METHODS IN MOLECULAR BIOLOGY™

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DNA Recombination

Methods and Protocols

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Preface

Homologous recombination has been intensively studied in budding yeast. I think we are extremely lucky to find that homologous recombination is exceptionally robust in this organism, making it an ideal model to study this process. Historically, the availability of powerful genetics in this simple, unicellular organism has enabled the isolation of genes that play key roles in homologous recombination, and we have learnt a lot about homologous recombination using this organism. Homologous recombination is important in various aspects of DNA metabolism, including damage repair, replication, telomere maintenance, and meiosis. We also now know that key players in homologous recombination identified and characterized in yeast, such as proteins encoded by the genes belonging to the so-called RAD52 group, are well conserved among eukaryotic species, including humans. This offers promise that further in-depth characterization of homologous recombination using yeast will help provide the basic framework for understanding the universal mechanism(s) of homologous recombination conserved in eukaryotes. When asked to edit a book about methods for studying homologous recombination, I decided to include chapters that cover recent techniques that best utilize the advantages of the yeast system, with the belief that yeast will keep serving as a great model organism to study homologous recombination.

On the other hand, there is a group of genes involved in recombination that are apparently found only in higher eukaryotes, such as BRCA2, indicating the presence of an extra layer of mechanistic complexity in these organisms. Obviously, the most straightforward approach to study these mechanisms is to use models in which these particular mechanisms exist. From this point of view, chapters for studying recombination using higher eukaryotes have also been included.

Although we have gained significant understanding of the entity underlying homologous recombination, I have to say that we still do not know much about it when we see it as a "micro machine" that is incredibly efficient at finding similarity between two DNA molecules inside a cell. Obviously, a necessary step in the direction of understanding this process is to isolate the machine and let it work in a test tube. Understanding the design by studying the appearance and behavior of the machinery as a single molecule will be an important milestone toward understanding the mechanism of action of the machinery. Almost as important is to learn how the machinery behaves inside living cells. In recent years, this approach has flourished due to advances in microscopy and the availability of various fluorescent proteins. Techniques covering these topics have been included.

Yeast genetics has successfully provided a framework for the mechanism of homologous recombination. Now the question is, what can we do next to bring it to the next level of understanding? This is a question I ask myself, but I believe it is more or less a question for anyone who is enthusiastic about understanding this very fascinating phenomenon. I hope this protocol book will prove useful for this purpose. Finally, I would like to thank all the contributors who willingly agreed to share their expertise/knowledge. Needless to say, this book would not exist without their effort.

Hideo Tsubouchi

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