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Original Article

Pattern regulation properties of a Hydra strain which produces additional heads along the body axis

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ABSTRACT The multiheaded one (mh-1) strain, isolated from inbred crossings of wild type *Hydra magnipapillata*, develops additional heads along the body axis. This strain reproduces asexually by budding like the wild type (wt) does. We found that young polyps have a wt-like shape and display wt-like properties. When they grow in size and before they produce extra heads along the body axis, the tissue between the head and the budding zone changes its property: in this region, where later on the extra heads preferentially form, foot regeneration is significantly delayed while head regeneration remains unaffected. Further, following various transplantations additional heads form under conditions under which the wild type did not. The observed changes in pattern control and regulation indicate a two-step process of pattern formation. Morphogenetic signalling is suggested to cause the positional value to increase slowly in the form of patches and preferentially in the region between the head and the budding zone. This increase causes an altered morphogenetic signalling, which is eventually responsible for additional head formation.

KEY WORDS: *Hydra, mutant, pattern formation, positional value.*

Introduction

A set of simple experiments forms the basis of our knowledge about the control of pattern formation in the fresh water polyp *Hydra*. One of these experiments shows that a *Hydra* regenerates both the removed head and basal disc (foot). The tissue lining the gastric cavity is obviously able to form both structures. This indicates an inhibition which prevents head and foot formation, respectively, in the remaining body column. Even cell aggregates of dissociated gastric tissue organize in such a way that finally normal shaped animals emerge (Gierer *et al.*, 1972). The way this is achieved is still a matter of research. At present there are several strategies to study the control of pattern formation in *Hydra*. They include the tracing of early changes in gene expression when new body parts form (Schummer *et al.*, 1992; Shenk *et al.*, 1993; Grens *et al.*, 1997; Martinez *et al.* 1997; Broun *et al.* 1999; Smith *et al.*, 1999). Various species of *Hydra* with an almost identical shape and identical properties are studied as well as mutants with a different morphology and a different response in regeneration and transplantation experiments (Sugiyama and Fujisawa, 1977b; Sugiyama, 1982; Achermann and Sugiyama, 1985; Kobatake and Sugiyama, 1989). Further, related species e.g. marine cnidarians are included (Müller *et al.*, 1986; Berking, 1991; Pfeifer and Berking, 1995; Walther *et al.*, 1996; Kehls *et al.*, 1999). The results obtained are used to question and to evolve the existing models of pattern formation in *Hydra*, which in turn stimulate the experimental approaches.

It is generally accepted that pattern formation in *Hydra* is governed by morphogens which control their own generation. In aggregates of isolated cells the generation of such morphogens starts from a rather uniform concentration level and ends in a concentration pattern which somehow directs the local development, e.g. at a certain place it causes head formation and at the same time prevents head formation in the surroundings. Thus, a short-range self enhancement (autocatalysis) of the generation of certain morphogens must be coupled to a long ranging inhibition of this autocatalysis, e.g., an activator stimulates the generation of an inhibitor which has a long range (Gierer and Meinhardt, 1972).

In *Hydra* the gastric tissue has different properties along its length axis as observed in regeneration and transplantation experiments. For instance, a tissue ring removed from the body column regenerates the head at the site of the original head and the foot at the opposite end, at whatever position the tissue ring has been removed from the animal. The tissue property responsible for the obtained result has been termed positional value (Wolpert *et al.*, 1971). Obviously, the positional value changes in form of a gradient along the body axis. The highest value is found in the tissue lining the mouth opening. It is further obvious that the positional value influences the generation of morphogens, because in a ring of the body column the

Abbreviations used in this paper: mh-1, multiheaded one strain; RF-amide, Arg-Phe-amide; wt, wild type strain.

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head is formed at the end with the highest positional value. The physical basis of the positional value is still unknown. Known is, however, that the positional value is not represented by a collinear orientation of polar cells but rather by a scalar property like the density distribution of certain cells or subcellular particles along the body axis (Gierer *et al*., 1972) or receptors for morphogens (Müller, 1990). When from an aggregate a normal animal develops, morphogens must control the formation of the appropriate positional values along the new body axis. Thus, there is a feedback control between morphogens and the positional value and *vice versa*.

The term positional value is not used in all models of pattern formation in *Hydra*. One reason is that its definition has changed (Wolpert *et al*., 1971, 1974). A further reason is that different terms allow to refer to specific details of the experimental procedure, for instance a restriction to head formation, or to include specific theoretical considerations. Thus, terms like source density (Gierer and Meinhardt, 1972) and head activation and head inhibition potential (Sugiyama, 1982) were introduced. We prefer the rather neutral term positional value and would like to summarize under this term the differential ability of a piece of the body column to form a head and a foot, respectively, in comparison with its surroundings. This structure formation was observed in the noted sectioning experiment or when the tissue is transplanted into various positions of the gastric column of a host animal (Berking, 1998).

For a deeper understanding of pattern formation and a further analysis at the biochemical level it is of particular importance to get an idea of how the hypothetical morhogens may act. Some authors proposed morphogens to exist which directly control head and foot formation (Meinhardt, 1993; Müller, 1993). An alternative is that pattern formation is hierarchically organized in such a way that the morphogens primarily control the positional value along the body axis. Therewith, these (primary) morphogens are not structure-specific. The locally attained value is suggested to control the local development by generation of secondary morphogens, which may be structure-specific (Berking, 1984, 1998).

