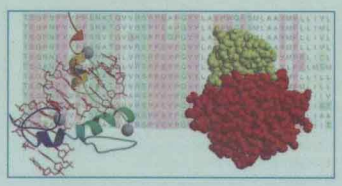
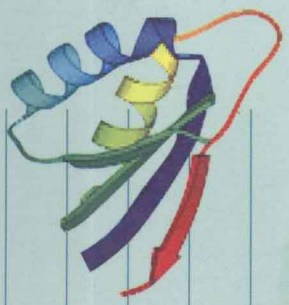
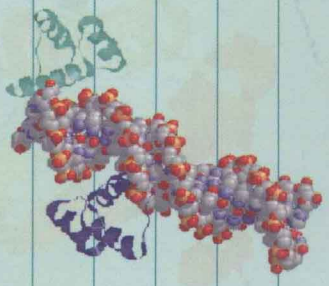


Protein-Protein Interactions
A Molecular Cloning Manual

蛋白质相互作用
分子克隆手册

Erica Golemis 编著





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北京

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Edited by Erica Golemis

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出 版 前 言

自 20 世纪以来，生命科学取得的巨大成就和进步，不仅使生命科学这门古老的学科焕发了青春，也使它在自然科学中的地位发生了革命性的变化。同时，生命科学也为物理学、数学、化学、信息科学、材料与工程科学注入了新鲜血液，科学家在研究生命科学的过程中提出了数不胜数的新问题、新概念、新思路。生命科学和其他学科之间广泛渗透、相互交叉、相互作用，从而极大地推动了科学的发展，生命科学已经成为带头学科之一，21 世纪将是生命科学世纪。生物技术是以现代生命科学理论为基础，利用生物体及其细胞、亚细胞、分子的组成部分，结合工程学、信息学等手段开展研究及制造产品或改造动物、植物、微生物等，并使其具有期望的品质、特性，从而为社会提供商品和服务的综合性技术体系。目前，生物技术已经广泛应用于医药、农业、环保、轻工、化工等重要领域。20 世纪 90 年代以来，以生物经济为重点的第四次科技革命正在形成。许多学者预测，生物经济必将在 21 世纪超过网络经济，成为世界经济的主导。

我国在生命科学和生物技术的研究和产业化方面取得了举世瞩目的成就。中国作为惟一的发展中国家参与了国际人类基因组计划，完成了 1%测序工作，中国科学家独立完成了杂交水稻父本 9311（籼稻）的基因组序列草图，在国际上首次定位和克隆了神经性高频耳聋基因、乳光牙本质Ⅱ型基因、汗孔角化症等遗传病的致病基因；我国生物技术产业已初具规模，目前涉足现代生物技术的企业约 500 家，从业人员超过 5 万人；我国研制的生物技术医药已经达 150 多种，基因工程干扰素等 21 种药品已经投入生产，生物医药制品 2000 年销售额已达 200 多亿元；中国的超级杂交水稻每公顷产量突破 12 吨；我国是世界上第二个有转基因抗虫棉花自主知识产权的国家，转基因棉花 2002 年的种植面积占棉花种植面积的 40%，5 年累积为农民增收 50 多亿元。

但我国的生命科学与生物技术和美国、日本等发达国家相比还有较大差距，存在生命科学基础理论研究不够深入扎实、学术创新能力不强、拥有自主知识产权的生物技术产品种类少、产量低、生物技术产业规模小、相关配套产业社会化程度低等问题。为了高等院校师生和广大科技人员借鉴和学习发达国家生命科学与技术的先进理论和技术，提高我国在生物经济方面的竞争力，我们精选了一批国外优秀生命科学与生物技术著作，组织专家进行了评阅和审核，希望能对高等院校师生和广大科技人员有所帮助，同时对我国生命科学和生物技术产业赶超世界先进水平起到一定的推动作用。

欢迎广大读者将使用本书的意见反馈给我们，更欢迎国内外专家、教授积极地推荐国外优秀的生命科学及生物技术著作，以便我们将工作做得更好。

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2003 年 7 月

Preface

UNDER THE GUIDANCE OF GENERAL EDITOR JOE SAMBROOK, Cold Spring Harbor Laboratory Press is launching a new series of advanced laboratory manuals for biological research that build on the 3rd edition of *Molecular Cloning: A Laboratory Manual.*

The major aims of *Molecular Cloning* are to provide researchers with protocols that work reproducibly and the background knowledge required to carry out these protocols intelligently. *Protein–Protein Interactions: A Molecular Cloning Laboratory Manual*, one of the first in the new series of advanced manuals, has similar goals: to provide a complete and current account of technical and theoretical issues in the study of protein associations, for an audience ranging from early graduate students to experienced investigators.

