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Original Article

A shoot meristem-like organ in animals; monopodial and sympodial growth in Hydrozoa

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ABSTRACT Thecate Hydrozoa produce stems from which polyps branch off. Similar to plants these stems form in two ways, either in a sympodial or in a monopodial type of growth. In the latter group a terminal organ develops which has similarities to a shoot apical meristem of higher plants: it elongates without a further differentiation. Similar to leaf formation in plants, thecate Hydrozoa produce polyps in a repetitive manner. This process continues during the whole life of the animal and has not yet been found to be limited by internal mechanisms. We studied the monopodially growing thecate Hydrozoon *Dynamena pumila* and suggest that the stem tip, the apical shoot meristem-like organ, is a polyp primordium hindered to develop into a polyp by the laterally developing polyps.

KEY WORDS: *Dynamena pumila, pattern formation, shoot apical meristem*

Introduction

Hydra, the best known hydrozoon, displays the basic morphology of a hydrozoa polyp: The body is tube-shaped with a ”head”, a mouth / anus opening surrounded by tentacles at one end, and a basal disc at the other end. The body wall consists of two layers, the ectoderm and the endoderm. These layers or “tissues” consist of various cell types which are separated by an extracellular matrix, the mesogloea. Most hydrozoa form colonies. The simplest types of colonies form by producing hollow tubes at the base of the polyps, termed stolons. The stolons are fixed to the substrate. They elongate like roots and give rise to further polyps some distance away from the original polyp. Solitary polyps like Hydra and complex colonies, in general, grow and reproduce asexually by forming buds whose tips have one out of two developmental fates: either the bud tip eventually transforms into tissue surrounding the mouth / anus opening of a polyp or the bud tip develops into a basal disc / stolo tip. This is also true for polyps of scyphozoa, like that of *Aurelia aurita* or *Cassiopea andromeda*. In addition, body sections of solitary polyps or colonies regenerate either a head or a basal disc / stolo tip at their wound. (In a few cases no structure forms and development ceases). Thus, it appears that the pattern forming mechanism is constructed in such a way that one of the alternatives has to be chosen, necessarily.

However, one group of hydrozoa appears to violate this rule, the monopodially growing thecate Hydrozoa. Thecate hydrozoa are covered with a rigid outer skeleton, termed perisarc. The perisarc covers the stolons as well as the stem and forms a housing for the polyp, the hydrotheca. Among the thecate hydrozoa one can distinguish monopodially and sympodially growing colonies (Kühn, 1909). The sympodial colonies grow in a conventional manner: A mesh of tubes, termed stolons, fixed to a substratum produce stems which bear polyps (Fig. 1). Starting from the roof of the stolo a polyp bud grows out which finally differentiates into a polyp. At a certain distance from the apical end of this polyp a bud forms, similar to that on the stolo roof. This bud grows out and differentiates into a polyp at its terminal end. Therewith, the colony grows step by step in form of repetitive units. Monopodially growing colonies have a further type of growing tip, the stem tip (Fig. 1). This tip (usually) neither ends as a stolo tip nor as a polyp, but rather grows to form a stem out of which laterally polyps grow out in a repetitive manner. Therewith, these animals have organs similar to both, the root and the shoot meristem of higher plants.

In higher plants, the shoot apical meristem consists of a small group of morphologically undifferentiated dividing cells laid out in an organised manner (reviewed in Evans and Barton, 1997, Brand *et al.*, 2001). Cells at the summit of the shoot apical meristem form the central zone (approximately four to six cells wide), which is the source for the peripheral zone where organ primordia develop and for the underlying rib zone. In *Arabidopsis* sp. the shoot apical meristem never develops into a flower but remains indeterminate until the apex senesces. *Arabidopsis* sp. belongs to the group of higher plants with monopodial growth whereas the tomato belongs to the group with sympodial growth. Certainly, plants and animals are differently organised but the principles of pattern formation may be similar.

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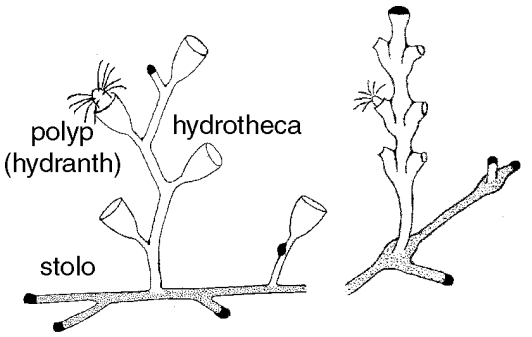

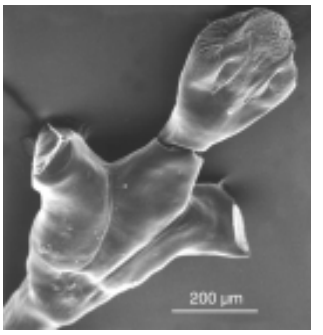

Fig. 1 (Scheme). Drawings of a sympodially (left) and of a monopodially (right) growing colony of thecate Hydrozoa. *The areas in black indicate where the outer skeleton, the perisarc tube elongates. The stippled area indicates the stolo.*

Fig. 2 (Photo). *Dynamena pumila*. *Stem (hydrocaulus) with two internodes. The furrow clearly seen between the youngest and the next older internode is called the node. At the time of fixation the youngest internode has not yet completed its development. The crumbling of the outer surface at the apical end of the youngest internode, an artefact of the preparation, indicates the soft perisarc. Hard perisarc is not crumbled.*

The hydrozoon *Dynamena pumila* forms polyps in a repetitive manner right and left to an elongating stem. Most of these so-called internodes look identical (Fig. 2) but some show severe malformations. For instance, instead of a stem the central primordium forms a polyp, as well. If possible, the malformations are followed by the most common, the normal internode form. Thus, (somatic) mutations can be excluded as a cause for the development of malformations. Rather, certain external conditions are suspected to somehow influence the pattern forming system in a reversible manner. Such conditions may include an extreme temperature or salinity during the hours the colonies may fall dry at low tide. These malformations are the basis for the present study. The aim of the present study is to better understand the stem tip of a monopodially growing hydrozoon.

