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Reversing the Life Cycle: Medusae Transforming into Polyps and Cell Transdifferentiation in *Turritopsis nutricula* (Cnidaria, Hydrozoa)

STEFANO PIRAINO[1], FERDINANDO BOERO[2], BRIGITTE AESCHBACH[3], AND VOLKER SCHMID[3]

[1]*Istituto Sperimentale Talassografico CNR "A. Cerruti," Via Roma 3, I-74100 Taranto, and Laboratorio di Ecologia del Benthos, Stazione Zoologica di Napoli "A. Dohrn," 80077 Ischia Porto (Naples), Italy;* [2]*Dipartimento di Biologia, Stazione di Biologia Marina, Universitá di Lecce, I-73100 Lecce, Italy; and* [3]*Institute of Zoology, University of Basel, Rheinsprung 9, CH-4051 Basel, Switzerland*

Abstract. Organisms develop through a series of stages leading to sexually mature adults. In a few cases ontogeny reversal is possible, but it does not occur typically after the onset of sexual reproduction. All stages of the medusa *Turritopsis nutricula,* from newly liberated to fully mature individuals, can transform back into colonial hydroids, either directly or through a resting period, thus escaping death and achieving potential immortality. This is the first metazoan known to revert to a colonial, juvenile morph after having achieved sexual maturity in a solitary stage. Selective excision experiments show that the transformation of medusae into polyps occurs only if differentiated cells of the exumbrellar epidermis and part of the gastrovascular system are present, revealing a transformation potential unparalleled in the animal kingdom.

Introduction

Hydrozoan life cycles are among the most varied of the animal kingdom, with a vast array of different patterns (Boero and Bouillon, 1993). The basic hydroidomedusan life cycle proceeds from the adult medusa (a solitary stage), to the planula larva, to the polyp (the hydroid, an asexual and often colonial stage). Medusae are produced asexually by polyps. In most cases, the medusae have a limited life span, with a growth phase leading to sexual maturity and liberation of gametes, sometimes in multiple episodes of gamete production, followed by cell disintegration and organismic death. Ontogeny reversal, with direct transformation of isolated medusa buds into polyps, has been demonstrated to occur in *Podocoryne carnea* (Müller, 1913; Frey, 1968; Schmid, 1972), *Eleutheria dichotoma* (Hauenschild, 1956), *Cladonema* sp. and *Cladonema uchidai* (Kakinuma, 1969), and *Perarella schneideri* (Piraino, unpubl. obs.). These isolated medusa buds can revert to polyp structures only at the beginning of their development, completely losing this ability before they are liberated. Recently, however, a unique case of ontogeny reversal has been reported by Bavestrello *et al.* (1992), in which newly released, sexually immature medusae of *Turritopsis nutricula* McCrady, 1859, regressed, settled onto a substrate, and gave rise to stolons and hydroid colonies. The onset of sexual reproduction has been hypothesized to be the point of no return in the ontogenetic sequence of any living organism (Stearns, 1992). We evaluate this hypothesis by studying in the laboratory both the potential for and the triggers of transformation throughout the life span of *T. nutricula* medusae.

We have also investigated the cellular basis of this transformation process. The medusa is distinguished from the polyp, not only by a different shape and anatomical organization, but also by a completely different set of somatic cells in the umbrella. A layer of mononucleated, striated muscle cells lines the subumbrellar cavity, and specialized nerve rings and sensory organs (ocelli and statocysts) are present only in the medusa (Tardent, 1978; Mackie, 1980; Werner, 1984). Differences in patterns of protein expression between polyps and medusae were detected by Bally and Schmid (1988) and, more recently, RNA fingerprinting data confirmed these distinctions at

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the genomic level (Acrne *et al.*, in press). In addition, the epidermal muscle cells of the hydroid produce a chitinous covering (perisarc), but the naked exumbrellar, nonmuscle cells of the medusa do not. Thus, transformation of the medusa into a hydroid must involve substitution of cell types and tissue regeneration and reorganization. Both hydroids and medusae can regenerate missing tissues and organs, but regenerative potential is not solely associated with the proliferation and differentiation of cycling embryonic cells (interstitial or I-cells). Regeneration in medusae is more likely to be due to the remarkable transdifferentiation potential of fully differentiated cells, as demonstrated for tissues of the medusa of *Podocoryne carnea* (reviewed in Schmid, 1992). Transdifferentiation is defined as a change of commitment and gene expression of somatic, well-differentiated, noncycling cells to other cell types directly or through the return to a condition of undifferentiated cells. In the past, this process was called metaplasia or cell transformation (reviewed in Okada, 1991). The pioneering work by Wilson (1907) opened a long-lasting debate on the reversibility of cell commitment and functional stability. In recent years, the pluripotency of nuclei of differentiated cells and the activation of silent genes have been demonstrated through transplantation studies and DNA-binding proteins (Di Berardino *et al.*, 1984; Di Berardino, 1988; Topscott *et al.*, 1988; Lassar *et al.*, 1989). The processes of transdetermination of embryonic cells and transdifferentiation of specialized somatic cells, having strong ties with regeneration processes both in invertebrates and vertebrates, demonstrate that cell commitment and functional stability are not consequences of loss or irreversible inactivation of genes (reviewed by Okada, 1991; and by Schmid, 1992).

From the foregoing considerations, the transformation process of *T. nutricula* medusae can be explained, at the cellular level, by two alternative, but not mutually exclusive, hypotheses. First, all differentiated somatic cells of the medusa degenerate, and the production of polyp cells is initiated by a set of undifferentiated reserve cells that were not irreversibly committed. Second, differentiated cells of the medusa might transdifferentiate to produce the requisite new cell types.

Materials and Methods

Rearing

About 4000 medusae of *Turritopsis nutricula* were obtained from hydroid colonies collected in the Gulf of Naples (Western Mediterranean) from June to October, in 1993 and 1994. The medusae were reared in the laboratory at 20°C in groups of 100 individuals in 500-ml beakers, and fed every other day with newly hatched brine shrimp (*Artemia salina*) until gonad maturation and spawning. Slight water circulation was maintained by a gentle stream of air blowing across the surface of the water.