This paper concerns a strain of *Hydra magnipapillata* which produces extra heads along the body axis. The strain is termed multiheaded 1 (mh-1). It was obtained by inbred crossing of specimens of *H. magnipapillata* collected from ponds (Sugiyama and Fujisawa, 1977a). Such animals are obviously a challenge to the existing models and may, therefore, help to get a deeper insight into pattern control. Based on transplantation experiments it has been proposed that both, head activation and head inhibition are changed in these animals. This was proposed to lead to the formation of buds outside the budding zone which do not detach from, but remain attached to the parent polyp (Sugiyama and Fujisawa, 1977a; Sugiyama, 1982). An mh-1-like phenotype has been obtained experimentally by daily pulse treatments of the standard wild type strain 105 (wt 105) of *Hydra magnipapillata* with the diagylclycerol diC_8 coupled to feeding (Müller, 1989, 1990) and by heavy feeding only (Kroiher, 1999).

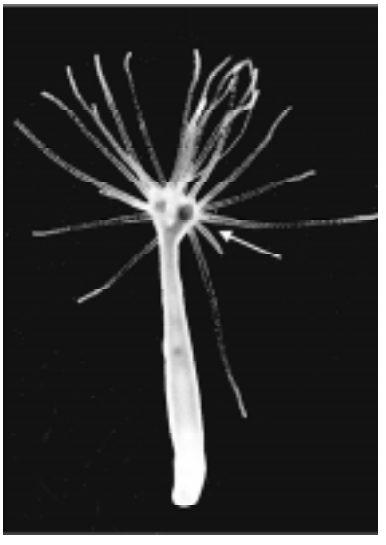


Fig. 1. An mh-1 with an additional head next to the first one. *The arrow indicates the second head.*

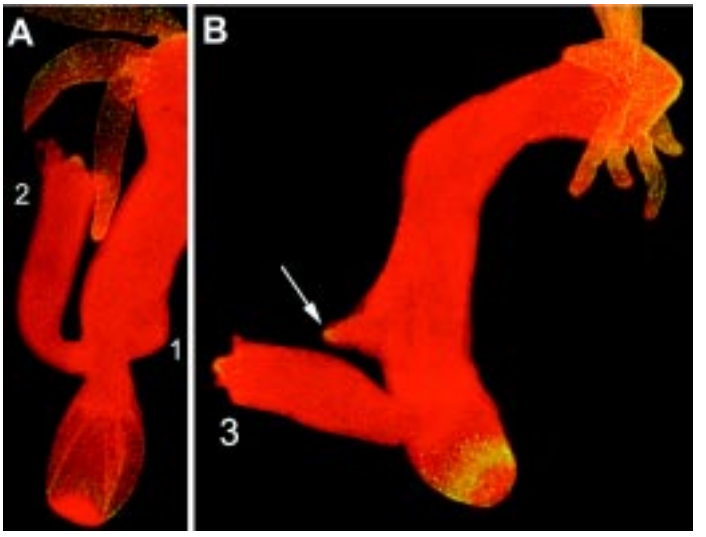


Fig. 2. Whole mounts of mh-1 stained with the RF-amide antibody. (A) *An animal bearing two buds, a stage 3-4 (10h) (1) and a stage 8-9 (55h) (2).* **(B)** *An animal with a stage 7-8 bud (31h) (3) and a developing additional head. The arrow indicates RF-amide positive nerve cells in the developing additional head.*

We performed regeneration and transplantation experiments with mh-1 and obtained different results with increasing age of the animals while the morphology of the animals looks still like that of the wildtype. It appears that before the mh-1 phenotype is expressed the tissue changes its property. We distinguish three different developmental stages of mh-1; 1) polyps with a wild type phenotype and a wild type behaviour; 2) polyps with a wild type phenotype and a mutant behaviour; and 3) polyps with a mutant phenotype and a mutant behaviour.

Results

The development of the multiheaded phenotype

Polyps of mh-1 which have just detached from their parents always bear only one head. In one experiment 21 of them were cultured for 36 days and compared to those of wt 105 (n=21) which never produce extra heads spontaneously. In both groups budding was found to start three days after they had separated from their parent animal. Bud formation starts with an evagination of both the ectodermal and the entodermal epithelial layer. The tip of the bud develops into a head and after the bud was grown out a foot forms at the bud's base next to the gastric tissue of the parent animal. Then a constriction forms basal to the bud's foot and the bud separates from the parent animal. The process of budding takes

