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Current Opinion in Genetics & Development

Regeneration in the spiny mouse, *Acomys*, a new mammalian model

Aaron Gabriel W Sandoval and Malcolm Maden

We describe the tissues and organs that show exceptional regenerative ability following injury in the spiny mouse, *Acomys*. The skin and ear regenerate: hair and its associated stem cell niches, sebaceous glands, dermis, adipocytes, cartilage, smooth muscle, and skeletal muscle. Internal tissues such as the heart, kidney, muscle, and spinal cord respond to damage by showing significantly reduced inflammation and improved regeneration responses. The reason for this improved ability may lie in the immune system which shows a blunted inflammatory response to injury compared to that of the typical mammal, but we also show that there are distinct biomechanical properties of *Acomys* tissues. Examining the regenerative behavior of closely related mammals may provide insights into the evolution of this remarkable property.

Address
Department of Biology & UF Genetics Institute, University of Florida, Gainesville, FL, USA

Corresponding author: Maden, Malcolm (malcmaden@ufl.edu)

Current Opinion in Genetics and Development 2019, 64:xx–yy

This review comes from a themed issue on **Cell reprogramming, regeneration and repair**

Edited by **Pentao Liu** and **Antonio Jacinto**

https://doi.org/10.1016/j.gde.2020.05.019

Introduction

If you pose the question – can mammals regenerate? – the typical reply is likely to be no. Certainly not compared to the prodigious powers displayed by zebrafish and salamanders. This impression is probably prevalent because we all have scars on our skin, the heart fibroses after a myocardial infarction and ends up killing us, and paraplegia is the likely result of spinal cord injury. But if we delve a little more deeply then there are actually some tissues with surprising regenerative powers even in mammals. Skeletal muscle regenerates extremely well after certain types of damage [1], the liver can regenerate provided it is not chronically damaged [2], deer antlers regenerate each year covered by full thickness skin [3], postnatal mice and rats can regenerate the heart even after amputation of the apex of the ventricle [4,5•], embryonic skin heals scarlessly [6], and childrens' fingertips can regenerate after amputation as can the tips of adult mouse digits [7,8]. Perhaps these are the remaining vestiges of a formerly widespread regenerative ability in mammals which has been lost in evolution. If so, perhaps not all mammals have lost regenerative ability and some have retained it. After all, we currently have surveyed very few of the 5400 species of mammals for regeneration so there may be some mammals that can regenerate very well but have yet to be discovered. We suggest that the spiny mouse of the genus *Acomys* is one such example of regenerative powers which have been evolutionarily conserved.

There are 18 species of *Acomys* in the genus which are characterized by spine-like hairs on the dorsum, and they are in the subfamily called Deomyinae. The closest relatives to this subfamily are the gerbils and not the old world mice and rats on which most mammalian regenerative studies are conducted. A phylogenetic study of the distribution of regenerative powers among rodents would be fascinating because of the remarkable regeneration that *Acomys* species display as we now describe.

Skin

Following full-thickness dorsal skin excisional wounds of 4 mm–1.5 cm, *Acomys* is capable of regenerating hairs, erector pili smooth muscle, sebaceous glands, panniculus carnosus (PC) skeletal muscle, adipose tissue, and dermis [9••]. The same wound in the lab mouse, *Mus*, forms a hairless scar with dense collagenous dermis and no PC muscle. After wounding, *Acomys* re-epithelializes the wound faster than *Mus*, an observation also seen in *in vitro* scratch assays using isolated keratinocytes [10]. The *Mus* wound ECM is dense and composed mostly of collagen I, whereas that of *Acomys* is porous and composed of collagen III [9••]. Microarray and RT-qPCR data have revealed that at least 8 collagen types are highly upregulated in *Mus*, with collagen XII being upregulated up to 30-fold after wounding. Comparatively, there are fewer collagens upregulated in the *Acomys* wound [11] and they display profiles that are reminiscent of fetal wounds, which highly express collagens III and V [12]. Proteomic analysis corroborated this and also revealed that *Acomys* wounds exhibit increased levels of extracellular matrix remodeling proteases [13]. Between three and four weeks post-injury, hairs regenerated in the *Acomys* wound bed, but none were found in *Mus* [9••]. Compared to *Mus*, uninjured *Acomys* skin contains a larger hair bulge with a high expression of the stem cell markers CD34, K15 and

Sox2 [14], and since hairs are regenerated in the *Acomys* skin it means that the new stem cell niches of the hair bulge, sebaceous gland and dermal papilla can be respecified, the former two presumably from the basal cells of the newly formed wound epidermis and the latter from the dermal fibroblasts (Figure 1).

