

Role of Heterotypic Tissue Interactions in Deer Pedicle and First Antler Formation—Revealed via a Membrane Insertion Approach

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ABSTRACT Heterotypic tissue interactions play an indispensable role in organ generation and regeneration. In contrast to the classic examples of tissue interactions prevailing in the formation of tetrapod limbs or pectoral fins that can only take place when the interactive tissues are in intimate contacts, the interactions in deer antler formation are novel in that the inducer and the responder are separated by a distance of 1–2 mm. This feature offers a unique opportunity to explore the mechanism underlying tissue interactions by permitting membrane insertion between the two interactive tissues. Four experiments were conducted in this study. The results showed that the impermeable membranes inhibited antler formation. In contrast, the permeable membrane (0.45 μ m in pore size) substantially slowed pedicle growth and antler initiation but did not stop them. Interestingly, the impermeable membrane/sheath only slightly retarded antler elongation. Overall, our results demonstrate that interactions between the two interactive tissues, antlerogenic tissue and the overlying skin, are indispensable for first antler initiation and are achieved through diffusible molecules rather than direct physical contact. As the heterotypic tissue interactions are only required during antler initiation but not elongation, they must be transient in nature, and thus differ from those operating in limb/fin formation that can only be sustained by continuous interactions. A system in which organ development is achieved only through transient tissue interactions must be novel, if not completely unique. Understanding this system will undoubtedly enrich the knowledge in the field of tissue interactions and organogenesis. *J. Exp. Zool. (Mol. Dev. Evol.)* 310B:267–277, 2008. © 2008 Wiley-Liss, Inc.

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Heterotypic tissue interactions play a crucial role in tissue/organ formation (Gilbert, 2003) and regeneration (Stocum, 2006). Practical model systems in which effects can be readily observed by interruption of these interactions are needed if the underlying mechanisms are to be understood. In this case, formation of the initial deer antler offers a prime example. The heterotypic tissue interactions in antler formation take place in postnatal life which is readily accessible for manipulation and evaluation; the distance between the two interacting tissue types is over 1 mm, an exceedingly long range in histological terms and hence possible to position barriers between tissue types, and yet potent enough to

trigger antler formation and transform the adult scalp skin epidermis into a unique pelage, the antler velvet (Li and Suttie, 2000).

Deer antlers are bony organs that are cast and fully regenerate each year from permanent bony protuberances on the frontal bone of a deer skull called pedicles. Pedicles start to develop when

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male deer approach puberty under the stimulation of androgen hormones (Li et al., 2003). When pedicles reach their species-specific height (about 5–6 cm high in red deer), first antlers generate spontaneously from apices of the pedicles (Li and Suttie, '94). It has been convincingly demonstrated by a combination of tissue deletion and transplantation studies that the antlerogenic potential is exclusively held in the periosteum overlying deer frontal crests. Removal of the periosteum abrogates future pedicle and antler formation from the original region; transplantation of the periosteum elsewhere on the deer body induces ectopic pedicle and antler to grow (Hartwig and Schrudde, '74; Goss and Powel, '85; Li and Suttie, 2001). However, transplantation of the skin overlying the periosteum elsewhere on the deer body fails to yield an ectopic pedicle and antler even if the grafted skin survives for many years (Goss, '72). Therefore, this periosteum has been termed "antlerogenic periosteum" (AP).

First antler transformation from a pedicle is considered a unique zoological phenomenon (Goss, '83), because although pedicles are permanent, antlers are deciduous. Thus far, the process of this transformation is not well understood. Through AP transplantation experiments, Goss ('87) found that ectopic antlers cannot be induced unless the antlerogenic tissue derived from the grafted AP becomes closely associated with the overlying skin, which led Goss to conclude that the close association is indispensable for first antler formation (Goss, '90). The results from detailed histological analysis of normal pedicle formation and transformation into first antlers (Li and Suttie, 2000) support this claim. However, experiments specifically designed to investigate the nature of these interactions, i.e., through physical contact or diffusible molecules, during first antler generation were, thus far, lacking.

The aim of this study was to take the approach of inserting permeable or impermeable membrane into the space between antlerogenic tissue and the overlying skin to determine whether (1) interactions between these two tissue types were realized through physical contact or diffusible molecules; (2) these interactions were continuous or transient in nature throughout the entire growing phase of first antler formation.

MATERIALS AND METHODS

This study consists of four experiments, which are presented in a chronological order. The first

three experiments were undertaken to determine whether pedicle/antler generation depends on the interactions between AP and the overlying skin, and if so, by what pathway (physical contact or diffusible molecules). Three experiments were conducted to achieve this aim only because the successful idea was evolved step by step. The fourth experiment was carried out to determine whether these interactions were still required following first antler initiation.

Each experiment had full approval from our AgResearch Invermay Animal Ethics Committee. Experimental surgery in these experiments was conducted under general anesthesia using intravenous fentanyl citrate/azaperone/xylazine hydrochloride (Fentazin 5, Parnell Laboratories Ltd., Auckland, New Zealand) at a dose rate of 1.8 mL/100 kg live weight. Frontal crest/pedicle on one side of each animal was randomly chosen for membrane/sheath insertion, whereas the contralateral side was used as the sham-operated control. Each operated site was thoroughly shaved, and then sterilized with 70% alcohol and 1% iodine tincture. All the skin incisions were closed using non-absorbable suture (2-0 black braided silk, Ethicon, NJ). The anesthesia was reversed with naloxone/yohimbine (Contran H, Parnell Laboratories Ltd., Auckland, New Zealand). Long-acting Penstrep LA (BOMAC Laboratories Ltd., Auckland, New Zealand) was injected subcutaneously following each surgery. Animals from each experiment were observed daily for 7 days after surgery, weekly thereafter, and photographed when necessary.

