of the histone proteins packaging DNA) complexes (Vickaryous and Whitelaw 2006; Waterland 2006; Ozanne and Constanci 2007). Imprinted genes are particularly vulnerable, as only the maternal or paternal copy of the gene is normally expressed (Fowden et al. 2006). There are now examples of epigenetic changes brought about in the embryo, fetus and neonate by maternal diet (Lillycrop et al. 2005; Drake et al. 2005; Kwong et al. 2006; Bogdarina et al. 2007), drugs such as cocaine (Zhang et al. 2007), endocrine disruptors (Chang et al. 2006) and, in the rat, maternal behaviour towards the neonate (Weaver et al. 2004, 2005, 2007; Meaney et al. 2007). So far, genes shown to be altered have been expressed in liver, brain, and adrenal but not the kidney. Nevertheless, as detailed in Sect. 3, numerous examples of altered gene expression have been found in the kidney as a consequence of some environmental event, particularly in the components of the RAS. It is most likely that these represent epigenetic changes brought about by the programming event, be it alterations in the protein content of the diet or exposure to excess natural or synthetic glucocorticoids (Moritz et al. 2005a).

8.10.2 Mitochondrial Dysfunction

Another potential molecular mechanism underlying the programming of adult diseases is mitochondrial dysfunction (McConnell 2006; Dickinson and Wintour 2007). There is evidence showing that mitochondrial DNA (mtDNA) mutations are linked to and precede the development of diabetes and hypertension in humans, implying a causal link (Song et al. 2001; Ballinger et al. 2002). Experimental manipulations of mtDNA in mice either in vitro in pancreatic cell lines or in vivo using conditional tissue-specific gene ablation approaches, show a direct link with altered mtDNA and altered pancreatic and cardiac function. Defective mtDNA-specific DNA polymerase has also been shown to lead to more rapid rates of ageing in mice (Trifunovic et al. 2004). In a rat model involving prenatal and suckling exposure to a diet rich in animal fat, which leads to whole body insulin resistance and pancreatic β-cell dysfunction in adulthood, there is evidence of reduced tissue mtDNA content and altered mitochondrial gene expression that precedes the insulin resistance (Taylor et al. 2005).

9 Future Directions

There are many areas ripe for research in the area of kidney development. With the current advances in technology available to image and examine the developing kidney along with transgenic animal technology, many new strategies can be employed to answer old questions as well as devising new ones. In the following sections, we consider a few very recent areas of interest in the field of renal development.

9.1 Clock Genes

It is now known that most cells in different organs have intrinsic clocks that regulate the functioning of the organ/system in a time-dependent fashion, mostly around a 24-h, circadian time frame. The level of expression of genes involved in these processes may change up to 20fold throughout the 24-h period (Boden and Kennaway 2006). These intrinsic clocks depend on the constant expression of a gene–*CLOCK* (circadian locomotor output cycle kaput)–whose protein product has to heterodimerize with that of a second gene, *BMAL1* (brain and muscle ARNT-like protein 1), to be effective. The dimer affects the expression of other timing genes such as the three *Period* (*Per*) genes and two *Cryptochrome* (*Cry*) genes. The protein products of the *Per* and *Cry* genes both have negative feedback effects on the primary genes and regulate the expression of other genes responsible for many metabolic, hormonal and other physiological functions (Kalsbeek et al. 2006; Boden and Kennaway 2006). These genes are autonomous, but they can be synchronized by a master clock in the suprachiasmatic nucleus (SCN) of the hypothalamus or by metabolic/other signals. Various disorders, particularly of early ageing and age-related pathologies have been seen in mice deficient in *BMAL1* (Kondratov et al. 2006), in Alzheimer's disease in humans (Wu et al. 2006) and in chromosome abnormalities in humans (De Leersnyder et al. 2006). In mice with the double knock-out of Cry1 and Cry2, mean arterial pressure was equal in both day and night periods, instead of being higher at night, and was higher at both time periods in the knock-out mice than the wild-type mice (Masuki et al. 2005). In addition, there were significant changes in baroreceptor sensitivity and response to adrenergic drugs. This establishes that abnormalities of some clock genes can produce hypertension.

