Xenopus a vertebrate model to study Ca^{2+} signalling involved in genetic diseases

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In recent years the field of organ engineering has advanced through the use of embryonic stem cells (ES). ES are pluripotent cells ie they have the potentiality to differentiate into multiple cell types depending on the culture conditions. This potentiality is confined to cell differentiation and does not extend to the differentiation of organs that have three dimensional shapes.

Amphibians such as *Xenopus* have several advantages over mammals as experimental models: a) They lay a large number of eggs at a time and the embryos develop outside the maternal body b) In vitro fertilizations are easy to perform c) According to the size of the embryos, tissues can be dissected, isolated, or transplanted with high precision and ease in these embryos; d) In addition, it is easy to manipulate the expression of gene products by injecting in-vitro transcribed RNAs into developing embryos, e) The recently sequenced genome of *X. tropicalis* affords unique opportunities for genomic studies, e) transgenesis on Xenopus is fully controlled and high throughput methods for transgenesis are available.

At blastula stage (7 hours post fertilization) the ectoderm of the early amphibian embryo is constituted of multipotent cell groups (animal caps) and can be considered as stem cells.

Animal caps are easily dissected from blastula stage embryo using fine needles. The animal caps can be kept several days in physiological saline for several days in absence of any extrinsic factors. In these conditions it gives rise to a mass of epidermal cells. However the animal caps exhibit plasticity and can differentiate into a variety of neural tissues (central nervous system organs, sensory organs such as eye and ear vesicle), mesoderm tissues (blood cells muscles, and functional organs such as heart, kidney) and/or endoderm derivatives (liver, pancreas) by addition of appropriate inducers in the culture medium.

Animal caps treated with activin and retinoic acid differentiates in vitro into a functional pronephros completed within 2 days (Moriya et al. 1993). Most of the genes necessary for the formation of the *Xenopus* pronephros are also crucial for the formation of the more complex mammalian metanephros.

We have recently provided direct evidences showing that an increase in intracellular Ca^{2+} concentration is a necessary signal in the process of pronephric tubule differentiation. This increase occurs in response to RA via calcium channels located at plasma membrane (Leclerc et al. 2008). Our hypothesis is that this calcium increase may occur via the complex polycystin 1 (Pc1) and polycystin 2 (Pc2). Pc2 also called TRPP2 belongs to the TRP family of cation channels. Pc1 would play a role of regulatory unit of this Ca^{2+} channel. These proteins are respectively encoded by 2 genes PKD1 and PKD2. Autosomal dominant polycystic kidney (ADPKD) (the most frequent human monogenic disorder) is caused by mutations in PKD1 and PKD2 and is characterized by progressive renal enlargement due to the formation of cysts leading to terminally kidney failure which characterize disorders in tubule differentiation. By identifying mechanisms involved in Ca^{2+} signalling in pronephros development we propose to transfer this knowledge to higher vertebrate systems. This work may provide understanding of the molecular basis of kidney diseases which is an important problem in public health. Animal caps may constitute a powerful model in high throughput assay for screening new therapeutics agents for ADPKD.

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Moriya N., Uchiyama H., Asashima M. (1993): Induction of pronephric tubules by activin and retinoic acid in presumptive ectoderm of *Xenopus laevis*. Development Growth Differentiation **35**, 123-128