PREFACE: THE ROLE OF RECONSTITUTION IN CYTOSKELETON RESEARCH

Reconstitution is an experimental strategy that aims to recapitulate a cellular activity outside of a cell so that it can be studied in a "test tube"-like environment. Being able to manipulate molecules *in vitro* provides an unparalleled opportunity to dissect mechanism in ways that cannot be easily achieved in a living cell. Many of the most important discoveries in cell and molecular biology in the twentieth century utilized reconstitution; examples include the classic mechanistic studies of DNA replication, ubiquitin-mediated protein degradation, protein insertion into the endoplasmic reticulum, and vesicular trafficking to the cell surface.

Reconstitution also has had a rich history in the cytoskeleton field. A notable early success was Albert Szent-Gyorgyi's *in vitro* reconstitution of the contraction of actomyosin threads in the early 1940s (Szent-Györgyi, 1942), which allowed him to dissect the molecules involved in muscle contraction. For cilia and flagella motility, Summers and Gibbons (1971) demonstrated microtubule sliding in permeabilized axonemes, which helped to understand how dynein motors drive axonemal beating. In the 1980s, Sheetz and Spudich (1983) directly observed the motility of myosin-coated beads along actin cables from dissected *Nitella* cells; shortly thereafter, the Spudich lab demonstrated myosin motility on purified actin filaments. At a similar time, several *in vitro* motility assays were developed for studying the molecular motors that drive the transport of membrane organelles in nerve cells (Vale, Schnapp, Reese, & Sheetz, 1985). Collectively, these *in vitro* assays were essential stepping stones for the single-molecule assays that soon followed. *In vitro* motility assays, single-molecule measurement techniques, and protein expression in bacteria or insect cells opened the doors to the study of many molecular motors that could not be easily studied in living cells. Thus, reconstitution was essential for broadening the investigation of cellular motility beyond the classical systems of muscle and cilia.

Understanding the dynamic properties of cytoskeletal polymers also was aided by reconstitution strategies. Shinya Inoue first observed the dynamic nature of the cytoskeletal fibers that comprise the mitotic spindle (Inoue, 1953). More than two decades later, the polymerization and depolymerization of purified tubulin were demonstrated *in vitro* when the correct buffer conditions were identified (Weisenberg, 1972). Later, Mitchison and

Kirschner (1984) demonstrated that microtubules grow slowly followed by catastrophic shrinkage. This unusual behavior (termed "dynamic instability") was first observed with purified tubulin and then later confirmed to occur in living cells. For the actin cytoskeleton, the first principles of polymerization of purified actin were derived by Oosawa and colleagues in the early 1960s (Kasai, Asakura, & Oosawa, 1960), and later Pollard and coworkers (Woodrum, Rich, & Pollard, 1975) developed a more detailed understanding of subunit addition to the two ends of an actin filament.

In addition to work on purified proteins, reconstitution strategies have been developed for complex systems of cytoskeletal filaments. For example, using frog egg extracts, Murray and colleagues (Shamu & Murray, 1992) observed mitotic spindle formation and anaphase movement of chromosomes, a complex system involving the orchestrated actions of hundreds of proteins. Heald et al. (1996) followed by demonstrating that DNA-coated beads can replace natural chromosomes in inducing the formation of a mitotic spindle. Success in reconstituting actin-based cell motility arguably has been even more dramatic. Actin-based polymerization at the leading edge is believed to drive cell migration. *Listeria*, a pathogenic bacteria, hijacks this actin polymerization system to propel itself through cytoplasm, and this process was reconstituted in Xenopus egg extracts (Theriot, Rosenblatt, Portnoy, Goldschmidt-Clermont, & Mitchison, 1994). Several years later in 1999, Carlier and colleagues (Loisel, Boujemaa, Pantaloni, & Carlier, 1999) demonstrated that a defined cocktail of actin, Arp2/3, and additional actin regulatory proteins can propel *Listeria in vitro*, and 2 years later, they replaced the *Listeria* itself with artificial beads.

Even in our modern era of "omics"-driven research, reconstitution is alive and well, and indeed flourishing as never before in cytoskeletal research. Building upon the earlier work described above, investigators are now pushing to reconstitute more complex systems and develop more sophisticated assays. Combining *in vitro* reconstitution with *in vivo* studies of living cells/organisms is proving to be a powerful overall approach for elucidating the roles and mechanisms of cytoskeletal proteins.

