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Genetic analysis of developmental mechanisms in hydra

XVI. Effect of food on budding and developmental gradients in a mutant strain L4

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SUMMARY

Effect of food was examined on the budding rate and the developmental gradients of a mutant hydra strain L4.

This mutant strain has very high levels of head-inhibition potential gradient along its body axis (Takano & Sugiyama, 1983). It also has a reduced budding capacity when it is cultured using brine shrimp nauplii as food, but its budding capacity is significantly improved when a small amount of tubifex worm tissue is added to its diet of brine shrimp (Takano, 1984).

To test whether or not this change of budding rate is correlated with the change in the levels of the head-activation or head-inhibition potential gradients, L4 animals were cultured on the diet of brine shrimp with or without addition of tubifex worm tissue and the budding rates and the gradient levels were examined in these animals.

The results showed that food affected the budding rate in L4 without affecting its gradient levels. This suggests that the gradient levels and the budding rate in L4 are uncorrelated to each other, and that therefore the high levels of head-inhibition potential are not the cause for the low budding rate in this strain (cf., Takano & Sugiyama, 1983).

INTRODUCTION

Strain L4 is a mutant strain of *Hydra magnipapillata* which has a defect in its budding mechanisms (Sugiyama & Fujisawa, 1979). Under the normal culture conditions, the budding rate of L4 is less than half the rate of the normal strain. This strain, however, has a nearly normal regenerative capacity after removal of the original head (Takano & Sugiyama, 1983) and its cells divide and proliferate at nearly the normal rates (Takano, Fujisawa & Sugiyama, 1980). It therefore appears that the low budding rate of L4 is produced not by the generally reduced physiological or morphogenetic activities but by a specific defect in the budding mechanisms in this strain.

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Three relevant observations have previously been made on L4. Cell cycle analysis by continuous labelling with tritiated thymidine has shown that both the epithelial cells and the interstitial cells in L4 divide and proliferate at nearly the same rates as the cells in the normal strain (Takano, 1980). This indicates that L4 animals produce enough cells to grow and produce buds at the same rates as the normal strain. However, a large part of these cells in L4 are not used for these purposes but apparently wasted away in some manner (Takano, 1984).

L4 has highly altered developmental gradients along its body axis (Takano & Sugiyama, 1983). Hydra tissue has the potential to stimulate, or activate, the formation of head structure (head-activation potential) and the potential to inhibit the head formation (head-inhibition potential). The levels of both potentials are high in the head and become progressively lower toward the foot, forming gradients along the body axis (for a recent review see Bode & Bode, 1983). It is thought that the interaction of these two potentials plays crucial roles in regulating the head structure formation (Meinhardt & Gierer, 1974; Wolpert, Hornbruch & Clarke, 1974; MacWilliams, 1982; Kemmner & Schaller, 1984). In normal tissue, the two potentials are in balance. Under some conditions, however, this balance is lost and the activation potential may become significantly higher than the inhibition potential. Head structure formation is thought to occur under such situations.

Takano & Sugiyama (1983) examined and compared the two potential levels in L4 and the normal strain by the lateral tissue transplantation procedure of Webster & Wolpert (1966). The results have shown that L4 has a nearly normal or a slightly lower head-activation potential but a much higher head-inhibition potential than the normal strain along its body axis. Takano & Sugiyama (1983) has suggested that this high head-inhibition potential is correlated with, and may be responsible for, the low budding rate in L4.

The budding rate of L4 is strongly affected by the type of food it eats. Brine shrimp nauplii are commonly used as food to raise hydra in laboratory (Loomis & Lenhoff, 1956). On this diet, the budding rate of L4 is less than half the rate of the normal strain (Sugiyama & Fujisawa, 1979). However, its budding rate is significantly improved when a small amount of tubifex worm tissue is added to the brine shrimp diet (Takano, 1984). This addition, however, produces little effect on the budding rate of the normal strain.

Why food affects the budding rate of L4 is not understood at present. One possibility is that food affects the developmental potentials in L4, and that this alteration of the potentials secondarily affects its budding rate. In other words, addition of tubifex tissue to the brine shrimp diet may either lower the very high levels of the inhibition potential in L4 and bring them down close to the normal levels, or raise the nearly normal levels of the activation potential to much higher levels. Such potential level alterations may be the cause for the change of the budding rate by food.

The present study was carried out to examine whether or not such potential level changes occur in the L4 animals by food. L4 animals were raised on a diet of brine

shrimp nauplii with or without supplementation of tubifex tissue. The potential levels in these animals were examined and compared by the lateral tissue transplantation procedure of Webster & Wolpert (1966).

It will be shown that the type of food significantly affects L4's budding rates (0·3–0·8 buds/polyp/day) but not its head-activation or head-inhibition potentials. This indicates that the suggestion previously made by Takano & Sugiyama (1983) was not correct. The high inhibition potential is uncorrelated with, and therefore not responsible for, the low budding rate in L4.