This book owes its existence to the valuable contributions of many talented people. First and foremost, I thank Cold Spring Harbor Laboratory Press for their interest in developing an advanced manual devoted to the topic of protein interactions, and then for allowing me to take an editorial role in the project. In this context, I gratefully acknowledge Patricia Barker, Mary Cozza, Judy Cuddihy, and most of all, Kaaren Janssen. Their collaboration in all aspects of this work cannot be overestimated. Separately, I thank Jan Argentine, John Inglis, and Joe Sambrook for setting such an inspiring precedent in *Molecular Cloning.*

It was a privilege to work with the many authors of this volume, and I greatly appreciate their efforts in constructing thoughtful contributions marked by creativity and clarity. I appreciatively acknowledge my past mentors, Nancy Hopkins and Roger Brent, for years of helpful discussion of issues related to organismal complexity, protein interactions, and the relationships between the two. I thank the members of my laboratory, past and present, for stimulating scientific discussions. I particularly thank Ilya Serebriiskii, whose oversight of ongoing protein interaction benchwork allowed me the time to concentrate on protein interaction editing.

I also acknowledge the rich intellectual and collegial environment at the Fox Chase Cancer Center, which has contributed immeasurably to the thinking behind this project. More pragmatically, Kathy Buchheit and Sarah Costello-Berman were of great assistance in many aspects of organization throughout the editorial process, which would have been much more difficult without their efforts. Finally, I particularly thank Michael and Ian Ochs, and Marion and Emanuel Golemis, for their constant and spirited support.

E.G.

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1 Toward an Understanding of Protein Interactions

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The goal of this book is to provide an overview and practical explication of critical technologies in the field of protein–protein interactions, describing both concepts of current importance in this area of research and technologies that enable the characterization and manipulation of proteins. Therefore, the goal of this introduction is to emphasize why an understanding of protein–protein interactions is an essential requirement of the modern molecular biologist, and the manner in which this volume aims to address this need.

SPECIFICS

Proteins control and mediate many of the biological activities of cells. Although some proteins act primarily as single monomeric units (for instance, enzymes that catalyze changes in small-molecule substrates), a significant percentage, if not the majority, of all proteins function in association with partner molecules, or as components of large molecular assemblies. Hence, to gain an understanding of cellular function, the function of proteins must be understood both in isolation and in the context of other interactive proteins. Complicating this process is the fact that a cell is not static: Both internally and externally generated signals induce changes in shape, division, viability, metabolism, and other intrinsic properties. Interpretation of, and response to, these signals requires changes in the protein composition of cells and the pattern of association between cellular proteins. Further complicating the analysis of cellular function is the fact that all cells are not equivalent. Within a single species, such as *Homo sapiens*, liver versus lymphoid versus neural cells contain very different populations of proteins and respond differently to identical external stimuli. Crossing species (for instance, *H. sapiens* to *Mus musculus* to *Gallus gallus*), even a given cell type, such as a lymphoid B cell, possesses significant differences in protein composition and func-

tional properties based on both differences in the genetic code specifying the proteins and changes in the expression levels of discrete proteins. Thus, the situation is highly complex.

To address this complexity, it is useful to generate multiple different classes of information about proteins. For any given protein, these classes of knowledge would include (1) the structural and sequence properties of the protein, including its possession of defined motifs that would allow assignment to a functional class, or be predictive of a certain pattern of protein–protein interaction; (2) the evolutionary history and pattern of conservation of the protein, to allow identification of critical residues; (3) the expression profile of the protein, including issues such as cell-type specificity and abundance, and changes in this profile in response to dynamic processes such as cell cycle progression or growth factor response; (4) the intracellular localization of the protein, and association with specific organelles or structures; (5) the forms of posttranslational regulation to which a protein is subject (phosphorylation, ubiquitination, acylation, and others); and (6) the other cellular proteins with which the protein associates. In fact, all of the first five points together contribute to the determination of the sixth; and given that proteins of linked function tend to associate in transient or stable complexes, determination of a profile of protein–protein interactions is an extremely important step toward the ultimate goal of identifying the functional significance of the activity of any given protein in a cell. Chapters 2 and 3 in Section 1—The Biological Context for Protein Interaction Studies—are essays focused on current topics in signal transduction and human genetics/pharmacogenomics, exemplifying analysis of protein interactions as an organizing principle for these disciplines.

