Induced segmental reorganization in sabellid worms

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SUMMARY

Processes of regeneration and reorganization are analyzed in two sabellid polychaetes. Abdominal pieces of Branchiomma nigromaculata, during head regeneration, ordinarily reorganize only a fraction of the number of segments typical of the thorax. In pieces transected in the vertical plane, but obliquely with regard to the main axis of the worm, the extent of reorganization is greatly enhanced. The same number of segments transform on the right and left sides, the surviving damaged segments transforming on one side and a corresponding number of more posterior, undamaged segments transforming on the other. Reorganization is shown, in abdominal pieces of Sabella melanostigma, to involve destruction and inversed dorso-ventral reconstitution of all parapodial structures in the segments affected, the conversion of intestine to thoracic stomach, and the invasion of previously abdominal segments by a pair of large nephridia which grow posteriorly after being formed in the basal portion of the head blastema. All three events exhibit a time-graded character, starting soonest at the anterior end and progressively later posteriorly, and apparently independently of one another. Abdominal type segments are formed only from the anterior region of the caudal, prepygidial zone of growth, successively, never by transformation. The dorso-ventrality of abdominal segments is the inverse of the thoracic, with the antero-posterior polarity unchanged, in all circumstances. Only during posterior regeneration from thoracic segments are thoracic segments produced from the posterior zone of growth, but only two or three are thus formed, the zone of growth then changing to the production of abdominal segments. It is concluded that the unique feature of sabellid-serpulid organization is the complete inversion of the dorso-ventrality of the posterior zone of growth as the result of emancipation from the generally dominating dorsal field emanating from the anterior end.

INTRODUCTION

Regeneration and segmental reorganization occur in challenging form in sabellid polychaetes. At all levels of the body, transversely cut pieces can regenerate a new head anteriorly and a new abdomen posteriorly. A head blastema forms as such independently of the character of the adjoining tissue and is evidently a self-organizing system, a term used by Coe (1934) to describe the regenerating head of a nemertean worm. It is strictly comparable to the ‘head-hump’ regenerate formed in nerveless, lateral fragments of the body of planarians (Sperry, Krystina & Tittel, 1973). It gives rise always to four head components: the tentacular crown (prostomium), collar (peristomium), atypical thoracic segment (partially fused to collar and bearing setae alone), and typical thoracic segment (with dorsal setae and ventral hooks) (Fig. 1). New thoracic structure results from reorganization of the more anterior abdominal segments already present, rarely by regeneration. The reorganization proceeds segment by segment commencing at the anterior end.

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Reorganization is seen externally to consist of destruction of abdominal parapodial structure (dorsal bristle bundle and ventral row of hooks) and its replacement with thoracic parapodial structure (dorsal row of hooks and ventral bristle bundle), the one being an inversion of the other. The present account relates to restriction and extension of the reorganizing influence, to thoracic reorganization of internal structure, and to formation of new abdominal segments at the caudal end of a regenerating abdomen. The underlying concept is of two ends of a worm in contention morphogenetically with one another (Abeloos, 1965).

Functional, developmental, and phylogenetic aspects of these features have been discussed elsewhere (Berrill, 1977).

MATERIAL AND METHODS

This study is based mainly on two contrasting species, Sabella melanostigma Schmarda and Branchiomma (Dasychone) nigromaculata Baird. Live specimens of both species were obtained from the Florida Keys, while Branchiomma nigromaculata was also found in abundance in Kaneohe Bay, Oahu, Hawaii. Sabella melanostigma is unusual in having from 12 to 20 thoracic segments, rather than the usual 7, and this number is restored during reorganization. Moreover, small individuals are comparatively transparent when examined by transmitted light with a low power microscope, so that changes in internal structure during reorganization can be followed. Branchiomma nigromaculata, while having the more typical number of thoracic segments, usually undergoes reorganization in only one or two anterior abdominal segments during regeneration of a head, although occasionally more. This restriction is experimentally modifiable.

Experiments consisted of transecting individuals at various levels along the antero-posterior axis in Sabella melanostigma, and at various angles to the axis in Branchiomma nigromaculata. Healed and regenerating pieces were kept in covered dishes containing sea water of only a few millimeters depth at 23–24 °C, the water having been obtained from the same locale as the worms, respectively. Specimens were examined regularly throughout periods of 3–4 weeks.