MATERIALS AND METHODS

Strains

Two strains belonging to *Hydra magnipapillata* were used. Strain 105 is the wild-type standard strain. Strain L4 is a naturally occurring mutant strain originally isolated from an outdoor pond. It has a large polyp size, a low budding rate (Sugiyama & Fujisawa, 1979), nearly normal cell cycles (Takano *et al.* 1980), and altered developmental gradients along its body axis (Takano & Sugiyama, 1983).

Foods

Two different brands of brine shrimp were used: Nissei brand (Nippon Jisei Sangyo, Tokyo) and San Francisco Bay brand (Brine shrimp Sales Co. Inc., Haywood, California). These two brands had significantly different characteristics as previously described (Takano, 1984). Newly hatched nauplii from these brine shrimp eggs were obtained as previously described (Takano, 1984).

Tubifex worm was purchased from a local pet shop. To remove parasites, worm was first briefly rinsed in 70% ethanol, next in 3% salt solution, and then thoroughly washed in the culture solution. To feed hydra, worm tissue was cut into small pieces (0·5–1 mm cube), and one such piece was individually given to each polyp. After confirming that it was firmly captured by an animal, a large number of brine shrimp nauplii were given to each animal.

Culture conditions

Culture and all the experiments were carried out in a constant temperature room maintained at 18 ± 1 °C, using modified M-solution (Muscatine & Lenhoff, 1965; Sugiyama & Fujisawa, 1977) as the culture solution.

Animals used for determination of developmental parameters (see below) were cultured individually in small plastic dishes as described by Sugiyama & Fujisawa (1979).

Animals used for tissue transplantation experiments (see below) were cultured under the rigorously controlled and standardized mass culture conditions described previously by Takano & Sugiyama (1983).

In transplantation experiments (see below), 105 polyps vitally stained with Evans blue were also used. These animals were cultured using the brine shrimp nauplii stained with Evans blue as food (Wilby & Webster, 1970*b*; Nishimiya, Wanek & Sugiyama, 1985) but otherwise in the same way as described above for other animals cultured for transplantation.

Developmental stages of polyps

Polyps in two different stages of development were used in experiments. One stage was very young polyps dropped from parents within the previous 24 h period (d in fig. 2 in Takano, 1984). They were termed newly dropped polyps.

The other stage was a little older polyps which were showing their first protrusion for budding (f in fig. 2 in Takano, 1984). They were termed protrusion-bearing polyps (previously called standard polyps).

Developmental parameters

The following four parameters of development were determined using populations of individually cultured animals (Sugiyama & Fujisawa, 1979).

Population growth rate (α) is defined as $dN/dt = \alpha N$, where N is the total number of polyps of an exponentially growing population at time t, and t is the time measured in days.

Budding rate is defined as the average number of buds produced per day per mature polyp.

Bud developmental rate is defined as the reciprocal of the number of days required by a newly formed bud to produce its own first bud. Completion of a basal disk while the bud is still attached to the parent is used as the criterion of a new bud formation.

Polyp size was measured by the total protein content per polyp. Individual polyps were dissolved in 0·2 ml of 0·5 N-NaOH–7 % glycerol solution and the protein content was determined by the method of Lowry, Rosenbrough, Farr & Randall (1951) using bovine serum albumin as standard (Takano, 1984).

Tissue transplantation

The levels of the head-activation and the head-inhibition potentials were examined using animals cultured under the standardized mass culture conditions by the tissue transplantation procedure described previously (Takano & Sugiyama, 1983).

The body column of a well-stretched protrusion-bearing polyp from the hypostome to the bud protrusion was divided into four equal lengths, the column from the protrusion to the basal disk was divided into the ratio of 1:2, and the four positions thus obtained were numbered from 1 to 4 as shown on left in Fig. 1 (Sugiyama, 1982). The body column of a well-stretched newly dropped polyp was divided into four equal lengths and the three positions thus obtained were marked u (upper), m (middle) and l (lower) (right in Fig. 1). These positions were used as the sites for comparing the potential levels.

The procedure used to determine the head-activation potential levels is schematically shown in Fig. 2. A ring of tissue was excised from one of the four (three) positions of a protrusion-bearing (newly dropped) polyp, and cut into two or three pieces containing about 5000 cells each. One such piece was then transplanted to the standard host site at position-3 or -4 of a protrusion-bearing 105 polyp vitally stained with Evans blue (standard host *a* or *b*, respectively in Fig. 2). The grafted animals were kept for 7–8 days to observe the head induction produced by the donor tissue. The relative levels of the head-activation potential at various positions were compared by the differences in the percentages of head induction which were produced when these tissues were transplanted to the same hosts.

The procedure used to determine the head-inhibition potential is schematically shown in Fig. 3.

