

The Role of the Nervous System in the Planarian Regeneration

II. Regeneration of the Body Fragments with the Reversed Nerve Cord

Shingo KURABUCHI and Yoshikazu KISHIDA

Department of Biology, Faculty of Science, Kanazawa University

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Abstract In order to determine the influence of the nerve cord on the polarity of the blastema regeneration in *Dugesia japonica*, three groups of isolated body fragments with the reversed ventral nerve cord were prepared; 1) fragments with one nerve cord intact and the other rotated; 2) fragments with only one nerve cord reversed; 3) fragments with three nerve cords which consist of two intact nerve cords and one reversed nerve cord transplanted between them. Although the experimental fragments developed into regenerates of various shapes, the results in all the experimental series agreed in that a head is regenerated from the posterior cut end of the fragments with the original anterior end of the rotated nerve cord. From these results, we discussed the role of the biological polarity of the nerve cord in determining the differentiation of the regenerating blastema.

Introduction

When some species of the freshwater planarians are cut transversely into pieces, in each cut piece a head is regenerated at the anterior end and the tail at the posterior. The most important interpretation of this phenomenon seems to be that of Child (1941), who concluded from an enormous number of experiments that quantitative gradients of metabolic activity, mainly oxygen consumption, along the antero-posterior axis of the planarian body determines the pattern of differentiation of the regenerating blastema. Later, Flickinger (1959), and then Flickinger and Coward (1962) proposed a fascinating assumption that there is an axial gradient of activity of protein synthesis in the planarian body and that head formation occurs at the anterior end of the isolated fragment where conditions for protein synthesis are more favorable.

There is, on the other hand, some evidence that the nervous system plays an important role in the planarian regeneration (Morgan, 1898; Sugino, 1964; Sperry, et al., 1973). Our earlier investigation (Kishida and Kurabuchi, 1978) has shown that the denervated fragments isolated from the planarian body develop a head from their

posterior end to give rise to bipolar heads in a large percentage, and we suggested that this effect might be brought about by the exclusion of the head-inhibiting factor which may be located in the nerve cord. However, the extirpation experiment of the nerve cord could not prove the possible presence of relationships between these two factors, the nerve cord and the biological polarity of the body, relationships which are likely to determine the differentiation of the regenerating blastema into either a head or a tail. Can the biological polarity relevant to the determination of the blastema exist in the nerve cord itself? If so, can the polarity of the nerve cord control that of the body and determine the blastemal differentiation or vice versa? Or else, can these factors exert independent questions?

In view of these considerations, the present study was carried out to investigate regeneration in isolated body fragments with the transplanted nerve cord oriented in the reversed direction, in an effort to obtain further information about the effect of the nerve cord on the polarity of planarian regeneration.

Material and Methods

The material used in this study was the asexual form of the planarian, *Dugesia japonica*, collected from a stream located in the suburbs of Kanazawa City. The worms, about 15 mm in length, were selected and kept in fasting for at least one week prior to the experiments. In accordance with the procedure described in the previous paper (Kishida and Kurabuchi, 1978), the worms were held facing upwards for operation on a piece of filter paper placed on a plate of ice. Three kinds of operations were performed; 1) a narrow rectangular strip containing a nerve cord in one side of a worm was cut off and was inserted into the original wound to be oriented in the reversed direction in relation to the anteroposterior body axis (Fig. 1a); 2) after a strip containing a nerve cord was discarded, the same operation as 1) was performed on the other nerve cord of the worm (Fig. 1b); 3) a rectangular strip containing one nerve cord was removed from a worm and then this was transplanted in the reversed direction into the median region between the nerve cords in another worm (Fig. 1c). The worms treated as above were covered with a piece of wet tissue paper and were immobilized by placing several small glass splinters around them. All operations were performed exclusively on the postpharyngeal region of the worms. The operated-upon worms were kept in a wet condition at 10°C in darkness for one day and then released in aged tap water at 18–20°C. After 2 days, they were amputated at the anterior and posterior levels of the operated region to make fragments of 1.5 mm in length. As a result, three kinds of fragments were made (Fig. 1). The first group consisted of fragments which had one intact nerve cord in the normal direction and the other nerve cord reversed. The second group was composed of fragments with only one rotated nerve cord. The third group was composed of fragments containing three nerve cords which were two intact nerve cords in the normal direction and one rotated nerve cord between them. As a control,

