CHAPTER 5

Branching in Colonial Hydroids

I.A. Kosevich

Abstract

Cnidarians are primitive multi-cellular animals whose body is constructed of two epithelial layers and whose gastric cavity has only one opening. Most cnidarians are colonial. Colonial hydroids with their branched body can be regarded as a model for the whole phylum and are the most- studied cnidarian group with respect to developmental biology. Their colonies are constructed by repetition of limited number of developmental modules. The new modules are formed in the course of activity of terminal elements – growing tips of stolons and shoots. The growing tips of cnidarians, in contrast to those of plants, lack cell proliferation and drive morphogenesis instead by laying down and shaping the outer skeleton and formation of the new colony elements. Cell multiplication takes place proximally to the growing tips. Branching in colonial hydroids happens due to the emergence of the new growing tip within the existing structures or by subdivision of the growing tip into several rudiments. Marcomorphogenetic events associated with different variants of branching are described, and the problems of pattern control are discussed in brief. Less is known about genetic basis of branching control.

Introduction

Cnidarians are generally considered to be the basic primitive group of multi-cellular organisms. The main feature of their general body plan is a two-layer body in a form of a blind sack with only one mouth opening; the body is composed of two tissue layers, ectoderm and endoderm, separated by extracellular matrix – the mesoglea. One of the most distinctive features is the presence of the nematocytes – epithelial cells containing sting capsules (cnidae or nematocysts) that are used for defence, capture of prey and temporary attachment. Cnidarians remain at the epithelial level of organisation – they have no real tissues or organs. The ectoderm and endoderm are composed of several cell types, namely epithelia-muscular cells with contractile processes at the base, several types of gland cells, nerve cells, nematocytes, and multipotent interstitial cells (i-cells). The whole diversity of cells types is maintained by the presence of three independent and self-supporting cell lineages – ectodermal epithelia-muscular cells, endodermal epithelia-muscular cells and i-cells that give rise to the nerve cells, different gland cells, nematocytes and germ cells.[1-4] It is believed that these cell lineages are determined during early stages of embryogenesis[5-7] and show no ability for reciprocal trans-differentiation under normal conditions.[1, 8-11]

The phylum Cnidaria is composed of four classes: Anthozoa, Hydrozoa, Scyphozoa and Cubozoa.[12, 13] With respect to the question of branching morphogenesis we will discuss the representatives of the class Hydrozoa, which have received most attention from developmental biologists. This group of cnidarians is characterised by metagenetic life-cycle: the larva undergoes metamorphosis into the polyp stage (mostly sessile and attached) and this stage sheds the motile planktonic medusae.[14] Polyps multiply asexually through different variants of budding, while medusae generally reproduce sexually. The ability of polyps to produce buds was the

Branching Morphogenesis, edited by Jamie Davies. ©2004 Eurekah.com.

basis for the development of colonial (or modular) organisation within the polypoid stage of cnidarians and hydroids in particular.[15]

Organisation of Hydroid Colony

The main parts of hydroid colony are the creeping hydrorhiza and the hydranths, or shoots, that protrude into surrounding water (Fig. 1). The hydrorhiza is composed of a net of the tube-like stolons. Hydranths are either located directly on the stolons (sessile hydranths) or have a pedicel. The shoots have a different structural organisation: they may have a stem and lateral branches of successive orders, and may bear numerous hydranths. Modular organisation of the organism implies that the its body is constructed by the repetition of the limited number of definite elements (modules). In the case of colonial hydroids, these modules are: stolon internodes, shoot internodes, hydranths, and growing tips of stolons and shoots. Commonly, the stolon internode is a section of the stolon tube between two adjacent bases of the sessile polyps or shoots (Fig. 2A). The organisation of the shoots is more complex in most cases. The simplest variant is repetition of almost identical shoot internodes (Fig. 2A, B). The branches and the shoot stem in that case are organised similarly. In more highly-integrated shoots, the internodes within the stem and branches may differ and are frequently complicated by formation of secondary (complex) internodes (Fig. 2C, D).

Schematically, a hydroid colony may be imagined as a system of branching tubes with hydranths at one end and growing tips at the others. The nongrowing terminus of the shoot either is occupied by the hydranth or has no specific structure. The nongrowing end of the stolon is a blind end of the tube without any specific structure either. The hydranths are organised more or less as a solitary polyp *Hydra* with one exception – they lack the foot structure and are connected to the tube of the colonial body tissue – the coenosarc. The coenosarc is a two-layer tube with practically unvarying organisation along its length. From the outside the coenosarc is covered with the outer rigid skeleton – the chitinious perisarc. The perisarc is used for tight attachment to the substrate along the stolons, gives some protection against predators, and provides mechanical support for soft tissue for development of the elevated structures of the shoots (Marfenin, Kosevich, in press).[16]

The presence of the hard skeleton (perisarc) and branching points along the colony limit the mode of elongation of the colony. Growth of the colony can be achieved only by the

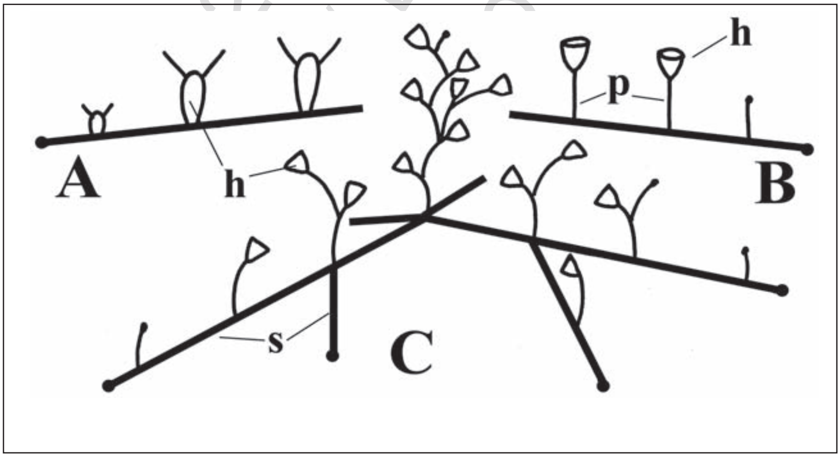

Figure 1. Scheme of the hydroid colony organisation.
A. Stolonal colony with sessile hydranths. B. Stolonal colony with hydranth with pedicels. C. Colony with sympodial shoots.
s – stolons, h – hydranths, p – hydranth pedicels.

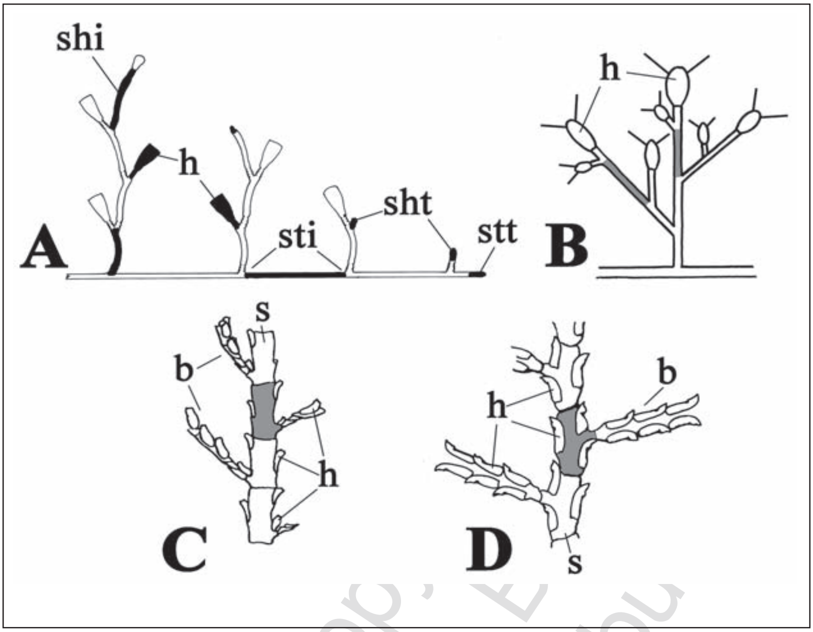

Figure 2. Main elements of hydroid colonies.
A. Colony with sympodial shoots. B. Colony with monopodial shoots with terminal hydranth. C, D. Parts of highly-integrated shoots with complex shoot internodes.
b – shoot branch, h – hydranths, s – stem of the shoot, shi – shoot internode, sht – shoot growing tip, sti – stolon internode, stt – stolon growing tip.

extension of tubes at their termini. The terminal part of the stolon tube is occupied by a growing stolon tip. The termini of the shoots are occupied either by shoot growing tips (Thecate hydroids) or by terminal hydranths (Athecate hydroids). The growing tip in colonial hydroids is a morphogenetic element whose job is to shape new colonial elements by laying down new portions of perisarc and to move ahead by repetitive growth pulsations – the series of elongation-contraction of growing tip with a periodicity of several minutes.[17-26] Morphologically, the growing tip differs from the rest of the coenosarc: its tissue has permanent contact with the perisarc tube and the cells of the growing tip have a characteristic organisation. The soft tissue extends within the part of the stolon or shoot between the growing tip and the last branching point (Fig. 3).[27-29] In those shoots where the termini are occupied by the hydranth, the soft tissue and new perisarc are added just under the hydranth's base (Fig. 4).

The material for elongation of the coenosarc tube comes from more proximal regions of the colony.[30-36] The growing tip completely lacks cell divisions and has relatively permanent cell composition. Proliferation has been observed in cells just behind the growing tip and proliferation is distributed more or less evenly, at least along the nearest 3-5 internodes.[37] Direct observation of the ectoderm revealed that single cells and entire tissue sheets move towards the growing tip. The speed of such migrations decreases with distance from the growing tip coming to nought within the third or forth internode. But within the most distal uncompleted internode just behind the tip, the speed of ectoderm cells' migration can even be higher than the movement of the tip itself.[33]

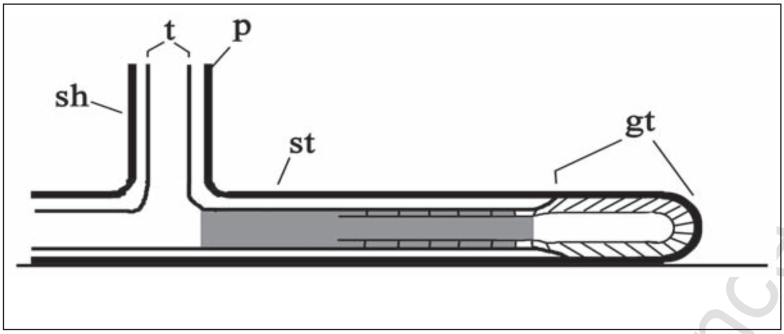

Figure 3. Scheme of the terminal part of the colony stolon showing the relative organisation of the outer skeleton and soft tissue. Only one tissue layer is marked.
gt – growing tip, p – perisarc (outer skeleton), sh – shoot base, st – stolon, t – tissue tube. The region of the tissue extension is shadowed.

