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Head formation in *Hydra* is different at apical and basal levels

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SUMMARY

***Hydra*'s head is a compound structure with a hypostome at the apical extreme and a circle of tentacles more basally. During head regeneration, it is thought (P. M. Bode, T. A. Awad, O. Koizumi, Y. Nakashima, C. J. P. Grimmelikhuijzen and H. R. Bode (1988) *Development* 102, 223-235; R. Weinziger, L. M. Salgado, C. N. David and T. C. G. Bosch (1994) *Development* 120, 2511-2517) that the conditions for tentacle formation are fulfilled before those for hypostome formation. Using a new hypostome-specific marker, we have found that the order of hypostome and tentacle formation is variable. In regenerating basal tissue, the hypostome marker is expressed before tentacles appear but in apical tissue, the tentacles appear first. This observation appears inconsistent with current views but can be explained by a hierarchical model (H. Meinhardt (1993) *Developmental Biology* 157, 321-333) in which tentacles require an inductive influence of the hypostome. In basal regenerates, the hypostome forms first and then induces tentacles. In apical regenerates, inductive factor remains from the amputated hypostome, and tentacle may form before the new hypostome. We have also observed that the mode of expression of the tentacle marker differs in basal and apical tissue. In basal tissue, the marker first appears in the definitive tentacle zone; in apical tissue, the marker first appears in the position of the presumptive hypostome and is then displaced to its final position, as described by previous workers. This observation is also expected according to the above-cited model.**

Key words:*Hydra*, regeneration, axis formation, pattern formation, gradient

INTRODUCTION

Axis formation is a crucial event in embryogenesis. Most metazoans are bilateral, that is, possess an anteroposterior and a dorsoventral axis. The freshwater polyp *Hydra* has a radially symmetrical body plan and thus a single axis; this extends between the two terminal structures, the mouth and the foot. Little is known about the formation of this apicobasal axis during embryogenesis, but studies of dissociated cells (Gierer et al., 1972; Sato et al., 1990, 1992; Technau and Holstein, 1992) show that the axis can be formed de novo. This process can be explained by theoretical models of self-organisation (Turing, 1952; Gierer and Meinhardt, 1972).

An apicobasal axis can also be formed in the process of regeneration. In this case, pattern formation does not occur de novo, but is guided by a pre-existing polarity of the tissue. Extensive transplantation studies (Browne, 1909; Webster 1966a,b; Wilby and Webster, 1970a,b; Webster and Wolpert, 1966; Webster, 1971; MacWilliams, 1983a,b) have led to formal models of this process. It appears that there is a continuous gradient of a property that has been called 'head activation' along the body axis (MacWilliams, 1982, 1983a,b). During head regeneration, the gradient level in the head anlage increases continuously until levels characteristic of the apical terminus are attained.

Hydra's head is a compound structure composed of an apical hypostome and a slightly more basal tentacle ring. The relation between the hypostome and tentacles is not completely clear. The currently prevailing view, which borrows from classical ideas of 'positional information', is that the hypostome is formed at the peak of the apicobasal gradient, whereas tentacles represent a somewhat lower level. According to this view, one would expect a regenerating hypostome to pass through a stage of tentacle identity before assuming hypostomal character. This idea is supported by the expression pattern of a tentacle-specific antigen, TS19, during head regeneration. TS19 antigen is first expressed at the apical tip, the position of the presumptive hypostome. Expression subsequently spreads to the presumptive tentacle area and is finally lost from the tip, persisting only in the emerging tentacles (Bode et al., 1988). A similar pattern was observed by in situ hybridisations directed against the RNA of the 'head specific' gene *ks1*(Weinzinger et al., 1994).

Heretofore only tentacle markers were available to investigate head formation. Recently, we identified a monoclonal antibody, L96, which recognises a population of specialised epithelial cells located around the mouth – at the most apical position in the hypostome (Technau and Holstein, 1995). In this paper, we have reinvestigated the relation between hypostome and tentacle formation during head formation using this marker as well as the pre-existing marker TS19. We found that the relation between tentacle and hypostome formation is different during regeneration from apical and basal starting levels. The control of tentacle formation thus appears more complex than previously envisioned; it is in accord, however, with a recently published model (Meinhardt, 1993).

MATERIALS AND METHODS

Animals

If not otherwise indicated, polyps of *Hydra vulgaris*, strain Basel, were used; this strain was isolated 1980 from a pond near Basel by T. Honegger. For ecto-endodermal chimeras, we used *Hydra vulgaris* Basel and a strain of *Hydra vulgaris* isolated by P. Tardent from Lake Zürich in 1962. Mass cultures were kept in M-Solution at 18±0.5°C and fed daily with *Artemia* brine shrimp nauplii (Loomis and Lenhoff, 1956; Muscatine and Lenhoff, 1965). Animals were starved for 24 hours before all experimental treatments.

Strain specificity of the staining pattern

The L96 antibody appears to be specific for *Hydra vulgaris* Basel and one other strain of *Hydra vulgaris* isolated in 1993 near Vienna; no staining was observed in 5 other strains of *Hydra vulgaris* isolated from different locations in Middle Europe. It is possible that L96 antigen-positive strains are a subspecies of *Hydra vulgaris* (see Holstein et al., 1990). In strain Basel, L96 monoclonal antibody specifically recognises, in addition to apical cells, a subset of neurons in the lower peduncle (Technau and Holstein, unpublished observations).

Construction of ecto-endodermal chimeras

To obtain large numbers of ecto-endodermal chimeras, we used the procaine method (Smid and Tardent, 1984), as modified by Bode et al. (1987), together with the reaggregation technique (Gierer et al., 1972). Ectoderm/endoderm separation occurs if isolated body columns are sequentially incubated in a solution of 0.5% procaine in dissociation medium and M-solution (2:1) at pH 2.5 (1 minute) and pH 4.5 (2-5 minutes) and subsequently transferred to dissociation medium. Using Inox 5 forceps, we carefully separated ectodermal and endodermal tissue from polyps of both strains (*Hydra vulgaris* Zürich and *Hydra vulgaris* Basel) and recombined ectoderm from *Hydra vulgaris* Zürich with endoderm from *Hydra vulgaris* Basel). After dissection into smaller fragments, we reaggregated the pieces as described previously (Technau and Holstein, 1992). Animals regenerating from these reaggregates were grown in mass cultures and used for further experiments.