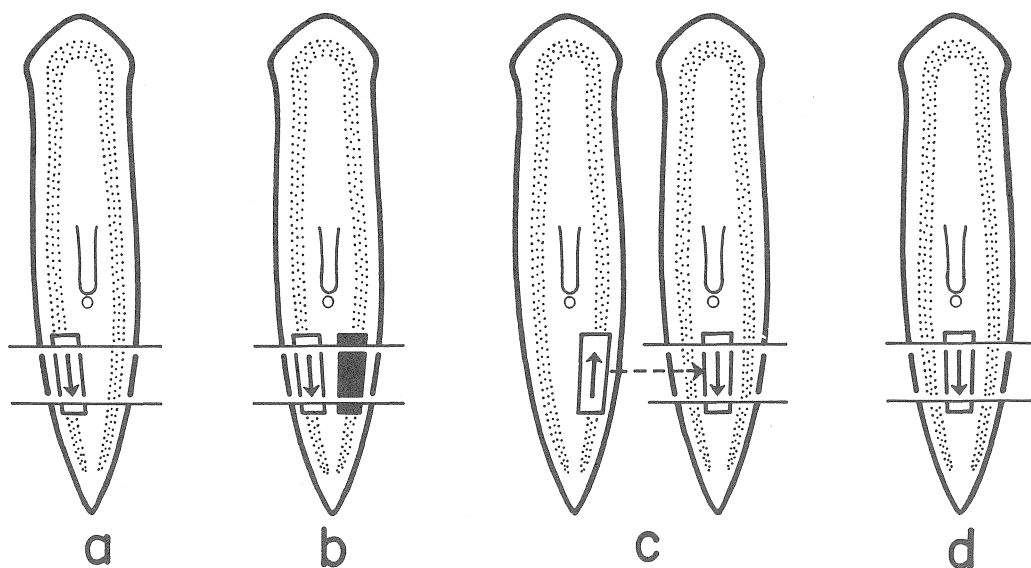


Fig. 1 Schematic drawings of the operation procedures to make the first group of fragments (a), the second group (b), the third group (c) and the control group (d). Dotted area: location of the nerve cord seen as a pigmented line in the ventral face of the worm. White rectangular area: grafted tissue strip. Arrow in this area shows orientation of the strip, which points to the anterior. Black rectangular area: discarded tissue strip. Transverse lines: cut levels to isolate the fragment.

fragments were prepared, in which the nerve cords were kept intact and the median tissue strip containing no nerve cords was cut off and reunited in the reverse direction

All fragments were allowed to regenerate in aged tap water at 18–20°C for 30 days. The aged tap water was changed every two days. Some specimens were treated with formic acid according to the method used by Kishida (1969) in order to make the pharynges in the regenerated worms visible, and their existence and direction were observed.

Results

In the present experiment, success was obtained in only 5–10% of all the operated worms, owing to the difficulty of the operation procedure used; the grafts in some cases degenerated or were absorbed due to their too small size, and in other cases, unions between the host and the graft tore off within several days of operation. Eight types of regenerates were distinguished in the experimental and control groups as is represented schematically in Figure 2. The results obtained after 30 days of regeneration are summarized in Table 1. Thirteen successful cases were obtained in the first group of the fragments which had one nerve cord intact and the other rotated. In these fragments, each blastema formation began at the anterior and posterior ends of the

nerve cord. In two of these cases, a head grew up at the posterior as well as the anterior end of the fragments, after the fusing of two blastemas at the median line, and they consequently became bipolar heads (type 5). In five of the cases, the anteriorly fused blastema resulted in one head but the posterior blastemas developed into a head and a tail (type 7). The other six cases developed simultaneously a head and a tail at both cut ends to give rise to a doublet type with opposing polarity (type 8). The second group of fragments, in which one nerve cord was reversed and the other was discarded, were successful only in 3 cases. A blastema appeared at a position of the fragment end which contained the cut end of the nerve cord in the beginning of regeneration. This

Table 1 Regeneration of the fragments with the nerve cord in the reversed direction

| Group of fragments | Types of regenerates* | | | | | | |
|--------------------|-----------------------|---|---|---|---|---|---|
| | 1 | 2 | 4 | 5 | 6 | 7 | 8 |
| 1 | | | | 2 | | 5 | 6 |
| 2 | | | | 3 | | | |
| 3 | | | | | 3 | 1 | |
| Control | 6 | 2 | 3 | 2 | | | |

* Types of regenerates are represented schematically in Figure 2.

type later formed a head at both the anterior and posterior ends to become what might be called a bipolar head (type 5). In 3 out of the 4 successful cases of the third group of fragments with two intact normally-directed nerve cords and one median nerve cord grafted in the reversed direction between them, their posterior ends formed separately three tiny blastemas at the cut ends of the three nerve cords and then the median blastema grew into a head and the lateral two into tails, while their anterior cut end developed one head (type 6). The last single case formed a head and a tail at the posterior end, perhaps because of the fact that the median and one lateral blastema fused together into one structure, which eventually became a head (type 7). In the fragments belonging to the control group where the median tissue strip containing no

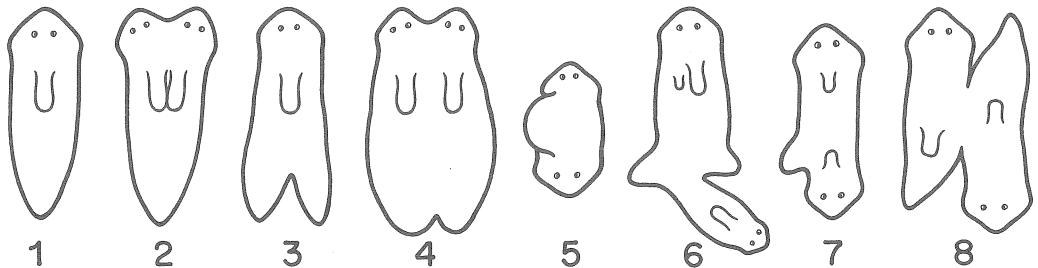


Fig. 2 Schematic representation of the types of regenerates (type 1-type 8) developed from the experimental and control groups of fragments.

