that the vertebrate skeleton is just as important, complex and, of course, as scientifically interesting as any of the other organs. As such *The Skeletal System* edited by Olivier Pourquié is published at a good time and offers both global and detailed views of our current understanding of skeletal evolution, development and biology at the molecular level.

It is my recommendation that every principal investigator in the skeletal biology field, whether the research is basic or clinically oriented, should be armed with a copy of this book

A distinct strength of the book is that it has 11 chapters all written by leaders in their fields. The book provides a broad coverage of skeletal biology but focuses on three major themes: skeletal development, covering skeleton formation in the limb, craniofacial region and spine; cell differentiation and proliferation in the developing and formed skeleton, and their regulation by signaling molecules and transcription factors; and skeletal mineralization and remodeling. The book also contains chapters on the evolution of the vertebrate skeleton and on human genetic diseases that affect the skeletal system.

For someone (such as a graduate student) who has never formally learned the biology of the skeletal system in depth but is interested in it, this book is a good place to start. The chapters of the book are organized in a way that fits the ontogeny of the skeleton, and are hence very easy to follow. The book first covers skeletal evolution, then patterning and cell differentiation. After describing remodeling, mineralization and extracellular matrix regulation, events that occur specifically and extensively during skeletal formation and homeostasis, it ends with a comprehensive and insightful chapter on skeletal diseases that are caused by abnormal regulation of the skeletal system. Thus, the book can be chosen as a textbook by those who teach graduate courses on this topic too.

For senior basic science researchers and clinicians who are thinking about getting into the skeletal field or who already have established careers in the field, this book is also a great reference. The authors of all of the chapters have provided comprehensive and updated overviews of the field, with much of the information presented concisely and critically summarized by schematic graphics. What is more important is that, along the way, the authors offer their unique vision about the significance, history and impact of the particular subjects that they cover. It is provocative to read their views, for example, on the future directions for the field, which are provided in the concluding remarks and perspective of some chapters. These sections, in particular, offer food for thought for other scientists in the skeletal field and will have a positive impact on the future development of skeletal research. It is my recommendation that every principal investigator in the skeletal biology field, whether the research is basic or clinically oriented, should be armed with a copy of this book.

From every aspect, this book is almost perfect for readers at different levels. However, the field of skeletal biology is moving at a very fast pace. In the past two years, rapid advances have been made in understanding the molecular mechanisms that underlie the role of bone as a crucial endocrine organ that regulates body metabolism and bone mass via the central and sympathetic nervous system. Although these new advances have been mentioned in a couple of chapters, there is obviously a need to expand them into a new chapter. I must say that such hindsight is completely beyond editorial control, but the field will be better served if this new chapter is added to any future edition of this book. In addition, the formation and homeostasis of the cartilage and bone, two major components of the skeletal system, are intimately linked at both molecular and cellular levels in most vertebrate species. As such, readers would have been better served if these two components of the skeletal system were not so clearly separated into distinct chapters or if the chapters had been synthesized in a slightly different way to give more emphasis to the connections between bone and cartilage. Along this line, readers might also want to have more discussion of how signaling molecules, as extrinsic factors, and transcription factors, as intrinsic factors, act together in the same context to regulate the skeletal system.

In summary, I highly recommend this book to researchers interested in exploring skeletal biology and diseases: it is an excellent source of information on the molecular and cellular biology of the skeletal system and belongs in every laboratory of skeletal research.

A guide to the productive poking, prodding and injection of cells

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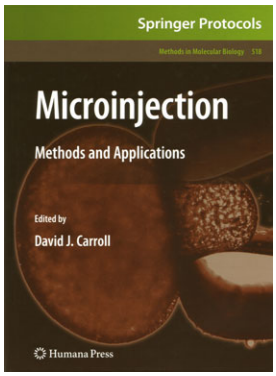

Microinjection: Methods and Applications (Methods in Molecular Biology Vol. 518)

Edited by David J. Carroll

Humana Press (2009) 224 pages
ISBN 978-1-58829-884-3
$99.50 (hardback)

This is just the type of book I would have liked to have had read before I began my own injection studies in the early 1980s. In *Microinjection: Methods and Applications*, David Carroll, the Editor, has assembled a knowledgeable group of practitioners, who describe clearly how to inject molecules, nuclei and cells (sperm) into a range of target cells. The difference between injection then and now, however, is not really how to inject, but what to inject and how to analyze the effects of such manipulations. Again, the chapter authors do a good job of describing various analytical methods, often in great detail.