Results

The Formation of an Internode

The stem or hydrocaulus of *D. pumila* consists of a sequence of so-called internodes (Fig. 2). In lateral position an internodium generally bears two polyps (hydranths) and their housing (the theca) and in the middle the rather unstructured stem. Under laboratory conditions, at 18^{0}C the formation of one internode took 36 to 72 hours.

The development of an internode is arbitrarily subdivided into three phases.

In phase 1 the stem primordium elongates. In phase 2 the stem divides into three primordia by furrows. In phase 3 the lateral primordia differentiate into hydranths while the central one does not differentiate but grows out.

Phase 1. In *D. pumila* as in other thecate Hydrozoa, the whole colony is covered with a chitinous extracellular matrix, the perisarc (cf. Fig. 3A). At the apex the material is soft and thin. Proximal to that it is thick and rigid. The ectoderm of the tip has a tight contact to the soft material. The ectoderm in a more proximal position has contact to the already hardened perisarc. Therewith, the perisarc is exclusively shaped by the activity and form of the tissue in the tip. A colony of thecate Hydrozoa elongates exclusively at its tips: The rigidity of the perisarc prevents an intercalary growth of the old perisarc tube. Within the tissue tube cell multiplication is expected to take place as it is observed in thecate hydrozoa so far analysed, e.g. in *Obelia loveni* by Kossevitch (1999). Therewith, an intercalary growth of the tissue tube is combined with a terminal elongation of the perisarc.

Early in phase 1 the apex of the stem has the shape of a hemisphere. During elongation the size of the distal surface of the stem increases but almost exclusively in the plane of the foregoing internode (Fig. 2). The ectodermal cells are thought to be the

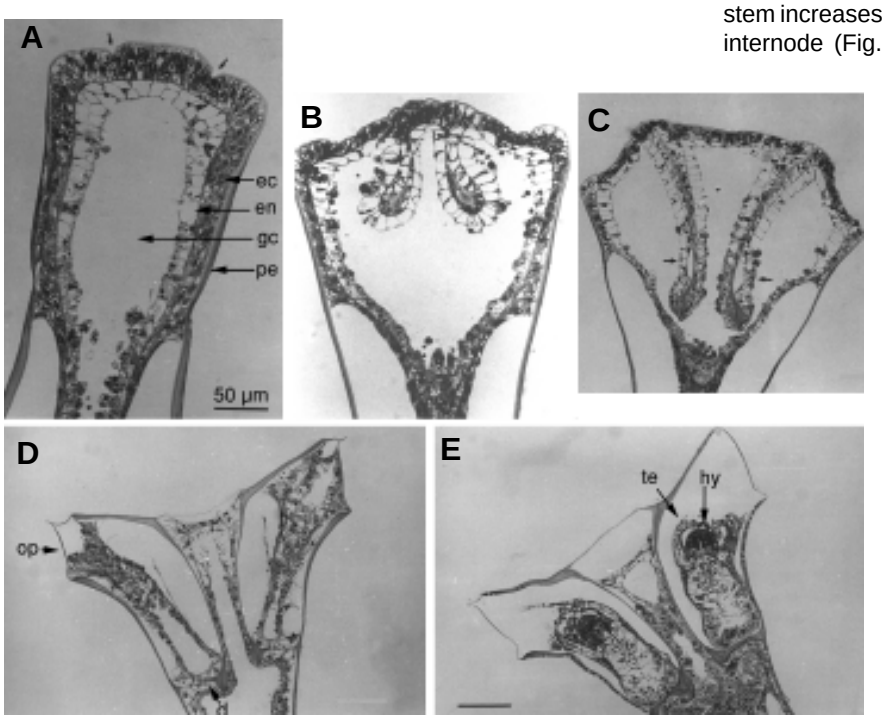

Fig. 3. Development of an internode in *D. pumila*. *Longitudinal sections.* **(A)** *The beginning of phase 2. Two furrows (arrows) form at the distal end indicating the visible initiation of the formation of three primordia out of one. ec, ectoderm; en, endoderm; gc, gastric cavity; pe, perisarc.* **(B)** *Middle of phase 2. Note the perisarc deposition in the proximal part of the furrow.* **(C)** *End of phase 2. Note the separation of the ectoderm from the perisarc in the furrow of the hydranth forming primordia (arrows). At the opposite site the ectoderm of the hydranth forming primordium has also lost contact with the perisarc.* **(D)** *Phase 3. The perisarc in the furrow has come into contact with the perisarc deposited at the distal surface. The head of the hydranth (polyp) starts to form and the diaphragm (d). An operculum (op) has formed.* **(E)** *End of phase 3. Hydranths are fully developed and are ready to stretch out of the hydrotheca, the housing of the hydranth. At the apical end the hypostomal cells (hy) and the tentacles (te) have differentiated. The central primordium is covered within thin perisarc. The retraction of the tissue from the apical perisarc is probably an artefact of the preparation.*

