. *J. Morph.* **121**, 19–28.

Campbell, R. D. (1973). Vital marking of single cells in developing tissues: India Ink injection to trace tissue movements in hydra. *J. Cell Sci.* **13**, 651–661.

Campbell, R. D. (1987). Organization of the nematocyst battery in the tentacle of hydra: Arrangement of the complex anchoring junctions between nematocytes, epithelial cells, and basement membrane. *Cell Tissue Res.* **249**, 647–655.

Diehl, F. A. and Burnett, A. L. (1964). The role of interstitial cells in the maintenance of hydra. I. Specific destruction of interstitial cells in normal, asexual, non-budding animals. *J. exp. Zool.* **155**, 253–260.

Dübel, D., Hoffmeister, S. and Schaller, C. H. (1987). Differentiation pathways of ectodermal epithelial cells in hydra. *Differentiation* **35**, 181–189.

Dunne, J. F., Javois, L. C., Huang, L. W. and Bode, H. R. (1985). A subset of cells in the nerve net of *Hydra oligactis* defined by a monoclonal antibody: Its arrangement and development. *Devl Biol.* **109**, 41–53.

Ewer, R. F. (1947). On the function and mode of action of the nematocysts of hydra. *Proc. Zool. Soc. London* **117**, 365–376.

Fujisawa, T. and David, C. N. (1982). Commitment during stenotele differentiation in hydra is localized near the S/G_2 boundary in the terminal cell cycle. *Devl Biol.* **93**, 226–230.

Ghysen, A. and Dambly-Chaudiere, C. (1989). Genesis of the *Drosophila* peripheral nervous system. *Trends Genet.* **5**, 251–255.

Hobmayer, E., Holstein, T. and David, C. N. (1990). Tentacle morphogenesis in hydra: I. The role of head activator. *Development* **109**, 887–895.

Holstein, T. and David, C. N. (1986). The properties of nerve cell precursors in hydra. *Devl Biol.* **115**, 18–26.

Hufnagel, L. A., Kass-Simon, G. and Lyon, M. K. (1985). Functional organization of battery cell complexes in tentacles of *Hydra attenuata*. *J. Morph.* **184**, 323–341.

KOIZUMI, O. AND BODE, H. R. (1986). Plasticity in the nervous system of adult hydra. I. The position-dependent expression of FMRFamide-like immunoreactivity. *Devl Biol.* **116**, 407–421.

KOIZUMI, O., HEIMFELD, S. AND BODE, H. R. (1988). Plasticity in the nervous system of adult hydra. II. Conversion of ganglion cells of the body column into epidermal sensory cells of the hypostome. *Devl Biol.* **129**, 358–371.

MCLAREN, D. (1976). Sense organs and their secretions. In *The Organization of Nematodes* (ed. N. A. Croll), pp. 139–162. London: Academic Press, Inc.

READY, D. F. (1989). A multifaceted approach to neuronal development. *Trends Neurosci.* **12**, 102–110.

STEINBRECHT, R. A. (1969). Comparative morphology of olfactory receptors. In *Olfaction and Taste*, vol. 3 (ed. C. Pfaffman), pp. 3–21. New York: Rockefeller University Press.

THURM, U. (1965). An insect mechanoreceptor. *Cold Spring Harbor Symp. Quant. Biol.* **30**, 75–82.

TOMLINSON, A. AND READY, D. F. (1987). Cell fate in the *Drosophila* ommatidium. *Devl Biol.* **123**, 264–275.

WEBER, E. (1964). *Grundriss der biologischen Statistik. 5. Auflage.* Jena: VEB Gustav Fischer Verlag.

WESTFALL, J. A. AND KINNAMON, J. C. (1978). A second sensory-motor-interneuron with neurosecretory granules in hydra. *J. Neurocytol.* **7**, 365–379.

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. *Devl Biol.* **114**, 225–237.

YU, S.-M., WESTFALL, J. A. AND DUNNE, J. F. (1985). Light and electron microscopic localization of a monoclonal antibody in neurons *in situ* in the head region of hydra. *J. Morph.* **184**, 183–193.

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