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ORIGINAL ARTICLE

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Deer antlerogenic periosteum: a piece of postnatally retained embryonic tissue?

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Abstract This article reviews the research findings on the piece of periosteum overlying the lateral crest of prepubertal deer frontal bone, known as antlerogenic periosteum (AP). AP was initially discovered by Hartwig and Schrudde in 1974 when searching for the tissue that gives rise to antlers. In their experiment, when AP was transplanted elsewhere on the deer body it formed ectopic antlers. This clearly shows that AP possesses full self-differentiating ability, an attribute that can only be paralleled by embryonic tissue in mammals, like lateral plate mesoderm (LPM). Studies along this line by Goss in the 1980s further demonstrated that AP also holds the patterning information for antler formation. In the 1990s, our group carried out a series of studies on this unique tissue. The results showed that some of the critical features of AP resemble those of embryonic tissues, such as the astonishing growth potential in vivo and in vitro, and rich glycogen content. Histological observations and cell lineage tracing using a genetic marker convincingly demonstrate that pedicles and antlers are the derivatives of AP. Based on these findings, we advanced a hypothesis that AP is a piece of postnatally retained embryonic tissue. Morphological and histological examinations on the presumptive antler growth regions in deer prenatal life showed that the growth of primordial pedicles is initiated in the early pregnant stage (about 55 days) but then ceases (about 100 days) and is subsequently repressed at the late stage of pregnancy. The epidermis overlying the primordial pedicles resembles the apical ectoderm ridge (multicellular layer). These results strongly support our hypothesis. The results from the specific comparison between deer antler formation (from AP in postnatal) and mammalian limb development (from LPM in prenatal) showed that the ontogeny of antlers and limbs are comparable, and that deer antler has the same level of regulative properties as mammalian limbs. We believe that revealing the mechanism underlying the retention of embryonic tissue properties by AP until deer postnatal life will have important implications in biomedical research. Antler formation from AP offers an ideal model to work with in investigating how a self-differentiating system functions.

Keywords Antler · Pedicle · Limb development · Lateral plate mesoderm · Self-differentiation ability

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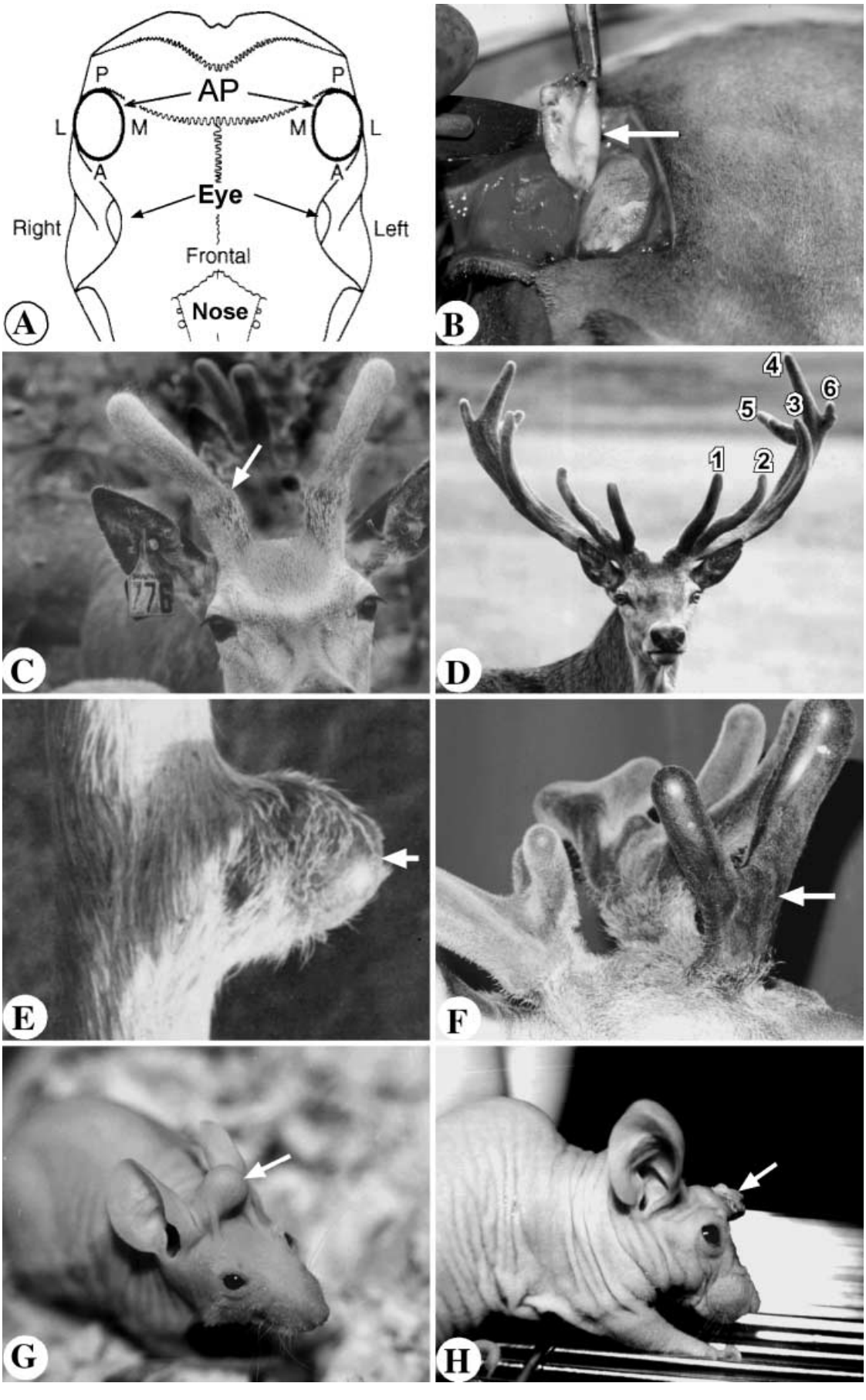
Introduction

Histogenesis of deer antlers and their antecedent pedicles relies on the periosteum overlying the lateral crests of deer frontal bone. Therefore, this periosteum is called antlerogenic periosteum (AP). AP possesses the ability of self-differentiation (see below), an attribute that can only be paralleled by embryonic tissue in mammals, like lateral plate mesoderm (LPM). Through reviewing the research findings on this unique tissue and followed by the report of morphological and histological examination results on the presumptive antler growth regions in deer prenatal life, we advanced an hypothesis that AP is a piece of postnatally retained embryonic tissue. The results from the specific comparison between deer antler formation (from AP in postnatal) and mammalian limb development (from LPM in prenatal) strongly support this hypothesis. If this hypothesis is correct, it would offer an ideal model for investigating how a self-differentiating system functions during embryo development.