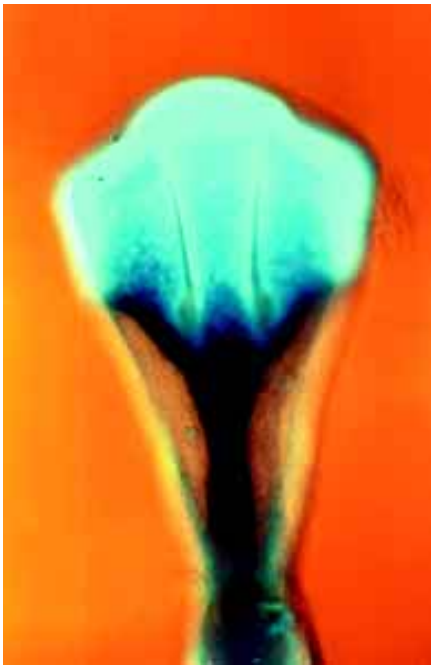

Fig. 4. Whole mount of an internode in the middle of phase 2 stained with Calcofluor White M2R. *The dye particularly stains amorphous chitin before its final hardening.*

driving forces for the elongation of the stem. The processes involve growth pulsations during which the vacuoles of the ectodermal cells change their volume strongly (Beloussov *et al.*, 1972, 1984, Wyttenbach 1974, Beloussov and Dorfmann, 1974). The endodermal cells are highly vacuolated where the ectoderm has a tight contact to the perisarc. At the proximal end of this region the vacuoles are smaller. In the narrow tissue tube, which follows in proximal direction, the endodermal cells do not contain vacuoles. This distribution of vacuoles is present in the following developmental phases as well. The ectodermal cells in the internode are generally less vacuolated than the endodermal cells. Vacuolated ectodermal cells are particularly concentrated on the distal surface where the tissue forms a strong convex curvature.

Phase 2. This phase starts when two transverse furrows form at the apical surface (Fig. 3A). The furrows visibly start at the outer surface of the ectodermal cell sheet. Initially, both the mesogloea (the extracellular matrix between the ectoderm and the endoderm) and the endoderm are not involved in furrow formation. It appears that the furrows are formed by stripes of hardened perisarc material which has contact to the hardened perisarc in the proximal part of the growing tip. Therewith, these stripes of hardened perisarc cause the sheet of tissue at the apex to protrude in three separated bulbs.

As a consequence, the mesogloea and the endoderm get involved in the process of tissue folding. In the proximal part of the furrows perisarc becomes visible (Fig. 3B). In the distal part of the furrow a separation into two ectodermal layers could not be traced with certainty. In addition, in this part of the furrow we even failed to detect soft perisarc material (Fig. 3 B,C). The perisarc in the furrow is confluent with the perisarc surrounding the internode. Therewith, in the proximal part there is a complete separation of the three primordia. In the most proximal part of the furrow the perisarc is the thickest of the whole internode, while the distal part of the furrow is apparently free of perisarc. Following a treatment with Calcofluor White M2R the primordia became stained. The dye preferentially stains various polysaccharides, including chitin, in particular before its crystallisation (Gooday, 1996). It appears, that perisarc material is secreted predominantly by that part of the ectodermal tissue which is in tight contact with the perisarc (Fig. 4).

At the end of phase 2 the ectoderm lining the proximal part of the furrow separates from the perisarc (Fig. 3C, arrows). Such a separation is observed where the distal part of the polyp will form. It does not separate from the most proximal part of the perisarc in the furrow where later on the diaphragm will form (see below).

Phase 3. Finally, thick perisarc is laid down in the distal part of the furrows and at the distal surface of the internode close to the position where the furrow ends. The reason may be that the centres of the lateral primordia have moved to a more peripheral position (Fig. 3D).

In the lateral primordia polyp differentiation visibly starts just before the primordia become isolated by deposited perisarc on the distal surface between them. At the base of the furrows the differentiation of a diaphragm starts: The two tissue layers protrude in form of a collar into the open space of the polyp primordium (Fig. 3D). At the distal end of a developing polyp the mouth and the tentacles differentiate while the ectoderm of this region is still in contact with the perisarc. Finally contact is lost (Fig. 3E). The polyp's housing opens distally and the polyp is free to stretch out. In the course of phase 3 the tissue of the central primordium has changed much less compared to that of the lateral ones.

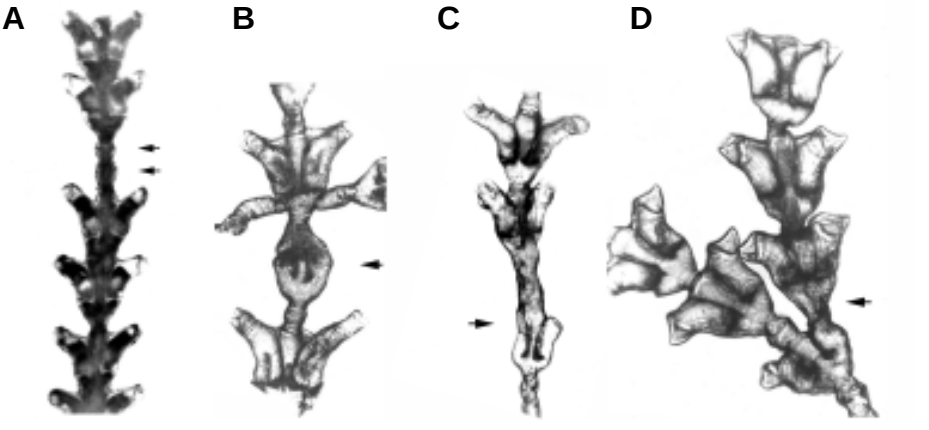

Fig. 5. Malformations of *D. pumila*. (A) *A hydrocaulus with long and short distances between internodes. Note the two bulbs (arrows).* **(B)** *The arrow indicates an internode (arrow) in which the three primordia have fused after a certain period of separated growth. Note the two stripes of perisarc between the primordia. The next internode is of normal shape. It formed two branches some time after having completed its development.* **(C)** *Two of the three primordia fused (arrow) while one developed into a hydranth and its housing.* **(D)** *The primordium on the right (arrow) has developed into a stem tip.*

