3D atlas of human embryology
*New insights in human development*
de Bakker, B.S.

Link to publication

*Citation for published version (APA):*
Bones can break, muscles can atrophy, glands can loaf, even the brain can go to sleep, without immediately endangering our survival, but when the kidneys fail to manufacture the proper kind of blood neither bone, muscle, gland nor brain can carry on."

*Homer W Smith, 1943*
Abstract

Contemporary papers and book chapters on nephrology open with the assumption that human kidney development passes through three morphological stages: pronephros, mesonephros and metanephros. Current knowledge of the human pronephros, however, appears to be based on only a handful of human specimens. Moreover, the ongoing inconclusiveness concerning the definition of a pronephros hampers interpretation of study results whereas there is an increased interest in using the anamniote pronephros as a genetic model for kidney organogenesis. We aimed to provide an overview of literature concerning kidney development, and to clarify the existence of a pronephros in human embryos.

We performed an extensive literature survey regarding vertebrate renal morphology and we investigated histological sections of human embryos between 2 and 8 weeks of development. To facilitate better understanding of the literature concerning kidney development, a referenced glossary with short definitions was composed.

The most striking difference between pronephros versus meso- and metanephros concerns nephron architecture. The pronephros comprises exclusively non-integrated nephrons with external glomeruli, whereas meso- and metanephros are composed of integrated nephrons with internal glomeruli. Non-integrated nephrons were not identified in histological sections of human embryos. Animals whose embryos have comparatively little yolk at their disposal and hence have a free swimming larval stage do develop a pronephros, dedicated to survival in aquatic environments. In embryos that have no free swimming larval stage, are supplied with a large amount of yolk, or develop within the body of the parent, the pronephros is usually absent, incompletely developed, and apparently functionless.

We conclude that a true pronephros is not detectable in human embryos. The most cranial part of the amniote excretory organ has often been confusingly referred to as pronephros. The term pronephros should be avoided in amniotes unless all elements for a functional pronephros are undeniably present.

Introduction

A kidney-related article or book chapter commonly starts with: “Human kidney development follows three separate stages: pronephros, mesonephros and metanephros (Fig. 1A)” (Vize et al. 1997; Hiruma and Nakamura 2003; Ryffel 2003; Solhaug, Bolger, and Jose 2004; Raciti et al. 2008; Michos 2009; Carev et al. 2006; Pole, Qi, and Beasley 2002; Nishinakamura 2003; Kuure, Vuoletenaho, and Vainio 2000; McCrory 1974; Wessely and Tran 2011; Sadler 2004; Cochard 2002; Patten and Carlson 1974; Bailey and Miller 1921; Moore 1988; Tuchmann-Duplessis and Haegel 1974; Prentiss and Arey 1917; Gerlach and Wingert 2013; Hohenstein, Pritchard-Jones, and Charlton 2015; Marra and Wingert 2014; Xing et al. 2014; Wang and Li 2015; Upadhyay and Silverstein 2014). Is this actually true? How sure are we that human embryos pass through a pronephric phase? Doubt on the existence of this structure might be inferred from its vague connotation as ‘transient’, ‘vestigial’ (Goodrich 1930), ‘nonfunctional’ or ‘aglomerular’ (Hamilton, Boyd, and Mossman 1972; Solhaug, Bolger, and Jose 2004; Fraser 1950; Goodrich 1930). Until the 1950s the pronephros, referred to as the first and most primitive embryonic kidney, was actively studied in various species and it recently regained attention because of the establishment of zebrafish and Xenopus laevis as vertebrate models to study human urogenital development. These animals display a transient but functional pronephros.
at some stage of their embryonic development (Kuure, Vuolteenaho, and Vainio 2000; Wessely and Tran 2011; Raciti et al. 2008; Drummond 2005; Jones 2005; Vize et al. 1997).

In 3-6 out of 1,000 human live births, the renal system is affected (Sanna-Cherchi et al. 2007; Schulman et al. 1993). As a possible cause of renal agenesis, Wallace and McCrory suggested that if the pronephros or mesonephros fails to form, the mesonephric duct, ureteric bud or ureter will be absent (McCrory 1974). Therefore, the pronephros as a model to study human kidney development and disease received increasing interest of researchers (Raciti et al. 2008). It is therefore not surprising that Wessely and Tran rightly stated in 2011 that “the golden age of pronephros development may just have begun” (Wessely and Tran 2011). Morphology and development of the renal system in chordates have however been subject of confusion and the literature remains inconclusive. The objective of this study was to clarify the existence of a pronephros in the various taxa and especially in humans, by means of a literature review and by exploring histological sections of human embryos between Carnegie Stage 9 and 23 (19-60 days of development).

Background

The pronephros; prone to confusion and inconclusiveness

Since Johannes Müller first discovered the pronephros and its associated excretory duct in frogs in 1829 (Vize et al. 1997; Balfour and Sedgwick 1878; Müller 1829; Müller 1830), and Bidder identified the glomus (i.e. a large external glomerulus that forms over 2-3 body segments) as its vascular component in 1846 (Vize et al. 1997; Raciti et al. 2008; Bidder 1846), many histological studies were performed in a range of animals: Amphioxis (Prentiss and Arey 1917), primitive jawless fish (Prentiss and Arey 1917), cartilaginous fish (Chimenti and Accordi 2011; Fraser 1950; Goodrich 1930; Kerr 1919; Vize, Woolf, and Bard 2003), bony fish (Hiruma and Nakamura 2003; Chimenti and Accordi 2011; Raciti et al. 2008; Nishinakamura 2003; Gilbert 2010; Szelenyi 1977; Prentiss and Arey 1917), amphibians (Vize et al. 1997; Hiruma and Nakamura 2003; Goodrich 1930; Chimenti and Accordi 2011; Raciti et al. 2008; Wrobel and Suss 2000; Nishinakamura 2003; Fraser 1950; Sedgwick 1881; Gilbert 2010; Prentiss and Arey 1917; Vize, Woolf, and Bard 2003; Rabl 1908), reptiles (Vize et al. 1997; Goodrich 1930; Chimenti and Accordi 2011; Fraser 1950; Vize, Woolf, and Bard 2003), birds (Vize et al. 1997; Hiruma and Nakamura 2003; Goodrich 1930; Davies 1950; Balfour and Sedgwick 1878; Sedgwick 1880, 1881; Gasser 1879; Balfour and Sedgwick 1879), and mammals (Vize et al. 1997; Goodrich 1930; Vize, Woolf, and Bard 2003). Although the presence of a pronephros in human embryos was already questioned by Fraser in 1950 (Fraser 1950), it remains inconclusive whether amniotes, mammals or humans actually do possess a pronephros in the embryonic stage, mainly due to confusing terminology and definitions. The nephrocoel for example (Goodrich 1930; Kerr 1919), a fluid filled cavity in which the external glomerulus or glomus of the pronephros protrudes, can also be named pronephric cavity (Vize et al. 1997), glomeral space (Vize et al. 1997), pronephric chamber (Goodrich 1930; Huettnner 1968), nephric chamber (Fraser 1950), or coelomic chamber (Davies 1951; Fraser 1950), depending on the source, era and background of the author. Even more confusing is the fact that sometimes one term is used for two completely different structures. The nephrocoel for instance has also incorrectly been named nephrotome (Davies 1951; Fraser 1950), while nephrotomes are in fact the mesodermal segments that form the precursors of individual pronephric branches. To facilitate better understanding of the literature concerning kidney development, a referenced
glossary with short definitions was composed (Supplementary data: Kidney development glossary).

Kidney architecture
The basic architecture of a nephron shows that it is one of the best evolutionary conserved structures in the vertebrate kingdom (Fox 1963) and to a certain extent also in several invertebrate clades (Ruppert 1994). Despite the anatomical differences (Fig. 2 and 3) and functions between the three kidney types, the nephron is more or less present in all of them (Wessely and Tran 2011). Each nephron is composed of three components; an initial filtering component (a more or less developed glomerulus), a waste collecting unit (coelom/nephrocoel/Bowman’s capsule/Bowman’s space) and a nephric tubule specialized for reabsorption of solutes and water, and secretion of wastes (Fraser 1950; Sanna-Cherchi et al. 2007). Although each kidney form differs in overall organization and complexity, they all have the nephron as their basic structural and functional unit (Raciti et al. 2008). Gérars & Cordier divided nephrons into two types, whether they are in open communication with the coelom (the non-integrated nephron, Fig. 3A), or separated from it (the integrated nephron, Fig. 3B) (Gérard and Cordier 1934a, 1934b; Fraser 1950; Dawson 1925; Lambert 1933). For further details on the terminology concerning kidney development, see the enclosed glossary (Supplementary data: Kidney development glossary).

Definition of a pronephros
The word ‘Pronephros’ is derived from the Greek and means ‘before kidney’ (Larsen 1993): the first and most primitive (Fox 1963; Hall 1904) kidney. The pronephros develops from mesenchymal buds of pronephric primordia, or nephrotomes (Vize et al. 1997; Sadler 2004) at the most cranial part of the mesodermal nephrogenic cord (Vize et al. 1997; Chimenti and Accordi 2011; Cochard 2002; Mathews 1976). These buds of pronephric primordia hollow out to form pronephric tubules (McCrorry 1974; Mathews 1976).

A typical pronephric nephron, as can be found in amphibian larvae and some adult teleosts (Fraser 1950; Hamilton, Boyd, and Mossman 1972), consists of the following functional units; an external glomerulus or glomus as vascular component that filters wastes into the coelom or nephrocoel as waste-collecting unit, from which a ciliated nephrostome leads to the pronephric tubule that drains into the pronephric duct (Fig. 2A and 3A) (Fraser 1920; Vize et al. 1997; Chimenti and Accordi 2011; Nieuwkoop and Faber 1994; Brandli 1999; Raciti et al. 2008; Davies 1950; Cho et al. 2011; Nishinakamura 2003; Wessely and Tran 2011; Fraser 1950; Dawson 1925; Lambert 1933; Vize, Woolf, and Bard 2003). Vize et al. stated that when the filtering vascular structure is one body segment in length it is referred to as a glomerulus, while if it extends over multiple body segments it is referred to as a glomus (Vize et al. 1997; Pole, Qi, and Beasley 2002). The pronephros proper secretes its filtered wastes from the glomerulus or glomus directly into the coelom (Fig. 4A) or in an anatomically more advanced pronephros in a nephrocoel, a fluid filled cavity contiguous with the coelom in which the external glomerulus or glomus of the pronephros protrudes to filter wastes (Fig. 4B) (Vize et al. 1997; Goodrich 1930; Chimenti and Accordi 2011; Fraser 1950; Huettner 1968). Although the glomus hangs freely in the coelom, it is intimately associated with the ciliated nephrostomes that transport the coelomic fluid towards the pronephric tubuli (Fig. 2B). By contrast, more advanced mesonephric and metanephric nephrons encompass a Bowman’s capsule or Bowman’s space respectively as waste-collecting unit, which is integrated in the tubule and is therefore called an integrated nephron (Vize et al. 1997; Fraser 1950; Davies 1950). Bowman’s capsule, together with the internal glomerulus, constitute a Malpighian body (Fraser
The Pronephros; a fresh perspective

The Malpighian body is regarded as a typical feature of the mesonephros (Wrobel and Suss 2000). The waste-collecting unit of the pronephros on the other hand is not integrated in the tubule, and is therefore referred to as non-integrated nephron (Fig. 2B and 3B) (Vize et al. 1997; Davies 1950; Fraser 1950; Dawson 1925; Lambert 1933).