Immunohistochemistry

Whole medusae were anesthetized in $MgCl_2$ (3.5% [w/v] in seawater), and fixed in 4% paraformaldehyde (PFA; pH 7.2) in phosphate-buffered saline (PBS, 150 m*M* NaCl, 10 m*M* Na_2HPO_4; pH 7.4) for 10 min. Then the medusae were washed twice for 5 min in PBS containing 0.05% Triton + 0.05% NaN_3 (PBS/Triton) and rinsed in two changes of PBS. The histological and anatomical organization of the medusa of *T. nutricula* was investigated by incubating for 1 h at room temperature in 1:100 dilution of either (a) rabbit polyclonal antibodies raised against RFamide positive nerve cells (antisera kindly provided by the laboratory of Prof. C.J.P. Grimmelikhuijzen), or against human "β1"-integrin; or (b) mouse monoclonal antibodies raised against smooth muscle cells (termed sm-1) or against striated muscle cells (termed 93, Schuchert *et al.*, 1993) of *P. carnea*. All showed cross-reactivity with *T. nutricula* tissues. Subsequently, the medusae were washed in PBS (10 min, two times), and incubated in FITC-conjugated goat anti-rabbit IgG (Sigma) or anti-mouse IgG (Sigma) diluted 1:20 with PBS, for 30–60 min at room temperature. Finally, the medusae were washed in PBS and stained with 4,6-diamidino-2-phenylindole 2 HCl (DAPI, 0.05 mg/ml; Serva) in PBS for 1 min, washed with PBS, mounted in 10:1 glycerine/PBS supplemented with 25 mg Diazabicyclol (2,2,2)-octane (Aldrich-Chemie) (DABCO/glycerine/PBS; Alder and Schmid, 1987), and observed with a fluorescence microscope.

Electron microscopy

At each stage of the transformation of medusae into polyps, sets of 10 specimens were fixed overnight in 2.5% glutaraldehyde in cacodylate buffer (pH 7.2) at 4°C, post-fixed for 1 h in 1% osmium tetroxide in cacodylate buffer before dehydration in an alcohol series, and embedded in Epon resin. Ultrathin sections were stained with 1% uranyl acetate and lead citrate for 15 min.

Isolation of tissues

To investigate the transformation potential of different medusa fragments, manubria and tentacular bulbs, as well as portions of radial and circular canals, tentacles, and portions of the interradial umbrella, were excised from *T. nutricula* medusae. In addition, the potential of exumbrellar epidermis, subumbrellar endoderm, and striated muscle was investigated as isolated tissue fragments or as a combination of these tissues (Fig. 1, A–I).

Tissue isolation was performed either mechanically with microscissors and watchmaker's forceps or by short incubation in collagenase (Schmid *et al.*, 1982). Isolation of striated muscle cells from *T. nutricula* proved to be harder than in *P. carnea*, probably due to stronger adhesion of the cells to the mesoglea (extracellular matrix, or

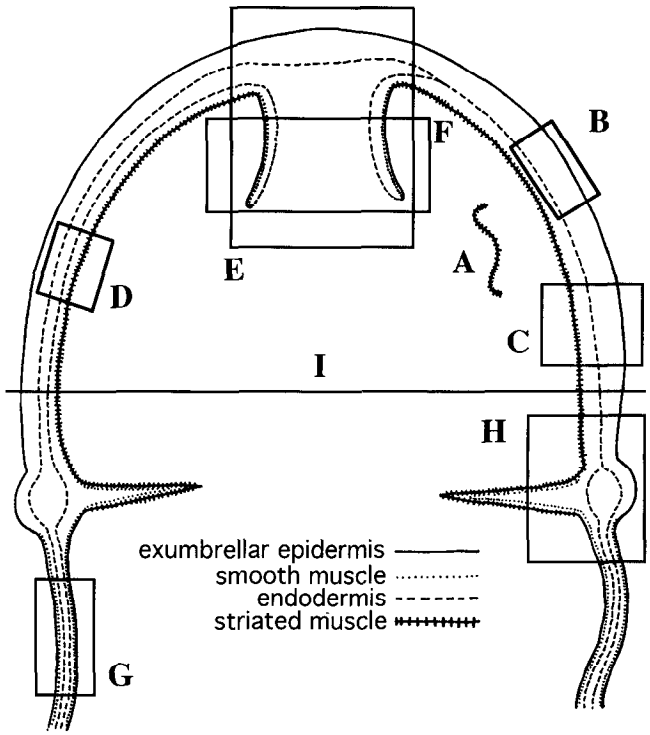

Figure 1. Scheme of dissection of tissues and fragments from *Turritopsis nutricula* medusae. A = fragments of isolated striated muscle tissue; B = exumbrellar epidermis + endoderm (interradial sectors); C = striated muscle + endoderm + epidermis (interradial sectors); D = striated muscle + endoderm of radial canals; E = manubrium + exumbrellar epidermis; F = manubrium only (middle-tip); G = tentacles; H = exumbrellar epidermis + velum + tentacular bulbs + tentacles; I = upper and lower halves of medusa.

ECM). The presence of occasional contaminating endodermal cells in the striated muscle isolates was tolerated because of their easy identification in both the light and the electron microscope (Schmid *et al.*, 1982). Tissue isolates or medusa fragments ($n = 20$) were cultured at 22°C in filtered (0.22 μm) seawater to which streptomycin (0.1 mg/ml), penicillin (100 U/ml), and chloramphenicol (0.01 mg/ml) were added. The culture media were changed daily. To induce transdifferentiation in some isolated portions of striated muscle, a post-isolation treatment with pronase (1.25 mg/ml seawater, Schmid, 1992) was also applied.

DNA replication

The pattern of DNA replication both in stable and in transforming medusae was determined to investigate whether differentiated cell types can resume cell cycle activity and be involved in the transformation process. Therefore, the rate and site of DNA replication in healthy and wounded medusae of both *T. nutricula* and *P. carnea* were investigated by pulse-labeling the animals or their fragments with 5-bromo-2-deoxyuridine (BrDU; Plickert and Krohier, 1988). For comparison, the same treatment was applied to entire *Bougainvillia muscus* medusae.