The major zeitgeber or synchronizer receives input by light, for example, and then establishes a day/night cycle of behaviour or hormone release. One of the major systems regulated by the SCN is the circadian synthesis and release of the hormone melatonin by the pineal gland (Jilg et al. 2005). Melatonin concentrations rise with the onset of darkness and maternal melatonin can cross the placenta and entrain at least some of the circadian systems in the primate fetus (Torres-Farfan et al. 2006). Thus the fetus can tell the time even before it is born.

In the rat fetus, the circadian network is not fully developed before birth (Sumova et al. 2006) and exciting recent results suggest that some of the long-term consequences of alcohol abuse during pregnancy may result because the alcohol permanently altered the expression of some clock-gene mechanisms governing the expression of stress-related hormones in the brains of the adult offspring (Chen et al. 2006a). Adult male rats, whose dams had been given a diet in which 33% of calories came from ethanol during days 10–21 of pregnancy, demonstrate abnormal circadian expression of the mRNA for *proopiomelanocortin*, encoding the β-endorphin protein in the hypothalamus. These male offspring brains also had abnormalities in the *Per* genes of the SCN and the β-endorphin-

containing neurons. This finding has implications beyond those of fetal alcohol exposure. With particular relevance to the kidney, it has been shown that the level of expression of the sodium transporter, NHE3, shows diurnal variation under the influence of clock genes (Saifur Rohman et al. 2005). Thus, it is of great importance in all developmental studies of renal gene expression that all tissues collected are obtained at the same time of day. Investigation of the expression of clock genes in the kidney may also provide some answers as to mechanisms of programming.

9.2 Renal Stem Cells and Renal Regeneration

Regenerative therapies for the treatment of chronic kidney disease has emerged in recent years as an exciting new research direction. It is well known that the kidney can undergo significant repair and regeneration following certain types of injury or disease (see Cochrane et al. 2005; Little et al. 2006). This repair may be the result of activity of resident kidney cells and/or non-renal cells, for example cells recruited from the bone marrow (reviewed by Anglani et al. 2004; Ricardo et al. 2005; Little et al. 2006). The adult kidney contains approximately 26 differentiated cell types. However, as described in Sect. 2.3, these cells arise from only two embryonic tissues: (1) the WD from which the UB and collecting duct system is derived; and (2) the MM which gives rise to the epithelial cells of the nephron, stroma, endothelial cells and smooth muscle cells. These two tissues (UB and MM) are believed to contain progenitor cells for these various cell types (reviewed in al-Awqati et al. 2002). However, the question remains whether these progenitors persist in the adult kidney and whether they play a role in the repair or regeneration of cell types following renal injury. Although adult renal stem cells located within the renal papilla, proximal tubules, and side population have been reported to express CD133, no definitive data exists that these cells contain all the characteristics of a stem cell such as pluripotency, self-renewal and clonogenicity (reviewed in Little et al. 2006).

9.3 New Renal Factors

It has recently been shown that the kidney produces a hormone called renalase that metabolizes catecholamines (Xu et al. 2005). In humans, renalase gene expression is highest in the kidney but is also detectable in the heart, skeletal muscle and small intestine. Plasma renalase concentrations are reduced in patients with end-stage renal disease. Administration of renalase lowers blood pressure and heart rate by metabolizing circulating catecholamines. Recent studies have shown abnormalities in the renalase pathway in animal models of chronic kidney disease and hypertension (Xu and Desir 2007). Therefore, in the future renalase may become an important therapeutic agent in the treatment of renal and cardiovascular disease.

In the last few years, a new component of the RAS, called ACE2, has been identified, and although tissue expression of ACE2 is now thought to be widespread, the kidney is a major site of production (reviewed in Hamming et al. 2007). ACE2 may play a pivotal role in controlling the balance between the vasoconstrictor effects of Ang II and the vasodilatory properties of other RAS components such as the angiotensin (1–7) peptide (Lazartigues et al. 2007). ACE2 has been implicated in cardiovascular and renal disease, diabetes, pregnancy, lung disease and, surprisingly, ACE2 also acts as a receptor for the SARS virus (Hamming et al. 2007; Lazartigues et al. 2007).