The pronephros is a relatively large excretory organ in basal chordates, such as jawless fish (Vize, Woolf, and Bard 2003; Prentiss and Arey 1917), teleosts (Vize et al. 1997; Vize, Woolf, and Bard 2003), lungfish (Fraser 1950; Vize, Woolf, and Bard 2003; Goodrich 1930) and amphibians (Vize et al. 1997; Hiruma and Nakamura 2003; Goodrich 1930; Chimenti and Accordi 2011; Raciti et al. 2008; Wrobel and Suss 2000; Nishinakamura 2003; Fraser 1950; Gilbert 2010; Prentiss and Arey 1917; Vize, Woolf, and Bard 2003; Rabl 1896). In the latter, it functions mainly during their larval stage in an aquatic environment (Vize, Woolf, and Bard 2003; Gaeth, Short, and Renfree 1999; Fraser 1950; Chimenti and Accordi 2011). Presence of a pronephros has also been reported in some reptiles (Vize, Woolf, and Bard 2003) and birds (Davies 1950; Gasser 1879; Balfour and Sedgwick 1878, 1879; Sedgwick 1880, 1881) like the green sea turtle (Wiedersheim 1890; Davies 1950), crocodilians (Vize, Woolf, and Bard 2003; Wiedersheim 1890; Davies 1950), chicken (Davies 1950; Szebenyi 1977; Sedgwick 1880, 1881; Kerr 1919; Vize, Woolf, and Bard 2003) and duck (Davies 1950; Sedgwick 1880; Mihalkovics 1885) and it is commonly assumed to be present in mammalian embryos including humans (Fraser 1920; Solhaug, Bolger, and Jose 2004; Carev et al. 2006; Kuure, Vuolteenaho, and Vainio 2000; McCrory 1974; Hamilton, Boyd, and Mossman 1972; Sadler 2004; Gasser 1975; Moore 1988; Tuchmann-Duplessis and Haegel 1974; Prentiss and Arey 1917; Felix 1912; Torrey 1954; Hiruma and Nakamura 2003; Gilbert 2010; Sadler 2015; Cochard 2012; Bailey and Miller 2011; Hamilton 1952; Hoadley 1926; Keith 1933; Abdel-Malek 1950). In a few species, such as the sea lamprey (Prentiss and Arey 1917), lancelet (Prentiss and Arey 1917), hagfish (Prentiss and Arey 1917), lungfish (Prentiss and Arey 1917) and some Teleosts like Fierasfer (Goodrich 1930), Zoarces (Goodrich 1930) and Lepadogaster (Guitel 1906), the pronephros remains functional through adulthood.

Pronephros in human embryos?

Existence of a pronephros has often been claimed in human embryos (Fraser 1920; Solhaug, Bolger, and Jose 2004; Carev et al. 2006; Kuure, Vuolteenaho, and Vainio 2000; McCrory 1974; Hamilton, Boyd, and Mossman 1972; Sadler 2004; Gasser 1975; Moore 1988; Tuchmann-Duplessis and Haegel 1974; Prentiss and Arey 1917; Felix 1912; Torrey 1954; Hiruma and Nakamura 2003; Gilbert 2010; Sadler 2015; Cochard 2012; Bailey and Miller 2011; Hamilton 1952; Hoadley 1926; Keith 1933; Abdel-Malek 1950) and nowadays still many kidney-related articles or book chapters open with the assumption that human kidney development passes through all three kidney forms. Keeping in mind the research era in which study designs were based on the idea that ontogeny recapitulates phylogeny (Smith 1953; Huettner 1968; Hiruma and Nakamura 2003; Solhaug, Bolger, and Jose 2004), it could have been condoned to study exclusively fish and amphibians, after which the findings were projected on the early stages of human development. To support this now refuted theory, one might have named the most cranial region of the human mesonephros ‘pronephric’ (Davies 1950; Fraser 1950). Add to this that research on human embryos has always been hampered by their scarcity. Therefore, recent literature is almost always directly or indirectly referring to the extensive study of the human pronephros by Felix in 1912 (Felix 1912). Since 1912, not many researchers studied the human pronephros in particular. Most text-books are referring to Lauri Saxen’s “Organogenesis of the
Kidney” (1987). In the corresponding chapter the author quotes another kidney scientist, Torrey, as his prime source for information on the pronephros, but it turns out that Torrey did not claim at all that human embryos have a pronephros (Torrey 1954; O’Rahilly and Müller 1987). As it appears, the current knowledge of the human pronephros is very limited, since it is based on a hand full of observations. Already in 2004 Solhaug et al. stressed the need for studies in human samples (Solhaug, Bolger, and Jose 2004). We therefore decided to investigate the development of the nephric system in the specimens of human embryos available to us.

Methods

Specimens
Images of serial histological sections of 43 human embryos from Carnegie stage 8 (17-19 days) till 23 (56-60 days) from the Carnegie Collection in Silver Spring, MD, USA were used to study kidney development. Details concerning the used specimens can be found in table 1 (Gasser et al. 2014; Lockett 2001; Morgan 2009a; O’Rahilly and Müller 1987; Streeter 1942, 1949, 1945, 1948, 1951). The pronephros is said to develop in the third week of human embryonic development and to disintegrate at the end of the fourth week (Solhaug, Bolger, and Jose 2004; Carev et al. 2006; Kuure, Vuolteenaho, and Vainio 2000; McCrory 1974; Hamilton, Boyd, and Mossman 1972; Sadler 2004; Gasser 1975; Moore 1988; Tuchmann-Duplessis and Haegel 1974; Felix 1912) Therefore, more specimens of Carnegie stage 8 (17-19 days), stage 9 (19-21 days) and stage 10 (21-23 days) were incorporated in this study (Table 1). From stage 11 (23-26 days) onwards, two specimens per stage were studied. Image acquisition and alignment of the images was done as previously described by de Bakker et al. (de Bakker et al. 2016; de Bakker et al. 2012).

Research method
Histological sections of human embryos belonging to Carnegie stages 8 (17-19 days) till 23 (56-60 days) were inspected with a focus on the intermediate mesoderm or nephrogenic cord (Hamilton, Boyd, and Mossman 1972; Sadler 2004; Tuchmann-Duplessis and Haegel 1974; O’Rahilly and Müller 1987), the region between the somites (i.e. paraxial mesoderm) and the lateral plate mesoderm, from which the urogenital tract develops. Following the previously formulated definition (Fraser 1920; Vize et al. 1997; Chimenti and Accordi 2011; Nieuwkoop and Faber 1994; Brandli 1999; Raciti et al. 2008; Davies 1950; Cho et al. 2011; Nishinakamura 2003; Wessely and Tran 2011; Fraser 1950; Dawson 1925; Lambert 1933; Vize, Woolf, and Bard 2003), pronephric nephrons were distinguished from mesonephric nephrons by the exclusive presence of external glomeruli in the former (Fig. 2 and 3).
The Pronephros: a fresh perspective

Results

The stage 8 embryos (17-19 days of development) portrayed only the three undifferentiated germ layers, i.e. endoderm, mesoderm and ectoderm. In stage 9 (19-21 days) the mesoderm could be differentiated into axial mesoderm (i.e. the notochordal plate), paraxial mesoderm (i.e. the somites), the lateral plate mesoderm and in-between these last two the intermediate mesoderm could be identified (Fig. 5A). In stage 10 (21-23 days; Fig. 5B) and stage 11 (23-26; Fig. 5C) however, the intermediate mesoderm stood out more clearly, as a mass of undifferentiated mesenchymal cells. The cranial margin of the nephrogenic cord was first identified in the intermediate mesoderm at the level of the 10th somite of stage 12 (26-30 days; Fig. 5D, G) human embryos which is not in the cervical region, but merely at the level of the umbilical cord and vitelline duct, far caudal of the developing heart and liver. The presence of a very primitive Bowman’s capsule around a glomerulus, without a connection to the coelom, qualifies these nephrons as mesonephric (Fig. 5G). No structures with pronephric characteristics (i.e. external glomeruli excreting directly into the coelom or nephrocoel) were seen in embryos of stage 12 nor in embryos of earlier or later stages.

The mesonephros with its mesonephric duct (Fig. 5D-O and Fig. 1D) is present from stage 12 onwards and remains present during the embryonic phase of development, at least up to 60 days of development. Already in stage 12 or 13, depending on the specimen, the mesonephric duct makes contact with the urogenital sinus.

In human embryos of stage 14 (31-35 days), the metanephros anlage was first recognized as mesenchymal packaging around the ureteric bud and in stage 15 (35-38 days) the metanephros was clearly present. Mesonephros and metanephros were histologically easily distinguishable from each other, based on the complexity of their nephrons. Due to embryonic growth, the mesonephros shrinks relatively in size, compared to the metanephros (de Bakker et al. 2016). At stage 17 already, the metanephros is found at its adult location at the level of the first lumbar vertebra. The caudal region of the embryo, including the inferior mesenteric artery, grows further into caudal direction, giving the erroneous impression that the kidneys migrate upwards during development (de Bakker et al. 2016).

Discussion

The aim of the current study was to clarify whether or not a pronephros in human embryos exists, by using histological sections. Based on these sections we can conclude that the pronephros is not detectable in human embryos of 3-4 weeks of development, the time frame in which we expected to find the pronephros according to literature, nor in earlier or later stages (Fraser 1920; Solhaug, Bolger, and Jose 2004; Davies 1951; Carev et al. 2006; Kuure, Vuolteenaho, and Vainio 2000; Felix 1912; Gilbert 2010; Hamilton, Boyd, and Mossman 1972; Sadler 2004; Tuchmann-Duplessis and Haegel 1974; Moore 1988).