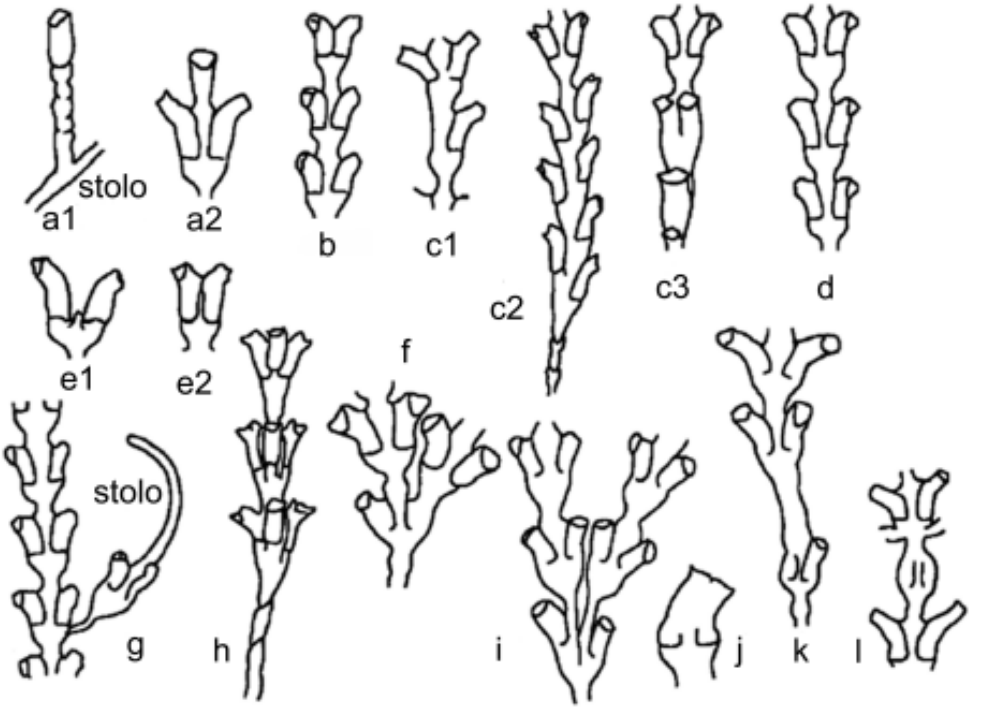

Fig. 6. Malformations of *D. pumila:* (a) *The stem transformed into a hydranth. (a1) A hydrocaulus (stem) emerging from a stolo directly developed into a hydranth. Shown is the hydrotheca. (a2) The central primordium developed into a hydranth. Note the symmetric form of the hydrotheca in (a1) and (a2).* **(b)** *Only two primordia have formed at the end, both developed into hydranths.* **(c)** *Two primordia formed, one of them developed into a hydranth, the other into the stem. (c3) After having formed two primordia per internode, three formed, but the first developed internode did not develop in a straight line.* **(d)** *The normal morphotype.* **(e)** *The primordium in the centre has stopped its growth.* **(f)** *One of the lateral primordia also developed into a stem (cf. Fig. 5D).* **(g)** *A branch developed into an internode in which the central primordium developed into a stolo.* **(h)** *Four primordia formed in a bundle. One of them which was close to the centre developed into a stem, the others into hydranths.* **(i)** *Four primordia formed. The two in the centre developed into stems.* **(j)** *Three primordia formed and fused after a period of separate growth to form a single hydranth.* **(k)** *Three primordia formed, two fused after some period of separate growth to form a stem (cf. Fig. 5C).* **(l)** *Three primordia formed and fused after a period of separate growth to form a single stem (cf. Fig. 5B). The figures of some malformations shown are obtained from Marfenin et al. (1995).*

Naturally Occurring Malformations

A variability in the architecture of internodes has been mentioned repeatedly. With respect to *D. pumila* the most detailed description was published by Marfenin *et al.* (1995) who analysed more than 200 000 internodes of colonies collected from the White Sea. We studied colonies of *D. pumila* collected from the North Sea at Helgoland and found almost the same malformations but a few others in addition. Between 1 and 0.1 % of the internodes were found to be different from the most abundant type.

The general impression is that the dimensions of the hydrothecas, the polyps' housing, are almost constant, but the length and the shape of the stem in phase 1 varies strongly: In Fig. 5A the distance between the youngest two internodes is very short, while the distance between the next older ones is very long. In the stem two transient widenings (arrows) can be seen proximal to the final widening which leads to the new internode. The following two types of malformations (Fig. 5 B,C) appear to be not yet described. Figure 5B shows an example in which all three primordia have fused after some period of separate elongation. The next internode formed a lateral branch. This occurs rather often and only takes place after several additional internodes have formed in the same line. In the case shown in Fig. 5C the subdivision into three primordia has taken place but two of them have fused again. The primordium on the right formed a hydranth, that on the left fused with the central one. Perisarc is deposited between the proximal parts of the primordium in the centre and the primordia on the left and on the right. In the specimen shown in Fig. 5D one of the lateral primordia transformed into a further stem primordium.