Immunocytochemistry of whole mounts

Animals were relaxed in 2% urethane in hydra medium for one minute and fixed in Lavdovsky's fixative for at least 24 hours (Technau and Holstein, 1995). After washing extensively in PBS (phosphate-buffered saline, pH 7.2), the polyps were incubated overnight in either the monoclonal antibody L96 or TS19 (H. Bode, Irvine; diluted 1:10000 in PBS/1% BSA/0.1% azide). To visualise the antibody L96, polyps were incubated for 2 hours with a FITC-conjugated goat anti-mouse antibody (Boehringer) diluted 1:50 in PBS/1% BSA/0.1%azide. TS19 antigen was visualised with a TRITC-conjugated goat anti-mouse antibody (Boehringer) diluted 1:50 in PBS/1%BSA/0.1% azide for one hour). For double staining experiments with two monoclonal antibodies (TS19 and L96), the antibody incubations were performed sequentially: 12 hours (overnight), monoclonal antibody L96; 2 hours, FITC-conjugated secondary antibody; 2 hours, monoclonal antibody TS19; 1 hour, TRITC-conjugated secondary antibody; between steps, polyps were thoroughly washed in PBS.

Definition of body regions

Regeneration (transplantation) sites were designated by the percentage of the body length between the site of regeneration (transplantation) and the apex (e.g. 50% body length, b.l.; MacWilliams, 1983a).

Transplantation experiments

Lateral grafting experiments were carried out in principle as outlined by MacWilliams (1983a). Large budless animals, starved for 24 hours, were used both for hosts and donors. There were four different kinds of grafts. For the first sort, heads were removed by cutting immediately under the tentacle ring (10% b.l., as in Fig. 2A) and the first eighth of the body column was isolated. For the remaining three sorts, animals were transected in the lower gastric region (70% b.l., as in Fig. 2C) and allowed to regenerate for 6, 9 and 12 hours, respectively; the regenerating heads were then isolated. All grafts were transferred to hosts incised in the middle of the gastric region (50% b.l.). After 2 days, the fraction of grafts that had developed head structures was scored.

RESULTS

The hypostome-specific monoclonal antibody L96 will be described in detail elsewhere (Technau and Holstein, 1995) (Fig. 1).

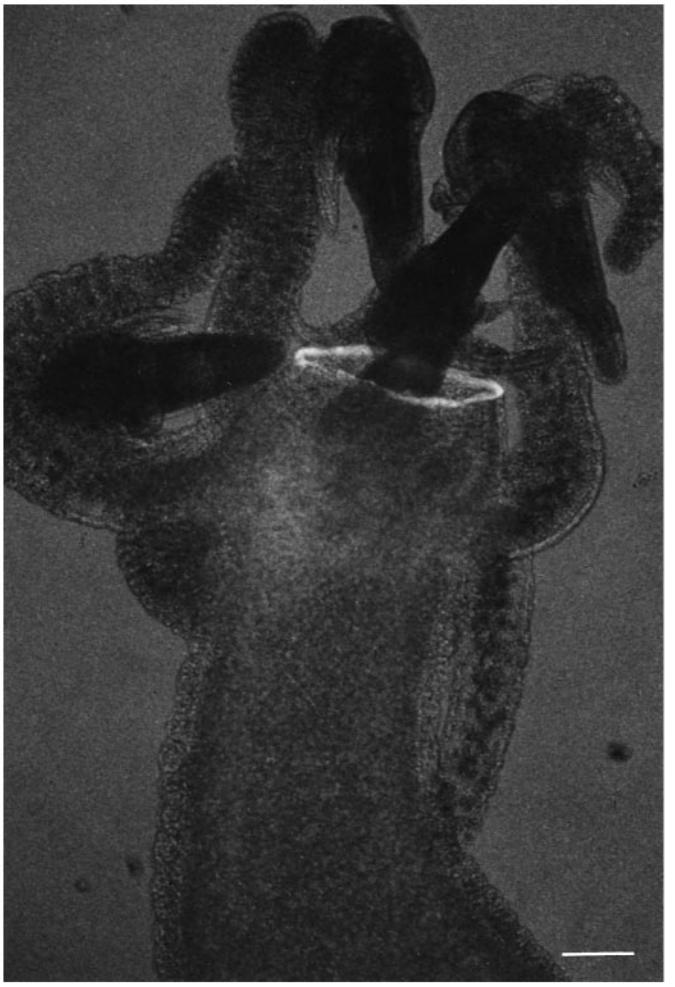


Fig. 1. Whole mount of a feeding polyp (*Hydra vulgaris* Basel). Polyps were fed with *Artemia* nauplii, fixed and prepared for immunocytochemistry using L96 monoclonal antibody (see Materials and Methods). The L96 antigen is expressed in a ring of endodermal epithelial cells surrounding the mouth opening, simultaneously defining the most apical position in the animal and the boundary between ectoderm and endoderm (for details see Technau and Holstein, 1995). Bar indicates 250 μm.

Hypostome and tentacle formation during regeneration from different levels

We followed the appearance of the L96 antigen and of tentacle protrusions during head regeneration from various axial levels. Budless animals were cut just below the tentacle ring (Fig. 2A), in the middle (Fig. 2B) or lower (Fig. 2C) gastric region, or slightly under the budding region (Fig. 2D); the apical portions were discarded. The earliest expression of the antigen was found in animals cut apically; animals cut more basally required progressively longer, so that there was a difference of about 12 hours between the apical (Fig. 2A) and basal fragments (Fig. 2C).

The gradient in time required for L96 antigen expression is reminiscent of the gradient in time required for 'head determination' reported in transplantation studies (Webster and Wolpert, 1966; MacWilliams, 1983b). We therefore remeasured the gradient in determination time in our animals (Table 1); the difference between the apical and basal fragments was 12 hours here as well. At all levels there is a constant interval between head determination and L96 antigen expression.

The formation of tentacles showed a variable relationship to the expression of L96 antigen. During regeneration from apical levels tentacles appeared about 12 hours before L96 antigen (Figs 2A, 3A) while, in regeneration from a basal level (Figs 2D, 3B,C), tentacles appeared slightly later than L96 antigen. At intermediate levels (Figs 2B,C), there was an intermediate result. This means that the delay between head determination

Table 1. Gradient in time for head determination

	Head formation frequency (%)	*n*
Fragment of the apical gastric region	42	72
Fragment of the basal gastric region regenerating a head after		
6 hours	10	49
9 hours	19	42
12 hours	40	52

Lateral grafts were prepared as described in Materials and Methods and outlined by MacWilliams (1983a). On day 2 after transplantation, the fraction of grafts that developed into secondary heads was scored (head formation frequency). In one set of grafts, the head formation frequency of apical gastric tissue was determined; in another set, the head formation frequency of basal tissue after 6, 9 and 12 hours of regeneration was measured (see Materials and Methods for further details of the transplantation procedure). The experiment indicates that basal tissue requires 12 hours to acquire the level of 'head determination' of apical tissue.