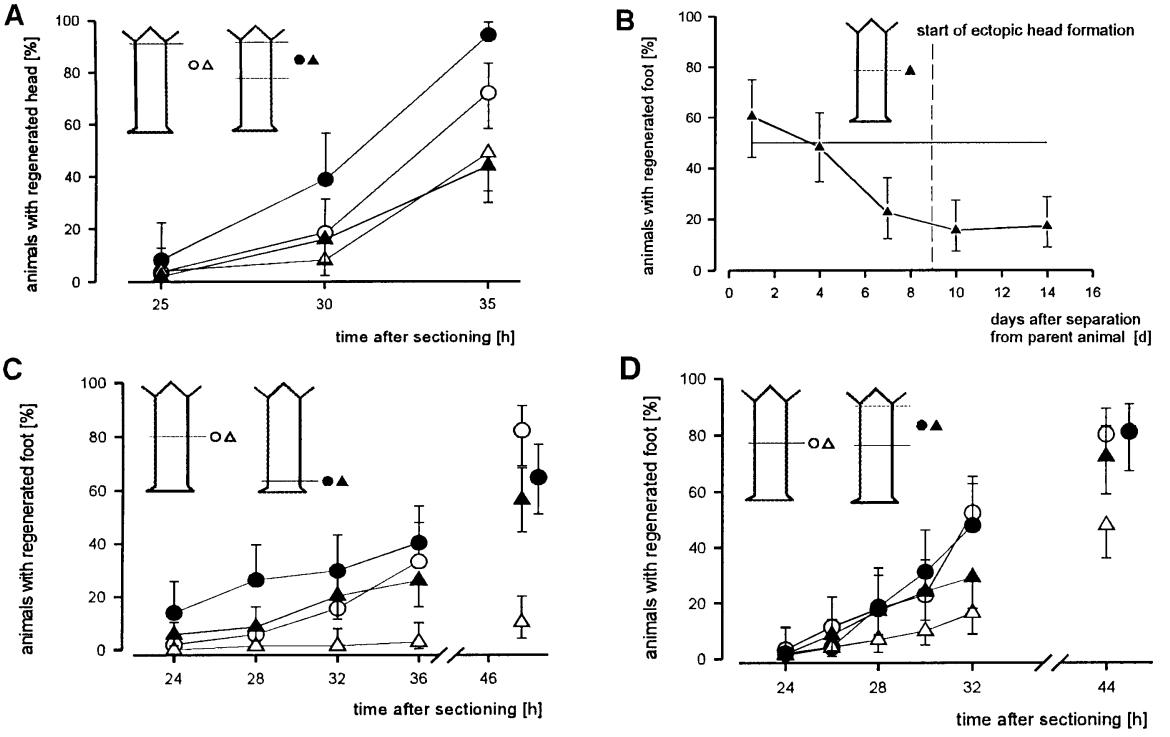


Fig. 3. Head and foot regeneration. *All experiments were performed with budless mh-1 and wt 105 respectively.* **(A)** *Head regeneration in the absence or presence of the foot. Budless mh-1 (> 10d) were sectioned either only just below the tentacle ring (open triangles) or in addition midway between the head and the foot (closed triangles). Wt 105 were sectioned in the same way, open and closed circles, respectively (n = 36-54).* **(B)** *Foot regeneration of mh-1 and wt 105 on different days after separation from the parent animal. Mh-1 and wt 105 were sectioned in the middle of their gastric region and monitored for foot regeneration. When 50% of the wt 105 had regenerated a foot the respective frequency for mh-1 was determined. The 50% values of wt 105 are indicated by a horizontal line. The respective values for mh-1 are given as closed triangles according to the results obtained (n = 53-64).* **(C)** *Foot regeneration of mh-1 and wt 105 (> 10d), sectioned at various positions. Mh-1 were sectioned below the budding zone (closed triangles) or in the middle of the gastric region (open triangles). Wt 105 were sectioned in the same way, closed and open circles, respectively (n = 51-69).* **(D)** *Foot regeneration of mh-1 and wt 105 in the absence or presence of a head (> 10d). Mh-1 were sectioned either only in the middle of the gastric region (open triangles) or in addition just below the head (closed triangles). Wt 105 were sectioned in the same way, open and closed circles, respectively (n = 48-73).*

three to four days. We found the rate of budding to be similar in both strains. During the first 18 days of observation the mh-1 produced 14.7 buds per animal while the wt 105 produced 12.6 per animal. In the mean, a freshly detached polyp of mh-1 bore more tentacles (6.4 ± 0.8; $n = 269$) than a polyp of wt 105 (5.7 ± 0.7; $n = 181$; $p<0.05$, Mann-Whitney-test). In both, wt 105 and mh-1 the buds were formed at almost the same relative distance to the head (at about two thirds of the total length) and a new bud always formed just above an existing older one.

TABLE 1

DETERMINATION OF THE AXIAL POSITION OF THE SECOND AND THE THIRD HEAD IN 79 BUDLESS MH-1 FROM A MASS CULTURE

	head	1	2	3	4	5	6	7	8	9	10	basal disc	sum
head	1				2			1					4
1								1					1
2				**1**	1	1		2					5
3				**1**	**1**		3	2	1				8
4				**1**	**3**	**6**	3	4	1				18
5				1	**1**	**4**	**5**		1				12
6			1	2		**1**	**6**	**3**	2				15
7			1	1	2		**1**	**2**	**2**				9
8				1	3		2						6
9	1												1
10													
basal disc													
sum	2		2	8	13	12	20	15	7				79

Ten equally sized regions between the head (hypostome and tentacle ring) and the basal disc were distinguished. Regions 7 and 8 include the budding zone. Numbers in bold indicate specimens which formed the additional heads in close proximity.

The first animal with a second head (the first additional one) was observed on the 9th day. 50% of the mh-1 had formed an additional head on the 33rd day. Within the period studied 13 (62%) of the 21 selected mh-1 formed additional heads. Buds were formed before, during and after additional head formation.

From a mass culture of mh-1, 79 polyps were collected which bore no buds, but one additional head (Fig. 1). It turned out that this second head can be formed at various positions but most were formed just above the budding zone. Very rarely an additional (the second) head was formed in or even below the budding zone or within the existing head (Tab. 1). Interestingly, a third head did not avoid the vicinity of the second, as indicated by the numbers in the grey areas in Tab. 1. These numbers should be close to zero if they kept the distance.

Kroiher (1999) found that wt 105 produced additional heads along the body axis after heavy feeding for several days with *Artemia* nauplii. This could be confirmed. However, a similarly heavy feeding of mh-1 did not result in a further stimulation of additional head formation (Tab. 2).