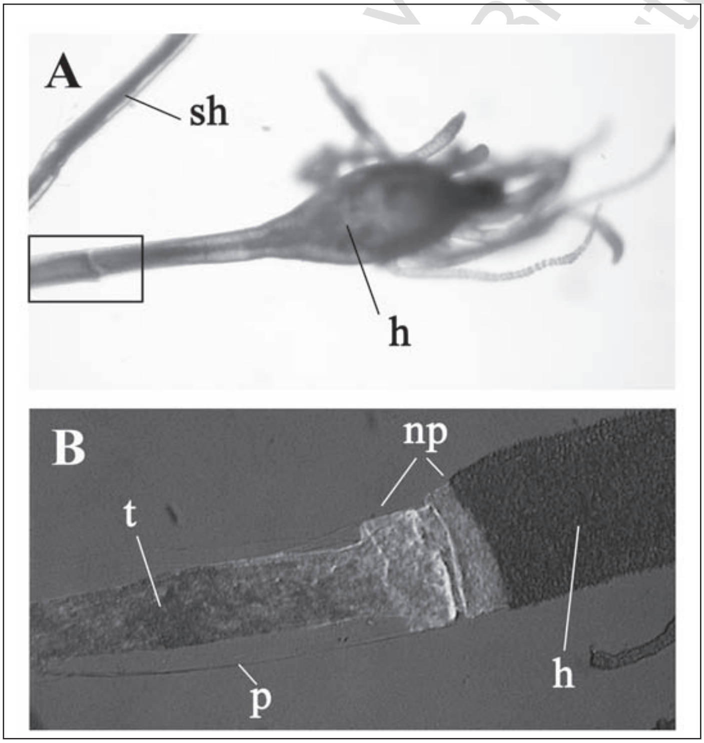

Figure 4. Place of the tissue and skeleton extension in shoots with terminal hydranths.
A. Photo of the terminal hydranth with part of the shoot. B. Magnified view of the hydranth base marked by the rectangle in A – white fluorescence corresponds to the newly laid perisarc (staining with Calcofluor White).
h – hydranth, np – newly laid perisarc (skeleton), p – old perisarc, sh – part of the shoot, t – soft tissue (coenosarc).

Branching in Colonial Hydroids

Each node within a stolon or a shoot is a branching point. This node can be formed either as a result of the appearance of a new growing tip upon the already formed structure, or in a form of the subdivision of the growing tips into two or more parts during the course of their growth. The first case is characteristic for stolon branching in most species, for the growth and branching in sympodial shoots and for other types of shoots with irregular mode of branching (Fig. 5A, B). Subdivision of the tip into several parts (rudiments) is an attribute of a monopodial type of the shoot growth with regular mode of branching (Fig. 5C).

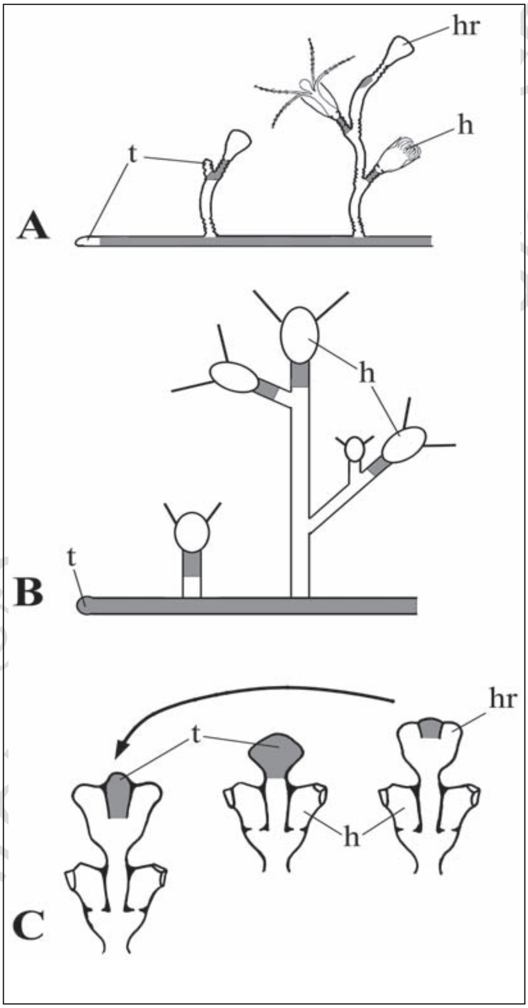

Figure 5. Zones of branching within different types of colonies.
A. Colony with sympodial shoots and irregular branching. B. Colony with monopodial shoots, terminal hydranth and irregular branching. C. Terminal part of the monopodial shoot with terminal growing tip and regular branching.
h – hydranths, hr – hydranth rudiment, t – growing tips. Zones of branching are shadowed.

Emergence of a New Growing Tip

The branching in stolons and shoots starts with the appearance of a new growing tip. In stolons and sympodial shoots, the new tip emerges from the coenosarc tissue which is similar in composition and is characterised by flattened ectodermal and loosely organised endodermal cells. The first visible changes are associated with formation of a plate of columnar ectodermal cells at the point of branching. It is very likely that this reorganisation of the ectoderm is linked with the simultaneous reorganisation of underlying endoderm cells, including their vacuolisation. Later, the plate starts to pulsate and forms out-folds (Fig. 6). During the course of the initial steps of growth, the new tip reaches its final dimensions and form and gradually gains the highest speed of its growth.

Emergence of a new tip requires that the existing perisarc tube must 'open'. Unfortunately the biochemical mechanism of this process is not known. No chitinase activity capable of digesting the chitinious matrix of the perisarc has been detected during new tip emergence (personal observations). From the outside view, it seems that in stolons and at least some of the species with monopodial shoots, the existing perisarc at the point of the new tip emergence 'melts' over the surface of the new tip. This process may be similar to the growth and budding of fungal cell walls: new portions of the polymers are added to the existing ones that constitute the matrix of the cell wall and this causes extension the surface of the cell wall which itself is not

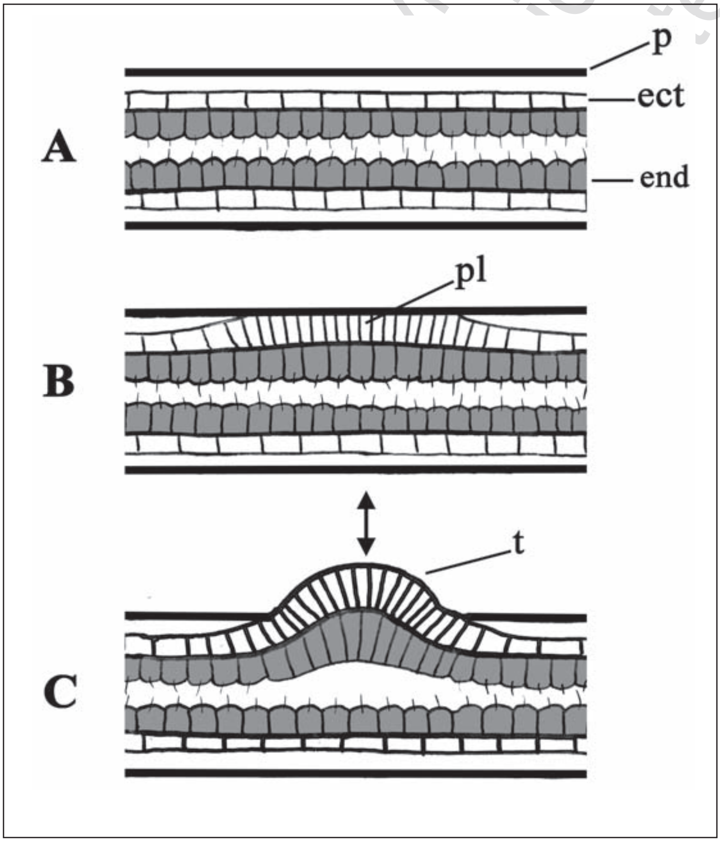

Figure 6. Scheme of the new growing tip emergence upon the stolon.
ect – ectoderm layer, end – endoderm layer of coenosarc, p – perisarc (skeleton), pl - plate of ectodermal cells, t – new growing tip. Arrows indicate direction of growth pulsations of the tip.

elastic.[38-43] If this is the case in such cnidaria, there would be no need for rupture of the old perisarc.

In the sympodial shoots of *Laomedea flexuosa* Hincks (Campanulariidae, Thecathora), the perisarc 'opening' is achieved in a different manner, but the basic biochemical machinery may be the same. At the very first moment of the tip emergence, when the ectodermal plate is just forming, the circular set of ectodermal cells start to release amorphous chitin, the precursor of the perisarc matrix. The release has been visualised by staining with Calcofluor White (Fluorescent Brightener 28)(Sigma)(Fig. 7), which stains various carbohydrate fibrils, including amorphous chitin.[44] Later, the entire apical surface of the growing tip releases the amorphous chitin and after the new tip has emerged one can see that the plate of the old perisarc is pushed out like the lid of a tin (Fig. 8) and the growing tip itself is covered by new perisarc.