Three standard donor tissues were employed. Head was removed from a protrusion-bearing 105 polyp vitally stained with Evans blue by amputation made at position-1. After a period of 0,

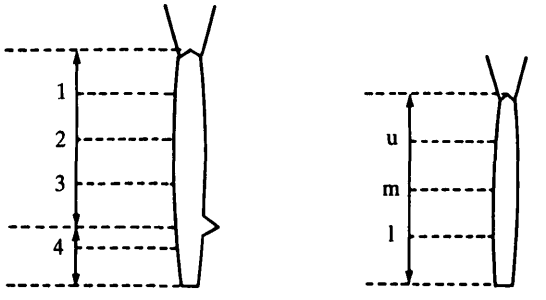

Fig. 1. Four positions on the protrusion-bearing polyp (left) and three positions on the newly dropped polyp (right). See Materials and Methods.

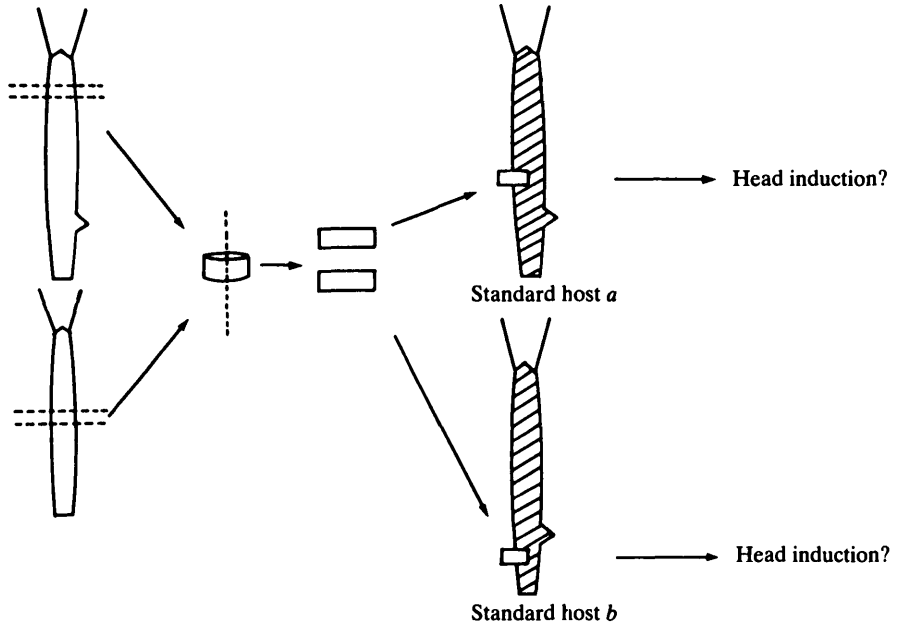

Fig. 2. Schematic representation of the procedure used to determine the head-activation potential levels of the experimental animals (unshaded). Shaded animals represent the standard host animals vitally stained with Evans blue. For detail see Tissue transplantation in Materials and Methods.

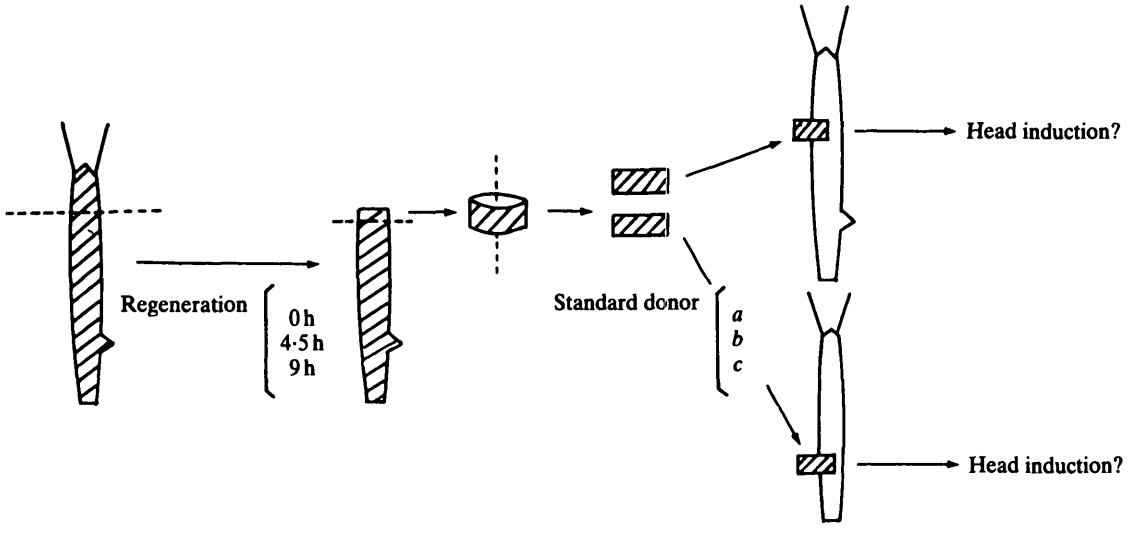

Fig. 3. Schematic representation of the procedure used to determine the head-inhibition potential levels of the experimental animals (unshaded). Shaded animal represents the polyp vitally stained with Evans blue which was used as the source of the standard donor tissues. For details see Tissue transplantation in Materials and Methods.

