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Distribution of Interstitial Cells and Differentiating Nematocytes in Nests in *Hydra attenuata*

CHARLES N. DAVID AND DIANE CHALLONER

Max-Planck-Institut für Virusforschung, Molekularbiologische Abteilung, 74 Tübingen, West Germany

SYNOPSIS. We have used tissue maceration to quantitate interstitial cell nests in *Hydra attenuata* and thiolacetic acid-lead nitrate staining to quantitate differentiating nematocytes. Most I-cell nests have 2, 4, 8, or 16 cells. Differentiating nematocytes occur in nests of 4, 8, 16, and 32. All classes of I-cell nests and nests of differentiating nematocytes are abundant in the gastric region. In contrast, the hypostome, basal disk and the regions immediately adjacent to them have no nests of differentiating nematocytes, virtually no nests of 4, 8, or 16 I-cells and reduced numbers of 1 and 2 I-cell nests. Since nests of 4 or more I-cells are found only where nematocyte differentiation takes place, these nest classes are probably nematocyte precursors. Since differentiation of nerves occurs in the hypostome and basal disk where only single or paired I-cells are found, these size classes include nerve precursors. The disproportionately large number of I-cell nests of 2 suggests that these include the autoreproductive stem cells responsible for maintaining the populations of I-cell derivatives in growing hydra.

Interstitial cells (I-cells) in hydra are rapidly proliferating, undifferentiated cells. They are widely acknowledged to be the precursors of nerve cells and nematocytes in asexual hydra. They are also precursors of sex cells under conditions of sexuality. Histological evidence for I-cell differentiation to other hydra cell types has often been reported (see Lentz, 1966), but this appears not to occur in rapidly budding *Hydra attenuata* in our laboratory (David and Campbell, 1972; David, unpublished) although it may occur during regeneration or nutritional stress.

I-cells are found primarily in the ectoderm of the gastric region of hydra wedged between epithelio-muscular cells. They occur as single cells or in clusters (nests). The cells in clusters are connected to each other by cytoplasmic bridges (Slautterback and Fawcett, 1959). Lehn (1951) observed that all cells in a cluster divide synchronously and postulated that the clusters arise from single cells by a series of divisions thereby giving rise to nests of exactly 2^n cells.

Nematocytes differentiate synchronously in clusters of 4, 8, 16, and 32 cells and are thus thought to be the products of the corresponding nests of I-cells (Lehn, 1951; Slautterback and Fawcett, 1959; Rich and Tardent, 1969). Whether I-cells other than nematocyte precursors occur in clusters is unknown. If not, nematocyte determination occurs in I-cells before proliferation of an I-cell to a nest; if so, determination could occur in a proliferating nest as late as immediately prior to capsule formation. A further question of interest is which of the non-nematocyte-forming I-cells are the precursors to nerve cells and which make up the proliferating stem cell pool.

Dissociation of hydra tissue into single cells by maceration in glycerol and acetic acid has proven an extremely useful technique for recognizing and categorizing I-cells and their differentiated products, nerves and nematocytes (Schneider, 1890; Hadzi, 1909; Davis et al., 1968; David, 1973). Based on cellular size and morphology two types of interstitial cells, big and little, have been distinguished. Photographs and extensive descriptions have been

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presented elsewhere (David, 1973). Big I-cells occur almost exclusively as single cells or nests of 2 and 4; little I-cells, by comparison, occur primarily in larger clusters (8 to 16 cells). Their morphological similarity to differentiating nematoblasts and their occurrence in larger clusters suggest that little I-cells are the precursors of nematocyte nests. Pulse-chase experiments with ^{3}H-thymidine support this interpretation (David and Gierer, 1974). The big I-cells, which must include the precursors to nests of little I-cells, probably also include nerve precursors and the proliferating stem cells necessary to maintain the population in growing hydra.

Further information about the role of the various types of I-cells in the differentiation of nerves and nematocytes has been obtained from the distribution among the various regions of hydra of I-cell nests and nests of differentiating nematocytes. The data presented here apply to rapidly proliferating *H. attenuata* maintained at 20 C and fed daily with *Artemia* (David and Campbell, 1972).

DISTRIBUTION OF I-CELL NESTS

The frequencies of I-cell nests of various sizes determined with the maceration technique are given in Table 1. Nests not containing 2^n cells are rare. This strongly suggests that the nest sizes observed in maceration preparations are not artifacts of breakage but accurately represent the situation in vivo. The maceration results are also supported by a study (David and Gierer, 1974) which indicates that the observed occurrence of I-cell nests corresponds closely with the distribution expected from nerve and nematocyte production rates and the cell cycle times of I-cells.