Antler, pedicle and antlerogenic periosteum

Antlers are male deer cranial appendages that are cast and fully regenerate each year. Antlers do not grow directly from the head of a deer, instead they form from the apices of pedicles. Deer are not born with pedicles, these start to develop when deer approach puberty. Pedicles are permanent protuberances that grow from the lateral crests of deer frontal bone (Goss 1983). It has been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convincingly demonstrated that it is the periosteum, called antlerogenic periosteum (AP), overlying the crests (Fig. 1A, B) that gives rise to pedicles and antlers (Hartwig and Schrudde 1974; Li and Suttie 1994). Both male and female deer possess AP, but in most species only male deer grow pedicles and antlers as the initiation of pedicle growth is triggered by androgen hormones (Suttie et al. 1995a). When a pedicle attains its full length, antler transformation spontaneously takes place from the pedicle tip. Normally the first antlers are unbranched, known as "spikes" (Fig. 1C). As growing antlers are covered with velvet-like skin, they are known as velvet antlers in the deer antler industry. When the rutting season comes, first antlers become calcified and the velvet skin is shed under the influence of high levels of androgen hormones. The exposed hard antlers drop off from the living pedicles the next spring and growth of new soft velvet antlers immediately follows. From then on, antler development enters a well-defined annual cycle. The subsequent regenerated antlers, unlike the first ones which are spikes, possess a species-specific pattern (Fig. 1D) that has been used as one of the criteria for distinguishing the different species (Li et al. 2000a).

The primacy of AP for the formation of pedicles and antlers was discovered by a combination of deletion and transplantation experiments. The presumptive pedicle growth region consists of bone, periosteum, connective tissue, dermis and epidermis. Goss et al. (1964) found when the skin overlying this region of a male white-tailed deer calf was removed, a pedicle and an antler developed normally after the wound healed. However, loss of the bony component of an incipient pedicle with or without the overlying skin resulted in failure of pedicle and antler formation. In addition, non-pedicle epidermis (such as ear epidermis) could differentiate into antler velvet (Goss 1964). These results indicated that it was the bone, not the skin that must be responsible for the initial development of pedicles and antlers.

Hartwig (1967) took a transplantation approach. He reported that when the scalp skin from the region of a male roe deer calf was grafted to the thigh autologously, no pedicle or antler was produced from the surviving grafted scalp skin. However, when the periosteum from the region was separated from the frontal bone and lifted as a flap graft to a site beneath the skin in the centre of the forehead, a pedicle and an antler subsequently developed from the implanted site, but not from the original region deprived of its periosteum. The most convincing experiment was that of Hartwig and Schrudde (1974), who transplanted the periosteum from the presumptive pedicle region of a roe deer subcutaneously onto a foreleg, as an autograft. They found that a pedicle and an antler were generated from the grafted site. These pioneering findings in roe deer were later confirmed and further extended by a series of transplantation experiments (Fig. 1E) in fallow deer (Goss and Powel 1985, Goss 1987, 1991).

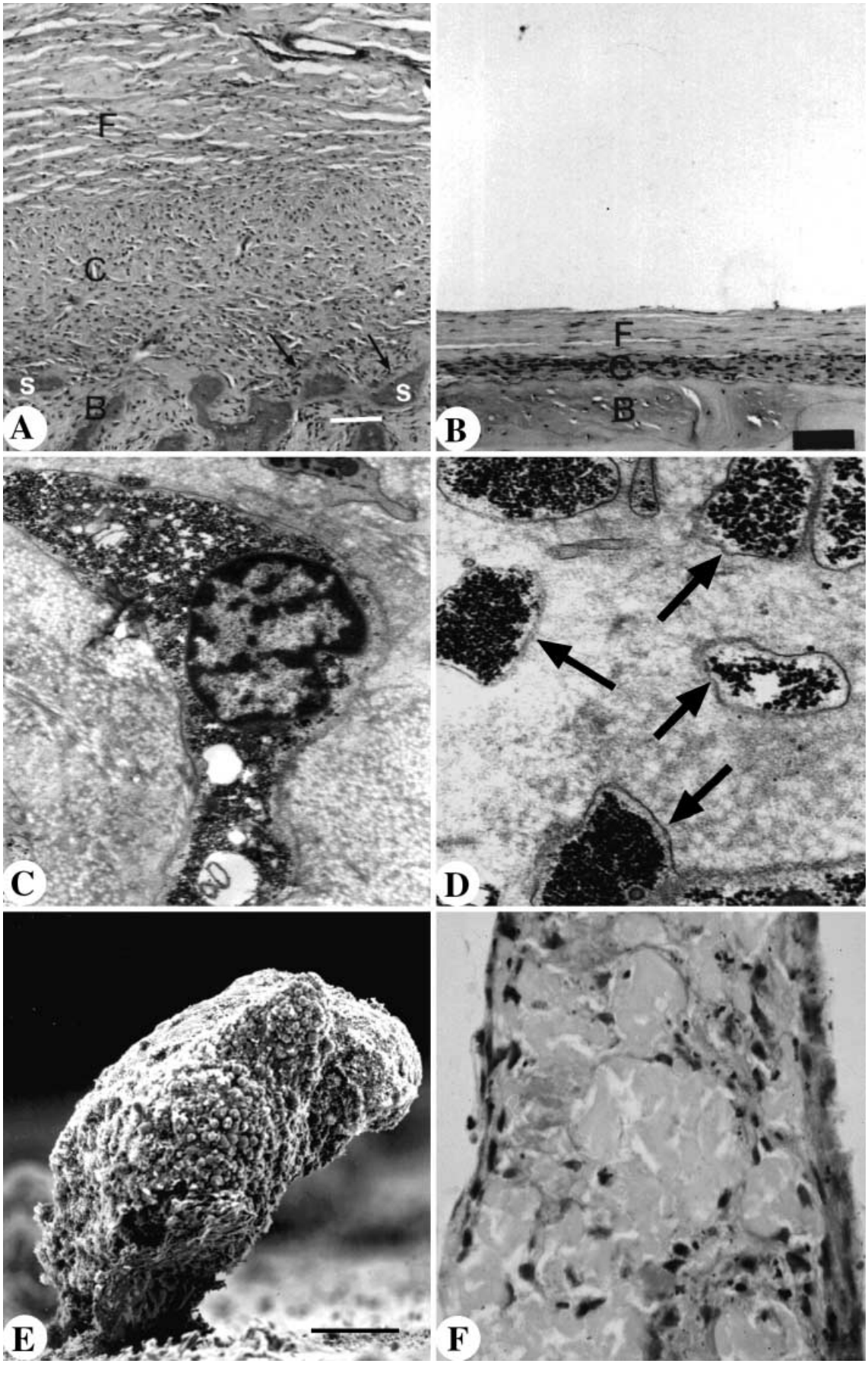
In 1998, we successfully induced ectopic pedicle and antler formation in red deer by transplanting the AP subcutaneously onto deer nasal bones (Fig. 1F; C. Li and J.M. Suttie, unpublished data). More impressively, pedicle-shaped (Fig. 1G) or even pedicle-antler-shaped (Fig. 1H) protuberances can be generated if a small piece of AP (2×2 mm^2) is subcutaneously transplanted onto the head of a nude mouse (Li et al. 2001a).

This astonishing self-differentiating ability shown by AP can only be paralleled, in mammals, by embryonic tissue, such as lateral plate mesoderm for limb development (Carlson, 1999). However, it is not known when AP acquires this self-differentiating ability during its ontogeny. If it is acquired postnatally, that means the cells in AP have dedifferentiated to embryonic stem-cell-like cells from the postnatal differentiated state. If prenatally, that means AP is a piece of residual embryonic tissue, but for some reason its developmental programme is not switched on until postnatal life when deer approach puberty. Either way, the phenomenon would be unique in mammals. Investigations on AP itself would provide further evidence as to whether AP possesses embryonic tissue features, whereas examinations on the presumptive antler regions of deer foetuses at different developmental stages would help us to understand how AP develops during deer prenatal life.