Experiments 1 and 2

Eight 8-month-old red deer (*Cervus elaphus*) stags were selected for each experiment. In Experiment 1, the deer were randomly allocated into two groups: Group 1 for insertion of impermeable membrane (Fig. 1A; surgical grade latex rubber sheet, 47 mm in diameter, 0.25 mm in thickness), and Group 2 for insertion of permeable membrane (LCR membrane, hydrophilized polytetrafluoroethylene (PTFE), 47 mm in diameter, 0.45 μ m pore size, Millipore). Experiment 2 was essentially the same as Experiment 1, but with three modifications. (1) An "O" ring was attached to each piece of membrane before surgical insertion in order to prevent the inserted membranes from being folded and/or pushed away. (2) Stags having just initiated pedicle growth were used, rather than before pedicle growth as in Experiment 1. The pedicles were 15–20 mm high when

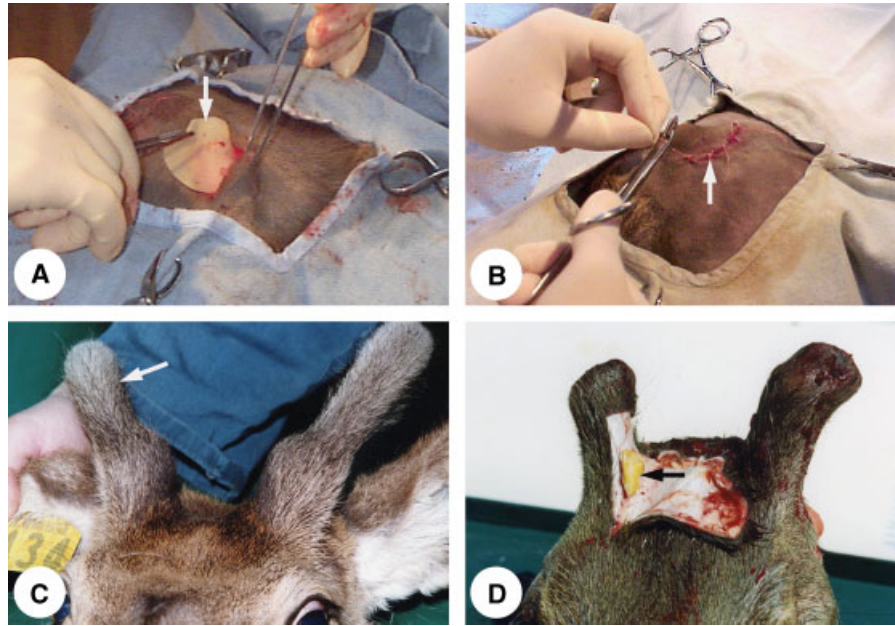


Fig. 1. Experiment 1: (A and B): membrane insertion surgery on 8-month-old red deer stags. (A) A piece of impermeable latex membrane (arrow) was placed on an exposed frontal crest. (B) Skin incision was closed with silk suture. (C) Pedicle and antler formation from the membrane-inserted and sham-operated pedicle growth regions. Notice that antler (arrow) formed from the membrane-inserted side is somewhat shorter than that of sham-operated side. (D) Dissection at the surgery site to expose the inserted membrane. Notice that the membrane (arrow) had been displaced from pedicle growth direction, folded, and rested on the lower part of the pedicle shaft.

the stags were selected, with these early pedicle bumps being useful as anchor points for the “O” ring-attached membranes (Fig. 2A and B). (3) A membrane-free “O” ring was inserted into the other side to serve as a sham-operated control. The “O” rings used in this experiment were made of stainless steel with 30 mm inner diameter, 1 mm wall thickness, and 3 mm height. A 0.5 mm deep groove was cut on the outer surface of each ring for firmly attaching the membrane.

Membrane insertion surgery was carried out before (Experiment 1) or just started (Experiment 2) pedicle initiation following the procedure reported by Li and Suttie (2003). A piece of membrane with or without an “O” ring was directly placed on top of each frontal crest using the crest’s posterior end as the central point. The wounds were then sutured (Fig. 1B). The same technique was applied to the contralateral side, but without inserting a membrane (Experiment 1), or with “O” ring insertion only. The experiments were terminated when formation of first antlers could be satisfactorily visualized.

Experiment 3

This experiment was conducted owing to the failure in the maintenance of the inserted im-

permeable membranes in place in Experiments 1 and 2. The problems encountered in the first two experiments were considered mainly owing to the angled topology of the presumptive region of pedicle growth, and the widespread and uneven distribution of antlerogenic potential in the region, with the lateral side having the greatest potential (Goss, '61). If these assumptions were correct, we reasoned that a membrane insertion experiment could be done successfully if the pedicle/antler growth region had a flat surface. To test this, we subcutaneously transplanted AP from its original site (angled topology; Fig. 3A) onto the deer forehead region (flat area; Fig. 3B). At the same time an “O” ring-attached impermeable or permeable PTFE membranes were inserted directly over the grafted AP. In this experiment, the membrane was tied onto an “O” ring to such a degree that, on the one hand, membrane was firmly held onto, and on the other hand, could be separated from the attached “O” ring without breaking it by the expanding tissue mass pushing upwards from the underneath.

