Regeneration science needs to broaden its focus to understand why some organisms can regenerate and others not

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When *Science* celebrated its 125th anniversary in 2005, the editors of the journal published a special issue in which they took a look at the most compelling questions faced by scientists. Among the top 25 questions was "what controls organ regeneration?" In the accompanying essay [1], *Science* argued that regenerative medicine will not be able to rebuild organs and tissues, unless researchers understand regeneration from a broad biological perspective – identifying the molecular and cellular signals that guide regeneration in regeneration-competent organisms, as well as those that prevent it in regeneration-incompetent organisms, including humans.

Such a call for a comparative strategy is diametrically opposite to the approach still employed by the overwhelming number of investigators in regeneration research, even 10 years after the publication of the special issue of *Science*. These investigators focus on a select few laboratory species, mostly mammals, which serve as models of regeneration-incompetence in humans. On the other hand, scientists who employ comparative strategies recognize the need to better understand the diverse forms of regeneration by studying a broad range of organisms, including such regeneration champions as the freshwater polyp *Hydra*, the flatworm *Planaria*, the hermit crab *Eupagarus*, lampreys, teleost fish and salamanders.

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It may come as a surprise to many that a scholar commonly associated with a one-model system approach – the use of the fruit fly *Drosophila melanogaster* to explore the role of chromosomes in heredity – pursued early in his career a comparative approach, with a remarkable diversity of organisms studied to elucidate mechanisms of regeneration. While still a graduate student, Thomas Hunt Morgan began to conduct experiments on the regeneration of earthworms, followed by a series of investigations on hydrozoans, planarians, sea urchins, crustaceans, killifish and amphibians, to name just a few. His book 'Regeneration', published in 1901 [2], was regarded as the most authoritative treatise of the subject at that time, and brought regeneration into the context of developmental mechanics. He argued that the study of a diversity of organisms was critical to the understanding of regeneration. At the same time, he was convinced that technological advances were key to any progress in this field [3]. Although Morgan remained interested in the phenomenon of regeneration throughout his life, it might have been the lack of suitable methods that led him to abandon research on regeneration, in favor of the study of genetics.

Today, more than a century after Morgan's ground-breaking work, unprecedented opportunities arise from the comparative study of organisms that can spontaneously regenerate. These opportunities are due to both the establishment of powerful comparative model systems, and the availability of modern biological methods, including big data analysis technologies, to study these systems. The overarching goal of these investigations is to identify the molecular signaling pathways shared by organisms that are able to regenerate successfully, and, by comparing them with regeneration-incompetent organisms, to establish the molecular and cellular differences that divide these two groups. The information extracted from using this approach will enable investigators to identify potential targets for the development of therapies so that, ultimately, the limits of regeneration-incompetent organisms can be overcome.

The comparison of regeneration-competent organisms with regeneration-incompetent organisms is particularly promising in cases of two closely related species because of the similarity of their molecular identities. Laboratory mice (genus *Mus*) and spiny mice (genus *Acomys*) both belong to the same taxonomic family – Muridae – but exhibit a fundamental difference in their ability to regenerate

skin. In its natural habitat, *Acomys* can shed parts of its skin to escape predation. The resulting wounds heal rapidly. Although certain strains of adult laboratory mice have also been reported to heal skin after injuries, the two healing processes differ significantly. As a result, in *Mus* scar tissue develops, while in *Acomys* the wounds heal by rebuilding the lost tissue through new cells arising from the proliferative activity of a blastema-like structure [4].

Similar to *Acomys*, adaptation to vulnerability caused by predation may have been one (although certainly not the only) important factor in the evolution of the regenerative potential of many organisms. Knifefish of the taxonomic order gymnotiforms are distinguished by their elongated, compressed caudal part of the body ('tail'), which includes a major portion of their spinal cord. In their natural habitat, these fishes often suffer from damage to, or loss of, parts of the tail because of predatory fish specialized in tail-eating. Perhaps in response to this selective pressure, gymnotiforms have developed an extraordinary ability to regenerate tails, including spinal cord tissue – even after repeated loss of the tail (for review, see [5]). The structural repair is achieved through activation of intrinsic stem cells that give rise to both neurons and glial cells, resulting within a few weeks in the full recovery of behavioral functions controlled by spinal motoneurons [6].

The capability of these fish for neural regeneration extends to the brain [7]. Equally remarkable, their neurogenic potential does not show any significant age-related decline in the brain [8]. This feature contrasts with the situation in mammalian species, which are characterized by a dramatic age-related depletion of endogenous stem cell populations and/or an increase in the number of stem cells entering a quiescent state [9,10]. This is likely the major causative factor preventing successful neural regeneration during adult stages of development in mammalian species. These findings not only demonstrate that the study of regeneration-competent model systems can lead to a better understanding of how tissues can perform self-repair, they are also likely to provide new insights into another issue of major biomedical relevance – how brains may be protected from senescing.

The notion of a close association of cellular senescence and the ability to regenerate tissue has recently received striking support through experiments performed using another well-established model of regeneration competence, limb regeneration in salamanders [11,12]. These experiments have shown that there is a significant induction of cellular senescence during the intermediate stages of regeneration. However, the senescent cells diminish subsequently through a macrophage-mediated clearance mechanism. This finding in a regeneration-competent system has important implications for the identification of potential targets to promote a regeneration-permissive environment in adult mammals.

Despite the exciting discoveries made in regard to the capabilities of regeneration-competent organisms, the identification of the molecular signaling pathways was, until recently, severely hampered by the lack of genomic resources. However, with the advent of relatively cheap high-throughput technologies, this drawback has been overcome (for review, see [13]). Reference transcriptomes are now available for a number of these nonmainstream model systems, including salamanders [14,15] and knifefish [16]. Proteomic analysis has led to the global identification of regeneration-associated proteins involved in successful brain and limb regeneration [17–19]. Comparative proteomic analysis of regeneration-competent and regeneration-restricted organisms has not only revealed commonalities among these organisms but also provided insights into the differences between them, which have enabled investigators to relate signaling pathways to the failure of the latter organisms to successfully regenerate [20].

The combining of high-throughput technologies with modern molecular approaches to modulate gene expression has created a powerful methodological arsenal to identify candidate molecules and to study their function. Together with the availability of well-characterized regeneration-competent and regeneration-incompetent model systems, we have now available all the tools that Thomas Hunt Morgan considered to be essential for the understanding of regeneration. Researchers and funding agencies alike should make it a priority to take advantage of this unique convergence. To not do so would be an unforgivable mistake.

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