Techniques to provide the classes of information listed above fall into three groups. In one class, the goal is to identify every possible interacting set of proteins for protein X of interest; in this case, physiological significance is temporarily downplayed in an effort to cast a broad net. In the second class, where interacting proteins of interest have been defined, the goal is to detail the biological function and impact of their interactions, i.e., to establish physiological significance. In this case, it is essential to be able to study the interaction under conditions that correspond as closely as possible to the endogenous situation. In the third class, an interaction has been identified, and validated as physiological, and is reasonably well understood; here, the goal is to devise high-throughput methodologies to identify agents that modulate the interaction in desirable ways. No single technique is optimal to address all the points of interest; however, by combining techniques, it is possible to make considerable progress. The techniques described herein bear on all three described classes. Although a complete review of the origins and nature of protein-interactive techniques is beyond the scope of this book, one excellent source of references to this end is provided in Phizicky and Fields (1995), along with an extensive discussion of parameters that can be critical in determining the ability of a specific technique to detect particular protein interactions. Finally, although we have tried to demonstrate the breadth of creativity and options in the protein interaction field, in the interest of space we have not addressed every possible interaction technology. For example, some traditional biochemical methodologies, such as column copurification and differential centrifugation, have been omitted, as have isothermal titration calorimetry and a number of recently developed technologies.

Following the introductory section noted above, this book has been organized into four methodological sections.

Section 2—Standard Technologies Used to Identify and Characterize Protein–Protein Interactions—presents six well-established approaches to the analysis of protein–protein interactions that exemplify standard molecular biological, biochemical, and microbiological approaches used in many laboratories. The use of in vitro GST-fusions in pull-down and far western overlay assays is described in Chapter 4, followed by the complementary methodologies required for coimmunoprecipitation analysis, in Chapter 5. Chapter 6 provides a thorough discussion of the issues involved in the empirical establishment of conditions suitable for protein cross-linking. Chapter 7 is devoted to yeast and bacterial two-hybrid approaches that use transcription-based readout. Chapter 8 details the uses of phage display to identify high-affinity interactions at high-

throughput, and Chapter 9 describes classic genetic approaches to discerning protein association based on functional interaction of gene products.

Section 3—Biophysical Approaches to Identify and Characterize Protein–Protein Interactions—in contrast, is devoted to technologies that have biophysical and cell biological roots. The technologies of FRET and PRIM, discussed in Chapters 10 and 11, allow analysis of the association of proteins in situ in either fixed cells or over real time in living cells, providing a unique insight into the changes in protein–protein association dependent on reaction to biological stimulus. Mass spectrometry has been an essential tool for proteomic analyses: Chapter 12 describes issues involved in using this technique to discern interactions between specific proteins in multicomponent complexes. Finally, Chapters 13 (atomic force microscopy), 14 (Biacore), and 15 (QCM biosensor) detail methodologies to obtain specific measurements of the forces involved in protein–protein or in some cases, protein–small molecule ligand interactions.

Section 4—Recent Developments and Other Tools: Reviews and Short Protocols—is devoted to innovative technologies, for the most part developed quite recently, that are either imaginative developments of "themes" related to the core technologies described in Sections 2 and 3, or are unique approaches to the analysis of protein interactions. Although the topics included in this section are quite diverse, they fall into several subgroups. The technique of protease fingerprinting described in Chapter 16 is complementary to protein cross-linking in its use of in vitro biochemical detection methods to identify essential contacts between associating proteins. Chapters 17–20 are related to GST pull-down and immunoprecipitation methodologies and describe means to facilitate purification based on in vitro physical association of proteins with target proteins of interest. Chapters 21–27 are devoted to different forms of two-component systems—in bacteria, yeast, and mammalian cells—for proteins expressed in the nucleus, cytoplasm, or at membranes. Although the ancestral concepts sparking the development of these systems are arguably the two-hybrid system and bacterial α-complementation paradigms, the actual manifestations of protein interaction detection machinery are highly evolved, with each offering unique advantages for different classes of proteins. Chapter 27, describing the screening of peptide aptamers, is a crossover chapter from an orientation of studying protein–protein interactions per se to using protein interaction technologies to develop tools that can be used to regulate protein interactions in vivo. Chapter 28, describing incremental truncation; Chapter 29, describing catalytic antibodies; and Chapter 30, describing protein bundling, suggest alternative technologies that might be valuable toward these ends. Chapters 31 and 32 describe high-throughput methodologies that facilitate the rapid screening of protein interaction pairs.