Early bud stages and growing additional heads show a different pattern of RF-amide positive nerve cells (Fig. 2). The sites of imminent additional head formation showed an accumulation of RF-positive nerve cells before tentacles and a hypostome actually emerged, while a bud bears such cells much later after it developed its head. Further, the position of additional heads and developing buds is different. Buds are always formed in the budding zone whereas the position of additional heads varies. Thus, the formation

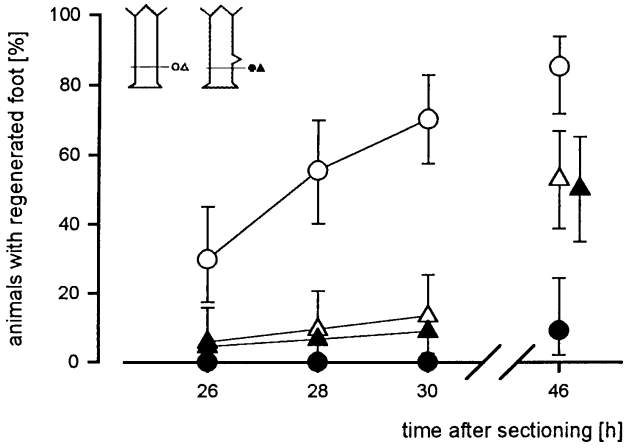


Fig. 4. Foot regeneration of mh-1 (> 10d) and wt 105 in the absence or presence of a bud. *Mh-1 with (closed triangles) and without a stage 3 bud (3-6 hour) (open triangles) were sectioned close to the bud between bud and foot or in the absence of a bud at the respective position. Wt 105 with a bud (closed circles) and without a bud (open circles) were transversely sectioned in the same way (n = 33-53).*

of additional heads and budding which both result in the formation of head structures at the distal end of the developing protrusion appear to be different from the outset.

Regeneration

Head regeneration

Budless mh-1 without additional heads and budless wt 105, which both detached from their respective parent animal ten days before, were sectioned at various positions and allowed to regenerate. When the upper body quarter was isolated by two cuts, an animal with normal polarity developed in most cases. A few developed a head at both ends (wt 105: 7%, 2 out of 28, mh-1 (13 %) 4 out of 30). However, when a piece of the body column between the two heads was cut out from mh-1 animals which bear a second head, it frequently (36 %, 8 out of 22) formed a head at both ends. It appears that in normally shaped adults the property of the tissue is not significantly different from that of wt 105, but it is changed when additional heads are present.

In *Hydra* the speed of head and foot regeneration depends on the culturing conditions. Thus, a measured difference in speed between species is only relevant, if the differences are maintained over weeks. The speed of head regeneration was studied in wt 105 and mh-1 in several experiments over a period of several months. A significant difference could not be detected. However, head regeneration was effected differently when the head and the foot were removed. The speed of head regeneration was increased in wt 105 but not in mh-1 (Fig. 3A).

Foot regeneration

In newly detached polyps of wt 105 and mh-1 the speed of foot regeneration was found to be equal when the animals were sectioned at the same position. However, mh-1 polyps, which had detached from their parent seven to 14 days before, showed a considerable delay in foot regeneration compared to wt 105 (Fig. 3B). This result shows that in mh-1 the delay in foot regeneration from gastric tissue does not occur from the outset. The decrease in the speed of foot regeneration was found in the apical half of the animal two to four days before the first animals of an unsectioned control group formed an additional head. The speed of foot regeneration depends on the position of the section. Sectioning at the apical half leads to a delay in foot regeneration while a section at the basal half does not (Fig. 3C). In wt 105 the position of the section does not have such a strong influence on the speed of foot regeneration (Fig. 3C). Thus, in mh-1 a delay or even an inability to regenerate a foot does not correlate with an acceleration of head formation. The following experiment may give a further insight into the control of regeneration. In mh-1 foot regeneration was accelerated when not only the foot but also the head was removed (Fig. 3D). In wt 105 the removal of the head had no influence on the speed of foot regeneration.

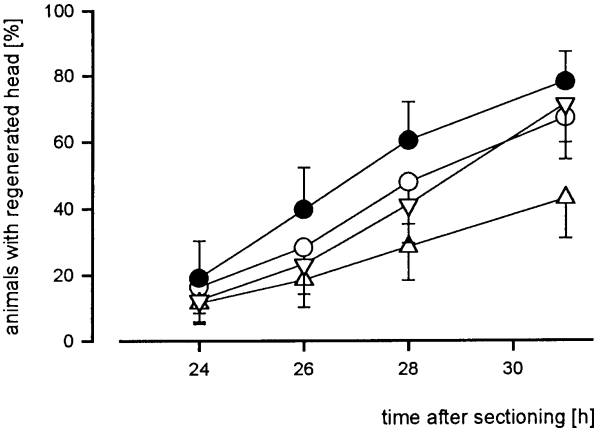


Fig. 5. Influence of compounds released by sectioned wt 105 and mh-1, respectively, on head regeneration in *Hydra vulgaris*. *For each experiment, which was repeated three times, 30 wt 105 and mh-1 were sectioned midway between the head and the foot in a dish containing 10 ml medium. The pieces remained in this medium for 30 minutes. Then they were replaced by freshly prepared Hydra vulgaris headregenerates, sectioned just below the tentacles (n = 67-73). Symbols: open triangles, double-headed mh-1; inverted open triangles; one-headed mh-1; open circles, wt 105; closed circles, hydra medium as control.*

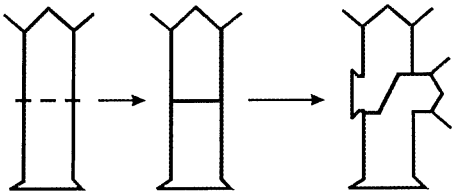
TABLE 2

INFLUENCE OF NORMAL AND HEAVY FEEDING ON THE FORMATION OF SECONDARY HEADS IN MH-1 AND WT 105

strain	number of animals	feeding modus	number of animals with additional heads at the 19th day (%)
mh-1	40	normal	13 (33)
mh-1	40	heavy	15 (37)
wt 105	40	normal	0
wt 105	40	heavy	8 (20)