Animals were pooled in two groups. Group A: 60 medusae of *T. nutricula* and 60 medusae of *P. carnea* were cut in two with scissors (upper half of the bell with manubrium, lower half with ring canal and tentacles) and maintained at room temperature (20°C). Group B: 100 medusae of *T. nutricula,* 100 medusae of *P. carnea,* and 20 medusae of *B. muscus* were maintained as controls at room temperature.

At 0, 4, 8, 12, 24, 36, and 48 h, entire animals or medusa fragments were incubated for 4 h in BrdU medium (200 μmol/l in FSW) and then fixed in Lavdowsky's fixative (5 ml formaldehyde 37%, 2 ml glacial acetic acid, 25 ml ethanol 96%, 20 ml distilled water) for 1 h at room temperature. Then specimens were given two 10-min washes in PBS/Triton, incubated in freshly made 2 N HCl, and again washed twice, 10 min each time, in PBS/Triton. The BrdU label was detected immunocytochemically by incubation in (a) anti-BrdU antibody and (b) FITC-conjugated anti-mouse IgG antibody (Boehringer). Before observation with the fluorescence microscope, specimens were stained with Evan's blue in PBS/Triton for 2 min and with DAPI in PBS for 1 min before they were mounted in DABCO/glycerine/PBS.

A 4-h incubation in BrdU medium was applied to a single set of 20 medusae of *T. nutricula* in which the transformation process was initiated by an increase in water temperature (27°C).

The tissue distribution of labeled nuclei was recorded under a fluorescence microscope for each set of animals or fragments. To facilitate identification of the nuclei of striated muscle cells the fixed specimens were also stained with mAb 93, which is specific for the myosin heavy chain (Schuchert *et al.*, 1993).

In five specimens of both *P. carnea* and *T. nutricula* medusae, all DAPI-stained nuclei in the bell were counted.

Results

Anatomy of the medusa of Turritopsis nutricula

The bell height of newly liberated, 8-tentacled medusae of *Turritopsis nutricula* was about 1 mm. Sexual maturity was reached after 25–30 days at 20°C (or 18–22 days at 22°C), at a 16-tentacle stage with bell height of about 1.8–2 mm. Higher numbers of tentacles were never observed in our laboratory rearings, and literature on plankton records in the Mediterranean sea lacks reports of the "oceanic," larger form of *T. nutricula* medusae with a higher tentacle number (Brooks, 1886; Russell, 1953).

As in *Podocoryne carnea,* a layer of mononucleated striated muscle cells, uncontaminated by other cell types, lines the subumbrellar cavity of *T. nutricula* medusae (Fig. 2). This layer is in contact with a thin inner mesogloea. An extremely flattened, interradial subumbrellar plate

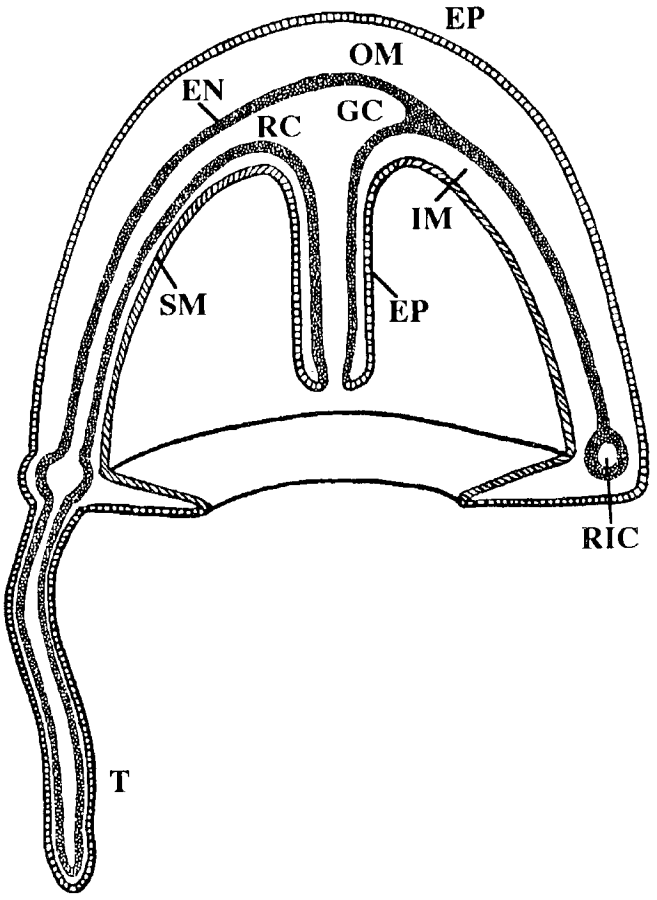

Figure 2. Anatomy of the medusa of *Turritopsis nutricula*. EP = epidermis, OM = outer mesogloea, EN = endoderm, IM = inner mesogloea, SM = striated muscle, GC = gastral cavity, RC = radial canals, RIC = ring canal, T = tentacle.

endoderm, connecting the endoderm of the radial canals (Delage and Hérouard, 1901; Bölsterli, 1977), divides the inner from the outer mesogloea (Fig. 2).

A first, dense nerve net of RFamide-positive cells is present in the epidermis of the manubrium lips. The net is connected through a few cells located above the radial canals to a second, larger network of nerve cells covering the circular canal, the tentacular bulbs and the tentacles. A second type of nerve cells was stained with the antibody against β1-integrin. These nerves form a ringlike structure that parallels the RFamide-positive nerve cells, but is restricted to the velar side of the circular canal and the tentacles. Smooth muscle cells were stained with mAb sm-1. The cells are present in the manubrium, the radial canals, the outer side of the velum, and the tentacles.

Transformation of medusae into stolons and polyps

Groups of 10 newly liberated medusae were separately reared and transformation was experimentally induced throughout development to sexual maturity either by (a) starvation, (b) sudden increase or reduction of water temperature (from 22°C to 17° or 27°C), (c) reduction of salinity (90% seawater, 10% distilled water, S = 33‰), or (d) mechanical damage of the bell with forceps or scissors. All immature medusae (up to a 12-tentacle stage) subjected to these different stresses regressed and transformed into stolons and polyps after expressing a cystlike stage. If reared under unstressed conditions, they never transformed spontaneously into cysts, stolons, or polyps. During gonad maturation (an irregular transition from 13- to 15-tentacle stages) the pattern of transformation was varied, and about 20%–40% of medusae skipped the cyst stage and transformed directly into stolons and polyps, no matter what the type of induction. Tentacle formation in this part of the cycle is rapid and changed even during the experiment. In contrast, all sexually mature medusae (at the 16-tentacle stage), even under the best culture conditions, regressed spontaneously and completely transformed into stolons and polyps.