Finally, the chapters in the last section of the book are forward-looking, based on the assumption that as we begin to acquire a comprehensive understanding of the complement of existing protein and their functions, we can constructively model this information, or employ it to other means. Hence, Chapters 33 and 34 are devoted to descriptions of computational tools and integrative approaches that enhance and organize many of the wet bench techniques described earlier. Chapter 35 describes some experimental efforts to apply protein–protein interactions as tools to manipulate biological systems via creation of synthetic transcriptional control systems in gene therapy applications. Such approaches are likely to become widely adapted in coming years and to allow elegant control of gene function (for other examples, see Gardner et al. 1998, 2000; Elowitz and Liebler 2000).

CONTEXT

Continuing this last theme, following the successful completion of the human genome sequence, the genomic decade of the 1990s spawned a series of derivative "-omic" disciplines, including analysis of the transcriptome (Velculescu et al. 1997), metabolome (Tweedale et al. 1998), and, most relevantly for this book, proteome (Wasinger et al. 1995). As of mid-2001, a search of

Medline using variants of the term "proteomics" identifies in excess of 1000 references, with more than 90% of these references derived from papers published within the last 2 years. The goal of these endeavors is to provide a complete and systematic description of the complete DNA, RNA, and protein content of living organisms to enable understanding of the dynamic regulation of these components. These are very large-scale endeavors and are frequently portrayed as setting the agenda for a new way of doing biological science in the 21st century. Balanced against these global approaches, a search of Medline for the term "protein" per se reveals in excess of 400,000 entries over the same 2-year period. Rather than dealing with global analysis of protein expression and function, these studies usually address the intrinsic mechanisms involved in the creation, modification, interaction, and function of either individual proteins of interest or small protein sets. On the basis of this ratio (1000:400,000), it is clear that whatever the future potential of global approaches, either there are still very significant issues in understanding protein activity on an individual basis, or, alternatively, the general mass of the scientific establishment is still working on a paradigm that will shortly be superseded. Hence, a question of direct relevance to this book is: What is the value of presenting techniques for studying the interactions of individual proteins in the year 2002?

On a fundamental level, biologists want to know first, what we are and how we got here; second, how we relate to a bewilderingly complex biosphere; and third, what actions can be taken to improve the duration and quality of life. Whereas the goal of scientific discovery has generally remained focused on answering these three basic questions, there have historically been very different intellectual approaches employed toward this end, which can be summarized as systemization (or synthesis) versus explication of mechanism (or reductionism). Scientists interested in systemization have focused efforts on the organization of very large quantities of observational results. For example, botanists and zoologists have directed efforts to achieving exact, ordered family relationships of existing flora and fauna. Building on this work, evolutionary biologists additionally delved into the fossil record to determine how life advances from simple to complex forms. In contrast to the historically more ancient systematic approach, the post-Enlightenment biological scientific establishment has attempted to intelligently probe these compendia of observations about nature by formulating and testing specific hypotheses. As one example, by creating targeted crosses of peas and then using the observed results of these crosses to discriminate between opposing models for heredity of traits, Mendel established a paradigm for subsequent work in genetics. As the modern molecular biological disciplines emerged, an earlier descriptive approach to viewing the world lost appeal in the face of new insights into the organism as machine, with key functions controlling inheritance, daily function, and disease recognized to be dependent on the action of biological molecules—nucleic acids, lipids, carbohydrates, and proteins.