nerve cord was rotated, all the regenerates showed the normal polarity by formation of a head at the anterior end and of a tail at the posterior, even though there were some cases which developed two structures from their cut end; six cases grew to be normal worms (type 1), two into regenerates with duplicate heads at the anterior end (type 2), three into duplicate tails at the posterior end (type 3) and two with anterior duplicate heads and posterior duplicate tails.

The pharynges formed in the old tissue of regenerates were made visible by the formic acid treatment. As shown in Figure 2, there was every indication that the developing pharynges, irrespective of the type of regenerates, were induced by regenerating heads, on one pharynx to one head basis, and their direction was oriented by the heads inducing them. Bipolar heads had quite incomplete pharynges or none at all. The regenerates of type 6 in which the duplicate pharynges were induced by one head developed from the anterior end, appear to be exceptional, but this may possibly be explained from the viewpoint that the anterior head was formed by fusion of two blastemas which were originally destined to develop into new heads.

Discussion

In our recent study (Kishida and Kurabuchi, 1978), we pointed out that the removal of the nerve cords stimulated the development of a bipolar head in a high proportion. This suggested that the nerve cord plays an important role in determining the differentiation of the regenerated blastema into either a head or tail. The results of the present investigation actually confirmed this supposition. Namely, about half of the members in the first group of fragments containing two nerve cords with opposite directions developed into the opposing doublet worms; the second group containing only one nerve cord with reversed polarity gave rise to typical bipolar heads; and the third group of fragments, which had a median reversed nerve cord and two intact nerve cords in the normal direction, developed a median head and two lateral tails at the posterior cut end of the fragment. Since the control fragments, whose median cordless strip was oriented reversely in relation to the antero-posterior axis, did not reversely in relation to the antero-posterior axis, did not show such polar abnormality, it is not surprising that the original anterior end of the nerve cord induced a head even at the posterior cut end of the fragment, and its original posterior cut end induced a tail at the anterior end of the fragment, though the latter effect did not appear so clearly as the former. This result is supported by the study done by Sugino in 1964. He undertook the experiment in which the ventral part containing a pair of nerve cords was taken off and fused onto the original wound with 180° rotation and the anterior end of the reversed ventral part was amputated. He obtained the result that a head regenerated at the anterior end of the reversed ventral part containing the nerve cord. It should be considered that the results of this study and those of Sugino's show the presence of biological polarity in the nerve cord which is capable of determining the direction of the blastema differentiation in the

regenerated planarians. Our earlier experiment (Kishida and Kurabuchi, 1978) suggested the existence of a head-inhibiting factor located at the nerve cord from the result that heads regenerated in a high percentage from the posterior cut end of the denervated fragments, as had been supposed in the experiment by Sperry et al. (1973) where the lateral cordless fragments regenerated into "head-hump syndrome". Biological polarity of the nerve cord which determines the polarity of the planarian regeneration, may represent the quantitative gradient of activity or an unknown substance connected with the head-inhibiting factor a great deal of which exists at the posterior end and decreases anteriorly, though one cannot assert it at present.

The strong tendency, however, to form a head at the anterior end of the fragment even in the presence of the original posterior cut end of the nerve cord, as is shown in the fact that a head appeared at the posterior end in only 4 cases of regeneration of type 8 among 18 successful fragments in all the experimental groups, cannot be explained by the existence of the biological polarity of the nerve cord alone. There must be thus other factors than the nerve cord which are effective in the determination of the regeneration blastema. Kanatani (1958, 1960) suggested that there is some physiological condition acting as a limiting factor to the realization of the determination of the blastema in his demecolchine treatment experiment, as did we, in our extirpation experiment of the nerve cords (1978). Such factors may include the respiration rate in the cut pieces of the worms as held by the quantitative physiological gradient theory of Child (1907, 1941) or a gradient of activity of the protein-synthesising mechanism in the body tissue as shown by Flickinger (1959) and others. However, the possibility that a gradient of respiration rate or activity of the protein-synthesising mechanism in the planarian body is controlled through the nerve cord cannot be denied either. On the other hand, Sauzin-Monnot (1972) indicated electron-microscopically that a large number of the neurosecretory granules of the nerve cord appeared at the anterior cut end. Marsh and Beams (1952) found that when the planarians settled in an electric field were isolated into fragments, they developed a bipolar head and, furthermore, there occurred the reversed polarity. Accordingly, we should think that polarity of the nerve cord might be related to the quantity of the neurosecretory substance or to the amount of the electric charge in the nerve cord. The identification of the factors for the blastema determination in the planarian regeneration is to be our further problem.

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