RESULTS

Comparison of thoracic and abdominal segments

Comparison of the parapodial pattern in thoracic and abdominal segments shows that the setigerous bulbs and hook-bearing tori are dorsal and ventral in thoracic segments, but are ventral and dorsal in abdominal segments (Fig. 1A) respectively. One is an inversion of the other, but not a reversion, since the hooks, wherever they may be, all point anteriorly, in all segments throughout the worm. There is an inversion of the dorso-ventral polarity but no change in the antero-posterior polarity.
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Fig. 1. Normal and regenerated structure of anterior end of Branchiomma nigromaculata. (A) Lateral view of thorax, including collar and anterior abdominal segments, from left side, showing continuous serial location of ocelli, reversal of parapodial organization in thoracic compared with abdominal segments, and antero-posterior gradation in size and hook number of thoracic tori. (B) regeneration stage at which all four antero-posterior components of developing head are recognizable, namely, crown, collar and two thoracic segments. c, Collar; cr, crown; oc, ocellus; pth, prethoracic segment; rp, palp; sb, setigerous bulb; ta, thoracic-abdominal junction; th, thoracic segment; tt, thoracic torus; vs, ventral shield.

Reorganization

When abdominal pieces of Branchiomma nigromaculata are isolated by transverse cuts, typical head and tail regenerates are produced but reorganization of the anterior abdominal segments in each piece is in most instances limited to one to three (Fig. 2A). No consistent differences are seen in either the
capacity for head regeneration or the extent of parapodial reorganization among pieces from successive levels along the antero-posterior axis. Significantly, regeneration occasionally occurs without any accompanying reorganization, while, conversely, reorganization may occur without any anterior regeneration.

Reorganization accompanying regeneration from anterior surfaces of ab-
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dominal pieces cut at various degrees and planes of obliqueness is surprisingly
different from reorganization associated with exactly transverse cuts.

Blastema formation and development proceed as in cases where the cut
surface is strictly transverse, but the extent and location of reorganization
varies with the plane and degree of obliqueness of the cut.

Category I. Reorganization following oblique cuts made in the vertical plane
with reference to the dorso-ventral axis, with various degrees of obliqueness
with regard to the bilateral organization of the worm, cuts which leave partial
segments on one side anterior to the mid-region of the cut, those of the other
side usually becoming resorbed (Fig. 2B-D). Reorganization is described with
reference to the location of the most anterior original segment to remain intact,
which is the first segment behind the most posterior edge of the oblique cut.
If this segment is taken as a transverse base line, reorganization in most cases is
seen to involve the same number of segments on the right and left sides, but
the segments affected are not the same on the two sides. If the oblique cut is
from right to left passing posteriorly, all the partial segments on the right side,
*anterior* to the base line, become thoracic. On the other side, a corresponding
number of segments *posterior* to the base line, which have not been damaged,
reorganize to become thoracic, each such segment remaining abdominal in its
opposite half. Left to right cuts of the same kind have the same result, apart
from being a mirror image. The above statement holds for all degrees of oblique­
ness, that is, if five to eight partial segments remain on one side anterior to the
base segment, five to eight more segments transform behind the base segment,
on the opposite side. Even if ten or more partial segments are left anteriorly on
one side, the same number are reorganized on the opposite side, posterior to
the base line (Fig. 2D).

Category II. Reorganization following horizontally oblique cuts made ventro­
dorsally, that is, cuts which remove more dorsal tissue than ventral, leaving the
ventral nerve cord intact in the anterior segments. Restitution of the damaged
segments occurs on both sides, but in most cases the abdominal character is
retained (Fig. 2E).

Category III. Reorganization following horizontally oblique cuts made
dorso-ventrally, cuts which remove more ventral tissue than dorsal, thus re­
moving the anterior nerve cord and, to a lesser extent, some of the intestine
from the most anterior segment of the surviving piece of worm. Reorganization
of segment from abdominal to thoracic character typically occurs in both sides
equally, and as far posteriorly as the posterior limit of the cut on the ventral
side (Fig. 2F). This can be interpreted as the result of elimination of the ventral
cord in these segments.

Two general observations can be made: (a) where oblique sectioning severely
damages or eliminates the nerve cord or segmental nerves, on one side, re­
organization of abdominal to thoracic structure occurs in the surviving parts
of the damaged segments. When the nerve cord remains intact, on the other side,
reorganization usually does not occur. (b) The reorganization always occurs, in all the above circumstances, as a bilaterally symmetrical extension of influence from the initial center of blastema formation, thereby including as much undamaged territory on one side as the damaged tissue on the other.

In experiments on *Sabella melanostigma* (Sperry, 1971, unpublished), the nerve cord was removed from 12-segment abdominal pieces by stripping, with sham experiments as controls. Segments, in every case, reorganized from abdominal to thoracic character, more or less simultaneously, and precociously. In all cases a head blastema formed at the anterior end and, in 50%, at the posterior end. It may be formed also from the posterior surface of abdominal segment pieces, that have been exposed for several days to sea water containing colchicine (Fitzharris & Lesh, 1969), in which the ventral nerve cord has become disorganized.

