Int. J. Dev. Biol. 62: 419-429 (2018)
https://doi.org/10.1387/ijdb.170335ce

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Kidney regeneration in fish

THOMAS BATES[#,1], UTA NAUMANN[#,1], BEATE HOPPE[#,1] and CHRISTOPH ENGLERT[*,1,2]

[1]*Leibniz Institute on Aging. Fritz Lipmann Institute (FLI) and*
[2]*Institute of Biochemistry and Biophysics, Friedrich Schiller University Jena, Germany*

ABSTRACT Age-related diseases, such as kidney diseases, are becoming more prevalent in aging societies. Currently, patients with reduced kidney function require dialysis or organ transplants. Those who suffer from kidney disease would benefit from regenerative therapies. Thus, one of the ultimate goals of regeneration research is to enhance an individual's capacity of self-repairing damaged tissue; something that fish models can contribute towards. Kidney structures are conserved among vertebrates highlighting the opportunities for fish to act as human disease models. Here, different species can offer respective advantages. An understanding of the different modes of regeneration can help to visualize the differences in mammalian and fish regenerative capacity. The remarkable regenerative capacity of fish is well known, but kidney regeneration is an understudied area. The kinetics of kidney regeneration allows one to investigate early damage responses, as well as the initiation and completion of repair. Age-related reductions in regeneration are an additional societal problem; again an area where fish models can be of help. Age-matched experiments between varied vertebrate species will help us to learn from those that do or do not exhibit age-related phenotypes. The goal of such experiments is not only to outline important age-related factors and pathways, but, in addition, to see if age-related decreases in regenerative capacity can be reduced. Widening our knowledge of this very complex process will help to address many of the unanswered questions in the field.

KEY WORDS: *kidney, regeneration, fish, disease, aging*

Introduction

The kidney is an indispensable organ that regulates metabolic waste removal and fluid balance. Mammalian kidneys have only limited proliferative and regenerative potential. Thus, kidney diseases can lead to life-threatening complications. In our aging society, the incidence of renal insufficiencies is predicted to increase (PHE, 2014) and highlights the need for new and efficient therapeutic approaches. Modeling human diseases in other vertebrate species can contribute to a better understanding of the underlying mechanisms and support biomedical research.

The kidney is an organized labyrinth of tubes. Kidneys of adult humans filter approximately 180 liters of blood daily, with subsequent efficient reabsorption of the filtrate retrieving it into the circulation. Among vertebrates, the basic composition of the kidney is conserved. In particular, the structure and the development of the filtering units, the nephrons, show high similarities. Various fish models have been recognized as valuable systems to investigate renal function and development, but also to model renal diseases such as acute kidney injuries. This review will focus on models of kidney disease in fish and how these can impact future clinical and basic research.

Comparative kidney structure and function

Mammalian kidneys have multiple roles. They excrete metabolic waste products into the urine, perform osmoregulation, reabsorb metabolites and balance acid-base levels. In addition, kidneys are capable of secreting hormones and regulating systemic blood pressure, bone calcification and the stimulation of red blood cell production (Ferenbach and Bonventre, 2016). The kidney in mammals has a highly organized structure that can be anatomically divided into the cortex and the medulla. The functional units of the

Abbreviations used in this paper: AKI, acute kidney injury; CKD, chronic kidney disease.

***Address correspondence to:** Christoph Englert. Leibniz Institute on Aging – Fritz Lipmann Institute (FLI), Beutenbergstrasse 11, 07745 Jena, Germany. Fax: +49 3641 656040. Tel: +49 3641 656042. E-mail: christoph.englert@leibniz-fli.de - web: http://www.leibniz-fli.de/research/research-groups/englert - https://orcid.org/0000-0002-5931-3189

[#] These authors contributed equally to this work

Submitted: 8 December, 2017; *Accepted:* 12 December, 2017.

ISSN: Online 1696-3547, Print 0214-6282

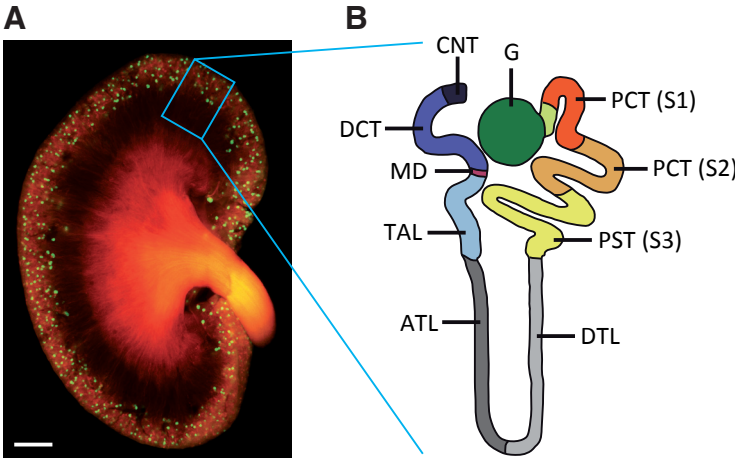

Fig. 1. Organization of the mammalian kidney and nephron. (A) *Fluorescent image of a two-month-old mouse kidney (Rosa26-flTomatofl-EGFP(B6);Pod-cre) where glomeruli are labeled in green and all other structures remain red.* **(B)** *Diagrammatic representation of nephron compartments. G, glomerulus; PCT, proximal convoluted tubule (Segments 1 + 2); PST, proximal straight tubule; DTL, descending thin limb; ATL, ascending thin limb; TAL, thick ascending limb; MD, macula densa, DCT, distal convoluted tubule; CNT, connecting tubule. Scale bar in A, 1 mm. Panel (B) adapted from (Li and Wingert, 2013).*

kidney are named nephrons and are mainly organized in the renal cortex. They show a specific segmentation (Fig. 1) responsible for the filtration of the blood, with subsequent reabsorption of 99% of the filtered electrolytes and water, and 1% excretion into the urine (Zhuo and Li, 2013).

Each segment of the nephron is composed of different cell types. The most proximal part is the glomerulus that consists of a capillary loop bound by mesangial cells, enveloped in podocytes, and enclosed by Bowman's capsule (Costantini and Kopan, 2010). Podocytes are specialized epithelial cells that possess a basket-like extension of cellular processes around the blood capillaries. The basement membrane between podocytes and capillary endothelial cells, together with specialized junctions between the podocyte processes form the blood filtration barrier. It allows passage of small molecules, ions and fluids, while retaining cells and macromolecules in the blood (Drummond and Davidson, 2010). The filtrate flows from the glomerular space into the joining nephron tubule. The tubule is composed as a pipe of mainly epithelial cells surrounded by a basement membrane (Li and Wingert, 2013) and is divided into multiple specialized segments. The epithelial cell populations that make up each tubule segment have a distinct cellular morphology and are characterized by an unique sets of solute transporters to perform discrete roles in modifying the glomerular filtrate (Davidson, 2008). In mammalian kidneys the 'Loop of Henle' (DTL and ATL see Fig. 1) is a specialized structure for concentration of urine.

When comparing the kidneys of mammals and fish species, the overall organization differs. In mammals, the kidneys are two bean-shaped organs found below the ribcage on either side of the spinal column. They display a high grade of organization, with aligned nephrons situated in defined regions of medulla and cortex, while fish kidneys are less organized. In both zebrafish (*Danio rerio*) and a distant relative the African Killifish (*Nothobranchius furzeri*) the kidney consists of a flattened structure located along the dorsal inner body wall. In *Danio rerio* there are morphologically separable head, trunk and tail kidney, in which nephrons can be detected. In contrast, in *N. furzeri*, there is only the head part of the kidney found, which is clearly enlarged compared to the zebrafish (Fig. 2) (Mccampbell *et al.*, 2015; Sander and Davidson, 2014).

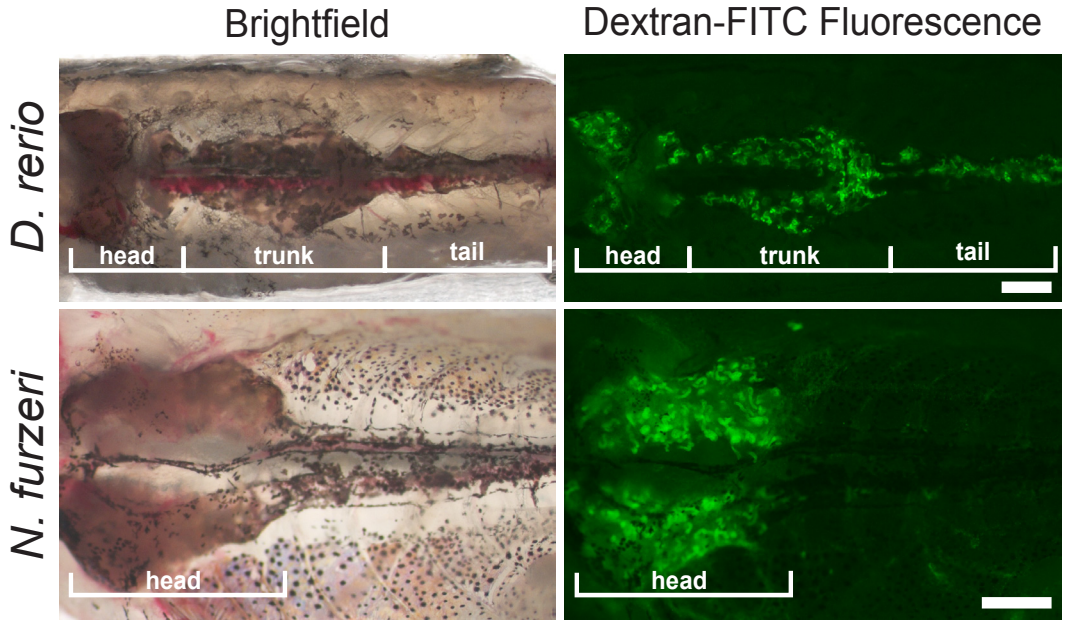

Fig. 2. Comparison of fish kidney structures. *Adult fish were injected with a 40 kDa fluorescently labeled Dextran in order to visualize the proximal tubule. Signals are observed throughout the head-, trunk- and tail portions of the* D. rerio *kidney, while in* N. furzeri, *tubules are mainly detected in the head portion. Figure adapted from Hoppe* et al. *(2015).*