4·5 or 9 h, a small piece of tissue was removed from the distal regenerating tip and cut into two or three pieces containing about 5000 cells. These pieces were termed the standard donor tissue *a*, *b*, or *c*, respectively. One of the pieces was then transplanted to one of the four (three) positions of the protrusion-bearing (newly dropped) polyp. The transplanted animals were kept for 7–8 days to observe the induction of the head structures by the transplanted tissue. The relative levels of the head-inhibition potential at various sites were compared by the differences of the percentages of head induction which were observed by transplanting the same donor tissues to these sites.

RESULTS

Effect of food on developmental parameters

Four different types of food were used to raise L4 animals in the present study. They were brine shrimp nauplii of Nissei brand and San Francisco Bay brand with or without addition of a small amount of tubifex worm tissue (Materials and Methods).

Table 1 shows the developmental parameters of L4 animals raised on these four types of food. It can be seen that food did not affect the polyp size (protein content per polyp), but strongly affected the population growth, the budding, and the bud developmental rates. These three rates of L4 were about or less than half the rates of the normal strain (105) when cultured on the diet of brine shrimp. Of the two brands of brine shrimp, Nissei brand produced somewhat better results than the other brand. Addition of tubifex worm tissue to both brands of brine shrimp diet significantly improved the three rates, and the differences observed when brine shrimp alone was used as food virtually disappeared.

Table 1 also shows the results for the normal strain 105 raised on the diet of Nissei brine shrimp. The other diets were not used for 105 in the present study since it was previously shown that the developmental parameters of this strain were uninfluenced by food (Takano, 1984).

Effect of food on the head-activation potential

The relative levels of the head-activation and the head-inhibition potentials in hydra tissue can be quantitatively compared by the lateral transplantation of tissue (Webster & Wolpert, 1966). When a small piece of tissue is excised from one polyp and transplanted onto another, it sometimes induces the formation of a secondary head on the host polyp. This is thought to occur when the head-activation potential level of the donor tissue is sufficiently higher than the head-inhibition potential level at the site of transplantation on the host polyp (Wolpert *et al.* 1974; MacWilliams, 1982).

This procedure has been utilized in recent years to examine the potential levels in various mutant and chimaeric strains (Rubin & Bode, 1982*a*,*b*; Sugiyama, 1982; Takano & Sugiyama, 1983, 1984; Achermann & Sugiyama, 1985; Nishimiya *et al.* 1985). The same procedure was used in the present study to examine the potential levels in the L4 animals raised on the four types of diet.

The results of comparing the head-activation potential levels in the protrusion-bearing L4 polyps are shown in Fig. 4. The procedure used was described in

Table 1. *Effect of food on growth parameters*

Strain	Food	Population growth rate		Budding rate		Bud developmental rate		Protein content per polyp			
								Newly dropped polyp		Protrusion bearing polyp	
		No. of determination	Rate (± S.D.)	No. of determination	Rate (± S.D.)	No. of determination	Rate (± S.D.)	No. examined	μg (± S.D.)	No. examined	μg (± S.D.)
L4	Nissei shrimp	6	0·13 ± 0·01	6	0·5 ± 0·1	60	0·08 ± 0·007	20	63 ± 12	20	145 ± 16
	S.F. Bay shrimp	6	0·07 ± 0·01	6	0·3 ± 0·1	60	0·05 ± 0·007	20	64 ± 10	20	168 ± 18
	Nissei shrimp +tubifex	6	0·19 ± 0·02	6	0·8 ± 0·1	60	0·11 ± 0·01	20	66 ± 10	20	155 ± 16
	S.F. Bay shrimp +tubifex	6	0·18 ± 0·02	6	0·7 ± 0·2	60	0·11 ± 0·01	20	61 ± 11	20	151 ± 13
105	Nissei shrimp	6	0·27 ± 0·02	6	1·1 ± 0·1	60	0·14 ± 0·01	(Not examined)		20	58 ± 9

Materials and Methods (also see Fig. 2). The figure shows the percentages of head induction which occurred when the four donor tissues taken from L4 were grafted to the standard host *a* (Fig. 4A) or *b* (Fig. 4B).

