Preface to the Special Issue on The Non-Coding Genome in Sex Determination

Francis Poulat^a Nitzan Gonen^{b, c}

^aInstitute of Human Genetics, CNRS UMR9002, University of Montpellier, Montpellier, France; ^bThe Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel; ^cInstitute of Nanotechnology and Advanced Materials, Bar-Ilan University, Ramat Gan, Israel

The process of sex determination involves the differentiation of an initially bipotential gonad into either a testis or an ovary. This remarkable process, driven by sex chromosomes in mammals, is first initiated within a somatic cell population called the supporting cell precursors. Once these differentiate into either Sertoli or granulosa cells, they instruct and direct other bipotential cell populations in the gonad, including other somatic progenitors but also the germ cells, to acquire the male or female path.

Much of the study in the last 3 decades has been focused towards identifying and understanding the function of many pro-testicular versus pro-ovarian factors involved in this process. Most of these are transcription factors and signalling pathway components expressed in various cell populations, but mostly in Sertoli and granulosa cells, which constitute the driving force of gonad differentiation. Human genetics studies of individuals with disorders of sex development (DSD) as well as mouse embryological and genetic studies provided much of the information about the genetic cascades controlling sex determination. Numerous mutations in protein-coding genes have been identified over the years which affect the process of sex determination, and yet, nowadays, more than 50% of 46,XY DSD and ~80% of 46,XX DSD remain unexplained following whole exome sequencing.

This indicates that the non-coding genome, which accounts for 98% of our DNA, also has wide involvement and implications on the process of sex determination.

Furthermore, another evident feature of sex determination is that this process relies on a very delicate balance of the gene expression levels of the many pro-testicular and pro-ovarian factors. It is estimated that in order to gain this high level of preciseness and complexity, many layers of gene regulation are involved, including 3D genome organisation, proper epigenetics landscape as well as involvement of regulatory elements and other non-coding RNAs.

This themed issue on “The Non-Coding Genome in Sex Determination” is dedicated to focus and summarize the most up-to-date existing studies and perspectives regarding the involvement of the non-coding genome in determining our sex. Altogether, the various chapters focus on different aspects of the non-coding genome like epigenetic landscapes, 3D genome organisation, and non-coding RNAs. They summarise what is known on the regulation of some of the key genes involved in sex determination like *SRY*, *SOX9*, and *FOXL2*. The chapters focus on different organisms as vertebrates, mice, and human, as well as on different gonadal cell types as somatic versus germ cells. The special issue ends up with a more evolutionary view of the sex chromosomes and how their

non-coding genome relates to the process of sex determination.

This issue opens with a chapter by Francis Poulat that reviews the most up-to-date view on the various pro-testicular and pro-ovarian transcription factors which play key roles during sex determination. These factors bind DNA to exert their regulatory roles and this chapter focuses on genomic approaches used to identify their targets. Next, a review by Dupont and Capel zooms out and explores the chromatin state that exist at the bipotential stage and how this landscape changes during the process of testicular or ovarian differentiation. They also describe the involvement of histone modifiers in establishing the specific chromatin state.

The next chapter by Ridnik, Schoenfelder, and Gonen describes recent advances in our understanding of the 3D genome organisation as well as function of cis-acting gene regulatory elements in general and more specifically during sex determination.

Burgos and colleagues describe many other types of non-coding genomic elements which can be transcribed as long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and PIWI-interacting RNAs (piRNAs) and their contribution to the process sexual development.

As much of the action during sex determination is mediated by transcription factors, the issue wanted to give a special emphasis to key, well-established transcription factors, and mostly on those of which more is known about their gene expression regulation, both in terms of the epigenetic landscape and regulatory elements involvement.

A chapter by Okashita and Tachibana reviews the transcriptional regulation of the *Sry* gene located on the Y chromosome and being at the top of the male developmental cascade in mammals.

This is followed by a review by Migale, Neuman, and Lovell-Badge that describes the long-range regulation of the Yin and Yang of the male and female master regulators: *Sox9* and *Foxl2*. These 2 genes are by far the most extensively studied genes in terms of gene expression regulation, and this is beautifully outlined in this chapter.

The main activity of the key pro-male and pro-female factors is to initiate a sex specific transcriptional output that will eventually lead to testis and ovary development. Among these sex specific transcriptional programs, it is very evident that alternative splicing plays an important

role. This aspect is summarized in the chapter by Gómez-Redondo and colleagues who explore the role of alternative splicing in sex determination in vertebrates with a focus on mammals.

Next, a driving force to better understand sex determination are DSD patients, individuals in which the chromosomal, anatomical, or phenotypic sex is atypical. While mutations in many different genes have been identified in DSD patients, we are still unable to provide a genetic diagnosis for many of these patients. The chapter by Atlas, Sreenivasan, and Sinclair provides an up-to-date summary on the techniques used to identify and characterize variants located in the non-coding parts of the genome as well as a clinical update on enhancers linked to DSD.

While the somatic cells drive gonad development, the main function of the gonads is to host, support, and guide the germ cells throughout life, while these produce the gametes needed for reproduction. The chapter by Ramakrishna and colleagues is focused on the germ cells and the extensive epigenetic regulation that occurs during their development and differentiation.

The last chapter of this issue closes the circle and comes back to the starting point: the sex chromosomes, and mostly the Y chromosome, that initiate sex determination. Maier and colleagues take an evolutionary point of view on sex chromosomes and the involvement of non-coding elements in their function.

We would like to deeply thank all the authors for their profound knowledge and very extensive chapters, each in its own field. We would also like to extend our gratitude to the reviewers for their insightful comments and suggestions. Lastly, we want to thank the Editorial Board of *Sexual Development* for providing us the opportunity to serve as guest editors of this fascinating issue.

We hope you find this issue interesting and mind-opening towards this up-and-rising field of the non-coding genome involvement during sex determination. We strongly believe this fascinating field will further grow and develop in the coming years in a way that will enlighten our understanding of sex determination and DSD pathologies.

Francis Poulat, University of Montpellier,
Montpellier, France

Nitzan Gonen, Bar-Ilan University, Ramat Gan, Israel
October 2021