The model proposed for the mechanism of the tip growth due to pulsations[18, 45] implies that the growing tip has a mechanical support from the hard (already hardened and practically

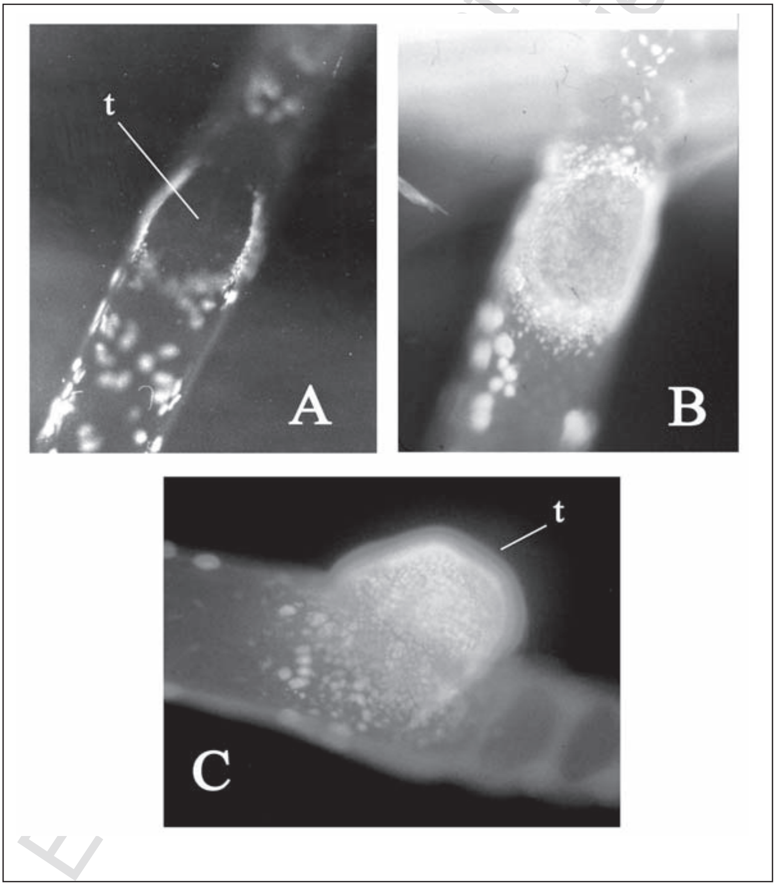

Figure 7. Staining for the amorphous chitin with Calcofluor White during the initial moments of the new tip emergence upon the shoot internode in *Laomedea flexuosa*. White fluorescence corresponds to the places of the amorphous chitin release.

A. Initial moment of the tip appearance – formation of the ectodermal plate (see Fig. 6B). B. About an hour later - new tip is formed but had not yet opened the old perisarc. C. The new growing tip get out of the old perisarc.

t – new growing tip.

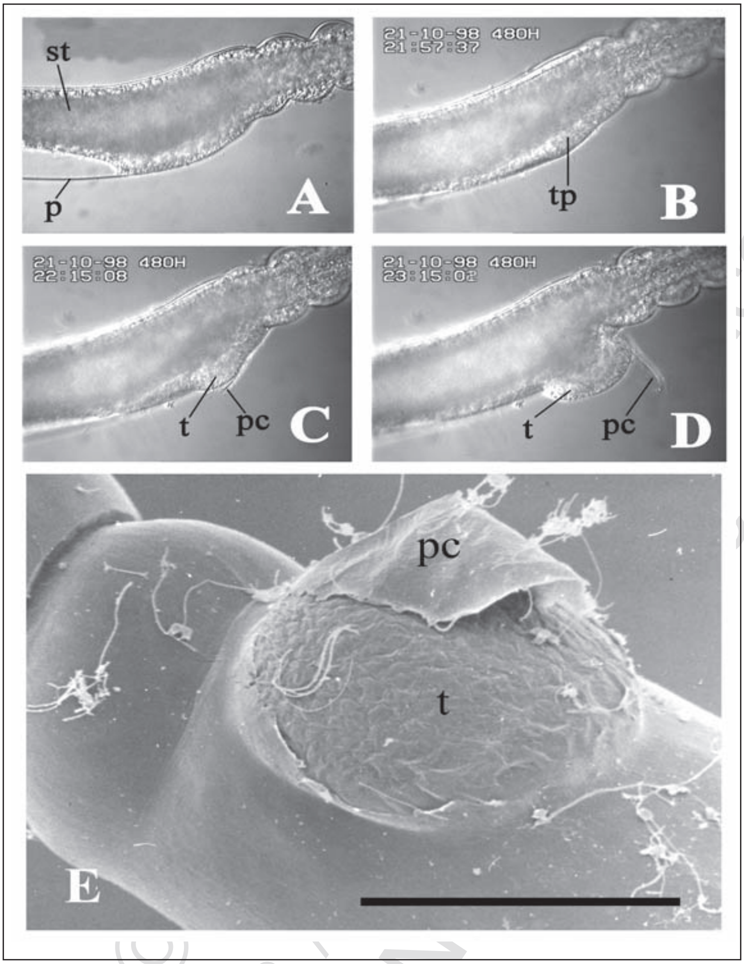

Figure 8. New growing tip emergence upon the shoot internode in *Laomedea flexuosa*.
A-D – sequence of events during new tip emergence (video microscopy - dissecting microscope). E – scanning electron micrograph of the newly emerged tip (corresponds to D). Scale bar – 100 mkm.
p – perisarc, pc – perisarc covering, st – soft tissue, t – new growing tip, tp – ectoderm plate at the beginning of the tip organisation.

not stretchable) perisarc that surrounds its circumference. In the course of growth, new soft perisarc is released by the tip tissue at its spherical apex. As soon as it is left behind by the advancing apex, the perisarc quickly hardens. So the growing tip forms the perisarc tube by itself and simultaneously uses it as a mechanical support for forward movement by growth pulsations.

Initially, the new tip on the sympodial shoot internode is supported by the old perisarc on only one side, the outer surface of the internode. That is why, after the onset of the pulsation and before the 'opening' of the maternal perisarc, the new tip has to form additional perisarc wall from the inside of the existing perisarc tube (Fig. 9A). This way, it obtains sufficient mechanical support along its circumference.

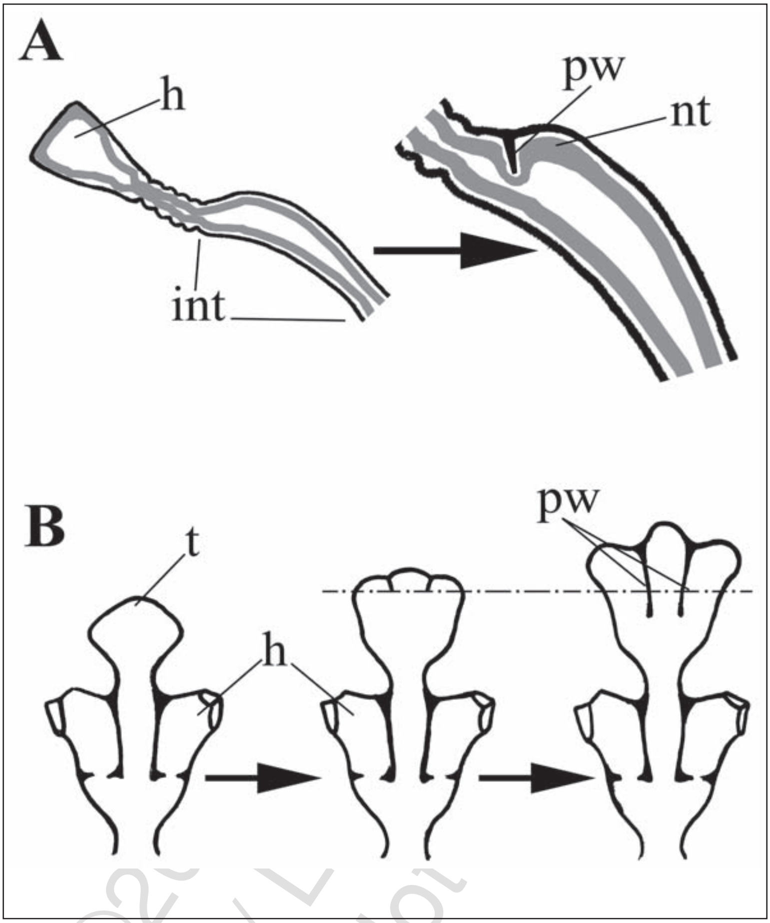

Figure 9. Formation of the additional perisarc walls from inside of the existing perisarc tube during new tip(s) emergence.
A. Scheme of the additional perisarc plate formation in sympodial shoots. Grey line shows the tissue. Perisarc is shown in black. B. Formation of the perisarc walls by ingrowth during the subdivision of the growing tip into 3 parts. Only perisarc is shown. Dashed line shows the primary level of the tip subdivision.
h – hydranth, int – shoot internode, nt – new tip, pw - additional perisare wall, t – growing tip.

Subdivision of an Existing Tip

The same is true for morphogenesis by subdivision of the growing tip into several parts (rudiments). For example, the growing tip of the monopodial shoot in *Dynamena pumila* L. (Sertulariidae, Thecaphora) has a morphogenetic cycle that includes the formation of a pair of oppositely- located hydranths with a growing tip between them. In the course of this cycle, the tip starts as a practically spherical bulb that later becomes oval in the plane of the shoot due to greater growth in the dimension of this plane. The apical surface of this growing tip then divides into three parts; lateral ones, which will form the hydranths, and the central one will produce the growing tip for the next cycle of shoot growth.[18, 46, 47] Subdivision of the entire growing tip is accompanied by formation of additional perisarc walls between the rudiments. These walls are formed not only along newly-grown lengths of the rudiment, but also by in-growth of the perisarc as the apical tissue divides (Fig. 9B). Such additional walls support the development of the rudiments without decreasing the speed of growth, and perhaps play certain role in determining the fate of the rudiment.[46]

Interaction of a New Tip With Adjacent Structures

In the majority of colonial hydroid species studied to date, the emergence of new growing tips is spatially connected to existing growing tips or to the bases of the hydranths (hydranth pedicels). The tip of a new shoot appears on the stolon just at the proximal part of the stolon tip.[48, 49] The new tip of the sympodial shoot (e.g., in *Laomedea flexuosa*, *Gonothyraea loveni, Obelia longissima*) emerges at the border of the smooth part and the hydranth pedicel.[48, 50-52] The lateral branches of the shoots in highly-organised species of the Sertulariidae family begin as a part of a morphogenetic cycle of the shoot growing tip in which the tip subdivides into several rudiments (Marfenin, Kosevich, in press). That means that the condition of the surrounding tissue is not homogenous along the circumference of the tip base. From the proximal side (along the axis of the maternal internode) the tissue is more stretchable in comparison with the tissue layer distal to the new tip base. Morphogenesis could be regulated simply by the extensibility of this tissue layer. New tips mechanically interact with one another, competing for the tissue and, because of the synchronous pulsations that take place just after subdivision, these daughter tips pull the same small portion of tissue connecting them. This may cause new tips to bend towards the existing one (Fig. 10A).[18] Later on, the distance between the daughter tips increases and they practically cease interacting mechanically.

