Interaction, assembly and processing
Life on an interface
Editorial overview

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Introduction

This section is originally entitled "Interaction, assembly and processing". However, the editors — respectively, a molecular biologist and a chemical biologist — originally had no idea what that title might mean. We therefore decided to come up with an interpretation we could at least understand and then applied our admittedly protein-centric bias to the enterprise. The title for this issue therefore should have been "Ways to use chemicals and proteins to study and manipulate interactions of proteins with other things". Although that title wasn't catchy, it did provide a framework that enabled us to solicit articles from authors whom we thought were doing interesting work.

We are cheerful about the result. As readers of this journal well know, biology is an enormously successful enterprise. But it is one that now encounters both difficult research questions (e.g. how groups of genes work together to produce effects) and a daunting mass of data (e.g. before this article is published, the complete DNA sequence of *Drosophila melanogaster* may be released to the public). To move from current molecular and cellular biology to a more genomic and more quantitative biology, new methods and new capabilities will be needed (see [1], for example). As some researchers, notably Richard Lerner, realized early on, some of the needed capabilities will come from the biology–chemistry interface.

In that interface lies both challenge and opportunity. Challenge, because chemists interested in making significant contributions to biology must absorb a daunting amount of new information and techniques while always remaining current in their core area of expertise; whereas molecular biologists who wish to make use of chemical methods must often relearn core concepts they have not encountered since undergraduate chemistry classes. Opportunity, because as the important issues in biology change from 'anatomical' issues, such as characterization of individual proteins and their membership in complexes, to 'physiological' issues of mechanistic and quantitative function, the mindset and capabilities that chemists bring to the table will become increasingly important. Opportunity also, because the combination of powerful new chemical techniques and our rapidly increasing knowledge of what interactions are critical for various biological events is creating a whole new field of biological manipulation in which researchers strive to manipulate biological pathways by either blocking or enhancing critical macromolecular interactions. Such 'biomanipulation' may lead to a new pharmacology in which the binding surfaces of macromolecules, rather than enzyme active sites, are the preferred targets. Biomanipulation is also proving to be a powerful engine for fundamental discovery [2–4].

The methodological firepower provided by chemical biologists and the new breed of technology-focused molecular biologists may well alter the way future biological research is performed. However, our view was that delineating the scope and possible goals of such research and setting goals for it was beyond the scope of this journal section, and that it would be more productive to focus on the technologies themselves.

Contents

In this issue, Brown (pp 16–21) reviews work to target both large and small molecules (proteins, genes and drugs) to specific cell types, tissues and organs. The oldest approaches to this problem used affinity reagents such as antibodies raised against a specific receptor chosen by the experimenter. Second generation approaches have used a conceptually different, unbiased approach in which a library of reagents is screened for those that show affinity for some cell type, tissue or organ, without knowledge of a particular molecular target. For example, phage display has been used to identify peptides that home in on particular cell types and organs [5,6]. This paper reviews past work and current efforts, which have expanded both the repertoire and the characterization of such targeting peptides.

Next, Gokhale and Khosla (pp 22–27) provide a careful case study of the function of linkers between modular functional domains in proteins. Early work on phage λ (see [7,8]) and lac established the importance of these protein regions. But the remarkable modularity and plasticity of many eukaryotic proteins, revealed for example by the construction of chimeric transcription factors [9], has resulted in a mentality in which investigators continually make new proteins by piecing together different protein modules without much thought to the connectors. Only now, as protein engineers are making large, combinatorial collections of multidomain proteins, for example to synthesize collections of new small molecules, are the

issues of how these connectors affect interaction and communication among modules being revisited.

Fancy (pp 28–33) reviews chemical methods (crosslinking and label transfer) used to examine the membership and spatial organization of protein–protein complexes. In this field, the state of the art has improved greatly in the past decade, and these chemical techniques at least promise to supplement information obtained from two-hybrid methods and other genetic techniques.

While most biomanipulation efforts have focused on developing molecules to modulate interactions between proteins (see below), reagents that allow one to control protein levels in a cell would represent a powerful complementary tool. Ideally, this manipulation would be done using molecules that modulate transcription levels from promoters native to the regulated genes [10]. Segal and Barbas (pp 34–39) describe work by their group and others that uses appropriate combinatorial libraries to generate new TFIIIA-like zinc-finger proteins that bind to different DNA sequences. Combinations of these binding modules allow a great deal of control over site specificity of the chimeric protein, and fusion of these binding moieties to appropriate transcription regulatory domains allows them to regulate gene expression.

