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Review

Deer antler – A novel model for studying organ regeneration in mammals☆

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ABSTRACT

Deer antler is the only mammalian organ that can fully grow back once lost from its pedicle – the base from which it grows. Therefore, antlers probably offer the most pertinent model for studying organ regeneration in mammals. This paper reviews our current understanding of the mechanisms underlying regeneration of antlers, and provides insights into the possible use for human regenerative medicine. Based on the definition, antler renewal belongs to a special type of regeneration termed epimorphic. However, histological examination failed to detect dedifferentiation of any cell type on the pedicle stump and the formation of a blastema, which are hallmark features of classic epimorphic regeneration. Instead, antler regeneration is achieved through the recruitment, proliferation and differentiation of the single cell type in the pedicle periosteum (PP). The PP cells are the direct derivatives of cells resident in the antlerogenic periosteum (AP), a tissue that exists in prepubertal deer calves and can induce ectopic antler formation when transplanted elsewhere on the deer body. Both the AP and PP cells express key embryonic stem cell markers and can be induced to differentiate into multiple cell lineages *in vitro* and, therefore, they are termed antler stem cells, and antler regeneration is a stem cell-based epimorphic regeneration. Comparisons between the healing process on the stumps from an amputated mouse limb and early regeneration of antlers suggest that the stump of a mouse limb cannot regenerate because of the limited potential of periosteal cells in long bones to proliferate. If we can impart a greater potential of these periosteal cells to proliferate, we might at least be able to partially regenerate limbs lost from humans. Taken together, a greater understanding of the mechanisms that regulate the regeneration of antlers may provide a valuable insight to aid the field of regenerative medicine.

This article is part of a Directed Issue entitled: Regenerative Medicine: the challenge of translation.

Contents

1. Introduction 112
2. The only complex mammalian organ that can fully regenerate 112
3. A single cell type-based process 113
4. The derivative of a postnatally retained embryonic tissue 114
5. Adult stem cells with embryonic stem cell features 115
6. The missing link 117
7. Stem cell-based *vs* classical blastema-based epimorphic regeneration 118
8. Lessons learnt from antler for regenerative medicine 119

Abbreviations: AP, antlerogenic periosteum; PP, pedicle periosteum; AGS, antler growth stimulus; IGF1, insulin-like growth factor 1; MAPK, mitogen-activated protein kinases; PI3K/AKT, phosphatidylinositol-3 kinases/AKT; AR, androgen receptor.

☆ This article is part of a Directed Issue entitled: Regenerative Medicine: the challenge of translation.

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1357-2725/

Conflicts of interest 120
Acknowledgements 120
References 120

1. Introduction

Deer are the only mammals that are capable of fully regenerating a complex organ, which they do annually with the growth of their magnificent bony headpieces, called antlers. Despite this unique position in mammalia, regeneration of antlers has largely gone unnoticed as a research model to attain the Holy Grail in the regeneration field – to grow a new limb on a mammal. Deer antlers are widely known for their traditional medicinal value, but few are aware of their unique biological features, which can be developed into multiple novel biomedical models (Goss, 1983; Li, 2012). The unprecedented growth rate (up to 2.75 cm/day) of antlers (Goss, 1970) provides a rare system in which fast cell proliferation is precisely regulated without becoming cancerous. The atypical features of antler "stem" cells, such as expression of key embryonic stem cell markers and pluripotency (Li et al., 2009), serve as an invaluable model for understanding how some of the attributes of embryonic stem cells can be retained in postnatal tissues. As a muscle- and joint-free bony cranial appendage (Banks and Newbrey, 1982; Kierdorf et al., 2003; Li et al., 2005), antlers provide a single system to investigate how bone is formed without the influence of external forces (except for gravity). The formidable size and complexity of antlers provides a powerful system to study how visual symbols can impact on animals' social ranking status (Bartos et al., 2012). Taken together, the annual renewal of antlers offers a unique opportunity to understand how nature has bestowed full regeneration of organs in mammals, which could, potentially, provide a solution to the regeneration of limbs.

2. The only complex mammalian organ that can fully regenerate

The ability to fully regenerate stands out as the most impressive feature of antlers. To do so repeatedly each year is even more remarkable, because mammalian appendages are generally considered as being incapable of regeneration. Yet deer grow antlers annually in defiance of what could be considered nature's rules (Goss, 1995).

The annual cycle of antler growth starts in spring (mainly based on red deer and sika deer), when the nascent antler regenerates from a permanent cranial bony protuberance, known as a pedicle, following casting of the previous hard antler. Rapid elongation of antlers and the formation of lateral branches, called tines, occur in summer. During this growth phase, antlers are enveloped with a special type of soft pelage, called velvet skin. In late summer/autumn, the regenerating antler attains its full size and becomes totally calcified and, in conjunction with the loss of blood vessels and nerves, the velvet skin is shed. In winter, the bare bony antlera are firmly attached to the living pedicle and is not "cast" until the following spring. Antler casting triggers another round of antler regeneration (Goss, 1983; Li, 2003).

Bleeding occurs on the cast plane of a pedicle stump immediately after casting of an antler (Fig. 2A and C). A scab is formed from the dried blood within 1–2 days of casting, and it occupies approximately half of the entire casting surface and is surrounded by a ring of shiny velvet-like skin, indicating that a new antler has commenced growth. As the wound healing process advances, the ring of velvet skin migrates centripetally and the scab and underlying scar become smaller (Fig. 2B). The transformation from pedicle skin to antler velvet is seen as a change in the thickness of epidermis (about 10 times thicker than pedicle skin) and the associated skin appendages (Li and Suttie, 1994). The change in skin appendages includes the loss of arrector pili muscles and sweat glands, and the gain of the large bi-lobed or multi-lobed sebaceous glands. The wound healing process over a pedicle stump is considered complete when the new skin has converged at the center to close the wound over the casting plane (Li et al., 2004b).

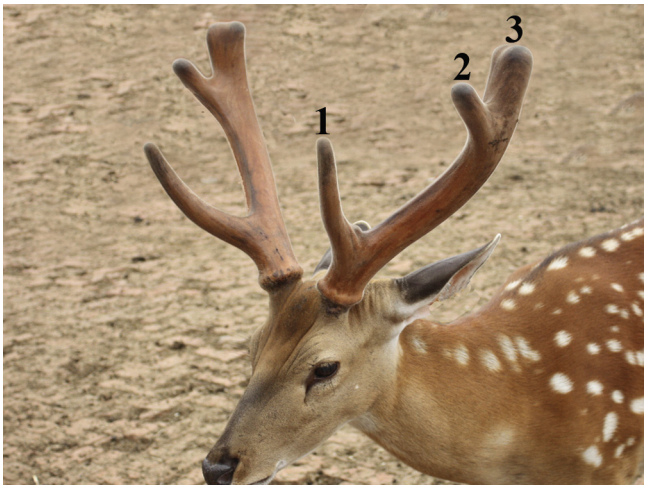

Fig. 1. Growing antlers on a male sika deer. 1, first tine; 2, second tine; 3, main beam.

The pedicle periosteum (PP), located at the rim of a pedicle stump and underling the new shiny skin during the early wound healing stage, becomes thickened after the activation and division of resident stem cells, and incorporation of their progeny (Kierdorf et al., 2003; Li et al., 2005). Subsequently, at the late wound healing stage, two crescent-shaped growth centers, one located anteriorly and the other posteriorly, are formed directly from the proliferation and differentiation of pedicle periosteal cells (Fig. 1D). Each center is made up of cartilaginous clusters that are capped by a layer of hyperplastic pedicle periosteum/perichondrium (Fig. 2D). Further augmentation of each growth center raises both the anterior and posterior portions of the pedicle stump to leave the central scab region behind. These posterior and anterior growth centers are the focal points from which the "main beam" and "first tine" of the antlers form (Fig. 1) (Li et al., 2005).

The main beam and the first tine regenerate from the pedicle simultaneously, and the centrally located scab is displaced by the eccentrically formed main beam and the first tine and eventually flakes off to reveal an underlying scar (Li et al., 2005). No causal relationship has thus far been detected between the shape of antlers and the integrity of the healing process over the cast plane of a pedicle stump. Therefore, the wound healing process may not contribute to the growth, final size and shape of antlers. Goss (1995) concluded that the wound healing over the top of a pedicle stump is a prerequisite for the initiation of antler regeneration. However, our membrane insertion experiment (see below) demonstrated that antler regeneration can take place without the pedicle skin participating and this resulted in a skin-less antler (Li et al., 2007b).

