DNA Science

DNA 科学

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DNA Science:
A First Course in DNA Technology
DNA 科学

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DNA Science:A First Course in DNA Technology

—— 重印版 ——

For Charles and Charlotte,
My connections to the past

For Richard, Marian, Carol, and Ann,
My connections to my childhood

For Dana, Alec, and Andrew,
My connections to the future

For Ellie Greenan,
Who will tell you that DNA is "the thing"

— DAVID A. MICKLOS

To my wife Joadie,
and my three sons Kurt, Eric, and Alec

— GREG A. FREYER

Preface

In 1984, THE LABORATORIES IN THIS BOOK were second nature to Greg, then a post-doctoral fellow with Rich Roberts at Cold Spring Harbor Laboratory. But to Dave they were the keys to the unseen world of DNA. At the time, there were virtually no simple experiments using these techniques that were aimed at advanced high school and beginning university students. So why not develop a lab sequence that would allow virtually any student the opportunity to make and analyze a recombinant DNA molecule?

Thus began this book, with Dave spending odd hours in the Roberts' lab trying and fleshing out a 14-page recipe book containing Greg's vision of a beginning laboratory course in recombinant DNA technology. In addition to Rich Roberts, who went on to win a Nobel prize, many prominent scientists were generous with their ideas and lab tips. Doug Hanahan shared with us his supremely simple method to transform *E. coli*. The hair dryers in Ed Harlow's lab provided a sensible, and stylish, means to dry a DNA pellet.

The result was a 124-page manuscript called "Recombinant DNA for Beginners," first tested with faculty and students on Long Island in summer 1985. By the following year, we had equipped the first of two "Vector Vans," crammed to the ceiling with centrifuges, pipettes, electrophoresis chambers, water baths, incubators, and reagents enough to clone a molecular genetics lab anywhere. For many years, summers became a blur of places and faces, as we traveled the United States training several thousand biology faculty to do the laboratories in this book. In the meantime, Greg left the Cold Spring Harbor campus ultimately taking a faculty position at Columbia University and Dave moved down the street to found the Dolan DNA Learning Center.

Jim Watson coined the term *DNA Science* over lunch in Cold Spring Harbor in 1988. For Jim, it was an everyday, throwaway comment—his world had revolved around DNA for 40 years by that time. But for us it captured the simplicity of the new DNA world we wanted to introduce to students: a science based on the molecule of life and a book to help bring that science to life for students.

In making this second edition, we preserved the successful formula of the first edition—one part well-tested laboratories and one part insightful, explanatory text. We maintained the core laboratory sequence, which first introduces the basic techniques of DNA restriction, transformation, isolation, and analysis, and then applies these techniques to the construction and analysis of a simple recombinant DNA molecule. We resisted the temptation to tinker very much with the laboratories. After all, they are the best-tested and most widely used teaching labs available on the basic techniques of gene manipulation. These labs, as well as numerous adaptations and analogs, provide biology students an introduction to molecular genetics at high schools and universities throughout the United States.

We have, however, included insights and refinements introduced in our teaching lab over the last several years. These include a method to spread *E. coli* with glass beads, shared by Steve Hughes of the National Cancer Institute, which puts an end to alcohol fires. We have included additional labs that focus on gene products to at least partially address the criticism that "there is more to biology than just DNA." One provides a simple colorimetric assay for the activity of β-lactamase (penicillinase), the enzyme produced by the ampicillin resistance gene. The other uses green fluorescent protein (GFP) to illustrate the principles of protein expression and purification (without the fuss of a column).

While maintaining the historical approach of the first edition, the text portion has been entirely reorganized and updated with almost 200 pages of new material—bringing it up to the minute with current research. More than a recitation of facts, the narrative takes students behind the scenes of modern research, introducing key people and their experiments. David Crotty, a molecular and developmental biologist, provided additional insight and material for the text portion.

The first three chapters cover essential principles of genetics, and DNA structure and function. Chapters 1 and 2 cover the historical foundations of DNA, by simply explaining "How We Learned That DNA Is the Genetic Material" and "How We Learned the Function of DNA." The third chapter, "How We Learned How Genes Are Regulated," moves from classic studies of the *lac* operon to the multiplicity of mechanisms that we now know to modulate gene expression.

