

LIFE AND DEATH IN REPRODUCTIVE BIOLOGY

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*What we call the beginning is often the end
And to make an end is to make a beginning
The end is where we start from*

TS Eliot

Life consists of a series of cycles, from the developmental cycle of the foetus, to our seemingly endless menstrual cycles and the whole grand cycle of birth, puberty, menopause and death. On a smaller scale we are surrounded with the rhythms of life. Our heart beats, our lungs replenish our oxygen supplies, our nerves send waves of electrical messages through our bodies and our hormones spurt from our glands in pulses. Our bodies are designed to respond best to change, rather than to a constant signal. Continuous stimulation of cells with a hormone leads to the turning off of the response - to desensitisation.

As I look back over my scientific career, I can see that it is this aspect of the ever-changing nature of life, and in particular endocrinology, which drives me on. In many ways my science parallels my life and I feel that it is no coincidence that I am a reproductive biologist. My son was conceived alongside my aspirations to do a PhD and as he has grown, I find myself asking more scientific questions about development and puberty and fewer about the more esoteric parts of neuropeptide biosynthesis, where I started.

Over the past six years I have turned my attention to the ovary. The mammalian ovary is a truly remarkable organ and an excellent model for studying cellular life cycles. Life is appropriately busy in this essentially female tissue: there are follicles growing and developing, maturing oocytes being bathed in nurturing fluid, follicles being "selected" for ovulation, ovulated follicles differentiating into corpora lutea and non-ovulatory follicles degrading and disappearing, in a process of programmed cell death or apoptosis, called atresia. All these processes occur side by side, stimulated by the cyclic ebb and flow of a multitude of hormones and growth factors from the pituitary gland and from within the ovary itself. In an average modern woman the cycle of follicle growth and ovulation occurs between 400 and 500 times in her reproductive life. One hundred years ago, when women conceived young, often had many children at regular intervals and died at a much younger age, their ovaries would have ovulated fewer than 50 of the 400,000 oocytes present at birth. This extra work that the ovary carries out in our lifetime puts it under additional

functional strain: every ovulation creates damage that needs to be healed, as well as another cohort of atretic non-ovulatory follicles.

My research has now focussed on this cycle of life and death within the ovary. I am interested in a family of growth factors which appear to be involved in follicle growth and atresia: the inhibins and activins. Inhibin is a classical endocrine hormone, consisting of two protein subunits (alpha and beta), which is made by specific cells in the growing ovarian follicles. Inhibin acts by switching off the synthesis and release of one of the gonadotrophins in the pituitary gland. Activins, on the other hand, consist of a dimer of two beta subunits. There are now four homologous beta activin subunits (beta A - D), although beta A and beta B activin are far better characterised physiologically. In my laboratory there is a major project underway investigating the expression of these four beta activin genes in sheep. I am interested in their tissue distribution, their developmental expression pattern, their gene sequences and homologies and, eventually, the transcription factors which control their synthesis. As we characterise these genes further, I hope to explore whether the proteins form specific combinations (beta A with beta C or D?, beta B with beta C?) and whether the possible combinations produce different biological activities. My initial interest in the activins came from work I did on the Booroola fecundity gene, which causes superovulation in gene carriers. Booroola ewes which are homozygous for the fecundity gene have significantly more messenger RNA for beta A activin than non-carrier ewes. In contrast, the Booroola mutation does not appear to affect the expression of the alpha inhibin subunit gene (1) .

While working on possible candidate genes for the Booroola mutation I became interested in a protein called clusterin. Clusterin is a highly sulphated glycoprotein which appears to have a variety of activities in the body. The clusterin gene is highly expressed in the gonads and in the testis the protein appears to bind to sperm at different stages of their development, at times acting in a protective role, possibly as an inhibitor of complement cytolysis, and at times helping to cluster and inactivate damaged or deformed sperm. My initial work on clusterin expression in the ovary showed that there was more clusterin mRNA in extracts of corpora lutea, than in the ovarian follicles or connective stromal tissue (2) . However the northern analysis of mRNA that I was using failed to identify the cells that synthesised the protein or the cells that were binding the clusterin protein. For those studies I needed to develop in situ hybridisation and immunohistochemical methods.

Recent studies in my laboratory in the Physiology Department of the Otago School of Medical Sciences by postdoctoral fellow Janice Bolter, have revealed a dual role for this protein in the ovary, similar to that seen in the testis. Immunohistochemical staining has shown that clusterin protein is found in the zona pellucida (the protective membrane coating) of oocytes from healthy, growing and atretic follicles. Clusterin protein also appears to help in the apoptotic process of atresia, binding to dead and dying follicle cells. Clusterin's role in programmed cell death is thought to be that of a biological detergent, helping to solubilise the membrane lipid and nucleic acids from the dead cells. Janice has also shown that the characteristic

DNA cleavage seen in apoptosis, precedes the presence of clusterin protein in atretic follicles. On going in situ hybridisation studies will reveal which cells are synthesising clusterin, both in growing and atretic follicles and in the corpus luteum.

Our continuing study of life and death processes in the ovary may help to uncover the mechanisms that determine which follicles are selected to mature and which ones are selected to die and this may help solve some of the ovarian problems of women today.

References

1. Fleming JS, Tisdall DJ, Greenwood PJ, Hudson NL, Heath DA , McNatty KP. Expression of the genes for alpha-inhibin, betaA inhibin and follistatin in the ovaries of Booroola ewes which were homozygotes or non-carriers of the fecundity gene Fec(B). *J Molec Endocrinol* 8:265-273, 1992.
2. Fleming JS, Greenwood PJ , Chen CLC. Expression of the clusterin gene in the tissues of Booroola sheep which were homozygotes or non-carriers of the fecundity gene FecB. *J Molec Endocrinol* 9:207-211, 1992.