The branching of antlers is undoubtedly under the control of genetic programming, as the shape of antlers is species-specific

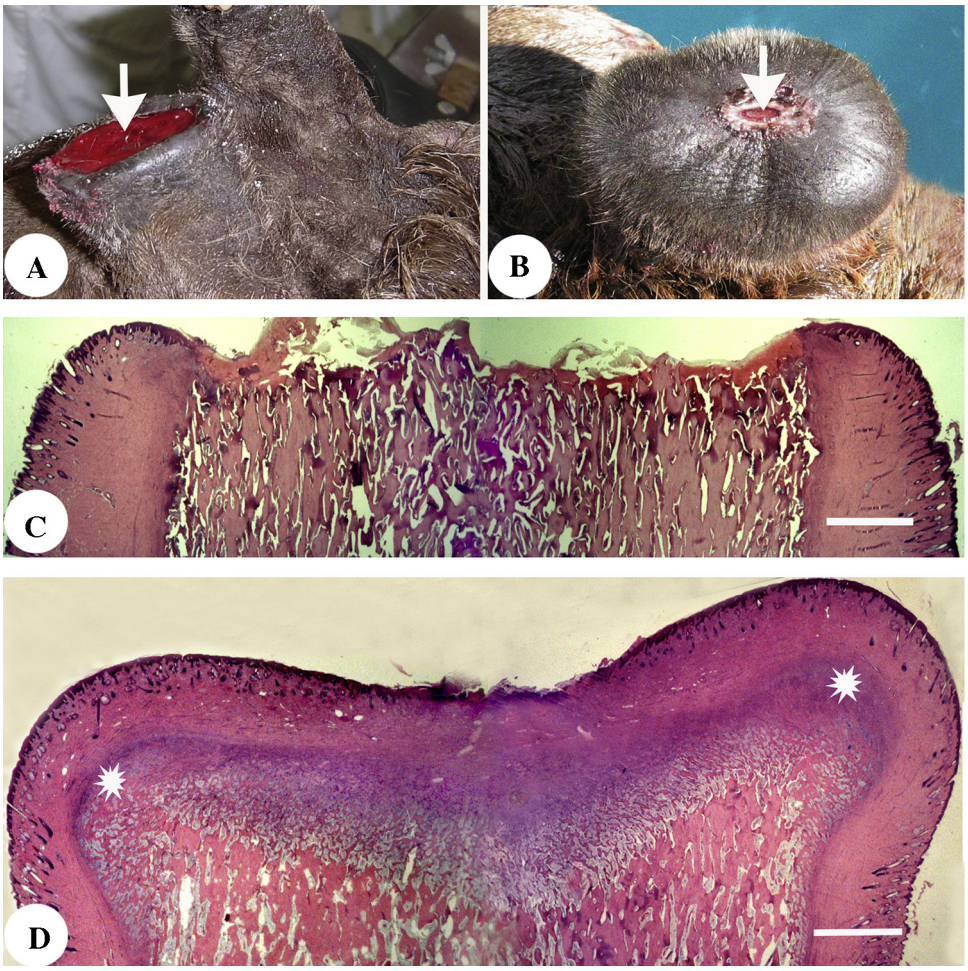

Fig. 2. Morphogenesis and histogenesis of deer pedicles and antlers. (A) Pedicle stump with a fresh casting surface. Notice that some blood was retained in the depressed central portion (arrow). (B) An incipient antler at the late wound healing stage. Notice that the scab (arrow; lost during sampling) becomes very small and is located in the center of the pedicle. (C) A pedicle stump sectioned sagittally immediately after antler casting. Notice that the rough surface of the casting plane of the pedicle stump and epithelium of the rim formed by the distal pedicle skin is thicker and has acquired some of the features of velvet skin. Bar = 3 mm. (D) A late wound healing stage pedicle stump sectioned sagittally. Notice that the anterior and posterior growth centers have formed (indicated by the stars). Bar = 3 mm.

(Chapman, 1975). However, it remains unclear how the branching process is initiated. An interesting finding was that blood vessel branches supplying the first, second and/or third tines from the superficial temporal artery were already present prior to the initiation of these antler tines. In contrast, the arteries supplying the terminal fork (royal tines) only begin to form just before these structures started to develop (Suttie et al., 1985a). This might be one of the reasons why the first two to three tines are predestined to form in regenerating antlers independent of environmental factors, whereas the number of royal tines that form depends, at least in part, on the level of nutrition (Ullrey, 1982).

In autumn, as the reproductive season approaches, antlers gradually become calcified. It is clear that the process of calcification is initiated from the base of the antler and proceeds up through the antler and finishes when the distal ends of the tines form sharp tips (Goss, 1983). After the calcification process has been completed, the velvet skin loses its shiny appearance and becomes dry and progressively peels off to expose the underlying bone. There does not seem to be a particular place on the antler surface from where the shedding of velvet is initiated. However, one can observe that some shedding processes are lengthy with no apparent bleeding, while in others the shedding is rapid and is accompanied by much bleeding. The exposed hard antlers are firmly attached to their living pedicles during winter, and are not cast until the following spring (Li et al., 2004b, 2005).

"The antlers of deer are so improbable that if they had not evolved in the first place they would never have been conceived even in the wildest fantasies of the most imaginative biologists" (Goss, 1983). However, deer antlers have indeed evolved, which challenges us to describe and explain this unique phenomenon.

3. A single cell type-based process

What cell type(s) give rise to regenerating antlers? This is a question that has challenged and frustrated generations of researchers. While the histogenesis of the pedicle may be assigned to periosteal osteoblasts, this is no guarantee that the antler *per se* is derived from osteoblasts of the pedicle periosteum. Goss (1995) thought that as histologically complex structures, antlers must surely have multiple origins for such tissue components as the epidermis, dermis, cartilage, bone, blood vessels, and nerves.

If the growth centers for antler regeneration are solely established from the distal pedicle periosteal cells (see above), antler regeneration would be a single cell type-based process. Mindful of the dangers of inferring dynamic processes from static representations (see above for morphological and histological descriptions), we carried out a functional analysis. In the analysis, the PP was either totally (Fig. 3A) or partially removed from a pedicle stump prior to antler regeneration to learn if antlers could regenerate from these PP-less pedicles (Li et al., 2007a). The results showed that

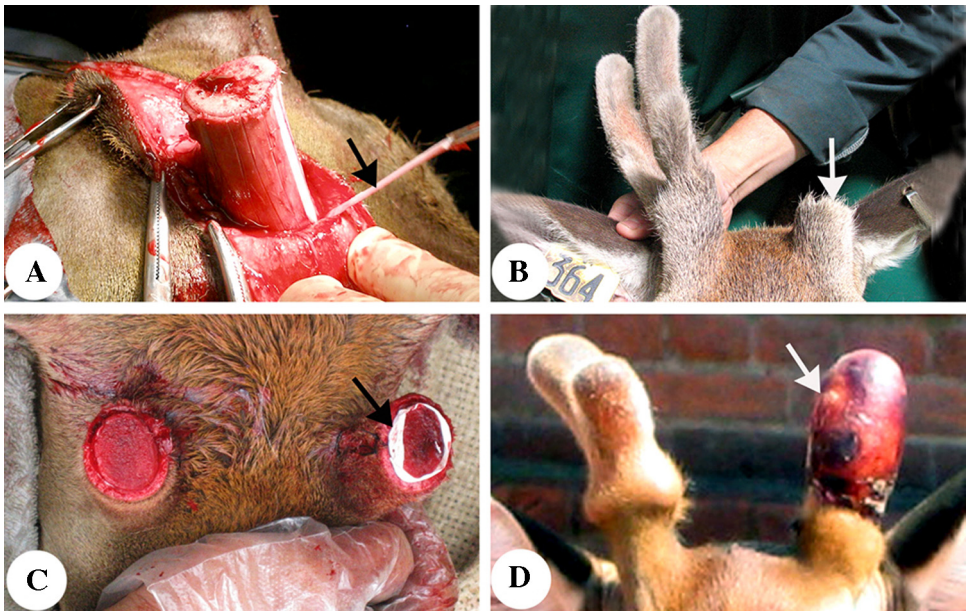

Fig. 3. PP deletion and membrane insertion. (A) PP strips were peeled off the pedicle bone with a pair of rat-toothed forceps (arrow). (B) No antler regeneration occurred on the PP-deleted pedicle (arrow), while the sham-operated pedicle formed a three-branched antler on the contralateral side. (C) A piece of impermeable Teflon membrane (arrow) was inserted into the space between the PP and the enveloping skin. (D) Skin-less antler (arrow) regenerated from a pedicle stump that had a membrane inserted. Notice that most parts of the antler were covered with a fresh blood-colored scab.

complete removal of the PP inhibits the pedicle stump from initiating regeneration of the antler (Fig. 3B). Furthermore, regeneration of antlers can only take place on the shaft of the partially PP-deleted pedicles from the location of the remnant PP, which is markedly distant from the pedicle apices where normal antler regeneration originates. Therefore, we were able to convincingly demonstrate that antlers are regenerated from cells derived exclusively from the PP.

While the PP is indispensable for regeneration of antlers, it is unclear whether or not the skin plays a role in the regeneration and growth of antlers because the pedicle skin is always an integral part of a regenerating antler (Li et al., 2007b). Whether regenerating antlers are of dermal or of periosteal origin has been an issue of considerable debate. Wislocki (1942), Wislocki and Waldo, 1953, Goss (1969, 1995, 1992) stated that after the previous hard antler is cast, the cells in the dermal layer of the pedicle skin give rise to the antler blastema (see below). Thus, it was argued, the dermis, which elsewhere in the body is responsible for producing the regeneration-inhibiting scar, is the very tissue that makes antler regeneration possible. However, other authors suggested that the PP (Kierdorf and Kierdorf, 1992), or the PP together with bone marrow on the immediate pedicle casting plane [Gruber (1937), cited in Kierdorf et al., 2003], may provide the main cell source for regenerating antlers. The controversy was resolved after a breakthrough was made by Li et al. (2007b) who inserted an impermeable membrane between the PP and skin to separate the contact between these two tissues at the onset of antler regeneration (Fig. 3C). Li et al. showed that an antler was regenerated despite the pedicle skin not participating in the process (Fig. 3D). However, the new antler was skin-less, which suggests that the skin accompanies new growth as a protective covering, but does not initiate it. Therefore, regenerating antlers arise from cells that are resident in the PP, but not in the pedicle skin.