For a summary of the differences between the three kidney forms see table 2 and figures 2, 3 and 4. The most striking difference between pronephros and meso- and metanephros is that the pronephros proper consists of non-integrated nephrons, whereas the mesonephros and metanephros consist of only integrated nephrons (Fig. 2B and 3B) (Vize et al. 1997; Davies 1950; Fraser 1950; Dawson 1925; Lambert 1933).
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### Table 1 continued. Overview of the studied human specimens

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Table 1 continued. Overview of the studied human specimens

CS: Carnegie stage, Year: Year of acquisition, CRL: Calculated crown-rump-length in mm, Day: days post ovulation, Z-res: Calculated Z-resolution in μm.

a Origin of the specimen: CC = Carnegie Collection: Human Developmental Anatomy Center at the National Museum of Health and Medicine in Silver Spring, Maryland, USA // BC = Boyd Collection: Department of Physiology, Development and Neuroscience, University of Cambridge, United Kingdom // AMC = Department of Medical Biology, Academic Medical Center, University of Amsterdam, The Netherlands // CU = Cambridge University, United Kingdom // HDBR = Human Developmental Biology Resource, Institute of Genetic Medicine, International Centre for Life, Newcastle Upon Tyne, United Kindom.(Streeter 1942, 1945, 1948, 1949, 1951; O’Rahilly and Müller 1987; Morgan 2009b; Gasser et al. 2014; Lockett 2001)
b Plane of sectioning: o = oblique, t = transversal, c = coronal

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**Fig. 1. Organization of kidney development.**

- **A:** Diagrammatic sketch illustrating the three sets of excretory structures as supposed to be present in a human embryo during the fifth week (about 32 days, Carnegie stage 14) (After Moore, 1988).(Moore 1988) Cr: cranial, Ca: caudal.
- **B:** Reconstruction of the urogenital system of a stage 16 human embryo specimen 6517 (37-42 days), including meso- and metanephros and their ducts (de Bakker et al. 2016). Cr: cranial, Ca: caudal, D: dorsal, V: ventral.
- **C:** Section through the mesonephric region of a stage 17 human embryo specimen 6521 (42-44 days).
The pronephros; a matter of definition?

Providing clear definitions on the developmental aspects of the pronephros has proven to be a challenge. The pronephros can be defined strictly as non-integrated nephrons, where others like Davies (1951) defined it as ‘the most cranial part of the amniote excretory organ’ (Pole, Qi, and Beasley 2002; Larsen 1993; Vize, Woolf, and Bard 2003). The notion put forward, mainly by early German researchers, and above all by Felix (1912), that the vertebrate excretory system was made up of three sets of organs, the pronephros, the mesonephros and the metanephros, which were laid down all along the trunk and succeeded one another in time (Fig. 1A), has long ago been shown to be merely a hypothesis for which no real proof has ever been found (Fraser 1950). The idea that vertebrates carry three sets of kidneys has first been explained by the existence of a common ancestral kidney, the archinephros, which becomes differentiated into pro-, meso-, and metanephros, according to the needs of the animal (Fox 1963; Goodrich 1930; Balfour and Sedgwick 1876; Renson 1883; Weldon 1883; Wiedersheim 1890; Field 1891; Price 1897, 1904; Brauer 1902; Kers 1907; Burland 1913; Borcea 1905; Kerr 1919). The obsolete terms holonephros (Price 1897; Brauer 1902; Torrey 1954) and mononephros (Audigé 1910) which have also been used in literature to indicate the entire excretory system (Smith 1953, 1943) have added to the confusion.

The exclusive existence of nephrostomes and peritoneal funnels have long been regarded as typical differences between pro- and mesonephros. However, literature remains inconclusive on which of the two features is typically pronephric or mesonephric, due to returning terminology issues. Peritoneal funnels and nephrostomes are both ciliated tubules, which complicates the distinction between the two (Goodrich 1930; Kerr 1919). A ciliated nephrostome links the waste collecting unit (i.e. the coelom (Fig. 4A), nephrocoel (Fig. 4B) or Bowman’s capsule (Fig. 4D)) to the proximal nephric tubule (Vize, Woolf, and Bard 2003), whereas a narrow ciliated peritoneal funnel links the coelom to the encapsulated glomerulus, the precursor of Bowman’s capsule (Fig. 4C). The ciliated nephrostome is always present in the non-integrated nephron of a pronephros (Fig. 4A and B) but can also be present in a mesonephros (Fig. 4D). As such, it does not discriminate between the two. The gradually ligating wide connection between coelom and nephrocoel in the pronephros has sometimes even been regarded as peritoneal funnel, but we recommend not to use this term if there are no cilia present.

The nephron in figure 4C, which includes both a peritoneal funnel and a nephrostome, as found in embryos of ruminants such as sheep and cattle, has been identified by Vize in the anterior-most tubules of the mesonephros (Davies 1951; Vize, Woolf, and Bard 2003; Wintour et al. 1996). To avoid ambiguity, we suggest to refer to these anterior-most intermediate nephrons (i.e. nor non-integrated, nor integrated nephrons) as an evolutionary transition zone between pro- and mesonephros (Fraser 1920; Mihalkovics 1885; Renson 1883; Sedgwick 1881; Davies 1950; Hiruma and Nakamura 2003), since the waste collecting unit is still in contact with the coelom (a typical pronephric feature) and the peritoneal funnel has confusingly been regarded as both pronephric (Davies 1951; Hamilton, Boyd, and Mossman 1972; Fraser 1950; Goodrich 1930) as well as mesonephric (Vize, Woolf, and Bard 2003). Therefore, we do not entitle the peritoneal funnel as an exclusive feature of pro- or mesonephros, but advocate this characteristic to be a reflection of a gradual evolutionary change from pro- to mesonephros, as Wiedersheim already postulated in 1890 based on his observations in crocodile and turtle embryos (Davies 1950; Hiruma and Nakamura 2003; Wiedersheim 1890).
Pronephros in amniotes

In amniote embryos that have no free swimming larval stage, which are supplied with a large amount of yolk, or which develop within the body of the parent (i.e. elasmobranchii, reptilia, aves and mammalia) the pronephros is usually not present or incompletely developed, and therefore functionless (Fraser 1950; Sedgwick 1881; Rabl 1896). However, functional pronephroi with external glomeruli, ciliated nephrostomes and three to four well-differentiated tubules, have been reported in some reptilia, e.g. turtles and crocodiles (Davies 1950; Vize, Woolf, and Bard 2003; Wiedersheim 1890). Nevertheless, since the main observation in reptiles date back to Wiedersheims work from 1890 (Wiedersheim 1890), it is advisable to reinvestigate these observations following the correct definition of a pronephros.

Based on a range of different definitions, the pronephros has often been described in aves (Gasser 1879; Balfour and Sedgwick 1878, 1876; Sedgwick 1880, 1881; Mihalkovics 1885; Kerr 1919; Huettner 1968; Jacobsen 1917; Patten and Carlson 1974; Hiruma and Nakamura 2003; Vize et al. 1997; Goodrich 1930; Davies 1950; Rabl 1896), but it is almost never regarded as functional (Vize et al. 1997; Hiruma and Nakamura 2003; Goodrich 1930; Davies 1950; Abdel-Malek 1950; Patten and Carlson 1974; Huettner 1968; Hamilton 1952; Rabl 1896; Sedgwick 1880). At the utmost, the avian pronephros is considered to have particular minor functions like secreting some wastes into the body cavity (Vize et al. 1997; Needham 1931; Waddington 1938; Jacob, Jabob, and Christ 1977), because the tubules are not hollow (Huettner 1968). Nevertheless, detailed descriptions of the external glomeruli in aves are given by Gasser (Gasser 1879), Balfour & Sedgwick (Balfour and Sedgwick 1878, 1876), Sedgwick (Sedgwick 1880, 1881), and Mihalkovics (Mihalkovics 1885).

The pronephros in mammals, including human, has most often been considered as vestigial and non-functional (Goodrich 1930; Chimenti and Accordi 2011; Solhaug, Bolger, and Jose 2004; Nishinakamura 2003; Fraser 1950; Gilbert 2010; Hamilton, Boyd, and Mossman 1972; Sadler 2004; Cochrard 2002; Prentiss and Arey 1917; Vize, Woolf, and Bard 2003; Rabl 1896; Sedgwick 1880), or not present at all (Davies 1951; Guitel 1906). On the other hand, there have also been authors describing a pronephros in embryos, not only of humans, as stated above, but also in mice (Nishinakamura 2003; Kobayashi et al. 2007; Kuure, Vuolteenaho, and Vainio 2000) and other mammals (Davies 1951; Fraser 1920; Goodrich 1930; Pole, Qi, and Beasley 2002; Vize et al. 1997; Vize, Woolf, and Bard 2003). The most cranial part of the amniote excretory organ is then often confusingly referred to as transient, vestigial (Goodrich 1930), nonfunctional or aglomerular (Goodrich 1930; Solhaug, Bolger, and Jose 2004; Fraser 1950; Hamilton, Boyd, and Mossman 1972) pronephros (Fraser 1920; Davies 1951; Fraser 1950; Goodrich 1930; Larsen 1993). Although in the absence of distinctive morphological characteristics, no real distinction can be made between remnants of an incomplete, vestigial pronephros and the gradually degenerating cranial nephrons of the mesonephros (Hiruma and Nakamura 2003; Keith 1933; Abdel-Malek 1950; Hamilton 1952; Hoadley 1926), the term pronephros should be avoided in amniotes (Fraser 1950) because the elements for a functional pronephros (including a fully developed external glomerulus, hollow ciliated nephrostomes and hollow pronephric tubules) are undeniably never present.
**Pronephros in anamniotes**

Animals whose embryos have comparatively little yolk at their disposal (mesotecithal) and pass through a free swimming larval stage develop a pronephros (Goodrich 1930; Sedgwick 1881; Rabl 1896), dedicated to water excretion (Vize, Woolf, and Bard 2003) and survival in aquatic environments (Howland 1916; Raciti et al. 2008; Prentiss and Arey 1917). In general, all anamniote embryos have well-developed pronephroi (Vize et al. 1997; Fox 1963; Goodrich 1930; Nieuwkoop and Faber 1994; Wrobel and Suss 2000; Nishinakamura 2003; Kuure, Vuolteenaho, and Vainio 2000; Wessely and Tran 2011; Fraser 1950; Gilbert 2010; Prentiss and Arey 1917; Huettnerr 1968; Vize, Woolf, and Bard 2003; Kerr 1919; Howland 1916; Jaffee 1963; Christensen 1964; Armstrong 1932; Howland 1921; Fales 1935; Holtfreter 1944; Brauer 1902; except for most sharks and rays (Vize et al. 1997; Goodrich 1930; Kerr 1919). In basal chordates, e.g. Amphioxus, Cyclostomes and Dipnoi, the pronephros functions generally as their adult kidney (Hamilton, Boyd, and Mossman 1972; Prentiss and Arey 1917; Vize, Woolf, and Bard 2003; Bertrand and Escriva 2011). In bony fish and amphibians, the pronephros functions as the embryonic kidney. However, in some Dipnoi (Prentiss and Arey 1917) and Teleosts, e.g. Fierasfer (Goodrich 1930), Zoarces (Goodrich 1930) and Lepadogaster (Guitel 1906), the pronephros remains functional through adulthood, often alongside the functional mesonephros (Hamilton, Boyd, and Mossman 1972). The number of pronephric nephrostomes differs between species. Anurans generally have three nephrostomes between coelom and pronephric tubules, most urodeles have two and teleosts usually have only one nephrostome connecting its single pronephric tubule (Vize et al. 1997).