Although the systematic (non-hypothesis-driven) and mechanistic (hypothesis-driven) approaches are sometimes portrayed as oppositional, they are more correctly perceived as complementary. Without an ample data set, there is nothing on which to base or interpret experiments, and without rigorous formulation and test of hypothesis, gathered data exist only as information, and fail to lead to knowledge or, ideally, wisdom. In a historical context, the "-omic" studies represent the work of a direct line of descendents of the Systematists, finally reestablishing their importance alongside the Mechanists. Through a combination of systemic and mechanistic analysis, in the next decades we may begin to gain a reasonable understanding of the composition and function of the human organism and other organisms, making progress on the first two fundamental questions noted above.

There remains the third question: How do we improve the quality of life? Inevitably, addressing this question will require the addition of a third element to the knowledge-gathering approaches described above: the science of productively manipulating biological systems. Although biological engineering has sometimes been dismissed as less challenging than "pure science" aimed solely at generating knowledge, in fact, the bioengineer exists in unique dialog with the pure scientist. Because the goal of this discipline is to be able to dissect and manipulate bio-

logical systems, of necessity, bioengineering starts with the study of naturally occurring control mechanisms through approaches such as those described in this work. This understanding is then creatively translated in two ways. One way is to adapt naturally occurring biological agents to make tools that can be applied for desirable goals. A considerable number of the protein–protein interaction systems described in this book have such origins. The second way is to identify critical control points utilized in biological regulatory systems and to generate de novo means to perturb them specifically to achieve desirable effects. This latter objective describes the general ambition of applied scientists, including gene therapists, pharmacogenomicists, and clinicians, and is facilitated by possession of a well-stocked toolbox to allow probing of protein interactions in as many ways as possible. In the year 2001, it is hoped that the apparent success of the protein-targeted anticancer drug Gleevac (Druker et al. 1996) may represent one of the first of many such intelligently designed therapies. Given the advancements in our understanding of the basic organismal building materials, the next decades hold promise for a great burst of ingenious exploitation.

In this contextual light, this book has three primary objectives. First, the intent is to introduce current critical issues in analysis of protein–protein interactions. Second, the book presents a number of widely differing approaches to the study of protein–protein interactions, providing insight into the range of tools available to address differing issues of protein interaction and demonstrating the dynamic interplay between the identification and manipulation of protein interactions. Third, and most practically, the included chapters provide clear and detailed technical description of the various protein interaction techniques. However, following in the tradition of the *Molecular Cloning* series, it seems reasonable to propose that a technique can best be practiced when presented in a context of historical development and most appropriate applications. Such a presentation has been attempted by the various authors who have taken part in this project.

A prime challenge for the future is to conduct targeted studies of proteins of interest while considering the larger context of whole organismal function; and conversely, to carefully validate macro-models of organismal function through individual test cases. The sensibility involved in this process is particularly compatible with studies of protein–protein interactions. It is hoped that the sum of these efforts will be to advance the pragmatic needs of the bench practitioner, while conveying a broader sense of the interest and importance of the field of protein interaction-based studies.

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2 Signal Transduction and Mammalian Cell Growth: Problems and Paradigms

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INTRODUCTION

To divide or to differentiate, to attach or to move, to survive or to die—these are among the key decisions cells must make during the development and adult life of a metazoan organism. Such decisions must be accurate and well coordinated and are dictated both by factors external to the cell and by internal cues. The process by which cells carry out these decisions is termed signal transduction. This chapter reviews emerging principles that govern signaling pathways germane to cell growth and division, with particular emphasis on the role of protein–protein interactions. In so doing, the crucial role of such protein interactions in mitogenic signal transduction, and the importance of emerging technology for their detection, will become apparent.

Mitogenic signaling pathways are complicated. With the completion of the human genome sequence, the number of recognizable signaling proteins will certainly increase, and the models of these pathways are apt to become more complicated still. Although many signaling pathways seem formidably complex when viewed as a whole, at closer inspection these pathways often can be described in terms of a series of simple interactions of one protein with another. Indeed, so fundamental are these interactions that it is not an exaggeration to say that they form the basis of all signal transduction machinery.