In another type of injury to the skin, that of a full thickness burn injury, *Acomys* was also found to regenerate perfectly in contrast to the hairless scar produced by *Mus* [15].

Ear punches

Following ear biopsy punches of 2 mm–8 mm, *Acomys* is capable of wound closure and regeneration of the missing tissue. In addition to the accessory organs that regenerated in the dorsal skin (above), *Acomys* also regenerated cartilage in its ears [9••,16]. Although ear cells re-enter the cell cycle in both *Acomys* and *Mus*, only *Acomys* cells progress through the cell cycle and ultimately proliferate. The regenerating *Acomys* ear demonstrated all the characteristics of a mammalian blastema including being able to form a wound epidermis, maintaining a pro-regenerative ECM, accumulating dividing mesenchymal cells, and becoming innervated. Thus, *Acomys* ear regeneration is epimorphic, similar to the blastema-mediated regeneration observed in salamander limb and zebrafish fin regeneration. Importantly, wound size does not impact regenerative ability, and all healthy *Acomys* adults are capable of regeneration [17] (Figure 1).

Skeletal muscle

Myotoxins which cause breakdown of the sarcolemma are typically injected into skeletal muscle to examine its regenerative ability. In response to this acute damage, *Acomys* tibialis anterior muscle regenerated in eight days, 2–3 days faster than *Mus*. RT-qPCR data showed that *Acomys* exhibited lower levels of NF-kB, indicative of inflammation, and TGFβ-1 and collagens, indicative of fibrosis, as well as higher levels Cxcl12, an anti-inflammatory cytokine. In response to chronic damage in the form of multiple rounds of repeated myotoxin injections (5 of them), *Acomys* was able to regenerate perfectly; however, *Mus* was unable to do so, instead producing adipocytes throughout the muscle, generating a histological picture reminiscent of Duchenne muscular dystrophy [18] (Figure 1).

Acomys also demonstrated the first known case of *de novo* regeneration of skeletal muscle in the absence of an existing, instructive extracellular matrix. This phenomenon is termed volumetric muscle loss (VML). Following dermal skin excision wounding, *Acomys* regenerates the panniculus carnosus (PC) of the skin which re-expresses adult muscle myosins and displays neuromuscular junctions. Immediately after injury, myogenic regulatory factors (MRFs) are downregulated in both *Acomys* and *Mus*. After two weeks, *Acomys* MRF levels increase to normal, indicating muscle regeneration. Histological analysis shows newly formed myofibers positive for embryonic myosin differentiating in the *Acomys* wound bed. Embryonic myosin is upregulated 450-fold, whereas in *Mus* it is barely detectable at the wound margins [19•] (Figure 1). Similar PC muscle regeneration was observed in *Acomys* following burn injury as described above [15].

Heart

Myocardial infarction (MI) was induced via ligation of the left anterior descending coronary artery. Following this injury, *Acomys* exhibited an infarct area that was fourfold smaller and displayed increased coronary microvasculature compared to *Mus*. In the left ventricle of *Acomys*, over twice as many BrdU-positive, proliferating cardiomyocytes were observed compared to *Mus*. Four weeks post-injury, MRI showed that *Acomys* left ventricular ejection fraction (LVEF) had returned to baseline levels observed in sham animals, whereas *Mus* levels remained significantly lower than their sham counterparts. Furthermore, wall ventricular thickness was significantly lower in *Mus* treated with MI compared to sham animals, but no such difference was observed between sham and treated groups in *Acomys*. Increased expression of angiotensin converting enzyme 2 and certain microRNAs, which could have cardioprotective effects, were observed in Acomys [20–22] (Figure 1).