Within all parts of the kidney the nephrons are homogeneously distributed, randomly oriented and interfused into hematopoietic tissue which forms a matrix around the nephrons. Although the kidney architecture varies between vertebrates, the specific segmentation of the nephrons is broadly conserved (Mccampbell and Wingert, 2014; Wingert and Davidson, 2008). As in mammals, zebrafish nephrons consist of a glomerulus, proximal and distal tubule segments and the collecting duct (Mccampbell *et al.*, 2015; Mccampbell and Wingert, 2014). However, zebrafish nephrons do not develop a loop of Henle but show a branching of the distal tubules (Mccampbell and Wingert, 2014). Furthermore, specialized cell types and orthologues of tubular segment marker genes are highly conserved between the species (Wingert and Davidson, 2008). Based on these similarities in nephron composition, fish species have emerged as favorable models to study human kidney diseases.

Types of regeneration

Vertebrate kidneys show specific regenerative responses after nephron damage. The first type of response to restore nephron functionality has been designated classically as "renal regeneration" or "tubular regeneration" (Mccampbell *et al.*, 2015; Reimschuessel, 2001). It describes a local self-repair of nephrons with intact basement membrane after a limited injury (Fig. 3). In this process, an initial phase of cell death and detachment of tubular cells from the basement membrane is followed by an initiation of cellular proliferation of either dedifferentiated cells or resident stem/progenitor cells and migration along the tubule, leading to a repopulation of the existing nephron (Davidson, 2011; Reimschuessel, 2001).

A second strategy of the vertebrate kidney to restore nephron function is called "compensatory renal hypertrophy", meaning an enlargement of kidney size by cellular proliferation (Fine, 1986; Kaufman *et al.*, 1975; Reimschuessel, 2001). After injury, surviving nephrons enhance the excretion of metabolic waste by increasing their single nephron filtration rate and undergo morphological changes, such as expanding their glomeruli diameter and proximal tubule cell volume (Davidson, 2011). This regenerative strategy mimics the enlargement of nephrons seen during postnatal growth in mammals, suggesting that both processes may be induced by a common mechanism (Davidson, 2011).

The last renal repair response designated as "neo-nephrogenesis" is a process of generating entire nephrons *de novo* after injury (Davidson, 2011, 2014; Reimschuessel and Williams, 1995). The source of newly forming nephrons was traced to proliferating mesenchymal cell clusters that once induced follow similar developmental patterns as observed during nephrogenesis in developing vertebrate kidneys, eventually forming a new functional nephron (Davidson, 2014; Reimschuessel, 2001).

The regenerative capacity of the mammalian kidney

When trying to better understand human disease, animal models have proven incredibly informative. Understanding the onset and subsequent response to damage and repair in an organismal setting is necessary for basic research. Mammalian models of acute kidney injury (AKI) have been performed in murine models (Shoskes *et al.*, 1990; Wei and Dong, 2012) and have included surgical approaches to induce ischemia/reperfusion injuries (Duffield *et al.*, 2006) and injection of nephrotoxins such as Cisplatin (Ramesh and Reeves, 2003). In addition, a more recent publication describes a renal cryo-injury model aimed at uncovering endogenous damage and repair processes (Abdulmahdi *et al.*, 2015).

Mammalian kidneys have developed regenerative strategies to restore their integrity after damage, but also to manage cell turnover to ensure continuous nephron maintenance. For mammals, it has never been reported that their kidneys have the capability to form new nephrons via "neo-nephrogenesis", once nephrogenesis is completed in the embryo or neonate (Fine, 1986; Hartman *et al.*, 2007; Larsson *et al.*, 1980). Instead, the answer to kidney damage is a combination of "tubular regeneration" responses, to repair the nephric epithelium along the intact basement membrane (Humphreys *et al.*, 2008), and "compensatory renal hypertrophy" to substitute for the loss of not repairable nephrons (Hayslett,

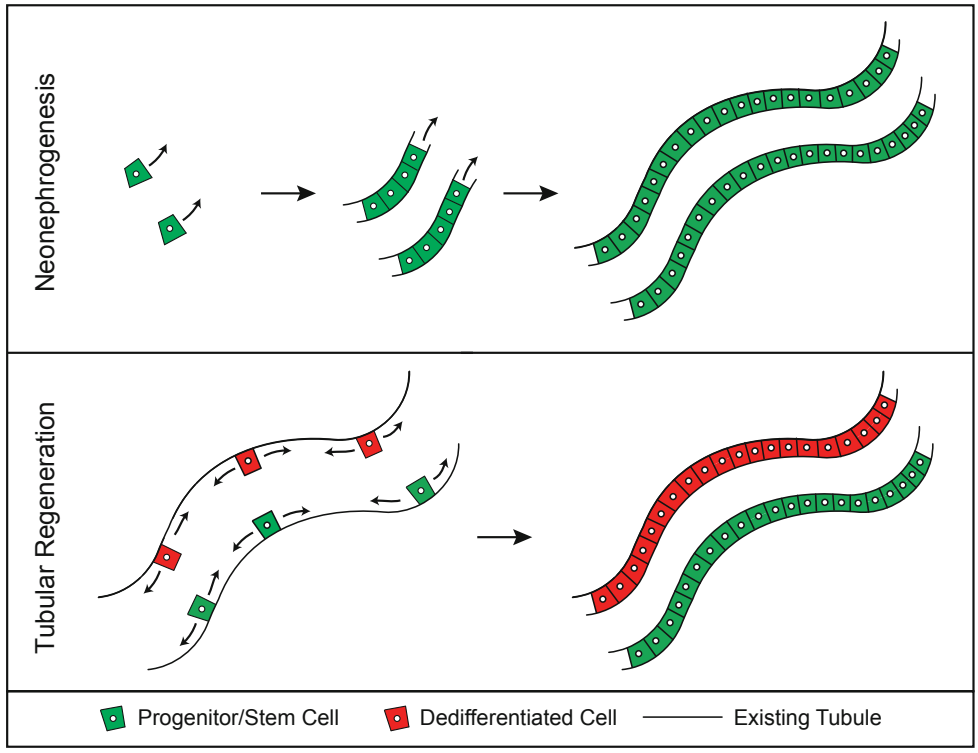

Fig. 3. Visualization of kidney regeneration models. *Neonephrogenesis is defined as the* de novo *formation of tubules from a resident progenitor/stem cell. Tubular regeneration describes a process where either existing tubular cells dedifferentiate or resident progenitor/stem cells within existing tubules repopulate damaged tissue.*

1979; Mccrory, 1972).

Both processes require proliferative cells. Although the source of cells has been demonstrated to be nephron-intrinsic (Humphreys *et al.*, 2008), their identity remains still controversial. There is given evidence, that reparative programs of the nephric tubules depend on surviving tubular cells that dedifferentiate and undergo epithelial to mesenchymal transition to become proliferative and migratory (Bonventre and Yang, 2011). Consistent with this, it was shown that the renal epithelium is not quiescent. Many tubular epithelial cells in uninjured nephrons are arrested in the G1 phase, waiting for a rapid recruitment into the cell cycle and allowing a renal repopulation after injury (Vogetseder *et al.*, 2008). However, this mechanism does not exclude the coexistence of specialized stem or progenitor cells within the nephron. Several candidate populations have been proposed. Researchers have identified renal progenitor cells scattered along the tubular epithelium and located within the Bowman's capsule of the glomerulus, that are suggested to give rise to tubular and glomerular cell types (Benigni *et al.*, 2010; Bussolati *et al.*, 2005; Hopkins *et al.*, 2009). In addition, Lazzeri *et al.,* has demonstrated existence of bipotent progenitor populations located at the urinary pole of the glomerulus that can give rise to both tubular epithelium as well as glomerular podocytes (Lazzeri *et al.*, 2010; Ronconi *et al.*, 2009).

Taken together, there are several indications that mammalian nephrons harbor cells with a robust proliferation capacity sufficient to perform epithelial replacement. While "tubular regeneration" and "compensatory renal hypertrophy" processes enable for a partial rescue from nephron damage, kidney regeneration remains a limited process in mammals and can even lead to insufficiencies in kidney function. A continuous loss of nephrons as well as prolonged hyperfiltration caused by extended hypertrophy eventually lead to glomerular injury, toxic proteinuria and kidney sclerosis (Brenner, 1985).

