

Preface

Immunocytochemistry of plant cells has come a long way from the first review on this subject by Bruce Knox in the early 1980s. In that early review, our only tools were fluorescein-labeled antibodies for light microscopy and ferritin-labeled antibodies for electron microscopic observation. Frankly, in many of these early localizations the resolution of the tissue or the specificity of the labeling left much to be desired. Many of my traditional plant biochemist/physiologist colleagues said things like “I don’t believe those immunocytochemical techniques Kevin”. One can understand this level of skepticism when organelles were not readily discernable and the label was hard to determine from background. Embedding and sectioning plant tissue embedded in Lowicryl resin was very difficult and the tissue appeared extracted after prolonged embedding. Only certain unique tissues such as germinating seeds were preserved sufficiently to allow for good resolution of structures. However, things started to improve dramatically for plant immunocytochemistry with the introduction of the London resins. These resins infiltrated plant tissues easily and could be polymerized with standard electron microscopy techniques used for epoxy-based resins. The other breakthrough was the development of gold-labeled secondary antibodies. Unlike ferritin, these antibody-probes could be prepared in a variety of sizes and the preparation of the particles themselves was not difficult and they became available from numerous commercial sources as well. In addition, gold probes could be used at both the light and electron microscopic levels so that a single specimen block could be used to localize at the tissue level with the light microscope and at the organelle and sub-organelle level with the transmission electron microscope.

My goal when I entered this area was to produce micrographs that had a high level of structural preservation and a convincing immunolocalization as well. When these papers started to appear in the early 1980s, I had a steady stream of visitors to the lab to learn the protocols and my laboratory phone was dubbed “the immunogold hotline” by my post docs in the lab! “Why don’t my localizations look like yours?” was the most frequent question. Luckily, this is not rocket science and most of my visitors and telephone correspondents after a bit of coaching were able to localize their protein of interest. A 1988 McKnight training

class at U. Georgia even resulted in a whole class full of students doing a successful electron microscopic localization even though most of the students had never performed electron microscopic studies previously.

Science is not done in a vacuum and certainly the development of techniques in my laboratory was heavily influenced by other plant and animal immunocytochemists. Prominent among those people that were influential in these projects are Dick Trelease, J. Paul Knox, John Harper, Roberto Ligrone, Andrew Staehelin, Karen Renzaglia, Tobias Baskin, and my former post-docs Andrew Bowling, John Hoffman, Timothy Sherman, Martin Vaughan, and Larry Lehnen. Each of these contributed a bit of knowledge or technique that helped these experiments progress and the protocols become more refined. I am also most grateful to my mentors, Martha Powell and Kenneth Stewart, in my initial training in microscopy while a graduate student at Miami University. I entered graduate school planning to be a geneticist but ended up a cell biologist thanks to Martha and Ken. Rex Paul, who maintained the microscopes at the Stoneville location for many years, kept the microscopes in impeccable shape and allowed a high productivity from my now retired Zeiss EM 10CR microscope with almost 38,000 micrographs produced. I am also indebted to several NRI funded proposals that allowed me to hire some of the above named post docs and to develop the techniques described in this volume. My retirement from the USDA in August 2010 has allowed me the time to focus more on the writing of this book, while the memories of the experiments and the many modifications we made over the years is still fresh in my mind. I thank my friends Paul Knox, Andy Bowling, Dave Collings, John Harper, Roberto Ligrone, Tobias Baskin, Lacey Samuels, and Bo Kwang for supplying me with a lovely set of micrographs to help illustrate this book.

Immunocytochemistry, like its predecessor cytochemistry, arose out of my frustration with trying to either use a very small amount of tissue (such as that occurring in variegated chimera plants) or to determine specific reactions in a subset of that tissue using biochemical methods. For example, the presence of RuBisCo in guard cell chloroplasts was the subject of much debate but immunocytochemical techniques allowed for unequivocal localizations. The development of immunogold-silver and immunofluorescence on semi-thin sections for light microscopy was similarly fruitful in answering some long-standing anatomical questions. Just in our lab, we have answered questions on the nature of gelatinous fibers in trees, the role of gelatinous fibers in vines, mechanisms for ballistic seed dispersal and leaf abscission. As more traditional anatomists embrace these techniques, I am sure that a number of other recalcitrant questions will be answered.

This book is organized essentially into two sections. The first chapter gives what we consider general protocols that work well on a variety of tissues and organelles, but also a number of variations that one might try in order to obtain a successful localization. Most of these were developed when the more standard protocols failed. The second portion of the book reviews by organelle of those techniques that may work better with that particular organelle, what unique immunocytochemical techniques can be used, and a review of some of the more

important studies on that organelle. Some of the chapters also address the questions that are still outstanding and which could benefit from immunocytochemical studies.

My hope with the protocols outlined in this book and the description of other studies that more people will attempt these techniques and that they become more widely adopted by the plant science community.

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