In certain situations there could be additional mechanical interaction between adjacent tips. For example, during the morphogenetic cycle of *D. pumila*, hydranth rudiments are forced to bend towards the central rudiment initially. After several growth pulsations, however, the parameters of pulsations change and instead of being synchronous they gradually switch so that the central rudiment pulsates in antiphase with the lateral rudiments. This means that as the central rudiment retracts and the tissue on its sides shifts disto-proximally, the lateral tips move forward pulling the tissue behind them. The tissue between the rudiments is therefore practically pushed in the direction of the hydranths tip by the central retracting rudiment. As a result the tissue on this side moves forwards more than on the opposite side and causes the tip itself to bend (Fig. 10B).[53] This might explain why the orifices of the hydrothecae in complex shoots with 'sunken' hydrothecae are always directed outwards, away from the axis of the shoot.

The mechanical interaction between adjacent tips explains why the axis at the base of a shoot is always bent towards the stolon tip. This is never seen in the primary shoots that develop from the settled planula larvae of frustules (small stolon-like pieces of the coenosarc separated from the colony for asexual reproduction). In primary shoots, the new growing tip is the only one and emerges in the centre of the structure, so the tissue state is symmetrical at the point of the tip emergence.

Branching control

The question of the branching morphogenesis in colonial hydroids is inseparable from the problem of pattern formation: what controls the distance between the adjacent structures (hydranths, shoots, branches) – the length of the internode, and how the fate of the new tip is determined?

Necessary Conditions

An important condition for new tip initiation is that there must be sufficient 'excess' production of new cells over and above that required by the colony for replacement of spent cells and maintenance of growth of existing tips. If the quantity of new cells exceeds these needs, then there will be sufficient for the initiation of a new tip. The presence of such condition is obvious but can be illustrated by the ratio between the number of growing tips and the length of the coenosarc (where the cell divisions take place) in the colony under different nutrition

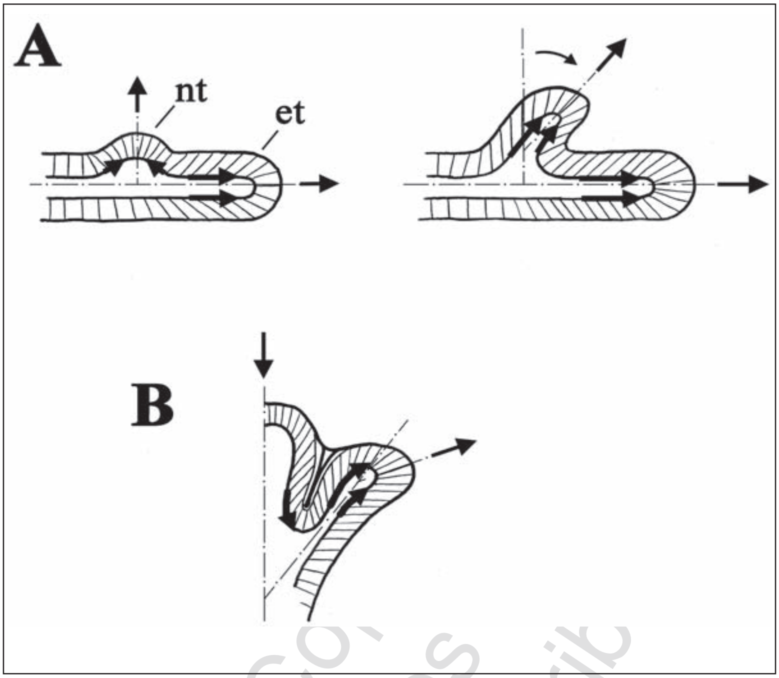

Figure 10. Schematic explanation of the bending of the growing tip due to interaction with adjacent tip. A. Initial bending towards the adjacent tip due to synchronous pulsations. B. Outward bending of the tip in the case of asynchronous pulsations.
et – existing growing tip, nt – new growing tip. Arrows inside the rudiment indicate the direction and magnitude of tissue movement during growth pulsations; arrows outside shows the direction of simultaneous growth pulsations. Dashed lines indicate primary axes of the tips growth.

levels. For *Gonothyraea loveni*, *Obelia longissima* and *Dynamena pumila,* even under most favourable conditions, the value of this ratio never exceeds 0.3. With decrease of nutrition the ratio diminishes.[49, 54, 55] Under starvation the branching and growth of the tips within the colony stops,[56] although the cell proliferation can still take place, as in *Hydra*,[57, 58] to replace of spent cells.

Control of Branch Spacing

We will discuss the determination of branching points within the stolon and shoots separately.

Branching of a Stolon

Emergence of the lateral stolon tips is the least regular branching process, at least for the majority of colonial hydroids. It strongly depends on the nutrition of the colony. In most athecate species, there is no exact spatial preference for the appearance of a new stolon tip. Generally, the new stolon tips appear in peripheral parts of the colony near the base of the sessile hydranths or shoots. When nutrition increases, however, or when there is a lack of free substrate, new stolon tips can emerge in the old part of the colony too.[59, 60] There appears to be only one rule: a lateral stolon branch will never be formed very close to the apex of existing tip. The smallest distance differs between species but approximately is about 200-300 micrometers. This could be explained by the inhibitory effect from the existing tip according to the predominant model of local activation and lateral inhibition.[61-67]

When the general arrangement of a colony is more regular, the stolon branching is too. This regularity is demonstrated by the appearance of points within the stolon at which branching is more probable. The simplest rule is that lateral stolons emerge close to the base of the shoots; but other positions remain possible (e.g *Laomedea flexuosa, Gonothyraea loveni*, *Obelia geniculata* (Campanulariidae) – species with smooth tube-form perisarc of the stolons). It is difficult to explain such predominance. One possibility is that it is somehow is connected with the peristaltic waves of the coenosarc contractions that provide the gastro-vascular flow within the colony.[59,68-74] At the base of the shoots the oppositely directed peristaltic waves could meet to produce a "standing wave" and therefore cause prolonged pressing of the coenosarc over the perisarc from inside. This may initiate the emergence of a new tip. The possible role of mechanical pressure upon the initiation of the new tip is supported experimentally.[64,65] Another possibility is that tip initiation is driven by an accumulation of 'free' cell material which arises from migration of cells into the stolon from the shoot.

In highly-integrated species (e.g., certain species of Campanulariidae, Sertulariidae, Plumulariidae families) the branching points are 'preformed' during the growth of existing stolon. Each stolon internode (segment between adjacent shoots) ends with formation of the wide plate at the base of the next shoot. In the simplest case, this consists simply of a widening of the stolon but in many species it becomes plate-like and the inner space is subdivided into regular pockets by perisarc partitions (Fig. 11). These pockets are the potential points of initiation of new stolon branches. Within one shoot base not all pockets will be used, which ones perhaps being determined by chance. In these species, stolon branching is restricted strictly to bases of the shoots.

Appearance of the New Hydranths or Shoots on the Stolon

New hydranths or shoots appear in a regular way during stolon growth. The emergence of the new tip on the upper side of the stolon takes place close to the stolon tip. Sometimes it appears that the stolon tip buds off the new hydranth tip on its upper side (Fig. 12A). In some simple colonies of athecate species, however, the stolon tip raises itself up from the substrate and transforms into the hydranth bud and, as it does so, a new stolon tip emerges from the point of bending up to continue the growth of the stolon (Fig. 12B-E). In these species, a stolon clearly has its own morphogenetic cycle that starts with tip emergence and ends with formation of a hydranth/shoot tip. In most of the colonial species, this sequence is secondary modified and the stolon tip has the appearance of a permanent element.

The distance between two adjacent hydranth/shoots is strictly controlled in most of colonial hydroids. It can vary under different nutrition conditions and can also be altered artificially by surgical operations.[48,75] In the predominant hypothesis about how spacing works – a reaction-diffusion model which includes local activation and lateral inhibition in different modifications[46,61-63,76-84] - the distance is controlled by some inhibitory effect from the existing hydranth/shoot that diminishes with distance from that shoot. When the concentration of inhibitor has fallen below some threshold level, a new hydranth/shoot tip can be initiated on the stolon. The main problem with this model is that no molecules responsible for it have been identified.

One proposal is that the changes in the value of ROX potential could play a decisive role.[85-89] At first glance the results of experimental perturbations and measuring of potential in colonial hydroid *Hydractinia* support this hypothesis, but it is difficult to separate the effect and the result. As most chemicals affect numerous targets the question remains: does the ROX state alter the colony proportions, or it is just the result of the altered colony composition?