Kidney diseases

Humans are affected by a wide spectrum of renal diseases, ranging from minor dysfunctions to kidney failure, which requires organ replacement therapies by dialysis or kidney transplantation. In general, a sudden damage of the kidney as induced by, e.g. ischemia reperfusion injury or nephrotoxic agents is termed "acute kidney injury" (AKI). Slow progressive loss of tubules and ongoing fibrosis can lead to "chronic kidney disease" (CKD). AKI is a life-threatening disease, characterized by an increase in serum creatinine levels and a decrease in urine volume. Due to secondary complications such as pulmonary edema, high potassium levels in the blood (hyperkalemia) and metabolic acidosis, AKI has a world-wide mortality rate of between 30% and 50% (Yaklin, 2011). In the United States of America the total number of deaths from AKI doubled from 18,000 to 39,000 between 2000 and 2009 (Hsu *et al.*, 2013). As such, AKI is a large socio-economical problem in our increasingly populated and aging society.

Morphologically, AKI manifests in different, overlapping phases starting with the appearance of dedifferentiated cells at the injury site, followed by morphological changes of the cells in the proximal tubule. As a consequence, cells detach from the basement membrane, undergo apoptosis or necrosis (in severe and rapid cases) and can be seen as cellular casts in the lumen causing tubular destruction downstream of the lesion site (Mccampbell *et al.*, 2014). Many molecular pathways are involved in the ensuing damage response; such as activation of transcription factors like hypoxia induced factor (HIF) and NF-kB, Toll-like receptor (TLR) signaling and inflammatory processes (Basile, 2007; Eltzschig and Carmeliet, 2011; Havasi and Borkan, 2011; Leemans *et al.*, 2005).

When loss of renal function proceeds over a long period, CKD develops, which can lead to end-stage renal disease (Chawla and Kimmel, 2012). The complex pathophysiology of CKD by progressive scarring is usually too severe to be repaired. Due to chronic lesions, remaining nephrons try to increase functionally by intra-glomerular hypertension and hyper-filtration (Benigni *et al.*, 2010). This leads to further renal damage, such as excessive accumulation of ultra-filtered proteins in the Bowman's space and the lumen of the tubules. As a consequence, mitochondrial oxidative stress, expression of epithelial growth factor receptors (EGFR) and other factors can lead to glomerulosclerosis (Bollee *et al.*, 2011; Daehn *et al.*, 2014). It is thought that one of the main causes of progression from AKI to CKD is an increase of reactive oxygen species (ROS). ROS are formed as a consequence of increased oxygen consumption in the proximal tubules, because of increasing levels of nephrotoxic indoxyl sulfate (Barreto *et al.*, 2009; Palm *et al.*, 2010). The progression from AKI to CKD will be a very important aspect of future research. However, this review will concentrate on models of AKI in fish species, both at larval and adult stages.

The remarkable regenerative capacities of fish

Compared to kidney regeneration in mammals, adult fish kidneys have a different strategy to maintain and restore their functionality. It has been demonstrated that different fish species like goldfish (Reimschuessel *et al.*, 1990b), trout (Reimschuessel *et al.*, 1993), catfish (Cormier *et al.*, 1995), toadfish (Brown and Reimschuessel, 1998), medaka (Watanabe *et al.*, 2009) and zebrafish (Diep *et al.*, 2011) have a remarkable regenerative ability to recover from an acute kidney injury. In response to nephrotoxins they undergo a two-phase repair mechanism (Reimschuessel *et al.*, 1990c). Initially, fish kidneys perform a repair of existing nephric tubules via "tubular regeneration", leading to the re-population of the basement membrane with epithelial cells consistent with what has been found in mammalian models (Augusto *et al.*, 1996; Reimschuessel, 2001; Reimschuessel *et al.*, 1990c, 1993). The origin of proliferating progenitor cells in this response has not yet been identified, but it is suggested that underlying mechanisms are similar to mammals (Kusaba and Humphreys, 2014).

The second repair response enables fish to compensate efficiently for the loss of nephrons after an acute injury. A major difference between fish and mammalian kidneys is their potential for ongoing nephrogenesis in adults (Reimschuessel, 2001). Fish continue to add nephrons throughout their lifespan and have the ability to significantly increase nephrogenesis after an injury (Augusto *et al.*, 1996; Brown and Reimschuessel, 1998; Elger *et al.*, 2003; Reimschuessel, 2001; Reimschuessel *et al.*, 1990c; Watanabe *et al.*, 2009; Zhou *et al.*, 2010). The capacity to generate entire nephrons *de novo* has been traced to nephrogenic aggregates that appear during kidney regeneration and give rise to new nephrons in a process that recapitulates developmental nephrogenesis (Diep *et al.*, 2011; Zhou *et al.*, 2010) but also follows the same pattern of development as is observed during nephrogenesis in developing

mammalian kidneys ((Reimschuessel, 2001). A major question that remains is about the origin and identity of cells that contribute to these cell clusters. Whether they arise from stem cells, progenitors cells or terminally differentiated cells, and if they are of intra- or extra-tubular origin has to be investigated.

Among animals that have been used for developmental and regenerative studies of the kidney, the zebrafish has become a favorable model organism (Mccampbell and Wingert, 2014). It has been shown that zebrafish embryos, larvae and adults can model kidney diseases of humans, including AKI caused by tubular or glomerular damage (Mccampbell and Wingert, 2014; Zhou and Hildebrandt, 2012), but also other common nephropathies like polycystic kidney disease (Drummond *et al*., 1998). This is based on the high conservation of nephron structures, cell types, development and functionality between mammals and fish (Drummond *et al*., 1998; Sander and Davidson, 2014; Wingert and Davidson, 2008; Wingert *et al*., 2007). In addition zebrafish harbor an amazing regenerative potential. This includes the ability to restore nephron epithelia and to form entirely new nephrons. Zebrafish show *de novo* formation of kidney structures after an injury, a major difference to mammals that can complement research in traditional AKI models such as the mouse and rat (Mccampbell and Wingert, 2014; Romagnani *et al*., 2013).

Zebrafish is a well-established genetic model with a steady increase of transgenic reporter lines and CRISPR/Cas9-mediated mutants. This allows studying of specific cell types and fates (Zhou and Hildebrandt, 2012) and supports transplantation assays to prove stem-cell capabilities (Diep *et al*., 2011). Analysis of kidney regeneration is much improved by newly established methods to determine the structure and functionality of nephrons (Mccampbell *et al*., 2015). This comprises standard histological stains, such as hematoxylin and eosin but also complex immunological stains with zebrafish specific antibodies, both on tissue section and in whole-mount. Furthermore, numerous solute transporters and renal transcription factors have been identified and assigned to individual segments of the zebrafish nephron (Wingert and Davidson, 2008; Wingert *et al*., 2007). One of these, the sodium-dependent phosphate transporter slc20a1a, is specifically found in the proximal tubule epithelium of zebrafish and mammalian kidneys, and *slc20a1a* transcripts are transiently abrogated after gentamicin administration (Diep *et al*., 2011). Similarly, alkaline phosphatase reactivity has been shown to be a specific feature of the luminal surface of proximal tubules in nephrons, meaning one can follow structural changes in this segment (Mccampbell *et al*., 2014, 2015). To visualize kidney functionality in the zebrafish, injections of 40 kDa Fluorescein-isothiocyanate-labeled dextran (Dextran-FITC) is a significant method. The fluorescent sugar is selectively reabsorbed in the proximal parts of the tubules. Functional destruction of this segment is indicated by an inability to reabsorb Dextran-FITC (Diep *et al*., 2011). Taken together, zebrafish represents a unique tool to investigate temporal and spatial progress of regeneration.

Modeling acute kidney injury in fish

In 1990, hexachlorobutadiene-induced nephrogenesis was used as a model of recovery in adult goldfish (Reimschuessel *et al*., 1990a), with newly formed nephrons marked as basophilic structures in H&E staining. Gentamicin, another injury model, is a nephrotoxic aminoglycoside (AG) antibiotic, which affects the proximal parts of the tubule leading to a dose-dependent apoptosis (Laurent *et al*., 1983) or necrosis of tubular epithelial cells (Edwards *et al*., 2007). Traditionally, AGs are used in infections with gram-negative bacteria and bacterial endocarditis (Chen and Kaye, 2009) and are, despite their side effects, the most effective treatment against germs insensitive to other antibiotics. Classical clinical manifestations of AG toxicity are, among others, proteinuria, increase in plasma creatinine and electrolyte alterations (Parsons *et al*., 1997), signs that are also observed during AKI.