**Evolutionary aspects of kidney development**

We sought to verify the existence of a pronephros in the different vertebrate taxa to get a grasp on the evolutionary aspects of the three subsequent kidney forms (see Fig. 6 and supplementary table 1). Although evolution has provided more advanced vertebrates with complex adult kidneys, they continue to utilize simple evanescent kidneys during embryogenesis (Vize et al. 1997). Basal vertebrates with simple adult kidneys use even more uncomplicated versions during early developmental stages (see also Figs. 6 and 7 and supplementary table 1) (Vize et al. 1997). In the end, it is much easier to form a pronephros, than it would be to form a more complex meso- or metanephros in a short period of time. The advantages of a simple temporary kidney to serve the free-swimming larva are obvious: provide time for a complex kidney to form. The same genes are involved in the development of all three vertebrate kidney forms (Nishinakamura 2003; Kuure, Vuolteenaho, and Vainio 2000; Carroll and Vize 1996; Heller and Brandli 1997). Among these genes are Pax8 (Bouchard et al. 2002; Kobayashi et al. 2007; Nishinakamura 2003; Kuure, Vuolteenaho, and Vainio 2000; Carroll and Vize 1996; Heller and Brandli 1997; Dressler et al. 1990; Dressler and Doughlass 1992), Pax8 (Bouchard et al. 2002; Kobayashi et al. 2007), Tbx2 (Cho et al. 2011), BMP (Gilbert 2010), Hey1 (Cho et al. 2011), Gremlin (Cho et al. 2011), Xlim1 (Nishinakamura 2003), and WT1 (Nishinakamura 2003; Kuure, Vuolteenaho, and Vainio 2000; Carroll and Vize 1996; Heller and Brandli 1997). No genes have yet been discerned that are exclusively involved in pronephric development. This strong genetic conservation of kidney organogenesis (Kuure, Vuolteenaho, and Vainio 2000) ironically hampers differentiation between pro-, meso and metanephros on a genetic level but also substantiates the theory that pro- and mesonephric development features merely a gradual evolutionary transition from external- to internal glomerulus.
4.1 Fig. 3. Detailed architecture of a pro- and mesonephric nephron. A: Pronephric anatomy: the non-integrated nephron of a pronephros in frogs. The glomus, a single glomerular unit that is formed over 2-3 body segments and later becomes more compact, is supplied by arterial sprouts from the dorsal aorta and filters wastes directly in the fluid of the coelomic cavity (Gerth, Zhou, and Vize 2005). The glomus is not integrated in the tubules, but it lies in the immediate vicinity of them, so there is almost no space between the glomus and nephrostomes. These ciliated nephrostomes collect the coelomic fluid and through the pronephric tubules the primitive urine will be collected via the pronephric duct in the cloaca. See also figure 3A. B: Mesonephric anatomy: the integrated nephrons of a mesonephros. The glomeruli are supplied by arterial sprouts from the aorta that pass behind the coelom and filter wastes into Bowman’s cavity. The glomeruli are therefore integrated in the tubules. The collected wastes are transported through the mesonephric tubules toward the mesonephric duct and collected in the urogenital sinus. See also figure 3B. The ureteric bud which initiates metanephric development and will become the ureter is an outgrowth of the mesonephric duct. The cranial-most metanephric nephrons degenerate over time. Note that these degenerate nephrons have often been wrongly presumed as pronephric. C: Metanephric anatomy: the integrated nephron. The mesonephric nephron with its Bowman’s space and extensive tubule is firmly embedded within the renal cortex (brown) and renal pyramids (lighter triangles). Urine is collected in the renal pelvis and transported through the ureter toward the bladder. A: aorta, B: bladder, C: coelom, Ca: caudal, Cr: cranial, DA: dorsal aorta, PD: pronephric duct, US: urogenital sinus, U: ureter, UB: ureteric bud, MD: mesonephric duct.
Fig. 4. Different manifestations of pronephric and mesonephric nephrons. The nephrons in A and B are purely pronephric as the external glomerulus or glomus hangs freely in the coelom (A) or nephrocoel (B). The nephrons in D and E are typical mesonephric because the connection with the coelom is lost and the internal glomerulus is enclosed by Bowman's capsule. The nephron in C, which includes both a peritoneal funnel (PF) and a nephrostome (NS), has been regarded by Vize as mesonephric (Vize, Woolf, and Bard 2003). To avoid ambiguity, we however suggest to refer to these nephrons as evolutionary transition zone (Fraser 1920; Mihalkovics 1885; Renson 1883; Sedgwick 1881; Davies 1950; Hiruma and Nakamura 2003), since the glomerulus is still in contact with the coelom (a typical pronephric feature) and the PF has been regarded both pronephric (Davies 1951; Hamilton, Boyd, and Mossman 1972; Fraser 1950) as mesonephric (Vize, Woolf, and Bard 2003), depending on the source. This kind of nephron might actually represent the gradual evolutionary change from pro- to mesonephros (Davies 1950; Hiruma and Nakamura 2003; Wiedersheim 1890). C: coelom, G: glomerulus, G/G: glomerulus/glomus, L: lateral, M: medial, NS: ciliated nephrostome, which links the coelom or nephrocoel with the proximal tubule, PF: ciliated peritoneal funnel, which links the coelom to the encapsulated glomerulus (primitive Bowman’s capsule), T: Tubule.

Fig. 5. Histological features of the human nephrogenic development (right page). A: Transverse section through the caudal region of a stage 9 (26-30 days) human embryo specimen H712. The intermediate mesoderm (IM) is still hard to discern from somite (paraxial mesoderm) and lateral plate mesoderm (LM) B: Transverse section through the caudal region of a stage 10 (21-23 days) human embryo specimen 5074. The intermediate mesoderm is recognizable as a clump of undifferentiated mesenchymal cells. C: Transverse section through the caudal region of a stage 11 (23-26 days) human embryo specimen 6344. The intermediate mesoderm is still undifferentiated. D: Transverse section through the caudal region of a stage 12 (26-30 days) human embryo specimen 8943. The nephrogenic cord (NC) is now present. E: Transverse section through the mesonephric region of a stage 13 (28-32 days) human embryo specimen 836. F: Transverse section through the mesonephric region of a stage 14 (31-35 days) human embryo specimen 6502. G: Enlarged part of the section in D. A very primitive glomerulus is recognizable, surrounded by a primitive Bowman’s capsule. Bowman’s capsule is not in contact with the coelom, also not on the adjacent and subsequent sections. The mesonephric duct is not yet lumenized at this stage. H: Enlarged part of the section in E. The glomerulus and Bowman’s capsule still appear in a primitive stage. The mesonephric duct becomes lumenized. I: Enlarged part of the section in F. The definitive morphology of the mesonephros is recognizable. The glomerulus and Bowman’s capsule together constitute the malpighian body. The mesonephric duct and tubule are well defined and lumenized. At this stage the mesonephros can be assumed to be in function. Between stage 14 and stage 17 (L,O) the histological features of the mesonephros remain constant. J: Transverse section through the mesonephric region of a stage 15 (35-38 days) human embryo specimen 721. K: Transverse section through the mesonephric region of a stage 16 (37-42 days) human embryo specimen 6517. L: Transverse section through the mesonephric region of a stage 17 (42-44 days) human embryo specimen 6520. M: Enlarged part of the section in J. N: Enlarged part of the section in K. Two Bowman’s capsules are present in this section. Also note the clear presence of a gonadal ridge. O: Enlarged part of the section in L. Two glomeruli can be appreciated in this section. A: Arteriole, AO: Aorta, BC: Bowman’s capsule, C: coelom, D: mesonephric duct, G: Gonadal ridge, GL: glomerulus, IM: intermediate mesoderm, LM: lateral plate mesoderm, N: notochordal plate (CS 9, 10, 11) or notochord (CS 12, 13, 14), NC: nephrogenic cord, NG: neural groove, NT: neural tube, PV: postcardinal vein, S: somite, T: mesonephric tubule.
Fig. 6. Overview of the appearance of a pronephros in animal species, presented as evolutionary cladogram. For details and references, see supplementary table 1. Yellow (Cephalochordata and Cyclostomata): pronephros functioning as adult kidney. Orange (Chondroichthyes, Actinopterygii, Sarcopterygii and Amphibia): pronephros functioning in the larval stage. However, the pronephros seems to be absent in those Elasmobranchii that have no larval stage, and in the Amniota which develop within the body of the parent (Fraser 1950). Blue (Squamata, Crocodylia and Aves): Although a pronephros has been described in embryos of some of these animals (supplementary table 1), the term pronephros should be used with much restraint (Fraser 1950). Further research in these species is needed to clarify the contradictions that appeared in the literature as a result of the use of different definitions. Green (Mammals): no pronephros is present.
**The Pronephros; a fresh perspective**

### Table: Pronephros, Mesonephros, Metanephros

<table>
<thead>
<tr>
<th></th>
<th>Pronephros</th>
<th>Mesonephros</th>
<th>Metanephros</th>
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<tbody>
<tr>
<td><strong>Jawless fish</strong></td>
<td><img src="image1.png" alt="Image" /></td>
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<tr>
<td><strong>Teleosts &amp; Anamnia</strong></td>
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<td><img src="image3.png" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td><strong>Amniotes</strong></td>
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<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
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</tbody>
</table>

**Fig. 7. General concept for renal evolution.** Vertical columns: different kidney forms (i.e. prone-, meso- and metanephros). Horizontal columns: different classes of species. The blue boxes indicate the larval or embryonic stage of a certain class, and the light brown boxes indicate adult stages of the respective classes. In most jawless fish, like Amphioxus and hagfish, the pronephros remains functional through adulcy, often alongside a functional mesonephros. Larvae of teleosts and anamnia (e.g. tadpoles) generally pass a pronephric stage, whilst adult specimens (e.g. frogs) use a mesonephros for secretion. Embryos of amniotes (e.g. humans) do not pass a pronephric stage, but do use the mesonephros during the embryonic phase and the metanephros through fetal development, in childhood and in adulcy. The used kidney form thus gradually shifts from simple pronephric kidney as used by adult jawless fish, via the intermediate mesonephric kidney in more basal vertebrates towards the intricate metanephric kidney as used by more advanced vertebrates.
The main tool of vertebrates to survive in varying circumstances, from fresh to salt water and from desert to rain forests, is the renal system which provides the vertebrates to either excrete large amounts of water or retain as much water as possible. The three vertebrate kidney forms are suitable for different habitats and are used in diverse combinations by the vertebrates with specific physiological requirements in the various stages of their life (Raciti et al. 2008). Water excretion seems to be the most common characteristic for species that show pronephros development (Vize and Smith 2004). This is in line with Fraser’s theory, that the pronephros seems to be absent in those Elasmobranchii that have no larval stage, and in the Amniota which develop within the body of the parent (Fraser 1950).