There are models other than the reaction-diffusion one. One feature that could play a role in determining the distances between adjacent hydranths/shoots might be the cell density immediately behind the stolon tip. As has been shown by several different approaches[28,33] the

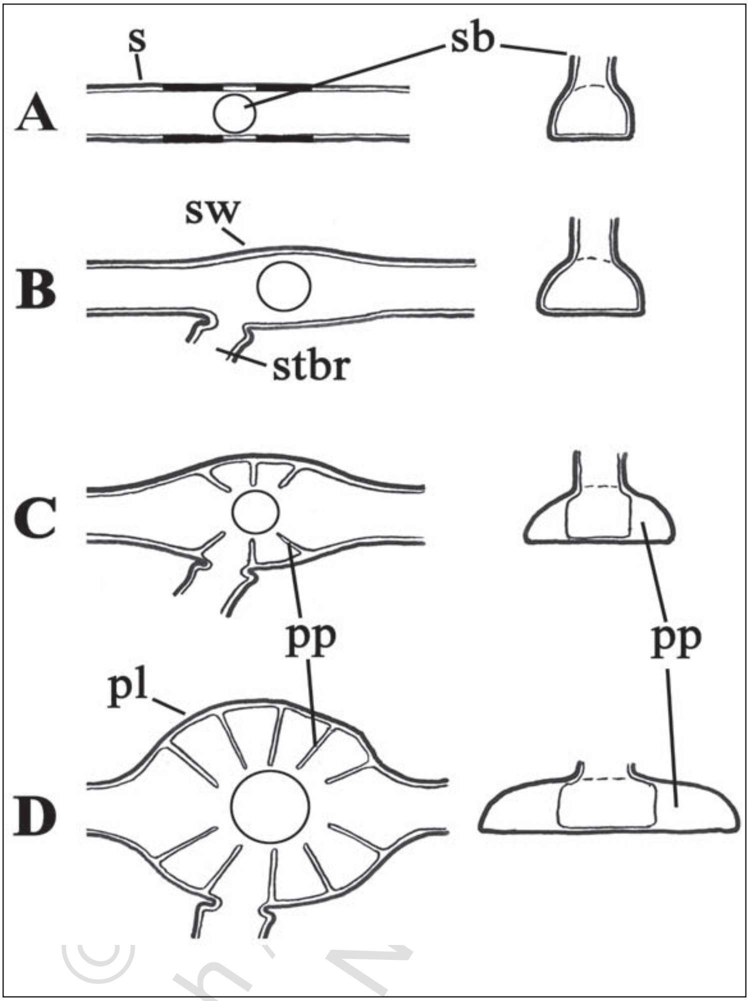

Figure 11. Schematic sketch of the variants of stolon shape at shoot base in different species of colonial hydroids.
Left column –view from above, left column – cross section through shoot base. Only the perisarc is shown. A. *Laomedea flexuosa* (Campanulariidae). Thicker line indicate the region of predominant stolon branching. B. *Obelia longissima* (Campanulariidae). C. *Dynamena pumila* (Sertulariidae). D. *Sertularia mirabilis* (Sertulariidae).
pl – stolon plate at the shoot base, pp – perisarc partitions, s – stolon, sb – shoot base, stbr – stolon branch, sw – stolon widening.

speed of cell movement towards the stolon tip is higher than the speed of the tip growth itself. This can only mean that the density of cells must rise behind the tip, and it has been suggested that this increase provides both the signal and the raw materials for new tip initiation. The problem with this model is that the distance between the hydranth/shoots remains approximately constant regardless of the nutrition of the colony and, even under starvation conditions when the stolon itself ceases its growth this distance is not affected.

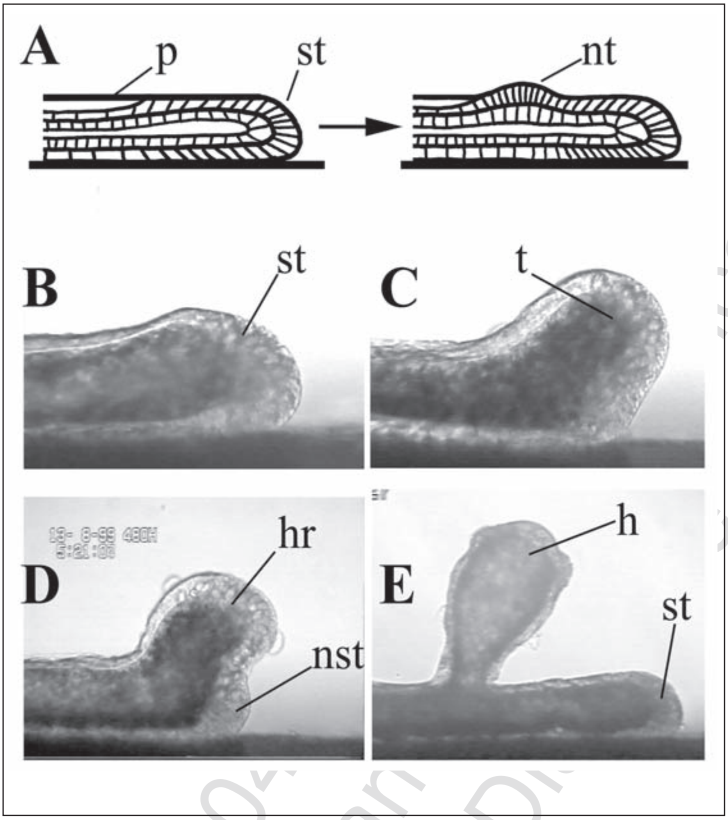

Figure 12. Appearance of the new hydranth/shoot tip on the stolon.
A. Scheme of the emergence of the new tip upon upper side of the existing stolon growing tip. B. Transformation of the stolon growing tip into the hydranth rudiment (video microscopy – dissecting microscope). h – hydranth, hr – hydranth rudiment, nst – new (next) stolon tip, nt – new tip, p – perisarc, st – stolon tip, t - former stolon tip.

Branching of the Shoots

Branching of shoots includes at least three processes: regular appearance of growing tips that continue elongation of sympodial shoot; subdivision of the tips into separate rudiments (with different fates in shoots with monopodial growth); and emergence of the lateral branches over the shoot stem. In many cases, elongation of the shoot is complicated by the general complexity of shoot morphogenesis (Marfenin, Kosevich, in press), and it becomes difficult to separate these processes. But the main rules seem to be the same, so we will examine several examples.

Emergence of the Next Tip in Sympodial Shoots

In all cases, a new tip appears after the maternal shoot internode has been formed. The completion of the internode is defined by formation of the hydranth. In some groups (e.g., the Campanulariidae, Campanulinidae families), the hydranths have an annulated pedicel, the distal portion of the internode below the hydrotheca. In others, the hydranth lacks such a pedicel and the internode perisarc gradually turns into the hydrotheca. In all cases, the new growing tip emerges close to the base of the hydranth (Fig. 13): in species with a hydranth pedicel this occurs at the border of the smooth part of the internode and the pedicel.

At least two hypothetical explanations can be proposed to account for this pattern. One is based on the hypothesis of positional information.[90-93] Briefly, it proposes that during the course of internode formation, the positional value of the tip tissue gradually increases from a basal value and causes the transition from development of one part of the internode to development of the other. Once the positional value reaches its highest possible value, the growing tip initiates the hydranth formation.[46] The next tip of the shoot has an innate tendency to be form within the tissue with highest positional value, but it is opposed in this by an inhibitory signal emerging from the hydranth. These two opposing tendencies result in the next tip being initiated at the border between the hydranth pedicel and the rest of coenosarc tissue.

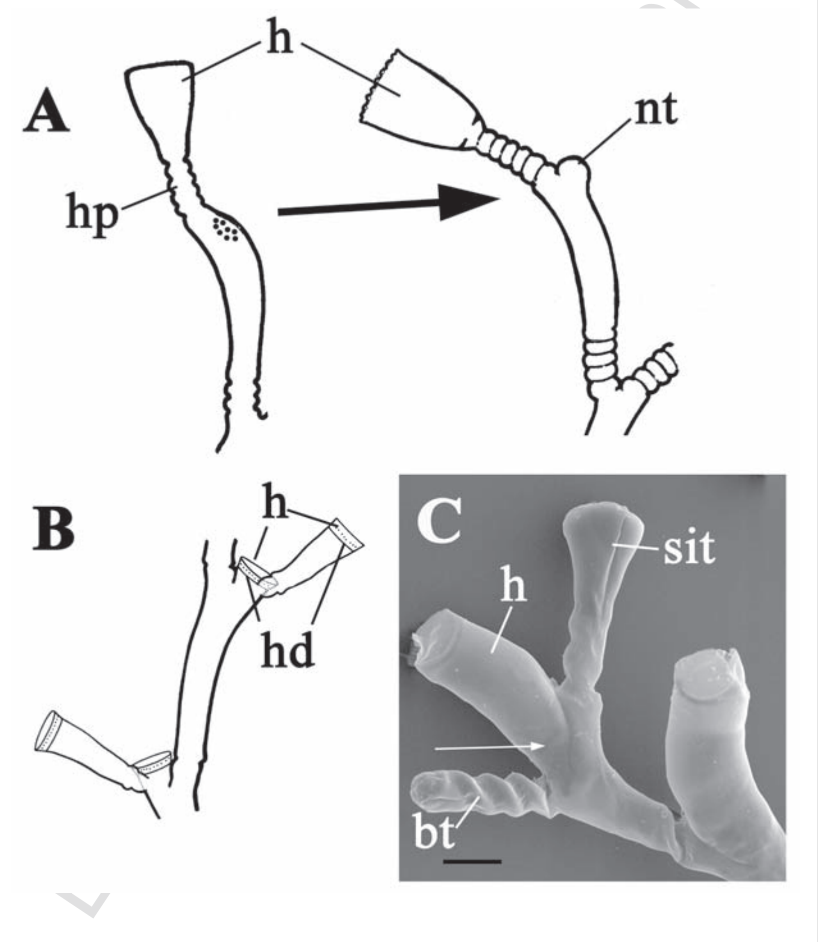

Figure 13. Places of the next shoot growing tip emergence in different hydroids with sympodial shoots. A. gg.*Obelia, Campanularia, Gonothyraea, Laomedea*, *Calicella*, etc. Dots indicate the place of the next tip appearance. B. g.*Halecium*. C. g.Sertularia (Scanning electron micrograph. Arrow indicates the level of the hydranth diaphragm. Scale bar – 100 mkm).
bt – branch tip, h – hydranth, hd – hydranth diaphragm, hp – hydranth pedicel, nt – next tip, sit – shoot internode tip.