For example, Fig. 4B shows that the donor tissues from position-1, -2, -3, and -4 of the L4 polyps raised on the diet of Nissei brine shrimp induced heads at 48, 28, 7 and 0%, respectively, on the standard host *b*. As already described, head induction is thought to be determined by the relative levels of the activation potential in the donor tissue and the inhibition potential of the host animal. Since the same host was used in the four grafts, the differences in the percentage values observed were due to the differences in the activation potential levels of the donor tissues. The results, therefore, indicated that the activation potential level in the L4 polyp was the highest at position-1 and became progressively lower toward position-4, forming a gradient along the body axis. This agrees well with the results previously reported (Takano & Sugiyama, 1983, 1984).

Fig. 4B also shows that the donor tissues taken from the same positions of the L4 animals raised on the four types of food induced heads at similar percentages. This

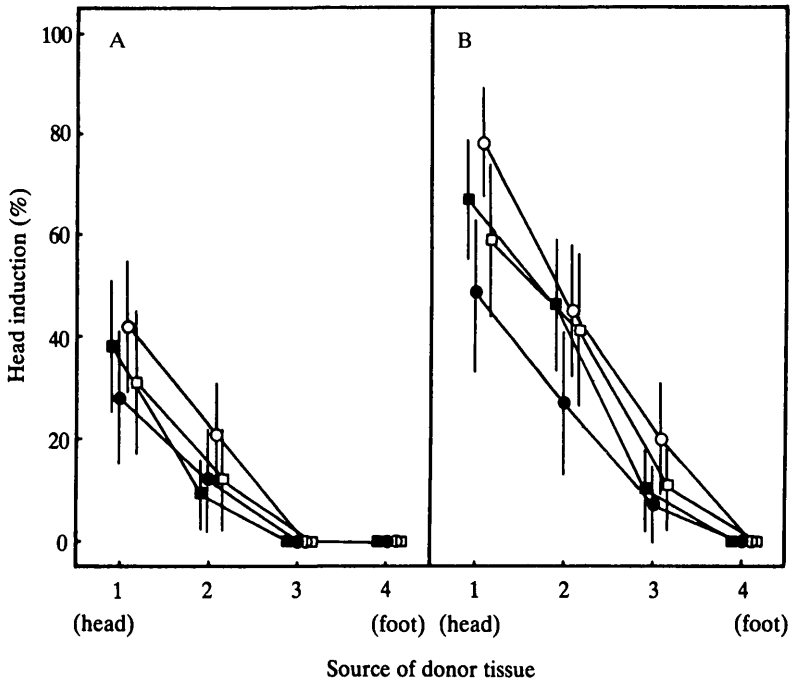

Fig. 4. Comparison of the head-activation potential levels in the protrusion-bearing L4 polyps raised on four types of food. The abscissa represents the four axial positions on the L4 animals raised on the diet of Nissei brine shrimp (closed circles), Nissei brine shrimp and tubifex tissue (open circles), San Francisco Bay brine shrimp (closed squares) and San Francisco Bay brine shrimp and tubifex tissue (open squares). The ordinate represents the percentages of head induction which were observed when the donor tissues obtained from the four positions on these animals were transplanted to the standard host *a* (A) or *b* (B). The vertical bars represent confidence limit ($P > 0{\cdot}95$) in this and the following three figures. (Sample size n = 44–57.)

indicates that these animals have similar gradients. The donor tissues taken from position-1 and -2 of the animals raised on Nissei brine shrimp induced heads at somewhat lower percentages than the corresponding tissues from the animals raised on the other three types of diet. However, the significance of this relatively small difference was uncertain (see Discussion).

The results of similar experiments using the standard host *a* are shown in Fig. 4A. The head-induction percentage values shown in this figure were generally much lower than those shown in Fig. 4B. This was because the head-inhibition potential level in the standard host animal used in Fig. 4A (position-3) was significantly higher than that in Fig. 4B (position-4 on the protrusion-bearing 105 polyp). No head was induced by the donor tissues from position-3 and -4 in Fig. 4A. This was because the head-activation potential levels in these donor tissues were not sufficiently higher than the head-inhibition potential level at the site of transplantation on the host animal used.

Except for these two differences, the results in Fig. 4A show the same general features as those in Fig. 4B. The L4 animals raised on the four types of food have very similar gradients of the head-activation potential along their body axis.