As mentioned before, the need for a pronephros also depends on the amount of yolk available for the embryo. Embryos supplied with large amounts of yolk (macrolecithal) generally show less developed pronephric tubuli, whereas embryos with comparatively little yolk at their disposal develop extensive and functional pronephroi (Fraser 1950; Rabl 1896; Sedgwick 1880). The placenta also influences the degree of kidney development. The mesonephros is less developed in species that exhibit an intimate relation between extraembryonic membranes and placenta (e.g. humans and mice), whereas species with less effective placental systems (e.g. pigs) show better developed mesonephroi (Nelson 1953; Vize et al. 1997; Carlson 1988). Thus, in the presence of a yolk sac or a placenta as efficient waste disposal systems, kidney development is not essential for waste disposal or osmotic regulation prior to birth (Vize, Woolf, and Bard 2003). It can therefore be reasoned that the appearance of the yolk sac and placenta featured the gradual disappearance of the pronephros in more advanced vertebrates. It would be interesting to study the presence of a pronephros in egg laying mammals (Protheria) since Fraser stated that Marsupialia (Metatheria) do develop a pronephros that functions in the larval stage (supplementary table 1). To better grasp the evolutionary development of the pronephros, more research is also needed to dispel the ambiguous presence of a pronephros in egg laying amniotes, i.e. birds and reptiles (supplementary table 1).

The fate of the pronephros

In both Teleost and Ganoid fish, the pronephric filtration unit relinquishes its excretory task to the mesonephros (Vize, Woolf, and Bard 2003), which leaves a lymphoid organ with hematopoietic function (Hansen and Kaattari 1996; Vize et al. 1997; Vize, Woolf, and Bard 2003). This transition from excretory to lymphoid organ occurs relatively late, beyond the sixth developmental week in zebrafish (Drummond 2000; Vize, Woolf, and Bard 2003). Further research is needed to seize the process which underlies the pronephric transition from excretory to lymphoid organ. The amphibian pronephros degenerates during metamorphosis (Vize, Woolf, and Bard 2003). By stopping the metamorphosis process, through blocking thyroid function (Fox and Turner 1967; Hurley 1958) or by thyroid- or hypophysectomy (Fox 1963), degeneration of the pronephros can be inhibited (Vize, Woolf, and Bard 2003). How the degeneration of the pronephros exactly occurs remains however unclear, since in neotene amphibians (e.g. Caudata like Axolotl or Olm salamanders) who stay in their larval phase, the pronephros is also only described in early life (Duellman 1994). Some authors proposed apoptosis as key element in this process (Chimenti and Accordi 2011; Ellis and Youson 1990; Pole, Qi, and Beasley 2002; Vize, Woolf, and Bard 2003), others suggested autolysis followed by phagocytotic activity of reticular macrophages and autophagic bodies (Fox 1970; Chimenti and Accordi 2011) or the breakup of rudiments into mesenchyme (Fraser 1950). Another argument could be that when the pronephros stops developing after the embryonic stage and other organs expand
towards their adult size, the pronephric remnants become untraceable, due to differential growth.

**Table 2. Similarities and differences between the excretory organs**

<table>
<thead>
<tr>
<th></th>
<th>Pronephros</th>
<th>Evolutionary Transition zone</th>
<th>Mesonephros</th>
<th>Metanephros</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nephron</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Filtering component</strong></td>
<td>Non-integrated External glomerulus/glomus/coelomic epithelium (Vize et al. 1997)</td>
<td>Intermediate Intermediate glomerulus</td>
<td>Integrated Internal glomerulus</td>
<td>Integrated Definitive internal glomerulus</td>
</tr>
<tr>
<td><strong>Waste collecting unit</strong></td>
<td>Pronephric coelom/nephrocoel</td>
<td>Primitive Bowman’s capsule</td>
<td>Bowman’s capsule</td>
<td>Bowman’s space</td>
</tr>
<tr>
<td><strong>Ciliated peritoneal funnel</strong></td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Nephrostome</strong></td>
<td>Connected to coelom/nephrocoel</td>
<td>Absent/connected to Bowman’s capsule</td>
<td>Absent/connected to Bowman’s capsule</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Architecture Complexity Collecting duct</strong></td>
<td>Segmental Simple Pronephric duct</td>
<td>Segmental Intermediate Pro-/Mesonephric duct</td>
<td>Segmental Intermediate Mesonephric duct</td>
<td>Branched Advanced Ureteric bud</td>
</tr>
<tr>
<td><strong>Cellular</strong></td>
<td>Parietal epithelial cells not yet described in the pronephric glomerulus (Wessely and Tran 2011).</td>
<td></td>
<td></td>
<td>Parietal epithelial cells present in Bowman’s space. (Wessely and Tran 2011) Dedicated cell types like pericytes and mesangial cells to form the filtration barrier (Vize, Woolf, and Bard 2003).</td>
</tr>
</tbody>
</table>
Conclusion

The aim of this study was to clarify the presence of a pronephros in human embryos. With our referenced glossary and extensive literature survey we strived to clarify the definitions concerning kidney development. The pronephros proper consists of non-integrated nephrons, whereas the mesonephros and metanephros consist of only integrated nephrons. We observed that the pronephros as such is not detectable in human embryos. The peritoneal funnel is not entitled as exclusive feature of pro- or mesonephros, but we advocate an evolutionary transition zone as representation of the gradual evolutionary change from pro- to mesonephros. Environmental conditions (i.e. life of water) and the rise of the yolk sac and placenta affected the gradual disappearance of the pronephros in more advanced vertebrates. Thus, as Elizabeth Frazer already stated in 1950, the term pronephros does therefore not apply to human or even mammalian embryos, and should be used with much restraint in other amniotes.

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Appendix I, Kidney development glossary

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**Archinephric duct:** (Archi-: Greek for original, ancient, primitive.) Duct of the archinephros (Kuhn, Stolte, and Reale 1975), a hypothetical common ancestral kidney (Fox 1963; Wessely and Tran 2011), which becomes differentiated into pro-, meso-, and metanephros (Goodrich 1930). The pronephric- and mesonephric ducts (Mathews 1976) are sometimes referred to as archinephric duct, but once the pronephros and mesonephros can be clearly segregated this term should be avoided.

**Archinephros:** (Archi-: Greek for original, ancient, primitive.) A hypothetical common ancestral kidney (Fox 1963; Goodrich 1930; Wessely and Tran 2011; Sanna-Cherchi et al. 2007) with an archinephric duct, which becomes differentiated into pro-, meso-, and metanephros, according to the needs of the animal (Goodrich 1930).

**Bowman’s capsule and space:** Fluids from the glomerular capillaries pass into the coelom in case of external glomeruli (pronephros), or into the cavity of Bowman’s capsule or Bowman’s space, in case of internal or definitive glomeruli (mesonephros and metanephros respectively) (Vize et al. 1997; Sanna-Cherchi et al. 2007). The nephrocoel is the pronephric precursor of the mesonephric Bowman’s capsule. (Goodrich 1930) The distal ends of the nephric tubules develop a Bowman’s capsule with a flat visceral epithelium enclosing the glomerulus (McCrory 1974; Fraser 1950). Parietal epithelial cells form the epithelial cell layer surrounding the metanephric Bowman’s space and are the only cell type not yet described in the pronephric glomerulus (Wessely and Tran 2011). Bowman’s capsule, together with the glomerulus and tubule is typically known as nephron (Fraser 1950; Sanna-Cherchi et al. 2007).

**Coelom:** (Greek for hollow or cavity.) Also: splanchnocoel (Fraser 1950)/splanchnocoele (Fraser 1950)/body cavity (Balfour and Sedgwick 1878; Fraser 1950)/coelome (Vize and Smith 2004; Huettner 1968). The coelom, or body cavity, develops in the lateral mesoderm by the confluence of coelomic vesicles. The coelomic cavities are filled with a clear fluid and are lined with a mesodermal derived serous epithelium. In external glomeruli, fluids from the glomerular capillaries pass directly into the coelom (Vize et al. 1997; Kuure, Vuolteenaho, and Vainio 2000; Sanna-Cherchi et al. 2007). When no external glomeruli are present, the coelomic epithelium might also excrete wastes into the coelomic fluid (Vize et al. 1997; Holz and Raidal 2006). Pronephric tubules are connected to the (pericardial) coelomic cavity (Zapata et al. 1984; Vize, Woolf, and Bard 2003) by thin ciliated epithelial tubules called nephrostomes to collect wastes excreted into the coelom by the external glomeruli and/or the coelomic epithelium (Vize et al. 1997; Holz and Raidal 2006).

**Connecting tubule:** The term connecting tubule has sometimes been used to refer to the common region of the proximal tubules in pronephric nephrons, or to link the distal segment of the pronephric tubules to the pronephric duct (Vize et al. 1997; Raciti et al. 2008; Vize, Woolf, and Bard 2003).

**Glomerulus:** Plural glomeruli. A glomerulus is the blood filtrating unit of a nephron, which contains of glomerular capillaries. The pronephros contains external glomeruli (Vize et al. 1997; Davies 1950, 1951; Sanna-Cherchi et al. 2007; Hamilton, Boyd, and Mossman 1972; Felix 1912; Vize, Woolf, and Bard 2003), the mesonephros contains internal glomeruli (Fraser 1920; Vize et al. 1997; Davies 1950; Wrobel and Suss 2000; Sanna-Cherchi et al. 2007; Hamilton, Boyd, and Mossman 1972; Vize, Woolf, and Bard 2003) and the metanephros contains definitive glomeruli.
The Pronephros; a fresh perspective

**Definitive glomerulus:** The metanephric glomerulus resembles that of the metanephros, though larger and more advanced (McCrory 1974), with a capillary tuft which forms a close network with dedicated cell types like pericytes and mesangial cells to form the filtration barrier. The definitive glomerulus is surrounded by Bowman’s space (Vize, Woolf, and Bard 2003).