The regression and transformation pathways of medusae into hydroid colonies are diagrammed in Figure 3, and they can be characterized as follows:

1. The developing medusa (Fig. 4) slowly enlarged its manubrium (Fig. 5), and the bell was either inverted (Fig. 6) or regressed without inversion, gradually contracting around the proximal base of the manubrium.
2. The tentacles and mesogloea degenerated and disappeared.
3. When transforming medusae were still immature, they regressed to a ball of tissue (a cyst) covered by perisarc (Fig. 7a). When cultured at 22°C, stolons were produced within 3 days (Fig. 7b). If temperature was progressively lowered to 14°C, the cysts rested for up to 3 months without losing their ability to produce stolons after the temperature was raised again to 22°C.
4. Mature medusae attached to the substrate by the apex (exumbrellar side) of the bell (Fig. 8). In general, spawning occurred shortly thereafter. A perisarc sheet and stolons soon appeared at the site of attachment.
5. Polyps were produced about 2 days after stolons had developed (Fig. 9a, b). The polyps were able to feed upon brine shrimp nauplii and to bud new medusae.

Transdifferentiation, regeneration, and transformation from isolated tissues and organs

To investigate whether the ability to transform to polyp structures is a property of the whole medusa or is restricted to only some of its organs and tissues, we dissected medusae into different body parts and cultured them (Fig. 1, A–I). Results of isolation experiments are summarized in Table I.

Medusa tissues and fragments unable to regenerate polyp tissues. Tissue fragments of mechanically isolated mononucleated cells of striated muscle (Fig. 1A; Table IA) occasionally transformed into flagellated cell types,

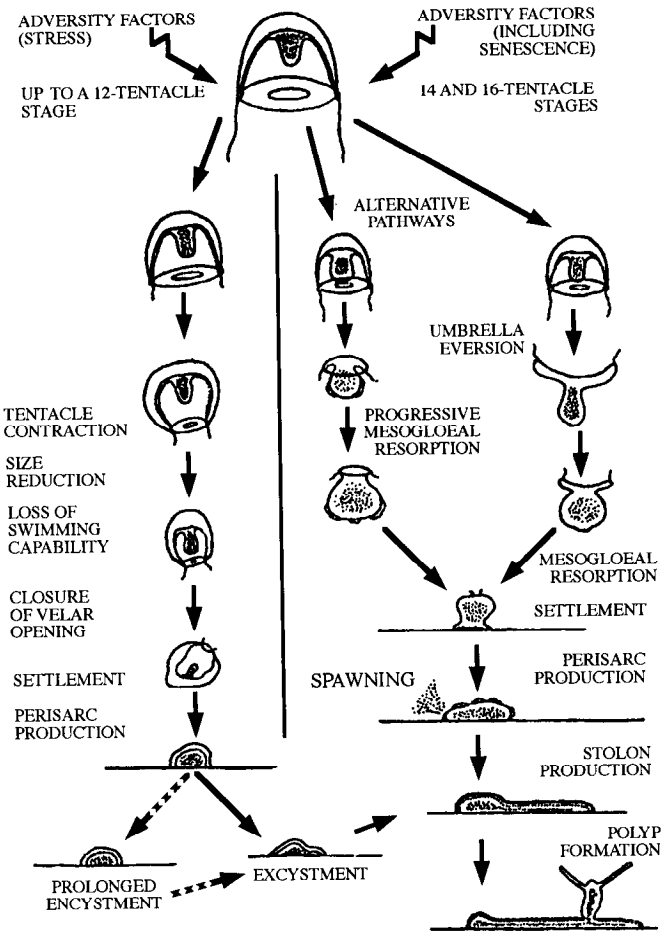

Figure 3. Pathways of transformation from medusa into polyp. Fate of stressed medusae up to 12-tentacle stage (left side), and alternative transformations of stressed or spawning medusae from a 14-tentacle or 16-tentacle stage (right side). The final product is always the polyp colony (bottom), directly or through a resting stage.

but polyp structures did not form. Myofibrils in these muscle cells progressively degenerated in the cytoplasm, while associated mitochondria clumped and degraded into lytic vesicles. However, mechanically isolated muscle cells (Fig. 1A) maintained their differentiated state (except in a few cases, see Table IA), even in the presence of radial canal endoderm (Fig. 1D, Table ID). On the other hand, the ECM-degrading pretreatment with collagenase and, overall, the pronase post-treatment induced a higher proportion of muscle cells to destabilize with degradation of striated myofibrils and transformation into flagellated cells (Fig. 10). As in *P. carnea* smooth muscle (Schmid and Alder, 1984), these transdifferentiating cells typically showed peripheral vacuoles filled with electron-dense material (Fig. 10).

When interradial fragments, consisting of exumbrellar epidermis and some endodermal cells (Fig. 1B; Table IB), were activated by collagenase treatment, flagella could form *de novo* (not shown). Occasionally, a thin perisarc layer was secreted (Fig. 11). However, stolon and polyp formation was never observed.

Interradial sections (Fig. 1C; Table IC) of the medusa bell containing striated muscle, endoderm of the subumbrella plate, exumbrellar epidermis, and both inner and outer mesogloea maintained a stable state for several days; cell dissociation then occurred, starting with the exumbrella cells, and the fragments fell apart.

Isolated manubria and tentacles (Fig. 1, F–G; Table I, F–G) did not transform into stolons and polyps. These fragments were highly stable for several weeks.

Medusan tissues and fragments regenerating polyp tissues. Full transformation into stolons and polyps was observed when excised manubria and tentacles additionally contained cells of exumbrellar epidermis and portions of the canal system (Fig. 1:E, H, I; Table I: E, H, I). In addition, bell fragments containing exumbrellar epidermis and portions of the radial or circular canal fully transformed into stolons and polyps (not included in Table I).