4. The derivative of a postnatally retained embryonic tissue

The remarkable ability of a finite number of PP cells to support the full regeneration of a complex mammalian appendage such as deer antlers, is not shared by any of the cell populations remaining in the stump of a lost deer leg, which at best, can only seal the open end of a long bone. We believe that the unique attributes of the PP cells result from their developmental origin, as direct derivatives of the antlerogenic periosteum (AP), a tissue that overlies each frontal crest in prepubertal deer.

Surgical removal of the AP from the presumptive sites from which pedicles will form on the frontal bone (Fig. 4A) abolishes both pedicle and subsequent antler formation, while subcutaneous transplantation of the AP elsewhere on the deer body, such as to the forehead (Fig. 4B), a foreleg or even the head of a nude mouse, induces ectopic antler growth (Goss and Powel, 1985; Hartwig and Schrudde, 1974; Li, 2003; Li et al., 2001a). In addition, when a small number of the AP cells were labeled using the LacZ gene during the initiation of pedicle growth, all types of tissues in the interior component of the resultant pedicle and the antler, including their periosteum/perichondrium, contained the progeny of the labeled AP cells (Li and Suttie, 2001). Therefore, deer pedicles and antlers are direct derivatives of the AP.

Although the importance of the AP in antlerogenesis is acknowledged, the embryonic origin of AP tissue remains unclear. Given that the remarkable ability of the AP tissue for self-differentiation (perhaps unique to adult mammalian tissues) is more reminiscent of the anlage of transient embryonic tissue, such as the lateral plate mesoderm, which predicates organogenesis during development, Li and Suttie considered that the AP may represent "a piece of postnatally retained embryonic tissue" (Li and Suttie, 2001). In support, Hall (1978) proposed a neural-crest-origin of AP tissue, and recently, Mount et al. (Mount et al., 2006) provided experimental evidence for an embryological link with the neural crest. Irrespective of its embryological heritage, the progenitor cell population responsible for antler generation and regeneration exhibites substantial developmental multipotency, if not pluripotency.

Like the role played by the lateral plate mesoderm in limb bud development, the AP is the bearer of the blueprint for antler formation. Pedicle development from the AP is activated (Fig. 4C) and arrested (Fig. 4D) prenatally and reactivated during the postnatal life of male deer to form complete pedicles and antlers (Li and Suttie, 1996; Lincoln, 1973). Of paramount importance is that the

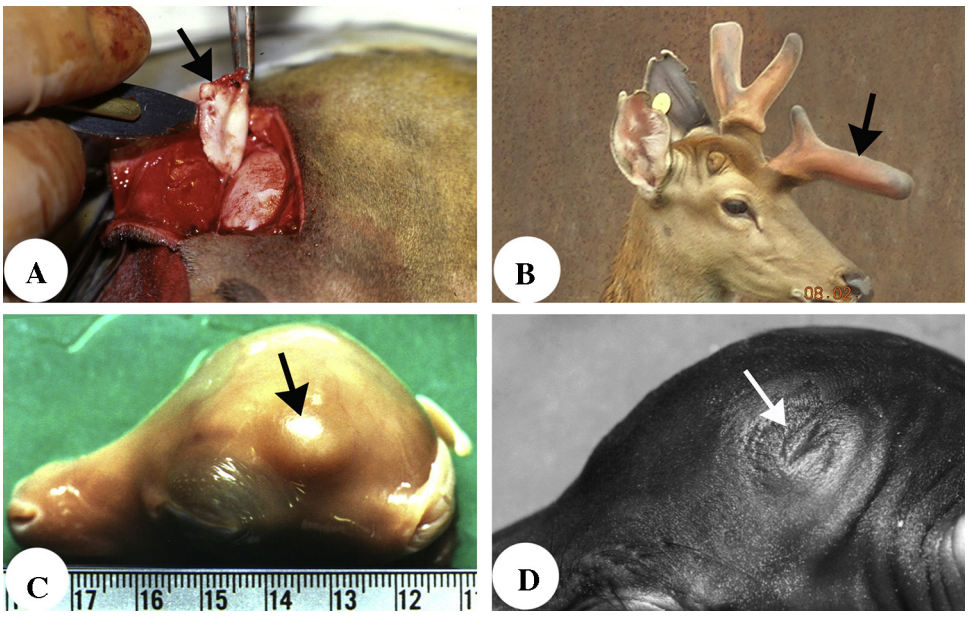

Fig. 4. Antlerogenic periosteum (AP) transplantation and prenatal development of deer pedicle/antler. (A) Surgical removal of the AP (arrow), which overlies the frontal crest of a male red deer calf. (B) An ectopic branched-antler (arrow) formed on the forehead of a three-year-old sika deer from the autologously grafted AP. (C) The head of a male fetus at about 100 days gestation. The arrow points to the pedicle primordia. (D) The head of a male fetus at about 220 days gestation. The arrow points to the repressed pedicle primordia.

AP can retain its embryonic stem cell features until postnatal life. These features include an ability to self-differentiate *in vitro* and *in vivo* (Goss, 1990a; Li and Suttie, 2001), an astonishing potential for growth (Goss, 1970) and a high concentration of intracellular glycogen (Li and Suttie, 1998). 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 legs, it may be unique to mammals. Therefore, the AP, which gives rise to the pedicle and first antler, is a piece of postnatally retained embryonic tissue, and the PP, which gives rise to subsequent regenerating antlers, is a direct derivative of the AP.

5. Adult stem cells with embryonic stem cell features

Both the AP and PP cells display an astonishing potential for population expansion. The AP, a tissue of around 2.5 cm in diameter and 2.5–3 mm in thickness, contains around five million cells that sustain the seasonal renewal of the entire antler for the extent of the deer's life (up to 28 rounds of antler regeneration). Once the AP gives rise to a pedicle, it transfers the antlerogenic potential to the PP. In each round of annual antler regeneration, the PP provides roughly three million cells, from which approximately 10 kg of antler tissue mass can be generated in 55–60 days (Li et al., 2009). Both the AP and the PP cell populations are, therefore, clearly capable of self-renewal.

To qualify as stem cells, the AP and the PP cells should express key stem cell markers and possess multipotency. We have demonstrated that both the AP and the PP cells express considerable concentrations of the embryonic stem cell antigen CD9 (Fig. 5A). The phenotypic fidelity of established stem cell lines is monitored by the characteristic expression of a defined set of transcription factors, which are thought to underpin the genetic hierarchy that maintains this unique phenotype (Ginis et al., 2004). Principal amongst these so-called 'pluripotency genes' are the POU domain family members Oct4, SOX2 and Nanog. Critically, all three genes were found to be present in both the AP and the PP cells (Fig. 5B) (Li et al., 2009; Rolf et al., 2012). Additionally, we have shown that the activity of telomerase (Fig. 5C) and nucleostemin genes was increased (Fig. 5D) in AP and PP cells (Li et al., 2009). Telomerase activity has been linked to an enhanced self-renewal in cells (Yang et al., 2008), which might explain why so few antler stem cells can form such an impressive amount of antler tissue mass within a very limited period. Expression of nucleostemin has been linked to controlling the proliferation of stem cells (Beekman et al., 2006) and regeneration of newt limbs (Maki et al., 2007). Recently, the PP cells have been shown to express Stro-1, a recognized marker of mesenchymal progenitor cells (Rolf et al., 2008). The range and nature of markers that we have observed in both the AP and the PP cells strongly suggests that these cell populations not only function as tissue specific stem cells in adult male deer, but that they retain characteristics of an embryonic origin throughout the life of the animals.

The differentiation potency of the AP and the PP cells has been investigated by several laboratories (Berg et al., 2007; Li and Suttie, 2006; Price et al., 2005). Clearly, both populations of cells can give rise to chondrocytes (Fig. 6A) and osteoblasts, respectively, when in micromass culture (Li and Suttie, 2006) and in media containing dexamethasone and ascorbate (Berg et al., 2007). As both the pedicles and antlers are composed of cartilage and bone, it was not surprising to learn that the AP and the PP cells were able to differentiate into chondroblastic and osteoblastic lineages (Kierdorf et al., 1994; Li and Suttie, 1994). In addition, when the cultured AP and PP cells were exposed to linoleic acid, or the AP to rabbit serum (Berg et al., 2007), they also differentiated into adipocytes (Fig. 6B). Furthermore, we were able to induce transdifferentiation of AP cells to multinucleated muscle precursor cells by co-culture with an established C2C12 myoblast cell line (Fig. 6C), or in medium supplemented with galectin 1. The AP cells have also been shown to form neuronal-like cells with neurite-like structures projecting from each cell body (Fig. 6D) when cultured in N2 medium, a formula that promotes neural differentiation. This result may not be totally unexpected as the AP cells are thought to be derived from the neural crest lineage (Mount et al., 2006). Overall, these observations offer the tantalizing possibility that at least the AP cells, harvested from an adult deer, may possess pluripotency, a capability previously thought improbable in postnatal mammals.