Figure 6 includes the normal type (Fig. 6d) and malformations observed by Marfenin *et al.* (1995) and us. Not all malformations listed by Marfenin *et al.* (1995) are included. Omitted are those which appear to have developed by regression and a new beginning, e.g. those which show a stolo or a second hydrotheca to emerge out of a fully developed hydrotheca. Further, the figure does not include malformations which show an unusual bending or twisting of the body or an incomplete development of the hydrotheca.

Discussion

Control of the Number and the Spatial Arrangement of the Primordia

When a stem of *D. pumila* grows in length, generally three primordia form out of the initial single one. The two lateral ones develop into polyps, the central one elongates, subdivides into three primordia, and therewith, starts a new cycle of internode formation. Occasionally, two or four primordia form. In most cases the primordia are arranged in a line, rarely are they clustered (Figs. 6 c3, h). We suggest the size and the shape of the distal surface (e.g. the region where the persisarc is thin and soft) to determine how many primordia form and how they are arranged (end of phase 1). Such self organisation processes are well studied by means of computer simulations. Two principles appear to be necessary: a self enhanced activation (autocatalysis) and an inhibition of that activation (lateral inhibition). The activation has a short range, the inhibition has a long range (Gierer and Meinhardt, 1972). The studies show that when a morphogenetic field grows in size the area of activation within this field does not increase accordingly, rather, in the available space additional areas of activation develop which are separated from each other due to lateral inhibition. If the space is just too small for three areas, it is the middle one which has a high chance to become finally extinct due to the inhibitory input from both sides. Malformations are found which appear to fit into

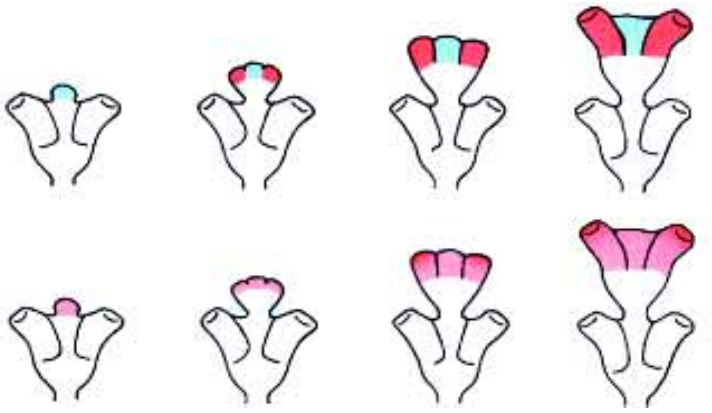

Fig. 7. Alternative models of pattern control for *D. pumila*. *Polyp and stem formations are controlled either by stem or polyp pattern forming systems (Upper panel) or by one pattern forming system (Lower panel).* **(Upper panel)** *Three primordia form out of one. Qualitatively different pattern forming systems (red for polyp, blue for stem) become active at the appropriate site and take over the control of the further development. Certain interactions address the correct system to every originating primordium.* **(Lower panel)** *One and the same (primary) pattern forming system controls the development of polyp and stem. All primordia generate an inhibitor with a long range. Due to its position the central primordium (faint red) is affected most strongly which hinders its development into a polyp (red). The stem is a polyp primordium which is hindered to develop into a polyp by the laterally developing polyp primordia.*

that: the middle one, the stem primordium, has regressed after a short period of growth (Fig. 6e).

Fate Control

With respect to *D. pumila*'s relative Hydra, the fate of the tissue has been proposed to be controlled by qualitatively different morphogens: a head activator and a head inhibitor on the one hand and a foot activator and a foot inhibitor on the other hand (Gierer and Meinhardt, 1972, Meinhardt, 1993). Models with different morphogens and different types of interactions have been subsequently put forward by others, (e.g. Müller, 1995). To explain pattern formation in *D. pumila* one may add a stem activator and a stem inhibitor.

Based on such assumptions (Fig. 7), there is still the problem of explaining how in *D. pumila* the appropriate sets of morphogens are initiated at the various sites and how at certain positions a certain set of morphogens can become extinct while a different one takes over. Apparently there is no simple solution to these problems.

We prefer an alternative explanation which assumes that all primordia are governed by the same set of morphogens (Fig. 7) because this alternative offers simple solutions to the two problems mentioned. Second, it allows to understand the list of malformations and the histological observations. Third, the alternative will help to understand sympodial growth, as well. We would like to emphasise that our thesis doesn't have to be introduced *ad hoc* to only explain pattern formation in *D. pumila* but has rather been applied successfully to understand processes of pattern formation in various cnidarians (Berking, 1998, Kehls *et al.*, 1999, Kroiher, 2000, Zeretzke and Berking 2001, 2002).

The alternative includes that all primordia are governed by the same set of morphogens (Fig. 7). The morphogens control a quantity, termed positional value. The maximal positional value causes hypostome formation, the minimal one basal disc / stolo tip formation. Values in-between cause the local development in-between these structures. (The local positional value may control the local development by means of secondary morphogens.)

In *D. pumila*, at the outset the three primordia may have almost identical positional values. According to our approach in the most abundant type the tip of both lateral primordia will reach the maximal positional value, which causes the formation of the hypostome of a polyp while in the tip of the central primordium the positional value will remain low which causes the tissue to remain of (almost) gastric tissue composition (Fig. 7).

