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MONOCLONAL ANTIBODIES: A NEW APPROACH TO THE STUDY OF HYDRA DEVELOPMENT

BY

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ABSTRACT

Monoclonal antibodies have been generated to aid in the study of two aspects of hydra development, the control of pattern formation and the regulation of interstitial cell differentiation. Antibodies which recognize restricted regions of hydra have been isolated. Two examples are described, one which binds specifically to battery cells of the tentacles, and another which labels only ectodermal epithelial cells of the body column. These antibodies will be useful markers in examining how patterning mechanisms regulate the formation of head structures from the body tissue.

The interstitial cells of hydra form a multipotent stem cell system, producing a variety of differentiated cell types. Several antibodies which mark specific subsets of the interstitial cell population and/or their derivatives are described. Some antibodies reveal the intricate pattern of nerve cells in the tissue, while others indicate intermediate stages in the formation of nematocytes. Several suggest the existence of subsets within the interstitial cell population which may have restricted developmental potentials. These antibodies will be important tools in analyzing the mechanisms which control interstitial cell proliferation and differentiation.

INTRODUCTION

The study of developmental biology requires a careful examination of the changes in organization which occur during morphogenesis. Hydra offers a unique opportunity to analyze these changes because, as first shown in Abraham Trembley's classic experiments on budding, grafting, and regeneration, hydra's tissue can be experimentally manipulated in a variety of ways. With hydra, we can examine the control of patterning at the tissue level, the mechanisms which regulate division and differentia-

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tion at the cellular level, and the interaction and feedback between these two levels. Recently, our approach has been to isolate monoclonal antibodies and to use them as specific labels to aid in studying the mechanisms underlying these biological properties.

There are several reasons why antibodies are useful experimental tools for these kinds of studies. (1) They exhibit a high degree of sensitivity and specificity, allowing the detection of small differences among otherwise identical cells. (2) They can serve as early markers of cell differentiation, yielding information about possible transition stages prior to establishment of the final pattern, and indicating the existence of lineage relationships between various cell populations. (3) They allow the easy visualization of cellular morphology and patterns of cell differentiation within the tissue, which might be difficult to detect or reconstruct by other methods. (4) They can be used to vitally stain cells, thus creating the opportunity for isolating distinct subpopulations and testing their developmental potential under different experimental conditions. In this paper we will describe several antibodies which exhibit patterns of labelling that are of interest for the study of hydra development.

MATERIALS AND METHODS

Hydra attenuata or *Hydra oligactis* were used for all labelling studies. Procedures for care and maintainence of stock cultures are detailed in Heimfeld and Bode (1984a). Procedures for the generation of monocolonal antibodies and their use in analysis are detailed in Dunne *et al.* (1985).

RESULTS AND DISCUSSION

Monoclonal Antibodies for Studying Pattern Formation

Patterning in hydra involves the formation of certain structures, the hypostome, tentacles, and foot (Fig. 1), from the tissue of the body column. Perhaps the best demonstration of this patterning ability is seen during regeneration. After amputation of the head or foot, the remaining tissue reforms the appropriate missing structures. Isolation of the body column reveals a polarity to regeneration, the head always forming at the apical end and the foot at the basal end of the tissue. This regenerative capacity holds when the isolated piece is only 1/40 the size of the original adult (Bode and Bode, 1980). Even more remarkable, the tissue can be dissociated into a cell suspension which, following aggregation, will regenerate new head and foot structures and eventually yield complete animals (Gierer *et al.,* 1972). These findings reveal the extraordinary capacity for reorganization and regulation of patterning in hydra.

Adult hydra continuously grow when well-fed, but do not increase in size. Instead, some of the excess tissue is used to replace the cells lost by sloughing from the head and foot regions (Campbell, 1967). The remaining extra tissue goes toward budding, a process of asexual reproduction by which tissue in the body column evaginates and subsequently forms head and foot structures to yield a smaller version of the parent animal (Trembley, 1744; Campbell, 1967). The net result of these tissue dynamics is that epithelial cells of the body column are displaced into the head or foot regions, and become integrated within those structures. Thus, even in the normal animal, the process of pattern formation occurs continuously.

