Preface

Starting in the 1950s, Briggs and King, and later John Gurdon and colleagues, showed that the transfer of a nucleus into an enucleated amphibian oocyte reconstitutes a cell that is capable of development at least part way to mature adulthood. This body of work showed that as an animal develops, despite the apparent loss of phenotypic potential that accompanies differentiation, the cell nucleus still contains sufficient genetic information to allow the cell to behave like a zygote and undergo embryonic development. We now know that nearly every animal cell, other than such odd exceptions as mature erythrocytes or lymphocytes with rearranged antigen receptors, contains a complete complement of the genetic information required to build another copy of the animal in question. However, the vast majority of cells in the adult are terminally differentiated with limited patterns of gene expression. These patterns of gene expression are conferred by both limitation of the repertoire of transcription factors expressed by the cell, and also by the chromatin configuration of the nucleus in that cell. This "nuclear program" defines the range of genes expressed by the cell, and hence its phenotype and function. Many of the molecular modifications conferring this program, DNA methylation, for example, are inherited epigenetically; that is, their inheritance is independent of the base pair sequence of the nuclear DNA. The sum total of all epigenetic marks in a cell comprises its epigenome.

Despite the early amphibian successes, it appeared that the more differentiated the cell that donated the nucleus, the poorer the efficiency of tadpole recovery, so that adult, terminally differentiated cells were much less efficient nuclear donors than early blastula cells. Early work in mammals, using enucleated mouse zygotes, reinforced this picture and for a long time it was thought to be impossible to transform the epigenome configuration of an adult terminally differentiated cell to that of a fertilized oocyte and thus reverse the linear, one-way process of differentiation to create a pluripotent, embryonic cell. More recently, the cloning from adult somatic cells using enucleated oocytes of several mammalian species, most famously the creation of the sheep "Dolly" at the Roslin Institute in 1997, showed that reversal of this process of increasing specification is possible. Because cloning of mammalian adults is achieved by nuclear transfer, we can conclude that the nucleus of the somatic donor provides all of the genetic information required, and that the mammalian oocyte contains an activity that acts on the donor nucleus to reprogram an embryonic state. This reassignment of a cell's nucleus from a somatic to an

embryonic program is an example of "nuclear reprogramming." Nuclear reprogramming is a complex process involving the restructuring of the chromatin in the nucleus, remodeling of such DNA modifications as methylation, and a major change in gene expression patterns. Nuclear reprogramming is obviously a major function of the oocyte, as it combines two haploid genomes from different sources into one diploid genome and then reprograms this new genome to form a pluripotent cell that then begins development. The oocyte is thus the canonical reprogramming cell. Nuclear reprogramming can also occur after fusion of cells of different types, where (usually) the less-differentiated cell reprograms the more-differentiated cell. Since this usually results in the loss of specific differentiated functions, this phenomenon was originally called extinction. In addition, events called transdifferentiation have been described, in which cells of one, possibly well-differentiated, type appear to differentiate into cells of another lineage, sometimes one derived from a different embryonic germ layer. Trans-differentiation events also almost certainly involve some level of nuclear reprogramming to change the phenotype and potential of the cell. At present, nuclear reprogramming is largely a "black box" and many of the events involved in a reprogramming event are either completely unknown or poorly characterized. How nuclear reprogramming is controlled is also unknown, but clues are beginning to emerge and it is a very exciting time to be working in this field.

It is therefore particularly well-timed of Humana Press to release *Nuclear Reprogramming: Methods and Protocols* devoted to research techniques in nuclear reprogramming. I hope that this book will be of interest to a variety of people, be they cloners interested in the generation of live animals from cells, perhaps endangered species, or medics interested in the generation of stem cells or other cell types for therapeutic purposes, or biochemists or molecular biologists interested in the mechanism of the reprogramming process itself. This volume includes chapters describing various methods of nuclear reprogramming, including nuclear transfer in several different species, both amphibian and mammalian; fusion achieved both by chemical treatment and by electrically shocking cells; quantitative fusion and reprogramming by in vitro treatment of cells with cell extracts. Isolation of an adult stem-cell type is included. Several different methods of monitoring nuclear reprogramming are described, including the use of transgenic markers to follow reprogramming after nuclear transfer, activation of telomerase as an ES-specific marker, observation of structural changes in the nucleus by both light and electron microscopy, and verification of stem cells' surface marker expression and differentiation potential. With respect to the biochemistry of nuclear reprogramming, methods for the examination of chromatin protein modifications,

nucleosomal footprinting, and transcription factor binding are included here, as are methods for the study of DNA methylation changes both at the specific locus level and, by microscopy, at the level of the whole nucleus.

I have enjoyed working on this project and I wish to take the opportunity here to express my gratitude to John Walker for the invitation to compile and edit this book, to Tom Lanigan and his team at Humana Press, and to the many scientists who have generously contributed chapters to this volume.

Steve Pells