The first published use of gentamicin's efficacy as a nephrotoxin in adult fish was performed in goldfish (Reimschuessel and Williams, 1995). Similarly to the earlier work with hexachlorobutadiene, gentamicin treatment induced an increase in developing tubules in adult fish. Gentamicin has also been used in larval zebrafish experiments (Cianciolo Cosentino *et al*., 2010; Hentschel *et al*., 2005). Injection into the larval bloodstream of zebrafish led to an increased occurrence of edema. In addition, with the application of a novel rhodamine-dextran clearance assay, it was shown that gentamicin reduced larval glomerular function (Hentschel *et al*., 2005). This approach also became the basis for a novel small molecule screen, searching for substances that could improve regeneration (Cianciolo Cosentino *et al*., 2013). It was not until 2010 that the regenerative response of the adult zebrafish to Gentamicin-induced kidney damage was described (Zhou *et al*., 2010). Utilizing two important zebrafish kidney markers, wt1b (developing tubules) and podocin (developed glomerulus), the authors showed that the number of tubules is positively correlated with body weight and age suggesting that neonephrogenesis is a life-long process in zebrafish (Zhou *et al*., 2010). In addition to the descriptive aspects of this work, functional AKI modeling with gentamicin in the *wt1b* and *podocin* reporter lines allowed certain populations of kidney cells to be investigated during adult kidney

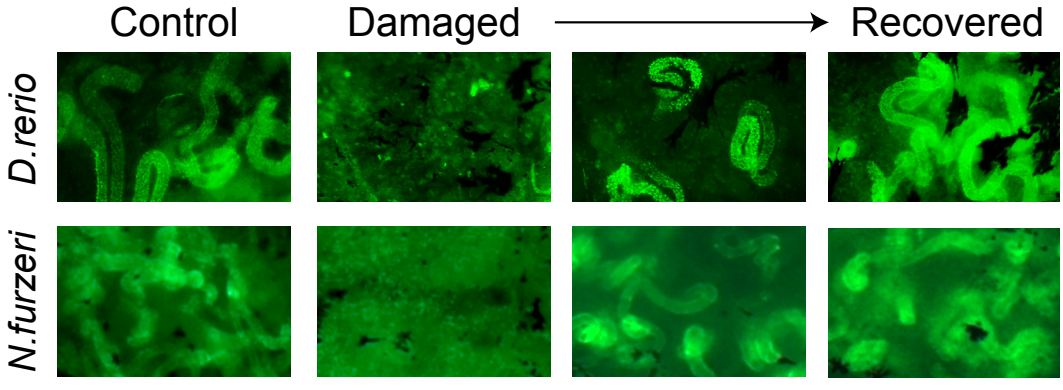

Fig. 4. Kidney regeneration kinetics in fish. *Example pictures of Dextran uptake in control, damaged and recovering animals. One can see that uptake is lost upon damage and recovered following regeneration. Scale bar, 1 mm. Part of the figure is adapted from Hoppe* et al. *(2015).*

regeneration. With significantly higher numbers of wt1b-positive nephrons seen throughout regeneration, wt1b became an important marker of tubular regeneration in zebrafish.

With the gentamicin protocol established, a more mechanistic study uncovered further aspects of kidney regeneration in the zebrafish (Diep *et al.*, 2011). With further data suggesting that new nephrons are created throughout life, the authors utilized a combination of methods to better describe in detail, the processes of regeneration. In addition, the presence of nephron progenitors was explored with transplantation studies. One very helpful tool was introduced in this publication; that the selective uptake of a 40kDa tagged sugar can be used as readout of regeneration (Fig. 4). Following this breakthrough discovery, additional publications have increased the tool kit of those investigating kidney regeneration in zebrafish. In 2014, an atlas of markers was published (Mccampbell *et al.*, 2014). Covering many aspects of histological staining of the adult kidney during regeneration, working markers for proliferation and cell death were combined with markers of both the proximal and distal tubules. In this readout of regeneration, an *in situ*-based quantification of tubular recovery allows researchers to look for differences in regeneration at different time points and under different experimental conditions (Mccampbell *et al.*, 2014). Another publication, which will contribute to the success of kidney regeneration experiments, was published in 2015. The work concentrates on how one can be sure of inducing AKI in the adult animal, without having to sacrifice the animal (Kamei *et al.*, 2015). It is now clear that following AKI, adult zebrafish excrete extra waste into the water. Due to death of the kidney epithelial cells, tubular renal casts are formed. These accumulate in the collecting ducts of the fish and are passed into the water by the fish. This screening possibility avoids the further use of non-injured fish in an experiment, ensuring the use of animals with high-level, non-lethal AKI (Kamei *et al.*, 2015).

Kinetics of regeneration

Shortly after application of the nephrotoxin gentamicin, epithelial cell death occurs in the proximal tubule segment of the zebrafish nephron (Verghese *et al.*, 2008), followed at one-day post injury (dpi) by an accumulation of intraluminal cellular debris and a disorganized proximal tubular epithelium (Mccampbell *et al.*, 2015; Zhou *et al.*, 2010). Cellular and functional disruption results in a failure to take up Dextran-FITC, that persists until 3 dpi (Diep *et al.*, 2011). Around 4 dpi, partial recovery of nephron function via "tubular regeneration" can be observed by appearance of few Dextran-FITC labeled nephrons (Diep *et al.*, 2011). This is caused by a repopulation of the basement membrane probably by proliferating tubular cells, a process similar to mammalian nephron regeneration (Diep *et al.*, 2011). At 5 dpi, cells within the proximal tubules start to display an epithelial organization as a single layer of cells and a restored luminal opening, leading to a complete recovery one week after gentamicin induced AKI (Mccampbell *et al.*, 2015).

"Neo-nephrogenesis" occurs in a partially overlapping time frame in order to replace severely injured nephrons that cannot be repaired (Mccampbell *et al.*, 2015). Beginning at approximately 5 dpi a small number of nephrogenic cellular aggregates begin to form by merging of nephron progenitors (Diep *et al.*, 2011). By 7 dpi, the number of aggregates increases and begins to show signs of proliferation and differentiation (Mccampbell *et al.*, 2015). The aggregates undergo a mesenchymal to epithelial transition to form an epithelial ball also referred to as renal vesicle (Diep *et al.*, 2011; Sander and Davidson, 2014). Eventually, the vesicles elongate showing comma- and S-shaped body stages and form a lumen within the second week after injury (Sander and Davidson, 2014). The immature tubules fuse into existing nephrons and mature in size and characteristics (Mccampbell *et al.*, 2015; Sander and Davidson, 2014). At about 14 dpi the nascent nephrons exhibit functional brush borders, shown by positive Dextran-FITC uptake, indicating proximal tubule character (Mccampbell *et al.*, 2015). The overall structure of the kidney is close to undamaged tissue, although immature nephrons can still be detected. At 21 dpi tissue staining reveals an absence of nephrogenic aggregates, suggesting that the functionality of the regenerated kidney in zebrafish is restored between 2 and 3 weeks following damage (Diep *et al.*, 2011; Mccampbell *et al.*, 2015).

The nature of progenitor/stem cells for kidney regeneration

The *de novo* formation of new nephrons is a feature, unique to fish, amphibians and reptiles. In zebrafish small cellular aggregates have been identified as the source of new nephrons. They are formed by the coalescence of multiple progenitor cells (Diep *et al.*, 2011; Zhou *et al.*, 2010). In goldfish, similar findings were made, identifying basophilic clusters of cells as source of new nephrons after kidney injury (Salice *et al.*, 2001). These clusters display an enlargement, lumen formation and maturation of nephrons. Similar observations have been made in medaka as well, where first repair processes of the damaged nephrons took place, followed by development of new nephrons (Watanabe *et al.*, 2009). Diep *et al.,* (2011) addressed the question about the origin and stemness of the cell clusters by analyzing a transgenic zebrafish reporter line for *lhx1a*, a transcription factor active in "pre-tubular" aggregates during mammalian nephrogenesis. Studies of kidneys in this reporter line revealed three distinctive cell populations: (I) single cells with mesenchymal morphology that are suggested to be migratory, (II) homogeneous aggregates comprising few *lhx1a* expressing cells, and (III) renal vesicle-like bodies. Interestingly, in transplantation assays, only the cellular aggregates could engraft in recipient fish and generate new functional nephrons, suggesting that these comprise nephron progenitors that are long-lived and possess extensive proliferative potential, consistent with stem-cell activity (Diep *et al.*, 2011). Because single cells failed to engraft, the question about the identity of the individual cells that form these nephrogenic clusters, and signals that regulates activation of *lhx1a* cells remains. It is also not clear whether *lhx1a* cells in zebrafish display an equivalent to mammalian nephron progenitors (Diep *et al.*, 2011; Kobayashi *et al.*, 2005).

A molecular understanding of the processes involved in regeneration has already been gained in zebrafish models. Targeted knockdown in adult zebrafish fin tissue also showed that miRNAs are required not only for regeneration to occur, but also that they are involved the correct amount of outgrowth (Thatcher *et al.*, 2008; Yin *et al.*, 2008). It would be interesting to investigate the role of miRNAs in zebrafish kidney regeneration, as *in vivo* abrogation of miR21 delayed regenerative responses of the kidney in the Killifish *Nothobranchius furzeri* (Hoppe *et al.*, 2015). With the explosion of genome modifying applications, comes the chance to look at the roles of specific genes and their requirements during regeneration. Although the signal that activates and modulates neo-nephrogenesis in fish is not known, there is some evidence

that systemic signals play a role (Elger, 2003). A partial unilateral nephrectomy caused induction in nephrogenesis of the contralateral portion of the kidney. The remote site of the affect suggests a circulating substance that activates or initiates regenerative processes. Comparative metabolomics analysis between damaged and control kidneys in the hours following kidney damage could highlight differences. With many candidates, the strength of large-scale zebrafish screens could help with a kidney-specific functional screen (Cianciolo Cosentino *et al.*, 2013).

Another aspect that should be considered is the ability of fish to detect changes in salt or fluid. Certain species of fish are able to survive in both fresh- and saltwater environments, as nephron numbers can adapt to the environments (Reviewed in (Davidson, 2011). Some authors discuss that although there are not many studies where the effect of the environment has been linked to kidney function, some species increase GFR when moved from fresh-to saltwater (Moriarty *et al.*, 1978). Some hypothesise (Davidson, 2011) that low blood pressure requires fish to have nephrogenic capacity in order to adapt. Measurements of glomerular filtration rates (GFR) are related to pressure (lower GFR = lower pressure) and in the river Lamprey (*Lampetra fluviatilis*), single nephron filtration rates were found to be roughly 4 times lower than the recordings from rat (Moriarty *et al.*, 1978). It is suggested that this reduces the ability of fish to increase pressure to adapt to reduced kidney function. These ideas could potentially be tested experimentally with kidney damage performed in murine models with lower blood pressure or conversely seeing if mild increases in the blood pressure of fish reduce their need or ability to perform nephrogenesis.