Table 2. Effect of tissue size on regeneration time in regenerates from apical levels

	Small-sized regenerates		Large-sized regenerates	
Regeneration time (hours)	Tentacles (%)	Hypostome (%)	Tentacles (%)	Hypostome (%)
24	35	0	27±2	0
30	100	0	88±5	0
48	100	100	98±3	85±20
72	100	100	100	100

Head regeneration from apical levels (cut at 10 % b.l.) was followed in small-sized (1/8 fragments of the body column) and large-sized (7/8 fragments of the body column) pieces of tissue. During 3 days following head removal, appearance of tentacles, as well as expression of L96 antigen in the hypostome was monitored. Data of large-sized tissue were taken from four independent experiments (mean and standard deviations).

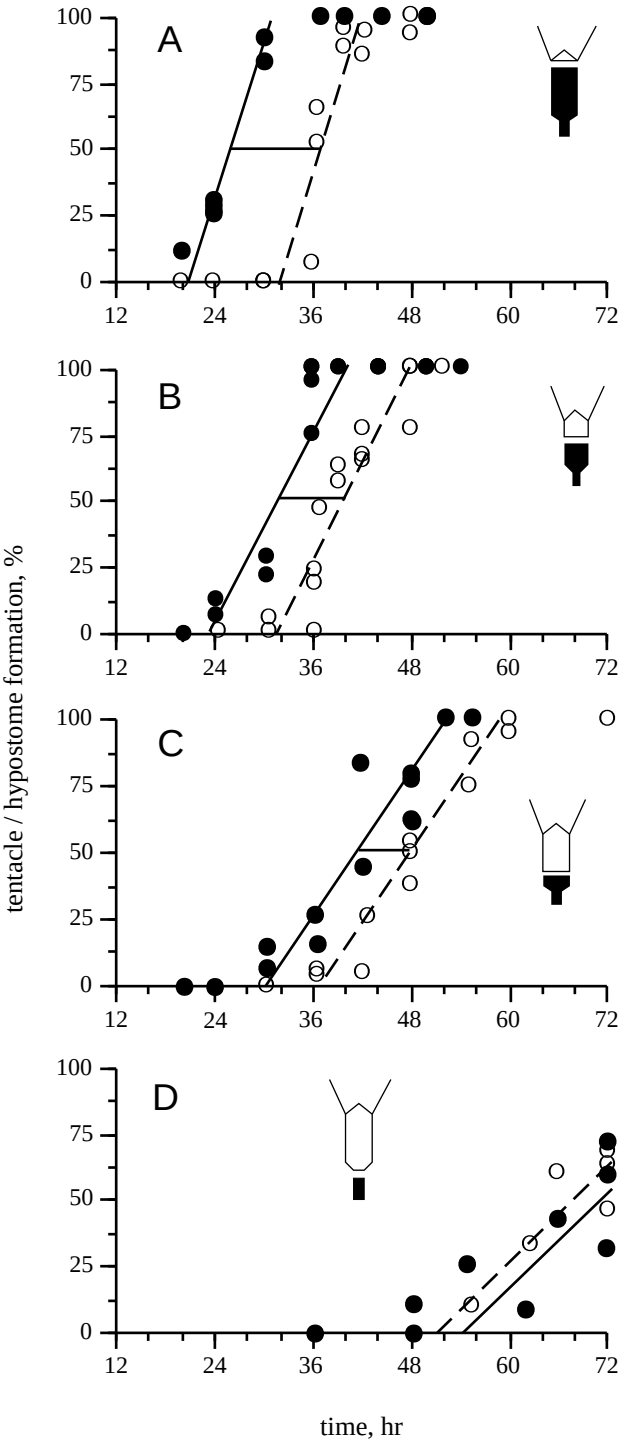


Fig. 2. Tentacle and hypostome regeneration of animals cut at various levels of the body column (for definition of body region see Materials and Methods). (A) Apical regenerate (10% b.l.); (B) mid-gastric regenerate (50% b.l.); (C) lower gastric regenerate (70% b.l.); (D) basal regenerate (80% b.l.). Symbols indicate ●, tentacles; ○, L96 antigen-positive hypostomal cells. Each data point corresponds to an independent experiment with 20-30 animals.

and tentacle formation is very different during regeneration from different levels.

The relationship between hypostome and tentacle regeneration formation is determined only by initial apicobasal value

Regenerating basal fragments (circa 800 epithelial cells) were about 10 times smaller than regenerating animals cut apically. Tissue size has been shown to influence regeneration (Shimizu et al., 1993); however, there was no effect of size on the kinetics of L96 antigen appearance and tentacle formation in apical fragments (Table 2). Furthermore, reaggregates of basal tissue containing about 8000 epithelial cells per reaggregate showed the same behaviour as basal regenerates (Fig. 4). Thus neither tissue size nor details that would be lost during disaggregation/reaggregation, such as gradient slope or shape, are important for the difference in behaviour of apical and basal regenerates.

Hypostome and tentacle formation in buds

We stained buds of different stages (Otto and Campbell, 1977a) for L96 antigen. L96 antigen expression was first seen 24-36 hours after the onset of budding (stage 5; Figs 5, 6A,B) before tentacle protrusions appeared. The antigen was expressed in all buds by the time the tentacles first became visible (stage 6; Figs 5, 6C,D). Head morphogenesis in buds thus resembled head regeneration in basal tissue.

L96 and TS19 antigen expression in single animals

TS19 antibody recognises tentacle cells only in the Zürich strain of *Hydra vulgaris*, whereas L96 antigen specifically binds to hypostomal cells only in the Basel strain. We wished to visualise both markers in the same animal and thus unequivocally establish their relative order of appearance. Since TS19 monoclonal antibody recognises an ectodermal antigen, while the L96 antigen-positive cells are endodermal, we constructed chimeras (Fig. 7) consisting of endoderm and ectoderm from the two different strains (see Materials and Methods).

For yet unknown reasons the regeneration of the chimeras from basal levels is slow and unreliable. It is possible, however, to compare apical regeneration in the chimeras with bud formation. We found that during apical regeneration TS19 antigen was expressed at the apical tip long before L96 antigen (Fig. 8A,B); it disappeared from

