Preface

This themed issue of *Sexual Development* on ‘Sexual Differentiation of Vertebrate Reproductive Organs’ continues and complements the story begun in the previous themed issue entitled ‘Control of Gonadal Development’ which focussed on the genetic control of gonadal development. This issue continues the story of the next steps in development, namely the transition from sex determination to sexual differentiation, and is divided into three broad sections. Many of these papers open up detailed information on less well-known species or ideas. Amongst mammals, since most work has been conducted on mice, it is not surprising that many generalisations have developed, some of which apply to all mammals while others differ quite significantly. There is still much to learn about sexual differentiation in the remaining 5,400 or so mammalian species. Birds do things quite differently; yet again our knowledge is derived from just a few species. Reptiles are even more diverse, but work is still in its embryonic stage of development, and there is just a ‘toe in the water’ about the variety of mechanisms of sexual differentiation in the vast numbers of fish.

The issue begins with three different mechanisms of sex determination in mice, reptiles and birds. Carré and Greenfield review the recent advances in mouse testis determination, showing a link between epigenetics and sex determination via the CBX2 proteins and their role in regulating *Sry* expression. They also highlight the importance of *GADD45γ* as an autosomal testis-determining gene via activation of *MAP3K4*. Kohno et al. discuss an

entirely different aspect of sex determination, i.e. that of temperature-dependent sex determination (TSD) in reptiles. Although many of the genes required for gonadogenesis are broadly conserved among vertebrates, in squamates, in many turtles, tuatara and all crocodilians studied so far, it is the temperature of egg incubation that determines sex, acting via steroid hormone signalling. There appears to be a conserved role for SOX9 and AMH in the early stages of testicular development in reptiles with TSD. Epigenetic mechanisms may be mediators between the environment and the developing phenotype. These authors conclude their review with a discussion of the endocrine-disrupting effects of environmental toxins that could induce permanent epigenetic changes during development. The third paper in this section by Guioli et al. addresses the curious phenomenon of left-right asymmetry in gonadal development in birds using the chicken as a model. The right female gonad does not develop into a functional ovarian structure in the adult and is considered to eventually atrophy. However, the removal of the left ovary in the young chick results in the differentiation of the right gonad into a testis-like organ. There is differential proliferation, possibly due to SF1 which up-regulates the expression of the cell cycle regulator CD1. Although both ovaries synthesise oestrogen in the developing chick, there is an early asymmetric pattern of ERα expression which could be its primary cause. *PITX2* emerges as a key gene in the left-right identity. The evolution of this asymmetry is less clear, but the authors posit

that there might have been a greater possibility of damage to a shelled egg on one side from the proximity of a contralateral shelled egg within the abdomen.

The middle section provides insight into the somatic lineages, gonadal differentiation and disorders of sexual development in mammals, fish and humans. Nicol and Yao summarise the current knowledge on the molecular pathways driven by *WNT4*, *RSPO1*, *FOXL2*, and other ovary-specific genes in the mouse ovary. These signalling molecules and transcription factors secure the ovarian fate by antagonising the testis pathway and at the same time, maintain the survival of female germ cells. The unique expression patterns of these genes imply the presence of subpopulations of granulosa cells and other somatic cell lineages. The origins of these somatic cells in the ovary are more complex than expected, with probably multiple sources for both granulosa cells and theca cells. In the paper by Nishimura and Tanaka, the lineage contributions to germ cells and somatic cells are highlighted in the teleost fish Medaka. In Medaka, the genetic regulation of sex differentiation precedes the control by hormones. *DMY/dmrt1bY* acting in supporting cells initiates the male pathway and the sex identity of male germ cells. Without *DMY/dmrt1bY*, the interstitial cells differentiate into female interstitial cells and express aromatase. In contrast to the testis, the establishment of female interstitial cells requires the feminised germ cells. Loss of germ cells results in the failure to form an ovary. Ohnesorg et al. then turn the focus back to mammals with the emphasis on disorders of sex development (DSD) in humans. The genes responsible for the DSD in humans are highly conserved in other mammalian species such as mice; however, the phenotypes of certain DSD cases are different between human DSD cases and mouse mutants. This phenomenon becomes even more striking when other nonmammalian species are considered, highlighting the complexity inherent in the evolution of sexual differentiation mechanisms.

The final section summarises some much neglected aspects of sexual development: the development and molecular control of the duct system in mammals and the development of the external genitalia in mammals and its evolution in reptiles. First, Shaw and Renfree describe the androgen-dependent formation and control of Wolffian duct development, with androgen receptors in the epithelium and mesenchyme of the duct. There is a complex morphogenetic program and interaction with a suite of genes including *EGFs*, *inhibin β -A* and *HOXs* to induce the coiling, septum formation and regional functional differentiation of this duct, which becomes the vas defer-

ens in the adult. Mullen and Behringer provide a beautifully illustrated and detailed description of the formation, regression and differentiation of the Müllerian duct that develops into the oviducts, uterus, cervix, and upper vagina and the molecular signalling that controls this process. The Müllerian ducts form in three distinct phases: initiation (with expression of *Lhx1*), invagination (which depends on expression of *Wnt4* in the mesonephric mesenchyme) and elongation when the invaginating tip of the Müllerian duct contacts the Wolffian duct. This process occurs in a rostral-caudal direction. Regression in the male depends on the anti-Müllerian hormone secreted by the Sertoli cells, and failure of this pathway leads to persistent Müllerian duct syndrome. Distal to the duct system are the intromittent organs. Ipulan et al. describe the formation and development of the bipotential genital tubercle as it transforms into the sexually dimorphic phallus. Androgen signalling and crosstalk with other signalling factors drives the sexual dimorphism of the male and female genital tubercle. SHH is a key protein in this process for both patterning and proliferation, acting in concert with other signalling networks including *WNTs*, *FGFs*, *BMPs* and *HOX* gene expression.

Despite the extraordinary diversity in the external genitalia in amniotes, studies of phallus development have lagged behind those of the gonad and duct systems. Gredler et al. redress this lack of information in lizards, snakes, alligators, and turtles and describe the homology of genital structures in these amniotes and their association with the cloaca. They show that all amniotes other than mammals have an open sulcus that became the enclosed tubular urethra in mammals and suggest that the intromittent phallus may be a shared, but derived, character of amniotes. Finally, this section ends with a summary of the discovery in marsupials, and confirmed amply in studies of birds and mice, that not all sexual dimorphisms are controlled by hormones of the testis, contrary to the Jost paradigm. Renfree et al. illustrate the many (and varied) examples of these hormone-independent sexually dimorphic characters.

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