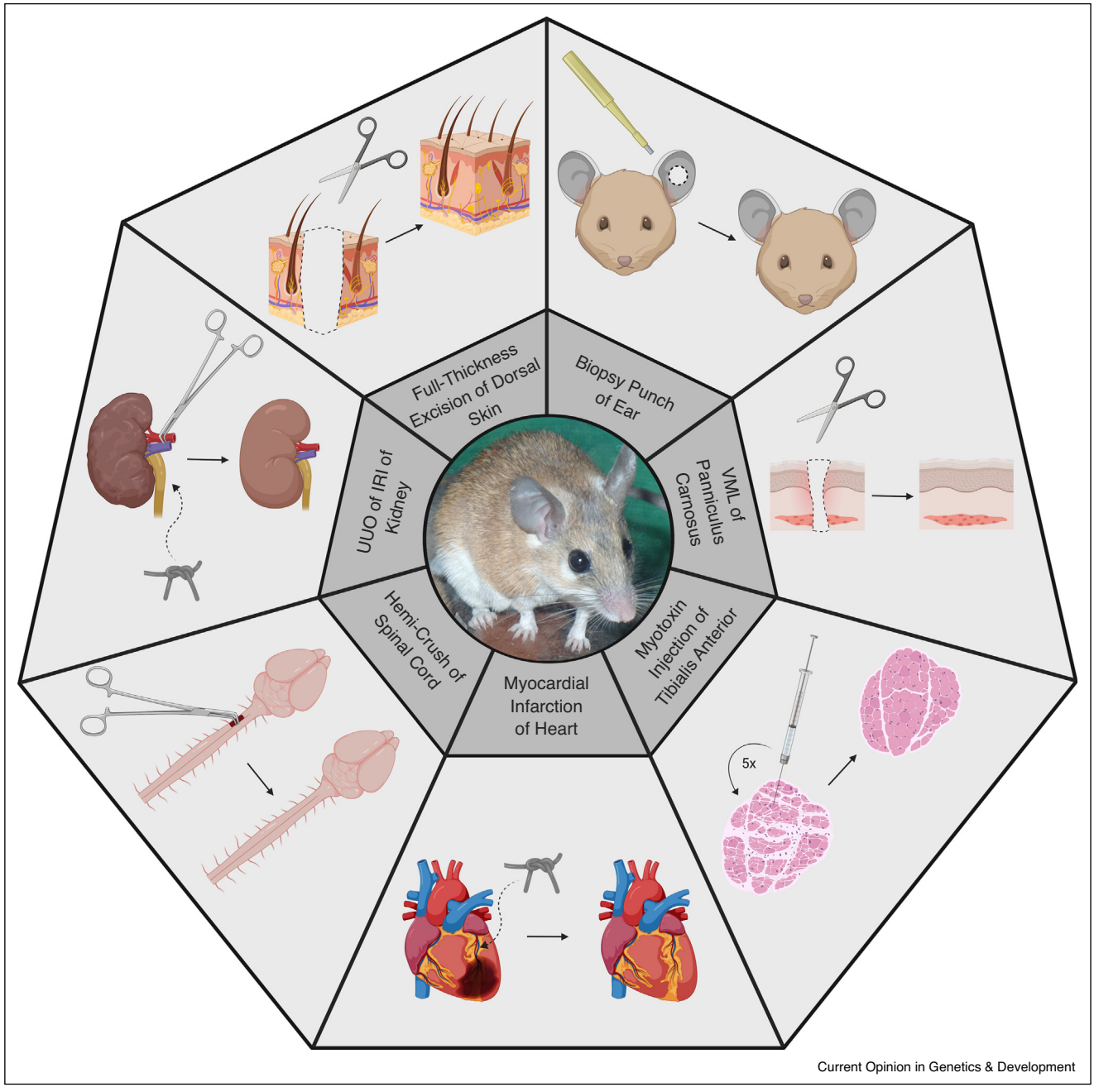
Spinal cord

A C3/4 lateral dorsal hemi-crush injury was induced using forceps to damage the spinal cord. *Acomys* exhibited lower levels of inflammation and scarring compared to *Mus*. *Acomys* resumed bladder voiding ability two days after injury, while it took over two weeks for *Mus* to regain the same ability. RT^2 Profiler PCR arrays (Qiagen) for wound healing and neurogenesis were used to assay both species. Generally, *Mus* upregulated more genes associated with wound healing, whereas *Acomys* upregulated more genes associated with neurogenesis. *Mus* upregulated pro-inflammatory, extracellular matrix remodeling, and fibrosis genes, while *Acomys* upregulated WNT-signaling, neural stem cell, and axonal guidance genes. Growth factor and *Tgfb1* genes were upregulated in both species. Compared to *Mus*, *Acomys* injuries exhibited decreased levels of collagen IV and GFAP immunostaining, two major components of the spinal cord scar [23] (Figure 1).

