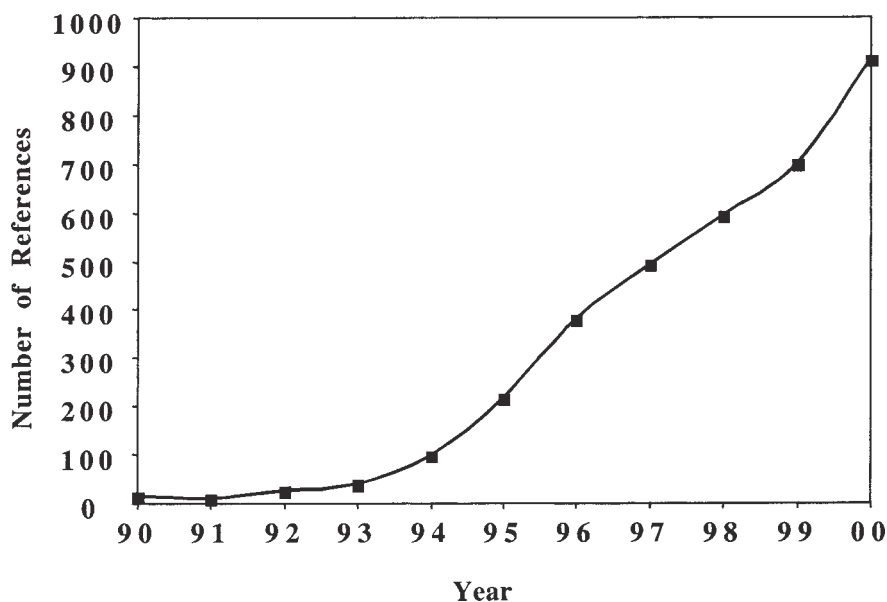


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## Preface

Many, if not all, essential biological processes require selective interactions between proteins. Complex signaling systems require sequential, ordered protein–protein interactions at essentially all levels of the signaling cascade. For example, peptide hormones interact with selective membrane receptor proteins, and autophosphorylation of the receptor then recruits other key regulatory proteins that initiate kinase cascades in which each phosphorylation event requires selective recognition of the protein substrate. The ultimate signaling effect, in many cases, is the regulation of RNA polymerase II-directed transcription in the nucleus, a process that involves numerous, multiprotein complexes important for transcription initiation, elongation, termination, and reinitiation. Defining, characterizing, and understanding the relevance of these protein–protein interactions is an arduous task, but substantial inroads have been made over the past 20 years. The development of more recent methodologies, such as mammalian expression systems, immunopurification schemes, expression cloning strategies, surface plasmon resonance (BiaCore), and nanosequencing technologies, has contributed a wealth of new insights into these complex multiprotein mechanisms and clearly accelerated the discovery process. Arguably, the yeast two-hybrid system has been one of the predominant and most powerful tools in this discovery process.

On a personal note, my specific interest in the yeast two-hybrid system developed in a manner probably not terribly different from that of many other investigators who were interested in the early 1990s in identifying and characterizing interactions between two proteins. While working in the laboratory of Mark R. Haussler, our interests centered on the vitamin D receptor (VDR), a member of the nuclear receptor family, and the mechanisms involved in VDR binding to DNA. Specifically, I was interested in identifying a nuclear factor that interacted with and conferred high-order binding of the VDR to DNA. We and other larger groups in the nuclear receptor field chose a traditional biochemical approach that focused on purifying and identifying the unknown nuclear accessory factor. Other laboratories used expression cloning strategies with purified radiolabeled proteins to screen cDNA expression libraries for clones encoding the interacting factor. Both approaches were comparatively large efforts at the time, requiring a tremendous number



**Fig. 1.** The number of publications over the past 10 years that were found in a search of PubMed using “two-hybrid” in the search window. The year 2000 value is projected based on the number of references found at the time of the search (September, 2000) and the number of remaining months in the year.

of person-hours. Both approaches eventually resulted in the successful identification of the factor as retinoid X receptor, a common heterodimeric partner for many of the class II nuclear receptors. Unfortunately, we were not one of the groups to first report the identification of RXR as the partner. Our smaller effort was, in no uncertain terms, “scooped.”