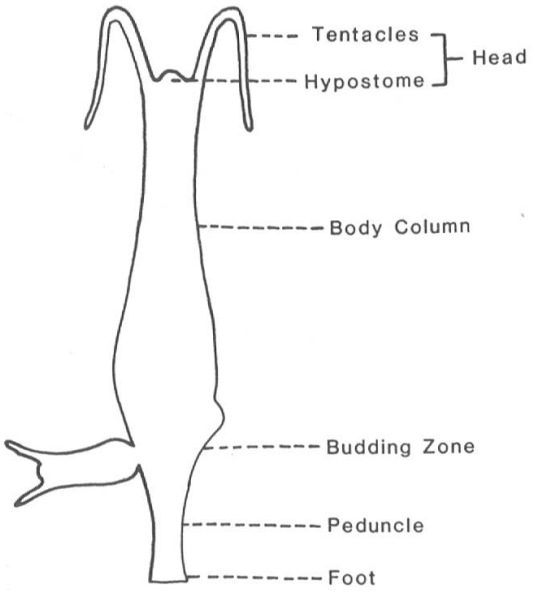

FIG. 1. — The different regions and structures of an adult hydra.

The ability of the body column to form or regenerate head and foot structures raises several questions. How do the cells know which type of structure to make and what controls the size and location of the head and foot regions when they form? There is an extensive amount of work which suggests the existence of developmental gradients distributed axially down the column, and models have been proposed to explain the role of these gradients in patterning (for review, see Bode and Bode, 1984). One of our approaches has been to look for antibodies which would show a graded distribution of label down the column. These might aid in studying the behavior of these developmental gradients during the processes of budding and regeneration. Additionally, we have screened for antibodies which distinguish between epithelial cells of the body column and epithelial cells which have undergone further differentiation and formed the structures of the head or foot regions. Such antibodies will allow us to analyze the early stages of regeneration, and possibly determine when tissue