**External glomerulus:** Also: pronephric capsule (Huettner 1968). A glomerulus is considered to be ‘external’ when it hangs freely into the coelom (Balfour and Sedgwick 1878, 1879; Davies 1950; Gasser 1879; Hamilton, Boyd, and Mossman 1972; Sedgwick 1880, 1881). External glomeruli are found in the region anterior to the first true Malpighian body of the mesonephros. The absence of external glomeruli in the mesonephros is the most important difference between pronephros and mesonephros. The most caudal external glomeruli are in some species (duck (Davies 1950; Hiruma and Nakamura 2003), turtle (Wiedersheim 1890), corocodile (Wiedersheim 1890)) continuous with the Malpighian bodies of the mesonephros through a short transition zone (Davies 1950; Fraser 1920; Mihalkovics 1885; Renson 1883; Sedgwick 1881).

**Internal glomerulus:** Also: integrated glomerulus (Vize, Woolf, and Bard 2003). A glomerulus is considered ‘internal’ when it lies enclosed within Bowman’s capsule in the nephrogenic cord (Vize et al. 1997; Davies 1950). Bowman’s capsule and the internal glomerulus together constitute a Malpighian body (Davies 1950; Fraser 1950). The absence of external glomeruli and the sole existence of internal glomeruli (Malpighian bodies) in the mesonephros is the most remarkable difference between pronephros and mesonephros (Davies 1950).

**Glomus:** Also: glomi, plural glomera (Vize et al. 1997). The pronephros’ glomerulus, or pronephric capsule, is considered to be an ‘external’ glomerulus (Balfour and Sedgwick 1878, 1879, 1876; Davies 1950; Mihalkovics 1885; Sedgwick 1880, 1881; Wiedersheim 1890), because its vascular tuft protrudes directly into the coelom, (Fraser 1950) without encapsulating sheath (Chimenti and Accordi 2011). Vize et al. stated that when the vascular structure is one body segment in length it is referred to as a glomerulus, while if it extends over multiple body segments it is referred to as a glomus (Vize et al. 1997; Pole, Qi, and Beasley 2002). In frogs, it develops over 2-3 body segments, and later compacts to about one segment which lies in the immediate vicinity of the pronephric tubules so there is almost no space between the glomus and nephrostomes (Gerth, Zhou, and Vize 2005). The glomus was first identified as the blood filtration unit of the pronephros by Bidder in 1846 (Adelmann 1966; Bidder 1846; Vize et al. 1997). As longitudinal vascular ridge (Goodrich 1930), it filters waste material directly into the fluid of the coelom (Vize et al. 1997; Raciti et al. 2008; Pole, Qi, and Beasley 2002; Kuure, Vuolteenaho, and Vainio 2000; Wessely and Tran 2011; Fraser 1950; Sanna-Cherchi et al. 2007) and is therefore besides the pronephric tubule and pronephric duct considered as one of the three distinct components of the pronephric nephron (Vize et al. 1997; Brandli 1999; Raciti et al. 2008; Cho et al. 2011; Nishinakamura 2003). Wessely and Tran stated in 2011 that the glomus is composed of at least three cells types (Fox 1963; Hall 1904; White et al. 2010; Gerth, Zhou, and Vize 2005): fenestrated endothelial cells (Doherty et al. 2007), mesangial-like cells and podocytes (Takahashi-Iwanaga 2002; Wessely and Tran 2011). Parietal epithelial cells, which form the epithelial cell layer surrounding Bowman’s space in the mesonephros and metanephros, are the only cell type not yet described in the pronephric glomus (Wessely and Tran 2011).
Holonephros (Fraser 1950; Hamilton, Boyd, and Mossman 1972; Price 1897; Torrey 1954): (Holo-: Greek for whole, entire, complete.) Prince first used this slightly obsolete term in 1897 to describe the entire excretory system of Bdellostoma embryos (Fraser 1950; Price 1897). The whole vertebrate excretory system is regarded as one unit that arises from the intermediate cell-mass (Fraser 1950; Sedgwick 1881; Balfour 1877), parts of which may develop more or less separately both temporally and spatially (Hamilton, Boyd, and Mossman 1972; Torrey 1954). There is striking uniformity in the structure and function of the various parts of the excretory system (Fraser 1950).

Malpighian body: Also: malpighian corpuscle (McCrory 1974). Bowman’s capsule, together with the (internal) glomerulus, constitute a Malpighian body (Davies 1950; Fraser 1950). The Malpighian body is regarded as a typical feature of the mesonephros. The completed mesonephric Malpighian body resembles that of the metanephros, though the latter is larger and more advanced (McCrory 1974).

Mesonephric duct: Also: Wolffian duct / nephric duct / (archinephric duct*). The excretory duct of the mesonephros is formed initially by the caudal growth of the pronephric duct towards the cloaca (Raciti et al. 2008; Pole, Qi, and Beasley 2002; Mathews 1976; Prentiss and Arey 1917). The mesonephric duct, or Wolffian duct, develops in craniocaudal direction from the intermediate mesoderm, thereby acting as a signaling center for the induction of nephric tubules in both the meso- and metanephric mesenchyme (Vize et al. 1997; Nishinakamura 2003; Kuure, Vuolteenaho, and Vainio 2000). In amphibians, the mesonephric duct becomes the duct of the adult kidney (opisthitonephros) and in amniotes it contributes to metanephros formation by forming the ureteric bud (Kobayashi et al. 2007; Nishinakamura 2003; Mathews 1976). The mesonephric duct mostly degenerates in adult female amniotes (Chimenti and Accordi 2011; Sainio et al. 1997), except for the rete ovarii (Mathews 1976; Vize, Woolf, and Bard 2003). In adult males, it constitutes the ductus epididymis (Chimenti and Accordi 2011; Michos 2009; Saxen and Sariola 1987), ductus deferens and seminal vesicles (Mathews 1976; Vize, Woolf, and Bard 2003). * The pronephric- and mesonephric duct are sometimes referred to as archinephric duct, but once pronephros and mesonephros can be clearly segregated this term should be avoided.

Mesonephros: (Meso-: Greek for middle, intermediate, between.) Also: thoracic kidney (Solhaug, Bolger, and Jose 2004)) / mid-kidney (Prentiss and Arey 1917; Gray 2010) / Wolffian body (Vize et al. 1997; Raciti et al. 2008; Foster and Balfour 1874) / urniere (DU) (Vize, Woolf, and Bard 2003) / oernier (Langman 1976) / intermediate kidney (Vize, Woolf, and Bard 2003) / opisthitonephros (Vize et al. 1997; Mathews 1976; Kerr 1919). The mesonephros is formed in all vertebrates, but while it degenerates and relinquishes its function to the metanephros in advanced vertebrates, it serves as the adult kidney in fish and amphibians (Wessely and Tran 2011). The mesonephros is developed from the intermediate mesoderm of the nephrogenic cord (Vize et al. 1997; Gilbert 2010; Hamilton, Boyd, and Mossman 1972; Sadler 2004), caudal to the pronephros, if present (Vize et al. 1997; Solhaug, Bolger, and Jose 2004; Kobayashi et al. 2007; Michos 2009; Pole, Qi, and Beasley 2002; Kuure, Vuolteenaho, and Vainio 2000). Nephrons arise in a craniocaudal direction (Vize et al. 1997) from a condensed mass of cells adjacent to the mesonephric duct (Nishinakamura 2003; Kuure, Vuolteenaho, and Vainio 2000; McCrory 1974). The mesonephric tubules do develop a Bowman’s capsule that encloses a vascularised glomerulus supplied by branches of the dorsal aorta (McCrory 1974). Bowman’s capsule, together with the (internal) glomerulus,
The Pronephros; a fresh perspective

constitute a *Malpighian body* (Fraser 1950). The *Malpighian body* is regarded as a typical feature of the mesonephros (Wrobel and Suss 2000). The mesonephros differs from the pronephros by the absence of *external glomeruli* (Vize et al. 1997; Chimenti and Accordi 2011; Davies 1950; Wrobel and Suss 2000; Davies 1951; Hamilton, Boyd, and Mossman 1972; Nelson 1953).

The mesonephros is well developed in basal vertebrates, where it is the adult kidney form (Vize et al. 1997). In these species, the mesonephros is sometimes referred to as the *opisthonephros*, to describe a mesonephros that functions as an adult kidney in animals that do not form the more complex metanephros (Vize et al. 1997; Mathews 1976). The mesonephros in birds is also well developed (Carlson 1988). The degree of mesonephric development seems to be correlated to the development of the placenta. In species where the extraembryonic membranes are intimately associated with the placenta (e.g. humans and mice), mesonephroi tend to be less well developed compared to species with a less intimate association, like the pig, which have extensive mesonephroi (Carlson 1988; Nelson 1953). The mesonephros, which is functional in human embryos, develops from embryonic day 26 and degenerates in early fetal life, after 60 days of development (de Bakker et al. 2016).

**Metanephros:** *(Meta-: Greek for after, behind.)* Also: *abdominal kidney* (Solhaug, Bolger, and Jose 2004) // *definitive kidney* (Solhaug, Bolger, and Jose 2004; Hamilton, Boyd, and Mossman 1972; Blechschmidt 2004; Langman 1976). The metanephros, the most complex kidney which is only found in amniota like reptiles, birds and mammals, serves as their adult kidney (Vize et al. 1997; Goodrich 1930; Wessely and Tran 2011). It develops in a yet more caudal portion of the *nephrogenic cord* (Hamilton, Boyd, and Mossman 1972) than the mesonephros and its *tubules* connect with a special duct, the *ureter*, which arises as a diverticulum from the *mesonephric duct* (Hamilton, Boyd, and Mossman 1972). The two essential components that trigger and sustain metanephric kidney formation are the outgrowth of the *ureteric bud* and its interaction with the surrounding mesenchyme to form the *nephrons* and *collecting ducts* (Solhaug, Bolger, and Jose 2004). Although similar cellular interactions, molecules, and signaling pathways are involved, the organization of the three kidneys is quite distinct (Vize et al. 1997). The metanephros differs from the pronephros and mesonephros by its branched architecture and its formation from the *ureteric bud* (Vize et al. 1997), whereas the pro- and mesonephros have a segmented architecture and comprise a *pro- or mesonephric duct*. An adult human metanephros contains almost one million *nephrons* (Vize et al. 1997; Smith 1951).