The pattern of DNA replication in different species of Anthomedusae

The location pattern of DNA-replicating nuclei in healthy, newly liberated or young (up to 10 days old) medusae of *T. nutricula* (n = 100), *P. carnea* (n = 100), and *B. muscus* (n = 19) was investigated by incubating animals of different ages every 4 h for 2 days in BrdU medium (see methods). No significant differences in the rate of DNA replication were observed in these 2 days.

Medusae of all three species showed DNA replication in the manubrium, the tentacular bulbs and, to some extent, the proximal part of the tentacles. No specimen of *B. muscus* showed DNA replication in the umbrella proper (without circular canal and manubrium). Umbrellar DNA replication was detected in 28% of *P. carnea* specimens, but only 3% of the animals had more than 5 labeled nuclei, out of a total of 2300 ± 250 bell nuclei (Fig. 12). On the other hand, more than 75% of *T. nutricula* specimens showed DNA-replicating nuclei in the umbrella, 5% having from 50 to 250 labeled nuclei out of 5000 ± 500 bell nuclei (Fig. 13). Although labeled nuclei of exumbrellar epidermis cells were easily identified (Fig. 14), generally the subumbrellar endoderm could not be clearly distinguished from the striated muscle cells in untreated animals, so the DNA replication potential for these two cell types could not be accurately evaluated. However, striated muscle cells must be considered basically out of cell cycle activity (Schmid, 1992).

The effect of adversities on the rate of DNA replication

To investigate the influence of mechanical stress on DNA replication, medusae of both *P. carnea* and *T. nutricula* were cut in half (Fig. 11) before BrdU incubation. No significant differences in the number of DNA-repli-

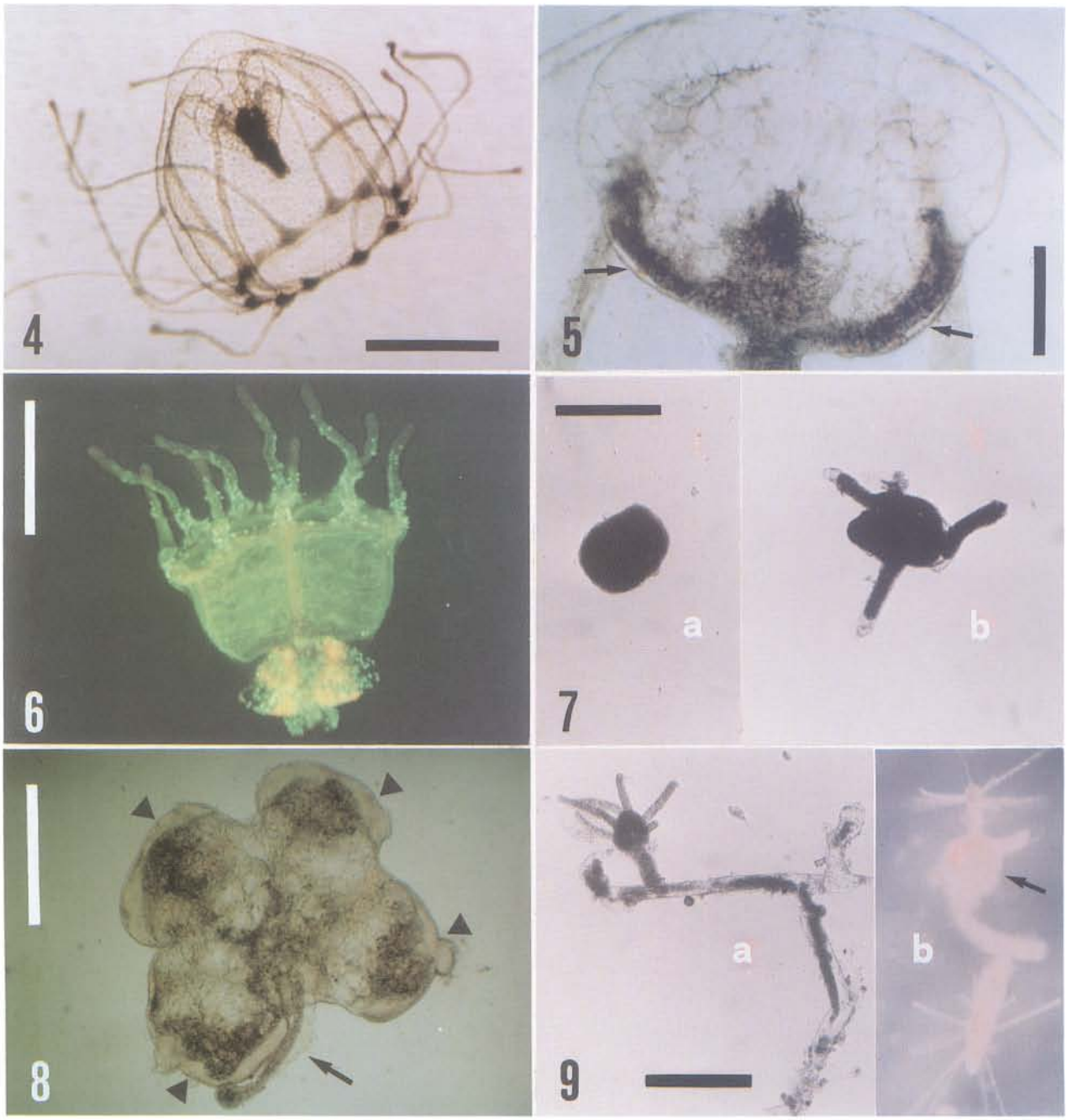

Figure 4. Medusa of *Turritopsis nutricula.* (12-tentacle stage). Scale bar: 1 mm.

Figure 5. Initiation of transformation: enlarged manubrium from a 16-tentacle medusa stage. Gonads in the process of maturation are already recognizable (arrows). Scale bar: 250 μm.

Figure 6. Everted medusa with extruded manubrium (bottom). Scale bar: 1 mm.

Figure 7. Encystment of an immature medusa of *T. nutricula:* (a) with outgrowing; stolons (b) 3 days after settlement of the medusa. Scale bar: 1 mm.

