

Developmentally regulated integration of PKA- and PI-3 kinase-mediated signalling on p70S6K by the FSH-R

Astrid Musnier¹, Charlotte Lécureuil², Sophie Tesseraud³, Thomas Boulo¹, Guillaume Durand¹, Hervé Guillou², Eric Reiter¹ and Pascale Crépieux¹.

¹ PRC, UMR 6175, INRA/CNRS/Université de Tours/Haras Nationaux, 37380 Nouzilly, France.

² The Inositide Laboratory, The Babraham Institute, Cambridge, CB2 4AT, United Kingdom.

³ Unité de Recherches Avicoles, UR 83 INRA, 37380 Nouzilly, France.

FSH is one of the hormones that regulate reproduction. In male, FSH triggers Sertoli cell development: proliferation around birth and differentiation before puberty. The efficiency of those responses directs the qualitative and quantitative spermatogenesis yield. Sertoli cell proliferation and differentiation are mediated by differential FSH-activated signalling pathways. As an example, Sertoli cell proliferation is dependent on the MAPK ERK1 and ERK2 phosphorylation. This pathway is switched off in differentiating cells (Crépieux et al., *Oncogene*, 2001, 20:4696-709). p70S6K (ribosomal S6 protein kinase, 70 kDa) was identified as a new target of FSH in granulosa cells (Alam et al., *J. Biol. Chem.* 2004, 279 :19431-40). In response to tyrosine kinase receptors, p70S6K is pluri-phosphorylated by the PI3K/mTOR pathway. Our group has shown that p70S6K was originally activated in differentiating Sertoli cells by a PKA-mediated dephosphorylation of the Thr421/Ser424 residues (Lécureuil et al., *Mol. Endocrinol.*, 2005, 19: 1812-20).

We then sought to determine if the particular p70S6K activation mode observed in differentiating cells was due to the Sertoli cell developmental stage or to the fact that the FSHR is a G protein-coupled receptor.

In proliferating Sertoli cells, we found that p70S6K activation implied the activation of both PKA and PI3K/mTOR pathways. PKA, which is activated by a Gs-dependent cAMP increase, induced Thr421/Ser424 dephosphorylation as in differentiating cells. But in contrast, this dephosphorylation inhibited p70S6K enzymatic activity and ribosomal protein S6 (rpS6) phosphorylation. Moreover, in proliferating cells, the PI3K/mTOR pathway was activated by FSH and induced an increase in PIP3 that led to mTOR phosphorylation whereas the same pathway was not activated upon FSH stimulation in differentiating cells. The PI3K/mTOR pathway maintained basal phosphorylation of both Thr421/Ser424 and Thr389 sites and induced Thr389 phosphorylation in response to FSH. p70S6K enzymatic activity and rpS6 phosphorylation were stimulated by PI3K/mTOR. Importantly, p70S6K activation in response to insulin at both developmental stages was consistent with the classical model described in the literature for this hormone.

In conclusion, p70S6K activation by FSH would depend on the developmental stage of Sertoli cells and on the class of receptor. The analysis of p70S6K global phosphorylation pattern in each of those conditions is currently underway in our laboratory.