Why are protein–protein interactions so important in mitogenic signaling? The binding of one signaling protein to another can have a number of consequences. For one, such binding can serve to recruit a signaling protein to a location where it is activated and/or where it is needed to

carry out its function. A relevant example that illustrates this phenomenon is the behavior of the protein kinase Raf, which, upon cell stimulation, is recruited from the cytoplasm to the plasma membrane by binding to the GTPase Ras (Avruch et al. 1994). A second consequence of protein interactions is that binding of one protein to another can induce conformational changes that affect activity or accessibility of additional binding domains, permitting additional protein interactions. Such is the case for signaling proteins such as p21-activated kinase, which, upon binding the GTPases Cdc42 or Rac, undergoes a profound conformational change that dislodges an autoinhibitory domain and thereby activates the kinase (Lei et al. 2000). Of course, stimulant-induced changes in protein location and conformation are not mutually exclusive. In many instances, recruitment to a signaling complex results in both relocation and enzymatic activation, as is the case, for example, with the protein tyrosine phosphatase SHP2, which is recruited from the cytoplasm to activated receptor tyrosine kinases (RPTKs) at the plasma membrane and is at the same time activated by the engagement of its SH2 domains to phosphotyrosine residues in the RPTK (Barford and Neel 1998; Hof et al. 1998).

THE SCAFFOLDING OF SIGNAL TRANSDUCTION

Binary protein–protein interactions are the cornerstone of signal transduction; however, an emerging theme in this research area is that higher-order assemblages are also critical for efficient transmission of signals. Scaffolds, adapters, insulators, and inhibitors are superimposed on the basic framework of the mitogenic signaling machinery. These additional layers of complexity have changed the way we look at signal transduction, and point to new ways to consider the organization of such pathways. For example, it has been known for some time that multicomponent complexes are assembled at activated RPTKs at the plasma membrane (Schlessinger 2000), whereas other complexes assemble at gene promoters on chromatin in the nucleus (Lee and Young 2000). It has now become clear that such multicomponent complexes also play a significant role in signaling in the cytoplasm and are critical for the regulation of mitogenesis. As one biologically important example, our understanding of the central Ras-Raf-Mek-mitogen-activated protein kinase (MAPK) pathway has evolved with the discovery that many of the elements of this complex are not only physically associated with one another, but also segregated from other cytoplasmic signaling proteins by several distinct scaffolding proteins (Garrington and Johnson 1999; Kolch 2000). These scaffolding proteins usually, but not always, lack catalytic function; however, they play key roles in signal transduction by virtue of their ability to complex with two or more elements of the Ras/MAPK pathway.

The use of scaffolding proteins in signal transduction is an evolutionarily conserved strategy. Examples of scaffolds for MAPK signaling modules have been found in all commonly studied eukaryotic organisms. At present, the function of these proteins is best understood in yeast (Fig. 1). In *Saccharomyces cerevisiae*, scaffold proteins function to segregate various common elements of MAPK modules. For example, the MAPK Ste11p participates in three distinct MAPK signaling modules: (1) the Ste5p scaffold coordinates components of the pheromone-response MAPK signaling module; (2) the Pbs2p scaffold coordinates components of an osmoregulatory MAPK signaling module; and (3) Ste11p also participates in a MAPK signaling module that regulates filamentation. In this last case, a scaffold protein has not been identified. In the absence of Pbs2p function, osmotic stress induces inappropriate activation of both the filamentous growth pathway and the mating pathway (O'Rourke and Herskowitz 1998; Davenport et al. 1999). Thus, in yeast it seems clear that one function of these scaffold proteins is to specify and insulate the signaling functions of MAPK modules.

In mammalian cells, scaffolds for MAPK modules include kinase suppressor of Ras (KSR) (Downward 1995), growth factor receptor-binding protein 10 (Grb10) (Nantel et al. 1998), and

