

# Phosphorylation as a basic step in human primary sex determination

## Introduction

Since 50s of the 20<sup>th</sup> century, for more than 60 years, I was interested in the study of human sexual development. The object of my studies was human embryos, fetuses as well as some patients affected by different anomalies. My preferential method of the embryologic histologic studies was a special dissection and staining of phosphatases activity and glycosaminoglycans. For fixation, I used the cold ca – formol followed by staining of the activity of the alkaline phosphatase using naphthyl AS phosphate as substrate. For the staining of glucose aminoglycans the 1% alcian at pH 2.0 and 4.0 was used. The method is described in my Atlas of Human Prenatal Morphogenesis (1983).<sup>1</sup>

The early experiences related to development of human gonads, genital ducts and external genitalia were summarized in my small monograph Development of the Genital System and Male Pseudohermaphroditism (1971). In this book, I defined genital and extra genital primordial germ cells differentiation of testicular cords tunica albuginea, and the Leydig' cells. I correlated the testicular morphogenesis with the morphogenesis of genital ducts and external genitalia and defined the testicular dysgenesis related to the Müllerian structures in man and the syndromes of androgen insensitivity. The origin of human primordial germ cells, as cells of extraectodermal origin, as well as the developmental staging of organs including gonads, were presented in the An Atlas of Human Prenatal Developmental Mechanics Anatomy and Staging (2004).<sup>2</sup>

In this contribution I am going to summarize changes of the activity of the ALP (non specific alkaline phosphatase) during testicular and ovarian development. The ALP is coded by the gene localized 1p36.1-p34, the gene maybe autosomal dominant or recessive, not specific to any tissue, interfering with gonadal development, ossification, renal development and others. The enzyme is involved in the formation of CpG islands connecting chromosomes and is related to methylation and activation of different genes. For testicular development the decisive genes are the SRY gene, SOX gene and genes related to synthesis and conversions of cholesterol and steroids. For a normal ovarian development, the presence of two complete X chromosomes is needed. Chromosomes and their role I discussed this subject in my contribution in GLOWM Jirásek, 2008.<sup>3</sup>

Related to the histogenesis of gonads and the activity of the ALP I observed following substantial differences.

## Primordial germ cells

Primordial germ cells their differentiation from the extraembryonal ectoderm of the yolk sac adjacent to the caudal rim of the bilaminar embryonal disk at day 14<sup>th</sup>, as well as their migration into the urogenital ridges is in both sexes (in embryos XY and XX) the same. The primordial germ cells join the other cells of the genital blastema.

## The gonadal blastema

The gonadal blastema contributing the genital ridges located on the medioventral surface of the mesonephric ridges is a cellular condensation of primordial germ cells, steroidogenic cells of celomic

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origin and desmogenic–collagen synthesizing cells of mesenchymal origin. The primary germ cells exhibit intensive activity of ALP and contain glycogen.

Within the gonadal blastema the germ cells divide mitotically. Related to the testicular differentiation in embryos 14–17mm long at the end of 6<sup>th</sup> week, the primary migrating pgc undergo apoptosis and liberate their ALP. The proteinaceous cytoplasmic contents of pgc exhibiting phosphatase activity cover all cells of the testicular blastema. Consequently in embryos 15-17mm long, week 17<sup>th</sup>, all phosphatase activity disappears from the blastema.

## Testicular cords of the embryonal testes

Testicular cords of the embryonal testes appear in embryos 17–25mm long from the week 8<sup>th</sup>. The testicular cords are constituted by spermatogonia originating from germ cells of the blastema and by embryonal Sertoli cells originating from the celomic steroidogenic cells. The spermatogonia are slowly mitotically proliferating and are joined by tied contacts with embryonic Sertoli cells.

## Inersitial mesenchyme of the embryonal testes

Coincidentally the testicular cords of the embryonal testes are delineated by desmogenic mesenchymal cells of testicular interstitial mesenchyme. Moderate activity of ALP is present in the embryonal spermatogonia during the whole prenatal period. The mitotic activity of spermatogonia is preserved for the whole life of man. There are no steroidogenic cells at the stage of embryonal testes. The interstitial mesenchyme of embryonal testes is rich in glugouseaminoglycane of prechondral type. The differentiation of this mesenchyme is related to regression of Müllerian ducts

## Leydig cells and the fetal testes

Leydig cells are steroidogenic cells present within the fetal testes they appear in the testicular interstitium in spaces between the propria of testicular cords and produce androgenic steroids converting indifferent external genitalia into male genitalia characterized by the presents of cavernous urethra. The characteristic enzyme related to the differentiation of Leydig cells is the acid phosphatase located in their paranuclear Golgi–vesicles.

## Ovarian differentiation and the germ cells

Within the genital blastema turning into the embryonal ovaries (in embryos with a normal XX karyotype. The primordial germ cells divide mitotically never spreading their phosphatase activity into their

vicinity. The strong phosphatase activity is evident only within the germ cells and is localized within the cell nuclei and cell membranes. The gonadal primordial germ cells located in contact with the surface epithelium of the blastema are in the cortical ovarian localization, while those located within the blastema are in the medullary ovarian localization. Consequently, the ovarian germ cells change into the oogonia, and enter intensive mitotic divisions. This mitotic division creates a cortical layer of oogonia. The repeated mitotic division exhausts all the mitotic activity of oogonia. The activity alkaline phosphatase disappears and the oogonia change into oocytes. Immediately the oocyte enters the meiotic prophase. The meiotic prophase begins in fetuses 12 weeks old within the medullary oocytes at twelfth week and reaches the surface of the ovary. The folliculo genesis continues till the end of the prenatal life. The folliculo genesis means formation a complete layer follicular-granulosa cells covering the whole surface of the meiotic oocyte. If this granulosa layer is incomplete the oocyte undergoes atresia, degenerates and disappears. Atresia is a special form of apoptosis (apoptosis of oocytes). Granulosa cells develop from steroidogenic cells of celomic origin. Granulosa cells join by gap junctions. Related to FSH stimulation they proliferated in growing follicles within the perinatal ovaries and constitute a multilayered granulosa of growing and cavitated follicles.

Differentiation of steroidogenic tissue within the perinatal ovaries is related to the growing cavitated follicles. The steroidogenic cells of perinatal ovaries develop from steroidogenic cells located within the mesenchymal connective tissue of the ovary.<sup>4-7</sup>

## Conclusion

We would like to conclude that many genes participate in the sexual differences. The primary differentiation is the methylation of chromatin in every cell by the product of the XIST located on the X chromosome (Xq13) This transcript is responsible for the formation

of sex chromatin in presence of two (or more) X chromosomes. The consequence is a different tri-dimensional arrangement of male and female genome. There is different tuning in male and female cells reflecting the different imprinting. The activation of genes is related to the CpG islands related to phosphorylation. In gonads the time table triggering the meiotic activity of germ cells in testes and ovaries maybe regulated by phosphorylation of decisive genes by ALP.

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## Conflicts of interest

The authors declare no conflicts of interest.

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