A better understanding of the molecular and cellular events underlying *de novo* formation of kidney structures in fish could help to resolve limitations of kidney regeneration in humans.

Age-related regeneration

In almost all organisms one can observe aging, being defined as gradual decline in physical, physiological and mental abilities, with homeostatic imbalances and a decreased capacity to respond to stress. On a cellular level, aging is thought to be the result of several factors, such as accumulation of ROS due to a decline in mitochondrial function (Harman, 1972), accumulation of DNA damage and telomere shortening (Hasty *et al.*, 2003), resulting in cellular senescence, increased inflammatory response and altered cellular response to injury. As our society ages, we are encountering increases in age-related kidney disease in aged populations (reviewed in (Schmitt and Melk, 2017)); and as research into aging and associated clinical problems increases, we are finally able to ask important socially relevant questions. In regards to kidney regeneration specifically, what can we learn from animal models that either exhibit or don't exhibit aging phenotypes, and how can we relate these questions to improve human health-span?

During aging a general decline of the regenerative response in tissues is observed, being the result of a decreased number of adult stem and progenitor cells. Next to their regenerative functions, these cells usually modulate maintenance and repair of tissues (Conboy and Rando, 2005). This age-dependent decrease is thought to be the result of changes in in the stem cell niche in addition to local and systemic factors, resulting in an inhibition and decline of regenerative activities. In 2005 Conboy and colleagues showed that parabiotic pairings of young and aged mice led to significantly improved regenerative capacity of muscle and liver in old mice. They could determine that pre-existing old muscle stem cells (satellite cells) are positively influenced by systemic factors of young mice (Conboy *et al.*, 2005). Due to changed gene expression and telomere shortening, cells reach a finite lifespan, termed as

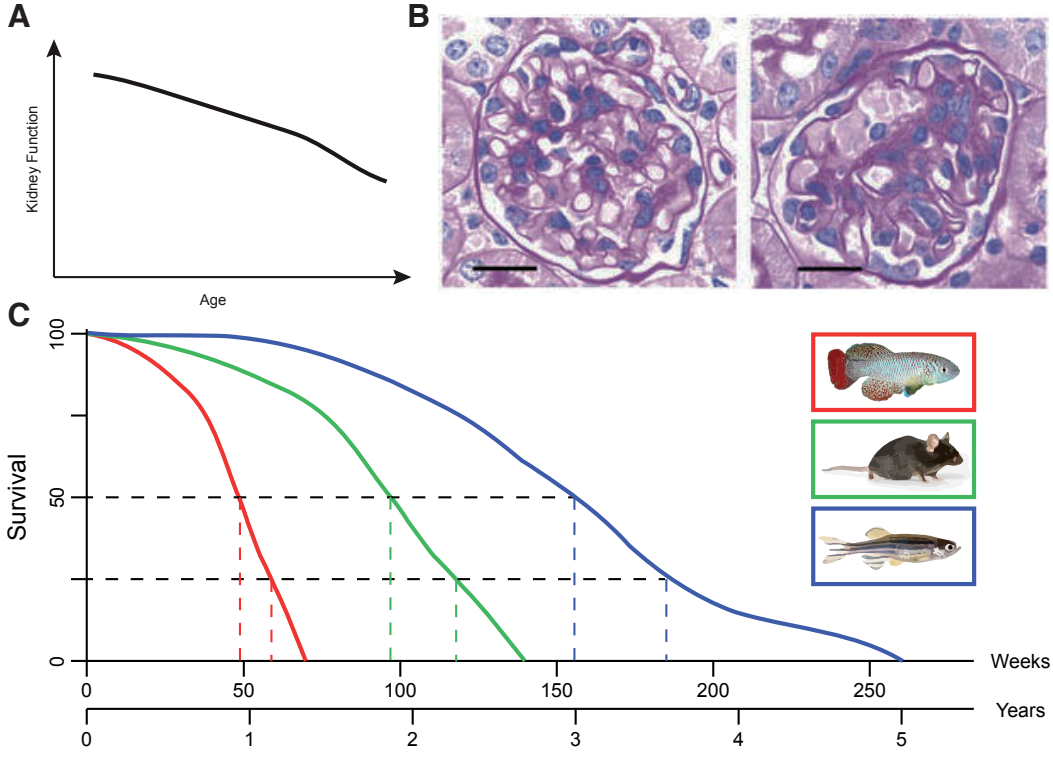

Fig. 5. Age-dependent changes in the kidney. (A) *Human kidney function reduces with age as seen in (Davies and Shock, 1950).* **(B)** *Human kidney diseases can be studied in animal models. Loss of Wt1 in the mouse can cause glomerulosclerosis as seen by an increase of sclerotic tissue (adapted from (Gebeshuber* et al.*, 2013)).* **(C)** *Graphical estimation of lifespan data for* N. furzeri *(red),* M. musculus *(green) and* D. rerio *(blue). Median and 75% lifespans were determined (see colored dotted lines) to highlight the large differences between these lab-based animal models. This data serves as a rough estimation of lifespans based upon data from (Gerhard* et al.*, 2002), C57BL/6J mouse lifespan from jax.org and (Wendler* et al.*, 2015). Animal pictures adapted from zdmsociety.org, FLI fish facility, jax.org.*

"cellular senescence" (Hayflick and Moorhead, 1961). Senescent fibroblasts for example are known to secrete epithelial growth factors, matrix metalloproteinases and inflammatory cytokines, leading to an alteration in the tissue structure and causing local inflammation (Campisi, 2005). These effects foster a microenvironment, promoting age-associated diseases and neoplastic transformations in old organisms, like atherosclerotic and hyperplastic epithelial lesions.

As aging effects the whole organism, clear signs of functional decrease can be observed in the kidney as well. In 1950, Davies and Shock reported an inverse relationship between glomerular filtration rate (GFR) and age (Davies and Shock, 1950) measuring the urinary clearance of inulin in individuals aged 24 to 89 years. More recently scientists have shown a significant reduction of the GFR by 22% in humans ≥55 years compared to younger individuals (Hoang *et al.*, 2003). One of the biggest studies in humans is the Baltimore Longitudinal Study of Aging (BLSA), measuring GFR by creatinine clearance, next to other aging associated factors. Combining data from the Baltimore Longitudinal Study of Aging (BLSA), a large-scale study of human aging (Ferrucci, 2008), biopsies of kidney donor transplantations and healthy kidney tissue of nephrectomy patients, Wheeler and colleagues analyzed gene expression and identified 630 genes being changed during aging. 101 of these genes showed expression-associated single nucleotide polymorphisms (SNPs) correlating with GFR decline (Wheeler *et al.*, 2009). Already in 2004, transcriptional profiling of the aging human kidney was done, identifying 985 genes that were changed with age, being associated with immune cell response and extracellular matrix (Rodwell *et al.*, 2004). Aging affects the kidney globally as decline in size is observed in cortex and medulla (Lindeman, 1990). Implying a general decline of the repair and regeneration processes, several diseases of the kidney are associated with aging, like focal segmental glomerulosclerosis, interstitial nephritis or renal stones (Fig. 5 A,B) (Nitta *et al.*, 2013).

Fish models of aging and regeneration

Only a few studies have tried to address the age-dependency of regeneration in the adult zebrafish, looking at the caudal fin and heart. Caudal fin amputation experiments showed that 28-month-old animals were able to regenerate to the same extent as both 4-month and 12-month-old animals (Shao *et al.*, 2011). These results were backed up by a further study that showed when comparing young fish (6-12 months) to older animals (26-36 months) similar regenerative capacities were described following fin amputation but also ventricular resection (Itou *et al.*, 2012). In contrast to zebrafish, *Nothobranchius furzeri* do show age-dependent differences.

Nothobranchius furzeri, also known as the turquoise killifish belongs to the group of teleost fish and has, with 3-12 months, the shortest reported lifespan of a vertebrate in captivity (Valdesalici and Cellerino, 2003). The short lifespan of *N. furzeri* is an adaptation to the special environmental conditions it has to deal with. *N. furzeri* shows clear signs of aging, such as accumulation of the aging markers lipofuscin in the liver and β-galactosidase in the skin (Terzibasi *et al.*, 2008), impairment of mitochondrial function (Hartmann *et al.*, 2011) and telomere shortening Hartmann *et al.,* (2009). Additionally, changes in learning/behavioral capabilities, weakening of the color and muscle dysplasia are typical signs of aging in this fish. In a recent study, recovery from caudal amputation was reduced by more than 50% in aged animals. These reductions are most likely due to reduction in proliferation and an increase in apoptosis, suggesting that the response to amputation is initiated earlier and is more efficient in young fish (Wendler *et al.*, 2015).

These findings suggest that while zebrafish do not exhibit age-related losses in regenerative capacity, *Nothobranchius furzeri* shows clear signs of aging and loss of regenerative capacity with age, and could be of help to understand missing signals in those animals that do exhibit such phenotypes. Comparative studies with both fish species could lead to highly informative results, which could prove helpful for future clinical therapies. When comparing the lifespans of *Nothobranchius furzeri* (Killifish), *Mus musculus* (Mouse) and *Danio rerio* (Zebrafish) one can see a large difference (Fig. 5 C is a graphical reconstruction of data from (Gerhard *et al.*, 2002), jax.org and (Wendler *et al.*, 2015)). With a median lifespan roughly three times longer than it's distant relative *N. furzeri*, aged or old zebrafish would ideally be older than 3 years (at least 36 months), for age-related experiments. And as this is not easy in a laboratory setting for time reasons, *Nothobranchius furzeri* can be used as an aged vertebrate in comparative studies. Age matched experiments in both fish species could help to understand the age-related regenerative capacities and help us to better understand increases in kidney disease.