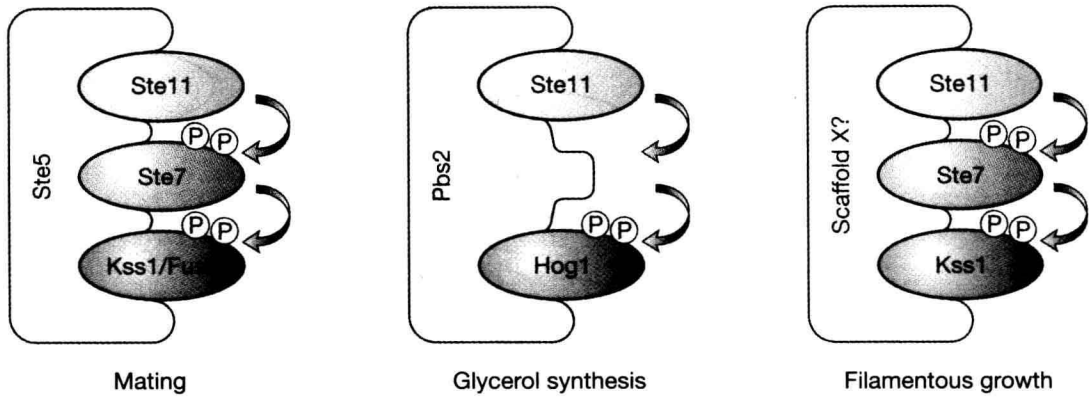

FIGURE 1. Scaffolds in budding yeast. *S. cerevisiae* uses similar signaling proteins toward different ends. The kinase Ste11p is involved in at least three distinct signaling cascades: mating, glycerol synthesis in response to hyperosmotic shock, and filamentous growth. It is thought that scaffolding proteins play a key role in signaling specificity, insulating Ste11p and downstream components from inadvertant activation by inappropriate stimuli. For the mating pathway, Ste5p binds three kinases, Ste11p, Ste7p, and Fus3p (and possibly Kss1p). In the case of hyperosmotic shock, the Ste11p target Pbs2p itself provides a scaffold function, binding both its upstream activator Ste11p and its downstream target Hog1p. It is not known whether the signaling machinery activated during filamentous growth requires an adapter protein.

Mek partner 1 (MP1) (Fig. 2) (Schaeffer et al. 1998). KSR binds to all three members of the canonical MAPK cascade: Raf, Mek, and Erk. Grb10 binds RPTKs, Raf, and Mek, but probably not at the same time, because a single SH2 domain in the carboxyl terminus of Grb10 mediates all these interactions. MP1 tethers the MAPK Erk1 to its activator MEK, and similar scaffolds (e.g,. JIP-1) are known for the stress-activated protein kinase Jnk and its upstream activators MKK7 and MLK (Yassuda et al. 1999). As in budding yeast, such a design may ensure efficient signal transmission and may also serve to prevent excessive interference, or cross-talk, from other signaling pathways in mammalian cells.

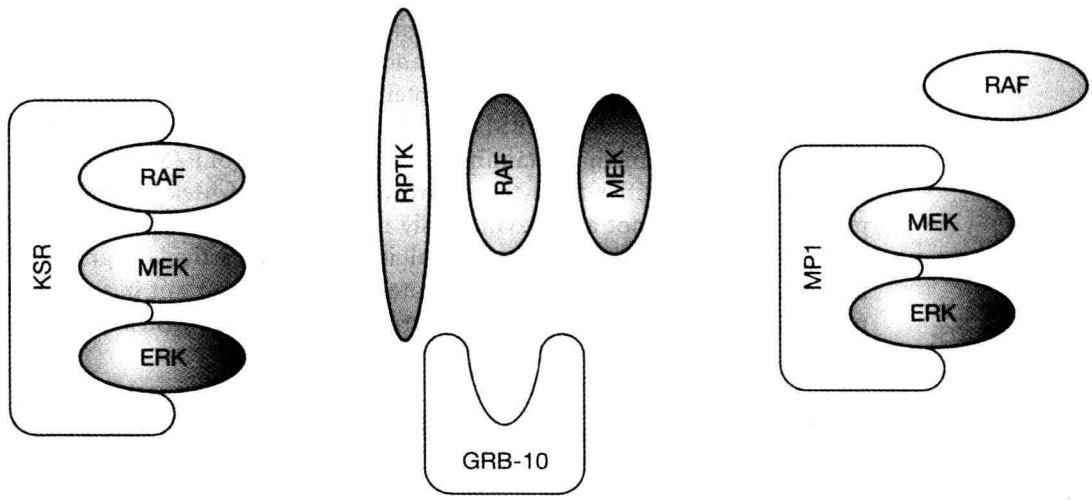

FIGURE 2. Examples of scaffolds in mammalian cells. As in yeast, mammalian signaling pathways also employ scaffolding proteins. KSR is functionally similar to budding yeast Ste5p, in that this protein can bind three members of a MAPK cascade. Grb10 represents a different type of adapter. This protein binds MAPK cascade members as well as RPTKs, but does so via a single binding domain and thus is unlikely to bind all three partners simultaneously. MP1 represents a third type of scaffold, linking together two elements of a MAPK cascade.