*Reorganization: extension of head field in Sabella melanostigma*

Abdominal pieces of individuals, 20 to 30 segments long, regenerate a new head and at the same time undergo extensive reorganization, in accordance with the comparatively large number of thoracic segments (Fig. 3A). The total time taken for complete reorganization in each such segment appears to be virtually the same in all. The morphological gradient reflects a temporal sequence *in the time at which the process begins* in the successive segments (Table 1).

An examination of young, living individuals of *Sabella melanostigma* shows a general relationship between external and internal features associated with 'thorax' and distinct from 'abdomen'. Internally, the intestine is seen to consist of three regions: an anterior oesophagus extending posteriorly from the mouth to about midway through the second fully thoracic segment, where a constriction occurs; a somewhat wider 'stomach' extending from the oesophageal junction to the thoraco-abdominal junction as indicated by parapodial inversion; and a relatively long, narrow intestine extending to the anus at the pygidium.

The thorax accordingly consists of co-extensive body wall and digestive canal structure. It also contains a pair of very large nephridial sacs which open together at the base of the collar and separately extend posteriorly in most individuals to the end of the 10th thoracic segment, rarely, if ever, to the posterior limit of the exceptionally long thorax.

Coinciding with the sequential conversion of parapodia from abdominal to thoracic type, the intestine enlarges from abdominal to thoracic dimensions in conformance with this posterior extension (Fig. 3B–D). The transformation process of the intestine also exhibits a temporal gradient, inasmuch as the region of the intestine involved is seen to taper from maximum width anteriorly to minimum width (original intestine) posteriorly, although it eventually becomes equally wide throughout. The antero-posterior length of the taper is short at first and slowly increases, in conformance with the progressively
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Fig. 3. Reorganization during anterior regeneration in *Sabella melanostigma*. (A) Gradient in onset of reorganization in series of thoracic segments. (B–D) Three stages of posterior extension of thoracic conversion of intestine and of pair of giant nephridia. (E) Diagram of reorganizing influence of anterior regenerating tissue through all body layers. _abd_, Unchanged abdominal segment; _d_, degenerating setigerous bulb; _ect_, ectodermal; _end_, endodermal; _gt_, thoracic gut; _mes_, mesodermal; _n_, nephridia; _nsb_, new setigerous bulb (setae); _nt_, new torus (hooks); _rhd_, regenerating head; short arrows indicate limit of transforming influence; dotted outline indicates regenerated structure, arrows indicate posterior limit of thoracic reorganization.
increasing posterior extent of the segmental parapodial reorganization. By the time the head blastema is full grown and differentiated, apart from further growth following functional completion, the new thoracic gut has attained full width throughout.

The formation and growth of the pair of large nephridial sacs are also seen during the reorganization period. The primary phenomenon here is the invasion of old structure by a component of the new anterior regenerate. The pair of nephridial sacs originate in the basal region of the developing blastema, in the regenerating thoracic-type segment adjoining the regenerating collar. They already extend into the most anterior one or two old abdominal segments at the regenerate stage shown in Fig. 3B. As the parapodial and intestinal transformation proceeds posteriorly, the pair of sacs also extend posteriorly, necessarily involving dissolution of septal membranes before them. This extension, however, cannot begin until the first setigerous segment of the new head is well formed.

Nephridial restoration usually falls short of the maximum extension seen in intact worms, in most cases extending through six to eight segments. In only one instance the nephridia extended through ten segments, the normal maximum, while in some others extension was four or five, and in two cases none (after 3 weeks), although parapodial reorganization and intestinal transformation involved 12 and 14 segments respectively.

Evidently, the transformation of anterior abdominal segments, during the process of regeneration of a new head, results from an influence extending posteriorly from the regenerating tissue through the adjoining abdominal segment, which affects epidermal structures, adjacent parapodial muscles, digestive canal, and nephridial growth. In other words, the anterior regenerate, during its own most formative period, has the property of a dominant morphogenetic field which extends progressively posteriorly through tissues of ectodermal, endodermal and mesodermal layers alike (Fig. 3E).