The results of comparing the head-activation potential levels in the newly dropped L4 polyps are shown in Fig. 5. Only the standard host *b* was used in this

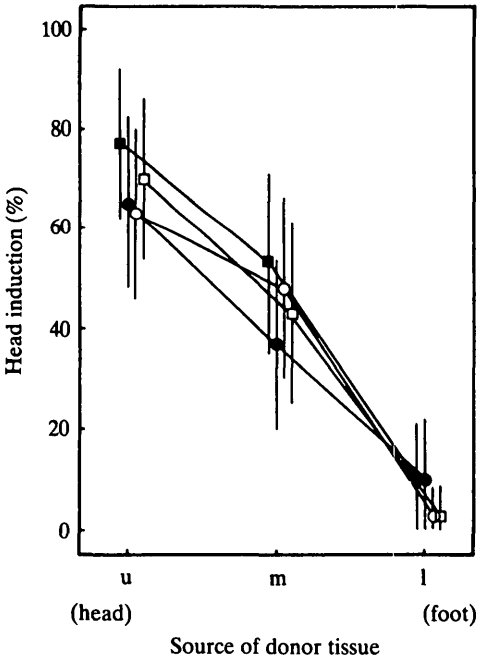

Fig. 5. Comparison of the head-activation potential levels in the newly dropped L4 polyps. The abscissa represents the three axial positions on the L4 animals and the ordinate represents the percentages of head induction which were observed when the donor tissues obtained from these positions were transplanted to the standard host *b*. The same symbol marks are used to indicate the food types as in Fig. 4. (n = 30–31.)

experiment. The same general features described for Fig. 4 can be observed in this figure. As in the protrusion-bearing polyp, the level of the head-activation potential also form a gradient from head to foot in the newly dropped polyp. Also, no significant differences in the activation potential levels exist in the animals raised on the four types of food.

Effect of food on the head-inhibition potential

Fig. 6 shows the results of comparing the head-inhibition potential levels in the L4 animals raised on the four different types of food. It shows the percentages of head induction which were observed when the standard donor tissue *a*, *b*, or *c* (Fig. 3) was transplanted to the four positions on the protrusion-bearing L4 polyps (Fig. 6A,B and C, respectively).

For example, Fig. 6B shows that the standard donor tissue *b* induced heads at 7, 17, 32 and 52 % when transplanted to position-1, -2, -3 and -4, respectively, of the protrusion-bearing L4 polyps raised on Nissei brine shrimp. Since the same donor tissue was used in these transplantations, the differences in the percentage values observed were due to the differences in the head-inhibition potential levels of the recipient sites. The results, therefore, indicated that the inhibition potential level

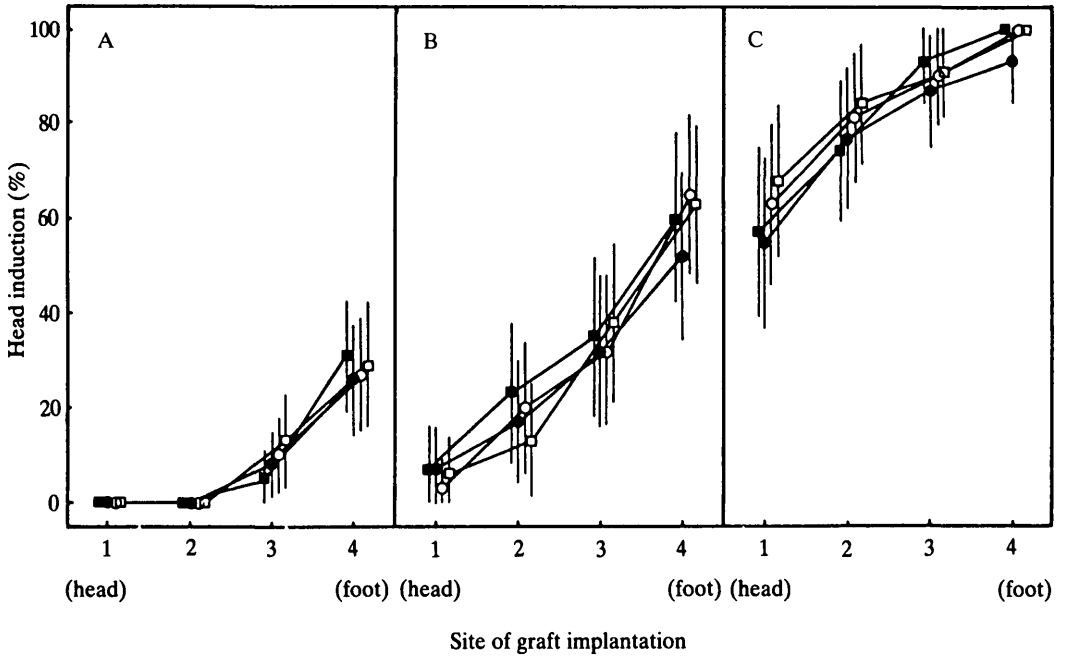

Fig. 6. Comparison of the head-inhibition potential levels in the protrusion-bearing L4 polyps. The abscissa represents the four axial positions on the L4 polyps used as the sites for transplantation. The ordinate represents the percentages of head induction which were observed when the standard donor tissue *a* (A), *b* (B) or *c* (C) were transplanted to these sites. The same symbol marks are used to indicate the food types as in Fig. 4. (n = 30–55.)

was the highest at position-1 and became progressively lower toward position-4, forming a gradient along the body axis. This agrees well with the results of similar experiments previously reported (Takano & Sugiyama, 1983, 1984).