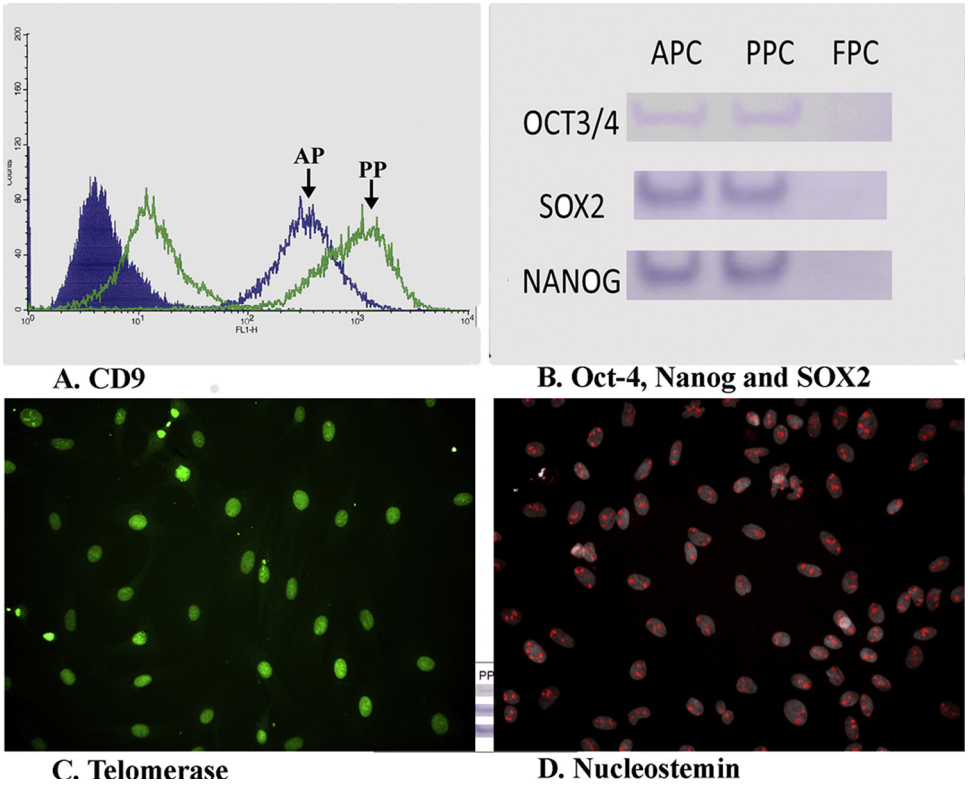

Fig. 5. Cell markers and genes associated with embryonic self-renewal and pluripotency in antler stem cell populations. (A) Cell surface expression of the embryonic marker CD9 in the AP (blue trace) and PP (green trace) cell populations. Notice that the peaks for each cell type have been offset for clarity, given the remarkable similarity in expression levels between AP and PP cells. (B) Detection of the 'pluripotency' genes Oct3/4, SOX2 and Nanog in antler stem cell populations (APC, antlerogenic periosteal cells; PPC, pedicle periosteal cells, FPC, frontal bone cells [control]). (C) Uniform expression of telomerase, the enzyme which regulates telomere length in AP cells. (D) Uniform expression of nucleostemin in AP cells. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

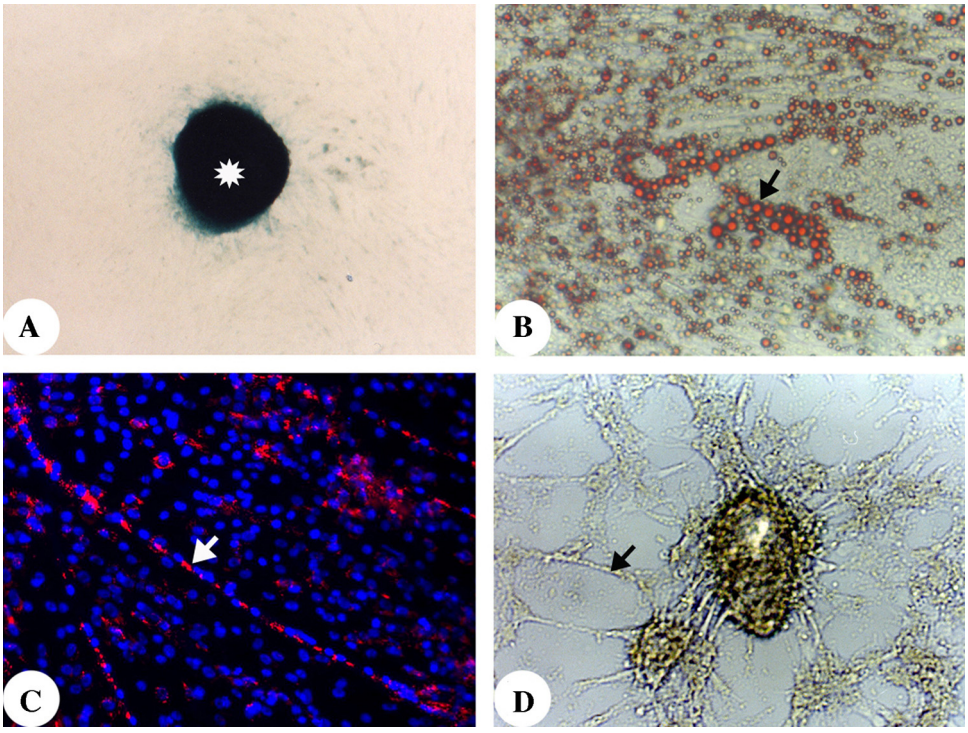

Fig. 6. Multipotency of antler stem cells *in vitro*. (A) Cartilage nodule (blue) formed by the PP cells in a micromass culture (asterisk). (B) Adipocytes differentiated from the PP cells in the culture medium containing linoleic acid (arrow). (C) Myotube (arrows) formed from AP cells (red color, labeled with fluorescent dye DiI) when co-cultured with C2C12 cells. (D) Neuronal-like cells differentiated from the PP cells when cultured in N2 medium. Notice the extended neurites (arrows) from each cell body. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

Interestingly, some biologists believe (Slack, 2000, 2008) that pluripotent cells reside in postnatal mammals, and that these derived from the neural crest, as these embryonic cells seem to undergo a more stochastic type of differentiation than other embryonic progenitor cells. Furthermore, these cells might represent a type of "embryonic remnant" comprising pluripotent cells left over from the early embryo. The collective body of work in antler research supports this "pluripotent adult stem cell" view.

6. The missing link

Deer antlers and their antecedents, the pedicles, are male secondary sexual characteristics, and as such their development is under the control of androgen hormones (Suttie et al., 1995a). The development of pedicles and antlers are closely associated with fluctuations in concentrations of testosterone. The growth of pedicles is initiated at puberty, when there is a rapid increase in circulating concentrations of testosterone, and growth continues while concentrations of testosterone remain high. In contrast, generation of the first antlers from fully formed pedicles (Li et al., 2003; Suttie et al., 1991, 1984) and regeneration of antlers in subsequent years from the pedicle stumps (Bubenik, 1982; Li et al., 1988) occurs when concentrations of testosterone are low. Indeed, antler growth occurs in a period when circulating concentrations of testosterone are barely detectable. Calcification of the antler and subsequent shedding of the velvet skin occurs when concentrations of testosterone increase sharply, just before the onset of the rutting season – a period of heightened sexual activity in males. Subsequently, concentrations of testosterone decline in spring, which is associated with the casting of hard antlers and the regeneration of new ones (Bubenik, 1982; Kierdorf et al., 1995; Li et al., 1988).

The close association of pedicle and antler development with changes in androgen hormones has been confirmed in studies that manipulated the availability of these hormones. Castration of pre-pubertal male deer abolishes the development of pedicles and subsequent antlers. This striking regulation by testosterone explains why female deer do not grow pedicles and antlers despite having a viable AP (Jaczewski, 1982). The abnormality of castration can be overcome by administration of exogenous testosterone (Jaczewski, 1982; Li et al., 2003; Wislocki, 1943). Interestingly, castration of male deer at a time when the growth of pedicles is nearing completion or just before the onset of regeneration of antlers does not affect antler generation or regeneration, respectively (Jaczewski, 1982; Li et al., 2003; Wislocki, 1943), which suggests that a transition from androgen-dependent to androgen-independent growth has occurred. After switching to an androgen-independent state, the growth rate (antler, up to 2.75 cm/day) becomes at least ten times faster than that (pedicle, approximately 1 mm/day) in the androgen-dependent state (Gao and Li, 1988; Goss, 1970).

Antlers are arguably the fastest growing mammalian organs and, therefore, nutrition (Ullrey, 1982) and growth factors (Suttie et al., 1985b) would inevitably play an indispensable role in the process. Based on his observations that antlers could continue to grow after castration, and antler generation and annual renewal take place when deer testes and seminal vesicles were the least active, Wislocki (1943) advanced the hypothesis that there must be a non-gonadal origin "antler growth stimulus (AGS)" for seasonal antler growth. In support, Suttie et al. (1985b) reported that circulating concentrations of IGF-1, a potent mitogen, are positively correlated with the rate of growth of pedicles and first antlers, which suggests that this growth factor may function as the putative AGS. Subsequently, the same team (Suttie et al., 1988) found that plasma concentrations of IGF-1 were significantly elevated in stags after their growing antlers were removed, indicating that antlers were the target, rather than the source of circulating IGF-1. Supporting this notion, both Type I and Type II IGF receptors have been identified in the antler growth centers (Elliott et al., 1992, 1993), and the mitogenic effects of IGF-1 have been shown to be dose-dependent on the growth of mesenchymal cells isolated from the antler tip (Price et al., 1994; Sadighi et al., 1994), the AP cells (Li et al., 1999, 2001b), and the PP cells in serum-free culture (unpublished data).