Our research findings on AP

Histologically, AP (Fig. 2A), like its somatic counterpart (Fig. 2B), consists of two layers: an inner cellular layer and an outer fibrous layer (Li and Suttie 1994). However, these layers are much thicker than those of a somatic periosteum. In red deer, the cellular layer is 3.7 times thicker than that of the facial periosteum (Fig. 2A and 2B). Inasmuch as the cellular layer accounts for bone formation (Ham and Harris 1971), the AP should possess a greater potential than facial periosteum to form bone. Both nuclear binding assays (Li et al. 1990) and autoradiographic localisation (Li et al. 1998a) have demonstrated that AP contains specific binding sites for testosterone. Therefore, these results support the notion that pedicle initiation results from direct androgen stimulation on AP (Fennessy and Suttie 1985). However, in

◀ **Fig. 1A–H** Antler, pedicle and antlerogenic periosteum (*AP*) in general. **A** Illustration of the location of antlerogenic periosteum on a deer head *A* anterior, *L* lateral, *M* medial, *P* posterior. **B** AP (*arrow*), which is being removed surgically for transplantation. **C** First antlers (spikes) from a 10-month-old red deer stag (in summer). The junction (*arrow*) between the pedicle and the antler can be easily distinguished by differences in skin appearance and hair type. **D** Fully grown regenerated antlers from an adult red deer stag (*1* brow tine, *2* bez tine, *3* trez tine, *4*, *5*, *6*, royal tines). **E** An ectopic antler (*arrow*) formed from the grafted AP on a foreleg of a fallow deer (reproduced with permission from Goss 1983, p 129). **F** An ectopic branched-antler (*arrow*) formed on the midline of frontal bone of a 3-year-old red deer stag by the autologously grafted AP. **G** A pedicle-shaped protuberance (*arrow*) formed on a nude mouse head by AP xenografts. **H** A pedicle-shaped protuberance with a piece of antler-like bony tissue (*arrow*) on its top formed by AP xenografts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vitro studies (Li et al. 1999; Li et al. 2001b) have shown that the primary cultured AP cells do not proliferate in response to testosterone, although these cells do respond to IGF1 in a dose-dependent manner. Ultrastructurally, the cellular layer cells are very rich in glycogen (Li and Suttie, 1998b, Fig. 2C, 2D). Thus, these cells closely resemble embryonic cells. The intracellular glycogen is known to be mainly used as a source of energy (Scott and Glimcher 1971) and for intracellular synthesis of mucosubstances (Cabrini 1961) in foetal osteoblasts. Interestingly, if these AP cells are left for an extended period in a culture medium, they form large bone nodules (Fig. 2E; C. Li and A. Harris, unpublished data). Histological examination shows that these nodules have a well-organised structure (Fig. 2F; C. Li and A. Harris, unpublished data) and resemble the bone trabeculae within a pedicle or a growing antler. That is the more differentiated cells are located in the centre and actively secrete extracellular matrix including collagens, whereas the less differentiated cells are found peripherally. Some of these features of AP, such as astonishing growth potential and rich glycogen content, can only be found in embryonic cells.

Histogenesis of pedicles and first antlers

Histologically, a pedicle and a growing antler consist of two components: an interior osseocartilage and an exterior skin. It is known that the interior component is formed from AP (refer to Li and Suttie 1994) and the exterior component is a derivative of scalp skin (refer to Li and Suttie 2000c).

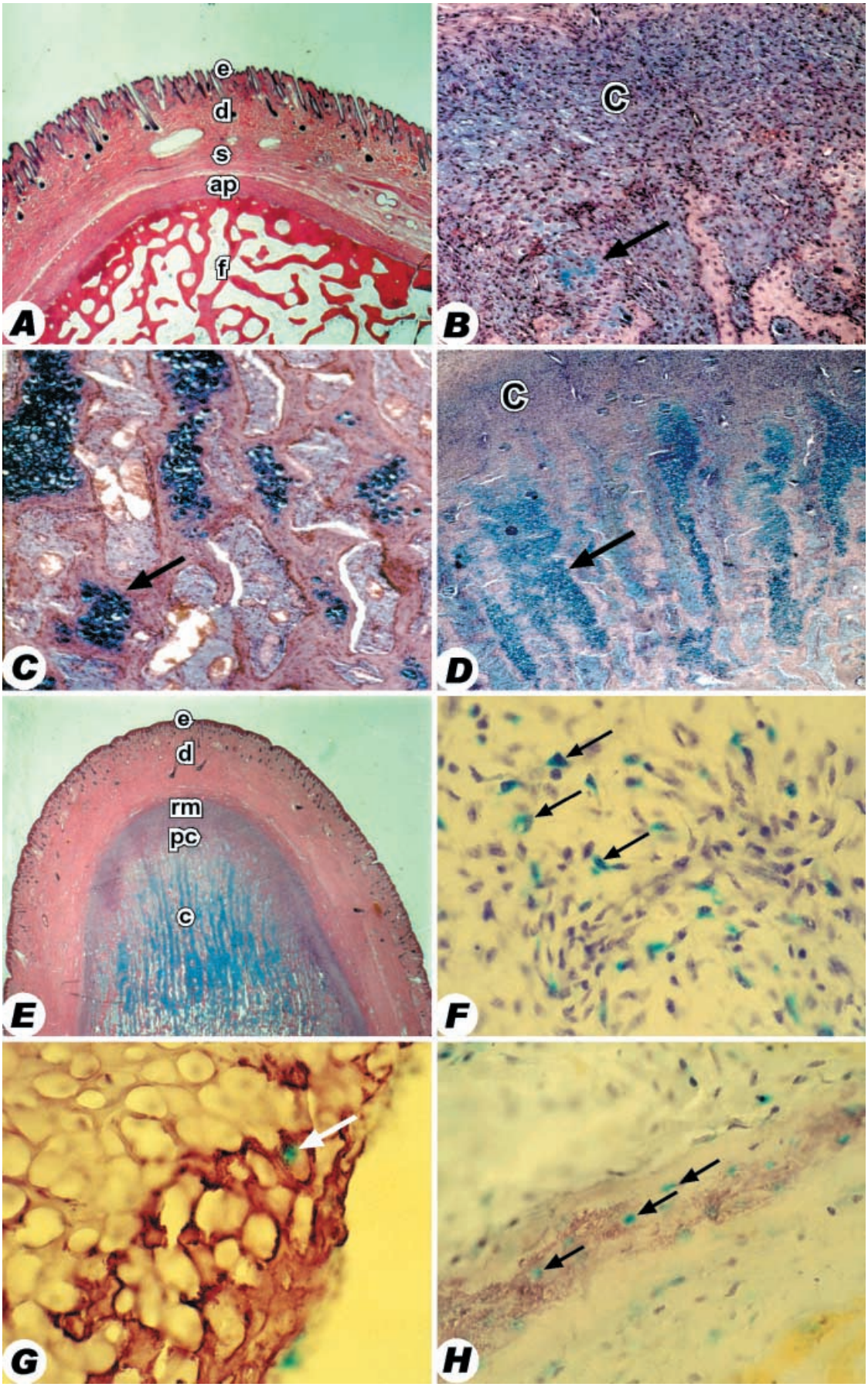
Pedicles develop from the lateral crests (Fig. 3A) of deer frontal bone. At the early pedicle initiation stage, AP cells residing in the cellular layer of the antlerogenic periosteum start to proliferate under the stimulation of androgen hormones and growth factors, and differentiate into osteoblasts. Thus, bone tissue is directly formed (Fig. 2A). This stage is called intramembranous ossification (IMO). When pedicles grow over 1 cm high, some AP cells in the central region of the pedicle apex start to change their differentiation pathway from forming osteoblasts to forming chondroblasts (Fig. 3B). As pedicles elongate, more and more antlerogenic cells switch their pathway to forming chondroblasts, and osseocartilaginous tissue is formed (Fig. 3C). This stage is called transitional ossification (OPC). When pedicles reach 2.5–3 cm high, AP cells solely differentiate into chondroblasts, and only cartilage is formed (Fig. 3D). This stage is called pedicle endochondral ossification (pECO). When pedicle growth proceeds into the antler stage, which is distinguished by skin change, the AP cells still maintain the same differentiation pathway to continuously form chondroblasts (Fig. 3E). This stage is termed antler endochondral ossification (aECO; Li and Suttie 1994).