At about this same time, reports from the Fields laboratory on the successful use of the yeast two-hybrid system began to emerge and more beneficial yeast strains and vectors were being developed. The power of the system was inspiring to anyone working on trying to identify protein interaction partners. Here was a simple, direct screening assay that could uncover novel factors that interacted with your protein of interest. Millions of cDNAs could be screened in a single experiment, in a relatively short time, and with comparatively less effort. Following the initial screen, the cDNA clones encoding the putative interactors were already in hand and they could be directly sequenced and identified. The playing field seemed somehow leveled a bit by the two-hybrid system. More than twelve years have passed since the original description of the yeast two-hybrid system was reported, and few would disagree that this system has had a

tremendous impact on virtually every field of modern biology. Continuous refinements and novel innovations of the original systems over the past decade have only strengthened the utility of the approach. As illustrated in **Fig. 1**, it is obvious that many groups continue to adopt the two-hybrid system as a new approach in their laboratories and this trend will only continue to expand in the future as the era of functional genomics unravels over the next century.

Therefore, the overall goal for *Two-Hybrid Systems: Methods and Protocols* is to introduce the yeast two-hybrid system to students, research assistants, research associates, and other more senior investigators considering this as a new approach in their laboratories and research projects. Toward this end, I have assembled a collection of detailed descriptions of basic protocols and a compendium of experimental approaches in different biological systems that I hope reflects the utility of the system and its variations in modern biomedical research. My hope is that this will also serve as a useful reference for those laboratories that have extensive experience with the two-hybrid system. Thus, I invited several authors to discuss in more general terms some of the problems and strategies involved in the yeast two-hybrid assay as well as some of the alternative systems that have evolved from the original system that may prove useful to those more experienced two-hybrid laboratories.

*Two-Hybrid Systems: Methods and Protocols* is divided into four main sections. The first section is a compendium of general methodologies that are used in the two-hybrid system. Here, the reader will find in-depth discussion and detailed methodologies that serve as the foundation on which successful yeast two-hybrid experiments rest. Since many laboratories beginning two-hybrid approaches have not worked with yeast to a significant extent, this first section begins with a general introduction to handling yeast, a detailed compendium of media formulations, as well as an overview of the common strains of yeast and plasmid vectors that are used for two-hybrid work. This section ends with three chapters that describe the basic methodologies involved in introducing plasmids into yeast, interaction assays, and recovering the plasmids from yeast. This first section was intentionally designed to be somewhat repetitive in nature with components of the subsequent application chapters. The intent was to provide more in-depth methodological detail and variations of these fundamental techniques that serve as the backbone of any two-hybrid assay as well as to illustrate how these techniques are incorporated into individual applications. One well-known, recurring drawback of the two-hybrid system is the potential for artifacts and false positives. Thus, Section II provides a discussion of the various classes of false positives and the common mechanisms through which false-positives arise. This section also includes two chapters that focus on general strategies and detailed

protocols to confirm the authenticity of the interaction using in vitro protein–protein interaction assays. Part III includes four application chapters that describe how the yeast two-hybrid system was applied in various systems to identify interacting partners in important biological systems including the Smad and nuclear receptor pathways. Finally, Part IV describes various alternative strategies that have arisen out of the original yeast two-hybrid paradigm. These alternative strategies include the one-hybrid, split two-hybrid, three-hybrid, membrane recruitment systems, and mammalian systems. These alternative systems serve to illustrate the flexibility and refinements that are possible with the basic two-hybrid approach.

The authors and I hope that *Two-Hybrid Systems: Methods and Protocols* will prove a valuable addition to any laboratory that is interested in studying macromolecular interactions between proteins.

I would like to express my sincere gratitude to all the authors for their valuable, insightful contributions and for their patience in seeing this project to fruition. This book is a testament to their breadth of knowledge on the topic and the power of the two-hybrid approach. It is evident that both the basic system, as well as its many variants, will continue to play a predominant role in the characterization and identification of protein–protein interactions in the genomic and proteomic arenas of the 21st century.

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