Table I also gives the distribution of I-cell nests in various regions of hydra. The gastric region and budding region contain the majority of all I-cells (Bode et al., 1973) including I-cell nests of all size classes. By comparison, the hypostomal region and proximal peduncle (including basal disk) have markedly reduced numbers of I-cell nests. Here nests of 4, 8, and 16 are absent or barely represented and the frequency of 2's is very low. The frequency of single I-cells, however, is only two- to threefold lower than in gastric tissue.

DISTRIBUTION OF NESTS OF DIFFERENTIATING NEMATOCYTES

Lehn (1951) first demonstrated that nematocytes differentiate in clusters con-

TABLE 1. *Distribution of I-cells in nests in macerations of hydra tissue.*

	I-cells per nest							
	1	2	3	4	5,6	7,8	9–13	14,15,16
Total hydra (excluding tentacles and buds)	14.5 ± 1.3	23.3 ± 1.5	0.9	5.9 ± 2.4	1.1	3.7 ± 1.3	0.4	1.2 ± 0.8
Hypostomal region + distal 1/10 body column	5.5	1.4		0.3		—		—
Gastric region	13.0	25.2		6.3		4.5		1.5
Distal half of peduncle	12.1	16.1		7.0		3.4		2.2
Basal disk + proximal 1/10 body column	2.7	2.9		0.8		0.2		—

The results are expressed as the frequency of a given nest per 100 epithelial cells. The values for total hydra are the average ± standard deviation of four independent preparations each containing 10 hydra; to obtain the uncertainty of the average, divide the quoted error by 2. The results for separate regions of hydra were obtained on one set of macerations using 10 animals. In all samples 500–1000 epithelial cells were counted.

