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OBITUARY

# Andrew Johnson (1958-2021)

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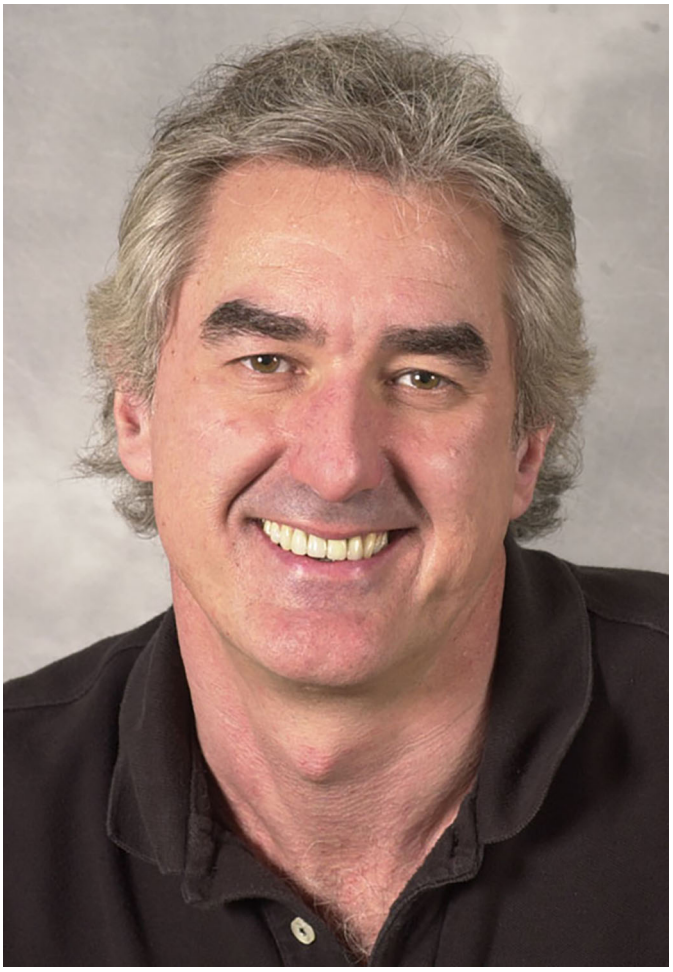
Andrew Johnson, a pioneer in the development of the amphibian axolotl as a model to study the early stages of metazoan development, died 15th September 2021. Known as 'AJ' by his family, and by his friends and colleagues, his older sister Pam referred to him as an unstoppable 'force of nature' who at the age of 9 or 10 said to her, 'I'm going to become a professor'. Here, we reflect on AJ's life and work, paying particular attention to his studies on the establishment of primordial germ cells in vertebrates.



AJ had a long-standing interest in the development of primordial germ cells (PGCs), the embryonic forerunners of adult gametes. Germ cells produce each new generation, but, in the embryo, they develop alongside the precursors of somatic cells, which comprise the present generation. What discriminates germ cells from somatic cells in early development is a question of enduring biological interest. After graduating from Hunter College, City University of New York in 1980 with a BA in Biology, AJ studied germ cell development both in the mouse (with Rosemary Bachvarova at Cornell University) and in *Xenopus* (with Dennis Smith at Purdue University), completing his PhD in 1990 under Smith's supervision.

Smith's seminal studies on germ plasm in frog embryos are cited to this day. He was influential in promoting the view that germ plasm, and cell-autonomous PGC specification in general, is conserved throughout the animal kingdom, an idea reinforced by the existence of germ plasm-like material in species as unrelated as nematode worms and flies. This hypothesis stood undisputed for decades until Pieter Nieuwkoop reported that axolotl (salamander) embryos do not contain germ plasm and, strikingly, that ectopic PGCs could be induced in the animal hemisphere (animal cap) of axolotl embryos (Sutasurya and Nieuwkoop, 1974). At that time, the specification of PGCs by induction had not been reported in any other experimental system, including mice, so the work was considered controversial, or a novelty of urodeles, and was largely disregarded.

On completion of his postdoctoral research, which he carried out with Paul Krieg at the University of Texas (1990-1994), AJ made what turned out to be an inspired decision. He abandoned the highly popular *Xenopus* model to focus on the less-tractable axolotl embryo, a move predicated on Nieuwkoop's classic studies. AJ recognized parallels between axolotl and mouse development and began work on a novel theory concerning how the germ line influences development of the soma. His studies of axolotl development were initiated during his first independent position at Florida State University (1994-2001) and were continued during his 20-year tenure at the University of Nottingham, UK (2001-2021).