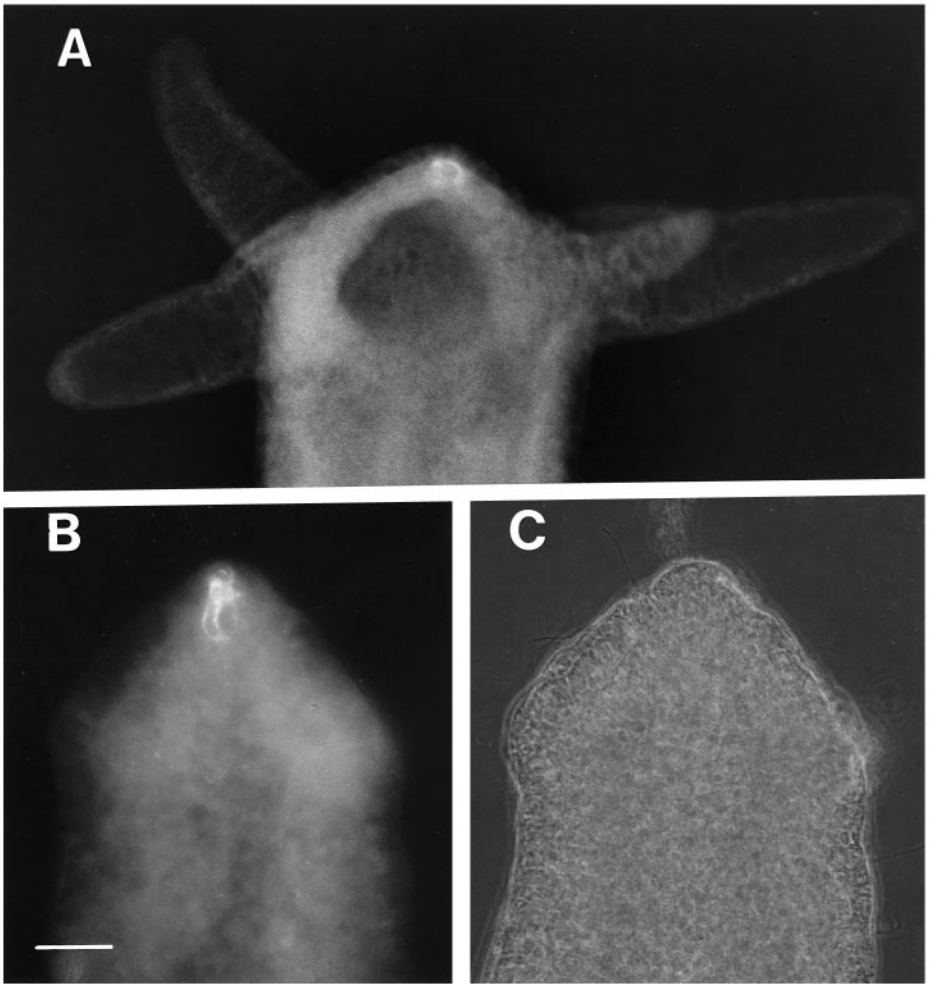


Fig. 3. L96 antigen expression in regenerates. (A) Whole mount of an apical regenerate (10% b.l.) at the onset of L96 antigen expression; tentacles are already well developed. (B) Basal regenerate (80% b.l.) with strong L96 antigen expression; phase-contrast image (C) shows that tentacles are still not formed. Bar indicates 65 μm.

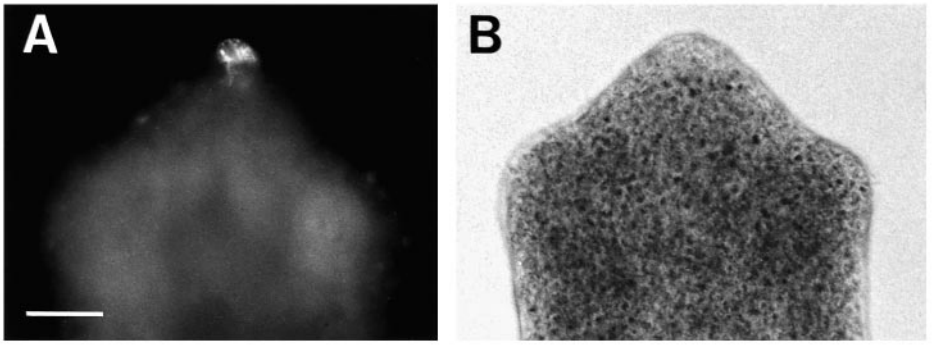
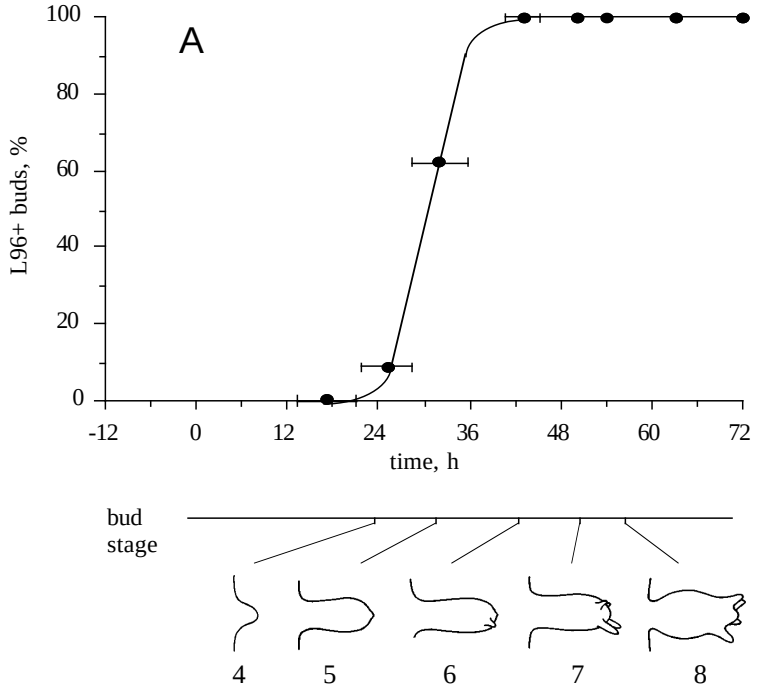


Fig. 4. L96 antigen expression in an aggregate (96 hours) of basal tissue; note that no tentacle rudiments are visible. (A) Indirect immunofluorescence; (B) bright-field image. Bar indicates 70 μm.



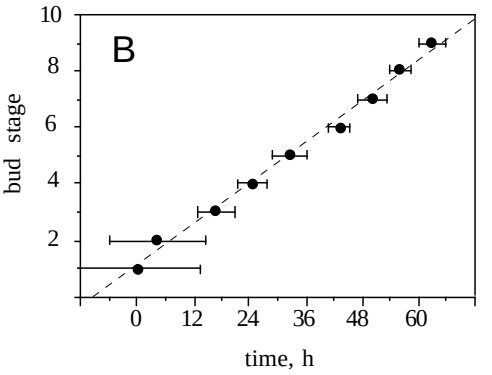


Fig. 5. L96 antigen expression during budding. (A) Kinetics of L96 antigen expression; buds of different stages were selected (Otto and Campbell,1977a), fixed, stained with monoclonal antibody L96, and the fraction of L96 antigen-positive buds was determined at the times indicated (*n*=15-20 buds; error bars were taken from B. (B) Calibration of bud stages; polyps were selected (*n*=10) at bud stage 1, observed individually and length of their bud stages was determined; bud stages were arranged on an absolute time scale by minimising the standard deviations.