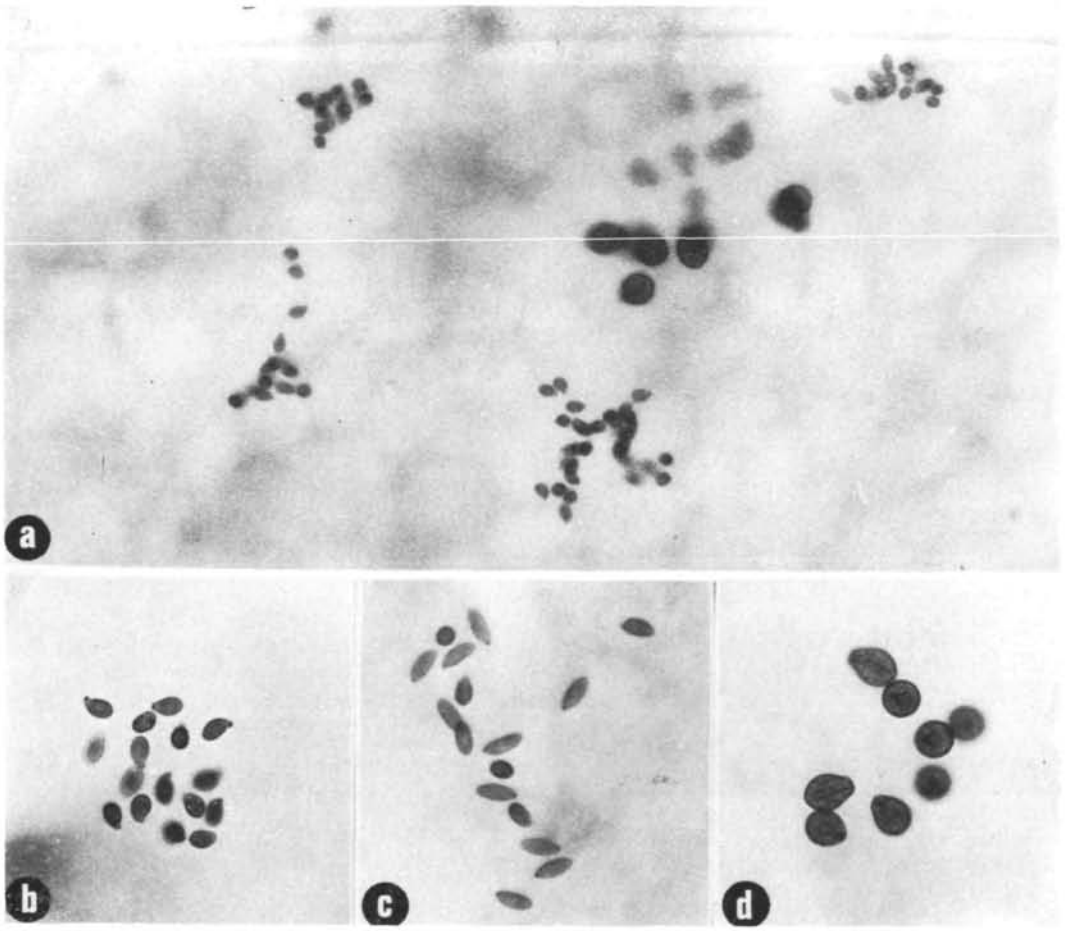

FIG. 1. Nests of differentiating nematocytes stained with thiolacetic acid and lead nitrate. Hydra were fixed in 96% ethanol for 10 min, rehydrated, and stained for 30 min at room temperature. The staining reagent, prepared immediately before use, contained 0.12 M thiolacetic acid (adjusted to *p*H 6.0 with NaOH), 0.005 M lead nitrate and 0.01 M cacodylate buffer *p*H 6.0. Following staining the hydra were washed, dehydrated through ethanol, cleared in xylene, and mounted in Cacdax. *a*, General view including the following nests: 8 stenoteles, 16 desmonemes (2 nests), 32 desmonemes, and 16 atrichous isorhizas. Some of the nematocytes in each nest are above or below the plane of focus and appear only as faint shadows. ×500. *b*, Nest of 16 desmonemes. ×750. *c*, Nest of 16 atrichous isorhizas. ×750. *d*, Nest of 8 stenoteles. ×750.

taining 4, 8, 16, and 32 cells by analyzing serial sections of hydra. More recently Rich and Tardent (1969) have obtained a similar result using preparations of ectodermal tissue peeled from hydra. We have taken advantage of the thiolacetic acid-lead nitrate staining procedure (Lentz and Barrnett, 1961) to selectively stain differentiating nematocytes in intact hydra. The procedure stains all types of nematocytes in *H. attenuata* and is highly selective for the late stage of nematocyte differentiation (Fig. 1). Early stages are not stained nor are mature nematocytes, either migrating to the tentacles or mounted in battery cells of the tentacles (Fig. 2). The background tissue also remains unstained. Thus, whole mounts can be prepared and the individual nests identified in all parts of an intact hydra.

Figure 2 shows a bud stained for differentiating nematocytes. The nests of nematocytes appear to be distributed uniformly throughout the tissue. At higher magni-

FIG. 2. Low magnification view of a bud stained with thiolacetic acid and lead nitrate. Nests of differentiating nematocytes are scattered throughout the tissue. Nematocytes in battery cells of the tentacles are not stained nor are migrating nematocytes. ×80.

fication individual nests can be distinguished and the number and type of the nematocyte determined (Fig. 1). The cells in a nest are usually tightly packed together. As the nematocytes complete their differentiation, the staining reaction becomes fainter and the nest begins to break up into individual, independently migrating nematocytes.

Table 2 gives a summary of the frequency of nests of 4, 8, 16, and 32 for each of the three types of nematocytes in *H. attenuata*. Desmonemes occur for the most part in nests of 16 cells although a few nests of 8 and 32 are found. Stenoteles and isorhizas both differentiate primarily in nests of 8 and 16 cells. These results are similar to those of Rich and Tardent (1969) who used less rapidly growing hydra. The low level of stenotele nests of 4 in the present data may reflect the different conditions of culture.

The absolute number of nematocyte nests per hydra varies over a twofold range in our animals. This variation affects all types about equally. Temperature and feeding also influence the number of nests per animal: growth at lower temperature (16 to 18 C) or less intense feeding or both can increase the number of nests per hydra as much as twofold.

LOCATION OF NESTS OF DIFFERENTIATING NEMATOCYTES

Our whole mounts demonstrate clearly the sites of nematocyte differentiation. Nests are abundant throughout the gastric region, budding region, and distal peduncle. By comparison, the region immediately proximal to the tentacle ring and the region immediately distal to the basal disk are almost completely free of nests

TABLE 2. *Distribution of differentiating nematocytes in nests.*

Nematocyte type	Nematocytes per nest			
	4	8	16	32
Desmoneme	—	5.7 ± 3.1	94.6 ± 27.3	9.5 ± 3.1
Stenotele	3.0 ± 3.2	34.3 ± 13.6	23.5 ± 11.8	—
Isorhiza (atrichous + holotrichous)	—	19.6 ± 12.3	13.9 ± 7.9	—

All nests on the upper surface of a whole mount (excluding buds) were counted, and the result multiplied by 2.2 to account for nests on the uncounted lower surface and sides. The rare nests with sizes other than 2^n were counted as nests of the next higher power of 2. The results given are the average ± standard deviation for 11 animals; the error quoted can be converted to the uncertainty of the average by dividing by 3.3.