Developing the axolotl as a molecular model proved challenging in more ways than one, especially as the discoveries AJ made, and the hypotheses he and his collaborators proposed, contradicted some well-established biological paradigms. At that time, the established opinion was that an almost invariant set of conserved mechanisms regulates vertebrate development, a point of view that did not align with Nieuwkoop's findings. At Florida State University, AJ set out to determine whether germ plasm was indeed conserved in axolotls and, if so, whether PGCs could be specified by induction. However, axolotl embryos were not a widely used model and had not yet been studied at a molecular level. Moreover, high-throughput sequencing technologies had not been

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invented. However, in 1994 Lawson and Hage published the fate map of mouse PGCs (Lawson and Hage, 1994), then Tam and Zhou demonstrated in 1996 that mouse PGCs are specified by induction (Tam and Zhou, 1996). Putting these observations together, AJ reasoned that axolotls might share conserved mechanisms of development with mammals, and as a rationale he postulated an evolutionary explanation: that axolotls resemble the amphibious ancestor of mammals. In fact, one of us (B.I.C.) was sitting on the beach with AJ when he brought up the idea that herpetologists and evolutionary biologists had got the relationships of frogs and salamanders wrong, and that salamanders were actually related to mammals! That comment kicked off the evolutionary aspect of AJ's work. Soon after this, we presented a poster at a national meeting that was a large single image of a phylogeny of a salamander, mammal and frog, with the salamander and mammal as sister taxa. Under the figure was merely a question mark. We, of course, were treated as cranks, but we knew then we needed to fill out the phylogeny to understand the mammal-salamander relationship. This was the impetus for AJ's singular effort to resurrect Nieuwkoop's long-forgotten work, and to establish axolotl embryos as a modern experimental system.

Using molecular markers to test Nieuwkoop's hypothesis, AJ demonstrated conclusively that axolotl embryos do not contain germ plasm (Johnson et al., 2001). Further, in the same paper and after evaluating the distribution of vertebrate morphological traits and established phylogenies, AJ proposed that germ plasm is not conserved. Rather, he concluded that induction is an ancestral (basal) mechanism, and that germ plasm evolved repeatedly, by convergence. This radical hypothesis, which was subsequently extended by others to include invertebrates (Extavour and Akam, 2003), ran contrary to thinking in the field. AJ therefore sought a rational explanation to account for divergent mechanisms of PGC specification in the animal kingdom. As germ plasm is found in even distantly related animal lineages, he reasoned that it must convey an evolutionary advantage. AJ applied comparative embryology across vertebrates to conclude that species with germ plasm had evolved novel embryological traits (Johnson et al., 2003b). Specifically, he reasoned that the relatively anteriorized morphology of frogs, teleosts and birds could not have evolved from embryos employing inductive PGC specification; it was enabled by germ plasm. On this basis, AJ proposed a theory in which germ plasm enhances the potential to accumulate heritable genetic mutations that alter the soma, thereby increasing genetic variation and phenotypic diversity within a population. This is achieved by insulating the development of PGCs from the effects of random mutations in the pathways that direct embryogenesis (Johnson et al., 2003b). In effect, therefore, germ plasm increases the likelihood that a genetic mutation is heritable. Because organisms with germ plasm can pass on higher levels of mutations, these species should evolve more rapidly and this would explain why germ plasm exists exclusively in the most speciose vertebrate clades (Crother et al., 2007; Evans et al., 2014).

AJ and his co-workers reasoned that the evolution of any mechanism that enhances genetic diversity should be favored in a biological system (e.g. Crother and Murray, 2018). Because the net effect of germ plasm is an increase in heritable mutations, it would increase the phenotypic variation upon which selection can act to evolve novel traits. Perpetuation of the germ line is the guiding biological principle underlying selection for somatic traits, as originally conceived by Richard Dawkins (Dawkins, 1976). Thus, the more malleable the soma, the more likely that selection will sculpt it towards efficient propagation of the germ line. This probably underlies the divergent morphologies observed in species with germ plasm.