Figure 8. Male medusa of *T. nutricula* (reduced to a manubrial vestige settled on a glass slide) budding a stolon (arrow) while still retaining mature gamete clumps on four lobes (arrowheads). Scale bar: 1 mm.

Figure 9. Polyps of *T. nutricula* on stolons developed from a transformed medusa. Preserved (a) and living specimens (b) with medusa cyst still recognizable (arrow). Scale bar: 1 mm.

cating nuclei were detected between cut and control specimens in either species.

Before transformation of wounded medusae into polyp tissues, mechanical stress caused umbrellar eversion of upper half-cut parts of *T. nutricula,* so that the striated muscle cells were stretched outwards (Fig. 15),

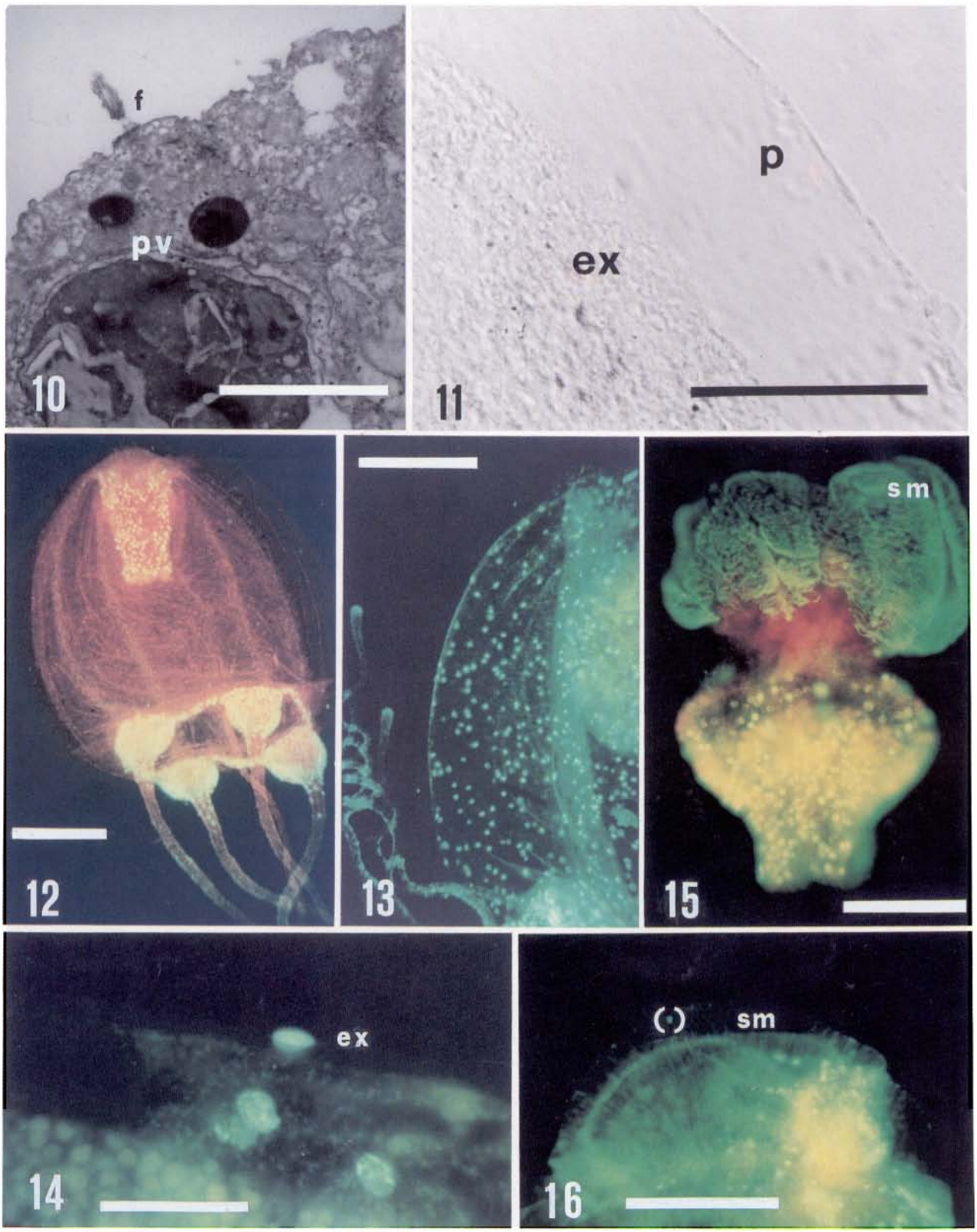

Figure 10. Electron micrograph of an isolated fragment of activated striated muscle tissue transforming into smooth-muscle-like, flagellated cells. pv = peripheral vesicles, f = flagellum. Scale bar: 5 μm.

Figure 11. Destabilized exumbrellar epidermis (ex) from transforming *Turritopsis nutricula* medusa, showing large perisarc secretion (p). Scale bar: 80 μm.

Figure 12. *Podocoryne carnea* medusa. BrdU staining of DNA replicating nuclei (courtesy of G. Plickert). Replicating nuclei are concentrated in the manubrium and tentacular bulbs. Scale bar: 500 μm.

Table I

Destabilization of and stolon formation from isolated medusa fragments (A–I, see Fig. 1)

Isolate composition	Isolation/treatment procedure	Destabilization	Stolon formation
A Striated muscle	Mechanical	+	–
	Collagenase	++	–
	Pronase*	+++	–
B Exumbrellar epidermis + endoderm	Collagenase	+	–
C Striated muscle + endoderm + epidermis (interradial sectors)	Mechanical	–	–
D Striated muscle + radial canals	Mechanical	–	–
E Manubrium + exumbrellar epidermis	Mechanical	+++	+++
F Manubrium only (middle–tip)	Mechanical	–	–
G Tentacles	Mechanical	–	–
H Ex. epidermis + tent. bulbs + tentacles	Mechanical	+++	+++
I Upper half of medusa	Mechanical	+++	+++
Lower half of medusa	Mechanical	+++	+++

Striated muscle was isolated either mechanically or with collagenase. Occasionally, mechanically isolated fragments were treated with pronase (*). Number of isolates: A–H = 20; I = 60. Destabilization was scored either by formation of flagella or by production of perisarc, adhesion of fragments to the bottom of culture plates, or both. Percentage of destabilized fragments: + = <5%; ++ = 5%–50%; +++ = 50%–100%.

being more distinguishable from the subumbrellar plate endoderm. In this case, DNA replication was easily detected in a few nuclei belonging to the striated muscle sheet (Fig. 16).