**Posterior regeneration**

Posterior regeneration in sabellids can occur from any level of the body with the exception of the most anterior level of the thorax. Cut epidermal and endodermal tissues fuse directly with each other, thereby restoring the anus
Fig. 4. Segment formation and growth during caudal regeneration. (A) Piece of abdominal region regenerating anteriorly a bilobed blastema, and posteriorly a bilobed pygidium together with extension of the intestine and initial mesodermal segmentation. (B) Lateral view of caudal extremity of a young regenerate showing pygidium of one side together with zone of growth and a delimiting segment, together with a number of differentiating and growing segments; (C) Lateral view of posterior regeneration from an original thoracic region, showing three regenerated thoracic segments and about 15 abdominal segments, with terminal pygidium. b, Bilobed anterior blastema; hk, hooks; int, intestine; ns, nest of hooks; p, pigment patch (or ocellus); py, pygidium; s, setae; sm, segmented mesoderm; tr, original thoracic segments; tr, regenerated thoracic segments; z, zone of growth (shaded).

immediately (Hill, 1970), and a small 'primary' blastema develops to form the bilobed pygidium, which is a well defined, postsegmental structure around the anus, as distinctive of caudal morphology as the presegmental prostomium and peristomium are of the head. Almost immediately, in regeneration from abdominal segments, the posterior blastema as a whole grows from the adjoining
old segment as a cone in which a slender new intestine extends from the old intestine to the anus (Fig. 4A). Extirpation of the posterior region of the intestine in the most posterior abdominal segments of an isolated piece either inhibits or greatly delays any posterior outgrowth. In typical posterior regeneration, the extension of the intestine supplies the necessary thrust upon which the posterior extension as a whole depends.

Mesoderm is initially seen as a thin strand along each side of the extending new intestine, a strand which very quickly exhibits division into six to eight segmental units. Additional mesodermal segments are added sequentially at a slowing rate from a zone of growth adjoining the pygidium. This zone of growth is a narrow transverse band of mesodermal tissue that grows posteriorly, as the intestine extends, and at the same time delineates one new segment after another from its anterior region (Fig. 4B).

In the series illustrated (Fig. 4B), the barely discernible zone of growth has no visible features. The youngest segment, adjoining it, has a minute pigment spot, in the mid-lateral region, which increases in size anteriorly throughout the segmental series in both Sabella melanostigma and Branchiomma nigromaculata, as pigment patch and ocellus respectively. In the next two older segments, a single recognizable hook lies dorsal to the pigment spot.

With subsequent growth of each segment, additional hooks appear successively adjacent to the median pigment spot, so that a row forms dorsally. At the same time, a setigerous bundle appears ventral to the median spot. The abdominal pattern of the segment is thus established.

The overall character of the abdominal segments, forming successively as a segregating anterior portion of the zone of growth, accordingly is imprinted with its distinctive pattern at the time of its initiation. Here is the crux of the problem. The production of new segments from a posteriorly located growth zone is basically the same as in all polychaetes. The unique abdominal feature is that from the time of initiation of such a segment, the pattern of segmental epidermal structure in sabellids (and the closely related serpulids) is inverted compared to that of all other polychaetes. However, caudally produced segments can be thoracic in character. This occurs when posterior regeneration originates from the posterior cut surface of a piece consisting entirely of thoracic segments. The first two or three segments regenerated posteriorly from such pieces are frequently thoracic in character (Fig. 4C), followed by regenerated abdominal segments, although all the posteriorly regenerated segments are produced by successive delimitations of the anterior portion of the zone of growth.

Accordingly, the direction of dorso-ventral polarity of segments, and therefore that of the zone of growth responsible for this direction, is determined by one of two opposing agencies. The abdominal type is associated with a relatively rapid outgrowth of the prepygidial intestine, associated with an abdominal segment as point of origin. The thoracic type is associated with a relatively
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delayed initial outgrowth of the intestine, together with association with a thoracic segment as point of origin.

DISCUSSION

The experimental results reported here show that thoracic organization extends posteriorly under the influence of the head, that it is modifiable, that individual segments can unilaterally change from abdominal to thoracic character but never from thoracic to abdominal, and that abdominal type segments form only from the anterior region of a posteriorly growing zone of growth. The picture emerging is of two ends of the worm in contention, a concept which Abeloos (1965, and earlier) has described as two ends in opposition with a neutral zone somewhere in the middle.

Earlier experiments on Sabella pavonina demonstrated that additional posterior extension of thoracic segmental character, imposed on further abdominal segments, follows repeated regeneration of head tissue, or during exposure to augmented visible light (Berrill & Mees, 1936a, b).

The modifiability of the extent of the dorsal field, from restriction in Branchiomma nigromaculata to a tenfold extension in Sabella pavonina (Berrill, 1931), suggests that this dorsal field may normally be comparatively weak, and is ineffective at the site of the caudal zone of growth.

The fact that the production of thoracic type segments from the posterior zone of growth occurs at first, and is quickly followed by production of abdominal type segments when a dorsal field is initially present, as in the case of caudal regeneration from a posterior thoracic surface, supports this contention. Moreover, the fact that reorganization to thoracic character immediately follows removal of the ventral nerve cord indicates that the cord and segmental nerves control at least the epidermal ventro-dorsal polarity.

REFERENCES


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