Antler Regrowth in Deer as a Model of Mammalian Tissue Regeneration.

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Introduction

Much of the work performed in the field of regenerative biology has been investigated using organisms such as the axolotl. Although the axolotl is capable of regenerating limbs there is a considerable phylogenetic distance between amphibians and mammals. This paper considers the ability of deer to re-grow antlers as a system of mammalian tissue regeneration that may have therapeutic potential.

Antler Phenotypes and Behaviour

Antlers are curious structures. Unlike horns, which are keratinised tissue growing from an underlying mesenchymal cell layer, antlers are organs. They have blood vessels, nerves, skin, cartilage and bone, and grow under strict endocrine control. They are shed each year, providing the only example of organ or limb regeneration in mammals. Furthermore, the correct signals for antler development are only turned on at puberty. Thus they are the only example of the development of an organ that is delayed (Price *et al.* 2005).

Figure 1. North American Reindeer, with large, highly branched velvet covered antlers. Credit Dean Biggins, http:images.fws.gov U.S. Fish & Wildlife Service Licence: Public Domain

Antlers are unique to deer, but not all species have them, nor is their biology the same across the species. Reindeer (*Rangifer tarandus*), for example, are the only species in which the females develop antlers (Cegielski *et al.* 2005).

Antlers come in many shapes and sizes, from a few centimetres long in the pudu (*Pudu puda*) to the magnificent palmated antlers up to 1.5 metres in length in the moose (*Alces alces*) (Price *et al.* 2005).

Antlers usually begin to grow in the spring of deer's second year, but moose, roe, white-tailed and mule deer fawns may produce tiny antlers during their first autumn, whilst reindeer calves start to grow antlers within weeks of being born (Putman, 1988).

Many mammalian species have evolved systems of masculine competition which minimise physical damage, such as the establishment of dominance purely by display. The size of antlers partially determines strength without the need for violence. Thus, antlers are condition-dependent indicators of fitness, in addition to their practical role for protection against predators (Andersson, 2006) (Putman, 1988).

Antler Growth and Development

Periosteum is a thick membrane that covers the surface of bones. Osteoblasts (cells that develop into bone) and other cells important in the maintenance and repair of bone, are associated with the periosteum. Antlers develop from pedicles (the base from which the antler grows) on the frontal bone of the skull from antlerogenic periosteum (AP) (Goss and Powell, 1985). Pedicles develop in the males around the time of puberty. Rising androgen levels in the blood activate periosteal cells in part of the frontal bone of the skull to form this tissue. The initial stage of development is characterised by the differentiation of antlerogenic cells into osteoblasts. When the pedicle is between 5 and 15 millimetres in height, cartilage is formed which ossifies (Li and Suttie, 1994).

Figure 2. Caribou antlers near Noatak River, Selawik National Wildlife Refuge, Alaska.
Credit, Jo Goldmann, U.S. Fish & Wildlife Service
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The annual regeneration of antlers begins in early summer when food is plentiful. The regrowth places a huge strain on the animal's mineral reserves, with 30kg of bone growing in just under three months. The skeleton undergoes a process of "cyclic reversible osteoporosis", occurring most notably in non-weight bearing bones, such as the ribs (Bubenik, 1983). Given that there is no resorption of the antler (apart from a negligible amount at the pedicle before shedding) the stag has to replace this quantity of tissue and minerals each year.

In the summer months stags are reproductively inactive, and circulating testosterone levels are low (Goss, 1983), suggesting antler regeneration is under non-gonadal control. IGF-1, a mediator of growth hormone, synthesised in the liver, may be the controlling influence. IGF-1 is circulating in high concentrations at this time (Suttie *et al.* 1985), IGF receptors can be found in the growing tip of the antler (Elliott *et al.* 1993) and IGFs have been seen to promote the proliferation of antler cells *in vitro*.

Shedding or casting of the antlers normally occurs every year in winter or early spring and is under the control of the sex steroid, testosterone. The circulating levels of testosterone change in response to increasing daylength. Antlers are usually cast within a day or so of each other, and the process if very tightly regulated. Osteoclasts (large multinucleate cells that resorb calcified bone) resorb bone in the distal pedicle (the root or base of the antler) causing the antlers to be shed (Goss and Powell, 1985).