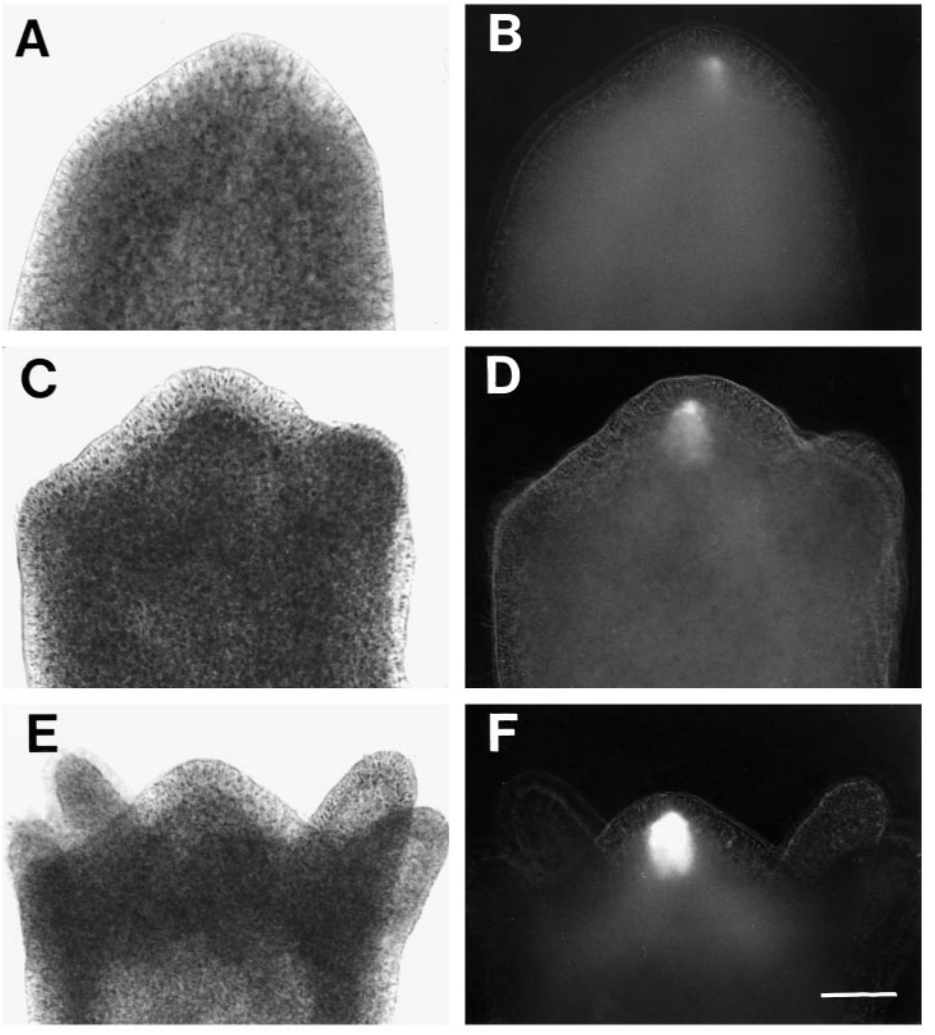


Fig. 6. L96 antigen expression in buds. (A,C,E) Bright-field images of immunofluorescence preparations shown in B,D and F, respectively. (A,B) Onset of L96 antigen expression at bud stage 5 before tentacle rudiments are visible; (C,D) bud stage 6 with 2 tentacle rudiments on the lower surface of the bud; (E,F) bud stage 8 when bud begins to separate from the parent by constriction; 4-5 tentacles have formed. Bud stages were classified according to Otto and Campbell (1977a). Bar indicates 80 μm.

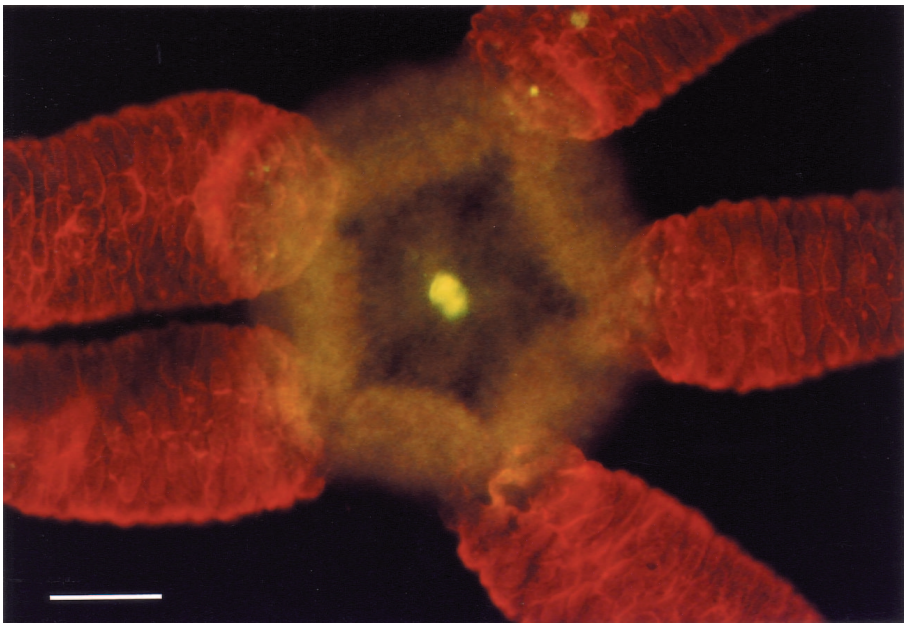


Fig. 7. Head of a chimera consisting of ectodermal tissue from *Hydra vulgaris* Zürich and endodermal tissue of *Hydra vulgaris* Basel (see text). Whole-mount preparation was double-stained with tentacle-specific monoclonal antibody TS19 (red TRITC-fluorescence) and hypostome-specific monoclonal antibody L96 (green FITC-fluorescence). Bar indicates 100 μm.