Nine 8-month-old sika deer (*Cervus nippon*) stags were selected for this experiment. The reason for using sika deer is that sika antler velvet is shiner and has more sparsely populated hairs than that of red deer. These features are

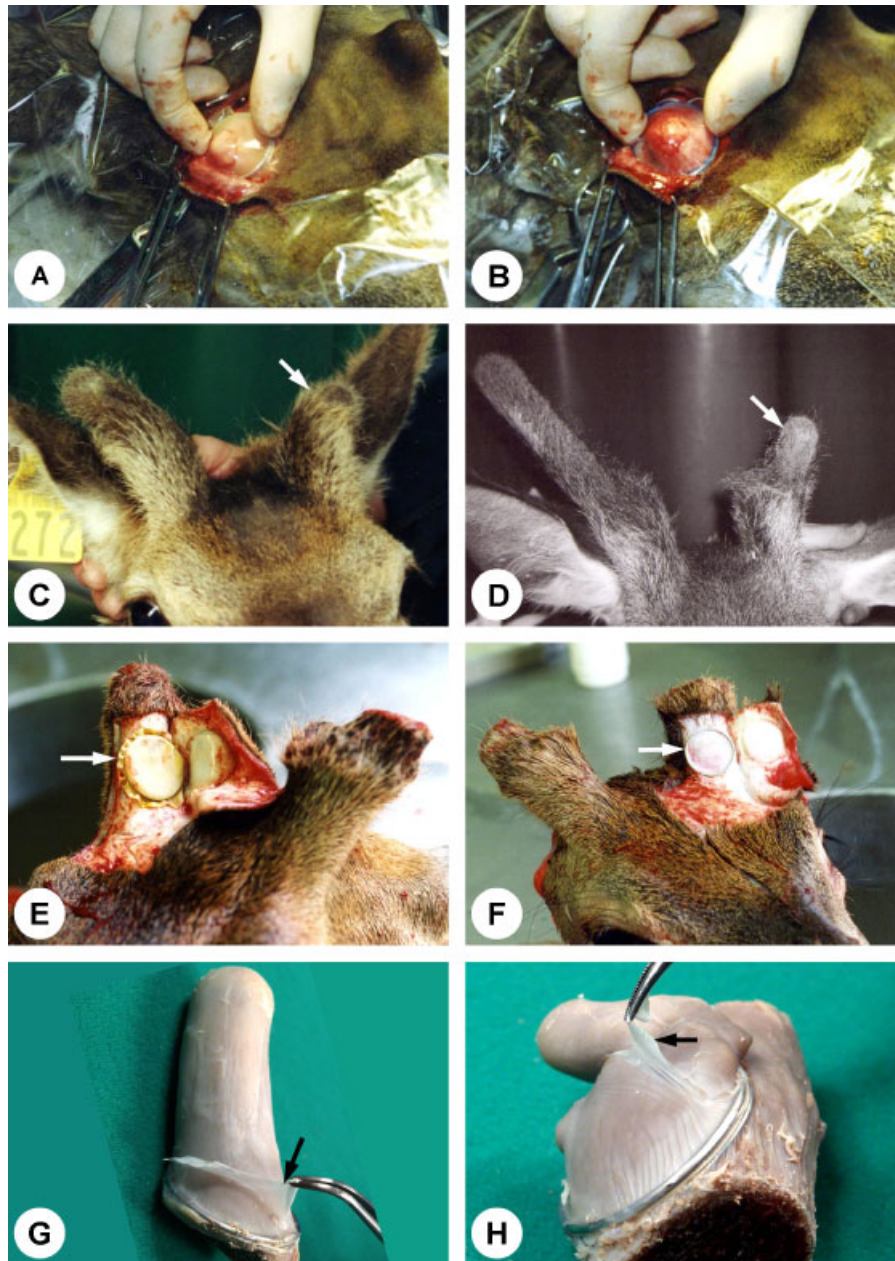


Fig. 2. Experiment 2: (A and B): membrane insertion surgery on 8-month-old red deer stags. A piece of “O” ring-attached impermeable latex membrane (A) and permeable PTFE membrane (B) were placed on an exposed frontal crest, respectively. (C and D) Pedicle and antler formation from the membrane-inserted (arrow) and sham-operated pedicle growth regions. Notice that antler formed from either impermeable (C) or permeable (D) membrane-inserted side is substantially shorter than that of its corresponding sham-operated side. (E and F) Dissection at either impermeable (E) or permeable (F) membrane insertion site to expose the inserted membrane. Notice that both types of “O” ring-attached membrane (arrow) had been displaced from pedicle growth direction and were found on the middle part of the pedicle shaft. (G) Antler that directly grew through an “O” ring-attached PTFE permeable membrane by breaking it (arrow). (H) Antler that had its growing tip been split into two uneven parts by the edge of the inserted ring. Notice that the bigger part grew laterally away from the membrane and the small part medially by breaking through the membrane (arrow). PTFE, polytetrafluoroethylene.