The other hypothesis is mechanical rather than biochemical. It is based on the observation that the tissue and cells actively migrate towards the growing tip, and the coenosarc tube shows peristaltic-like waves of contraction and expansion. As the shoot internode develops, the tip moves forward to shape new parts of the perisarc and directs and uses cell material for formation of the new coenosarc. When the hydranth bud at the distal terminus of the internode reaches its final dimensions it ceases to consume cell material and therefore results in an accumulation of cell material still being delivered. If the conditions are favourable, the dense accumulation of cells forms a new tip. The initial stimulus that determines the general location of tip emergence therefore comes from the asymmetry in mechanical forces within the tissue layer during interaction between the coenosarc and perisarc. Periodically the coenosarc is pressed over the perisarc from inside, and the border between the hydranth pedicel and the rest of the perisarc is the most curved region of the internode. This curvature will cause local mechanical stress, and fixes the precise place of tip emergence (Fig. 13A).[94] Experimental alteration of the position of maximal curvature of the perisarc results in emergence of a new tip at the new position of maximal curvature, providing strong support for this hypothesis. [52]

Subdivision of a Shoot Tip

The best hypothesis for growth, morphogenesis and subdivision of the tip into several rudiments in monopodial shoots with terminally located tips, was proposed by L.Beloussov.[95,96] The central idea of this hypothesis is that the transition from one form to the other in thecate hydroids is based of the shifts of the region of the maximal active stretching of the rudiment (tip) surface in basicoapical direction within one growth pulsation. These shifts cause symmetric or asymmetric narrowing or widening of the tip. In the case of successive widening the tip would subdivide into several parts according to mechanical properties of such structures. The forms of the rudiments predicted from this theory fit well with the main types of branching actually seen in thecate hydroids (Fig. 14). The relative activities of the ectoderm and endoderm in the tip are considered to be the main mechanism of such shifts,[97] and the 'physical' properties of the tissue layers – quasi-elasticity and mechanical cell-cell interaction – are used as the main varying parameters of the model.[96]

An additional condition of the model is that the successive changes in the shape of the developing tip have to be fixed by the perisarc and changes in tip form are possible only during growth pulsation.[98] If the border between the already-hardened and still-soft perisarc (which is released on the apical surface of the growing tip) shifts closer to the tip apex, the tip becomes narrower. If the border shifts away from the tip instead, the tip expands in width. Spherical tips become intrinsically unstable as the tips enlarge,[18] leading to the splitting of the tip into several rudiments. Alternatively it is possible that asymmetry in the local rate of hardening of the perisarc would provoke subdivision of the tip.

Emergence of the Lateral Branches

There are two main variants of branching in colonial hydroids: 1) the process of branching is not regular and the branches appear on an already-formed stem; 2) the branching is regular and the next branch tip is formed by subdivision of the stem tip in the course of shoot internode development. In the latter case, the cyclic morphogenesis of the shoot becomes more complicated by inclusion of branch tip formation into the growth cycle: the secondary morphogenetic cycle now includes formation of several internodes and one branch (Marfenin, Kosevich, in press) (Fig. 15).

If the branch is started later, the main rules will perhaps be the same as those for the appearance of a new growing tip in sympodial shoots. The branching point is close to the base of the hydranth and the relative state of the soft tissue and the outer skeleton provide necessary conditions for initiation of the new tip (Fig. 16A). There are still many unanswered questions. For example, in most species with compact shoot stem and no regular branching, the bases of

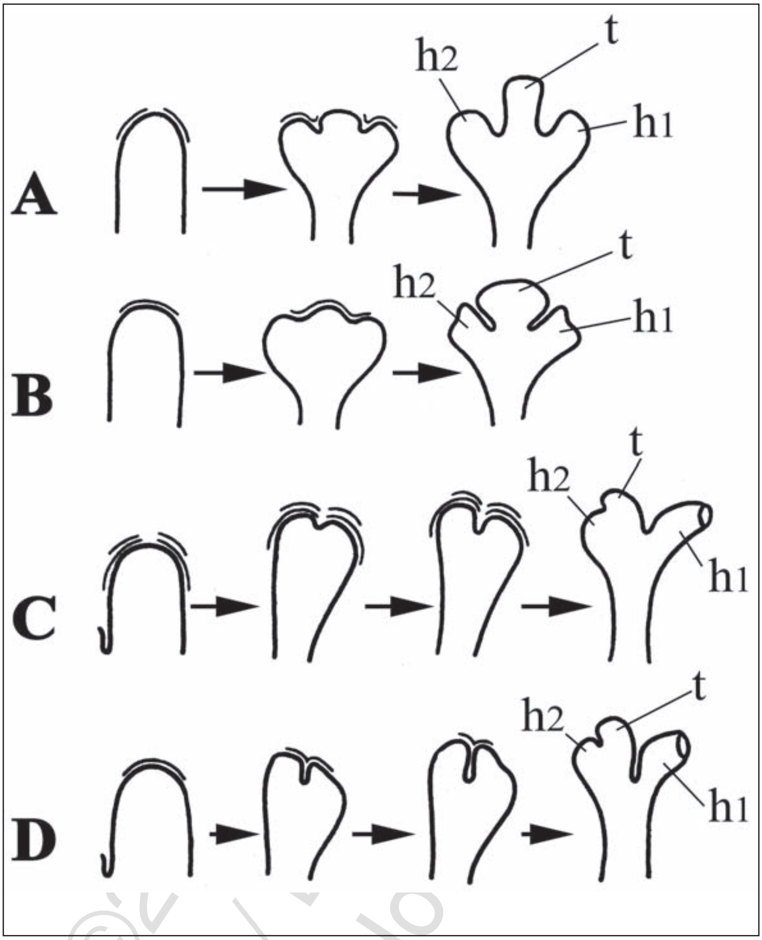

Figure 14. Schematic illustration of the different fates of the tip growth and development in the case of the basicoapical shifts of the regions of the maximal active stretching of the tip surface (shown by second contour) during growth pulsations.
A, B. Symmetric tips (rudiments). C, D. Asymmetric tips (rudiments).
h1 – first hydranth, h2 – second hydranth, t – growing tip. (Modified after Belousov, 1975.)

branches are localised on one side of the stem rather than being symmetrical with respect to the axis of the shoot (Fig. 16B). Nothing is known about why.

Control of Developmental Fate

When a tip subdivides into rudiments that give rise to different structures, such as hydranth, lateral branch, stem *etc.*, some system must act to regulate the developmental fate of each. The models that seem most reasonable are based on the variations of the hypothesis of local activation and lateral inhibition.[46,80,90,99,100] These models describe the determination of the rudiment fate on the bases of distance control, and are founded on the interaction of three mutually dependent 'players'; one activator and two inhibitors. This models fit most of the observed data and experimental results on branching processes in cnidaria and in plants and they set out an agenda for experimental identification of their molecular components. The

Figure 15. Microphotograph of the terminal part of *Abietinaria abietina* (Sertulariidae) shoot illustrating complex shoot internodes that include obligatory lateral branch formation.
b – branch, bt – branch growing tip, h – hydranth, st – shoot stem, stt+h – shoot tip in the course of subdivision into the shoot stem tip and hydranth rudiment. Arrows indicate the boarders of the internodes (marked by light furrows).

models do have various problems, however, in the case of certain highly-integrated species of colonial hydroids and will require improvements or introduction of additional parameters.

The Genetic Basis of Branching in Cnidarians

The genetic and molecular basis for branching in cnidarians remains unclear, mainly because cnidarian genomes have not yet been studied in detail. In *Hydra,* the Wnt signalling pathway has been shown to control formation of head structures.[101-103] The budding that results in the organisation of a second axis and head structures in *Hydra* can be compared with branching in colonial forms. Some conserved genes are expressed at early stages of bud formation.[102-110] In *Hydractinia* the gene *budhead*, a *fork head* homologue, seems to be involved in

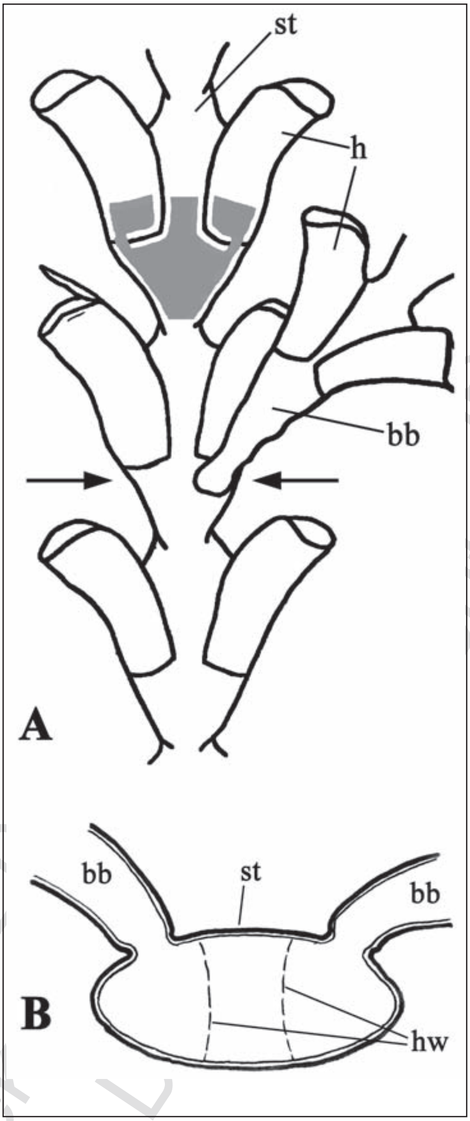

Figure 16. Places of the lateral branches formation upon shoot with irregular branching.
A. Part of the shoot of *Diphasia fallax* with the base of the lateral branch. Shadowed area shows relative position of the soft tissue within the skeleton. B. Cross-section through the shoot stem at the level of the branches bases displaying their asymmetric position.
bb – branch base, h – hydranth, hw – relative position of the inner walls of the upper hydrothecae, st – shoot stem.

the earliest stages of the polyp head determination during larva metamorphosis.[111] Some genetic information is now being obtained for the fate control between stolon and shoots (or hydranths); in *Hydractinia,* the gene *Cn-ems*, an *empty-spiracle* homologue, is expressed in the head region of gastrozooids (feeding polyps) and not in blastostyles (reproductive polyps);

Cnox-2 expression differs between polyp types and between polyps and stolons, implying possible specificity in expression during development of different elements within the colony.[111-113] Nevertheless, the really important genetic questions remains open: what is the primary signal that starts the whole sequence of events leading to the initiation of the new tip (new axis) formation? What determines the fate of a new axis? The expression patterns of all of the genes studied to date are simply consequences, and not causes, of these unknown regulatory events.