The influence of budding on foot regeneration

Early bud stages of *Hydra vulgaris* antagonized foot regeneration when the section, which cut off the foot, was made close to the bud (Tripp, 1928). We obtained the same result for wt 105 when we sectioned animals bearing a stage 3 bud (bud stages according to Otto and Campbell, 1977) just below the bud (Fig. 4). In mh-1 such an inhibitory interaction was not observed. The parent animal regenerated a foot in all cases and the buds detached from



strain (age of animals after separation)	number of grafts	number of animals with additional head (H) and foot (F)			
		2nd day		5th day	
		H	F	H	F
mh-1 (1-3d)	10	0	0	2	1
mh-1 (>10d)	15	13	0	13	3
wt 105 (> 10d)	10	0	0	0	0

Fig. 6. Head and foot formation at the junction in reconstituted homografts of mh-1 and wt 105 sectioned in the middle of the body column. *The head formed exclusively at the apical end of the basal piece and the foot formed exclusively at the basal end of the apical piece.*

the parent after they had completed their development, irrespective of whether the parents were sectioned or not. In wt 105 most parents did not regenerate a foot. Further, 85% (28 out of 33) of the buds did not separate. Each bud developed into a secondary axis with initially less tentacles a bud of wt 105 usually has (data not shown). 71% (20 out of 28) of these secondary axes formed a patch of foot tissue at the bud's base instead of a ring shaped foot which is necessary for the separation from the parent.

Repeated decapitation of mh-1

A newly detached mh-1 has properties similar to those of the wild type. With age or growth the mh-1 phenotype (formation of additional heads) develops. One possible explanation for this change of morphology may be that in the head of a mh-1 certain cells show signs of senescence, causing a reduced head inhibitory activity, whereas in wt 105 they do not. To test this hypothesis the head was removed repeatedly and allowed to regenerate from the body column. Thus, new head specific cells developed repeatedly. The head was removed on the second, fifth, eleventh and the seventeenth day. Up to the twenty-third day the 10 repeatedly decapitated animals developed four additional heads while the 10 controls formed 7 additional heads. Thus, the data do not support the hypothesis that an ageing of head specific cells is the cause of the development of the mh-1 phenotype.

The release of a head inhibiting activity

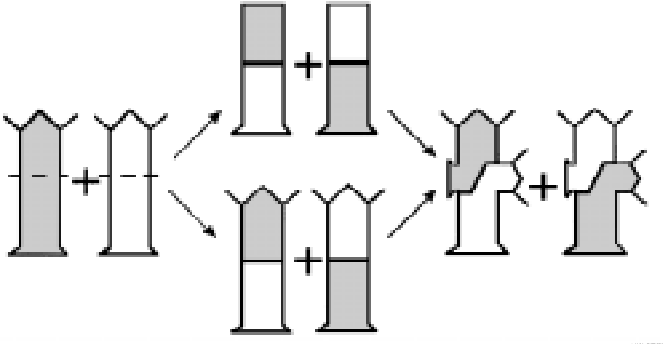
Schaller (1976) showed that *Hydra vulgaris* releases a head inhibiting activity after sectioning. In order to test whether wt 105 and mh-1 release a different amount of that activity, animals of various ages were sectioned once in a defined volume of culture medium. The culture medium was subsequently tested by using the head regeneration assay with *H. vulgaris*. There was no significant difference when the activity released was related to the size of the activity-producing animals (as measured by the protein content, data not shown). With respect to the number of sectioned animals per millilitre medium and, therewith, to the number of releasing sites, the inhibitory activity released by mh-1 (3d old) and wt 105 (10d old) was found to be similar and weak, while the activity released by mh-1, which bore a second head at the time of sectioning, displayed a stronger effect (Fig. 5).

Transplantation

In *Hydra* transplantation experiments were found to allow an insight into pattern control (e.g. Webster and Wolpert, 1966; Webster, 1966). By axial and lateral grafting it was detected that cells have a memory of their axial position along the body column. The property mediating this memory was termed positional value (Wolpert *et al.*, 1971). If body parts of different positional values are brought into contact, a head or a foot may form. If there is no difference the implanted tissue may preserve its identity. We used the axial grafting procedure for our experiments.

Homografts

We sectioned animals which had detached from the parent ten days before. A transversal section midway between the head and the



origin of the tissue		number of grafts	number of animals which formed a head (H) and a foot (F)			
apical	basal		not decapitated		decapitated following transplantation	
			H	F	H	F
mh-1	mh-1	10	10	1	10	4
wt 105	wt 105	10	0	0	7	0
mh-1	wt 105	9	7	4	9	8
wt 105	mh-1	9	8	5	8	5

Fig. 7. Head and foot formation at the junction in homo- and heterografts of mh-1 and wt 105 sectioned in the middle of the body column on the 5th day after transplantation. *The head formed exclusively at the apical end of the basal piece and the foot formed exclusively at the basal end of the apical piece. Decapitation was performed below the tentacle ring.*