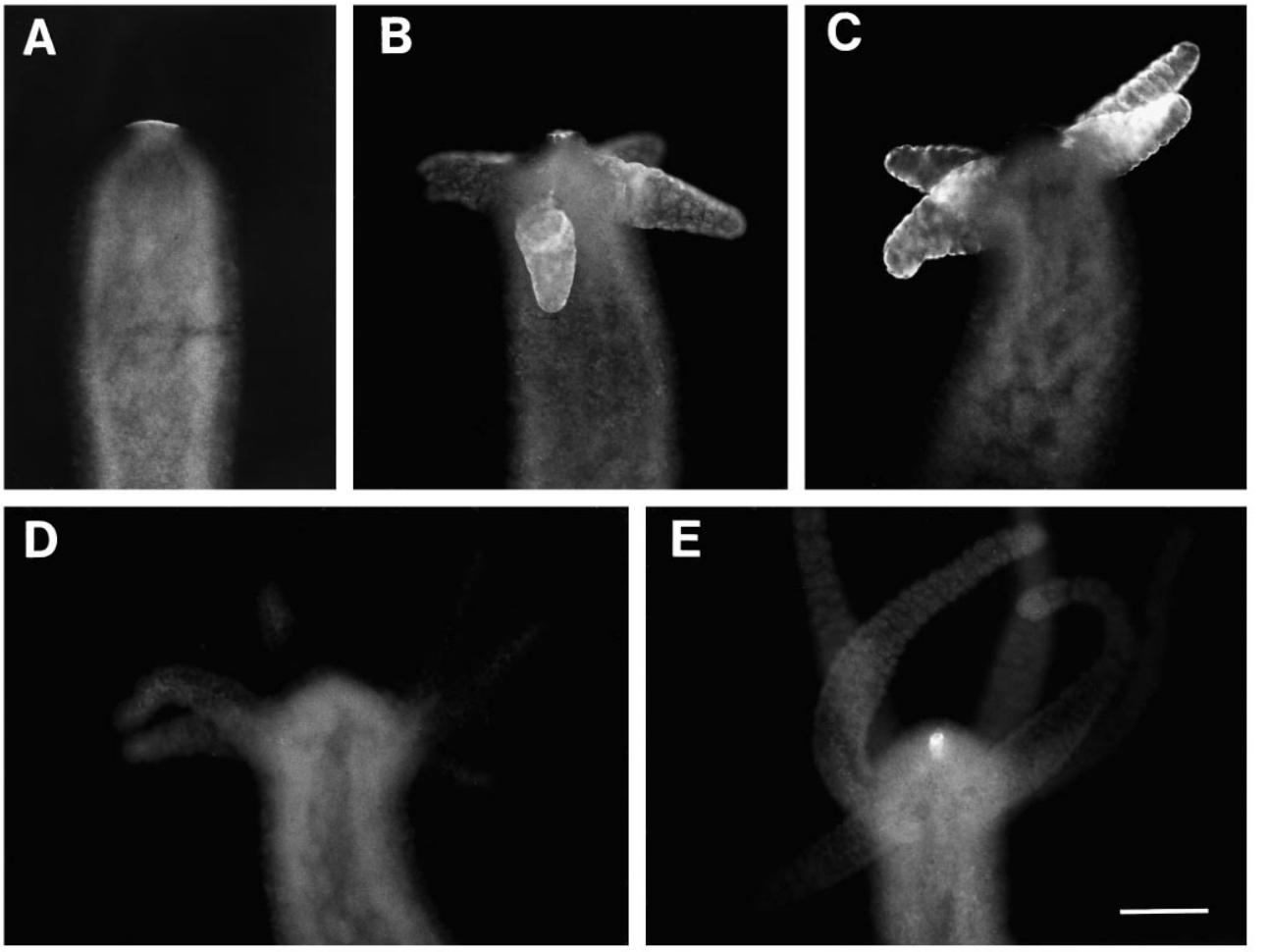


Fig. 8. TS19 and L96 antigen expression in regenerates from apical tissue (10% b.l.). Whole mounts of chimeras (consisting of ectodermal tissue from *Hydra vulgaris* Zürich and endodermal tissue of *Hydra vulgaris* Basel; see also Fig. 7 and Material and Methods) were fixed and stained with TS19 monoclonal antibody after 30 hours (A), 36 hours (B) and 48 hours (C) of regeneration; with L96 monoclonal antibody after 54 hours (D) and 60 hours (E). Note that tentacle-specific TS19 antigen expression first appears at the hypostomal tip and is displaced to the tentacle zone when L96 antigen expression becomes visible. Bar indicates 220 μm.

the tip shortly before L96 antigen appeared (Fig. 8C-E). During bud formation, by comparison, TS19 and L96 antigen appeared almost synchronously, just before tentacle evagination at stage 5 to 6 (Fig. 9).These data thus confirm the conclusions reached above, that the relation between L96 and TS19 antigen expression differs in tissue of different apicobasal starting levels.

The pattern of TS19 antigen expression in basally derived tissue

In the buds of the chimerical animals, we never detected TS19 antigen at the apical tip; expression always began in the definitive tentacle formation zone (Fig. 9B). This result appears to conflict with general views about TS19 antigen expression in head formation (Bode et al., 1988). To exclude the idea that some peculiarity of the chimeras is responsible for this behaviour, we reexamined TS19 antigen expression in buds of normal animals. Here too, there was never an indication of TS19 antigen expression at the apical tip (Fig. 10).

DISCUSSION

Hydra has a simple body plan with a single axis between two terminal structures, the head and the foot. It is not yet clear whether this axis is homologous to the anteroposterior or the dorsoventral axis of bilaterians, although recent findings on the expression of the antennapedia-like *Cnox-2* (Shenk et al., 1993a,b) point to a homology with the anteroposterior axis.

Hydra's head, located at the apical terminus, consists of at least two parts: the apical dome-shaped hypostome and the more basal ring of 4-8 tentacles. Several molecular markers for tentacles have been described (Bode et al., 1988; Weinzinger et al., 1994). We recently found that the monoclonal antibody L96 specifically recognises a small population – about ten cells – which form a ring, one cell thick, around the mouth opening. This is arguably the most apical position in the animal (Technau and Holstein, 1995).

Hypostome and tentacle formation is different in apical and basal regenerates

In classical studies of head regeneration, Webster and Wolpert (1966) found a correlation between the time required for the appearance of a morphologically recognisable head and the starting level on the apicobasal axis. Tissue from a more basal position required longer than apical tissue, and there was a continuous gradient in between. These studies also established that the time required by the regenerating tissue to become determined as head is graded along the body column. The delay between determination and formation of a recognisable head – plausibly the time required for head differentiation – was not constant at all body levels, suggesting that the control of head differentiation is complex.