References

1. Fujisawa T, Sugiyama T. Genetic analysis of developmental mechanisms in Hydra. IV. Characterization of a nematocyst-deficient strain. J Cell Sci 1978; 30:175-85.
2. Bode H, Dunne J, Heimfeld S et al. Transdifferentiation occurs continously in adult Hydra. In: Yamada Science Foundation, ed. Current Topics in Developmental Biology. Japan: Academic Press, 1986:20:257-80.
3. Smid I, Tardent P. Migration of I-cells from ectoderm to endoderm in *Hydra attenuata* Pall (Cnidaria, Hydrozoa) and their subsequent differentiation. Dev Biol 1984; 106:469-77.
4. Teragawa CK, Bode HR. Migrating interstitial cells differentiate into neurons in hydra. Dev Biol 1995; 171:286-93.
5. Van de Vyver G. Etude du developpment embryonnaire des hydraires athecates (Gymnoblastiques) a gonophores. 2. Formes a planula. Arch Biol (Paris) 1967; 78:451-518.
6. Bodo F, Bouillon J. Etude histologique du developpment embryonnaire de queiques Hydromeduses de Roscoff: *Phialidium hemisphaericum* (L.), Obelia sp Peron et Lesueur, *Sarsia eximia* (Allman), *Podocoryne carnea* (Sars), *Gonionemus vertens* Agassiz. Cah Biol mar 1968; 9:69-104.
7. Lenhoff HM. Our link with the Trambleys - Abraham (1710-1784), MAurice (1874-1942) and Jean-Gustave (1903-1977). In: Tardent P, Tardent R, eds. Developmental and cellular biology of coelenterates. Amsterdam: Elsevier/N. Holland Biomed Press, 1980:xvii-xxiv.
8. Burnett AL. A model of growth and cell differentiation in Hydra. Amer Naturalist 1966; 100:165-89.
9. Marcum BA, Campbell RD. Developmental roles of epithelial and interstitial cell lineages in hydra: Aanalysis of chimeras. J Cell Sci 1978; 32:233-47.
10. Rose PG, Burnett AL. The origin of secretory cells in *Cordylophora caspia* during regeneration. Wilhelm Roux's Arch EntwMech Org 1970; 165:192-216.
11. Rose PG, Burnett AL. The origin of the mucous cells in *Hydra viridis*. II. Mid-gastric regeneration and budding. Wilhelm Roux's Arch EntwMech Org 1970; 165:177-91.
12. Werner B. New investigations on systematics and evolution of the class Scyphozoa and the phylum Cnidaria. Publs Seto Mar Biol Lab 1973; 20:35-61.
13. Werner B. Die neue Cnidariaklasse Cubozoa. Verh Dtsch Zool Ges 1976; 230.
14. Werner B. Life cycles of the Cnidaria. In: Tardent P, Tardent R, eds. Development and Cellular Biology of Coelenterates. Elsevier / North-Holland: Elsevier / North-Holland Biomedical Press 1980; 3-10.
15. Beklemishev VN. Principles of comparative anatomy of Invertebrates. Edinburgh and University of Chicago Press: Oliver & Boyd Ltd 1970; 1-490.
16. Marfenin NN. Evolution of colonial organisation in hydroid order Leptolida. Transactions of Paleontological Institute, Ac Sci USSR 1987; 222:4-19.
17. Beloussov LV. Growth and morphogenesis of some marine hydrozoa according to histological data and time-lapse studies. Publ Seto Mar Biol Lab 1973; 20:315-66.
18. Beloussov LV, Dorfman YaG. On the mechanics of growth and morphogenesis in hydroid polyps. Amer Zool 1974; 14:719-34.
19. Beloussov LV, Badenko LA, Labas JuA. Growth rhythms and species-specific shape in Thecaphora hydroids. In: Tardent P, Tardent R, eds. Developmental and Cellular Biology of Coelenterates. Amsterdam: Elsevier/North-Holland Biomedical Press, 1980; 175-8.
20. Beloussov LV, Kazakova NI, Labas JuA. Growth pulsations in hydroid polyps: Kinematics, biological role, and cytophysiology. In: Rensing L, ed. Oscillations and Morphogenesis. New York: Marcel Dekker Inc., 1993:183-93.
21. Beloussov LV, Labas JuA, Badenko LA. Growth pulsations and rudiment shapes in hydroid polyps. Zhurnal Obshchej Biologii 1984; 45:796-806.
22. Crowell S. Morphogenetic events associated with stolon elongation in colonial hydroids. Amer Zool 1974; 14:665-72.
23. Hale LJ. Contractility and hydroplasmic movements in the hydroid *Clytia johnstoni*. Quarterly J Microsc Sci 1960; 101:339-50.

24. Wyttenbach ChR. The dynamics of stolon elongation in the hydroid, *Campanularia flexuosa*. J Exp Zool 1968; 167:333-52.
25. Wyttenbach ChR, Crowell S, Suddith RL. The cyclic elongation of stolons and uprights in the hydroid, *Campanularia*. Biol Bull 1965; 129:429
26. Wyttenbach ChR, Crowell S, Suddith RL. Variations in the mode of stolon growth among different genera of colonial hydroids, and their evolutionary implications. J Morphol 1973; 139:363-75.
27. Braverman M. Studies on hydroid differentiation. VII. The hydrozoan stolon. J Morphol 1971; 135:131-52.
28. Hale LJ. Cell movement, cell division and growth in the hydroid *Clytia johnstoni*. J Embryol Exp Morph 1964; 12:517-38.
29. Hale LJ. The pattern of growth of *Clytia johnstoni*. J Embryol Exp Morphol 1973; 29:283-309.
30. Braverman M. Studies on hydroid differentiation IV. Cell movements in *Podocoryne carnea* hydranths. Growth 1969; 33:99-111.
31. Braverman M. The cellular basis of hydroid morphogenesis. Publ Seto Mar Biol Lab 1973; 20:221-56.
32. Crowell S, Wyttenbaàæ ChR, Suddith RL. Evidence against the concept of growth zones in hydroids. Biol Bull 1965; 129:403
33. Kossevitch IA. Cell migration during growth of hydroid colony. Zhurnal Obshchej Biologii 1999; 60:91-8.
34. Suddith RL. Cell proliferation in the terminal regions of the internodes and stolons of the colonial hydroid *Campanularia flexuosa*. Amer Zool 1974; 14:745-55.
35. Wyttenbach ChR. Sites of mitotic activity in the colonial hydroid, *Campanularia flexuosa*. Anat Rec 1965; 151:483
36. Wyttenbach ChR. Cell movements associated with terminal growth in colonial hydroids. Amer Zool 1974; 14:699-717.
37. Marfenin NN, Burykin YuB, Ostroumova TV. Organismal regulation of the balanced growthy in hydroid colony *Gonothyraea loveni* (Allm.). Zhurnal Obshchej Biologii 1999; 60:80-90.
38. Gooday GW. An autoradiographic study of hyphal growth of some fungi. J Gen Microbiology 1971; 67:125-33.
39. Mulisch M. Chitin in Protistan organisms. Distribution, synthesis and deposition. Europ J Protistol 1993; 29:1-18.
40. Katz D, Rosenberger RF. Hyphal wall synthesis in *Aspergillus nidulans*: Effect of protein synthesis inhibition and osmotic shock on chitin insertion and morphogenesis. J Bacteriol 1971; 108:184-90.
41. Robertson NF. The growth process in fungi. A Rev Phytopath 1968; 6:115-136.
42. Stratford M. Another brick in the wall? Recent developments concerning the yeast cell envelope. Yeast 1994; 10:1741-52.
43. Wessels JGH, Sietsma JY, Sonnenberg ASM. Wall synthesis and assembly during hyphal morphogenesis in *Schizophyllum commune*. J Gen Microbiology 1983; 129:1607-1616.
44. Compere P. Cytochemical labelling of chitin. In: Giraud-Guille MM, ed. Chitin in Life Sciences. Lyon, France: Andre Publisher, 1996:66-87.
45. Zaraisky AG, Beloussov LV, Labas JuA et al. Studies of cellular mechanisms of growth pulsations in hydroid polyps. Russian Journal of Developmental Biology 1984; 15:163-169.
46. Berking S, Hesse M, Herrmann K. A shoot meristem-like organ in animals; monopodial and sympodial growth in Hydrozoa. Int J Dev Biol 2002; 46:301-308.
47. Marfenin NN. The phenomenon of coloniality. Moscow: Moscow State Univ Publisher, 1993:1-239.
48. Kosevich IA. Regulation of formation of the elements of the hydroid polyps colony. Russian Journal of Developmental Biology 1996; 27:95-101.
49. Marfenin NN, Kosevich IA. Colonial morphology of the hydroid *Obelia loveni* (Allm.)(Campanulariidae). Vestnik Moskovskogo Universiteta Biologia 1984; 2:37-45.
50. Kosevich IA. Development of stolon's and stem's internodes in hydroid genera *Obelia* (Campanulariidae). Vestnik Moskovskogo Universiteta Biologia 1990; 3:26-32.
51. Kosevich IA. Regulation of the "giant" shoot structure in the colonial hydroid *Obelia longissima* (Campanulariidae). Russian Journal of Developmental Biology 1991; 22:204-210.
52. Kossevitch IA. Role of the skeleton in determination of the branching points in hydroid colonies. Zhurnal Obshchej Biologii 2002; 63:40-49.
53. Belousov LV, Dorfman YaG. Mechanisms of growth and morphogenesis in hydroid polyps by the data of time lapse microcinematography. Russian Journal of Developmental Biology 1974; 5:437-445.
54. Kosevich IA, Marfenin NN. Colonial morphology of tyhe hydroid *Obelia longissima* (Pallas, 1766)(Campanulariidae). Vestnik Moskovskogo Universiteta Biologia 1986; 3:44-52.
55. Marfenin NN. The hydroid colony as an organism: Regulation of growth in the entire colony. Proceedings of the 6th International Conference on Coelenterate biology 1995; 315-320.