This book captures the excitement and progress on reconstituting cytoskeletal processes. It begins with new methods of assaying polymer dynamics, including powerful computational methods (Prahl et al.) and ways of modulating experimental conditions using microfluidics (Carlier et al.). In addition to the classic systems of actin and microtubules, the *in vitro* polymerization of actin-like proteins from bacteria can now be studied as well (Petek and Mullins). The nucleation and dynamics of cytoskeletal filaments are

controlled by numerous regulatory proteins that interact with actin and microtubules, and these mechanisms can be dissected by *in vitro* experimentation. The nucleation step is controlled by large, multi-subunit protein complexes. In this volume, Chen et al. describe how to prepare the WAVE complex (a regulator of Arp2/3 and actin nucleation) using a very clever protein expression method that may prove generally useful for other multi-subunit protein machines as well. Our understanding of tubulin nucleation lags behind that of actin, but Choi et al. have made progress in isolating an active γ-tubulin ring complex and assaying its nucleation of tubulin *in vitro*. Other proteins act as polymerases that add subunits to the filament end, and Mizuno and Watanabe (formin) and Al-Bassam (TOG domain proteins) describe elegant means of assaying these activities by microscopy. Other enzymes posttranslationally modify the filament after it has been assembled, and Vemu et al. describe how tubulin-modifying enzymes can be purified and their activities measured.

As described earlier, molecular motor proteins have been a focus for single-molecule investigations. A recent trend has been to develop motility assays that reflect greater and more physiological complexity. For example, many cargos contain multiple, rather than single, motor proteins and even can bind different types of motors that pull the cargo in opposite directions (e.g., kinesin and dynein). What are the properties of such complex multimotor systems? To answer such questions, one approach is to use scaffolds where the numbers and types of bound motors can be precisely controlled. To achieve this, Rogers et al. as well as Goodman and Reck-Peterson attach motors to DNA through precisely controlled linkages. To mimic other scenarios in which motors are bound to the cell cortex and pull on microtubules (causing displacement of the centrosome), Roth et al. attached dynein motors to the walls of microfabricated chambers. It is also important to study how ensembles of motors work on natural cargos. Both Hendricks et al. and Barak et al. describe methods for isolating membranous organelles and assaying their movement along microtubules *in vitro*.

Another challenge is to reconstitute complex cytoskeletal processes using networks of interacting purified proteins. Both Murrell et al. and Boujemaa-Paterski et al. describe amazing self-organization patterns, motility, and contractile properties of actin networks, assembled with different geometric constrains and protein mixtures. Substructures and reactions in the mitotic spindle are also proving amenable to reconstitution. Driver et al. describe methods for isolating kinetochores and studying their interactions with microtubules, and Fourniol et al. have reconstituted the basic

ingredients of the microtubule overlap zone that forms during anaphase. And why not start to bridge the worlds of actin and microtubules? By adding proteins that interact with both actin and microtubules, Preciado López et al. have developed assays where the interactions and self-organization of actin and microtubules can be studied *in vitro*.

For some activities, it is not yet possible (and perhaps not even desirable, since regulatory mechanism might be lost) to achieve a complete reconstitution with purified proteins. In such a case, reconstitution can be performed in a crude extract, which still enables types of experiments that cannot be easily achieved with intact cells (e.g., immunoprecipitations, certain drug treatments, kinetic experiments, etc). The Xenopus egg extract, because of its very high protein concentration, has been a remarkable source for reconstitution experiments. In this volume, Groen et al. and Field et al. describe very useful tricks for preparing different types of extracts and ways in which these extracts can be used to study the self-organization of microtubules or actin.

From examining these chapters, it is apparent that new trends are emerging in reconstitution work. First, the reconstitution of cytoskeletal functions is attracting and benefitting from the interdisciplinary research of biologists, chemists, physicists, and engineers. For example, the microfabrication of chambers, lithography, and microfluidics are involved in many of the methods described in this volume. Furthermore, the self-organization and mechanical properties of the cytoskeleton *in vitro* are attractive experimental and theoretical problems for physicists (many of the authors in this series were originally trained as physicists). A second trend is the use of light microscopy for the vast majority of the assays in this book. Microscopy-based assays provide much more detailed information and require less material than bulk population measurements using fluorimeters or spectrophotometers. For such microscopy assays, one has to prepare specific types of chambers, clean and passivate the glass, and attach specific proteins of interest to the surface. Most of the chapters describe such methods, which are often derived from painstaking development work. There is a wealth of information in browsing and comparing the methods for surface preparation in these different chapters.

For students and postdocs reading this volume, reconstitution, while very powerful, requires attention to detail; protein handling, buffers, and concentrations matter a great deal. However, in this era of "black-box," commercial "kits," it is particularly rewarding to work out, step by step, a reconstitution method, with the reward being seeing a biochemical process

"come to life" or witnessing the self-organization of molecules and polymers into larger structures. Exciting challenges also lie ahead for young scientists in this field. Might it be possible to build something as complex as a mitotic spindle? An axoneme? A microvillus? A centrosome? Perhaps, one day, these far-fetched dreams may not be so far-fetched after all.

ACKNOWLEDGMENT

I would like to thank Peter Bieling for his helpful suggestions in the planning of this volume.

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