more helpful for the visualization of the transformation from scalp skin to antler velvet. Moreover, as the sika deer we selected for the experiment lived in Northern Hemisphere, we could com-

mence the experiment half a year earlier, hence advance the progress of the study. Most importantly, there is essentially no difference in the ability to induce ectopic antler formation by

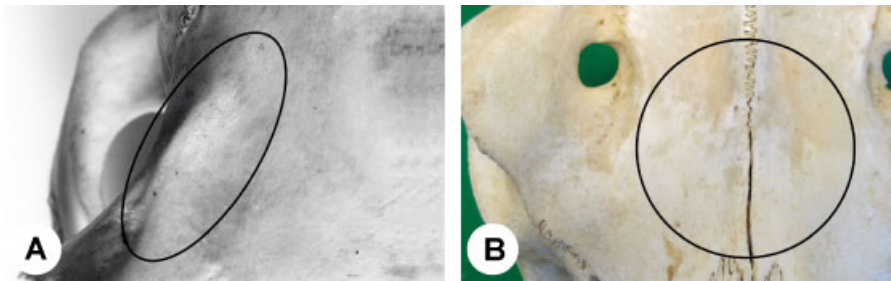


Fig. 3. Topology of the presumptive pedicle growth region (A) and the forehead region (B) to which antlerogenic periosteum and PTFE membrane were transplanted. Notice that the pedicle growth region has uneven surface (circle) and the forehead region has flat surface (circle). PTFE, polytetrafluoroethylene.

transplanted AP between red deer and sika deer (Li and Suttie, 2001, unpublished data). The sika deer were randomly allocated into three groups: Group 1 for the insertion of the “O” ring (same as in Experiment 2)-attached impermeable PTFE membrane (3 inch wide PTFE membrane, Alltech, Auckland, New Zealand), which was selected to replace the impermeable rubber membrane used in Experiments 1 and 2, as PTFE material is more compatible with implantation; Group 2 for the insertion of the “O” ring-attached PTFE permeable membrane (same as in Experiment 2); and Group 3 for the insertion of the “O” ring alone without membrane.

Surgeries for AP transplantation and membrane insertion were carried out before pedicle initiation (with 5–10 mm high frontal crests). Following the procedure used in Experiment 1, the frontal crest of each future pedicle growth region was exposed. AP (25 mm in diameter) was then peeled from the underlying bone following the procedure described by Li and Suttie (2003). For transplantation surgery, a 20 mm skin incision was made coronally on the deer forehead region along the line between the two eyes. A subcutaneous pocket was made anteriorly through the incision by blunt dissection, into which a piece of peeled AP was inserted (Fig. 4A). Before suturing the skin incision, either an “O” ring-attached impermeable membrane (Group 1), an “O” ring-attached permeable membrane (Group 2), or a membrane-free “O” ring (Group 3) was inserted into each pocket and directly placed over each transplanted AP (Fig. 4B).

Experiment 4

Twelve 8-month-old red deer (*C. elaphus*) stags were selected for this experiment. The deer were randomly allocated into three groups: Group 1 for the insertion of the impermeable PTFE membrane (same as in Experiment 3); Group 2 for the

insertion of the PTFE permeable membrane (same as in Experiment 1); and Group 3 for the insertion of the impermeable rubber sheath (surgical grade latex, 0.25 mm in thickness). An extra group deer was used to have right-shaped rubber sheath insertion, because we could not obtain right-shaped PTFE sheath. By so doing, we would double the chance of success, if the inserted PTFE membrane was displaced during the course of antler growth. One of the deer from Group 3 died from an accident 4 days after surgery, and was excluded from the experiment.

Membrane/sheath insertion surgery was carried out when pedicles of the experimental animals had grown to around 35 mm height, at which point first antler transformation from a pedicle has taken place in red deer (Li and Suttie, '94). Following the procedure used for sampling pedicle apical perichondrium (Li and Suttie, 2003), the apex of each pedicle bone was exposed. Either an impermeable PTFE membrane (Fig. 5A), permeable PTFE membrane (Fig. 5D), or impermeable rubber sheath (Fig. 5G) was capped onto each growing pedicle tip and the skin incisions closed. In order to hold the impermeable PTFE membrane in place (slippery surface), a PTFE thread was used for tying each membrane around the pedicle shaft (Fig. 5A).

RESULTS

Experiments 1 and 2

Wounds healed around 7 days after surgery for both the membrane (impermeable and permeable) inserted (with or without “O” ring attachment) and the sham-operated presumptive pedicle growth sides. No difference in the rate of healing was observed between the two sides. There was no obvious difference in timing of subsequent pedicle and first antler initiation between the membrane-inserted and the sham-operated sides. In