In this paper, we describe similar experiments using the new

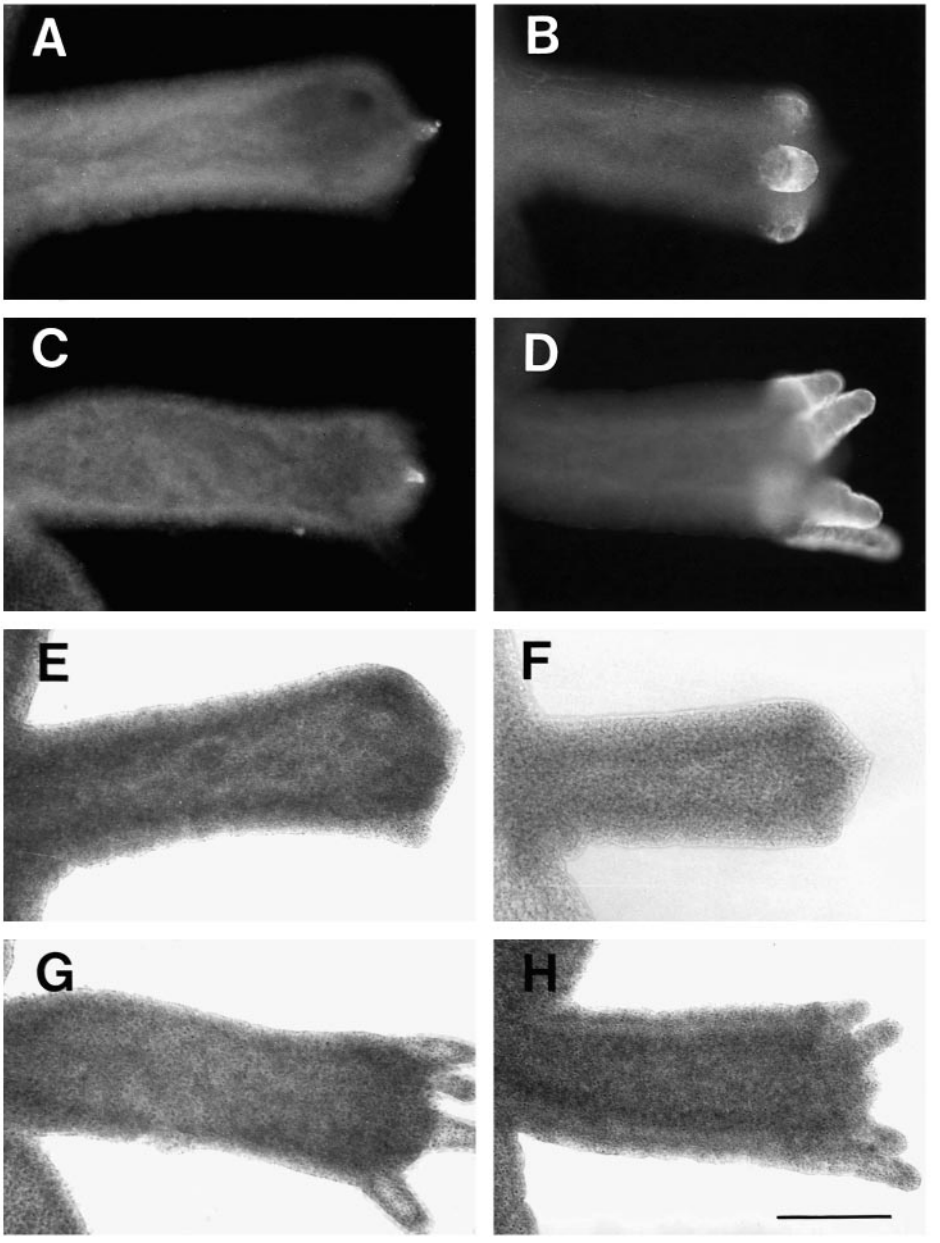


Fig. 9. L96 and TS19 antigen expression in bud tissue. Staged buds of chimeras (see Fig. 7 and Material and Methods) were selected, fixed and stained with L96 and TS19 monoclonal antibody. (A-D) Immunofluorescence preparations shown in corresponding bright-field images (E-H). (A,B) Buds at stage 5-6, when L96 antigen is expressed at the hypostomal tip (A), while early TS19 antigen expression is strictly confined to the site of presumptive tentacle formation (B); increased expression of L96 (C) and TS19 (D) antigen at bud stage 8. Bar indicates 300 μm.

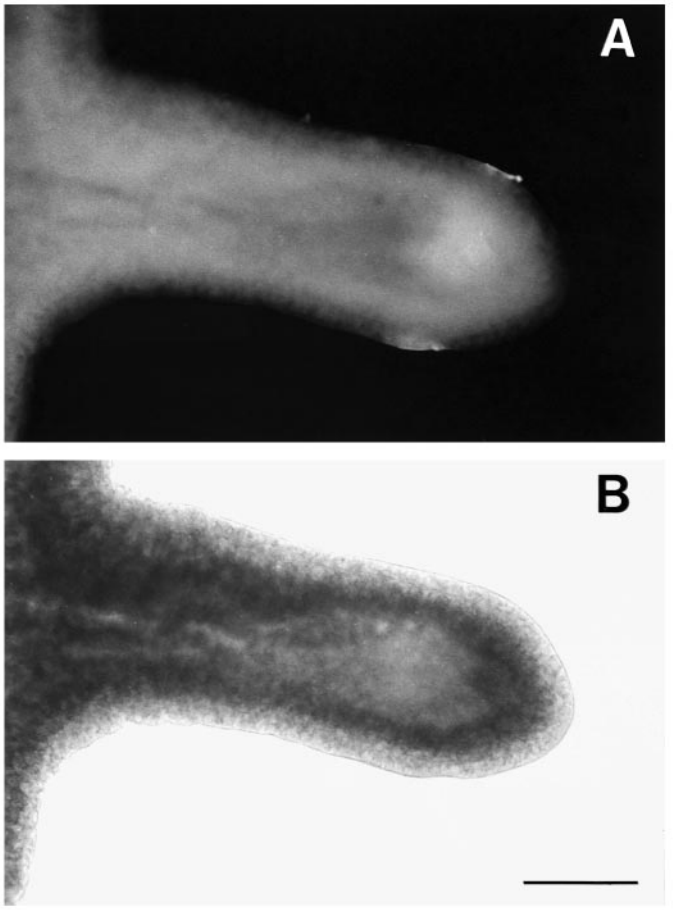


Fig. 10. TS19 antigen expression in bud tissue of *Hydra vulgaris* strain Zürich. TS19 antigen expression begins at buds stage 5 and is directly confined to the presumptive tentacle region; note that there is no antigen expression in the presumptive hypostomal region at the tip. (A) Indirect immunofluorescence; (B) bright-field image. Bar indicates 170 μm.

hypostome marker L96. Contrary to the results based on head morphology, we find a constant delay between head determination and L96 antigen expression at all body levels. This aspect, at least, of head morphogenesis may thus be under relatively simple control.

Studies of the appearance of tentacles in the same regenerates reveal that the relationship between head determination and tentacle morphogenesis is anything but constant. In head formation from apical levels, tentacles appear well before L96 antigen. Starting from basal levels, however, the structures appear simultaneously or even in reverse order. Experiments with regenerating fragments of various sizes and with tissue reaggregated from single cells show that this difference is due only to apicobasal level and does not depend on tissue size or gradient shape.