Kidney

Unilateral uretal obstruction (UUO) was conducted by ligating the left ureter with 4−0 silk. After two weeks of obstruction, *Acomys* maintained normal anatomic structure and kidney weights, while *Mus* kidney weights rapidly declined due to fibrosis. Extensive interstitial matrix fibrosis was observed in *Mus*, but none was evident in *Acomys*. Even after three weeks of obstruction, there was no difference in total collagen levels between

Figure 1



Summary of the organs and tissues in *Acomys* that have been investigated for regenerative ability and the results of those experiments. In the centre is an *Acomys cahirinus*. The experiments described in the text are shown here. From the top going clockwise are: a biopsy punch through the ear shows a 4 mm circle through the left ear regenerating perfectly; a VML injury of the skeletal muscle in the skin (the panniculus carnosus) is induced when full thickness skin is removed and the diagram shows that it can regenerate; when a myotoxin such as cardiotoxin is injected into the skeletal muscle and this is repeated five time the muscle still regenerates perfectly; when a myocardial infarction is induced by tying off the left descending coronary artery then the damage can be partially repaired; when a hemi-crush of the spinal cord is performed there is far less fibrosis at the site of injury than normal and a different set of genes are induced; when the kidney is damaged by obstructing the ureter or by inducing temporary ischemia then there is far less fibrosis; when full thickenss skin is removed then all of the components including hairs and sebaceous glands can regenerate without a scar. VML = volumetric muscle loss, UUO = unilateral ureteral obstruction, IRI = ischemia reperfusion injury.

obstructed and contralateral kidneys in *Acomys*. Cdh1 protein levels were used to measure tubular integrity, a surrogate for kidney function. *Acomys* Cdh1 was maintained in the injured kidney, whereas *Mus* Cdh1 levels progressively declined. Unilateral ischemia reperfusion injury (IRI) was also conducted by placing a vascular clamp on the left renal pedicle for 40 min. Histologically and functionally, *Acomys* and *Mus* were equivalently injured when analyzed at 24 hours and 72 hours postsurgery Again, fibrosis was observed in *Mus*, while *Acomys* was able to recover both kidney structure and function. After 16 days, *Acomys* exhibited normal blood urea nitrogen levels, while *Mus* exhibited extremely elevated levels indicative of kidney failure [24•] (Figure 1).

What is the cellular and molecular basis of this regenerative behavior?

There are two clear physiological differences between *Acomys* and *Mus* that are systemic and, therefore, relevant to all the tissues described above. These distinctions may begin to explain the basis of regenerative behavior versus fibrosis. The first is the immune system and its components since a strong inflammatory response prevents infection by killing pathogens but can also damage tissue, induce fibrosis, and inhibit regeneration. A blunted immune system is characteristic of the mammalian fetus and lower vertebrates capable of regeneration, and the inverse relationship between advanced immune systems and the ability to regenerate is well recorded [25].

In line with this concept, *Acomys* has a relatively dampened response following injury, characterized by low or absent expression of inflammatory chemokines and cytokines. In contrast, *Mus* strongly expresses several inflammatory pathway genes and proteins after wounding [11–13]. *Acomys* blood also has a lower proportion of neutrophils and a higher proportion of lymphocytes compared to *Mus* [11]. The differential blood compositions necessitate that each species defends against pathogens differently. In *Mus,* neutrophils play an important role in killing bacteria. To compensate for its deficiency of neutrophils, the serum of *Acomys* blood predominantly kills bacteria, which decreases inflammation from neutrophils while still protecting against infection [26]. Moreover, different T cell populations were found to infiltrate regenerating and non-regenerating wounds. In *Mus,* inactivated T helper cells accumulate. However, there is an early influx of cytotoxic and regulatory T cells in *Acomys*, which could potentially be modulating inflammation and encouraging regeneration rather than fibrosis [27].