**Mononephros:** *(Mono-: Greek for one, alone, single.)* Audigé lanced in 1910 the term mononephros, in preference to the term *holonephros*, used by Prince since 1897 (Fraser 1950; Price 1897; Audigé 1910). The term mononephros as it was used by Audigé describes the entire teleost kidney (Fraser 1950; Audigé 1910).

**Nephrocoel:** Also: *nephrocoele* (Goodrich 1930; Kerr 1919) // *pronephric cavity* (Vize et al. 1997) // *glomerular space* (Vize et al. 1997) // *pronephric chamber* (Goodrich 1930; Huettner 1968) // *nephric chamber* (Fraser 1950) // *coelomic chamber* (Davies 1951; Fraser 1950) // *(nephrotome)* (Fraser 1950; Rückert 1888). The nephrocoel or *pronephric chamber* is a fluid filled cavity in which the *external glomerulus* or *glomerus* of the pronephros protrudes to filter wastes (Vize et al. 1997; Goodrich 1930; Chimenti and Accordi 2011; Fraser 1950; Huettner 1968). It is initially contiguous with the *coelom* in both amphibians and fish but later they separate into distinct cavities (Vize et al. 1997). The *nephrocoel* is the pronephric precursor of the mesonephric *Bowman’s capsule* (Goodrich 1930). When the nephrocoel communicates with the *coelom* by a
narrow ciliated peritoneal funnel (Fraser 1950; Patten and Carlson 1974; Bailey and Miller 1921; Huettner 1968) we call it primitive Bowman’s capsule. These nephrons belong to the transition zone. The pronephric tubule is an outgrowth from the dorsal wall of the nephrocoel and runs downstream into the pronephric duct (Fraser 1950).

* The nephrocoel is sometimes incorrectly named nephrotome. Nephrotomes are the mesodermal segments that form the precursors of individual pronephric branches, which are preferably referred to as pronephric primordia, or anlagen (Vize, Woolf, and Bard 2003).

**Nephrogenic cord:** Also: Wolffian ridge. The intermediate mesoderm gives rise to a nephrogenic cord; a solid, initially unsegmented mass of mesenchymal tissue, from which the excretory organs and their ducts develop (Vize et al. 1997; Chimenti and Accordi 2011; Sadler 2004; O’Rahilly and Müller 1987). The nephrogenic cord differentiates progressively from the cervical to the caudal region and cleaves into nephrotomes (Tuchmann-Duplessis and Haegel 1974). The most cranial segments roughly constitute the pronephros, the intermediate segments the mesonephros, and the most caudal segments the metanephros (Hamilton, Boyd, and Mossman 1972; O’Rahilly and Müller 1987; Sadler 2004; Tuchmann-Duplessis and Haegel 1974).

**Nephron:** (Nephros: Greek for kidney.) Each nephron is composed of three elements; an initial filtering component (a more or less developed glomerulus), a waste collecting unit (coelom/nephrocoel/Bowman’s capsule/Bowman’s space) and a nephric tubule specialized for reabsorption of solutes and water and secretion of wastes (Fraser 1950; Sanna-Cherchi et al. 2007). Although each kidney form differs in overall organization and complexity, they all have the nephron as their basic structural and functional unit (Raciti et al. 2008). Gérars & Cordier (Gérard and Cordier 1934a, 1934b) divided nephrons into two types, whether they are in open communication with the coelom (the non-integrated nephron), or separated from it (the integrated nephron) (Fraser 1950; Dawson 1925; Lambert 1933).

**Integrated Nephron:** Integrated nephrons are found in the mesonephros and metanephros, where the glomerulus is directly integrated into the kidney tubule within the nephrogenic cord, through Bowman’s capsule or space respectively (Vize et al. 1997).

**Nonintegrated (simple) Nephron:** This form of nephron is found only in the pronephros, where the capillary filtration unit (glomus or glomerulus) hangs freely in the coelom or nephrocoel and is therefore not directly linked to the kidney tubule (Vize et al. 1997; Pole, Qi, and Beasley 2002). The structure and number of nonintegrated nephrons varies between species, amphibians for example usually have two to three nephrons (Kuure, Vuolteenaho, and Vainio 2000) or the pronephros is merely seen as one large, nonintegrated nephron (Vize et al. 1997).

**Nephrostome:** Also: nephrocoelostome (Goodrich 1930) / nephrostomal funnel. From the dorsal wall of the coelom, nephrocoel or Bowman’s capsule arises a thin ciliated tubular outgrowth, the nephrostome (Fraser 1950; Vize, Woolf, and Bard 2003), which connects the coelom, nephrocoel or Bowman’s capsule with the tubule (Fraser 1950; Bailey and Miller 1921; Huettner 1968; Vize, Woolf, and Bard 2003). As ciliated epithelial tubule, it draws in dissolved wastes from the waste collecting unit into the direction of the pronephric or mesonephric tubules (Vize et al. 1997; Davies 1950; Fraser 1950; Mathews 1976; Prentiss and Arey 1917; Huettner 1968; Tytler 1988; Marshall and Smith 1930). Nephrostomal tubules are narrower in overall diameter.
The Pronephros; a fresh perspective

than the proximal tubules (Vize et al. 1997). A nephrostome is not a typical feature of the pronephros, since it has also been observed in the mesonephros (Vize, Woolf, and Bard 2003). It is therefore important to make a clear distinction between nephrostomes and peritoneal funnels, since they are both ciliated (Goodrich 1930; Vize, Woolf, and Bard 2003; Kerr 1919). A peritoneal funnel links the coelom to the primitive Bowman’s capsule in the transition zone, whereas the nephrostome links the coelom or nephrocoel in the pronephros or Bowman’s capsule in the mesonephros to the nephric tubule (Vize, Woolf, and Bard 2003). Another important point of terminology is the distinction between nephrostomes and nephrotomes. While nephrostomes are ciliated tubules, nephrotomes are the mesodermal segments that form the precursors of individual pronephric branches (Vize, Woolf, and Bard 2003).

Opinephros: Graham Kerr (1919) stated that: ‘In many of the lower vertebrates there is no separation between mesonephros and metanephros, the two forming a continuous structure which acts as the functional kidney. Such a type of renal organ consisting of the series of tubules corresponding to mesonephros together with metanephros may conveniently be termed opinephros’ (Kerr 1919; Fraser 1950). However, basal vertebrates do not possess a metanephros that resembles the final kidney of advanced vertebrates so this term is obsolete and one must only speak of a mesonephros as the final kidney in basal vertebrates.

Opisthonephros: (Opistho-: Greek for backward; behind, at the back, after, posterior.) The term opisthonephros was first used by Kerr in 1919 (Kerr 1919) to describe a mesonephros that functions as an adult kidney in amphibians, in contrast to the mesonephros of advanced vertebrates, which will later relinquish function to the metanephros (Vize et al. 1997; Mathews 1976). Opisto- means behind or posterior and refers to the development of the mesonephros, which develops from the entire posterior region of the nephrogenic cord, whereas in advanced vertebrates part of this cord is reserved for the metanephros (Vize et al. 1997).

Peritoneal funnel: A primitive Bowman’s capsule sometimes communicates with the coelom by a narrow, ciliated peritoneal funnel (Goodrich 1930; Fraser 1950; Vize, Woolf, and Bard 2003). Peritoneal funnels and nephrostomes are both ciliated, which complicates the distinction between the two (Goodrich 1930; Kerr 1919). A peritoneal funnel links the coelom to the primitive Bowman’s capsule, whereas the nephrostome links the coelom or nephrocoel in the pronephros or Bowman’s capsule in the mesonephros to the nephric tubule (Vize, Woolf, and Bard 2003). We reckon nephrons contemplating peritoneal funnels in the transition zone in-between pronephros and mesonephros.

Pronephric primordia: Also: (nephrotome*) / pronephric anlage. The intermediate mesoderm lateral to the somites and at the cranial end of nephrogenic cord cleaves into nephrotomes*, the pronephric primordia (Vize et al. 1997; Tuchmann-Duplessis and Haegel 1974; Prentiss and Arey 1917). These blocks are thought to be mesodermal segments which hollow out to form tubules, being the precursors of individual pronephric nephrons (Mathews 1976; Tuchmann-Duplessis and Haegel 1974; Vize, Woolf, and Bard 2003). The pronephric duct precursors arise from the posteriorventral portion of the pronephric primordia (Vize et al. 1997).

*nTo avoid confusion with the term nephrostome, a ciliated tubule that swipes fluids from the coelom/nephrocoel/Bowman’s capsule into the pro- and mesonephric tubules, Vize suggested to avoid the term nephrotome by referring to these mesodermal masses as the pronephric primordia, or anlagen (Vize, Woolf, and Bard 2003).


**Pronephric duct:** Also: primary nephric duct / segmental duct / head-kidney duct / primärer Harnleiter / primary mesonephric duct / collecting duct / (archinephric duct*).

The pronephric duct forms ventral to the most cranial somites from a cell cord in the intermediate mesoderm (Vize, Woolf, and Bard 2003; Hiruma and Nakamura 2003; Gilbert 2010). The cranial region of the duct induces the adjacent mesenchyme to form the pronephros (Gilbert 2010). The caudal free end of the duct then extends lateral to the nephrogenic cord in caudal direction, until it reaches, and perforates, the cloaca or urogenital sinus (Pole, Qi, and Beasley 2002; Mathews 1976; Prentiss and Arey 1917). The pronephric duct thereby serves as an inducer of the mesonephric mesenchyme and the ureteric bud, a caudal outgrowth of the mesonephric duct, initiates metanephros formation (Vize et al. 1997).

Holtfreter (1944) and Vize et al. (1995) have refuted the common theory that the pronephric duct forms by fusion of the terminal ends of the pronephric tubules, since they both showed that the pronephric duct could form in the complete absence of any pronephric tubules (Vize et al. 1997; Vize, Jones, and Pfister 1995; Holtfreter 1944). The current view is that the pronephric duct and pronephric tubules form simultaneously from pronephric mesoderm, and that the duct receives additional contribution from the neural crest (Vize, Woolf, and Bard 2003).

In larval stages of basal vertebrates, with a functioning pronephros (Fraser 1950; Mathews 1976), molecules and fluids not resorbed in the pronephric tubules are transported through the pronephric duct towards the cloaca or urogenital sinus (Vize et al. 1997; Mathews 1976). Then, instead of forming a duplicate duct, the succeeding kidney (mesonephros) adopts the pronephric duct to transmit its excretory products (Huettner 1968). Therefore, the pronephric duct automatically becomes the mesonephric duct (Wolffian duct) with the arrival of the mesonephros (Huettner 1968).

* The pronephric- and mesonephric duct together are sometimes referred to as archinephric duct, but once pronephros and mesonephros can be clearly segregated this term should be avoided.