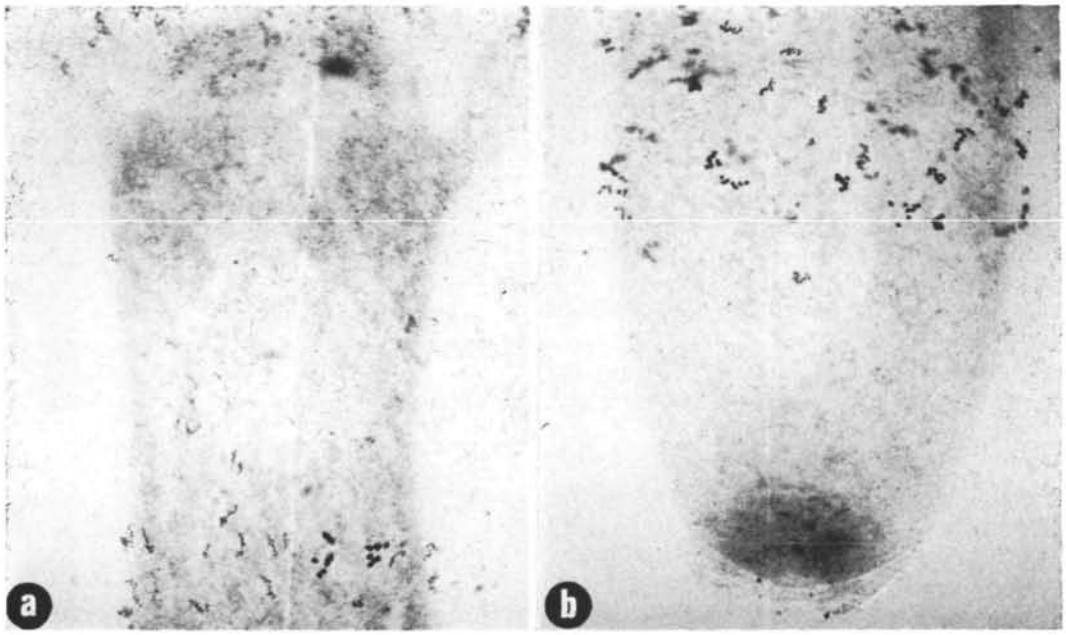

FIG. 3. Whole mount of hydra showing "clear zones" containing no nests of differentiating nematocytes in tissue proximal to hypostome (*a*) and distal to basal disk (*b*). Stained with thiolacetic acid and lead nitrate. ×80.

of differentiating nematocytes (Fig. 3). The size of these "clear zones" varies from animal to animal but is generally about $\frac{1}{10}$ of the total length.

The developing bud hypostome, in marked contrast to the hypostome of the parent, has no subjacent clear zone. Nests of differentiating nematocytes often appear, in fact, on the very tip of the developing bud even at stages of bud development as late as tentacle formation. A similar situation exists in the newly formed basal disk of the bud.

DISCUSSION–CONCLUSIONS

In asexually growing hydra, I-cells have three principal functions: (i) as precursors for nematocyte differentiation, (ii) as precursors for nerve cell differentiation, and (iii) as an autoreproductive stem cell population which must increase in size as the hydra grows. The results on the distribution of I-cell nests and nests of differentiating nematocytes in conjunction with results on nerve cell differentiation in hydra permit the assigning of these functions to certain I-cell nests.

The spatial distribution of nests of 4, 8, and 16 I-cells in hydra is correlated with the occurrence of nests of 8, 16, and 32 differentiating nematocytes. This coincidence suggests that these nests of I-cells are the precursors for nests of nematocytes.

Both the hypostome and basal disk, where nests of 4, 8, and 16 I-cells do not occur, are sites of active nerve cell differentiation (David and Gierer, 1974). This indicates that nests of 4, 8, and 16 I-cells are not precursors for nerve cell differentiation. Rather, the nerve precursors are single I-cells or possibly nests of 2.

The present results do not permit a direct identification of the stem cell population with one of the classes of I-cells. Nevertheless, the disproportionately large number of nests of 2 compared to nests of 4 suggests the possibility that stem cells are included in this population. A quantitative study to be presented elsewhere demonstrates that a large proportion of

nests of 2 are in fact stem cells (David and Gierer, 1974).

Lehn (1951) and Rich and Tardent (1969) have presented evidence for the synchronous multiplication of I-cells to form nests which then differentiate synchronously into one specific type of nematocyte. From their results, however, they could not decide whether determination occurred prior to I-cell proliferation to form a nest or at the final cell cycle prior to nematocyte differentiation. The present experiments do not provide a complete answer. They do suggest, however, that determination as nematocyte rather than nerve or stem cell occurs at least as early as the 2-cell stage in order to achieve the production of nests of 4 I-cells.

Such an early determination event provides a ready explanation for the observation that the bud hypostome 2 days after bud initiation still does not have a clear zone subjacent to it. If the hypostome blocks nematocyte determination before the 4-cell stage and does not affect later stages of I-cell nest proliferation or nematocyte differentiation, then clear zones cannot be expected until several days after the initiation of the bud hypostome.

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