Pedicle skin forms from the scalp overlying the deer frontal lateral crest (Fig. 3A). The first sign of pedicle skin initiation is the change in thickness of the subcutaneous loose connective tissue (SLCT). At the IMO stage, the SLCT is a very loose and thick layer. As pedicle growth proceeds, this layer becomes thinner and denser. At the aECO stage, the layer is only a thin strip. The change in configuration of the overlying epidermis starts in the late OPC stage when the subcutaneous loose connective tissue is substantially compressed. At the IMO stage, the epidermis has a very undulating profile (Fig. 3A). At the pECO stage, undulation of the epidermis is hardly detectable. At aECO stage, the epidermis is essentially flat (Fig. 3E). The change in epidermis thickness commences at the mid pECO stage when the epidermis becomes more or less flat. At the IMO stage, the epidermis is very thin (Fig. 5D). As pedicle growth proceeds, the epidermis becomes thicker and thicker. At the aECO stage, the thickness of the epidermis becomes about six times thicker on average than that at the IMO stage (Fig. 5C). Antler velvet transformation, which occurs in the late pECO stage, is mainly associated with alterations in the skin appendages. These alterations include the loss of arrector pili muscle and sweat glands, and the gain of the large bi- or multi-lobed sebaceous glands.

The results show that formation of the exterior skin of a pedicle and antler velvet covers four distinctive stages. These are compression of SLCT, stretching of the overlying epidermis, neogenesis of pedicle skin and generation of antler velvet. The histological results seem to suggest that pedicle formation may be caused by mechanical stretching, although antler velvet generation may require additional factors, as mechanical stretch can create new skin but cannot alter skin type.

In order to confirm our histological findings that pedicles and antlers are the derivatives of AP, we recently carried out an experiment to trace AP cell lineage using a genetic marker. In this experiment, some of the AP cells were labelled using the LacZ gene in vivo during the pedicle initiation. The pedicle and early antler formed from the genetic-marker-labelled AP were biopsied, processed and stained using X-gal. The results clearly show that all types of cells in the interior component of pedicle and antler are the progeny of AP cells. Therefore, we conclude that deer pedicles and antlers are the derivatives of AP (C. Li et al., unpublished data). We think the annual antler casting from a pedicle may create

◀ **Fig. 2A–F** Antlerogenic periosteum (AP) and antlerogenic cells from red deer. **A** AP and underlying bone from a 4-month-old male calf (*F* fibrous layer, *C* cellular layer, *B* bone). The bony spicules (*S*) were covered with active osteoblasts (*arrows*). *Bar* 0.1 mm. **B** Facial periosteum and the underlying bone from a 4-month-old male calf. *Bar* 0.1 mm. **C**, **D** Intracellular glycogen of antlerogenic cells from the cellular layer. × 25,257. **C** The cytoplasm of a cell densely occupied by glycogen granules. **D** A section of a group of glycogen-filled processes of a cell or cells. **E** A bone nodule formed in a culture dish from the cultured antlerogenic cells. *Bar* 0.1 mm. **F** Longitudinal section of a bone nodule formed in a culture dish from the antlerogenic cells. Note that more differentiated cells are in the centre, whereas less differentiated cells are mainly found peripherally. ×630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an opportunity to recruit antler-stem cells from the retained embryonic tissue (peripheral periosteum of the pedicle stump) for new antler formation.

Prenatal development of pedicle primordia

Previously published results

As early as 1973, Lincoln (1973) noticed the primordial pedicles of red deer showed a transitory enlargement in early male fetal life. The primordia became apparent at about 60 days of pregnancy. From 75–100 days they were particularly conspicuous. This precocious development of the pedicles coincided with a phase of increased testicular activity. He thought that formation of the primordial pedicles was triggered by a surge of testosterone, and considered that the phenomenon whereby the primordia became less obvious in the late pregnancy period was probably due to differential growth of the surrounding tissue. However, in his study, he did not carry out histological examinations on these pedicle primordia. Therefore, the extent to which the AP participates in the formation of these primordia is not known.

Our findings

In order to determine whether AP participates in the formation of pedicle primordia, we collected red deer foetuses at different developmental stages and examined the presumptive pedicle regions of these foetuses morphologically and histologically (C. Li, unpublished data). The results show that morphologically primordial pedicle initiation (Fig. 3A, B) is about 2 weeks later than deer limb-bud formation, which becomes apparent at about 35–48 days of gestation (McMahon, 1989). The primordial pedicles show a transitory enlargement between 55–150 days of pregnancy, and from 75–100 days become particularly obvious (Fig. 3C, D). Interestingly, these primordial pedicles repress in the later stages of gestation (Fig. 3E, F). Histological examinations show that the epidermis (Fig. 3G) overlying the primordia resembles the apical ectoderm ridge (AER) and is much thicker than its surrounding counterpart (Fig. 3H). Apical ectoderm ridge is a multicellular layer structure overlying a developing limb bud (Carlson 1999). In contrast, no obvious histological difference can be detected between the periosteum (future AP) underneath the AER-like epidermis and its surrounding counterpart (data not shown).

If pedicle formation is initiated during deer embryo development, why this process should then cease and repress is not known. A possible reason could be that if pedicles and antlers had developed during deer prenatal life the deer could not have been born, as these cranial appendages, unlike arms or legs, are rigid structures. Therefore, the phenotypic attributes associated with antler forms found in deer could not have been transmitted from one generation to the next, and so evolved into the form we see today.