The mechanism proposed is simple: One of the morphogens generated by all primordia is an activator which causes an increase of the positional value. An inhibitor with a long range antagonises this increase. The central primordium is affected most strongly: its positional value does not increase up to the maximal value (see appendix).

Our approach is mainly based on the malformations observed and in particular on the malformations not found though more than 200,000 internodes have been studied. Marfenin *et al.* (1995) and also we have never observed the central primordium to develop into a polyp while the lateral ones develop into stems or stolons. Further, it has not been observed that the central one develops into a stem while the lateral ones develop into stolons. (Stolons can emerge out of a fully developed theca, the polyp's housing after the regression of the polyp.) The absence of these malformations indicate a rule: The tip of the central primordium usually develops a lower, rarely an equal but never a higher positional value than the tip of a lateral primordium. This indicates that the primordia form so close to each other that they influence each others fate. Guided by the histological sections (see below) we further assume that in the

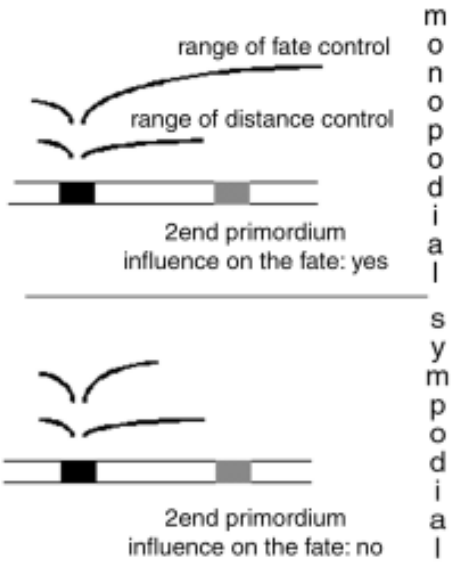

Fig. 8. Two types of dominance. *A primordium (black area) generates two types of inhibitory signals: one which in close vicinity hinders the formation of a second primordium (grey area) and one which can antagonise an increase of the positional value. The local positional value determines the local fate. The differential range of the two inhibitory influences, therewith, controls the fate of the second primordium. For reasons of clarity the signals generated by the second primordium are omitted.*

course of growth and development the central primordium becomes increasingly isolated from the lateral one which allows the positional value to increase again. Therewith, in the central primordium the positional value oscillates once in a node internode-cycle without reaching the maximal and the minimal values (see computer simulation in the appendix). Such a mechanism has an inherent instability which may explain the high number and the nature of malformations observed in *D. pumila*: quantitative changes of external influences cause a quantitative change of the „lateral dominance". A small dominating influence allows the central primordium to develop into a polyp. A very strong one causes the central primordium to develop into a stolo.

When the (usual) three primordia form they are covered with a common thin and soft perisarc. Then, the perisarc starts to harden between them. Transverse stripes of hardened perisarc appear to be the cause for the tissue to protrude in three columns (cf. arrows in Fig. 3A). However, distal to these stripes the folded tissue meets again (Fig. 3H). Hardening of perisarc between the folded tissue takes place but for some time the protrusion of the tissue is faster than the hardening of the perisarc. Up to this stage signalling substances which are generated in the tip of a primordium may easily reach the neighbouring primordium by diffusion. The covering of all three tips with the sheet of soft perisarc material facilitates that communication. During this phase of growth the lateral primordia are suggested to increase their positional value and at the same time to hinder the central one to increase its positional value too. Then, communication of the primordia by diffusible substances becomes increasingly reduced. According to our proposition, this allows the positional value to increase again in the central primordium: (1) Thick perisarc is deposited in the distal part of the furrow. (2) The tips of the lateral primordia have moved to a more peripheral position. (3) The ectodermal layer of the developing polyp loses the tight contact to the perisarc (end of phase 3). (4) The newly formed diaphragm reduces the fluid exchange between the polyp and the central primordium. (5) The primordium in the centre starts to grow out and (6) the housing of the polyp opens to allow the polyp to stretch out into the surrounding water. It turns out that though the physical distance between the primordia does not change strongly, the interaction by diffusible molecules is reduced significantly.

Some Additional Minor Points

(1) Our proposition concerning the fate control in developing stems may also explain the observation that a stolo tip can transform into a stem tip and *vice versa*. In *D. pumila* a stolo tip was found to transform into a stem tip which subsequently results in internode formation (own observation, unpublished). In Plumulariidae (thecate Hydrozoa with monopodial type of growth) v. Schenck (1965) reports that it is widespread that both, a stolo tip is able to directly transform into a stem tip and a stem tip into a stolo tip. The malformations shown in Fig. 6g result from such a switching: The observed qualitative change of the fate is suggested to be caused by external influences which quantitatively change the concentrations of those morphogens which control the positional value in the growing tip.

(2) In *D. pumila* the polyp's housing is strongly asymmetric. However, when a single housing is formed it looks rather symmetrical (Fig. 6 a,j). We suggest the noted interaction between the primordia to cause this asymmetric form. A similar proposition was made for the dorsiventral organisation of leaves in seed plants: radial or centric leaves were obtained by isolating the presumptive leaf from the apical meristem by an incision close to the leaf site (see Sussex (1955) for literature).

(3) In histological sections the sheet of soft perisarc material covering the distal surface of a growing internode could easily be seen while the soft material in the distal part of the furrow could not be traced. One may argue that sea water contains substances which speed up the hardening. In the furrow these substances are rare. Possibly Ca^{2+}-ions are involved.