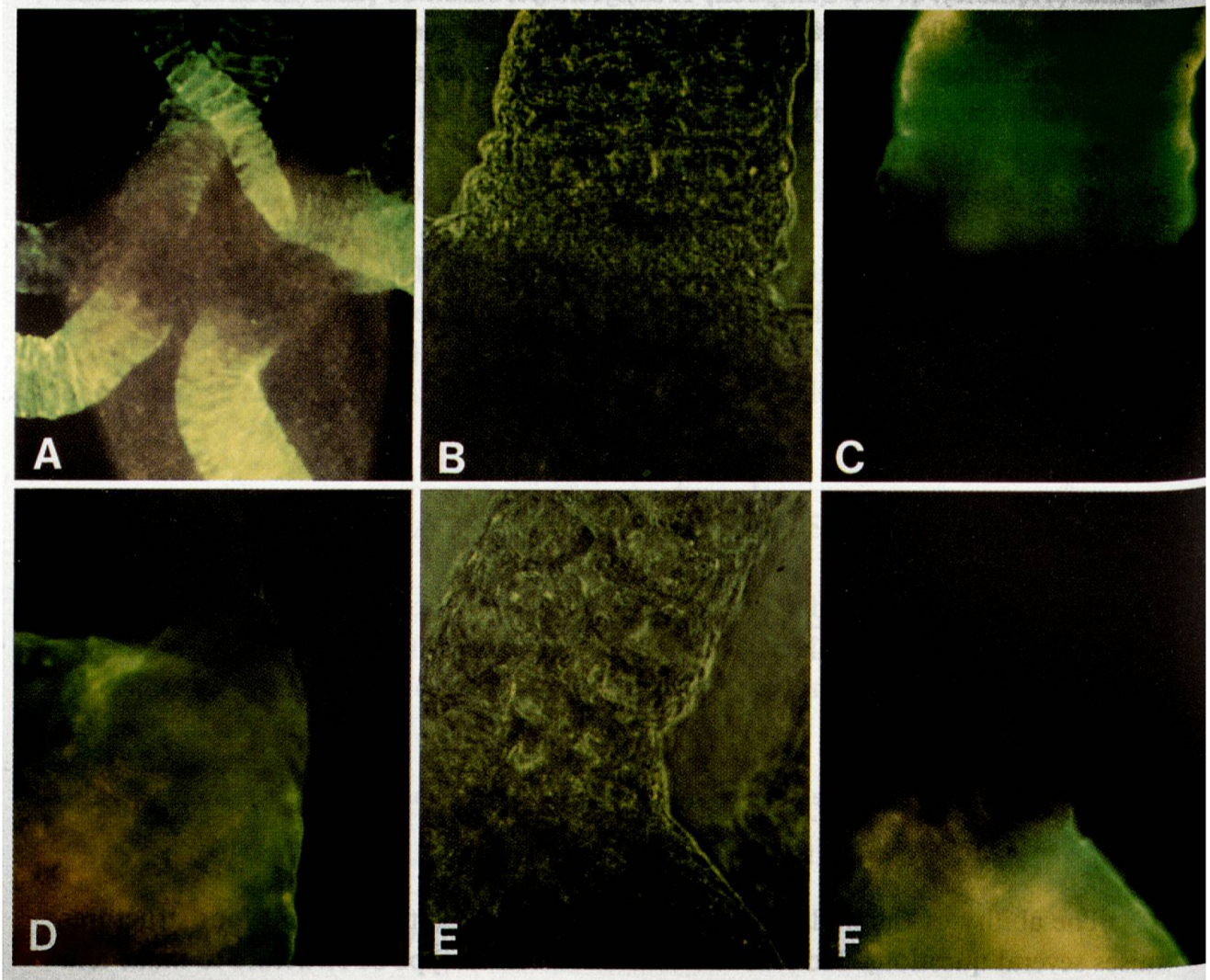

FIG. 2. — The staining pattern of antibody TS 19 (A, B, C) and TS 12 (D, E, F), visualized on whole-mounts with indirect immunofluorescence. The bright green represents areas of binding, while the duller green is background. (A) the upper half of an adult animal, looking down on the hypostome and tentacles; Phase contrast (B) and fluorescent (C) images of the boundary between the body column and a tentacle. (D) the upper half of a young bud, viewed from the side; Phase contrast (E) and fluorescent (F) images of the boundary between the body column and a tentacles. Magnifications are 100-500X. See text for detailed descriptions.

is first set aside for patterning. We have isolated several antibodies which show these regional restrictions, and two of these are described in detail below.

One, TS 19, labels epithelial battery cells of the tentacles, as shown in Fig. 2A. This is a photograph of a whole-mount of the upper half of a hydra, looking down on the hypostome and tentacles. All of the tentacles are equally stained, although differences in the plane of focus give the illusion of variable intensity. Fig. 2B shows a phase image of tissue at the junction between a tentacle and the hypostome, while Fig. 2C shows this same region viewed with fluorescence. The two pictures indicate that TS 19 is highly specific for the tentacles. There does not appear to be any binding to the epithelial cells of either the body column or the hypostome. TS 19 also sometimes labels a very restricted band of epithelial cells just above the foot region. Binding seems to be to the cell surface, although this is hard to demonstrate conclusively in whole-mounts. The fact that the label extends to the base of the tentacles suggests that when body epithelial cells are displaced onto the tentacles they rapidly shift their differentiation behavior.

Another antibody, TS 12, binds only to ectodermal epithelial cells of the body column. Fig. 2D shows a whole-mount of a young bud stained with this antibody. The tissue of the body column is easily visible, while the two tentacles of this bud are barely apparent. Fig. 2E and 2F show a part of the junction between the tentacles and the body column, under phase and fluorescent optics, respectively. As with TS 19, labelling with TS 12 reveals a sharp boundary between the two regions, lending further support to the idea that the conversion of epithelial cells from the body column to those of the tentacles is a rapid and abrupt process. TS 12 does not bind in the hypostome or foot regions, which suggests that body epithelial cells which become displaced into those regions also alter their differentiation behavior.

In keeping with the theme of this symposium, it is worth noting that Abraham Trembley, through his detailed experiments, observed that the tentacles differed from other tissues of the body column in that only the tentacles would not regenerate. Our studies with TS 19 and TS 12 refine Trembley's original observations, pointing out subtle molecular differences between the epithelial cells of the body column and the tentacles.

Monoclonal antibodies, hopefully, will help us to examine the developmental gradients which regulate the patterning processes. TS 12 and TS 19 should allow us to examine the processes by which regions and structures become specified, and may also yield information about the molecular events which accompany changes in morphology. More likely, the antibodies will reveal new characteristics and properties of pattern formation which have not been seen previously, thus raising new questions, but still bringing us closer to an understanding of how development of form occurs in hydra.

Monoclonal Antibodies for Studying Interstitial Cell Regulation

Another important question in the study of development is how specific patterns of cell differentiation are established. The interstitial cell system in hydra, because it can be manipulated and modified so readily, provides a convenient model in which to investigate this question. Interstitial cells are multipotent stem cells (David and Murphy, 1977), which give rise to a variety of terminally differentiated products (Fig. 3). Similar to other stem cell systems, the proliferation and differentiation behavior of the interstitial cells seems to be regulated (for review, see Bode and David, 1978). To study such a stem cell population, and to ask specific questions about control of commitment and differentiation, it would be helpful to be able to separate and independently examine the various components of the interstitial cell system. A number of monoclonal antibodies have been isolated for this purpose, some of which are described in detail in the following paragraphs.