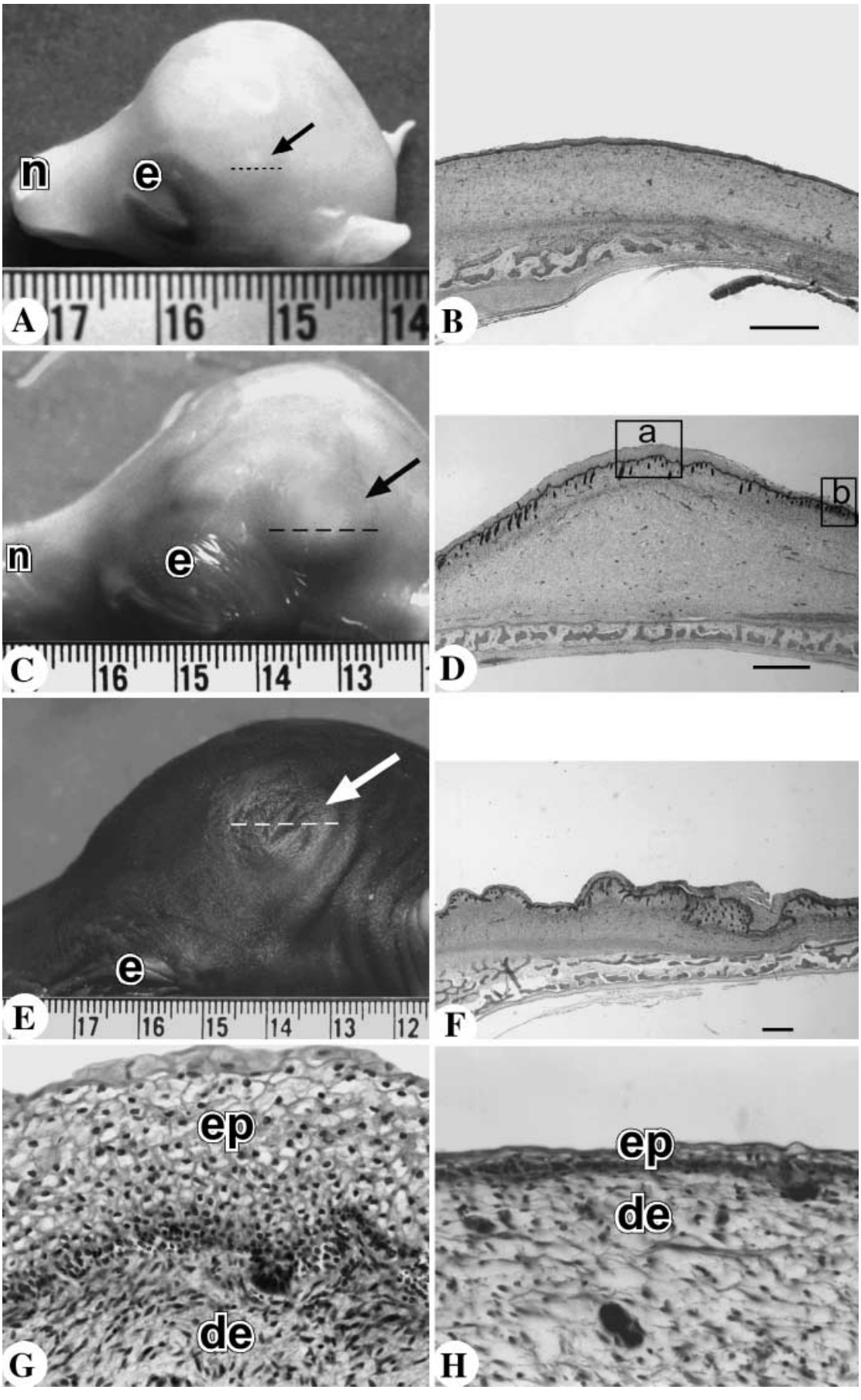
Comparison between antler and limb formation

In order to further support our hypothesis that AP is a piece of postnatally retained embryonic tissue, we would like to make a specific comparison between antler and limb formation. Because limb buds form from lateral plate mesoderm (LPM), a piece of embryonic tissue (Carlson 1999), any demonstration that antler development resembles limb formation would certainly provide evidence for our claim that AP is a piece of residual embryonic tissue. The references relating to limb development are cited here from the textbook by Carlson (1999), unless otherwise indicated.

Histogenesis

Prior to the initiation, both future limb and antler growth regions consist of two tissue components. These are LPM and the overlying ectoderm for limb, and mesoderm (or neural crest?)-derived lateral crest periosteum (AP) and the overlying ectoderm-derived skin epidermis for antler (Li and Suttie 1994). Both LPM and AP are the primary bearers of the blueprints for limbs and antlers respectively, because if LPM or AP is deleted, the limb or antler fails to form (Fig. 4A). However, if LPM is transplanted to the flank of an embryo or AP transplanted elsewhere on a deer body, a supernumerary limb (Kieny 1968) or an ectopic antler (Fig. 4A) forms at the transplantation site. Limb bud development begins with the activation of a group of mesenchymal cells in LPM (Todt and Fallon 1984). Likewise, antler pedicle formation starts with the proliferation of the mesenchymal cells in AP (Li and Suttie 1994). In contrast, both the

◀ **Fig. 3A–H** Histogenesis of a pedicle and first antler in red deer (vertical sections). **A** A frontal lateral crest cut coronally through the integument and underlying tissue (*e* epidermis, *d* dermis, *s* subcutaneous connective tissue, *ap* antlerogenic periosteum, *f* frontal bone. ×14. **B** A palpable pedicle cut through the cellular layer and underlying osseocartilaginous tissue during the early transitional ossification stage. Notice a colony of chondrocytes (*arrow*) appearing under the cellular layer (*C* cellular layer of antlerogenic periosteum). ×100. *C* Osseocartilaginous tissue of an incipient pedicle at late transitional stage. It shows the bony trabeculae having discrete cartilaginous cores (*arrow*). ×200. **D** A pedicle cut through the cellular layer and continuous cartilaginous columns (*arrow*) at the pedicle endochondral ossification stage. ×50. **E** Tip of a growing first antler (*rm* reserve mesenchyme, *pc* precartilage, *c* cartilage). ×3.85. **F** Cells from the cellular layer of antlerogenic perichondrium of an early growing first antler. Notice that some of the cells (*arrows*) stained with X-gal were expressing the *LacZ* gene. ×250. **G** A part of a cartilaginous column from an early growing first antler. Notice that a chondrocyte (*arrow*) stained with X-gal was expressing the *LacZ* gene. ×400. **H** A part of lamellar bone from an early growing antler. Note that most of the cells in the bone were stained with X-gal and expressing the *LacZ* gene. ×100.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overlying ectoderm and the ectoderm-derived skin epidermis are only secondarily co-opted into their own respective system. If the overlying ectoderm or the overlying skin is removed, new ectoderm or new skin heals the defect and a limb or an antler forms (Goss et al. 1964). If the overlying ectoderm is transplanted to the flank of an embryo or the overlying skin to elsewhere on a deer body (Hartwig 1967), no limb or antler (Fig. 4B) forms from the surviving graft. There are no nerves participating in the early limb bud development and those originating in the spinal cord enter the limb bud during the fifth week of limb development. Likewise, a pedicle and an antler can form from a denervated presumptive antler growth region (Li et al. 1993; Suttie et al. 1995b). Although developing limbs contain a muscle component and growing antlers do not, the mesenchymal component of an initial-forming limb bud consists only of skeleton, connective tissue, and blood vessels, which are derived from LPM. The musculature of a limb is formed from myogenic cells that migrate into the limb bud from the lateral somites after the initiation of limb bud formation. Antler development takes place postnatally when deer reach puberty. The formation of new muscle fibres typically ceases at or shortly after birth. That is probably the reason why a growing antler excludes skeletal muscle tissue.