Thus far, the mechanism underlying the action of nutrition/growth factors in concert with sex hormones on the transformation from pedicle to antler is unknown. In order to determine how androgen hormones and nutrition/growth factors work synergistically on the transformation from pedicles to antlers, we believe that efforts should focus on the endocrine regulation *in vivo* in combination with studies targeting mechanisms that direct differentiation of the AP and the PP stem cells into their respective lineages. Some signal transduction pathways are known to operate in the regeneration of organs and/or appendages. These are determined through proteomic (Bartos et al., 2012) and transcriptomic approaches (unpublished data), where two transduction pathways, MAPK and PI3K/AKT, are activated in the PP cells at the initiation stage of antler regeneration. Mourkioti and Rosenthal (Mourkioti and Rosenthal, 2005) reported that IGFs stimulate both proliferation and subsequent differentiation of muscle progenitor cells during muscle regeneration, through activation of different signaling pathways after binding and activating type 1 IGF receptors. Specifically, MAPK signaling induced proliferation, while PI3K/AKT signaling induced differentiation (Stoick-Cooper et al., 2007). Therefore, it is possible that IGF-1 stimulates antler regeneration and growth also through the activation of MAPK and PI3K/AKT signaling pathways.

Transcripts of androgen receptors (AR) were also identified in our recent transcriptome study (unpublished data). This finding, together with the results obtained using autoradiography (Li et al., 1998) and an *in vitro* binding assay (Li et al., 2001b), demonstrate that androgen signaling is also involved in antler regeneration through binding to AR. However, the mechanism conveying the communication between androgens and growth factors in regulating the transformation from pedicle to antler is not known.

The transition from androgen-dependent to androgen-independent growth also occurs in prostate glands, another organ in males that is associated with secondary sexual characteristics, when a gland becomes cancerous (Chen et al., 2004). At the initial stage, human prostate cancer regresses dramatically after castration to remove androgen, but recurs frequently with androgen independence. The crosstalk between androgen and PI3K/AKT signaling pathways has been demonstrated to be crucial for this transition, as a synergy between PI3K/AKT and AR signaling pathways on proliferation of cells overrides the effect of castration (Guo et al., 2009; Kaarbo et al., 2010; Xin et al., 2006). Likewise, the growth of pedicles is sustained by high concentrations of androgen hormones, and antler generation/regeneration takes place at a time when concentrations of androgen hormones decrease to being barely detectable when the growth of the pedicles has been completed. Based on the findings from these aforementioned studies, we know that both androgen and PI3K/AKT signaling pathways are activated in the antler stem cells just prior to regeneration, hence the transition from androgen-dependence to – independence during regeneration might result from the crosstalk between these two pathways. The synergistic effects of PI3K/AKT and AR signaling in the transition from pedicle to antler may also override the absence of androgens. Interestingly, androgen-independence in the antler tissue is only restricted to the growth phase, because growing antler tissue still reacts to high concentrations of androgen hormones during the ossification phase, a means through which antler overgrowth is effectively

controlled before becoming detrimental to deer themselves, such as antleromas (Goss, 1990b) or peruke (Chapman, 1975), which form after castration. It would be interesting to see if high concentrations of testosterone would provide a viable treatment option for prostate cancer cells.

7. Stem cell-based *vs* classical blastema-based epimorphic regeneration

The apparent resemblance between regeneration of antlers and newt limbs has prompted some biologists to suggest that regeneration of antlers is realized through the same mechanism as that operating in lower vertebrates. Goss and Holt (1992) stated that "very much the same mechanism is utilized in the epimorphic regeneration of all appendages. In each case, be it the fin of a fish, the limb of an amphibian, the tail of a lizard, or the antler of a deer, regeneration is made possible by the development of a blastema. The existence of a blastema is indicative of epimorphic regeneration". Because the formation of a blastema is the hallmark of epimorphic regeneration, this mode of regeneration is also referred to as a "blastema-based" process. A blastema has been classically defined as the cone-shaped mass of dedifferentiated cells from diverse origins on the stump remaining after amputation of an appendage (Meschaks and Nordkvist, 1962).

Interestingly, findings from recent studies have allowed a reappraisal of the classical view of a blastema to now include multipotent resident stem cells to give rise to a blastema, rather than dedifferentiation of post-mitotic cells alone (Brockes and Kumar, 2008; Kragl et al., 2009; Morrison et al., 2006). Consequently, Bely and Nyberg (2010) have redefined "blastema" as "The blastema arises through proliferation of undifferentiated cells, either dedifferentiated cells or stem cells". In this review, we have called the previous view for blastema formation as "classical blastema-based" to distinguish the current definition of a "blastema". In order to confirm that antler regeneration is a bona fide stem cell-based process but not a classical blastema-based process, we carried out a series of studies to compare the regeneration of antlers with that of newt limbs, a gold standard for the classical blastema-based regeneration (Li et al., 2004b, 2005, 2009). Collectively, these comparisons demonstrate that regeneration of antlers is a stem cell-based process and no dedifferentiation process is involved, which is fundamentally different from that of the classical blastema-based, epimorphic regeneration of newt limbs.

Regeneration of a newt limb proceeds through four distinct morphological stages, referred to as initial wound healing, cone (blastema), palette (the flattened cone) and notch (2–3 digits) stages (Wallace, 1981). In contrast, the contour over the distal end of a pedicle stump during the course of wound healing and early antler regeneration undergoes characteristic changes from flat to deeply concave, due to the rapid augmentation of tissue mass from the anterior and posterior growth centers (Li et al., 2004b). No cone-shaped structure is formed during the initial period of antler regeneration on a pedicle stump, in contrast to the regeneration of a newt limb.

Wound healing has been considered to be a prerequisite for regeneration of newt limbs. The continuous interaction between the new epithelium covering the wound and the mesenchyme on the amputated surface initiates the epimorphic regeneration (Goss, 1972; Stocum, 2006). Indeed, in the absence of the healing wound epithelium, regeneration of newt limbs does not occur (Tsonis, 2000). In contrast, we have found that regeneration of antlers occurs even if the pedicle skin is physically prevented from participating in the healing process by inserting an impermeable membrane between the skin and the PP (Li et al., 2007b). Hence, wound healing is not an obligate requirement for regeneration of antlers.

The initial formation of a blastema on the stump of newt limbs is a nerve-dependent process, and denervation at this stage completely inhibits this epimorphic regeneration (Mescher, 1996). However, denervation of the future antler growth regions (Li et al., 1993; Suttie et al., 1995b) does not affect the formation of pedicles and first antlers, indicating that innervation is dispensable for antler development. In addition, regeneration of antlers from the denervated pedicles takes place in a similar manner to that from the non-denervated pedicles (Li et al., 1993; Wislocki and Singer, 1946). Therefore, both generation and regeneration of antlers is not dependent on a nerve supply, which provides a further distinction from the classical blastema-based regeneration of newt limbs.

It is known that the formation of a classical blastema requires all cell types in the immediate amputation plane of a stump to participate in the growth of a newt limb (Mescher, 1996). This does not seem, however, to be the case for regeneration of antlers. In a typical deer farm practice, velvet antlers are sometimes removed 2 cm above the junction between the pedicle and antler in order to maximize economic returns at their mid-late growth stage. The remaining antler stumps may still retain the potential for partial antler regeneration. In such cases, the antlers always develop from the distal antler/pedicle periosteum and are enveloped by velvet skin without any contribution from the central bony tissue of the antler stumps (Li et al., 2004b), which again, departs from regeneration of newt limbs, which require all tissue types in the stump to participate in the process.

Regeneration of newt limbs through the formation of a blastema is a scar-less process (Wallace, 1981). In contrast, regeneration of antlers leaves an obvious scar on the antler from the wound healing process (Li et al., 2009). The blastema formed on the stump of a newt limb is avascular and capillaries do not invade until the blastema is fully formed (Mescher, 1996). In contrast, early regenerating antler buds are richly vascularized (Li et al., 2005). In the newt limb stump, proliferating cells are evident throughout the blastema (Wallace, 1981). In marked contrast, in the early regenerating antler buds, dividing cells are found predominantly in the mesenchymal layer and in the vascular walls of the precartilage zone, suggesting that a regenerating antler bud contains localized growth centers, which is again departs from the classical blastema-based regeneration process.

The ability to delay formation of the basal lamina until after a blastema has fully formed on a newt limb is the feature that distinguishes regenerative from non-regenerative appendages (Mescher, 1996). The basal lamina is a thin layer located between the epidermis and the dermis. Therefore, if antler regeneration were to take place through the formation of an initial blastema, it might be expected that the basal lamina is absent from the healing skin over a pedicle stump. However, this is not the case, and a well-developed basal lamina is evident throughout the healing skin over the pedicle stump (Kierdorf et al., 2007; Li et al., 2004a).