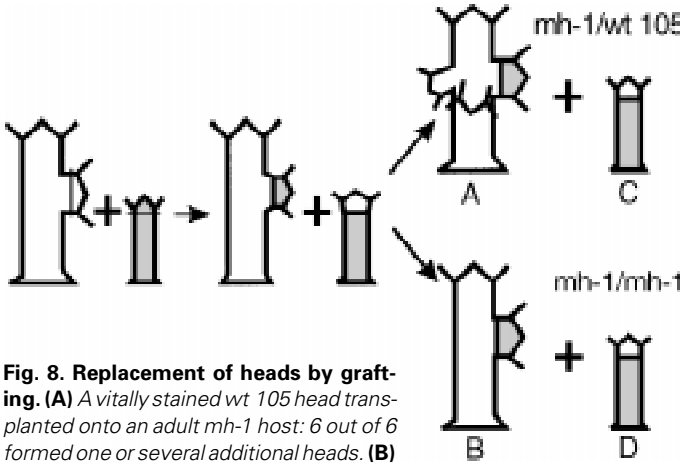


Fig. 8. Replacement of heads by grafting. (A) *A vitally stained wt 105 head transplanted onto an adult mh-1 host: 6 out of 6 formed one or several additional heads.* **(B)** *A vitally stained mh-1 head transplanted onto an adult mh-1 host: 0 out of 6 formed a head.* **(C)** *An mh-1 head (the removed additional one of an adult animal) transplanted onto a vitally stained young wt 105 host: 0 out of 6 formed an additional head.* **(D)** *An mh-1 head (the removed additional one of an adult animal) transplanted onto a vitally stained young mh-1 host: 0 out of 6 formed an additional head.*

foot, followed by the immediate rejoining of the separated parts caused the formation of an additional head in almost all mh-1 at the former wound two days after the reconstitution. Wt 105 of the same age and also mh-1, which had detached from the parent three days before, did (almost) not respond with additional head formation (Fig. 6). It should be remembered that in mh-1, which had detached from their parent animal ten days before, the ability of the gastric region to regenerate a foot is strongly decreased. We observed that five days after transplantation three of the 13 rejoined mh-1 with additional heads separated at the former wound. This splitting was enabled by a ring-shaped foot, which had previously been formed by the apical part at the junction. In the other group consisting of ten mh-1 animals which were sectioned three days after detachment from their parents, only two formed an additional head and one of them separated in the same way. A possible explanation may be that in these transplants the contact between the tissue pieces was initially not very tight all around the circumference, but was so in wt 105. Alternatively, in mh-1 the processes which eventually led to head and foot formation, respectively, had started earlier than in wt 105 tissue. A further alternative can be excluded, namely that a head simply causes foot formation in close proximity. According to the general tissue polarity the foot should be formed below (basal to) the new head but in all cases it formed out of the apical piece on the other side of the junction. As a further control we made an incision at those sites where the treated polyps had been sectioned transversely. In no case the incision was found to cause an additional head, neither in wt 105 nor in mh-1 (not shown).

Heterografts

In a further type of experiment we performed the four combinations of axial homo- and heterografts of mh-1 and wt 105 (Fig. 7). We used animals which were budless, had no additional heads and had separated from their parent animals ten days before. The animals were sectioned transversely in the middle of the body column. In addition, one half of the animals was decapitated (just below the tentacle ring) immediately after grafting to increase the chance of additional head formation at the former wound. The idea was based on findings by Webster (1966) who had shown that in *Hydra littoralis* decapitation increases the frequency of head formation from implants which had a higher positional value than their new surrounding when the operation was made not too far away from the head. Consistently, we found additional head formation in the decapitated wt 105 / wt 105 combination, indicating a long range inhibitory effect of the existing head on additional head formation at the transplant junction or alternatively a long-range supporting effect of the open wound and the regeneration process, respectively.

The wt 105 / mh-1 heterotransplants formed an additional head in high frequency. Decapitation did not further stimulate additional head formation. In all cases the additional heads were formed from the basal piece at the former wound. Thus, the additional heads did not derive from cryptic head anlagen possibly present in the gastric region. In the wt 105 / mh-1 chimeras neither a wt 105 head was able to antagonize head formation in mh-1, nor was a mh-1 head able to antagonize head formation in wt 105. A wt 105 head was only able to antagonize head formation at the former wound of a wt 105 stump. Thus, a simple assignment of “weak“ and “strong“ to the inhibitory signalling from the head and the resistance of the tissue to that signalling, respectively, appears to not give a satisfying explanation.

Several of the grafts formed a foot, which was always formed at the base of the apical piece of the transplant. Finally, these animals separated into two animals. In no case a foot was formed without a head at the opposite side of the junction. Homo- and heterografts responded differently. The homografts rarely formed a foot and did not separate (within five days following transplantation), but most of the heterografts did (Fig. 7): 15 of the 20 mh-1 homografts formed a head only, five formed a foot in addition. In the heterografts it was just the reverse: seven formed a head only, while 22 formed a head and a foot additionally. That may have a rather trivial cause: In heterografts there may be a reduced contact or a certain delay of contact between the different parts which allows both tissues to start the regeneration of a head and a foot, respectively. This may explain why we failed to assign the properties “weak“ and “strong“ to wt 105 and mh-1, with respect to inhibition of head formation and resistance to that inhibition.

Mh-1 / wt 105 heterotransplants, which contain a wt head and the body column of mh-1, were cultured for 4 weeks and found to produce additional heads along the body column in a frequency, which did not differ from that of mh-1 animals (not shown). It appears that a wt 105 head is unable to prevent additional head formation in the mh-1 body column. However, due to cell migration, the wt 105 head has become chimerical gradually.

Primary and secondary heads

Using the axial grafting procedure we replaced the second head of two headed mh-1 animals by a head taken from a freshly detached polyp (mh-1 or wt 105) (Fig. 8). As a control we simply removed the additional head and found the head to be regenerated in almost all cases (14 out of 15). The specimens with a transplanted additional head behaved differently depending on the origin of the transplant. When a homograft was made the mh-1 host never produced an additional head (6 grafts). In contrast, in all cases (6 out of 6) up to four additional heads were formed when a heterograft was made (Fig. 8A). This appears to fit the proposition of a reduced or delayed contact between the pieces when a heterograft is made. The other combination, an additional mh-1 head transplanted onto the young mh-1 or a

wt 105 host, did not result in additional head formation. This fits the observations made with other types of experiments that a just detached polyp of mh-1 responds like wt 105.