AJ went on to address the question of how germ cells might be specified in those species that retain the ancestral, inductive mechanism for distinguishing these cells from those destined to form the soma. Using the axolotl system, he and his collaborators discovered a stochastic mechanism that specifies PGCs. Specifically, they showed that PGC induction in this system is entirely reliant on signaling epistasis, indicating that mutations within signaling pathways would abrogate the germ line and so could not evolve (Chatfield et al., 2014). The tenuous nature of the germ line in this context is therefore a genetic constraint, and it maintains the precisely orchestrated embryology that is required to produce PGCs. Importantly, phylogenetic analysis suggests that this is the ancestral mode of germ line development in vertebrates (Johnson et al., 2003a,b), and the conserved morphogenetic movements that it requires underpin the well-documented conservation of the body plan throughout vertebrate natural history.

Another major breakthrough to emerge from AJ's group was the discovery of a novel tissue in axolotl that is an intermediate on the path to PGC induction, which they called pluripotent mesoderm (Chatfield et al., 2014). This tissue is induced by FGF and BMP signaling, and it gives rise to somatic lineages as well as germ cells. Importantly, efforts to derive human PGCs *in vitro* had suggested to AJ that equivalent tissue is induced as a prelude to PGC specification in human embryos, which is entirely in line with AJ's predictions (Johnson et al., 2003a; Johnson and Alberio, 2015). AJ subsequently used the axolotl system to investigate how a germ line-competent pluripotent fate can be maintained within specified mesodermal tissue, and how PGCs are derived from it. Crucially, equivalent tissue does not exist in *Xenopus* or zebrafish embryos, highlighting the significance of the axolotl experimental system. AJ's use of axolotls, and his focus on the ancestry of vertebrate development, led him to challenge the accepted wisdom that core gene regulatory networks (GRNs) for early vertebrate development are incapable of change. Indeed, his group showed that the GRNs for mesoderm are not conserved between axolotls and *Xenopus*, as would normally be expected (Swiers et al., 2010). Rather, his group found that GRNs are conserved between axolotls and mammals, as AJ had predicted. Pluripotency is also not conserved in *Xenopus* or zebrafish, so it was assumed to be a mammalian innovation. But AJ's group showed that the GRN that governs mammalian pluripotency is conserved in axolotls (Bachvarova et al., 2004; Dixon et al., 2010), consistent with the ability to induce ectopic PGCs in this species. AJ used the axolotl system to understand pluripotency in its simplest form, devoid of the complexities that evolved to support uterine development in mammals. He felt that such studies were destined to point the way to a deeper understanding of how cell fate specification is controlled in human embryos.

In the years leading to his death, AJ began to look into the macro-evolutionary implications of germ line-soma specification mechanisms. He was particularly struck by the work of Mike Benton, an expert on vertebrate paleontology. Over 90% of all species were eliminated during the Permian-Triassic extinction (Benton, 2016), which occurred after vertebrates had evolved germ plasm. As vertebrates repopulated the earth, there was no evidence for competitive replacement, i.e. the competitive dominance of one group of species over another. Rather, the fossil record suggests that surviving vertebrate lineages expanded into a barren ecoscape without competition. Into this void, the fossil record indicates that birds, teleosts and frogs – the lineages containing germ

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plasm – accumulated at a faster rate throughout the vertebrate recovery, which supported the conclusions of AJ's group, especially his paper with Crother and White (Crother et al., 2016). What remained unclear to paleontologists is why these vertebrate lineages, and not others, expanded most rapidly into empty niche space.

AJ was also working on the idea that when existing vertebrate populations were extinguished, survivors that evolved rapidly would most efficiently reoccupy empty niche space. Importantly, this model does not suggest that species with germ plasm would outcompete other organisms within a population at equilibrium, i.e. by selective advantage. However, they would be more successful at establishing new populations in a void, expanding into empty niches under non-competitive conditions, as had already been described by Benton. In this context, the genetic flexibility afforded by germ plasm would render individual animal lineages more fit for expansion than related creatures without germ plasm. Fitness here is thus defined by the ability to diversify, so the relative advantages of germ plasm are evident in the preponderance of species that have evolved across the vertebrate landscape, as AJ and colleagues had noted before.

In developing the axolotl embryo as a model system, AJ established a unique conceptual paradigm: he showed that even in closely related species, such as frogs and salamanders, mechanisms of development are not conserved. This necessitates a re-evaluation of the fundamental assumptions underpinning the model system approach to developmental biology, which has been dominant for decades. Indeed, it is increasingly clear that the mechanisms governing the earliest stages of development in humans and mice have diverged, consistent with AJ's predictions. AJ was clearly at the forefront of this field, working to explain how the mechanisms for vertebrate development have evolved. He anticipated the patterns of divergence and he emphasized a search for the embryological principles that underpin vertebrate evolution. His work was leading in a single direction: axolotls and humans share the basal embryological mechanisms that have been conserved since the emergence of the earliest vertebrates. This places the axolotl system that he pioneered in a unique position to unpick how the mechanisms that direct human development evolved.