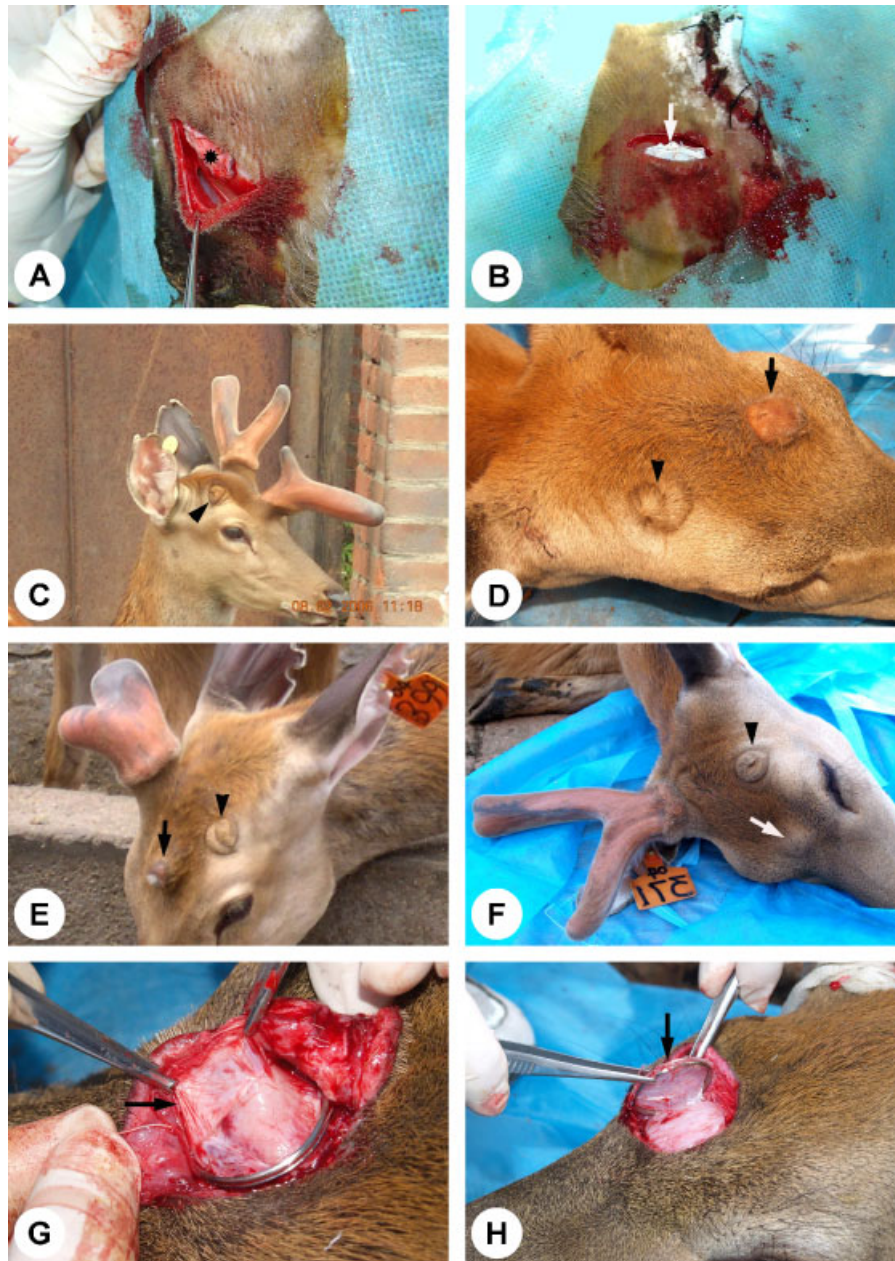


Fig. 4. Experiment 3: (A and B): membrane insertion surgery on 8-month-old sika deer stags. (A) A piece of antlerogenic periosteum (AP, asterisk) was inserted into a skin pocket made on the deer forehead. (B) An “O”-ring-attached permeable PTFE membrane (arrow) was placed over the inserted AP. (C) Pedicle and antler formation from the control group (transplanted AP+membrane-free “O” ring) in the second year. Notice that a two-branched ectopic antler was generated from the surgery site, but no pedicle or antler tissue was formed from the original pedicle growth region (arrowhead), from which AP had been removed. (D–F) Dome-shaped bumps (arrow). Each was formed on a deer forehead, onto which AP+permeable (D and E) or impermeable (F) membrane had been transplanted. Notice that the skin of the bumps in the permeable membrane group had transformed into the typical sika antler velvet, but in the impermeable group still remains in the scalp skin feature. No pedicle or antler tissue was formed from the original pedicle growth regions (arrowhead), from which AP had been removed. (G and H) Dissection on the dome-shaped bumps formed from either permeable (G) or impermeable (H) membrane implantation groups. Notice that these PTFE membranes (arrow) were all intact after 2 years implantation. PTFE, polytetrafluoroethylene.

Experiment 1, antlers formed from the membrane-inserted sites were visibly shorter than those from the control sides (Fig. 1C). In Experiment 2,

growth of the first three pedicles from the permeable membrane-inserted sides was substantially retarded compared to the control sides (Fig. 2C

and D). This was not seen in the animal in which the membrane had been totally displaced, which was identified by "O" ring palpation.

The experiments were terminated either at 140 days (Experiment 1) or 95 days (Experiment 2) after membrane insertion surgery when the last pedicle in the experiment had visibly transformed into antler tissue. The experimental animals were slaughtered for postmortem examination. Dissection at the membrane insertion sites on each recovered deer head revealed that in Experiment 1, eight out of eight inserted membranes (four impermeable and four permeable) were displaced medially by the upwardly growing bony pedicle. Each membrane was found folded and moved to the lower part of the medial surface of each pedicle bone shaft (Fig. 1D). In Experiment 2, all the "O" ring-attached impermeable membranes were found in the middle region of the medial surface of each pedicle shaft (Fig. 2E). For the "O" ring-attached permeable membranes, one was located in the middle region of the medial-anterior surface of the pedicle shaft (Fig. 2F) as had happened for the impermeable membrane group. One remained at the original insertion site with the pedicle bone growing directly through it by breaking the membrane (Fig. 2G). Two were partially tilted in the medial direction and had the lateral ring edge resting on each pedicle apex. As a result, each ring edge had split each growing pedicle tip into two unequal parts: the larger part grew laterally away from the membrane and the smaller part grew medially by breaking through the membrane (Fig. 2H).