It is well established that metalloproteinase enzyme MMP9 plays a major role in histolysis (Mescher, 1996), and is crucial for cells at the amputated surface of the stump of a newt limb to migrate, dedifferentiate and accumulate under the wound epithelium, to form the classical blastema. The wound-healing phase in newts is, therefore, always associated with high expression of the MMP9 gene (Mescher, 1996). In contrast, MMP9 mRNA is not detectable in the region of wound healing over the cast surface of a pedicle stump (Li et al., 2004a). These observations further support the notion that migration and dedifferentiation of the PP cells is not a prerequisite for regeneration of antlers.

After a carefully review of the relevant literature, it seems that there is a tendency for stem cell or progenitor-based processes to operate more in the regeneration of simple structured

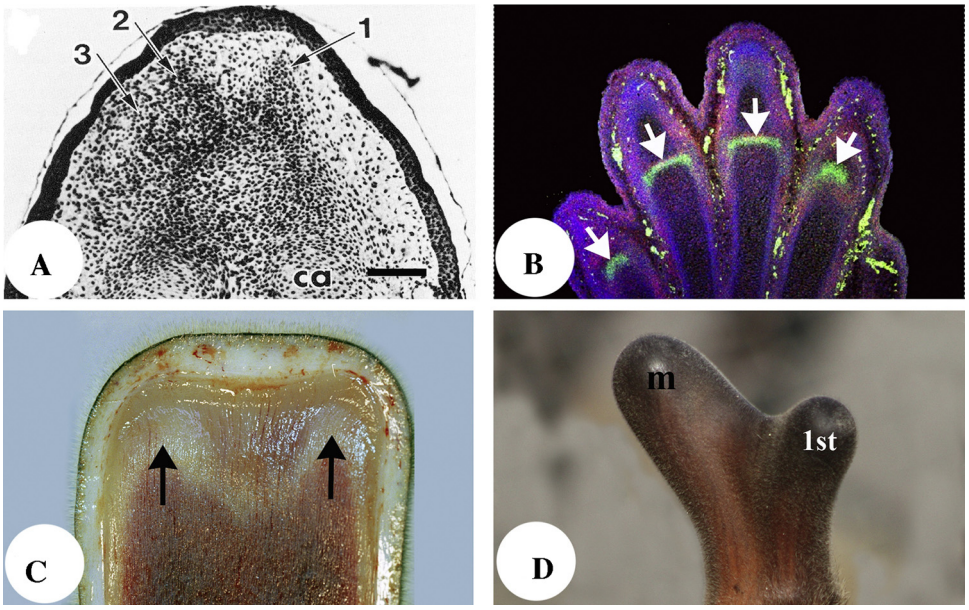

Fig. 7. Blastema-based *vs* stem cell-based epimorphic regeneration. (A) Blastema-based limb regeneration over a newt limb stump (reproduced with permission from Neufeld, 1982; Devel Biol. 93:36–42). Notice that at the early stage (notch) the miniature organ has already taken shape (three toes labeled using 1, 2, 3). Bar = 0.2 mm. (B) Joint formation in a hand through a blastema-based process (arrows). (C) Stem cell-based antler regeneration. The species-specific shape is gradually unfolding during antler regeneration as the antler grows proximodistally. Notice that a new growth center has formed for the first tine and is separated from the growth center of the main beam of the antler before the tine is visible externally (arrows). (D) A two-tined antler with the main beam (m) and the first tine (1st).

systems, and that the classical blastema-based process is associated more with the regeneration of complex structures like organs and/or appendages. Daily turnover of cells to counteract wear and tear (*e.g.* renewal of blood cells or epidermis), and compensatory growth in response to the increased functional load (*e.g.* removal of one kidney or hepatectomy) are typical examples of stem cell or progenitor-based regeneration (Goss, 1983; Stocum, 2002). Regeneration of limbs and other structurally complex body parts in urodele amphibians is a classical blastema-based process (Carlson, 2007; Stocum, 2006). A plausible explanation for this is that a classical blastema-based process allows for a miniature prototype-structure of the lost part to be formed (Fig. 7A). This process compliments that of ontogeny, wherein a mini-organ, including joints, is developed at the initial stage (Fig. 7B), and then development progresses to match the size of the organ that was lost. In contrast, a stem cell-based process builds up the missing tissue mass through proliferation and differentiation of the resident stem cells, and as such, it may not be compatible for the formation of morphologically and structurally complex organs and/or appendages. Again at odds with that dogma, the regeneration of deer antlers, which are morphologically complex mammalian appendages, is a stem cell-based process. The encoded morphogenetic blueprint of species-specific antlers is unfolded as the appendages elongate (Fig. 7C and D) and is driven by the multiplication of stem cell-derived mesenchymal cells in the apex of the growing antler. Despite the impressive regenerative capabilities of antlers, it remains unclear if such a process can cope with the regeneration of joints and muscles, as these are absent from antlers. Perhaps this reflects a limitation of this type of regeneration.

8. Lessons learnt from antler for regenerative medicine

The ultimate goal from studying regeneration of antlers is not to satisfy one's curiosity about this unique phenomenon, but to learn whether it can be used as a suitable model for regenerative medicine. Through the course of evolution, vertebrates have lost the ability to replace their missing appendages (Wallace, 1981), such that mammals now retain only a negligible potential to regenerate the tips of digits after damage (Han et al., 2005). The consequences of lost mammalian limbs have been studied histologically in some detail using mice (Neufeld, 1985). Externally, the full thickness of skin heals the stump of the wound, with the formation of a scar being the final outcome. Internally, the distal periosteal cells of the stump are activated by the mechanical trauma and enter a rapid phase of proliferation and differentiation. Subsequently, a significant amount of cartilage is formed around the distal end of the stump by these activated periosteal cells (Fig. 8A). At the same time, a limited number of fibroblasts migrate centripetally over the amputated plane to seal the open end of the long bone. Shortly thereafter, the newly formed cartilage is quickly remodeled into bone (Fig. 8B). Interestingly, the processes occurring in the stage of early antler regeneration are surprisingly similar to those observed in the healing processes over the wound of the stump of an amputated mouse limb (Neufeld and Aulthouse, 1986). In both cases, (1) the wounds over the stumps are healed with the full thickness of skin and with the formation of a scar, although in most cases the scars that form over a pedicle stump are less obvious (Li et al., 2004a); (2) cells from the periostea of the distal stumps of both a pedicle and a mouse limb are activated to enter a mode of rapid proliferation and differentiation to form cartilage; and (3) significant amounts of cartilaginous tissue are formed, which surround the distal ends of the stumps, and a very limited amount of cartilage is found to cover the amputation/cast plane (Fig. 8A and C). The most notable difference to set these two processes apart is the potential of the periosteal cell populations to proliferate. In mouse limbs, proliferation ceases as soon as the newly formed cartilage seals the open end of the amputated long bone, and subsequently the nascent cartilage formed is remodeled to bone (Fig. 8B); whereas, deer PP cell populations continue to produce cartilage tissue until the entire antler is fully regenerated (Fig. 8D).

Full regeneration of antlers is solely achieved through the proliferation and differentiation of a single cell type, the PP cells, on the cast plane of a pedicle stump. Through these comparisons, we can see that the limb stumps of mammals, as observed in mice, cannot

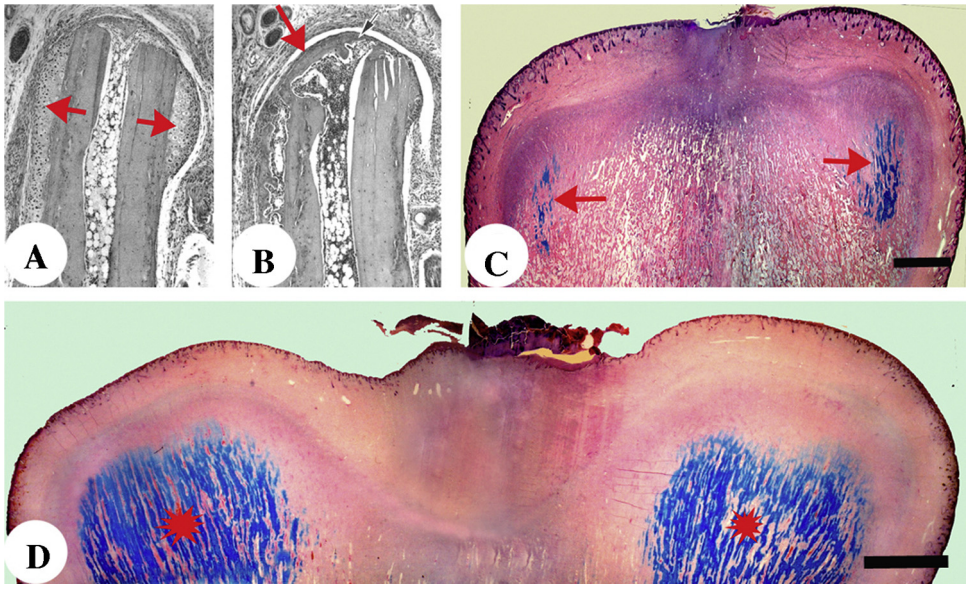

Fig. 8. Proliferative potential of periosteal cells and regeneration of appendages. (A and B) Bone healing over the stump of a mouse limb (vertical histological sections; reproduced with permission from Neufeld, Anat Rec 1985; 211(2): 156–165). (A) A week after amputation, periosteal cells have entered a phase of rapid proliferation and differentiation to form a substantial amount of hyaline cartilagesurrounding the distal end of the stump of the bone (arrows). (B) After 18 days, the healing process has stabilized and all the differentiated cartilage tissue has remodeled into bone (arrow). (C and D) Early regeneration of a deer antler (vertical histological sections). (C) A substantial amount of cartilaginous tissue has formed from the proliferation and differentiation of the PP cells in the newly established anterior and posterior growth centers (arrows). Bar = 2 mm. (D) The growth centers of the antler first tine and the main beam (stars), which develop from the initial anterior and posterior growth centers, respectively, through continuous proliferation and differentiation of the PP and PP-derived cells. Bar = 3 mm.

