PATHWAYS USED BY ANDROGENS OR FSH TO REGULATE TESTIS MATURATION

Sambroni E.[1] Rolland A.[2] Goupil AS.[1] Lareyre J.J.[1] Le Gac F.[1],

[1]INRA UR 1037, SCRIBE, [2]INSERM U625, 35042 Campus de Beaulieu, Rennes, France
Fax : +33223485020 email : florence.legac@rennes.inra.fr

Introduction:

In fish, gonadotropin hormones and sex steroids are known to trigger the initiation of testicular puberty and to promote final maturation of the gametes. In a series of studies, the molecular mechanisms involved were investigated through identifying androgen and FSH/LH modulated gene expression in the trout testis.

Methods:

A search for androgen dependent genes was initiated in pre-pubertal rainbow trout treated with physiological doses of androgens. Groups of seven to ten immature male trout were treated with testosterone (T) or 11-ketotestosterone (11KT) by implantation for 7-14 days. The treatment induced large increases in androgen plasma levels but these remained within the physiological range for adult male trout. FSH-regulated genes were also identified from trout testicular explants in early stages of development (I-II: spermatogonia only, III: initiation of meiosis) cultured in the presence of salmonid FSH at 500ng/ml. RNA was extracted from all testicular samples. In transcriptome studies using trout cDNA chips, the transcript accumulation for 9000 probes, corresponding to ~6000 non-redundant genes was measured. A limited number of candidate genes were also confirmed by *in situ* hybridisation and qPCR.

Results and discussion:

1) Up or down regulations of specific mRNA levels were observed after the androgen treatments or in response to LH /FSH. Early changes appeared to affect gene expression mainly in the somatic compartment, where receptors for these hormones have been described in fish. For many of these genes, significant developmental expression patterns during the annual maturation of the trout testis were observed. Some changes also corresponded well with the natural increases in circulating hormone levels during the reproductive cycle, reinforcing the idea that the hormone induced changes that we describe are *physiologically* relevant.

2) Conversely to mammals, FSH and LH had similar effects on one third of gonadotropin regulated transcripts. These genes are candidate targets for FSH physiological action during the pubertal transition since only FSH is detected at these stages of testis development. Interestingly, we also found that about 80 candidates appeared regulated exclusively or much more efficiently by FSH (rather than by LH) which should help to clarify the respective roles of the 2 gonadotropins in fish.

3) Data mining of the candidates revealed that hormones affected somatic genes involved in germ cell sustaining proliferative activity or evading growth suppressors, or differentiation.

- In prepubertal testis FSH and/or androgens affected the expression of several transcripts coding for paracrine factors of somatic origin, like GSDF, AMH, inhibin, ,Follistatin, BMP7, which are all potentially involved in germ cell proliferation or differentiation
- In addition to modifying gene expression in supporting somatic cells, androgen treatment resulted in a shift in germ cell gene expression profiles, with a decrease in several genes preferentially expressed in spermatogonia (like *noc2l*) and an increase in transcripts with a meiotic/post meiotic profile (like *morn3, rsph3, bty*). This reinforces the involvement of androgens in the transition from spermatogonia towards more differentiated germ cells expression profiles.
- Two transcripts encoding **G2/mitotic-specific cyclins** were up regulated by FSH/LH; one, annotated ccnb3 is known as involved in early meiosis in mouse; the second one, annotated *ccnb1* and expressed in late germ cells responded better to FSH, with a stronger effect in stage III (in contrast the G1/S-specific cyclin-E1 was found down regulated by the gonadotropins).
- Gonadotropins up regulated some germinal or somatic genes encoding intracellular factors known to take part, directly or indirectly, in germ cell fate, for example *nanos3* (FSH and LH), *dmrt1* (FSH and LH) or *sox8* (LH) and also affected genes encoding factors involved in other developmental processes like apoptosis (*casp8*, *faf1* and *clu1*) or testicular fluid homeostasis (iron/ion transporters *slc30a1*, *fth1*).
- FSH regulated several transcripts encoding proteins involved in microtubule and cytoskeleton rearrangement (calponin2 *cnn2*, desmin *des*, cytokeratins *krt18*, *krt8*, microtubule-associated protein *mapre1*) and proteins of the extracellular matrix (collagen *col1a1*, *col1a2*, proteases *mmp19*, *mmp9* and protease inhibitors, *timp2*) important for cell adhesion and migration and cell junctions.

4) The steroidogenic pathway was of course affected by the hormone treatments: the gonadotropins were generally stimulatory (*star, hsd3b1*), while *in vivo* treatment with high levels of androgens were inhibitory

(indirectly, through an FSH inhibition, or directly, via a short loop feed-back on steroidogenic enzymes).

5) Furthermore, a specific group of the regulated transcripts probably take part in the morpho-functional changes in the testis and sperm duct during spermiation in trout. We found that sex steroids possibly control these genes involved in the regulation of water exchanges (*aqp1, aqp4, vt1*) and in proton and potassium regulation in seminiferous tubules (*cahz, vt1, slc26a4, atp1b1, lgi1)*, and are therefore relevant concerning sperm maturation and excretion.

Conclusions:

We provide meaningful information on a large set of transcripts implicated in the testicular somatic cell response to gonadotropin and/or androgens. These include growth factors, extra-cellular matrix components and intracellular pathways which are potentially involved in the mechanisms by which reproductive hormones direct germ cell renewal or differentiation at puberty, then sperm maturation and excretion at spawning time in adult fish.