Kidney regeneration is a fascinating but understudied research field. The source of the regenerative capacity in fish must be further investigated, in order to see whether the resident progenitor cells are sufficient, or if as yet non-described stem cells are important for the initiation of regeneration. Secondly, an in-depth analysis of the age-dependency of regeneration is necessary. Learning which changes cause animal models to exhibit age-related phenotypes, whilst also finding out what can allow other models to have life-long regeneration is a vital future direction. A field-wide definition of aged-fish will be important for further research. Fish models have the opportunity to be the basis for further human clinical treatments (Cao *et al.*, 2009), with cell- and tissue-specific genome editing required in order to find the crucial signals for kidney regeneration. Additional molecular studies are needed to find important pathways and signaling, which can also give insights for future pharmacological targets for human therapies. In the coming years, kidney regeneration research will become increasingly important, as the kidney is one of the organs most affected by the increase in human lifespan.

Acknowledgements

Some of the work from this review is based upon thoughts and ideas from the doctoral theses of U.N. and B.H. In such a review, we are unable to include all of the excellent work from the field. To those authors whose work we have not included, we apologize. Birgit Perner (FLI) took the fluorescent picture in figure 1.

References

ABDULMAHDI, W, ZULLO, J, NESI, L, GOLIGORKSY, M S, RATLIFF, B B (2015). Charting the course of renal cryoinjury. *Physiol Rep* 3: pii: e12357.

AUGUSTO, J, SMITH, B, SMITH, S, ROBERTSON, J, REIMSCHUESSEL, R (1996). Gentamicin-induced nephrotoxicity and nephroneogenesis in Oreochromis nilotica, a tilapian fish. *Diseases of Aquatic Organisms* 26: 49-58.

BARRETO, F C, BARRETO, D V, LIABEUF, S, MEERT, N, GLORIEUX, G, TEMMAR, M, CHOUKROUN, G, VANHOLDER, R, MASSY, Z A, EUROPEAN UREMIC TOXIN WORK, G (2009). Serum indoxyl sulfate is associated with vascular disease and mortality in chronic kidney disease patients. *Clin J Am Soc Nephrol* 4: 1551-1558.

BASILE, D P (2007). The endothelial cell in ischemic acute kidney injury: implications

for acute and chronic function. *Kidney Int* 72: 151-156.

BENIGNI, A, MORIGI, M, REMUZZI, G (2010). Kidney regeneration. *Lancet* 375: 1310-1317.

BOLLEE, G, FLAMANT, M, SCHORDAN, S, FLIGNY, C, RUMPEL, E, MILON, M, SCHORDAN, E, SABAA, N, VANDERMEERSCH, S, GALAUP, A, RODENAS, A, CASAL, I, SUNNARBORG, S W, SALANT, D J, KOPP, J B, THREADGILL, D W, QUAGGIN, S E, DUSSAULE, J C, GERMAIN, S, MESNARD, L, ENDLICH, K, BOUCHEIX, C, BELENFANT, X, CALLARD, P, ENDLICH, N, THARAUX, P L (2011). Epidermal growth factor receptor promotes glomerular injury and renal failure in rapidly progressive crescentic glomerulonephritis. *Nat Med* 17: 1242-1250.

BONVENTRE, J V, YANG, L (2011). Cellular pathophysiology of ischemic acute kidney injury. *J Clin Invest* 121: 4210-4221.

BRENNER, B M (1985). Nephron adaptation to renal injury or ablation. *Am J Physiol* 249: F324-337.

BROWN, M, REIMSCHUESSEL, R (1998). Nephron neogenesis in an aglomerular fish. *FASEB J.* 12: A800-A800.

BUSSOLATI, B, BRUNO, S, GRANGE, C, BUTTIGLIERI, S, DEREGIBUS, M C, CANTINO, D, CAMUSSI, G (2005). Isolation of renal progenitor cells from adult human kidney. *Am J Pathol* 166: 545-555.

CAMPISI, J (2005). Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* 120: 513-522.

CAO, Y, SEMANCHIK, N, HUN LEE, S, SOMLO, S, EMILIO BARBANO, P, COIFMAN, R, SUN, Z (2009). Chemical modifier screen identifies HDAC inhibitors as suppressors of PKD models. *Proc Natl Acad Sci USA* 106: 21819-21824.

CHAWLA, L S, KIMMEL, P L (2012). Acute kidney injury and chronic kidney disease: an integrated clinical syndrome. *Kidney Int* 82: 516-524.

CHEN, L F, KAYE, D (2009). Current use for old antibacterial agents: polymyxins, rifamycins, and aminoglycosides. *Infect Dis Clin North Am.* 23: 1053-1075.

CIANCIOLO COSENTINO, C, ROMAN, B L, DRUMMOND, I A, HUKRIEDE, N A (2010). Intravenous microinjections of zebrafish larvae to study acute kidney injury. *J Vis Exp.* 4: pii: 2079.

CIANCIOLO COSENTINO, C, SKRYPNYK, N I, BRILLI, L L, CHIBA, T, NOVITSKAYA, T, WOODS, C, WEST, J, KOROTCHENKO, V N, MCDERMOTT, L, DAY, B W, DAVIDSON, A J, HARRIS, R C, DE CAESTECKER, M P, HUKRIEDE, N A (2013). Histone deacetylase inhibitor enhances recovery after AKI. *J Am Soc Nephrol* 24: 943-953.

CONBOY, I M, CONBOY, M J, WAGERS, A J, GIRMA, E R, WEISSMAN, I L, RANDO, T A (2005). Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* 433: 760-764.

CONBOY, I M, RANDO, T A (2005). Aging, stem cells and tissue regeneration: lessons from muscle. *Cell Cycle* 4: 407-410.

CORMIER, S M, NEIHEISEL, T W, WERNSING, P, RACINE, R N, REIMSCHUESSEL, R (1995). New nephron development in fish from polluted waters: a possible biomarker. *Ecotoxicology* 4: 157-168.

COSTANTINI, F, KOPAN, R (2010). Patterning a complex organ: branching morphogenesis and nephron segmentation in kidney development. *Dev Cell* 18: 698-712.

DAEHN, I, CASALENA, G, ZHANG, T, SHI, S, FENNINGER, F, BARASCH, N, YU, L, D'AGATI, V, SCHLONDORFF, D, KRIZ, W, HARALDSSON, B, BOTTINGER, E P (2014). Endothelial mitochondrial oxidative stress determines podocyte depletion in segmental glomerulosclerosis. *J Clin Invest* 124: 1608-1621.

DAVIDSON, A J (2008). Mouse kidney development, *StemBook, Cambridge* (MA).

DAVIDSON, A J (2011). Uncharted waters: nephrogenesis and renal regeneration in fish and mammals. *Pediatr Nephrol* 26: 1435-1443.

DAVIDSON, A J (2014). Kidney regeneration in fish. *Nephron Exp Nephrol* 126: 45.

DAVIES, D F, SHOCK, N W (1950). Age changes in glomerular filtration rate, effective renal plasma flow, and tubular excretory capacity in adult males. *J Clin Invest* 29: 496-507.

DIEP, C Q, MA, D, DEO, R C, HOLM, T M, NAYLOR, R W, ARORA, N, WINGERT, R A, BOLLIG, F, DJORDJEVIC, G, LICHMAN, B, ZHU, H, IKENAGA, T, ONO, F, ENGLERT, C, COWAN, C A, HUKRIEDE, N A, HANDIN, R I, DAVIDSON, A J (2011). Identification of adult nephron progenitors capable of kidney regeneration in zebrafish. *Nature* 470: 95-100.

DRUMMOND, I A, DAVIDSON, A J (2010). Zebrafish kidney development. *Methods Cell Biol* 100: 233-260.

DRUMMOND, I A, MAJUMDAR, A, HENTSCHEL, H, ELGER, M, SOLNICA-KREZEL, L, SCHIER, A F, NEUHAUSS, S C, STEMPLE, D L, ZWARTKRUIS, F, RANGINI, Z, DRIEVER, W, FISHMAN, M C (1998). Early development of the zebrafish pronephros and analysis of mutations affecting pronephric function. *Development* 125: 4655-4667.

DUFFIELD, J S, HONG, S, VAIDYA, V S, LU, Y, FREDMAN, G, SERHAN, C N, BONVENTRE, J V (2006). Resolvin D Series and Protectin D1 Mitigate Acute Kidney Injury. *J. Immunology* 177: 5902-5911.

EDWARDS, J R, DIAMANTAKOS, E A, PEULER, J D, LAMAR, P C, PROZIALECK, W C (2007). A novel method for the evaluation of proximal tubule epithelial cellular necrosis in the intact rat kidney using ethidium homodimer. *BMC Physiol* 7: 1.

ELGER, M (2003). Nephrogenesis Is Induced by Partial Nephrectomy in the Elasmobranch Leucoraja erinacea. *J. Am. Soc. Nephrol.* 14: 1506-1518.

ELGER, M, HENTSCHEL, H, LITTERAL, J, WELLNER, M, KIRSCH, T, LUFT, F C, HALLER, H (2003). Nephrogenesis is induced by partial nephrectomy in the elasmobranch Leucoraja erinacea. *J Am Soc Nephrol* 14: 1506-1518.