Way (pp 40–46) reviews means to generate small molecules that can block protein–protein interactions. Heretofore, most of the success stories in this area have represented special cases where the small molecule occupies a molecular cavity such as protease active site and thereby blocks binding of the substrate. In contrast, there are not many examples of small molecules that bind to larger, flatter surfaces typical of those generally employed by proteins to interact with one another [11]. Way's piece advocates a particular approach to solving this problem. We hope that this original idea will inspire useful debate.

Next, Crews and Mohan (pp 47–53) review successful uses of small molecules to block interactions among proteins that are needed for cell-cycle progression. The proteins involved in this process span a gamut of biological functions, and the fact that so many of these functions can be targeted by small molecules gives a measure of hope for such approaches in general

Colas (pp 54–59) reviews peptide aptamers and other affinity reagents isolated from combinatorial libraries. *In vivo*, these aptamers are proving to be powerful agents to block specific protein–protein interactions, to interact with specific allelic forms of different proteins, and to identify new protein targets in complex pathways.

Finally, Doyle, Mangelsdorf, and Corey (pp 60–63) describe an impressive combination of protein engineering and pharmacology to create nuclear hormone receptors whose DNA binding and gene regulation are controlled by molecules chosen by the experimenter rather than nature. This work bears some conceptual similarity to the quest of Shokat and co-workers [12] to engineer protein kinases that work with novel ATP analogs. In each case, the ability to mutate target proteins, if infused with enough structural insight to guide the choice of pharmacophores, has the potential allow the function of the target protein to be specifically probed and exploited.

Discussion

The pieces in this section illustrate a number of general points. First, note that each of them describes useful — or at least potentially useful — molecular tools. Like biology, chemistry is tenacious, in that it never completely abandons methods or abilities once those are attained. So even though these tools were hard to come by, now that they have been built, they will be used and, if ever they are used with conspicuous success, they will be used thereafter again and again.

Second, with all the tools, there seems to be great room for improvement. Note that, in each case the authors are attempting to observe, manipulate, or exploit the ability of proteins to interact with other macromolecules. The very number of ideas for probing and manipulating protein–protein and protein–DNA interactions shows there now exist good, general purpose solutions to controlling macromolecular associations.

Third, the reviews suggest obvious areas where new thinking might provide fruitful research directions. Because of the readership, here we wish to mention in particular different tactics in small molecule chemistry. For example, although Way's article is certainly a polemic, it does make the point that there are small molecule approaches (in this case species able to bind covalently to their targets) to manipulating protein interactions that may have been neglected because of the conservative requirements of the main producers and consumers of new bioactive molecules, the pharmaceutical industry. This industry uses comforting precedent to help it choose the kinds of molecules that it wishes to test and, eventually, introduce into humans. However, the requirements for molecules used as tools for discovery biology and for drugs are quite different [13]. One imagines that academic researchers, free of the constraints that come with making hundred-million-dollar bets on drug candidates, would be happy to explore fundamentally different kinds of protein-binding small molecules if they had the resources to pursue such studies.

Not so many years ago, it seemed that only big pharmaceutical companies, with their armies of medicinal chemists, could muster the resources necessary to develop specific protein-binding small molecules. The advent of combinatorial chemistry and the more recent development of sophisticated synthetic methods with which to build structurally interesting libraries has changed this picture. Inside and outside of industry, it thus seems reasonable to expect a

rapid increase in the generation of different sorts of molecules that will extend the range of manipulations biologists would like to be able to perform. In addition to the modestly reactive compounds proposed by Way, it seems to us that it should be possible to generate libraries of large, flat molecules, and libraries of small molecules designed to homo- and hetero-oligomerize to cover more area on proteins. The technology exists to test such approaches, if the will is there.

Fourth, the articles highlight the contrast between the diversity of synthetic chemicals and the diversity of DNA-encoded proteins. The diversity offered by the biological medium is easy diversity. That is, for trained researchers, DNA is now, for all practical purposes, infinitely malleable. The ability to make this manipulatable, programmable, stuff into mRNA, and then into proteins, using what is currently the world's only programmable nanotechnological assembler (the ribosome), is utterly without parallel in the synthetic chemical realm. But synthetic chemistry can deliver far more chemical diversity than that provided by biology. So chemistry needs more work. We anticipate that, in the next few decades, the advantages inherent in biological approaches (including, but not limited to, cheap, easy manipulations at non-extreme temperatures and conditions, self replication, mutation, clonal selection, and accurate nanoscale assembly of molecules by general purpose programmable assemblers) will be combined with the ability to use a much greater number of chemical building blocks.

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