**Pronephric sinus:** The pronephric sinus describes the venous network that surrounds the pronephric tubules. Resorbed nutrients and water are returned into the circulation through this venous sinus. It receives blood from various sources, in particular the vas efferentia of the glomus, the anterior and posterior cardinal veins, the branchial vein, and the external jugular vein. Blood exits the pronephric sinus through the duct of Cuvier (Vize, Woolf, and Bard 2003; Viertel and Richter 1999).

**Pronephric tubules:** Also: glandular tubules (Vize et al. 1997) / glandular tube (Goodrich 1930). The pronephros, when functional, consists of paired, segmentally arranged convoluted pronephric tubules that perform the resorptive and excretory functions of the pronephros (Vize et al. 1997; Chimenti and Accordi 2011; Raciti et al. 2008; McCrory 1974). Pronephric tubules can be subdivided into broad proximal and narrow distal tubules (Vize et al. 1997; Raciti et al. 2008; Vize, Woolf, and Bard 2003). The pronephric tubules are upstream (proximal tubule) connected to the nephrocoel or coelom (Mathews 1976) by thin ciliated epithelial tubules called nephrostomes and downstream (distal tubule) connected to the cloaca or urogenital sinus by the pronephric duct (Raciti et al. 2008; Mathews 1976; Prentiss and Arey 1917).

Pronephric tubules collect wastes excreted into the coelomic fluid by the coelomic epithelium or external glomerulus through the nephrostone (Vize et al. 1997; Pole, Qi, and Beasley 2002). Fluids are moved by ciliary action through the nephrostone into the pronephric tubules (Vize et al. 1997; Davies 1950; Fraser 1950; Mathews 1976; Prentiss and Arey 1917; Huettner 1968; Tytler 1988; Marshall and...
The Pronephros; a fresh perspective

Smith 1930). The tubules are surrounded by a blood sinus (pronephric sinus) through which reabsorbed nutrients and water recovered from the filtrate are returned to the blood stream (Vize, Woolf, and Bard 2003; Viertel and Richter 1999). Remnants are disposed via the pronephric duct (Kuure, Vuolteenaho, and Vainio 2000).

Pronephric tubules arise from the pronephric primordium (Vize et al. 1997; Tuchmann-Duplessis and Haegel 1974; Prentiss and Arey 1917). Buds of pronephric primordia hollow out to form tubules (Vize et al. 1997; Mathews 1976). It is likely that the pronephrogenic mesenchyme is induced by the surrounding tissues (Vize et al. 1997; Chimenti and Accordi 2011) to form the tubules as an outgrowth from the dorsal wall (Fraser 1950) of the nephrocoel or coelom. It was thought that the distal ends of the pronephric tubules linked together to form the pronephric duct, but Holtfreter (1944) and Vize et al. (1995) proved that even in the complete absence of pronephric tubules, a pronephric duct could develop (Holtfreter 1944; Vize, Jones, and Pfister 1995). The current view is that the pronephric duct and pronephric tubules form simultaneously from pronephric mesoderm, and that the duct receives additional contribution from the neural crest (Vize, Woolf, and Bard 2003).

**Pronephros**: (Pro-: Greek for before, for. (Larsen 1993)) Also: cervical kidney (Solhaug, Bolger, and Jose 2004) / head-kidney (Balfour and Sedgwick 1878; Sedgwick 1880; Gray 2010) / first kidney (Larsen 1993; Kuure, Vuolteenaho, and Vainio 2000; Cocharid 2002; Kobayashi et al. 2007) / Müllerian body (Balfour and Sedgwick 1878) / Vorniere (Balfour and Sedgwick 1878) / Kopfniere (Balfour and Sedgwick 1878) / voornier (Langman 1976). Plural, pronephroi. The first and most primitive (Fox 1963; Hall 1904) kidney. Johannes Müller discovered the head-kidney in amphibian larvae in 1829 (Vize et al. 1997). It was spoken of as the Müllerian body (Balfour and Sedgwick 1878) before it was named pronephros. The pronephros is a large organ in basal vertebrates and functions during their larval stage in an aquatic environment (Vize, Woolf, and Bard 2003; Gaeth, Short, and Renfree 1999; Fraser 1950; Chimenti and Accordi 2011).

The pronephros develops from mesenchymal buds of pronephric primordia, or nephrotomes (Vize et al. 1997; Sadler 2004) at the most cranial part of the mesodermal nephrogenic cord (Vize et al. 1997; Chimenti and Accordi 2011; Cocharid 2002; Mathews 1976). These buds of pronephric primordia hollow out to form pronephric tubules (McCorry 1974; Mathews 1976). One end of each tubule is connected through a nephrostome with the coelom or nephrocoel into which penetrates an external glomerulus (Goodrich 1930; Chimenti and Accordi 2011; Fraser 1950; Mathews 1976; Prentiss and Arey 1917; Huettner 1968). The tubules connect on the other end and drain into the pronephric duct (Mathews 1976), which grows dorsally along with the somites towards the cloaca (Mathews 1976; Prentiss and Arey 1917; Pole, Qi, and Beasley 2002).

A typical pronephric nephron, as can be found in amphibian larvae and some teleosts (Fraser 1950), consists of the following functional units; the coelom or nephrocoel with an external glomerulus or glomus, from which a ciliated nephrostome leads into the pronephric tubule that lastly drains into the pronephric duct (Fraser 1920; Vize et al. 1997; Chimenti and Accordi 2011; Nieuwkoop and Faber 1994; Brandli 1999; Raciti et al. 2008; Davies 1950; Cho et al. 2011; Nishinakamura 2003; Wessely and Tran 2011; Fraser 1950; Dawson 1925; Lambert 1933). Parietal epithelial cells, which form the epithelial cell layer surrounding of Bowman’s capsule or space in the internal glomerulus in meso- and metanephros, are the only cell type not yet described in the pronephros (Wessely and Tran 2011). In other words; the pronephros proper consists of non-integrated nephrons, whereas the mesonephros and metanephros consist of only integrated nephrons (Vize et al. 1997; Davies 1950; Fraser 1950;
According to this definition, “the term pronephros should only be applied to the organ in larval anamnia and to that of a few teleosts”, as Fraser stated already in 1950 (Fraser 1950).

Note on the amniote pronephros: The most cranial part of the amniote excretory organ is often referred to as transient, vestigial (Goodrich 1930), nonfunctional or agglomerular (Goodrich 1930; Solhaug, Bolger, and Jose 2004; Fraser 1950; Hamilton, Boyd, and Mossman 1972) pronephros (Larsen 1993). Though no real distinction can be made between the actual remnants of an incomplete, vestigial pronephros and the gradually degenerating cranial nephrons of the mesonephros (Hiruma and Nakamura 2003; Keith 1933; Abdel-Malek 1950; Hamilton 1952; Hoadley 1926), the term pronephros should not be used in amniotes (Fraser 1950).

Transition zone: Also: intermediate zone (Davies 1950; Hiruma and Nakamura 2003). At this level, external glomeruli of the pronephros and internal glomeruli of the mesonephros are homodynamous with each other and nephrons share both pronephric as mesonephric characteristics (Fraser 1920; Mihalkovics 1885; Renson 1883; Sedgwick 1881). We call these nephrons intermediate nephrons since their intermediate glomeruli are not exclusively external, nor exclusively internal and they comprise a ciliated peritoneal funnel, connecting the coelom with the primitive Bowman’s capsule. Wiedersheim noted in 1890 that the transition zone is well developed in the crocodile and turtle where it involves about fifteen tubules, including the pronephros (Wiedersheim 1890). Based on his observations he considered the transition zone to represent a gradual cranio-caudal transition from the pronephros to the mesonephros (Davies 1950; Hiruma and Nakamura 2003; Wiedersheim 1890).

Tubules: Tubules perform the resorptive and excretory functions of the nephron (Vize et al. 1997; Chimenti and Accordi 2011; McCrory 1974). Pronephric and mesonephric tubules can be subdivided into broad proximal tubules, situated downstream of the nephrostomes (also: nephrostomal tubules), and narrower ‘S-shaped’ distal tubules (Vize et al. 1997; Balfour and Sedgwick 1878; O’Rahilly and Müller 1987), linking the proximal tubules to the nephric duct (Vize et al. 1997; Raciti et al. 2008; Vize, Woolf, and Bard 2003). Pronephric intermediate tubules, situated between the proximal and distal tubules, have also been mentioned in amphibians and reptiles (Raciti et al. 2008; Chimenti and Accordi 2011). See also Pronephric tubules.

Ureteric bud: Also: metanephric diverticulum. In amniotes, the mesonephric duct contributes to the metanephros by forming the ureteric bud (Solhaug, Bolger, and Jose 2004; Kobayashi et al. 2007; Michos 2009; Kuure, Vuolteenaho, and Vainio 2000; McCrory 1974; Hamilton, Boyd, and Mossman 1972; Mathews 1976). Its outgrowth triggers and sustains metanephric kidney formation and by its interaction with the surrounding metanephric mesenchyme the nephrons and collecting ducts are formed (Vize et al. 1997; Solhaug, Bolger, and Jose 2004; Saxen and Sariola 1987). A branched architecture and its formation from the ureteric bud is unique for the mesonephros, as opposed to the pro- and mesonephros, which have a segmented architecture and comprise a pro- or mesonephric duct (Vize et al. 1997; Michos 2009). If ureter formation fails, the kidney will be absent and often, the development of the lung and the testes, epididymis and vas deferens in males or the ovaries and Gartner’s duct in females will be affected (McCrory 1974).
The Pronephros; a fresh perspective

**Waste collecting unit:** Wastes and fluids are filtered by the *glomerulus*/*glomus* and collected into the *coelom/nephrocoel* in the pronephros and in *Bowman’s capsule or -space* in the meso- and metanephros respectively, before they are transported towards the *tubules* (Vize et al. 1997; Sanna-Cherchi et al. 2007). The serous coelomic epithelium might also excrete wastes directly into the coelomic fluid (Vize et al. 1997; Holz and Raidal 2006). Depending on the type of kidney, the waste collecting unit is the *coelom* (Vize et al. 1997; Kuure, Vuolteenaho, and Vainio 2000; Sanna-Cherchi et al. 2007) or *nephrocoel* (Vize et al. 1997; Goodrich 1930; Chimenti and Accordi 2011; Fraser 1950; Huettner 1968) (in the pronephros), *Bowman’s capsule* (Vize et al. 1997; McCrory 1974; Sanna-Cherchi et al. 2007) (in the mesonephros) or *Bowman’s space* (Vize et al. 1997; Sanna-Cherchi et al. 2007) (in the metanephros).
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