The next three chapters introduce small- and large-scale methods for analyzing DNA. Chapter 4, "Basic Tools and Techniques of DNA Science," provides the theory behind the laboratories in this book. Chapter 5, "Methods for Finding and Expressing Important Genes," explains the arsenal of modern techniques for investigating individual genes. Chapter 6, "Modern Methods for Analyzing Whole Genomes," documents the race to sequence the human genome and new methods for working with hundreds of genes simultaneously.

The final chapters focus on human issues. Chapter 7, "The DNA Science of Cancer," describes the origin of the war on cancer and recent strides in fighting this most-dreaded disease. Chapter 8, "Applying DNA Science to Human Genetics and Evolution," explores the molecular basis of human variation, and its relation to human disease and our emergence as a species. This chapter also contains the first substantial treatment of American eugenics available in a general biology text.

Although much has changed in biology since the first edition, the ideas and techniques in this book are still the minimum requirements for any degree in DNA manipulation. At a time when molecular biology is increasingly accomplished on sequencers and microchips, agarose gel electrophoresis still provides a door through which anyone can enter the fraternity of DNA. Despite a growing emphasis on higher organisms, *E. coli* still provides insights to anyone with a warm incubator and a few hours to spare.

As with the first edition, we hope this second one correctly anticipates and explains trends that will occupy scientific thought for many years. We hope that *DNA Science* continues to provide a simple roadmap for beginning an exploration of the molecule of life—one that will take on added importance as more and more biology teachers around the world realize the value of giving students freedom to get their hands dirty with DNA.

November 2002 DAVE MICKLOS AND GREG FREYER

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A FIRST COURSE

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Christiane Nüsslein-Volhard
(Courtesy of Cold Spring Harbor Laboratory Archives.)

Eric Wieschaus
(Reprinted, with permission, ©The Nobel Foundation.)

Edward B. Lewis
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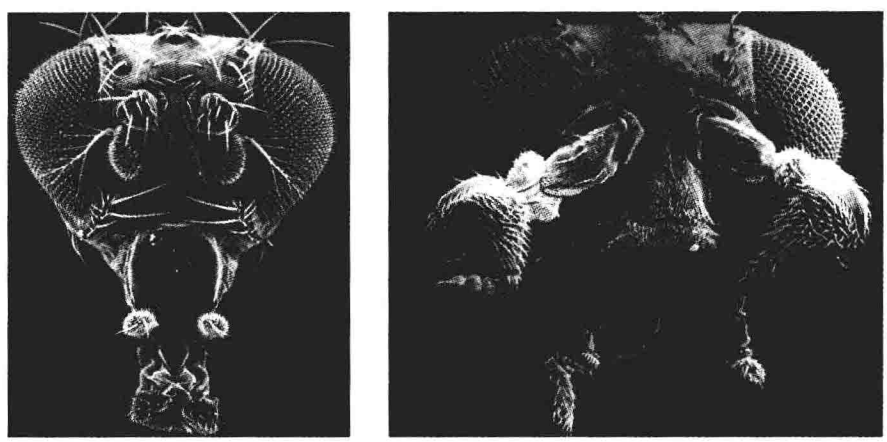
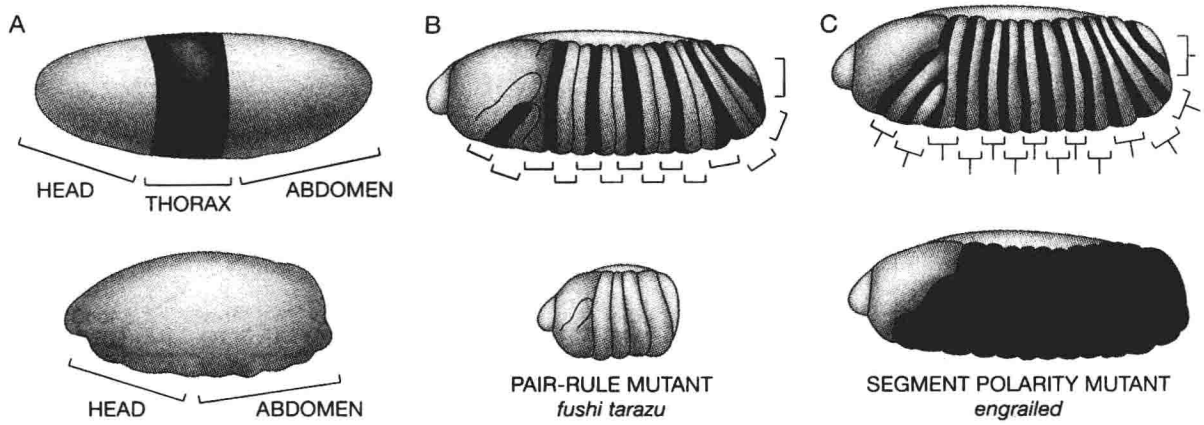
In the late 1970s, Christiane Nüsslein-Volhard and Eric Wieschaus, at the European Molecular Biology Laboratory, identified key genes that guide the spatial development of segments in the *Drosophila* embryo. Soon after fertilization, several sets of genes are differentially expressed to establish anterior-posterior (i.e., head-to-tail) and dorsal-ventral (i.e., back to abdomen) orientation, which guides the development of bilaterally symmetrical parts. For example, the concentration of the bicoid protein decreases from anterior to posterior, whereas the concentration of the Dorsal protein grades from ventral to dorsal. The Torso protein is concentrated at the anterior and posterior ends, whereas oskar and nanos proteins are concentrated exclusively at the posterior end. The relative mix and concentration of these proteins activate three types of genes (Gap, pair rule, and segment polarity genes), which work together to determine the identity of body segments.