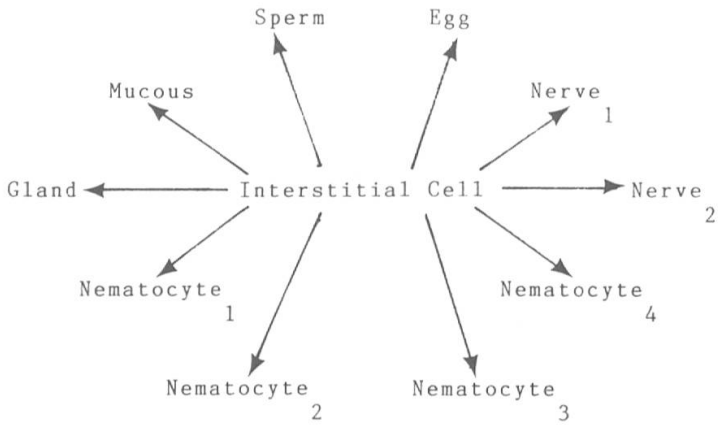

FIG. 3. — The variety of differentiated cell types produced by the multipotent interstitial cell population.

One antibody, JD 1, binds specifically to a subset of nerve cells that are restricted to the hypostome and tentacles. These cells are almost all sensory nerve cells (Dunne *et al.,* 1985). The network of nerve cell bodies and their processes in a tentacle is shown in Fig. 4A. This is an example where staining with antibody has revealed a pattern of differentiation within the tissue which otherwise would be almost impossible to detect. The existence of regionally restricted subclasses within the nerve cell population was unexpected. It implies that simply regulating the commitment of an interstitial cell to nerve cell differentiation is insufficient. Both the location and type of nerve cell to be formed must also be designated.