AJ very much wanted to write a book as a capstone to present his ideas and evidence. He felt certain that what he and his colleagues had learnt would provide a new foundational developmental explanation for the diversity of life. To that end, AJ and one of us (B.I.C.) initiated a working outline for the book in which AJ even wanted to pay homage to Darwin by including a most fitting quote from *On the Origin of Species*:

> '*We have reason to believe, as stated in the first chapter, that a change in the conditions of life, by specifically acting on the reproductive system, causes or increases variability; and in the foregoing case the conditions of life are supposed to have undergone a change, and this would manifestly be favourable to natural selection, by giving a better chance of profitable variation occurring; and unless profitable variations occur, natural selection can do nothing.*'
>
> (Darwin, 1859)

In conclusion, it is probably fair to say that AJ's research established a model that revealed how the dynamic relationship between the germ line and the soma sculpted the patterns of metazoan natural history. His discoveries and ideas demonstrated how he was not at all afraid to think outside the box, feeling that orthodoxy was there to be challenged, and that a good scientist should cast a jaundiced eye on ideas that smelt of 'group think'. He had the least respect for those critics who made no effort to provide tenable alternatives or to formulate alternative hypotheses when faced with new information and novel interpretations with which they disagreed.

AJ thrived during his 20 years at Nottingham. With initial start-up funds from the university and seed capital from local entrepreneurs, he quickly established a colony of axolotls and secured substantial grants from the UK Medical Research Council to support a flow of postdoctoral researchers and PhD students, all well marshalled by his long-serving technician Jodie Chatfield. He also attracted a steady stream of final year undergraduates wishing to gain research experience in his laboratory, drawn by his inspirational lectures on developmental biology. He was a born teacher and communicator. Anyone visiting him in his office would be welcomed with a huge smile and a firm handshake, and would rarely be able to leave without having been engaged in a lengthy discourse on one scientific topic or another, or debating at length the significance of the latest result from his laboratory, which was often his own finding; he was a superb cellular microscopist. His infectious enthusiasm was difficult to resist, but also sometimes infuriating as new ideas and hypotheses flowed so freely that it was difficult to pin him down to publishing his findings. Indeed, some might say that his tardiness in this respect meant that the originality and significance of his work is less widely appreciated in some quarters than it deserves to be.

To colleagues, AJ was an outstanding mentor. His radical thinking, incredible dedication, and his laser sharp focus on getting to the truth was a source of inspiration to many young scientists. One of the authors of this piece (R.A.), at the start of his independent scientific career, was inspired by the idea of selective advantage in species developing novel PGC specification mechanisms and, together with Azim Surani's lab, he showed that PGC specification and epigenetic reprogramming in pigs and humans differs from that in mice (Kobayashi et al., 2017; Zhu et al., 2021). These findings support the idea proposed a few years back that mice might be to mammals what frogs are to amphibians (Johnson and Alberio, 2015). Notably, precocious lineage restriction in the mouse may have enabled the evolution of the characteristic cup-shaped embryo, which contrasts with the archetypical embryonic disc of large mammals.

AJ was born in Brooklyn, NY (before Brooklyn became cool!), one of five children born to Edward and Margaret Johnson. He lived in several places in the USA before settling finally in the UK. As a child, AJ followed and played a lot of sports. He was a pretty good athlete, had loads of friends and was always uniquely hilarious, but a little different – hence, 'I'm going to be a professor'. Always the 'big picture'; he simply put his nose to the ground and just went for it. He succeeded. He retained an enormous sense of humor right to the end. He had an infectious, *joie de vivre*. If you wanted a party to get going, AJ was your man.

The COVID-19 pandemic proved particularly difficult for AJ. He was unable to continue his laboratory studies, finding himself instead confined to work at home. As the most gregarious of individuals, he found himself 'imprisoned'. His funeral was held on 20th October 2021, followed by a large gathering of family, friends and colleagues from the UK and the USA, at the Admiral Rodney in Wollaton, one of AJ's favored watering holes. He had a great send-off. As his sister said at his funeral, AJ was very much a force of nature, unstoppable and unforgettable.

AJ is survived by his sisters Pam and Irene, his brothers Eddie and Chris, and his daughters Molly, Tessi and Maggie. He will be missed.

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