Gap genes guide the differentiation of segments along the head-to-tail axis, leading to head, thoracic, and abdominal regions. For example, *Krüppel* is a Gap gene expressed mainly in the thoracic segments, and thus *Krüppel* mutants are missing those segments. Gap proteins, in turn, control expression of pair rule genes that define each segment. For example, *fushi tarazu* (*ftz*) is expressed in the boundaries between segments, so *ftz* mutants are missing every other segment. *engrailed* is an example of a segment polarity gene, which regulate the anterior/posterior orientation within each segment. In *engrailed* mutants, the posterior end of each segment is a mirror image the anterior end.

With work that began in the 1950s at the California Institute of Technology, Edward Lewis had a key role in understanding how the *Drosophila* body plan is determined. He studied how homeotic genes control the specialization of body parts within the segments. Homeosis describes the process in which one body part becomes like another by assuming its identity. For example, in the homeotic mutant *Ultrabithorax* (*Ubx*), the halteres (a type of small modified wing that helps the fly balance) of the third thoracic segment have been converted into a second pair of wings, mimicking the normal situation in the second thoracic segment. In the *Antennapedia* (*Antp*) mutant, antennae in the head segment are converted into an extra pair of legs, normally found in the second thoracic segment.



Antennapedia Mutation
(*Left*) Wild-type *Drosophila* head. (*Right*) *Antennapedia* mutant fly. Note the presence of legs where antennae normally form. (Reprinted, with permission, courtesy of F.R. Turner, Indiana University, http://flybase.bio.indiana.edu.)