ELTZSCHIG, H K, CARMELIET, P (2011). Hypoxia and inflammation. *N Engl J Med* 364: 656-665.

FERENBACH, D A, BONVENTRE, J V (2016). Kidney tubules: intertubular, vascular, and glomerular cross-talk. *Curr Opin Nephrol Hypertens* 25: 194-202.

FERRUCCI, L (2008). The Baltimore Longitudinal Study of Aging (BLSA): A 50-Year-Long Journey and Plans for the Future. *J Gerontol A Biol Sci Med Sci* 63: 1416-1419.

FINE, L (1986). The biology of renal hypertrophy. *Kidney Int* 29: 619-634.

GEBESHUBER, C A, KORNAUTH, C, DONG, L, SIERIG, R, SEIBLER, J, REISS, M, TAUBER, S, BILBAN, M, WANG, S, KAIN, R, BOHMIG, G A, MOELLER, M J, GRONE, H J, ENGLERT, C, MARTINEZ, J, KERJASCHKI, D (2013). Focal segmental glomerulosclerosis is induced by microRNA-193a and its downregulation of WT1. *Nat Med* 19: 481-487.

GERHARD, G, KAUFFMAN, E, WANG, X, STEWERT, R, MOORE, J, KASALES, C, DEMIDENKO, E, CHENG, K (2002). Life spans and senescent phenotypes in two strains of Zebrafish (Danio rerio). *Exp Gerontol* 37: 1055-1068.

HARMAN, D (1972). The biologic clock: the mitochondria? *J Am Geriatr Soc* 20: 145-147.

HARTMAN, H A, LAI, H L, PATTERSON, L T (2007). Cessation of renal morphogenesis in mice. *Dev Biol* 310: 379-387.

HARTMANN, N, REICHWALD, K, LECHEL, A, GRAF, M, KIRSCHNER, J, DORN, A, TERZIBASI, E, WELLNER, J, PLATZER, M, RUDOLPH, K L, CELLERINO, A, ENGLERT, C (2009). Telomeres shorten while Tert expression increases during ageing of the short-lived fish Nothobranchius furzeri. *Mech Ageing Dev* 130: 290-296.

HARTMANN, N, REICHWALD, K, WITTIG, I, DROSE, S, SCHMEISSER, S, LUCK, C, HAHN, C, GRAF, M, GAUSMANN, U, TERZIBASI, E, CELLERINO, A, RISTOW, M, BRANDT, U, PLATZER, M, ENGLERT, C (2011). Mitochondrial DNA copy number and function decrease with age in the short-lived fish Nothobranchius furzeri. *Aging Cell* 10: 824-831.

HASTY, P, CAMPISI, J, HOEIJMAKERS, J, VAN STEEG, H, VIJG, J (2003). Aging and genome maintenance: lessons from the mouse? *Science* 299: 1355-1359.

HAVASI, A, BORKAN, S C (2011). Apoptosis and acute kidney injury. *Kidney Int* 80: 29-40.

HAYFLICK, L, MOORHEAD, P S (1961). The serial cultivation of human diploid cell strains. *Exp Cell Res* 25: 585-621.

HAYSLETT, J P (1979). Functional adaptation to reduction in renal mass. *Physiol Rev* 59: 137-164.

HENTSCHEL, D M, PARK, K M, CILENTI, L, ZERVOS, A S, DRUMMOND, I, BONVENTRE, J V (2005). Acute renal failure in zebrafish: a novel system to study a complex disease. *Am J Physiol Renal Physiol* 288: F923-929.

HOANG, K, TAN, J C, DERBY, G, BLOUCH, K L, MASEK, M, MA, I, LEMLEY, K V, MYERS, B D (2003). Determinants of glomerular hypofiltration in aging humans. *Kidney Int* 64: 1417-1424.

HOPKINS, C, LI, J, RAE, F, LITTLE, M H (2009). Stem cell options for kidney disease. *J Pathol* 217: 265-281.

HOPPE, B, PIETSCH, S, FRANKE, M, ENGEL, S, GROTH, M, PLATZER, M, ENGLERT, C (2015). MiR-21 is required for efficient kidney regeneration in fish. *BMC Dev Biol* 15: 43.

HSU, R K, MCCULLOCH, C E, DUDLEY, R A, LO, L J, HSU, C Y (2013). Temporal changes in incidence of dialysis-requiring AKI. *J Am Soc Nephrol* 24: 37-42.

HUMPHREYS, B D, VALERIUS, M T, KOBAYASHI, A, MUGFORD, J W, SOEUNG, S, DUFFIELD, J S, MCMAHON, A P, BONVENTRE, J V (2008). Intrinsic epithelial

cells repair the kidney after injury. *Cell Stem Cell* 2: 284-291.

ITOU, J, KAWAKAMI, H, BURGOYNE, T, KAWAKAMI, Y (2012). Life-long preservation of the regenerative capacity in the fin and heart in zebrafish. *Biol Open* 1: 739-746.

KAMEI, C N, LIU, Y, DRUMMOND, I A (2015). Kidney Regeneration in Adult Zebrafish by Gentamicin Induced Injury. *J Vis Exp.* e51912.

KAUFMAN, J M, HARDY, R, HAYSLETT, J P (1975). Age-dependent characteristics of compensatory renal growth. *Kidney Int* 8: 21-26.

KOBAYASHI, A, KWAN, K M, CARROLL, T J, MCMAHON, A P, MENDELSOHN, C L, BEHRINGER, R R (2005). Distinct and sequential tissue-specific activities of the LIM-class homeobox gene Lim1 for tubular morphogenesis during kidney development. *Development* 132: 2809-2823.

KUSABA, T, HUMPHREYS, B D (2014). Controversies on the origin of proliferating epithelial cells after kidney injury. *Pediatr Nephrol* 29: 673-679.

LARSSON, L, APERIA, A, WILTON, P (1980). Effect of normal development on compensatory renal growth. *Kidney Int* 18: 29-35.

LAURENT, G, MALDAGUE, P, CARLIER, M B, TULKENS, P M (1983). Increased renal DNA synthesis *in vivo* after administration of low doses of gentamicin to rats. *Antimicrob Agents Chemother* 24: 586-593.

LAZZERI, E, MAZZINGHI, B, ROMAGNANI, P (2010). Regeneration and the kidney. *Curr Opin Nephrol Hypertens* 19: 248-253.

LEEMANS, J C, STOKMAN, G, CLAESSEN, N, ROUSCHOP, K M, TESKE, G J, KIRSCHNING, C J, AKIRA, S, VAN DER POLL, T, WEENING, J J, FLORQUIN, S (2005). Renal-associated TLR2 mediates ischemia/reperfusion injury in the kidney. *J Clin Invest* 115: 2894-2903.

LI, Y, WINGERT, R A (2013). Regenerative medicine for the kidney: stem cell prospects & challenges. *Clin Transl Med* 2: 11.

LINDEMAN, R D (1990). Overview: renal physiology and pathophysiology of aging. *Am J Kidney Dis* 16: 275-282.

MCCAMPBELL, K K, SPRINGER, K N, WINGERT, R A (2014). Analysis of nephron composition and function in the adult zebrafish kidney. *J Vis Exp*, e51644.

MCCAMPBELL, K K, SPRINGER, K N, WINGERT, R A (2015). Atlas of Cellular Dynamics during Zebrafish Adult Kidney Regeneration. *Stem Cells Int* 2015: 547636.

MCCAMPBELL, K K, WINGERT, R A (2014). New tides: using zebrafish to study renal regeneration. *Transl Res* 163: 109-122.

MCCRORY, W W (1972). *Developmental nephrology.* Harvard University Press, Cambridge, Mass. USA.

MORIARTY, R J, LOGAN, A, RANKIN, J C (1978). Measurement of single nephron filtration rate in the kidney of the river lamprey, Lampetra fluviatilis L. *J Exp Biol* 77: 57-71.

NITTA, K, OKADA, K, YANAI, M, TAKAHASHI, S (2013). Aging and chronic kidney disease. *Kidney Blood Press Res* 38: 109-120.

PALM, F, NANGAKU, M, FASCHING, A, TANAKA, T, NORDQUIST, L, HANSELL, P, KAWAKAMI, T, NISHIJIMA, F, FUJITA, T (2010). Uremia induces abnormal oxygen consumption in tubules and aggravates chronic hypoxia of the kidney via oxidative stress. *Am J Physiol Renal Physiol* 299: F380-386.

PARSONS, P P, GARLAND, H O, HARPUR, E S, OLD, S (1997). Acute gentamicin-induced hypercalciuria and hypermagnesiuria in the rat: dose-response relationship and role of renal tubular injury. *Br J Pharmacol* 122: 570-576.

PHE (Public Health England) (2014). Chronic kidney disease prevelence model. *PHE publications* gateway number: 2014386

RAMESH, G, REEVES, W B (2003). TNFR2-mediated apoptosis and necrosis in cisplatin-induced acute renal failure. *Am J Physiol Renal Physiol* 285: F610-618.

REIMSCHUESSEL, R (2001). A fish model of renal regeneration and development. *ILAR J* 42: 285-291.

REIMSCHUESSEL, R, BENNETT, R, MAY, E, LIPSKY, M (1990a). Development of newly formed nephrons in the goldfish kidney following hexachlorobutadiene-induced nephrotoxicity. *Toxicol Pathol* 18: 32-38.