Antler Regeneration and Limb Development

Antler regeneration appears to have more similarities with the development of limbs in a mammalian foetus than with the regeneration of the limb of urodeles. Antler regeneration involves the development of bone, cartilage, skin, nerve tissue and vasculature from the antlerogenic periosteum (AP) which may be postnatally retained embryonic tissue (Li and Suttie, 2001).

Li and Suttie (2001) suggested similarities between the complex regulatory processes behind limb and antler development. Transplantation of both Lateral Plate Mesoderm (LPM) in mice from which limbs develop and the antlerogenic periosteum (AP) in deer results in the development of limbs or antlers, respectively. Both limb bud and antler pedicle development starts with cell proliferation from one form of embryonic tissue the mesenchyme (Li and Suttie 1994). AP contains all the information for antler development in the same way the LPM does for limb development (Li and Suttie, 2001).

One of the main agreements in the field of antler research is that the ability of the antler to regenerate is held within the entire pedicle (Li et al. 2007), as transplantation experiments have shown. However, it was not known which specific tissue from the pedicle was required for regeneration, nor was it known whether regeneration was dependent on the interactions of different tissue types between the bone and the covering skin. Li et al. 2007 determined that the pedicle bone and not the pedicle skin was the tissue giving rise to the regenerating antler, and that the interactions between the skin and bone were vital for regeneration, but only in a priming capacity. The pedicle skin played no role in the following regenerative process (Li et al. 2007).

Molecular Pathways involved in Antler Growth

Different chemical signals interact to form an antler from the embryonic like tissue of the pedicle. There is an increasing body of evidence showing that the molecular regulation of regeneration in amphibians and antlers, are recapitulations of pathways involved in embryonic development (Mount *et al.* 2006).

There are many signalling pathways, acting both locally and systemically, acting on stem and progenitor cells to produce an organ. Two important factors (among several others) are retinoic acid and parathyroid hormone related peptide.

Retinoic acid (RA) regulates skeletal development and regeneration in urodele amphibians such as the axolotl (Scadding and Maden, 1994). *In vivo* studies performed in the 1990s provided evidence that exogenous RA application influenced the development of the first antler, and led to acceleration and alteration of the direction of the growth, whilst increasing the volume of the primary antler (Kierdorf and Kierdorf, 1998) (Kierdorf and Bartos, 1999). Findings published by Allen *et al.* (2002) confirmed the role of RA in the regulation of cell differentiation in the

growing antler. Levels of RA were measured in antler tissues during all the stages of differentiation, and were comparable to levels found in developing limb buds and regenerating urodele limbs (Price and Allen, 2004) (Scadding and Maden, 1994). RA may also regulate the early stages of antler regeneration at the site of wound healing (Price and Allen, 2004).

Parathyroid hormone related peptide (PTHrP) plays an important role in a number of developmental systems. *In vivo* models using knockout mice have confirmed that PTHrP is essential for the development of a normal bone mass (Amizuka *et al.* 2000). Acting through its receptor (PPR), it regulates the differentiation of cells that are the pre-cursors of cartilage, the chondrocytes, in the developing skeleton (Kronenberg, 2003).

In antler studies, PTHrP has been shown to increase proliferation and decrease differentiation of chondrocytes (Faucheux and Price, 2001) and PTHrP is expressed in high levels in the tissues forming cartilage in antlers (Price and Allen, 2004). Antler osteoblasts also express PTHrP and its receptor, suggesting it has a role in the regulation of the differentiation of osteoblasts, an idea supported by several *in vitro* models (Price and Allen, 2004).

Future Developments

Antler re-growth appears to rely upon the maintenance of an embryonic like set of cells within the pedicle that are spurred into action on an annual basis by a circulating growth factor. A cocktail of local hormones, proteins and other growth factors maintains and directs growth. The challenge lies in understanding the way in which all the different signalling pathways interact to form an antler.

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Author Profile

Daniel is 21 years old and began studying in the School of Biosciences after completing his A-Levels, graduating in 2007 with a 2:1 BSc (Hons) in Animal Science. As part of his dissertation, Daniel attended the sixth International Deer Biology Congress in Prague, thoroughly enjoying the chance to attend such an event, finding the whole experience invaluable. Whilst studying at the School of Biosciences, Daniel was particularly interested in the photoperiodic control of the endocrine system. Daniel will begin to study for a degree in medicine in 2007 and hopes to enjoy a career combining both research and patient contact.