Experiment 3

Wound healing of all the skin incisions was completed around 10 days after surgery and no obvious difference was observed between groups or treatments. In the control group (AP+"O" ring only), spike antlers were formed from the AP-grafted sites in the first year and two-branched antlers (Fig. 4C) in the second year.

In the permeable PTFE membrane insertion group, each AP-grafted site ("O" ring+permeable membrane) formed a small dome-shaped bump in the first year, which was covered with typical scalp skin. In the second year, the overlying skin was transformed into typical velvet either totally (Fig. 4D) or partially (Fig. 4E). In the impermeable membrane insertion group, each AP-grafted site formed a small dome-shaped bulge, which was covered with normal scalp skin in the first year. In

the second year, no obvious change was visualized in either the skin type or the bulge size (Fig. 4F) compared with those in the first year. No antler tissue was formed from the AP-deleted original pedicle growth regions in either the permeable or impermeable group. However, one of three deer grew a small spike antler in the second year from the original pedicle growth region from which AP had been removed.

The experiment was terminated 912 days (August, in Northern hemisphere) after the membrane insertion surgery. Dissection of the membrane insertion sites of each deer head showed that both inserted permeable (Fig. 4G) and impermeable (Fig. 4H) membranes were intact, although the permeable membranes had been partially lifted from the "O" rings (Fig. 4G) by the underlying expanding tissue mass.

This experiment showed that interactions between AP and the overlying skin were critical for first antler formation, as insertion of an impermeable membrane could abrogate the process, and that these interactions might be achieved through diffusible molecules, as a permeable membrane could only retard the process, but could not stop it.

Experiment 4

Wound healing was completed around 10 days after membrane insertion surgery. No obvious difference in wound healing rate was detected among three groups and between treatment and sham-operated sides. Antler growth from the membrane/sheath-treated pedicles was impaired compared with that from the control pedicles (Fig. 5B, E, and H). At the end of this experiment, antlers formed from the treated pedicles in most cases were substantially shorter than those from the control sides, except for one from the rubber sheath-treated group, in which the treated and sham-operated antlers reached the same length. The data from the rubber sheath insertion group are presented in Table 1. Statistic analysis of the tabulated data by paired *t*-test showed that there was no significant difference in antler growth rate ($P = 0.258$) between the rubber sheath-inserted (0.138 cm/day) and the sham-operated (0.213 cm/day) sides.

The experiment was terminated 78 days after membrane/sheath insertion surgery. Dissection of each deer head showed that in Group 1, two impermeable membranes were partially shifted medially, and the growing antler tips emerged from the space lateral to the edge of the medially

shifted membrane (Fig. 5C). Two impermeable membranes were totally displaced from the direction of antler growth. These membranes were

found in the lower medial surface of each antler. In Group 2, four out of four permeable membranes (PTFE thread was not used) were fully displaced, and were found in the lower part of the medial surface of each antler (Fig. 5F). In Group 3, all the rubber sheaths remained in place (Fig. 5I).

Overall, this experiment demonstrated that physical separation of antlerogenic tissue from the overlying skin would not seriously affect antler elongation.

DISCUSSION

As an organ, deer antler formation must rely on heterotypic tissue interactions (Gilbert, 2003). This study has experimentally demonstrated that

TABLE 1. Pedicle and antler height (cm) in rubber sheath insertion group

Deer	Side	Days after surgery			
		0	15	52	78
577	Treated	3.8	4.5	10.5	12.5
	Control	3.8	5	10.5	12.5
580	Treated	3.4	4.5	11.5	13.2
	Control	3.4	5.3	13.0	18.0
579	Treated	3.9	5.3	9.5	18.0
	Control	3.9	5.5	15.0	31.0