(4) The endodermal cells are highly vacuolated in that part of the tissue tube which has contact to the perisarc. In the proximal endoderm the vacuoles are much smaller. This distribution of vacuoles is present in all phases. The increased size of the endodermal cells may help to press the ectodermal cells to the perisarc, the soft and the hard ones. Therewith, the endodermal cells may strongly contribute to the forces which in phase 1 cause a widening of the distal soft surface of the growing internode. A differential distribution of the size of the vacuoles in the circumference may cause a differential pressure which in turn is able to shape the perisarc before it becomes sclerotized.

(5) Due to external influences, adjacent primordia fuse in a group of malformations and form a single primordium which either has the quality of a stem (Fig. 6 k,l) or forms a hydranth (Fig. 6j). What this fusion is driven by is an open question. A transient reduced secretion of soft perisarc material in the distal part of the furrow may be at least necessary. The positional values of the fused primordia equalise, either before or after fusion, because following fusion the resulting primordium has not a mixed but rather a uniform fate.

Sympodial Growth

In sympodial growing colonies the stem grows by means of a tip which eventually transforms into a polyp. Then a new tip forms which takes over the elongation (Fig. 1). Kosevich (1991, 1996) showed that in the sympodially growing *Obelia loveni* the tip of an internode bud isolated by sectioning forms the identical sequence of pattern elements up to polyp formation as it does when it is not separated from the stem. Thus, there is no indication of an influence on the patterning within the new internode by signals originating from the old internode. That means, the primordium forms outside the range of that activity which is able to decrease the positional value. The positional value increases from the outset of primordium formation and in the course of that increase the pattern elements are laid down according to the locally attained positional value. In *monopodially* growing colonies the activity which is able to decrease the positional value is proposed to have a longer range than the one which controls the distance between primordia (Fig. 8). In *sympodially* growing colonies this is proposed to be just the other way around. This difference is suggested to be the essential one in the primary pattern forming systems of monopodially and sympodially growing Hydrozoa.

Application to Growth of Seed Plants

In seed plants meristems are well studied. However, the control of their origin in embryogenesis and their maintenance during growth are still unknown. Leaf primordia have been proposed to form and to keep distance from each other by autocatalyis and lateral inhibition (Meinhardt, 1998). If the shoot apical meristem is

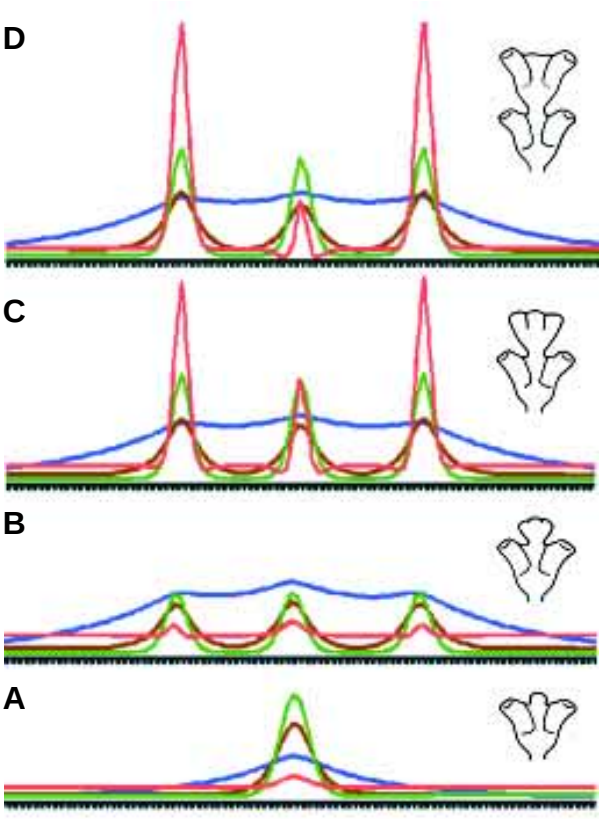

Fig. 9. Computer simulation of monopodial growth. *Simulated is the fate of a row of cells at the apex of a growing internode. The simulation is based on the equations given in the appendix. Green: activator, A; brown: inhibitor of autocatalysis, B; blue: inhibitor C which is involved in the control of the positional value D, i.e. control of the fate; red: positional value, D. All cells at the apex are assumed to generate the morphogens and initially, all cells are identical with respect to the morphogens they contain and generate.* **(A)** *In a small region at the apex the removal rate of the inhibitor B, r_b, is set higher than in the adjacent region (ratio: 1 to 0.67). The therewith specified region may represent the area where the outer skeleton is found to be thin and soft. The simulation leads to the generation of morphogens from a small region in the centre of the apex.* **(B)** *With growth an increasing larger part of the apical outer skeleton remains soft and thin. This is suggested to allow a widening of the apex and the formation of three primordia. The simulation represents this development by the formation of two additional groups of cells with an increased generation of morphogens. The formation of these two additional groups derives from the enlargement of the region in which the removal of inhibitor B, r_b, is higher than in the adjacent region (from 8 to 50 cells).* **(C)** *The simulation is carried on which causes the positional value to increase in all primordia but mostly in the lateral ones. According to the attained positional value, secondary systems are suggested to start which control the differentiation of the tissue as hypostome, tentacle and gastric tissue. In the lateral primordia, polyps develop whereas the tip of the central primordium reaches the positional value and thus the quality of gastric tissue. When at this stage the central primordium is getting isolated from the inhibitory influences of the lateral ones its positional value will increase and thus will develop into a polyp as well (one possible interpretation of Fig. 6 a2).* **(D)** *When there is no isolation, the positional value decreases in the central primordium. When later on the central primordium is getting isolated from the inhibitory influences of the lateral primordia a new cycle of development will start. However, without an isolation of the central primordium from the lateral ones, i.e. when the simulation is carried on, the positional value in the centre drops down to almost zero (not shown) which is assumed to cause the formation of a stolo (cf. Fig. 6g). Diffusion rates: $D_a = 0.01$, $D_b = 0.1$, $D_c = 0.4$, $D_d = 0.00002$; Removal rate: $r_a = 0.015$, $r_b = 0.015$, $r_c = 0.0015$, $r_d = 0.00001$; basic production: $b_a = 0.004$, $b_b = 0.001$, $b_c = 0$, $b_d = 0.0000015$; constants: $s_a = 0.015$, $s_b = 0.017$, $s_c = 0.006$, $s_d = 0.000075$, $s_e = 0.0036$. The parameters r_d, b_d, s_d and s_e have been made very small to cause a slow change of the parameter D (positional value) compared to the others (diffusible morphogens). D_d is assumed to be very small but not zero. This may represent some diffusion or a slow and limited mixing of cells. The technical basis of this simulation has been developed by H. Meinhardt (1995).*