Fig. 6B also shows that the same donor tissues induced heads at similar percentages when transplanted to the same positions of the animals raised on different types of diet. This indicates that these animals all have similar gradients.

The same features can also be seen in Fig. 6A and C. The general differences observed in the three figures in Fig. 6 were due to the differences of the head-activation potential levels in the standard donor tissues used. The standard donor tissues *a*, *b* and *c* have gradually higher levels of the potential in that order (Takano & Sugiyama, 1983, 1984), and these donor tissues produced gradually higher percentages of head induction as shown in Fig. 6A,B and C, respectively. No head induction occurred at position-1 and -2 when the standard donor tissue *a* was used (Fig. 6A). This was because the level of the activation potential in this donor was not sufficiently higher than the inhibition potential levels at these two recipient sites.

Thus, Fig. 6A,B and C all show the two general features. The head-inhibition potential level forms a gradient from head to foot along the body axis, and no significant differences exist in the potential levels in the animals raised on the four types of food.

Fig. 7 shows the results of examining the head-inhibition potential levels in the newly-dropped L4 polyps. Only the standard donor tissue *a* was used in this experiment. The same general features described for Fig. 6 can be seen in this figure. As in the protrusion-bearing polyp, the head-inhibition potential level also forms a gradient from head to foot in the newly dropped polyp, and the standard donor tissues induced heads at approximately the same rates when transplanted to the same positions of the polyps raised on different types of diet.

DISCUSSION

We previously made a suggestion that the very high levels of the head-inhibition potential in the mutant strain L4 are probably responsible for the reduced budding capacity of this strain (Takano & Sugiyama, 1983).

This suggestion, however, is not supported by the results of the present study. We thought that food might affect L4's budding rate by altering its potential levels, and compared the potential levels of L4 animals raised on different types of food. The results obtained, however, were contrary to our expectation. Addition of tubifex to the brine shrimp diet significantly affected L4's budding rate (Table 1), but not its potential levels (Figs 4–7). Particularly, the head-inhibition potential levels were equally very high in the animals raised on the diet with or without tubifex addition. This was true both in the presumptive budding zone (position-1) in young animals (Fig. 7) and also in the regions just above (position-3) or below (position-4) the budding zone in nearly mature animals (Fig. 6).

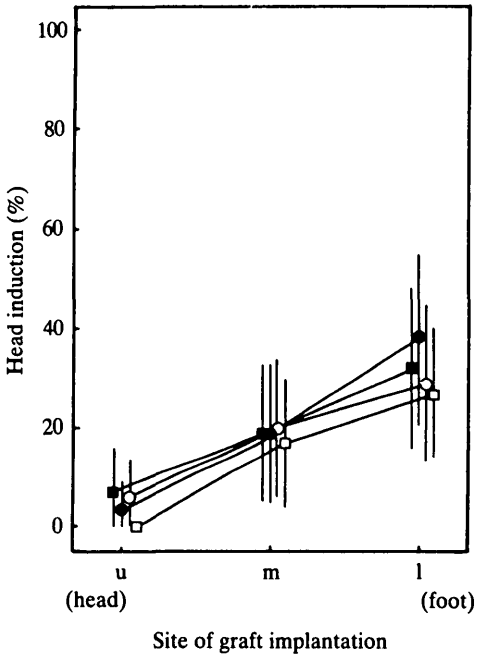

Fig. 7. Comparison of the head-inhibition potential levels in the newly dropped L4 polyps. The abscissa represents the three axial positions on the L4 polyps used as the sites for transplantation. The ordinate represents the percentages of head induction which were observed when the standard donor tissue *a* was transplanted to these sites. The same symbol marks are used to indicate the food types as in Fig. 4. (n = 30–32.)

In one experiment, a small increase in the activation potential levels was observed by addition of tubifex to one brand of brine shrimp (Fig. 4B). However, the significance of this observation was uncertain since a similar effect was not observed in other experiments (Fig. 4A and B).

The results, therefore, indicate that food affects the budding rate of L4 not by altering its potential levels but by some other mechanisms. They also suggest that the high head-inhibition potential levels in L4 is not correlated with, and therefore not responsible for, its low budding rate.

A similar suggestion has been recently made from another line of study. Takano & Sugiyama (1984) constructed chimaeric strains which consisted of various combinations of the cell lineages derived from 105 and L4. Analyses of the inhibition potential levels and the budding rates in these strains showed no correlation between the two. For example, the chimaera consisting of the wild-type ectodermal epithelial cell lineage and the mutant endodermal epithelial and interstitial cell lineages had a normal budding rate but very high head-inhibition potential levels.