Morphogenesis

Configuration

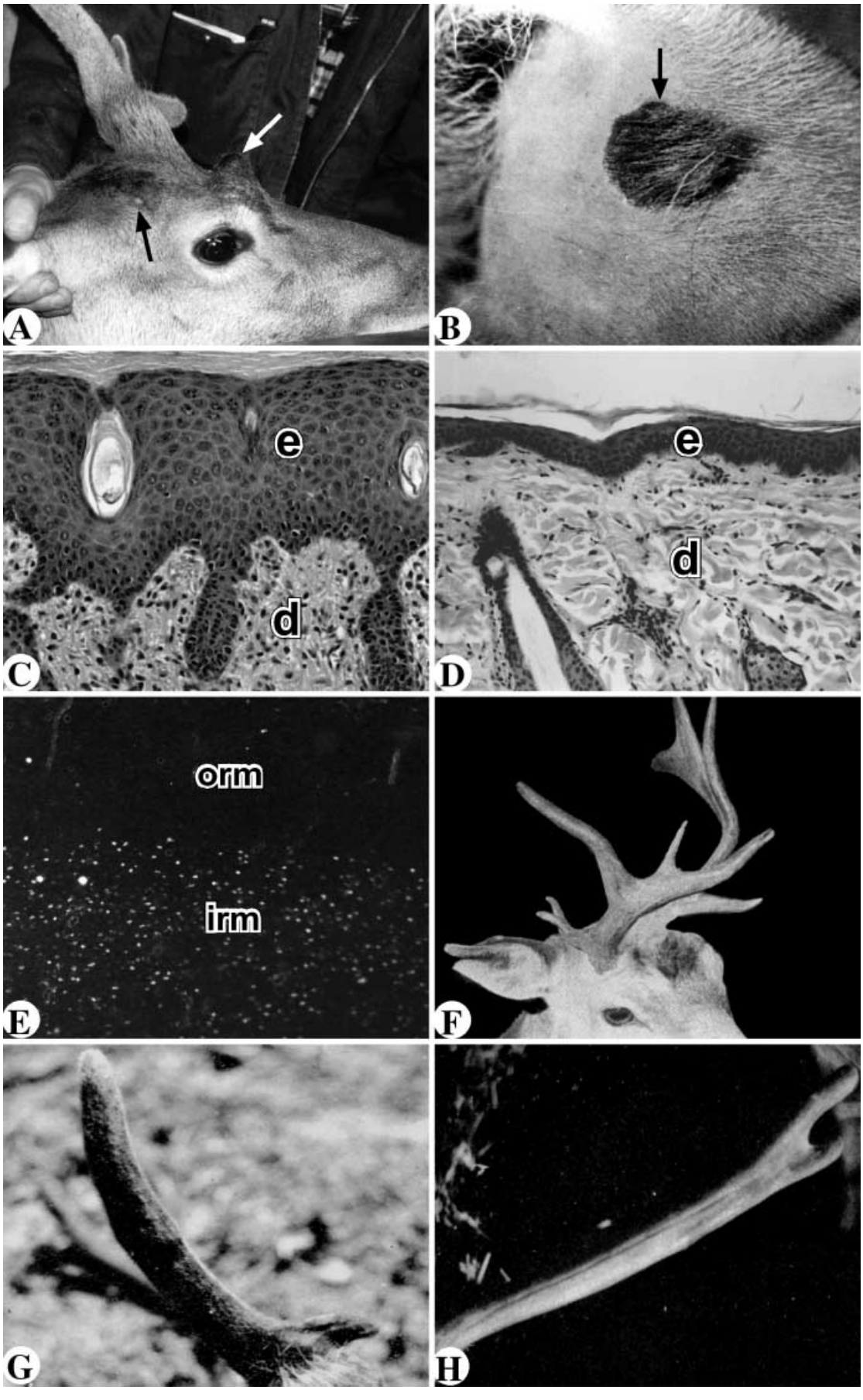
The organisation of the limb development is commonly related to three linear axes, as evident in the development of human hands: (a) the proximodistal axis, which extends from the base of the limb to the tips of the digits; (b) the anteroposterior axis, which runs from the thumb to the little finger in a hand; (c) the dorsoventral axis, which goes from the back of the hand to the palm. Likewise, antler development can also be described using three axes (refer to Fig. 1D) and the axial terminology used for limbs can be applied to antlers. That is: (a) the proximodistal axis from the base to the tips of the tines; (b) the anteroposterior axis from the first formed brow tine to the last ramified main beam tine; (c) the dorsoventral axis from the lateral surface to the medial surface.

Signalling centres

The elongation of the proximodistal axis in limb is achieved through the interactions between LPM and the overlying AER. Apical ectodermal ridge is a multilayered epithelial structure (Cohn 1996) that is induced from the single-layered overlying ectoderm by the activated LPM. Apical ectodermal ridge interacts with the subridge mesoderm to promote outgrowth of the developing limb through expressing members of the FGF gene family, particularly FGF10 (Ohuchi et al. 1997) and FGF8 (Crossley et al. 1996). Apical ectodermal ridge ensures cells in the distal mesodermal region remain in an undifferentiated state and divide actively. This region is called the progress zone. Likewise, the elongation of the antler proximodistal axis is also achieved through the communication between AP and the overlying apical velvet skin (Li and Suttie 2001a). This has been demonstrated by Goss (1990) who, having transplanted a piece of AP intramuscularly, found no pedicle or antler could be generated as the intervening muscle blocks the interactions between the transplanted AP and the overlying skin (Goss 1990). If a piece of AP is transplanted upside down, a normal antler would generate, which strongly supports the hypothesis that interactions between AP and the overlying skin control the growth of the proximodistal axis (Goss 1990). Antler velvet skin is velvet-like skin with very thick epidermis, which is induced from scalp skin by the underlying fast-growing antlerogenic tissue. The epidermis of antler velvet skin is about six times thicker than that of the skin from which antler velvet skin derives (Fig. 4C, D; Li and Suttie 2000c). Antler velvet skin, an AER-like structure, promotes antler outgrowth, which may be also through the same pathway as AER does, because the expression of FGF8 has also been detected in antler velvet skin (de Alwis et al. 1996; Ashrey 1999). Underneath antler velvet skin, there is also a progress-zone-like layer, known as inner reserve mesenchyme (Li et al. 2000b). In this layer, almost all the cells remain in the mitotic state (Fig. 4E).

The formation of the anteroposterior axis is controlled by a group of mesenchymal cells along the posterior border of a limb, which is known as the zone of polarising activity. The *Sonic Hedgehog* gene expressed in the zone encodes a key inductive signal for controlling the patterning of this axis (Riddle et al. 1993). Likewise, the anteroposterior axis of antler is also controlled by a group of mesenchymal cells of the AP. When the AP is rotated