56. Leontovich AA, Marfenin NN. Connection of major intercolonial processes at branching in colonial hydroids. Zhurnal Obshchej Biologii 1990; 51:353-362.
57. Bode HR. Activity of *Hydra* cells in vitro and in regenerating cell reaggregates. Amer Zool 1974; 14:543-550.
58. Bosch TC, David CN. Growth regulation in *Hydra*: Relationship between epithelial cell cycle length and growth rate. Dev Biol 1984; 104:161-171.
59. Blackstone NW. Gastrovascular flow and colony development in two colonial hydroids. Biol Bull 1996; 190:56-68.
60. Blackstone NW. Dose-response relationships for experimental heterochrony in a colonial hydroid. Biol Bull 1997; 193:47-61.
61. Gierer A, Meinhardt H. A theory of biological pattern formation. Kybernetik 1972; 12:30-39.
62. Meinhardt H, Gierer A. Applications of a theory of biological pattern formation based on lateral inhibition. J Cell Sci 1974; 15:321-346.
63. Meinhardt H. Models of biological pattern formation: Common mechanism in plant and animal development. Int J Dev Biol 1996; 40:123-134.
64. Muller WA, Plickert G. Quantitative analysis of an inhibitory gradient field in the hydrozoan stolon. Wilhelm Roux's Arch. 1982; 191:56-63.
65. Plickert G. Mechanically induced stolon branching in *Eirene viridula* (Thecata, Campanulinidae). In: Tardent P, Nardent R, eds. Developmental and cellular biology of Coelenterates. Amsterdam: Elsevier/North-Holland Biomedical Press, 1980:185-190.
66. Plickert G. Low-molecular-wight factor from colonial hydroids affect pattern formation. Wilhelm Roux's Archiv Dev Biol 1987; 248-256.
67. Plickert G, Heringer A, Hiller B. Analysis of spacing in a periodic pattern. Dev Biol 1987; 120:399-411.
68. Dudgeon SR, Buss LW. Growth with the flow: On the maintenance and malleability of colony form in the hydroid *Hydractinia*. Amer Naturalist 1996; 147:667-91.
69. Dudgeon SR, Wagner A, Vaisnys RJ et al. Dynamics of gastrovascular circulation: Clues to understanding colony integration and morphogenesis in hydrozoans. In: Grassle JP, Kelsey A, Oates E, Snelgrove PV, eds. Twenty Third Benthic Ecology Meeting. New Brunswick, Nj, USA: Rutgers the State Univ Inst Marine Coastal Sciences, 1995:5.
70. Dudgeon S, Wagner A, Vaisnys JR et al. Dynamics of gastrovascular circulation in the hydrozoan *Podocoryne carnea*: The one-polyp case. Biol Bull 1999; 196:1-17.
71. Fulton C. Rhythmic movements in *Cordylophora*. J cell comp Physiol 1963; 61:39-52.
72. Karlsen AG, Marfenin NN. Hydroplasm movements in the colony of hydroids, *Dynamena pumila* L. and some other species taken as examples. Zhurnal Obshchej Biologii 1984; 45:670-680.
73. Marfenin NN. The functioning of the pulsatory-peristaltic type transport system in colonial hydroids. Zhurnal Obshchej Biologii 1985; 46:153-164.
74. Winkle Van DH, Blackstone NW. Video microscopical measures of gastrovascular flow in colonial hydroids. Invertebrate Biology 1997; 116:6-16.
75. Marfenin NN. Study of the integration of the colony of *Dynamena pumila* (L.) using quantitative morphologival criteria. Zhurnal Obshchej Biologii 1977; 38:409-422.
76. Berking S. Metamorphosis of *Hydractinia* echinata. Insights into pattern formation in Hydroids. Roux's Arch Dev Biol 1984; 193:370-378.
77. Berking S. Hydrozoa metamorphosis and pattern formation. Curr Top Dev Biol 1998; 38:81-131.
78. Berking S. A model for budding in hydra: Pattern formation in concentric rings. J Theor Biol 2003; 222:37-52.
79. Leontovich AA. Regularities in spatial distribution of hydranth and stolons in a hydroid, *Cordylophora inkermanica* (Hydrozoa, Clavidae). Zhurnal Obshchej Biologii 1991; 52:534-550.
80. Meinhardt H. A model for pattern formation of hypostome, tentacles, and foot in hydra: How to form structures close to each other, how to form them at a distance. Dev Biol 1993; 157:321-333.
81. Meinhardt H. Organizer and axes formation as a self-organizing process. Int J Dev Biol 2001; 45:177-188.
82. Meinhardt H, Gierer A. Pattern formation by local self-activation and lateral inhibition. Bio Essays 2000; 22:753-760.
83. Pfeifer R, Berking S. Control of formation of the two types of polyps in *Thecocodium quadratum* (Hydrozoa, Cnidaria). Int J Dev Biol 1995; 39:395-400.
84. Walther M, Ulrich R, Kroiher M et al. Metamorphosis and pattern formation in *Hydractinia echinata*, a colonial hydroid. Int J Dev Biol 1996; 40:313-322.
85. Blackstone NW. Morphological, physiological and metabolic comparisons between runner-like and sheet-like inbred lines of a colonial hydroid. J Exp Biol 1998; 201:2821-2831.

86. Blackstone NW. Redox control in development and evolution: Evidence from colonial hydroids. J Exp Biol 1999; 202(24):3541-53.
87. Blackstone NW. Redox control and the evolution of multicellularity. BioEssays. 2000; 22:947-953.
88. Blackstone NW. Redox state, reactive oxygen species and adaptive growth in colonial hydroids. J Exp Biol 2001; 204:1845-1853.
89. Blackstone NW. Redox signaling in the growth and development of colonial hydroids. J Exp Biol 2003; 206:651-658.
90. Wolpert L. Positional information and pattern formation. Philos Trans R Soc Lond B Biol Sci 1981; 295:441-4450.
91. Wolpert L. The evolutionary origin of development: Cycles, patterning, privilege and continuity. Development 1994; Supplement:79-84.
92. Wolpert L. One hundred years of positional information. Trends Genet 1996; 12:359-364.
93. Kerszberg M, Wolpert L. The origin of metazoa and the egg: A role for cell death. J Theor Biol 1998; 193:535-537.
94. Beloussov LV. Patterns of mechanical stresses and formation of the body plans in animal embryos. Verh Dtsch Zool Ges 1996; 89:219-229.
95. Belousov LV. Possible ontogenetic mechanisms governing formation of principal morphogenetic types of thecaphoran hydroids. Zhurnal Obshchej Biologii 1975; 36:203-211.
96. Beloussov LV, Grabovsky VI. A Geometro-mechanical model for pulsatile morphogenesis. Comput Methods Biomech Biomed Engin 2003; 6:53-63.
97. Beloussov LV. Basic morphogenetic processes in Hydrozoa and their evolutionary implications: An exercise in rational taxonomy. In: Williams RB, Cornelius PFS, Hughes RG, Robson EA, eds. Coelenterate Biology: Recent Research On Cnidaria And Ctenophora. Dortrecht: Kluwer Acad Publ., 1991; 61-67.
98. Kossevitch IA, Herrmann K, Berking S. Shaping of colony elements in *Laomedea flexuosa* Hinks (Hydrozoa, Thecaphora) includes a temporal and spatial control of skeleton hardening. Biol Bull 2001; 201:417-423.
99. Wolpert L. Positional information revisited. Development 1989; (Supplement):3-12.
100. Wolpert L, Stein WD. Positional information and pattern formation. In: Maqlacinski GM, Bryant SV, eds. Pattern formation. A primer in developmental biology. New-York: Macmillan Publishing Company, A Division of Macmillan Inc., 1984:3-21.
101. Hobmayer B, Rentzsch F, Kuhn K et al. WNT signalling molecules act in axis formation in the diploblastic metazoan *Hydra*. Nature 2000; 407:186-189.
102. Gauchat D, Kreger S, Holstein T et al. prdl-a, a gene marker for hydra apical differentiation related to triploblastic paired-like head-specific genes. Development 1998; 125:1637-1645.
103. Technau U, Cramer VL, Rentzsch F et al. Parameters of self-organization in *Hydra* aggregates. Proc Natl Acad Sci USA 2000; 97:12127-12131.
104. Broun M, Sokol S, Bode HR. Cngsc, a homologue of goosecoid, participates in the patterning of the head, and is expressed in the organizer region of *Hydra*. Development 1999; 26:5245-5254.
105. Gauchat D, Mazet F, Berney C et al. Evolution of *Antp-class* genes and differential expression of *Hydra Hox/paraHox* genes in anterior patterning. Proc Natl Acad Sci USA 2000; 97:4493-4498.
106. Hermans-Borgmeyer I, Schinke B, Schaller HC et al. Isolation of a marker for head-specific cell differentiation in hydra. Differentiation 1996; 61:95-101.
107. Martinez DE, Dirksen ML, Bode PM et al. Budhead, a fork head/HNF-3 homologue, is expressed during axis formation and head specification in hydra. Dev Biol 1997; 192:523-536.
108. Technau U, Bode HR. HyBra1. a Brachyury homologue, acts during head formation in *Hydra*. Development 1999; 126:999-1010.
109. Shenk MA, Bode HR, Steele RE. Expression of Cnox-2, a *HOM/HOX* homeobox gene in hydra, is correlated with axial pattern formation. Development 1993; 117:657-667.
110. Shenk MA, Gee L, Steele RE et al. Expression of Cnox-2, a *HOM/HOX* gene, is suppressed during head formation in *Hydra*. Dev Biol 1993; 160:108-118.
111. Mokady O, Dick MH, Lackschewitz D et al. Over one-half billion years of head conservation? Expression of an ems class gene in *Hydractinia symbiolongicarpus* (Cnidaria: Hydrozoa). Proc Natl Acad Sci USA 1998; 95:3673-368.
112. Cartwright P, Buss LW. Colony integration and the expression of the *Hox* gene, *Cnox-2*, in *Hydractinia symbiolongicarpus* (Cnidaria: Hydrozoa). J Exp Zool 1999; 285:57-62.
113. Cartwright P, Bowsher J, Buss LW. Expression of a Hox gene, *Cnox-2*, and the division of labor in a colonial hydroid. Proc Natl Acad Sci USA 1999; 96:2183-2186.