Also, DNA replication was unequivocally resumed in some nuclei of well-differentiated muscle cells (Fig. 17a, b) from unwounded medusae, whose transformation was induced by heat stress.

Discussion

Does death occur in Turritopsis nutricula?

Turritopsis nutricula is a unique medusa and its transformation potential is unparalleled within the vast array of life-cycle patterns found in cnidarians. This is the first known case of a metazoan being capable of reverting completely to a clonal life stage after having achieved sexual maturity in a solitary stage.

Because all *T. nutricula* medusae regularly underwent transformation, we must assume that organismic death does not occur in this species. Hydrozoans are modular organisms in their polyp stage, but the medusa stage is unitary. Modular organisms have a potentially indefinite life span, but this is usually impossible for nonmodular ones, in which the onset of sexual reproduction ultimately leads to death (reviewed in Stearns, 1992). In planarians, aging is retarded through reduction in size, or degrowth, the regressing adults resembling juveniles in both morphology and physiology (reviewed in Calow, 1978). In bryozoans, colonies rejuvenate by dismantling their bodies and rebuilding them from undifferentiated cells (Gordon, 1977). Colony size is generally reduced in social and compound ascidians by the resorption of zooids, leaving potential buds of presumptive germinative tissue that quickly develop into new blastozooids under favorable conditions ("survival budding" as overwintering tactic: Nakauchi, 1982). All these processes, however, imply only a reorganization of the original morph. Instead, the transformation of *T. nutricula* may be considered a metamorphosis, though in a direction opposite to the usual ontogenetic path. In this same sense, a case of reversible metamorphosis was previously reported in corals in which primary polyps revert to "secondary" planula larvae when suitable conditions for

Figure 13. *T. nutricula* medusa, portion of bell shown. BrdU staining of DNA replicating nuclei (incubation: 30 min). Besides manubrium (arrow) and bulbs (arrowheads), labeled nuclei are widespread throughout the whole umbrella. Scale bar: 500 μm.

Figure 14. DNA-replicating cell of exumbrellar epidermis (ex) from the margin of *T. nutricula* medusa bell. Scale bar: 50 μm.

Figure 15. Half-cut, upper part of BrdU-incubated medusa of *T. nutricula* with extruded manubrium (bottom) and outwardly strained striated muscle sheet (sm). Striated myofibrils stained with mAb 93. Scale bar: 40 μm.

Figure 16. Outwardly strained striated muscle sheet (sm) from half-cut, upper part of BrdU-incubated medusa of *T. nutricula*. Brackets point out a DNA-replicating nucleus from the striated muscle sheet. Scale bar: 200 μm.

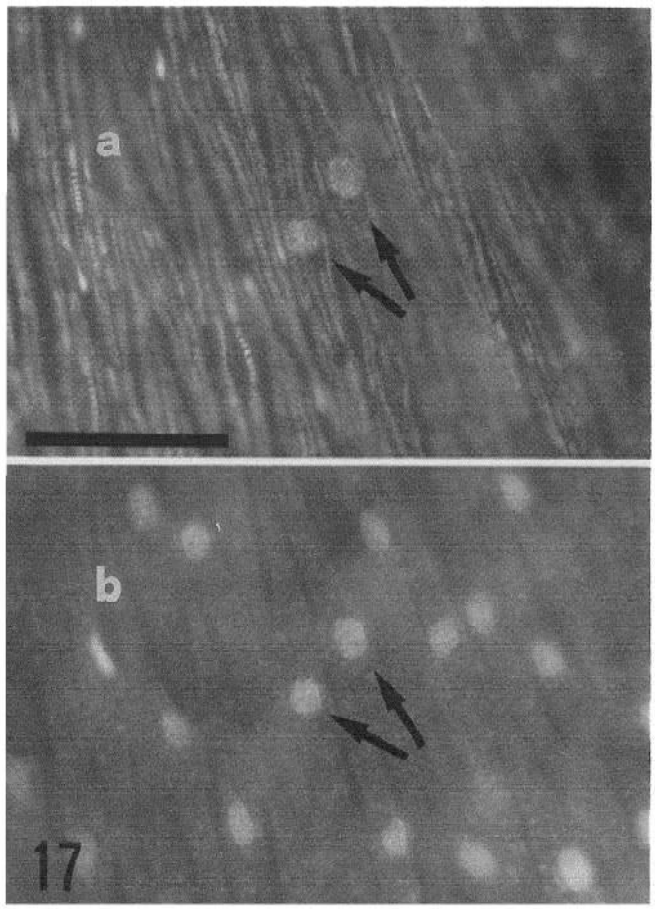

Figure 17. Striated muscle cells from BrdU-incubated, whole medusa of *Turritopsis nutricula* undergoing transformation into polyps after rearing at T = 27°C. (a–b) same frame: (a) two DNA-replicating, BrdU-labeled nuclei (arrows), with mAb 93-stained myofibrils running around them; (b) DAPI staining showing the two BrdU-labeled cells (arrows) surrounded by nonreplicating, striated muscle cells. Scale bar: 50 μm.

polyp colony growth are not encountered after "primary" planulae settlement (Richmond, 1985). However, that ontogenetic reversal occurs at the beginning of development, before colony growth and sexual reproduction, and does not involve a return to clonal or larval stages from aclonal ones. Similarly, scyphozoan ephyrae are reportedly able to transform into polyps under starvation conditions (Hadzi, 1912). As already mentioned, this "metamorphic" potential is present in the first stages of medusan development of other hydroidomedusan species, but is lost before the medusa detaches from the polyp (Müller, 1913; Hauenschild, 1956; Frey, 1968; Kakinuma, 1969; Schmid, 1972). In contrast, in *T. nutricula* each stage of medusa development can shift to hydroid structures as a response to adverse conditions, including senescence. This is an exceptional situation, like that of a hypothetical insect imago able to reverse to a larval stage after sexual reproduction.