Three Types of Body Patterning Mutations in *Drosophila*

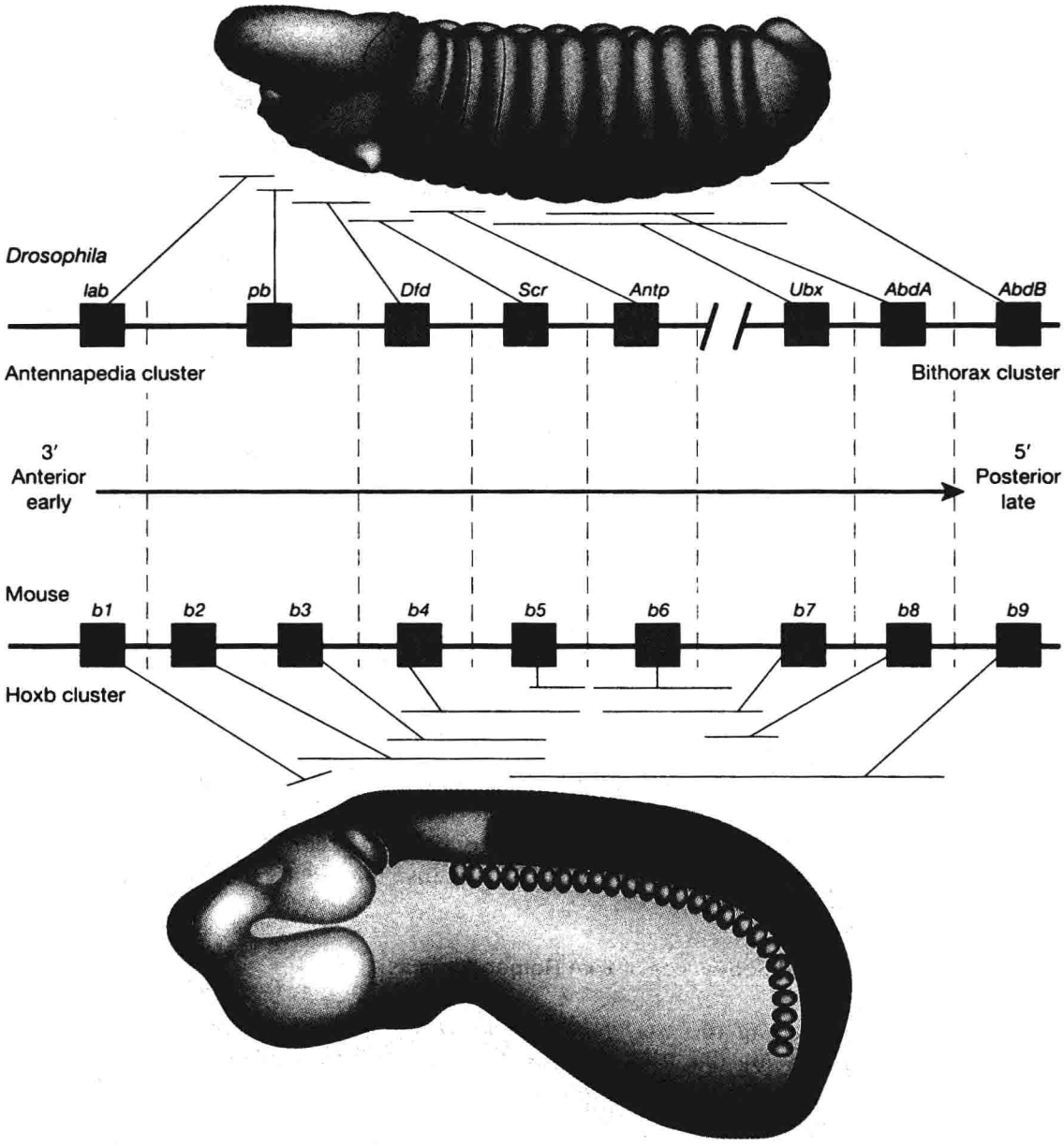
(*A*) Gap genes control the differentiation along the head to tail axis. In this case, a *Krüppel* mutation leads to the loss of the thoracic segments. (*B*) Pair-rule genes define each segment. A mutation in *fushi tarazu* leads to the loss of every other segment. (*C*) Segment polarity genes regulate the anterior/posterior axis within each segment. Each segment of *Drosophila* normally has an anterior and a posterior half. Mutations in *engrailed* lead to flies where every segment is entirely anterior.

Homeotic genes are found in two clusters in *Drosophila*. The bithorax cluster includes *Ultrabithorax* and two other genes, whereas the *Antennapedia* cluster includes five genes. These genes are involved in controlling the specialization of segments and are laid out on the chromosome in the same spatial order they are expressed during development. Genes at the 3′ end of a complex are expressed earlier, and toward the head of the animal, whereas genes at the 5′ end of the complex act later and more posteriorly. This arrangement, in which the physical order of the genes reflects the physical order of their activity, is called "colinearity." Homeotic control is not just limited to fruit flies. Vertebrates have