formed and maintained in a way similar to that proposed for monopodially growing hydrozoa, the meristem has to be understood as a leaf primordium which is hindered to develop into a leaf by the laterally developing leaf primordia.

Materials and Methods

Animals

Colonies of *D. pumila* were collected from the North Sea at Helgoland.

Histology

One day after the last feeding the animals were fixed with 5% formaldehyde, stained with haemalaun and transferred via alcohol and epoxypropane into araldite. Sections of 1µm thickness were stained with toluidineblue. Whole mount staining with Calcofluor White M2R: A stock solution containing 5% Calcofluor White M2R (Sigma) was diluted 1:800 with sea water containing pieces of the colony of *D. pumila* (according to Kosevich *et al.*, 2001). After 5 to 15 minutes the pieces were looked at under UV-light. The dye preferentially stains various polysaccharides including chitin in particular before its crystallisation (Gooday, 1996). It prevents the formation of chitin fibrils in such a way that the excreted material remains filamentous (Mulisch, 1996).

Appendix

We made a computer simulation for a linear row of epithelial cells on the surface of a growing stem of *D. pumila*. The formation and the fate of the developing primordia is assumed to be controlled by three substances, one activator and two inhibitors. The aim of the simulation is a rather restricted one: we would like to show that (1) one primordium becomes accompanied by two additional ones when the apical surface is enlarged (Fig. 9 A,B) and that (2) the lateral primordia dominate the original now central one in such a way that its positional value decreases (Fig. 9D). We assume that the maximal positional value determines (by means of secondary morphogens) the formation of the mouth opening of a polyp. A slightly lower one causes the ring of tentacles to form, and so on. The lowest one causes the formation of the basal disc / stolo tip.The simulations are made with the following equations:

$$\frac{\delta a}{\delta t} = s_a \frac{a^2}{b} - r_a a + db_a + D_a \frac{\delta^2 x}{\delta t^2}$$

$$\frac{\delta b}{\delta t} = s_b a^2 - r_b b + b_b + D_b \frac{\delta^2 x}{\delta t^2}$$

$$\frac{\delta c}{\delta t} = s_c a^2 - r_c c + b_c + D_c \frac{\delta^2 x}{\delta t^2}$$

$$\frac{\delta d}{\delta t} = s_d \frac{a^2}{c} - r_d d + b_d - s_e \left(s_a \frac{a^2}{b} + db_a \right) + D_d \frac{\delta^2 x}{\delta t^2}$$

r_x = removal rate
b_x = basic production
D_x = diffusion constant
s_x = constant

Following the widely accepted rules in physical chemistry and models proposed for pattern formation, the capitals A, B, C and D refer to the morphogens and the positional value, respectively, the small letters a, b, c, d denote the respective concentrations (used mainly in the equations). A diffusion constant is denoted by D_x.

Activator A stimulates its own generation / release from cells. Inhibitor B antagonises the generation of this activator. Generated activator A also stimulates the production of activator A within the cells. Therewith, the positional value D increases. Inhibitor C antagonises the increase of the positional value. The loss of the activator from the cells decreases the positional value. The range of inhibitor C is larger than the range of inhibitor B. (If the range was smaller the lateral primordia would not dominate the central one and all primordia would develop into polyps as it is observed in sympodial growth.) For further details see the legend to Fig. 9.

The removal rate r_b of inhibitor B is assumed to be larger at the apical surface of a primordium - where the perisarc is soft and thin - than in the periphery (ratio 1 to 0.67). Initially, this apical area with a soft perisarc is small, allowing the formation of only one primordium (Fig. 9A) out of a homogenous distribution of all substances (not shown). Subsequently the area is enlarged (by the operator) allowing the formation of two additional primordia (Fig. 9B). In these new primordia the positional value (D = red) increases and overcomes the positional value in the central one (Fig. 9C). Finally, in the central primordium the positional value decreases (Fig. 9D). When at this stage an isolation with respect to inhibitory influences of the central primordium from lateral ones takes place, the central one will start a new cycle of internode formation. When such an isolation does not occur the positional value in the central primordium decreases down to almost zero (not shown) which is suggested to cause stolo formation (cf. Fig. 6g). The technical basis of the presented simulation is the excellent program H. Meinhardt (1995) developed for the calculation of patterns on sea shells.

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