Cellular injection, which ranges from the injection of somatic cells growing in vitro, to injecting into much larger cells, such as fertilized eggs, has a long history. Its roots are in the work of early cell biologists,

perhaps best epitomized by Robert Chambers (1881-1957), who used a range of microknives and microneedles to probe the physiochemical nature of the living state. The design and fabrication of improved micromanipulators made possible a wide range of subtle perturbations of cells and intracellular structures (see Chambers, 1918; Chambers, 1922). The best-appreciated 'neoclassical' examples of such techniques include the pioneering studies of Briggs and King (Briggs and King, 1952) and Sir John Gurdon (Gurdon, 1960; Gurdon and Byrne, 2003), who used nuclear transplantation to explore the nature of the epigenetic changes associated with embryonic development, and those of R. B. Nicklas (Nicklas and Staehly, 1967; Nicklas, 1967) and others, who used micromanipulation to characterize the mechanical and adaptive properties of the eukaryotic spindle. Injection and cellular dissection methods were also key to defining the properties of nuclear-cytoplasmic targeting systems (Goldstein and Prescott, 1967; Paine and Feldherr, 1972).

The 'modern' age of cellular manipulation began with the introduction of more versatile and specific tracers and perturbants, including fluorescently labelled proteins (Taylor and Wang, 1978) and function-blocking antibodies. Using the injection of an anti-myosin antisera, Mabuchi and Okuno resolved the role of actin-myosin in cytokinesis (as distinct from mitosis) (Mabuchi and Okuno, 1977). Shortly thereafter the intracellular injection of monoclonal antibodies was found to be the first specific method to disrupt intermediate filament organization (Lin and Feramisco, 1981; Klymkowsky, 1981; Klymkowsky, 1982). Since then, many new experimental reagents have become available, including anti-sense and microRNA-based approaches, together with the expression of wild-type, epitope-tagged, and engineered polypeptides.

In my own case, mastering injection involved assembling an injection system (including a customized stand to hold a Leitz micromanipulator, and a large bore glass syringe and heavy rubber bands as a 'controlled' pressure source). These days there are many commercially available injection systems that significantly lower the activation energy involved in beginning and successfully completing such studies. Once convinced that mammalian cells could survive injection (by me), experiments became routine (aside from the frustration of clogged needles). While each investigator has to have their own 'conversion experience', they will be greatly helped by the clarity of the presentations provided in *Microinjection: Methods and Applications*.

The book is organized into 13 chapters that cover the injection of oocytes, eggs, embryos and germ lines in *C. elegans*, sea urchin, starfish, frog, zebrafish, mouse and human. Each chapter deals with a particular organism and a particular experimental manipulation. For example, the four chapters on the clawed frog *Xenopus laevis* are focused on mRNA and antisense (morpholino) oligonucleotide injection in embryos, mRNA injection/protein expression in oocytes, and the generation of transgenic organisms. Each chapter contains a number of helpful illustrations, as well as a 'notes' section that addresses specific experimental issues. The book's audience, primarily novices who seek guidance (and some reassurance) on how to make this experimental approach work for them, should find it quite helpful. One useful addition would be the inclusion of more videos (such as those referenced in Douglas Kline's chapter). I know from my own experience with students that showing them (by using a video system) exactly what injection looks like greatly reduces the time it takes for them to become proficient injectors.

This is just the type of book I would have liked to have had read before I began my own injection studies in the early 1980s

While the chapters are uniformly thorough, I did find myself taking exception to, or wanting more discussion about, some points – my comments are restricted to the six chapters on mammalian cells and *Xenopus*, the systems with which I have direct experience. In contrast to what is stated in the chapters on mammalian cell injection, only a few of the commonly studied cell types require glass cover slips to be treated to ensure the cells adhere to them, and there are simpler methods to hold cells during injection than Rose chambers. More importantly, there is no justification for the suggestion that miRNA-type reagents are, per se, more or less specific than injected antibodies. While an antibody can exhibit 'cross-recognition of other proteins within the cell' (p. 78), RNA reagents can also display off-target effects (Qiu et al., 2005). With respect to intranuclear injection, I have often wondered, while watching the flow of injected solutions, whether it might lead to breaks in genomic DNA, and as such had hoped that in the chapter written by Sebastien Chenuet et al. this possibility would have been discussed.