Extensive Homology in Amino Acid Sequences of Five Homeodomains

	1																			20
Mouse *MO*-10	Ser	Lys	Arg	Gly	Arg	Thr	Ala	Tyr	Thr	Arg	Pro	Gln	Leu	Val	Glu	Leu	Glu	Lys	Glu	Phe
Frog *MM3*	Arg	Lys	Arg	Gly	Arg	Gln	Thr	Tyr	Thr	Arg	Tyr	Gln	Thr	Leu	Glu	Leu	Glu	Lys	Glu	Phe
Antennapedia	Arg	Lys	Arg	Gly	Arg	Gln	Thr	Tyr	Thr	Arg	Tyr	Gln	Thr	Leu	Glu	Leu	Glu	Lys	Glu	Phe
Fushi tarazu	Ser	Lys	Arg	Thr	Arg	Gln	Thr	Tyr	Thr	Arg	Tyr	Gln	Thr	Leu	Glu	Leu	Glu	Lys	Glu	Phe
Ultrabithorax	Arg	Arg	Arg	Gly	Arg	Gln	Thr	Tyr	Thr	Arg	Tyr	Gln	Thr	Leu	Glu	Leu	Glu	Lys	Glu	Phe
	21																			40
Mouse *MO*-10	His	Phe	Asn	Arg	Tyr	Leu	Met	Arg	Pro	Arg	Arg	Val	Glu	Met	Ala	Asn	Leu	Leu	Asn	Leu
Frog *MM3*	His	Phe	Asn	Arg	Tyr	Leu	Thr	Arg	Arg	Arg	Arg	Ile	Glu	Ile	Ala	His	Val	Leu	Cys	Leu
Antennapedia	His	Phe	Asn	Arg	Tyr	Leu	Thr	Arg	Arg	Arg	Arg	Ile	Glu	Ile	Ala	His	Ala	Leu	Cys	Leu
Fushi tarazu	His	Phe	Asn	Arg	Tyr	Ile	Thr	Arg	Arg	Arg	Arg	Ile	Asp	Ile	Ala	Asn	Ala	Leu	Ser	Leu
Ultrabithorax	His	Thr	Asn	His	Tyr	Leu	Thr	Arg	Arg	Arg	Arg	Ile	Glu	Met	Ala	Tyr	Ala	Leu	Cys	Leu
	41																			60
Mouse *MO*-10.	Thr	Glu	Arg	Gln	Ile	Lys	Ile	Trp	Phe	Gln	Asn	Arg	Arg	Met	Lys	Tyr	Lys	Lys	Asp	Gln
Frog *MM3*	Thr	Glu	Arg	Gln	Ile	Lys	Ile	Trp	Phe	Gln	Asn	Arg	Arg	Met	Lys	Trp	Lys	Lys	Glu	Asn
Antennapedia	Thr	Glu	Arg	Gln	Ile	Lys	Ile	Trp	Phe	Gln	Asn	Arg	Arg	Met	Lys	Trp	Lys	Lys	Glu	Asn
Fushi tarazu	Ser	Glu	Arg	Gln	Ile	Lys	Ile	Trp	Phe	Gln	Asn	Arg	Arg	Met	Lys	Ser	Lys	Lys	Asp	Arg
Ultrabithorax	Thr	Glu	Arg	Gln	Ile	Lys	Ile	Trp	Phe	Gln	Asn	Arg	Arg	Met	Lys	Leu	Lys	Lys	Glu	Ile

From W.J. Gehring. 1985. *Sci. Am. 253/4:* 159.



Comparison of Homeotic Gene Clusters in *Drosophila* and Mouse

The *Drosophila Antennapedia* and *Bithorax* clusters are shown aligned with the mouse *Hoxb* cluster (mice have four such clusters, *Hoxa, Hoxb, Hoxc,* and *Hoxd*). The relative position of expression for each gene along the body axis is indicated.

homeotic genes, called *Hox* genes, which are arranged in clusters and expressed in the same colinear manner as in *Drosophila*.

Sequence analysis showed that homeotic genes from *Drosophila* and vertebrate animals share a 180-nucleotide region, called the homeobox. The homeobox encodes a DNA-binding region that is structurally related to other transcription factors. Homeotic proteins have been shown to have a role in transcriptional regulation, activating or repressing other genes, which is likely to explain how they function to regulate development. Homeobox-containing genes have been found in every eukaryotic species tested, from hydra to humans.

There is still much to be understood as to how these families of genes work together to provide the information that determines the position and identity of regions within a developing embryo.

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