Therefore, two independent lines of evidence suggest that cause–effect link does not exist in the high head-inhibition potential levels and the low budding rate

in L4. This is rather puzzling since it is contrary to various lines of evidence which indicate that budding is affected by the inhibition potential (for a recent review see Bode & Bode, 1983). For example, a small hydra does not bud. As it grows larger, budding occurs in a budding zone which is located some distance away from head. Budding is enhanced by removal of head. It is also enhanced by increase of distance from head to budding zone by addition of extra gastric tissue from another animal to the gastric region between head and budding zone (Webster, 1971). In contrast, budding is reduced by decrease of distance from head to budding zone by removal of a part of the gastric region (Burnett, 1961). When head is transplanted to the proximal end of the gastric region and the host head removed, the budding zone moves to the old distal end of the axis (Wilby & Webster, 1970*b*). Budding is inhibited by head inhibitor, a morphogenetic substance which exists at a very high level in head and in gradually lower levels in the tissue along the body column (Berking, 1974; Schaller, Schmidt & Grimmelkhuijzen, 1979). These observations indicate that budding is affected by the inhibiting capacity emanating from the head. This, however, is contrary to the present observations which show that the inhibition potential level is not correlated with the budding rate in L4.

At present we have no definite explanation for this contradiction. Two alternative possibilities can be considered. The simplest explanation is to assume that budding frequency is not affected by the inhibitory signal from head, although budding location may be affected by it (see below).

An alternative explanation is to assume that the inhibitory capacity from head consists of two (or more) signals, and that budding and head induction by transplanted tissue have different sensitivities to these signals. For example, one signal may be very effective in inhibiting only head induction whereas the other may be very effective in inhibiting only budding. If both signals are strong when L4 is cultured on the diet of brine shrimp but the latter becomes weak by tubifex addition to the diet, then food can affect L4's budding rate without affecting the inhibition potential levels measured by transplantation.

The existence of two inhibitory signals does not necessarily imply that hydra has two inhibitory substances. The head inhibitor isolated from hydra tissue inhibits budding at extremely low concentrations ($<10^{-8}$ M). This substance presumably plays an important role in determining the inhibition potential level of the tissue, although there is no definite proof for it at present. Assuming that this substance is the key factor in the inhibitory system, the two signals can be produced by a difference in the state of its form (free *vs* bound form) (Berking, 1974) or by a difference in its cellular localization (in nerve *vs* epithelial cells). For example, budding may be sensitive to the free form, whereas head induction by transplanted tissue may be sensitive to the bound form.

Alternatively, it is also conceivable that hydra uses two different substances for the two inhibitory signals. 'Head inhibitor' isolated by two laboratories appears to have generally similar but slightly different properties (Berking, 1983; Schaller, 1984). It seems possible that these workers have isolated two closely related

substances which have slightly different properties (although a real explanation for the differences may be more trivial in nature).

The discussions given above were concerned with the frequency of budding in L4. It may be worthwhile to briefly discuss the location of budding in the same strain. As already mentioned, head has a capacity to inhibit budding, and budding appears to occur in the area (budding zone) where the tissue has escaped the reach of this effect (Bode & Bode, 1984). However, the results of analyses of chimaeric strains produced from L4 and the normal strain indicate that budding position and inhibition potential levels are uncorrelated in these strains (Takano & Sugiyama, 1984). The lengths from head to budding zone or to basal disk in L4 are nearly twice as long as those in the normal strain (Takano & Sugiyama, 1983). These lengths in the chimaeras are determined primarily by the ectodermal epithelial cell lineage, whereas their inhibition potential levels are determined primarily by the endodermal and the interstitial cell lineages in them. For example, the chimaera which consists of the normal ectodermal epithelial cell lineage and the mutant endodermal and interstitial cell lineages has normal lengths but very high levels of the inhibition potential (Takano & Sugiyama, 1984). This indicates that the high head-inhibition potential levels are not the cause for the long length from head to budding zone in L4. It is conceivable that budding position, budding frequency and head induction by transplanted tissue are all affected by inhibitory signals emanating from head, but they have different sensitivities to different signals.

Whether or not hydra indeed have multiple signals in its head-inhibiting (and head-activating) system remains to be seen. In pursuing this problem, mutant strains which have altered levels of developmental gradients such as the 'aberrant' (Rubin & Bode, 1982) and reg-16 (Achermann & Sugiyama, 1985) may prove to be useful. In these strains, the levels of head-inhibitor activity found in homogenate and the levels of the head-inhibition potential measured by tissue transplantation do not agree. The former strain has a lower than normal level of inhibitor activity (Schaller, Schmidt, Flick & Grimmelikhuijzen, 1977) but higher than normal levels of inhibition potential (Rubin & Bode, 1982). After head removal of the latter strain, the inhibitor activity is not released into the culture solution (Kemmner & Schaller, 1981), but the inhibition potential drops slowly in steps (Achermann & Sugiyama, 1985). These differences may arise due to the presence of multiple signals which have differential effects on various morphogenetic processes.

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