grow beyond the wound-healing phase to even partially replace their lost organ because of the limited potential of periosteal cells in long bones to proliferate. If we can impart a greater potential of these periosteal cells to proliferate to a similar extent to that of the PP cells through a means of dedifferentiation/reprogramming (Takahashi and Yamanaka, 2006) or transdifferentiation (Thorel et al., 2010), we might be able to realize the dream of regenerating limbs in humans. Indeed, an overgrowth of bone over the limb stump sometimes occurs in subjects after an amputation transects the long bone (Firth et al., 2011). This phenomenon occurs most commonly in children under 12 years of age, but never after a person reaches skeletal maturity, which indicates that as long as the periosteal cells of the long bone possess the ability to proliferate, they would be able to extend a stump further. If we can properly control and manage this partial regeneration, we might be able to further enhance the functionality of amputees and achieve a better outcome beyond that of wound healing alone. Overall, a better understanding of the mechanisms that regulate the regeneration of antlers, the only mammalian organ that can fully regenerate, may provide a valuable insight to aid in the development of future treatment options in the rapidly developing field of human regenerative medicine.

Conflicts of interest

The authors have no conflicts of interest to disclose.

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References

Banks WJ, Newbrey JW. Light microscopic studies of the ossification process in developing antlers. In: Brown RD, editor. Antler development in Cervidae. Kingsville, TX: Caesar Kleberg Wildlife Research Institute; 1982. p. 231–60.
Bartos L, Bubenik GA, Kuzmova E. Endocrine relationships between rank – related behavior and antler growth in deer. Front Biosci (Elite Ed) 2012;4:1111–26.
Beekman C, Nichane M, De Clercq S, Maetens M, Floss T, Wurst W, et al. Evolutionarily conserved role of nucleostemin: controlling proliferation of stem/progenitor cells during early vertebrate development. Mol Cell Biol 2006;26:9291–301.
Bely AE, Nyberg KG. Evolution of animal regeneration: re-emergence of a field. Trends Ecol Evol 2010;25:161–70.
Berg DK, Li C, Asher G, Wells DN, Oback B. Red deer cloned from antler stem cells and their differentiated progeny. Biol Reprod 2007;77:384–94.
Brockes JP, Kumar A. Comparative aspects of animal regeneration. Annu Rev Cell Dev Biol 2008;24:525–49.
Bubenik GA. Endocrine regulation of the antler cycle. In: Brown RD, editor. Antler development in Cervidae. Kingsville, TX: Caesar Kleberg Wildlife Research Institute; 1982. p. 73–107.
Carlson B. Principles of regenerative biology. New York: Academic Press; 2007.
Chapman DI. Antlers-bones of contention. Mammal Rev 1975;5:121–72.
Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med 2004;10:33–9.
Elliott JL, Oldham JM, Ambler GR, Bass JJ, Spencer GS, Hodgkinson SC, et al. Presence of insulin-like growth factor-I receptors and absence of growth hormone receptors in the antler tip. Endocrinology 1992;130:2513–20.
Elliott JL, Oldham JM, Ambler GR, Molan PC, Spencer GS, Hodgkinson SC, et al. Receptors for insulin-like growth factor-II in the growing tip of the deer antler. J Endocrinol 1993;138:233–42.
Firth GB, Masquijo JJ, Kontio K. Transtibial Ertl amputation for children and adolescents: a case series and literature review. J Child Orthop 2011;5:357–62.
Gao Z, Li C. The study on the relationship between antler's growth rate, relative bone mass and circulation testosterone, estradiol, AKP in sika deer. Acta Vet Zootech Sin 1988;19:224–31.
Ginis I, Luo Y, Miura T, Thies S, Brandenberger R, Gerecht-Nir S, et al. Differences between human and mouse embryonic stem cells. Dev Biol 2004;269:360–80.
Goss R. Regeneration versus repair. In: Cohen I, Diegelmann R, Lindblad W, editors. Wound healing – biochemical and clinical aspects. Philadelphia: Saunders; 1992. p. 20–39.
Goss RJ. Principles of regeneration. New York: Academic Press; 1969.
Goss RJ. Problems of antlerogenesis. Clin Orthop 1970;69:227–38.
Goss RJ. Wound healing and antler regeneration. In: Maibach HI, Rovee DT, editors. Epidermal wound healing. Chicago, IL: Yearbook Medical Publishers; 1972. p. 219–28.
Goss RJ. Deer antlers. Regeneration, function and evolution. New York, NY: Academic Press; 1983.
Goss RJ. Of antlers and embryos. In: Bubenik G, Bubenik A, editors. Horns, pronghorns, and antlers. New York: Springer-Verlag; 1990a. p. 299–312.