Current views on head formation (Bode et al., 1988; Weinzinger et al., 1994) are based on a gradient concept; the hypostome is thought to represent the gradient peak, while tentacles are formed at somewhat lower levels. This suggests a simple, unitary control of both tentacle and hypostome formation. Our data suggest, in contrast, that hypostome formation is under relatively simple control, while the control of tentacle formation is more complex.

Current views further suggest that the apical tip of a regenerating animal will pass through the tentacle-specifying level on its way to hypostome identity; thus a transient expression of tentacle markers is expected. This appears to be supported by experiments using both the tentacle-specific antigen TS19 (Bode et al., 1988) and the tentacle-specific cDNA *ks-1* (Weinzinger et al., 1994). We have confirmed this result in apical regenerates, but we see no transient expression during head formation from basal starting levels. It is not clear that this observation can be reconciled with current models.

Features of an alternative model

An alternative to the current view has recently been presented by Meinhardt (1993). In this model, hypostome and tentacles are defined by separate reaction-diffusion systems that are hierarchically linked (Fig. 11A). The hypostome, at the top of the hierarchy, can form in tissue that has never had tentacle identity. The hypostome modifies the surrounding tissue by elevating a parameter, called 'source density' by Meinhardt, which is essentially the apicobasal gradient value (MacWilliams, 1982). The tentacle system can only be active at high values of source density, and thus depends on the hypostome.

In head formation starting from basal levels (low source density), tentacle formation cannot begin until a hypostome has formed and has elevated the source density to a sufficient level (Fig. 11C). Thus the hypostome must be established at the apical tip before tentacle formation begins. Since the hypostome locally excludes the tentacle system, it is expected that tentacle markers will never be expressed in the hypostomal area; this is exactly what we found using TS19 monoclonal antibody (Figs 9A,B, 10).

In head formation from apical levels, the situation is more complicated, because the source density is a relatively stable parameter and the sources that were created by the preexisting hypostome suffice to trigger the tentacle system (Fig. 11B). This leads to a comparatively rapid expression of tentacle markers. Since the hypostome has not yet appeared, the tentacle system first establishes itself at the highest available gradient value, which is found at the apical tip of the regenerate (Fig. 8A). When the hypostome subsequently forms, it displaces the tentacles to a more basal position (Fig. 8C).

From this perspective, head regeneration from basal levels most closely portrays the true workings of the system; apical regeneration is a comparatively complex situation, dominated by the remaining tentacle-organising signal from the now-amputated head.

To what extent do our data support the Meinhardt model?

When, as in the present situation, data agree with a theoretical model, it is important to ask whether the model's prediction is 'robust', i.e. whether the model necessarily predicts the observations or whether these features of the model's behaviour are trivial ones, which would not appear with another choice of parameter values.

The phenomenon described here – the difference between regeneration from apical and basal levels – results in the model from the fact that the levels of source density required for

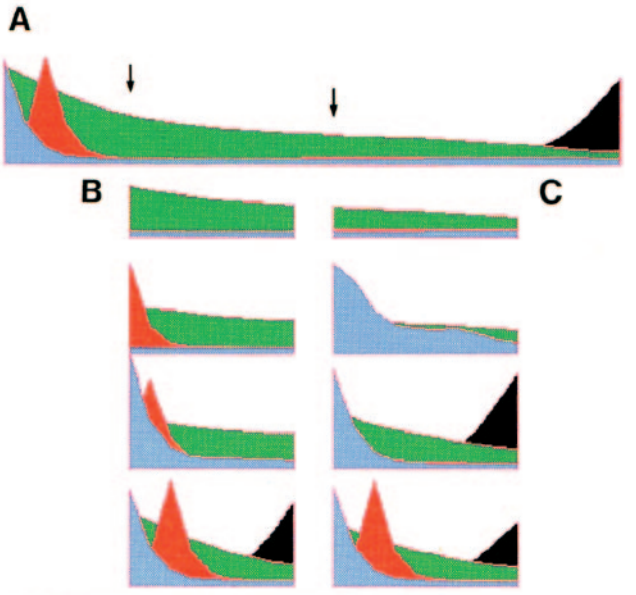


Fig. 11. Simulation of head and foot regeneration at apical and basal levels using the model of Meinhardt (1993) (700 iterations between each step). (A) Gradients of hypostome activation (blue), tentacle activation (red), foot activation (black) and source density (green) in a budless polyp; arrows indicate position of head regeneration from apical levels and from basal levels shown in B and C respectively. Dynamics of hypostome-, tentacle- and foot activation, and source density are simulated at subsequent stages of regeneration. The primary hypostome system excludes the tentacle system and increases the local source density. Activation of the tentacle system requires a certain threshold of source density. During head regeneration from apical levels the tentacle system is rapidly activated at the apical tip due to the high source density level in this region (B). Activation of the hypostome system is slower and occurs later at the tip, displacing the tentacle system to the 'next best position' more basally. During head regeneration from more basal levels (C), the hypostome system is activated at the apical tip before the tentacle system because the source density is below the threshold for tentacle activation. The hypostome system subsequently elevates the local source density and tentacle formation ensues.

tentacle formation are found in a region significantly larger than the region that the tentacles actually occupy. The region of tentacle competence clearly cannot be smaller than the tentacle zone. The competent region could in principle have exactly the correct size, but this model would thus lose an essential advantage of its hierarchical structure, namely local control that obviates the need for precision in inductive events.

Could the competent region encompass the entire animal, with the result that apical and basal regeneration would be similar? In the Meinhardt model, tentacle formation is limited to a single ring by lateral inhibition via a tentacle inhibitor; if the region 'competent' for tentacle formation were to encompass the entire animal, the tentacle inhibitor would require a long diffusion range. The allowable range of the tentacle inhibitor is constrained, however, by the role of the diffusion range in determination of the 'chemical wavelength' which is responsible for intra-tentacle spacing (see Harrison, 1982; MacWilliams, 1991). A tentacle inhibition of very long range would rule out the formation of a ring of closely spaced tentacles.

It thus appears to us that the Meinhardt model makes a relatively strong prediction of the phenomenon that we have described, and that our data thus support this kind of model in a meaningful way.

Interestingly, among the Cnidaria, species are found with two or more rings of tentacles, which are located below the subhypostomal region (Hydrozoa: *Tubularia*, *Coryne*, *Zanklea*) (Riedl, 1983; Brusca and Brusca, 1990; Ruppert and Barnes, 1994). Even *Hydra* with secondary tentacle rings have been observed as consequence of excessive feeding (Otto and Campbell, 1977b) or after treatment with LiCl (our unpublished observations). In contrast to traditional models, in which additional sets of tentacle-formation thresholds would be required, models of the Meinhardt type may be able to explain these structures as a simple consequence of an increase in the size of the zone 'competent' for tentacle formation.

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