◀ **Fig. 4A–H** Pedicle primordia and AER-like structure of red deer foetuses. **A** The head of a male foetus at about 50 days gestation. *Arrow* points to the presumptive pedicle growth region. *Dotted line* goes sagittally through the centre of the left side region (*e* eye, *n* nose). **B** Sagittal section cut through the left side presumptive pedicle growth region (see the dotted line in **4A**) of a male foetal head similar to **4A**. *Bar* 0.8 mm. **C** The head of a male foetus at about 100 days gestation. *Arrow* points to the pedicle primordia. *Dotted line* goes sagittally through the centre of the left side primordia (*e* eye, *n* nose). **D** Sagittal section cut through the left side pedicle primordia (see the dotted line in **4C**) of a male foetal head similar to *4C*. (*a* apical skin of the primordia, *b* marginal skin of the primordia). *Bar* 1 mm. **E** The head of a male foetus at about 220 days gestation. *Arrow* points to the repressed pedicle primordia. *Dotted line* goes sagittally through the centre of the right side primordia. (*e* eye, *n* nose). **F** Sagittal section cut through the left side repressed pedicle primordia of a male foetal head similar to *4E*. Notice that the region has an undulated surface, which has resulted from the primordial shrinkage. *Bar* 0.6 mm. **G** Enlargement of the area similar to the box *a* in Fig. **4D**. Notice the multicellular layer of epidermis (*ep* epidermis, *de* dermis). ×190. **H** Enlargement of the area similar to the box *b* in Fig. **4D**. Note the thin layer of epidermis. ×190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180° prior to antler pedicle formation, antlers form in reversed anterior-posterior orientation (Fig. 4F; Goss 1991). This group of mesenchymal cells is also likely to reside in the posterior region of AP, because if the posterior half of an incipient antler is removed, the rest of the antler is truncated above the brow tine (Fig. 4G). However, the removal of the anterior half of the incipient antler causes only a minor disturbance in the development of the antler (Fig. 4H; Goss 1961). In addition, *Sonic Hedgehog* may also play a critical role in controlling the formation of this axis as the expression of *Sonic Hedgehog* has been detected in antler tissue (de Alwis et al. 1996).

The signalling centre for the dorsoventral axis initially resides in the mesoderm before initiation of a limb bud. However, this is soon transferred to the dorsal ectoderm, which is characterised by the expression of WNT-7a (Riddle et al. 1995). Unfortunately, so far no effort has been made to determine whether an equivalent signalling centre controlling the dorsoventral axis of antler exists. This is partially because antlers lack clear-cut landmarks distinguishing the inside (ventral) from the outside (dorsal).

In addition, application of retinoic acid to either limb bud (Tickle et al. 1985) or incipient pedicle (Kierdorf and Kierdorf 1998) causes morphological deviation of limb or antler formation.

Regulative properties

Both early limb bud (refer to Carlson 1999) and incipient antler are highly regulative structures as indicated by the following points.

1. If part of a limb primordium is removed, the remainder reorganises to form a complete limb. In the case of antler, the casting of the previous antler can be considered as an equivalent event that is followed by the full regeneration of a new set of antlers (Fig. 6A, B). In the velvet antler industry, removal of antlers in their growing phase is the common practice. Generally antler regeneration from the sawed stumps takes place immediately after wound healing. These second sets of antlers also have commercial value and are known as “regrowth”.

2. If a limb primordium is split into two halves and these are prevented from fusing, each half gives rise to a complete limb. Likewise, if an anterior-posterior cleft is made on an incipient antler, two separate antlers can form and grow to a height similar to that of normal control antlers (Fig. 6C, D) (Goss 1961).

3. If two equivalent limb discs are superimposed, they reorganise to form a single limb. Likewise, when the left-side periosteal discs are grafted onto the periostea of the presumptive regions on the right side, all of the right-side regions grow normal antlers (Fig. 6E; Goss 1991).

4. If two equivalent halves of a limb primordia are juxtaposed, one complete limb forms. So far, this has not been tested experimentally on antlers. However, fused antlers sometimes occur naturally in roe deer. This is because the left and right pedicles of roe deer often are so closely approximated that the antlers themselves become fused at their base with the result that one antler is formed (Fig. 6F; Whitehead 1993).

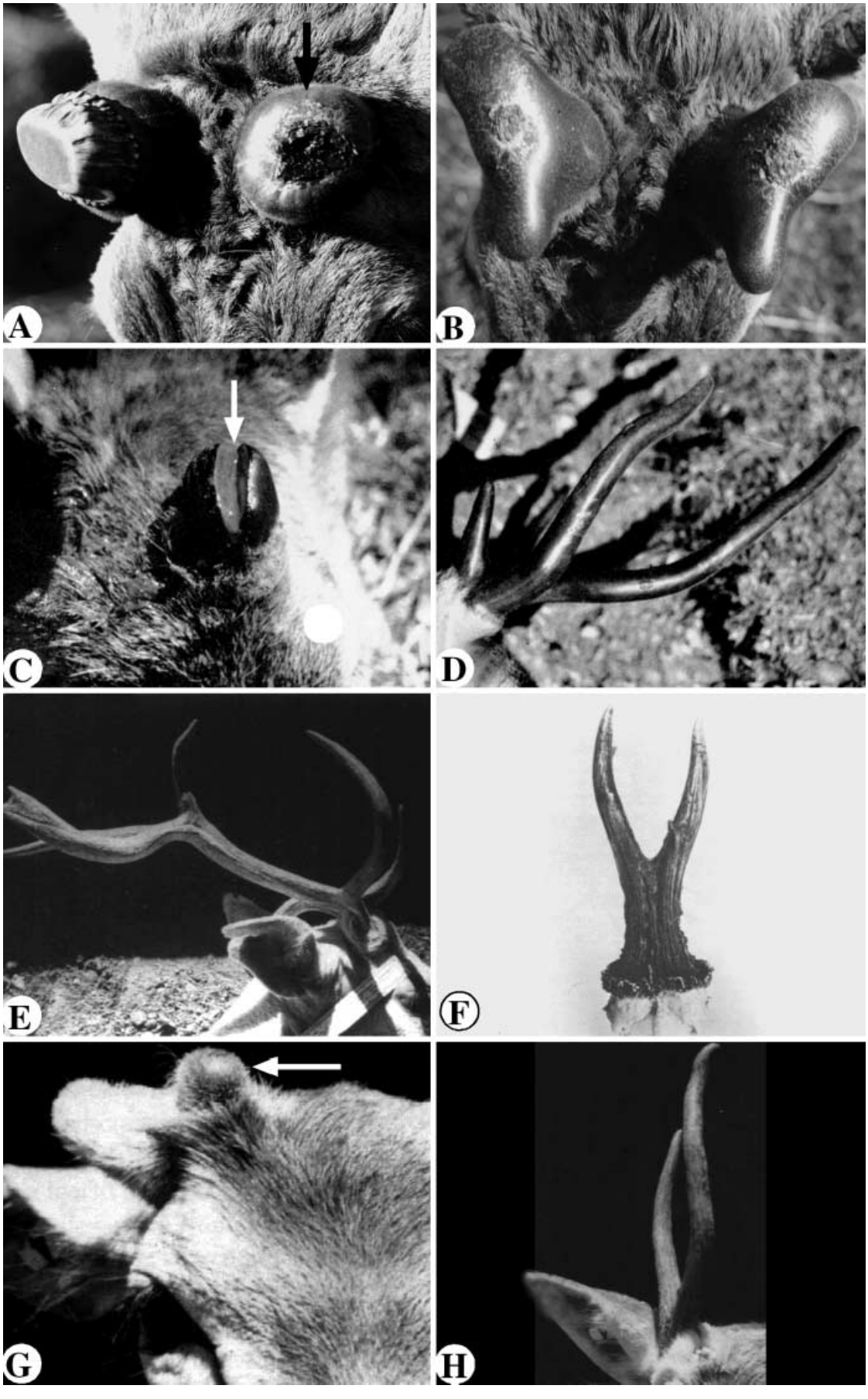
5. In some species, disaggregated limb mesoderm can reorganise and form a complete limb. Thus far, similar results have not been reported in antler development. However, the minced AP is capable of producing an organised antler (Fig. 6G, H; Goss 1991).