Stem cells (interstitial cells) and transdifferentiation

The change of medusae into polyps requires differentiation of new cell types and major reorganization of tissues. New cell types could arise either through a differentiation of uncommitted stem cells of the medusa or by cellular trandifferentiation of differentiated medusa cells.

The presence of a population of continuously proliferating cells (stem cells, interstitial or I-cells) was long thought to play a key role in asexual reproduction, in regenerative processes, and in the apparent lack of senescence observed in both colonial and solitary cnidarians (Brien, 1953; Crowell, 1953; Comfort, 1956; Brock and Strehler, 1963; Toth, 1969; Hughes, 1987; Buss, 1987). These cells are characterized by their histological features, but their state of commitment cannot be determined by isolation and cloning experiments. The role of interstitial cells is best understood in *Hydra* spp. in which these cells are able to differentiate into nematocytes, nerve cells, and germ cells (Tardent, 1978; Werner, 1984). It seems that, as a gross morphological category, interstitial cells include partially differentiated stem cells (Bode, 1992), but the question of whether their repertoire includes secretory cells is still unresolved (Smid, 1984). In any case, their influence on morphogenetic processes seems to be rather limited. Interstitial-cell-free *Hydra* polyps are able to regenerate fully and reproduce asexually (Brien, 1955; Diehl and Bouillon, 1966; Marcum and Campbell, 1978).

Recruitment of differentiated cells for transdifferentiation and regeneration processes was demonstrated in the medusa *Podocoryne carnea* (for review see Schmid, 1992). In that case, isolated and activated endoderm or striated muscle cells can resume DNA replication, transdifferentiate to several new cell types, and regenerate *in vitro* medusa organs. However, they are unable to regenerate polyp organs such as stolons or perisarc-secreting tissues (Schmid, 1992). Transdifferentiation in hydroidomedusae might be a common phenomenon in budding and regeneration, especially where no or few I-cells are located (Brien, 1941). Some hydroidomedusae belonging to quite different families (*e.g., Zanclea prolifera, Teissiera australis, Bougainvillia vervoorti, Proboscidactyla ornata, Cytaeis* spp., *Eirene elliceana, Eucheilota paradoxica, Clytia mccradyi*) have the ability to bud (but not to metamorphose into) polyp structures before sexual reproduction. Also, direct medusa budding from liberated medusae is known in several species at three alternative sites: (1) manubrial wall (*e.g., Sarsia gemmifera, Eucodonium brownei, Podocoryne minima, Bougainvillia niobe, Rathkea octopunctata, Lizzia blondina*); (2) radial canals (*e.g., Proboscidactyla ornata, Eucheilota paradoxica*); and (3) tentacle bulbs (*e.g., Niobia dendrotentaculata, Hybocodon prolifer, Sarsia prolifera*). These observations led to the assumption that both epidermis and endoderm are basically totipotent (Berrill, 1950). Indeed, manubrial budding

of medusae in *Rathkea octopunctata* seems to derive from the sole contribution of differentiated epidermal cells, with no aid from the I-cell compartment (Bouillon, 1961–1962). However, in all the other cases, the contribution of transdifferentiation processes is still unstudied.

Transformation of T. nutricula *medusae*

The excision experiments and the DNA-replication pattern in the nontransforming and the transforming medusa indicate that the transformation process does not depend exclusively on stem cells. In fact, isolated manubria, containing a large population of replicating stem cells (Tardent, 1978), are unable to tranform into stolons and polyps. Transformation occurs only in fragments that contain tissue of the exumbrella and of the canal system (the radial canals or the ring canal). Interstitial cells do not occur in the exumbrellar epidermis and are not prominent in the canals. The exumbrellar epidermis is the only tissue able to transform into the perisarc-secreting, epidermal tissue of the stolons; thus, we must conclude that the exumbrellar epidermal cell type clearly undergoes transdifferentiation. However, its potential is limited because the fragments are not able to reconstitute the endoderm needed to complete the transformation process. The endodermal lining of the canals evidently harbors the cells that give rise to the endoderm of stolons and polyps.

Therefore, the main question is what cell type (or types) in the transforming medusa or fragment is able to generate the missing cell types (sensory cells, myoepithelial cells, cnidocytes) needed to complete the transformation process. The presence of a few scattered interstitial cells cannot be excluded, and these cells could be responsible for the completion of the cell-type inventory. However, the available information is insufficient to allow discussion of this possibility. On the other hand, transdifferentiation from one or several differentiated cell types (secretory or digestive cells) could be an additional alternative. Transdifferentiation in general requires DNA replication (for review see Okada, 1991). Compared to the bell of *B. muscus,* where no DNA replication was shown, and of *P. carnea,* where DNA replication was scarce, in *T. nutricula* the cells of the bell can still undergo intensive DNA replication. This is especially true for the cells of the exumbrella, the endoderm of the radial canals, and those of the subumbrellar plate endoderm. Traumatization and heating experiments in *T. nutricula* showed DNA replication to occur in striated muscle nuclei too, suggesting that even some of these cells can contribute by transdifferentiation to the transformation process.

Conclusions

According to our results, the transformation of mature medusae of *T. nutricula* into stolons and polyps is an established trait in the life cycle of this species. It is not known whether these processes occur under natural conditions, because they take place in a rather short time and are difficult to observe in the field. Laboratory observations, however, demonstrate that *T. nutricula* has a transformation potential that has never been recorded in any other cultured species, and it seems improbable that such potential is expressed only under laboratory conditions. Excision experiments demonstrate that cells of the exumbrella and of the gastrovascular system are required for transformation. The perisarc-secreting cells of the stolons appear to be formed by transdifferentiation of some cells of the exumbrella. A contribution by differentiating stem cells (interstitial cells) cannot yet be ruled out. Comparative studies on the pattern of replicating nuclei among different species demonstrate that the cell types of the bell, including well-differentiated striated muscle cells, in *T. nutricula* have retained the ability to replicate. Further studies are required to investigate the role of DNA replication in the transformation process and the mechanisms that regulate this life-cycle reversal at the molecular level.

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