Discussion

In mh-1 three different developmental stages can be distinguished: (1) the just detached polyp, which has wild type-like properties. (2) The adult polyp, with a wild type-like body shape and a wild type-like budding behaviour, but with an increased capacity to form a head in transplantation experiments and a decreased capacity to regenerate a foot. (3) The adult polyp, with additional heads and eventually secondary axes caused by the outgrowing additional heads. As indicated by the distribution of RF-amide positive cells, from the outset the additional heads look different from developing buds, even if they developed in the same body region.

Such animals also had a changed property: they released more inhibitory activity, showed a reduced foot regeneration and an increased head formation in transplantation experiments, but budding occurred still wild type-like. The changed property was (almost) restricted to the tissue between the head and the budding zone. In most cases the additional heads formed in the lower half of this region. In mh-1 a bud appears to form similar to a bud of wt 105 and other *Hydra* species by recruiting gastric tissue (Sanyal, 1966; Otto and Campbell, 1977) which is or becomes wild type-like when the bud detaches normally.

The transplantation experiments

Head removal allowed head regeneration from the body column. In mh-1 the transplantation of the removed head back to its original position (homograft) prevented head formation at the junction when newly detached polyps were used, but did not prevent head formation when adults were used. A defect in healing, caused by the mutation(s) is, therewith, excluded. Thus in adult compared to young animals, either (1) communication across the junction is delayed, or (2) the inhibitory signalling by the head is weaker, or (3) at the wound the resistance to inhibition increases faster.

With respect to (1): A delay of communication is expected to increase the frequency of structure formation at both sides of the junction: head formation from the basal part and foot formation from the apical part. Most homografts form only a head but no foot. Thus, in mh-1 homografts made of adult polyps, a delay in communication does not appear to be the main reason for additional head formation. However, most heterografts produced both structures. It appears that the way and the dynamics of how contacts are made in the grafts are slightly different in mh-1 and wt 105. The reason for that difference is unknown. A histoincompatibilliy may play a role. Thus, the results obtained by wt 105 / mh-1 heterografts cannot be used for an analysis of the changed pattern control in mh-1.

(2) It appears to be unlikely, as well, that an adult head of mh-1 generates a considerably reduced inhibitory signalling compared to a young head (under transplantation conditions): If the inhibitory signalling of an adult head is strongly reduced compared to a young one an adult head transplanted to a basal part of a young animal should not be able to hinder head formation at the junction, but it did. The assumption of a considerably reduced inhibitory signalling by adult heads also does not fit the observation that additional heads are formed in animals of which the (original) head had been removed repeatedly and which repeatedly regenerated a new one at that position. Further, a wt 105 head was found to be unable to prevent additional head formation in the mh-1 body column of wt 105 / mh-1 heterotransplants.

(3) We favour the thesis that in adult polyps of mh-1 the resistance to inhibition is stronger. We proposed that the threshold above which head formation can no more be hindered at the former wound by a transplanted head is attained faster following sectioning compared to young mh-1 and wt 105. We suggest that the property which includes this threshold, the positional value, is affected quantitatively: it is increased. This proposition is consistent with that made for adult animals with additional heads by Sugiyama (1982).

A hypothesis for pattern control in mh-1

In a first approach we disregard that we do not know whether mh-1 differs from wt 105 in one or several mutations and assume a simple cascade of events:

(1) In mh-1 the signalling by morphogens is altered.
(2) The altered signalling allows an unusual increase of the positional value in the gastric region.
(3) The increased positional value allows additional heads to form.

(1) A reduced range of an inhibitory signalling may explain that the additional heads preferentially form in the lower half of the gastric tissue between the head and the budding zone. However, a head appears to form at a random position within that area and in a few cases an additional head is even formed very close to the original head. A similar pattern has been observed following repeated daily treatment of wt 105 with the diacylglycerol diC_8 accompanied by feeding (Müller, 1989, 1990) or heavy feeding only (Kroiher, 1999). Consequently, in mh-1 the additional heads may not be caused by a reduction of the range of inhibition to a fixed shorter range but rather a more dynamic property appears to be affected, at least in addition. The local level of inhibition and activation may change like the water depth at a windy shore. To explain the altered pattern in growing up mh-1 we suggest a transient overshooting activator generation starting in a body region where the level of inhibition is transiently low. This may occur rarely and only due to external influences, e.g. feeding, and may not occur in newly detached polyps but in animals after they have grown up to a certain length. The transient locally increased activator concentration does not cause head formation in a one-step process, rather it is suggested to cause an increase of the positional value.

An argument for a reduced range of an inhibitory signalling may be obtained from an experiment in which the foot was removed just below a young bud. This experiment was made with a body part which appears to remain wild type-like in adults. Hence the influence of the primary defect may be studied. In mh-1 the foot was regenerated and the bud developed normally, while in the wt 105 animals foot regeneration was hindered. Further, most buds did not form a normal foot and the bud's head produced less tentacles. Similar results have been obtained with other *Hydra* species (Tripp, 1928). Thus, in wt 105 an inhibitory interaction has taken place which involves both, the head and the foot. These observations suggest that the inhibitory signals are neither head nor foot specific but rather hinder the formation of both body parts. They may act by antagonizing a change of the positional value which was suggested to be a prerequisite for both head and foot formation (Berking, 1998). In mh-1 the range of the respective signals appears to be shorter.