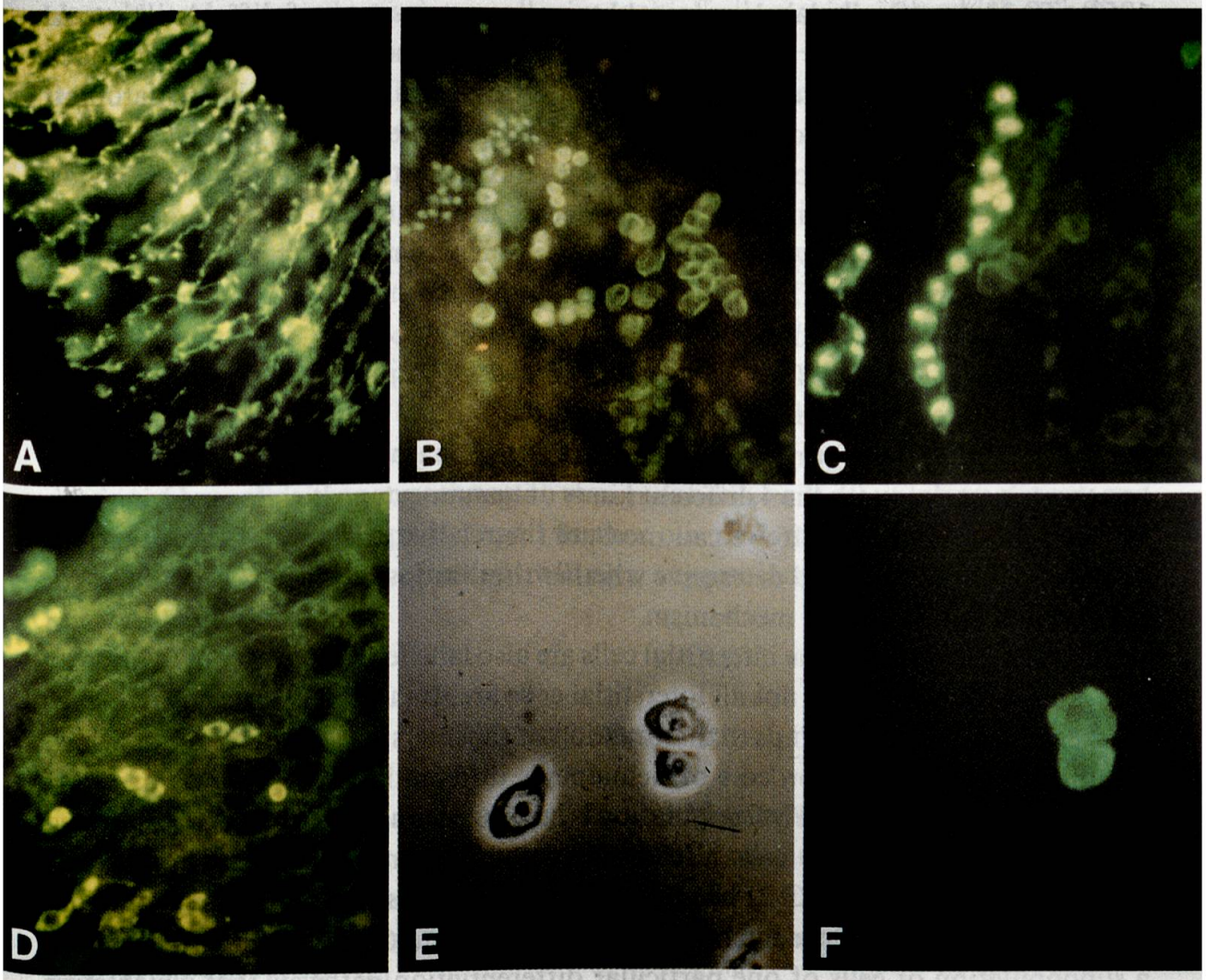

FIG. 4. — The staining pattern of antibodies which recognize subsets of the interstitial cell population or their derivatives. (A) a single tentacle labelled with JD 1; (B) body column labelled with TS 23; (C) body column stained with TS 16; (D) body column near the peduncle region labelled with CP 4; Phase contrast (E) and fluorescent (F) images of a macerated preparation of hydra tissue, showing a single and a paired nest of large interstitial cells stained with AC 2.
Magnifications are 250-1000X. See text for detailed descriptions.

Formation of nematocytes, the stinging cells characteristic of the coelenterates, occurs by incomplete synchronous divisions of a single interstitial cell to form a syncytium or nest of 4-32 cells. Each cell, termed a nematoblast, then produces a nematocyst capsule, a collagenous-polysaccharide shell which contains a thread that can be discharged under the appropriate conditions. There are 4 different types of nematocytes in hydra, distinguished by the shape of the capsule they contain (Kanaev, 1952). TS 23 specifically labels nematoblasts at a certain stage of differentiation, as shown in Fig. 4B. This is a whole-mount of the body column which has been stained with this antibody, revealing the separate capsules forming within the nematoblast cells. TS 23 appears to recognize each type of nematocyte, binding to the capsule at a stage before formation of the thread has been completed. With this antibody we can easily quantitate the amount of nematocyte differentiation which is occurring, and measure how this may change in response to experimental manipulation of the interstitial cell population.

In contrast to TS 23, antibody TS 16, shown in Fig. 4C, labels some other structure within the nematoblasts. The cells which stain with this antibody seem to be at an earlier stage in nematocyte formation, before the capsule has taken final shape. The pattern of TS 16 label is variable between different nematoblast nests. This variability appears to correlate with the 4 different kinds of nematocytes which form. Using antibodies like TS 23 and TS 16 we can measure the relative amounts of the 4 types of nematocytes produced, and determine whether these ratios are fixed or can be regulated through some control mechanism.

As seen in Fig. 4C some interstitial cells are also labelled, although not as brightly as the nematoblasts. Since not all interstitial cells are stained, TS 16 may be a lineage marker which selectively binds to that fraction of the interstitial cell population which has already initiated the process of nematocyte differentiation. If this is correct, it suggests that the interstitial cell population is heterogeneous, containing uncommitted stem cells as well as some cells already restricted to nematocyte formation.

Another antibody, AC 2, has provided data that also supports the possibility of heterogeneity within the interstitial cell population. AC 2 binding is restricted to a subpopulation of cells of one particular differentiation pathway, that which gives rise to sperm (Littlefield *et al.,* 1985). Fig. 4E shows a macerated preparation of hydra cells, a procedure by which the tissue is simultaneously fixed and dissociated (David, 1973). The large single cell and the paired cells are big interstitial cells, and these are generally assumed to contain the stem cell population. In this example, one can see that the single cell and the nest of two cells appear morphologically similar, but AC 2 binds only to the paired cells (Fig. 4F). This is a case where the use of an antibody has indicated a difference among cells which would have been classified as identical by previous criteria. AC 2 allows us to analyze these committed sperm cell precursors independently of the remainder of the interstitial cell population (Lifttlefield *et al.,* 1985).