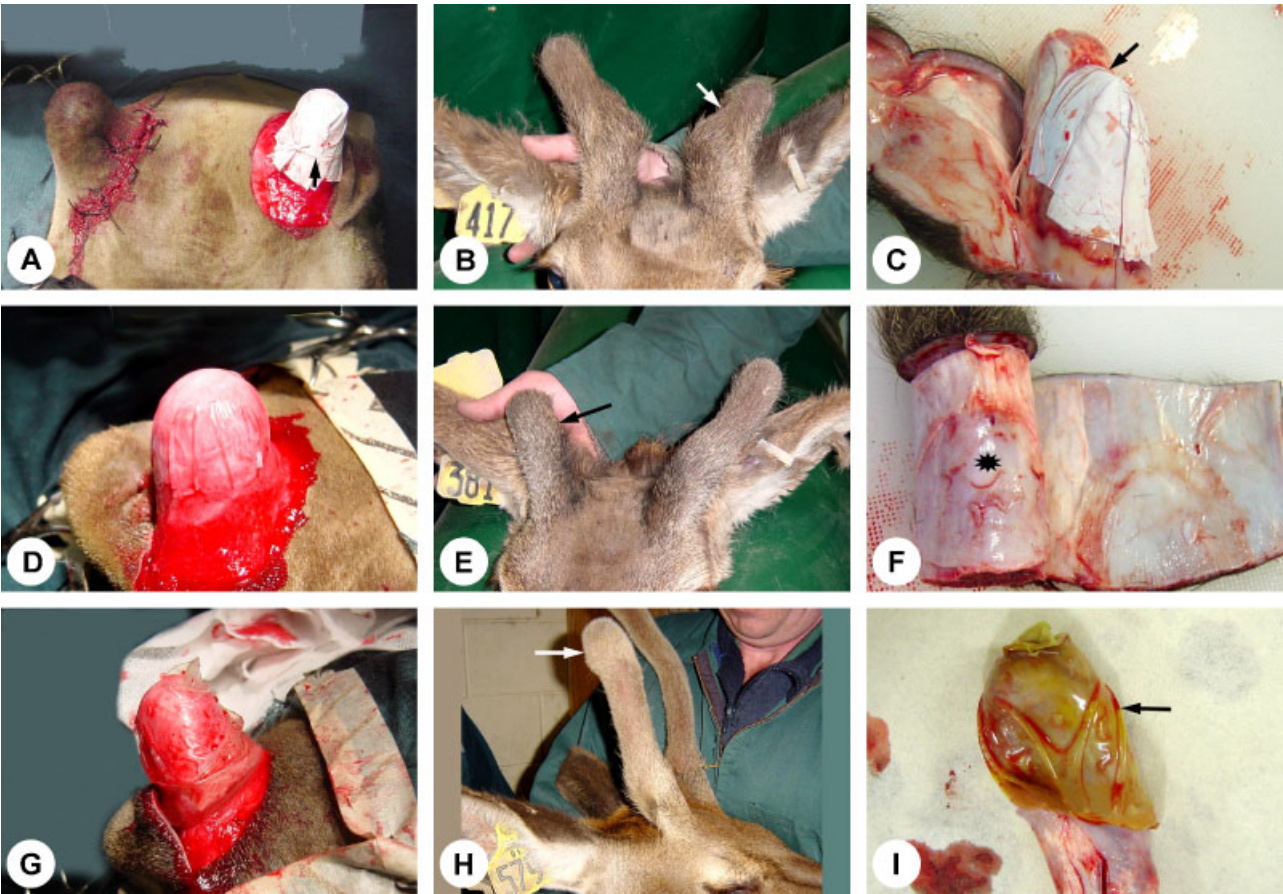


Fig. 5. Experiment 4: (A, D, and G): membrane/sheath insertion surgery on 8-month-old red deer stags. Impermeable (A), permeable (D) PTFE membrane, or latex rubber sheath (G) was capped onto an exposed pedicle tip. PTFE thread (arrow) was used to tie the slippery impermeable PTFE membrane onto each exposed pedicle bone. (B, E, and H) Antler formation from the membrane/sheath-capped pedicles. Notice that antlers (arrow) formed from the impermeable (B), permeable (E) PTFE membrane-capped, and latex (H) rubber sheath-capped pedicles are all somewhat shorter than those of sham-operated control group. (C, F, and I) Dissection on the antlers formed from the impermeable (C), permeable (F) PTFE membrane-capped, and latex (I) rubber sheath-capped pedicles. Notice that the capped impermeable PTFE membrane had been partially displaced by the upwardly growing antler tissue (arrow), permeable PTFE membrane (no PTFE thread was used) was completely pushed away and was found on the shaft of the antler (asterisk), and rubber sheath stayed in place (arrow). PTFE, polytetrafluoroethylene.

(1) interactions between antlerogenic tissue derived from AP and the overlying skin were indispensable for first antler generation, as the interposing impermeable membrane had completely inhibited antler initiation; (2) these interactions were realized through diffusible molecules rather than direct physical contact, as the permeable membrane only slowed the process of first antler initiation but did not stop it; (3) these interactions are transient in nature, rather than continuous. The interactions between antlerogenic tissue and the overlying skin are no longer required for antler elongation once antler generation had been initiated.

Antlerogenic tissue and skin interactions and first antler formation

Discovery of one way induction during first antler formation should be credited to Hartwig and Schrudde ('74) when they found that the ectopically transplanted AP transformed the overlying somatic deer skin into antler velvet. In an extension to this finding, Goss ('87) reported that, besides the grafted AP, ectopic antler formation also required a competent skin. Some AP-grafted regions, such as ventral tail surface and nose snout, failed to give rise to antlers, suggesting that skin types in these regions failed to respond to the initial induction from the grafted AP. Goss ('90) further stated that ectopic antler generation involves the operation of inductive interactions between the grafted AP and the overlying skin. To allow these interactions to proceed, antlerogenic tissue and dermis of the skin must come into close contact with each other. A detailed histological analysis of pedicle formation and first antler generation by Li and Suttie (2000) strongly supported Goss's ('90) statement. Antler generation from the apex of a pedicle takes place only when the interposing subcutaneous loose connective tissue (SLCT) layer is fully compressed into a narrow band. This study demonstrated that disruption of the interactions between antlerogenic tissue and skin can result in total inhibition (impermeable membrane insertion) or substantial delay (permeable membrane insertion) of antler formation. Therefore, we experimentally confirmed that heterotypic tissue interactions are indispensable for first antler generation.