REIMSCHUESSEL, R, BENNETT, R O, MAY, E B, LIPSKY, M M (1990b). Development of newly formed nephrons in the goldfish kidney following hexachlorobutadiene-induced nephrotoxicity. *Toxicol Pathol* 18: 32-38.

REIMSCHUESSEL, R, BENNETT, R O, MAY, E B, LIPSKY, M M (1990c). Renal Tubular Cell Regeneration, Cell-Proliferation and Chronic Nephrotoxicity in the Goldfish Carassius-Auratus Following Exposure to a Single Sublethal Dose of Hexachlorobutadiene. *Dis. Aquat. Organ.* 8: 211-224.

REIMSCHUESSEL, R, BENNETT, R O, MAY, E B, LIPSKY, M M (1993). Pathological Alterations and New Nephron Development in Rainbow-Trout (Oncorhynchus-Mykiss) Following Tetrachloroethylene Contamination. *J. Zoo Wildlife Med.* 24: 503-507.

REIMSCHUESSEL, R, WILLIAMS, D (1995). Development of new nephrons in adult kidneys following gentamicin-induced nephrotoxicity. *Ren Fail* 17: 101-106.

RODWELL, G E, SONU, R, ZAHN, J M, LUND, J, WILHELMY, J, WANG, L, XIAO, W, MINDRINOS, M, CRANE, E, SEGAL, E, MYERS, B D, BROOKS, J D, DAVIS, R W, HIGGINS, J, OWEN, A B, KIM, S K (2004). A transcriptional profile of aging in the human kidney. *PLoS Biol* 2: e427.

ROMAGNANI, P, LASAGNI, L, REMUZZI, G (2013). Renal progenitors: an evolutionary conserved strategy for kidney regeneration. *Nat Rev Nephrol* 9: 137-146.

RONCONI, E, SAGRINATI, C, ANGELOTTI, M L, LAZZERI, E, MAZZINGHI, B, BALLERINI, L, PARENTE, E, BECHERUCCI, F, GACCI, M, CARINI, M, MAGGI, E, SERIO, M, VANNELLI, G B, LASAGNI, L, ROMAGNANI, S, ROMAGNANI, P (2009). Regeneration of glomerular podocytes by human renal progenitors. *J Am Soc Nephrol* 20: 322-332.

SALICE, C J, ROKOUS, J S, KANE, A S, REIMSCHUESSEL, R (2001). New nephron development in goldfish (Carassius auratus) kidneys following repeated gentamicin-induced nephrotoxicosis. *Comp Med* 51: 56-59.

SANDER, V, DAVIDSON, A J (2014). Kidney injury and regeneration in zebrafish. *Semin Nephrol* 34: 437-444.

SCHMITT, R, MELK, A (2017). Molecular mechanisms of renal aging. *Kidney Int.* 92: 569-579.

SHAO, J, CHEN, D, YE, Q, CUI, J, LI, Y, LI, L (2011). Tissue regeneration after injury in adult zebrafish: the regenerative potential of the caudal fin. *Dev Dyn* 240: 1271-1277.

SHOSKES, D, PARFREY, N, HALLORAN, P (1990). Increased Major Histocompatability Complex Antigen Expression in Unilateral Ischemic Acute Tubular Necrosis in the Mouse. *Transplantation* 49: 201-207.

TERZIBASI, E, VALENZANO, D R, BENEDETTI, M, RONCAGLIA, P, CATTANEO, A, DOMENICI, L, CELLERINO, A (2008). Large differences in aging phenotype between strains of the short-lived annual fish Nothobranchius furzeri. *PLoS One* 3: e3866.

THATCHER, E J, PAYDAR, I, ANDERSON, K K, PATTON, J G (2008). Regulation of zebrafish fin regeneration by microRNAs. *Proc Natl Acad Sci USA* 105: 18384-18389.

VALDESALICI, S, CELLERINO, A (2003). Extremely short lifespan in the annual fish Nothobranchius furzeri. *Proc Biol Sci* 270 Suppl 2: S189-191.

VERGHESE, E, WEIDENFELD, R, BERTRAM, J F, RICARDO, S D, DEANE, J A (2008). Renal cilia display length alterations following tubular injury and are present early in epithelial repair. *Nephrol Dial Transplant* 23: 834-841.

VOGETSEDER, A, PICARD, N, GASPERT, A, WALCH, M, KAISSLING, B, LE HIR, M (2008). Proliferation capacity of the renal proximal tubule involves the bulk of differentiated epithelial cells. *Am J Physiol Cell Physiol* 294: C22-28.

WATANABE, N, KATO, M, SUZUKI, N, INOUE, C, FEDOROVA, S, HASHIMOTO, H, MARUYAMA, S, MATSUO, S, WAKAMATSU, Y (2009). Kidney regeneration through nephron neogenesis in medaka. *Dev Growth Differ* 51: 135-143.

WEI, Q, DONG, Z (2012). Mouse model of ischemic acute kidney injury: technical notes and tricks. *Am J Physiol Renal Physiol* 303: F1487-1494.

WENDLER, S, HARTMANN, N, HOPPE, B, ENGLERT, C (2015). Age-dependent decline in fin regenerative capacity in the short-lived fish Nothobranchius furzeri. *Aging Cell* 14: 857-866.

WHEELER, H E, METTER, E J, TANAKA, T, ABSHER, D, HIGGINS, J, ZAHN, J M, WILHELMY, J, DAVIS, R W, SINGLETON, A, MYERS, R M, FERRUCCI, L, KIM, S K (2009). Sequential use of transcriptional profiling, expression quantitative trait mapping, and gene association implicates MMP20 in human kidney aging. *PLoS Genet* 5: e1000685.

WINGERT, R A, DAVIDSON, A J (2008). The zebrafish pronephros: a model to study nephron segmentation. *Kidney Int* 73: 1120-1127.

WINGERT, R A, SELLECK, R, YU, J, SONG, H D, CHEN, Z, SONG, A, ZHOU, Y, THISSE, B, THISSE, C, MCMAHON, A P, DAVIDSON, A J (2007). The cdx genes and retinoic acid control the positioning and segmentation of the zebrafish pronephros. *PLoS Genet* 3: 1922-1938.

YAKLIN, K M (2011). Acute kidney injury: an overview of pathophysiology and treat-

ments. *Nephrol Nurs* J 38: 13-18; quiz 19.

YIN, V P, THOMSON, J M, THUMMEL, R, HYDE, D R, HAMMOND, S M, POSS, K D (2008). Fgf-dependent depletion of microRNA-133 promotes appendage regeneration in zebrafish. *Genes Dev* 22: 728-733.

ZHOU, W, BOUCHER, R C, BOLLIG, F, ENGLERT, C, HILDEBRANDT, F (2010). Characterization of mesonephric development and regeneration using transgenic zebrafish. *Am J Physiol Renal Physiol* 299: F1040-1047.

ZHOU, W, HILDEBRANDT, F (2012). Inducible podocyte injury and proteinuria in transgenic zebrafish. *J Am Soc Nephrol* 23: 1039-1047.

ZHUO, J L, LI, X C (2013). Proximal nephron. *Compr Physiol* 3: 1079-1123.

Further Related Reading, published previously in the *Int. J. Dev. Biol.*

Preformed Wolffian duct regulates Müllerian duct elongation independently of canonical Wnt signaling or Lhx1 expression
Masahiko Chiga, Tomoko Ohmori, Takashi Ohba, Hidetaka Katabuchi and Ryuichi Nishinakamura
Int. J. Dev. Biol. (2014) 58: 663-668
https://doi.org/10.1387/ijdb.140261rn

Induction of intermediate mesoderm by retinoic acid receptor signaling from differentiating mouse embryonic stem cells
Shiho Oeda, Yohei Hayashi, Techuan Chan, Minoru Takasato, Yuko Aihara,Koji Okabayashi, Kiyoshi Ohnuma and Makoto Asashima
Int. J. Dev. Biol. (2013) 57: 383-389
https://doi.org/10.1387/ijdb.130058ma

Characterization of an lhx1a transgenic reporter in zebrafish
Lisa M. Swanhart, Nobuhiro Takahashi, Rachel L. Jackson, Gregory A. Gibson, Simon C. Watkins, Igor B. Dawid and Neil A. Hukriede
Int. J. Dev. Biol. (2010) 54: 731-736
https://doi.org/10.1387/ijdb.092969ls

Isolation and expression analysis of foxj1 and foxj1.2 in zebrafish embryos
Emil Aamar and Igor B. Dawid
Int. J. Dev. Biol. (2008) 52: 985-991
https://doi.org/10.1387/ijdb.072477ea

Cadherin-6 is required for zebrafish nephrogenesis during early development
Fumitaka Kubota, Tohru Murakami, Kenji Mogi and Hiroshi Yorifuji
Int. J. Dev. Biol. (2007) 51: 123-129
https://doi.org/10.1387/ijdb.062200fk

The long and winding path to understanding kidney structure in amphioxus - a review
Nicholas D. Holland
Int. J. Dev. Biol. (2017) 61: 683-688
https://doi.org/10.1387/ijdb.170196nh

pdzrn3 is required for pronephros morphogenesis in Xenopus laevis
Silvia Marracci, Alberto Vangelisti, Vittoria Raffa, Massimiliano Andreazzoli and Luciana Dente
Int. J. Dev. Biol. (2016) 60: 57-63
https://doi.org/10.1387/ijdb.150381ld

5 yr ISI Impact Factor (2016) = 2.421