In contrast, antibody CP 4 binds to a large proportion of the cells of the interstitial cell system, including those within the nematocyte pathway. An area of the body column, near the peduncle region, which has been stained with this antibody is shown in Fig. 4D. As the concentration of interstitial cells and nematoblasts is greatly reduced in the peduncle, one can easily visualize the individual cells within this tissue. Some big interstitial cells, both single and paired, are labelled, along with a nest of nematoblast cells. Also stained are some smaller interstitial cells, not in nests, which we believe may be committed precursors to nerve cell differentiation (Heimfeld and Bode, 1984b). Because it stains nearly all of the interstitial cell population, CP 4 will be useful for measurements of growth rates and the study of the control of interstitial cell proliferation under different environmental conditions.

One of the most conspicuous features of the interstitial cell system is that the type of differentiated product formed is position-dependent. Nerve cells are primarily made in the head and foot regions, while nematocyte differentiation is confined to the body column (Bode *et al.,* 1973; David and Gierer, 1974; Yaross and Bode, 1978). This raises the intriguing possibility that the regulatory mechanisms which govern pattern formation in hydra are also responsible for controlling proliferation and differentiation of the interstitial cell population. The use of monoclonal antibodies, in combination with other experimental manipulations, will allow us to dissect and analyze both of these properties, and further our understanding of hydra development. Hopefully, these new findings will also suggest interesting new approaches to pursue in future studies.

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REFERENCES

BODE, H., S. BERKING, C. N. DAVID, A. GIERER, H. SCHALLER and E. TRENKNER (1973). Quantitative analysis of cell types during growth and morphogenesis in *Hydra*. *Wilhelm Roux Arch. EntwMech. Org.* 171, 269-285.

BODE, H. R. and C. N. DAVID (1978). Regulation of a multipotent stem cell, the interstitial cell of *Hydra*. *Progr. Biophys. Mol. Biol.* 33, 189-206.

BODE, P. M. and H. R. BODE (1980). Formation of pattern in regenerating tissue pieces of *Hydra attenuata*. I. Head-body proportion regulation. *Dev. Biol.* 78, 484-496.

—— (1984). Patterning in *Hydra*. In Primers in Developmental Biology, Vol. 1: Pattern Formation (G. Malacinski and S. Bryant, ed.), Macmillan. New York.

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

DAVID, C. N. (1973). A quantitative method for maceration of hydra tissue. *Wilhelm Roux Arch. Entw-Mech. Org.* 171, 259-268.

DAVID, C. N. and A. GIERER (1974). Cell cycle kinetics and development of *Hydra attenuata*. III. Nerve and nematocyte differentiation. *J. Cell Sci.* 16, 359-375.

DAVID, C. N. and S. MURPHY (1977). Characterization of interstitial stem cells in hydra by cloning. *Dev. Biol.* 57, 372-383.

DUNNE, J. F., L. C. JAVOIS, L. W. HUANG and H. R. BODE (1985). A subset of cells in the nerve net of *Hydra oligactis* defined by a monoclonal antibody: its arrangement and development. *Dev. Biol.* 109, 41-53.

HEIMFELD, S. and H. R. BODE (1984a). Interstitial cell migration in *Hydra attenuata*. I. Quantitative description of cell movements. *Dev. Biol.* 104, 1-9.

—— (1984b). Interstitial cell migration in *Hydra attenuata*. II. Selective migration of nerve cell precursors as the basis for position-dependent nerve cell differentiation. *Dev. Biol.* 104, 10-17.

KANAEV, I. I. (1952). *Hydra: Essays on the Biology of Freshwater Polyps* (Translated by E. J. Burrows and H. M. Lenhoff). Edited and published by H. M. Lenhoff, 1969.

LITTLEFIELD, C. L., J. F. DUNNE and H. R. BODE (1985). Spermatogenesis in *Hydra oligactis*. I. Morphological description and characterization using a monoclonal antibody specific for cells of the spermatogenic pathway. *Dev. Biol.* 110, 308-320.

TREMBLEY, A. (1744). Mémoires, pour servir à l'histoire d'un genre de polypes d'eau douce, à bras en forme de cornes. Verbeek. Leide.

YAROSS, M. S. and H. R. BODE (1978). Regulation of interstitial cell differentiation in *Hydra attenuata*. III. Effects of i-cell and nerve cell densities. *J. Cell Sci.* 34, 1-16.