Similarly, my concerns with respect to the four chapters on *Xenopus* are largely minor and technical. For example, in Chapter 1 (written by Jill Sible and Brian Wroble), I was surprised by the absence of a discussion of hormone-regulated proteins (delivered by RNA injection). This method, introduced by Hollenberg et al. (Hollenberg et al., 1993) and popularized by Kolm and Sive (Kolm and Sive, 1995), is simple, works with many different types of proteins, including non-transcription factors (Zhang et al., 2006), and together with targeted blastomere injection allows the investigator to control where and when a protein becomes active. Combined with the use of protein synthesis inhibitors, it provides initial (albeit not definitive) evidence about whether a regulatory interaction is direct or indirect. On a related note, while these authors recommend a commercial source for the pSP64T plasmid, they fail to note that other plasmids are readily obtained from members of the *Xenopus* community through the XenBase web resource. Given the high level of experimental detail in this and other chapters, one might have expected discussions of: (1) the typical (that is, reasonable) amounts of antibodies, RNAs, DNAs and morpholinos to be injected; (2) the use of GFP-chimeras to monitor RNA-directed expression and morpholino activity; and, importantly, (3) the fact that injection into a single blastomere does not lead to a uniform and global distribution of the injected molecule or its product. Particularly in the case of polypeptide encoding RNAs, post-injection diffusion can be quite limited, presumably because of interactions with ribosomes – typically uniform expression requires injection of both blastomeres at the two-cell stage, or of all four blastomeres at the four-cell stage, something that can be readily demonstrated through whole-mount immunocytochemistry.

In Chapter 3 (by Jeffrey Lau and Anthony Muslin), the authors describe the use of immunoblot analysis to characterize the effects of translation blocking morpholinos. They probably should have noted: (1) that such morpholinos have been reported to stabilize target RNAs (Gene-Tools web site and our own experimental observations), which can make in situ hybridization and RT-PCR analysis methods problematic; and (2) that

immunoblot relies on the availability of a good antibody against the target protein. Alternatives are available; for example, one can use in vitro translation systems or RNAs that contain the target sequence and encode an epitope-tagged version of the protein (see Zhang et al., 2006). Conversely, if RNA splice-blocking morpholinos are used, RT-PCR analysis is straightforward. Finally, the method for generating transgenic *X. laevis* embryos described by Bryan Allen and Daniel Weeks (Chapter 9) is clearly exciting, but it is worth noting that since it was first described in 2005 (Allen and Weeks, 2005) no other lab has (apparently) published using the technique. In this light, a general review of other transgenic methods (Ogino et al., 2006; Waldner et al., 2006; L'Hostis-Guidet et al., 2009), as well as a discussion of the application of these methods to *X. tropicalis*, a diploid relative with a shorter generation time and a sequenced genome, would seem to be both appropriate and useful.

All of which is to say that, while this book provides a useful guide to experimental design, the savvy investigator should take the time to explore the various ancillary technical issues associated with their specific project.

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An essential glycobiology resource for developmental biologists

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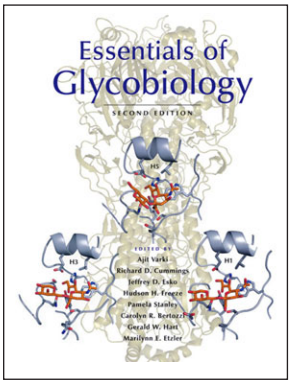

Essentials of Glycobiology, Second Edition

Edited by Ajit Varki, Richard D. Cummings, Jeffrey D. Esko, Hudson H. Freeze, Pamela Stanley, Carolyn R. Bertozzi, Gerald W. Hart and Marilynn E. Etzler

Cold Spring Harbor Laboratory Press (2009) 784 pages
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During the development of multicellular organisms, the formation of complex organs and tissues requires cell-cell and cell-matrix interactions. Cells in a developing embryo carry large quantities of carbohydrates known collectively as glycans that can serve as signaling effectors, recognition markers and structural components. Understanding the functions and regulation of glycans in growth and development is therefore a key issue in developmental biology. The second edition of the book *Essentials of Glycobiology*, written and edited by glycobiology experts, provides a comprehensive and updated overview of glycan structures, biosynthesis and functions. This book is an invaluable resource for both students and established investigators who are interested in glycan-related processes in development, as well as in many other areas of basic research.

The book consists of 51 chapters, which are divided into six sections: (1) General Principles, (2) Structure and Biosynthesis, (3) Organismal Diversity, (4) Glycan-Binding Proteins, (5) Glycans in Physiology and Disease, and (6) Methods and Applications. Together, the editors attempt to provide extensive coverage of glycobiology-related subjects using up-to-date information derived from a variety of research fields.

So, what's new in the second edition? The first edition of this book provides basic information needed to understand the fundamentals of glycobiology along with a summary of the state of the field of glycobiology in the 1990s. While the overall organization of the book has not changed, the second edition includes several new chapters, in which significant advances in the glycobiology field, which have occurred since the publication of the first edition, are presented. These chapters are integrated into three sections: a genomic view of glycobiology; the section on organismal diversity, which contains several new chapters; and the last section, which contains a new chapter on glycomics, which is analogous to genomics and proteomics, and which aims to