◀ **Fig. 5A–H** Antler development. **A** An ectopic antler formed on a male red deer nasal bone (*white arrow*) by a piece of grafted antlerogenic periosteum (AP) from the right side presumptive pedicle growth region (*black arrow*). Note that no pedicle or antler formed from the original region after the removal of AP. **B** Graft of a piece of pedicle skin (*arrow*) on the inner surface of a deer ear. Note that no pedicle or antler formed from the surviving pedicle skin (reproduced with permission from Goss, 1983, p 143). **C** Apical antler skin from a 60-day-growing antler of a 3-year-old red deer stag. ×115. **D** Pedicle skin on the shaft. ×115. **E** Two layers of the reserve mesenchyme from the tip of a growing antler: the outer reserve mesenchyme (*orm*) and the inner reserve mesenchyme (*irm*). Note “orm” was nearly devoid of dividing cells, whereas “irm” was intensively labelled by BrdU. ×32. **F** An antler with reversed anterior-posterior orientation formed on the antler growth region where AP was rotated 180^0 (reproduced with permission from Goss 1991). **G** The antler formed from the antler bud missing its posterior half (2.54 cm in height) in sika deer. Note that only a proper brow tine was formed. According to the author the absence of the posterior half caused serious disturbance in antler formation. (Both **G** and **H** are reproduced with permission from Goss 1961). **H** The antler formed from the antler bud missing its anterior half (2.54 cm in height) in a sika deer. Note that main beam with a branch was formed. According to the author, the absence of the anterior half caused only minor disturbances in antler formation

Discussion

Like the role played by lateral plate mesoderm (LPM) in limb bud development, antlerogenic periosteum (AP) is the bearer of the blueprint for antler formation. Pedicle development from the AP is activated and arrested prenatally and reactivated during deer postnatal life to form complete pedicles and antlers (Lincoln 1973; C. Li and J. M. Suttie, unpublished data). The most impressive feature of all is that AP can retain its embryonic stem tissue features until deer postnatal life. These features are: a self-differentiating capability, astonishing growth potential and richness in intracellular glycogen. Therefore, we conclude that AP is a piece of postnatally retained embryonic tissue.

The discovery that AP is a piece of postnatally retained embryonic tissue undoubtedly has very important implications. Although the phenomenon that an appendage develops from a piece of residual embryonic tissue is not without precedent in the animal kingdom, e.g. tadpoles wait until a premetamorphic period to sprout their legs, it may be unique to mammals. In addition, if we know an antler develops from a piece of residual embryonic tissue, we would certainly benefit from the findings made on other appendages formed from embryonic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tissue prenatally, such as limb development. By making comparisons between antler development and limb bud formation, we have already obtained significant insights. For example, we have detected the expressions of the following genes during antler development: *FGF8*, *FGF2*, *FGF4*, *BMP4*, *IGF1*, *Sonic Hedgehog* (*SHH*) (de Alwis et al. 1996; Ashery 1999). These genes are all critical developmentally-regulated genes that are expressed in limb bud formation. In addition, it is known that *FGF10* is, thus far, the earliest expressed mesodermal factor. The current working model for limb bud formation (Martin 1998) is that prior to limb initiation, *FGF2*, *FGF4* and *FGF8* are expressed in the intermediate mesoderm adjacent to the limb-forming LPM. The proteins of these growth factors are able to induce *FGF10* expression at the same level in the LPM in a diffuse pattern. The adjacent FGF8 (and probably other FGFs) signals to the LPM to maintain and restrict expression of *FGF10*. Once *FGF10* expression has been restricted to the LPM of the presumptive limb areas, it operates on the overlying ectoderm to induce the expression of *FGF8* and AER formation. Expression of *FGF8* in the AER is required for the maintenance of *FGF10* in the nascent limb mesenchyme and the localization of *SHH* to the posterior margin of the limb bud. Recently, it has been reported that three WNT factors are also involved in both limb initiation and AER induction (Kawakami et al. 2001). Thus, it would be reasonable to hypothesise that *FGF10* and the three WNT factors are expressed in early antler development as well.

Antler biologists can learn from limb development that, among other things: (1) limb formation has been successfully explained by the positional information theory – can the theory be employed to explain antler development?; (2) disaggregated lateral plate mesoderm cells have formed supernumerary limbs (Carlson 1999) – can disaggregated AP cells form antlers after implantation back into the deer?; (3) The AER of a limb bud is characterised by the presence of gap junctions – are gap junctions also features of the thickened apical velvet skin on antler? We have detected the expression of connexin 43 using the technique of suppression subtractive hybridisation. Connexin is the main component of connexon, which is the structural subunit of a gap junction.

Embryologists may learn from antler development: (1) If deer antlers can fully regenerate, why can human arms and legs not do so? Can revealing the underlying mechanism of antler regeneration help us to understand why other mammalian appendages cannot regenerate? (2) Deer antler pedicles can transiently develop and cease this development prenatally, then are reactivated to form antlers postnatally. Can the underlying mechanism of the reactivation of antler formation be applied for some clinical purposes? One possibility would be in limbless syndrome, where early limb development is normal, but later the AER disappears and further development ceases. Can these prenatally transiently developed limb buds be reactivated postnatally? (3) It appears the majority of the cDNA transcripts in the tip of a growing antler are much smaller in size than those from deer somatic tissue. This result is obtained by using agarose gel electrophoresis of the cDNA generated from the mRNA, which is extracted from growing antler tip tissue and nine different types of deer body tissue by our antler research group. Can the same be said for cDNA transcripts in some type of embryonic tissue, like LPM? If the same were true for general embryonic tissue, it would be interesting to discover that whether the small transcripts are casually related to rapid tissue growth and differentiation.

In conclusion, we confidently predict that an understanding of the underlying mechanism as to how the AP can retain its embryonic tissue features until deer postnatal life will have important implications in biomedical research. Formation of a pedicle and an antler during deer postnatal life from AP, a piece of postnatally retained embryonic tissue, would be an ideal model to work with in investigating how a self-differentiating system functions.

◀ **Fig. 6A–H** Regulative properties of deer antlers. **A** Antler autotomy in a 3-year-old red deer stag. The left hard antler button (*arrow*), the remnant hard antler remaining following surgical velvet antler removal in the previous season, has been shed and the wound has healed with new velvet antler regeneration taking place. The right button is yet to cast. Normally both would cast within 24 h. **B** Early regenerating antlers from a 3-year-old red deer stag. **C** Appearance of a sika deer following removal of a segment (*arrow*) of tissue slightly lateral to the mid-line of the left antler bud (2.54 cm in height). (Both **C** and **D** are reproduced with permission from Goss 1961). **D** Final shape of the antlers formed from the manipulated antler bud in **6C**. Notice that double antlers formed. **E** A reasonably normal antler formed from the antler growth region where the periosteal discs were doubled by grafting one on top of the other with their axes co-ordinated (reproduced with permission from Goss 1991). **F** Fused antler in roe deer (reproduced with permission from Whitehead 1993). **G** An antler bud formed from the antler growth region where AP was removed, minced and grafted back (*arrow*). (Both **G** and **H** are reproduced with permission from Goss 1990). **H** Final form of the antlers formed from the minced AP of the same deer in **6G**

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