Pathway through which interactions between antlerogenic tissue and skin are realized

Although the interactions between antlerogenic tissue and skin in first antler generation are

required, the pathway through which these interactions are realized is unknown. Inductive interactions involved in organogenesis are classified into two categories: juxtacrine and paracrine interactions (Gilbert, 2003; Singh et al., 2007). The former requires physical contact between the interactive tissue/cell types (ligand proteins on one cell surface interact with receptor proteins on an adjacent cell surface), and the latter involves diffusion of proteins synthesized by one cell type to induce changes in immediate neighboring cells. This study has demonstrated that diffusible molecules are involved in the interactions operating in first antler formation (Experiment 3), which partially fulfils the criteria of the paracrine mode. However, our results cannot determine whether the diffusible molecules act on their distant target tissue layer (epidermis) indirectly through the classic paracrine pathway, i.e., the adjacent tissue to "relay" the signal, or directly via long distance diffusion to reach the target (epidermis).

It is known that there are two tissue layers interposed between AP (inducer) and epidermis of the overlying skin (responder). These two layers are SLCT and dermis (Goss, '95; Li and Suttie, 2000). It is unlikely that SLCT is involved in these interactions, as removal of the SLCT and much of the dermis did not prevent epidermis and partial dermis of deer scalp skin from being transformed into antler velvet when subcutaneously co-transplanted with AP into a nude mouse (unpublished data). In contrast, dermis (at least partial dermis) may play an important role in relaying the AP signal to epidermis, as the phenotype of the epidermis is locally controlled by the underlying dermis (Gilbert, 2003). Moreover, it is unlikely that putative diffusible molecules, which are likely to be in low abundance, can penetrate the over 1 mm thick dense dermis layer (Li and Suttie, 2000) without being deflected before reaching the epidermis. In addition, AP consists of two sub-layers: the outer fibrous layer and the inner cellular layer (Li and Suttie, '94). It is currently unknown whether the instructive message originates from the cellular layer, fibrous layer, or from both. We propose that it is the cellular layer that produces the inductive diffusible molecules based on the following evidence. (1) An ectopic antler can be readily induced by a piece of grafted AP with its cellular layer facing the overlying skin (Goss, '91). (2) Wounding the skin and the underlying AP (breaking the fibrous layer) before pedicle initiation can precociously trigger pedicle and antler formation (Jaczewski, '82; Goss, '91).

(3) AP cellular layer cells form the tissue of pedicles and antlers (Kierdorf et al., '94; Li and Suttie, '94). Based on these analyses, our working hypothesis is that the cellular layer cells of AP produce the instructive diffusible molecules that traverse the periosteal fibrous layer and SLCT layer to reach and act on the dermis. The altered dermis tissue exerts its influence on the overlying epidermis to transform it into antler velvet. In turn, the transformed epidermal cells send instructive feedback signals to the dermis that relays the signal to the periosteal cellular layer to initiate first antler formation. The existence of the interposing layers (periosteal fibrous layer and SLCT) between the interactive tissue types may explain why occasionally first antler initiation is substantially delayed, as reported by Kierdorf and Kierdorf (2000) who found an antler bud initiated 9 years after ectopic pedicle formation through AP transplantation. Therefore, if antler interactions are to be classified as the paracrine, they can only be a special type of paracrine interactions, given that the inducer and the responder are markedly distant, rather than immediately adjacent. If this is the case, the special paracrine interactions operating in first antler generation are truly impressive as to be capable of triggering pedicle/antler formation and transform the adult scalp skin into a unique pelage, the antler velvet.

Nature of the interactions operating in first antler formation

First antler generation has been thought to be comparable to limb formation (Li and Suttie, 2001). However, the results from this study do not fully support this claim. It is known that growth of a tetrapod limb (Carlson, '99) or zebrafish pectoral fin (Nomura et al., 2006) is driven by continuous interactions between the apical ectodermal ridge (FGF4/FGF8) and the subridge mesoderm (FGF10). This study showed that interactions between antlerogenic tissue (mesodermal derivative) and the overlying skin (ectodermal derivative) are only required for first antler initiation but not for further growth. Therefore, these interactions are only transient in nature, and thus differ from those operating in limb/fin formation that can only be sustained by continuous tissue interactions. Similar set of molecules involving the interactions in limb/fin development may also be implicated in the first antler formation, as expressions of FGF10 (Anderson, 2002) and FGF8 (Ashery, '99) have

been detected in the antlerogenic tissue and the overlying skin, respectively, using reverse transcription polymerase chain reaction by our group. Interestingly, the transient nature of the tissue interactions prevailing in first antler formation is recapitulated in subsequent antler regeneration (Li et al., 2007). Therefore, both antler generation and regeneration only require transient heterotypic tissue interactions. A system in which organ generation and regeneration are achieved only through transient heterotypic tissue interactions must be novel, if not completely unique. This system surely has advantages over a system that requires continuous interactions to sustain as far as regenerative medicine is concerned.

Overall, in this study we experimentally demonstrated that interactions between antlerogenic tissue derived from AP and the overlying skin are indispensable for first antler generation; these interactions are achieved through diffusible molecules, rather than physical contact; and these interactions are only required for antler initiation but not elongation. This means that they are transient in nature. Understanding the system may have implications in the field of regenerative medicine.

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