Goss RJ. Tumor-like growth of antlers in castrated fallow deer: an electron microscopic study. Scanning Microsc 1990b;4:715–20.
Goss RJ. Future directions in antler research. Anat Rec 1995;241:291–302.
Goss RJ, Holt R. Epimorphic vs. tissue regeneration in Xenopus forelimbs. J Exp Zool 1992;261:451–7.
Goss RJ, Powel RS. Induction of deer antlers by transplanted periosteum. I. Graft size and shape. J Exp Zool 1985;235:359–73.
Guo Z, Yang X, Sun F, Jiang R, Linn DE, Chen H, et al. A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. Cancer Res 2009;69:2305–13.
Hall BK. Developmental and cellular skeletal biology. New York: Academic Press; 1978.
Han M, Yang X, Taylor G, Burdsal CA, Anderson RA, Muneoka K. Limb regeneration in higher vertebrates: developing a roadmap. Anat Rec B: N Anat 2005;287:14–24.
Hartwig H, Schrudde J. Experimentelle untersuchungen zur bildung der primaren stirnauswuchse beim Reh (*Capreolus capreolus* L.). Z Jagdwiss 1974;20:1–13.
Jaczewski Z. Brown RD, editor. The artificial induction of antler growth in deer. Kingsville, TX: Caesar Kleberg Wildlife Research Institute; 1982. p. 143–62.
Kaarbo M, Mikkelsen OL, Malerod L, Qu S, Lobert VH, Akgul G, et al. PI3K-AKT-mTOR pathway is dominant over androgen receptor signaling in prostate cancer cells. Cell Oncol 2010;32:11–27.
Kierdorf H, Kierdorf U. State of determination of the antlerogenic tissues with special reference to double-head formation. In: Brown R, editor. The biology of deer. New York: Springer-Verlag; 1992. p. 525–31.
Kierdorf U, Kierdorf H, Knuth S. Effects of castration on antler growth in fallow deer (*Dama dama* L.). J Exp Zool 1995;273:33–43.
Kierdorf H, Kierdorf U, Szuwart T, Gath U, Clemen G. Light microscopic observations on the ossification process in the early developing pedicle of fallow deer (*Dama dama*). Ann Anat 1994;176:243–9.
Kierdorf U, Kierdorf H, Szuwart T. Deer antler regeneration: cells, concepts, and controversies. J Morphol 2007;268:726–38.
Kierdorf U, Stoffels E, Stoffels D, Kierdorf H, Szuwart T, Clemen G. Histological studies of bone formation during pedicle restoration and early antler regeneration in roe deer and fallow deer. Anat Rec 2003;273A:741–51.
Kragl M, Knapp D, Nacu E, Khattak S, Maden M, Epperlein HH, et al. Cells keep a memory of their tissue origin during axolotl limb regeneration. Nature 2009;460:60–5.
Li C. Development of deer antler model for biomedical research. Recent Adv Res Updates 2003;4:256–74.
Li C. Deer antler regeneration: a stem cell-based epimorphic process. Birth Defects Res C Embryo Today 2012;96:51–62.
Li C, Harris AJ, Suttie JM. Autoradiographic localization of androgen-binding in the antlerogenic periosteum of red deer (*Cervus elaphus*). In: Milne JA, editor. Recent development in biology of deer. Scotland: Edinburgh; 1998.
Li C, Harris AJ, Suttie JM. Tissue interactions and antlerogenesis: new findings revealed by a xenograft approach. J Exp Zool 2001a;290:18–30.
Li C, Littlejohn RP, Corson ID, Suttie JM. Effects of testosterone on pedicle formation and its transformation to antler in castrated male, freemartin and normal female red deer (*Cervus elaphus*). Gen Comp Endocrinol 2003;131:21–31.
Li C, Littlejohn RP, Suttie JM. Effects of insulin-like growth factor 1 and testosterone on the proliferation of antlerogenic cells in vitro. J Exp Zool 1999;284:82–90.
Li C, Liu Z, Zhao S. Variation of testosterone and estradiol levels in plasma during each developmental stage of sika deer antler. Acta Theriol Sin 1988;8:224–31.
Li C, Mackintosh CG, Martin SK, Clark DE. Identification of key tissue type for antler regeneration through pedicle periosteum deletion. Cell Tissue Res 2007a;328:65–75.
Li C, Sheard PW, Corson ID, Suttie JM. Pedicle and antler development following sectioning of the sensory nerves to the antlerogenic region of red deer (*Cervus elaphus*). J Exp Zool 1993;267:188–97.
Li C, Suttie JM. Light microscopic studies of pedicle and early first antler development in red deer (*Cervus elaphus*). Anat Rec 1994;239:198–215.
Li C, Suttie JM. Histological examination of the antlerogenic region of red deer (*Cervus elaphus*) hummels. N Z Vet J 1996;44:126–30.
Li C, Suttie JM. Electron microscopic studies of antlerogenic cells from five developmental stages during pedicle and early antler formation in red deer (*Cervus elaphus*). Anat Rec 1998;252:587–99.
Li C, Suttie JM. Deer antlerogenic periosteum: a piece of postnatally retained embryonic tissue. Anat Embryol 2001;204:375–88.
Li C, Suttie JM. Recent progress in antler regeneration and stem cell research. In: Bartos L, Dusek A, Kotrba R, Bartosova-Vichova J, editors. Advances in deer biology. Prague; 2006. p. 18.
Li C, Suttie JM, Clark DE. Deer antler regeneration: a system which allows the full regeneration of mammalian appendages. In: Suttie JM, Haines SR, Li C, editors. Advances in antler science and product technology. Mosgiel, New Zealand: Taieri Print Ltd.; 2004a. p. 1–10.
Li C, Suttie JM, Clark DE. Morphological observation of antler regeneration in red deer (*Cervus elaphus*). J Morphol 2004b;262:731–40.
Li C, Suttie JM, Clark DE. Histological examination of antler regeneration in red deer (*Cervus elaphus*). Anat Rec A Discov Mol Cell Evol Biol 2005;282:163–74.
Li C, Wang W, Manley T, Suttie J. No direct mitogenic effect of sex hormones on antlerogenic cells detected in vitro. Gen Comp Endocrinol 2001b;124:75–81.
Li C, Yang F, Li G, Gao X, Xing X, Wei H, et al. Antler regeneration: a dependent process of stem tissue primed via interaction with its enveloping skin. J Exp Zool A: Comp Exp Biol 2007b;307:95–105.
Li C, Yang F, Sheppard A. Adult stem cells and mammalian epimorphic regeneration-insights from studying annual renewal of deer antlers. Curr Stem Cell Res Ther 2009;4:237–51.
Lincoln GA. Appearance of antler pedicles in early foetal life in red deer. J Embryol Exp Morphol 1973;29:431–7.
Maki N, Takechi K, Sano S, Tarui H, Sasai Y, Agata K. Rapid accumulation of nucleostemin in nucleolus during newt regeneration. Dev Dyn 2007;236:941–50.
Meschaks P, Nordkvist M. On the sexual cycle in the reindeer male. Acta Vet Scand 1962;3:151–62.
Mescher A. The cellular basis of limb regeneration in urodeles. Int J Dev Biol 1996;40:785–95.
Morrison JI, Loof S, He P, Simon A. Salamander limb regeneration involves the activation of a multipotent skeletal muscle satellite cell population. J Cell Biol 2006;172:433–40.
Mount J, Muzylak M, Allen S, Okushima S, Althnaian T, McGonnell IM, et al. Antlers may regenerate from persistent neural crest like stem cells. In: Bartos L, Dusek A, Kotrba R, Bartosova-Vichova J, editors. Advances in deer biology. Prague; 2006. p. 161.
Mourkioti F, Rosenthal N. IGF-1, inflammation and stem cells: interactions during muscle regeneration. Trends Immunol 2005;26:535–42.
Neufeld DA. Bone healing after amputation of mouse digits and newt limbs: implications for induced regeneration in mammals. Anat Rec 1985;211:156–65.
Neufeld DA, Aulthouse AL. Association of mesenchyme with attenuated basement membranes during morphogenetic stages of newt limb regeneration. Am J Anat 1986;176:411–21.
Price J, Faucheux C, Allen S. Deer antlers as a model of Mammalian regeneration. Curr Top Dev Biol 2005;67:1–48.
Price JS, Oyajobi BO, Oreffo RO, Russell RG. Cells cultured from the growing tip of red deer antler express alkaline phosphatase and proliferate in response to insulin-like growth factor-I. J Endocrinol 1994;143:R9–16.
Rolf HJ, Kierdorf U, Kierdorf H, Schulz J, Seymour N, Schliephake H, et al. Localization and characterization of STRO-1 cells in the deer pedicle and regenerating antler. PLoS ONE 2008;3:e2064.
Rolf HJ, Niebert S, Niebert M, Gaus L, Schliephake H, Wiese KG. Intercellular transport of Oct4 in mammalian cells: a basic principle to expand a stem cell niche? PLOS ONE 2012;7:e32287.
Sadighi M, Haines SR, Skottner A, Harris AJ, Suttie JM. Effects of insulin-like growth factor-I (IGF-I) and IGF-II on the growth of antler cells in vitro. J Endocrinol 1994;143:461–9.
Slack JM. Stem cells in epithelial tissues. Science 2000;287:1431–3.
Slack JM. Origin of stem cells in organogenesis. Science 2008;322:1498–501.
Stocum D. Regenerative biology and medicine. New York: Academic Press; 2006.
Stocum DL. Regenerative biology and medicine. J Musculoskelet Neuronal Interact 2002;2:270–3.
Stoick-Cooper CL, Moon RT, Weidinger G. Advances in signaling in vertebrate regeneration as a prelude to regenerative medicine. Genes Dev 2007;21:1292–315.
Suttie J, Fennessy P, Mackintosh C, Corson I, Christie R, Heap S. Fennessy P, Drew K, editors. Sequential cranial angiography of young red deer stags. Royal Society of New Zealand; 1985a. p. 263–8.
Suttie JM, Fennessy PF, Gluckman PD, Corson ID. Elevated plasma IGF 1 levels in stags prevented from growing antlers. Endocrinology 1988;122:3005–7.
Suttie JM, Fennessy PF, Lapwood KR, Corson ID. Role of steroids in antler growth of red deer stags. J Exp Zool 1995a;271:120–30.
Suttie JM, Gluckman PD, Butler JH, Fennessy PF, Corson ID, Laas FJ. Insulin-like growth factor 1 (IGF-1) antler-stimulating hormone. Endocrinology 1985b;116:846–8.
Suttie JM, Kostyo JL, Ebling FJ, Wood RI, Bucholtz DC, Skottner A, et al. Metabolic interfaces between growth and reproduction. IV. Chronic pulsatile administration of growth hormone and the timing of puberty in the female sheep. Endocrinology 1991;129:2024–32.
Suttie JM, Li C, Sheard PW, Corson ID, Waldrup KA. Effects of unilateral cranial sympathectomy either alone or with sensory nerve sectioning on pedicle growth in red deer (*Cervus elaphus*). J Exp Zool 1995b;271:131–8.
Suttie JM, Lincoln GA, Kay RN. Endocrine control of antler growth in red deer stags. J Reprod Fertil 1984;71:7–15.
Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–76.
Thorel F, Nepote V, Avril I, Kohno K, Desgraz R, Chera S, et al. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. Nature 2010;464:1149–54.
Tsonis PA. Regeneration in vertebrates. Dev Biol 2000;221:273–84.
Ullrey D. Nutrition and antler development in white-tailed deer. In: Brown RD, editor. Antler development in Cervidae. Kingsville, TX: Caeser Kleberg Wildlife Research Institute; 1982.
Wallace H. Vertebrate limb regeneration. Chichester: John Wiley & Sons; 1981.
Wislocki GB. Studies on the growth of deer antlers. I. On the structure and histogenesis of the antlers of the Virginia deer (*Odocoileus virginianus* borealis). Am J Anat 1942;71:371–451.
Wislocki GB. Studies on growth of deer antlers. II. Seasonal changes in the male reproductive tract of the Virginia deer (*Odocoilius virginianus* borealis), with a discussion of the factors controlling the antler-gonad periodicity. Essays in biology in Honour of H M Evans. Berkeley/Los Angeles: University of California Press; 1943. p. 631–53.

Wislocki GB, Singer M. The occurrence and function of nerves in the growing antlers of deer. J Comp Neurol 1946;85:1–19.

Wislocki GB, Waldo CM. Further observations on the histological changes associated with the shedding of the antlers of the white-tailed deer (*Odocoileus virginianus* borealis). Anat Rec 1953;117:353–75.

Xin L, Teitell MA, Lawson DA, Kwon A, Mellinghoff IK, Witte ON. Progression of prostate cancer by synergy of AKT with genotropic and nongenotropic actions of the androgen receptor. Proc Natl Acad Sci U S A 2006;103:7789–94.

Yang C, Przyborski S, Cooke MJ, Zhang X, Stewart R, Anyfantis G, et al. A key role for telomerase reverse transcriptase unit in modulating human embryonic stem cell proliferation, cell cycle dynamics, and in vitro differentiation. Stem Cells 2008;26:850–63.