(2, 3) The transient overshooting activator generation may start at a random position, but preferentially in a region where the positional

value is high and the inhibitory influence from the head is low, i.e. in the lower half of the body column between the head and the budding zone. This event is suggested to cause a small local increase of the positional value. When subsequently the conditions favour a further overshooting activator generation, the region with a locally increased positional value will win the race. Thus, the positional value will increase step by step in form of patches along the body axis. This process takes time during which the patches spread due to the multiplication of epithelial cells which occurs within the whole gastric region (David and Campbell, 1972). When a certain high positional value is attained RF-amide positive nerve cells form. Finally, at a certain site the generation of an activator becomes stable in time which causes the positional value to increase to its maximal value. According to the local positional value secondary morphogens may control tentacle and hypostome formation. Based on this proposition the activator in question is not a head specific activator which cause the head to be formed when a certain threshold concentration of the activator is reached but rather an activator which causes an increase of the positional value, which in turn causes tentacle and hypostome formation when a certain threshold of the positional value is reached.

A hypothesis for control of budding in mh-1

Buds are different from additional heads by at least two processes: the visible onset and the eventual separation from the parent. When the tip of a hypostome was transplanted to various body regions secondary heads and subsequently secondary axes were formed, which did not detach in cases the hypostome was transplanted to the gastric region above the position at which buds normally form. However, buds were formed which detached normally when the tip of a hypostome was transplanted into the budding region and, most interestingly, below the budding region down to just above the basal disc (Berking, 1979). Thus, bud induction is suggested to be not different from (secondary) head induction. Further, a secondary axis detaches from the parent as a bud only if the tissue at its base which is recruited from the parent by the outgrowing bud has a sufficiently low positional value. This proposition was confirmed by other experimental approaches (Tardent, 1972; Berking, 1979; Pérez and Berking, 1994). We suggest that in mh-1 buds form as long as the tissue is recruited from the surroundings of the outgrowing tip which eventually forms the base of the secondary axis has a sufficiently low positional value. Therewith, a bud and a newly detached mh-1 have a wt 105 like property. If at the future bud´s base the positional value is not low enough, the process of secondary axis formation may start bud-like i.e. in the form of a small tip, but the axis will not detach eventually.

In summary, we suggest that in mh-1 a hypothetical activator can be generated autocatalytically enhanced not only in the budding region but rather in a much larger region. Apical to the budding region the generation is not stable in time due to the vicinity of the head and due to the high local positional value, but in mh-1 (compared to wt 105, where it may also occur) the generation is long enough stable to cause a locally restricted small increase of the positional value. By repetition of this process eventually additional heads form. In the budding region, which is characterized by a certain low positional value and a certain distance to the head the occasional generation of activator becomes stable in time, due to the low level of inhibition. The activator causes in the tip of the outgrowing axis in a continuous process an increase of the positional value up to the maximal value. If at the base of the axis the recruited tissue has a sufficient low positional value, the axis detaches as a bud, otherwise not detached or partially detached polyps form with a foot in form of a patch. The formation of a bud close to an additional head may indicate that the local basal production of inhibitor according to the local positional value of the body column (Gierer and Meinhardt, 1972) has a strong influence on the survival of the autocatalytically produced activator. Further, the range of inhibition appears to be shorter in mh-1 as indicated by the reduced inhibitory interaction between a regenerating foot and a developing bud.

The experiments with mh-1 did not indicate the existence of head and foot specific activators and inhibitors, respectively, to play a leading role in pattern formation in *Hydra*. Rather, the results obtained favour the proposition that the first step in pattern control is the control of the positional value by means of diffusible morphogens including activators and inhibitors which regulate their own generation. The second step may be the regional differentiation as hypostome, tentacles and basal disc formation under the control of secondary morphogens which are generated according to the local positional value (Berking, 1998).

Materials and Methods

Animals

Two strains of *Hydra magnipapillata* were used, strain 105 and strain multiheaded-1 (mh-1) (Sugiyama and Fujisawa, 1977a). Strain 105 is commonly used as the standard wild type strain (wt 105). Strain mh-1 is a mutant strain which was originally produced through sexual inbreeding (F_1) of wild *Hydra*. It has been maintained clonaly by asexual propagation (budding). In addition we used for some experiments the Zurich strain of *Hydra vulgaris*, which was isolated by Pierre Tardent in 1966. The animals were maintained at 20°C in culture medium (1 mM $CaCl_2$; 0.5 mM $MgCl_2$; 0.1 mM $KHCO_3$; 0.35 mM $NaHCO_3$ and 0.025 mM EDTA). They were fed five times a week with nauplii of *Artemia salina*. In one experiment the animals were fed heavily (procedure according to Kroiher 1999). The animals were fed with 20 nauplii/hydra, while the control animals were fed normally (5 nauplii/hydra). All animals used in the experiments were starved for 24 hours prior to the start of the experiment.

Head and foot regeneration

Head and foot regeneration was studied in transversal sections generally of budless wt 105 and mh-1. The sections were made either below the tentacle ring or at one of the three positions which can subdivide the body into four equally sized pieces. Immediately after cutting, the tissue pieces were transferred to petri dishes, approximately 30 per dish, containing 10 ml medium. The criterion for head regeneration was the development of a hypostome and tentacles, the criterion for foot regeneration was the ability to stick to a surface.

Axial grafting

Axial grafting was done according to Browne (1909). To distinguish between the tissue of different origin we used vitally stained animals (according to Wilby and Webster (1970) modified by Berking (1979)).

Immunochemistry

Immunochemistry whole mount staining with an RF-amide antibody was done according to Grimmelikhuijzen (1985).

Statistics

Statistics in the graphs the means of three experiments are shown. The vertical bars indicate the respective confidence interval (95%-level).

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