Macrophage profiles following injury differ between *Acomys* and *Mus*. *In vitro* experimentation has shown that F4/80, a pan macrophage marker in *Mus*, only marks M1 macrophages in *Acomys* [28]. Following skin excision, burn, ear punch, and myotoxin injection injuries, no F4/80-positive M1 macrophages are found in the *Acomys* wound, whereas F4/80 staining is abundant in *Mus* throughout the wound healing process [11,15,18,28]. Following kidney injury, F4/80 levels are significantly lower in *Acomys* than in *Mus* [24•]. While CD86-positive M1 macrophages were present in *Acomys* burn wounds and ear punches [15,28], none were found in *Acomys* myotoxin wounds [18]. However, *Acomys* and *Mus* wounds have similar numbers of CD206-positive M2 macrophages [18,19•,28]. This lack of M1 but abundance of M2 macrophages during regeneration may be explained in part by the fact that *Acomys* upregulates Il10, which facilitates macrophage transition from M1 to M2 [19•]. When macrophages are depleted via clodronate liposomes, *Acomys* ear punch closure is inhibited, signifying that macrophages are necessary for regeneration. Macrophages are a source of reactive oxygen species (ROS) production after injury, which may serve as a pro-regenerative signal in *Acomys*. ROS production is higher and prolonged in *Acomys* [28], and *Acomys* fibroblasts are resistant to the ROS-induced cellular senescence and decreased proliferation observed in *Mus* [29•].

The second systemic difference between *Acomys* and *Mus* tissues is a mechanical one. *Acomys* demonstrates the first known instance of mammalian skin autotomy, allowing its skin to be sloughed off in order to escape predation [9••]. Accordingly, *Acomys* skin is 20 times weaker than *Mus* skin, requiring 77 times less energy to break. *Acomys* has a thicker layer of adipose than *Mus*, and it is postulated that this could contribute to its observed weakness [14]. But this softness is not only a property of the skin but also of skeletal [18] and cardiac muscle, and biomechanical differences are also apparent in cultured cells [10]. In response to a wound, fibroblasts are activated to become myofibroblasts expressing increased levels of aSMA. *In vitro*, *Mus* fibroblasts can be led down this path by increasing substrate stiffness; however, *Acomys* fibroblasts do not become activated when cultured on stiffer substrates. Furthermore, *Acomys* fibroblasts generate lower contractile forces than *Mus* fibroblasts. Finally, compared to *Mus* fibroblasts, *Acomys* fibroblasts produce much less collagen *in vitro*. In three-dimensional culture, *Mus* fibroblasts remodeled their surroundings, whereas *Acomys* fibroblasts did not. Thus, the resulting matrices were stiffer for *Mus* fibroblasts than *Acomys* [10].

Conclusion

Each tissue in *Acomys* that has been examined, as described above, shows a non-fibrotic, regenerative response to damage suggesting that this genus has evolved a property which affects all the tissues of the body. It will be important, therefore, to continue the survey of regenerative abilities across further tissues and organs of the body such as the brain, retina, and long bone cartilage which are subject to traumatic damage and of medical relevance to determine whether the regenerative response is a body-wide property. This will give us

important clues for deciphering the underlying mechanisms. It will also be important from an evolutionary point of view to examine the regenerative abilities of closely related genera such as *Lophuromys* (the brush-furred mouse), *Deomys* (the Congo forest mouse) or *Uranomys* (Rudd's mouse) to determine whether regenerative ability is more widely distributed than *Acomys* and to make genomic comparisons to identify mechanisms. Hopefully, these future investigations will provide some mechanistic answers to how *Acomys* regenerates so that we can translate these findings to stimulate human tissue regeneration.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Aaron Gabriel W. Sandoval: Writing - original draft. **Malcolm Maden:** Writing - review & editing.

Acknowledgements

Work from the authors' lab has been funded by W.M. Keck Foundation, National Science Foundation (1636007) and National Institutes of Health (1R21 0D023210, 1R21 0D028209).

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