生物实验室系列

Arabidopsis
a Laboratory Manual

拟南芥实验手册

（英文影印版）

Detlef Weigel and Jane Glazebrook

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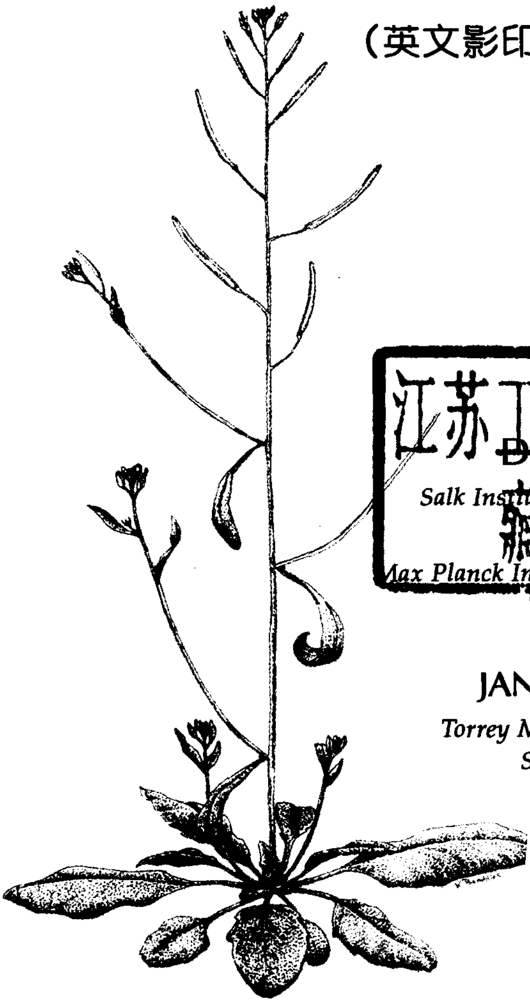

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内 容 提 要

拟南芥作为一种典型的模式植物，具有生长周期短、个体形态小和基因组小的特点，在植物分子生物学研究中已经成为非常重要的工具。在植物基因定位方法的研究中——这些基因往往对于生长发育、抗病抗逆性和产生有用的化学物质具有重要作用　拟南芥是重要的研究手段之一。除此以外，拟南芥还是一种珍贵的实验模型，对于理解光合作用和其他植物特有的功能，以及在分子和细胞水平上理解高等生物共有的基本过程具有重要意义。

本手册涵盖了关于拟南芥的基本实验方法。著者对这些方法进行了系统的搜集整理和认真审核，对实验过程进行了详尽的分步介绍，并就如何解决常见问题给出了非常有效的建议，可操作性和指导性较强。对于开展植物分子和细胞生物学、植物遗传和发育研究具有较高的参考价值。

生物实验室系列图书

2004年陆续出版的书目如下

- 发酵工程实验技术（2004年3月）
- 生物化学实验技术（2004年4月）
- 拟南芥实验手册［影印］（2004年5月）
- 现代生物科学仪器分析入门（2004年7月）
- 分子生物学与蛋白质化学实验方法［译］
- 转基因动物技术手册［译］
- 基因表达分析技术手册［译］
- PCR最新技术原理、方法及应用
- 蛋白质与蛋白质组学实验指南［译］
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- 分析信息处理技术

出版者的话

21世纪是生命科学的世纪，这已成为人们的共识。

生命科学随着人类对自身和自然的认识、探索而萌芽，随着人类生产和科学实践的进步而发展。现代生命科学包括生物学、医学、农学等传统学科领域，以及生物学、生物技术与环境科学乃至社会科学等其他学科相互渗透、交叉而产生的新型学科体系。20世纪后叶现代生物科学尤其是分子生物学取得了一系列突破性成就，使得生命科学在自然科学体系中的位置发生了革命性的变化，成为21世纪的带头学科。人们对生命科学也寄予了无限的期望，希望能够解决人类社会所面临的人口膨胀、资源匮乏、疾病危害、环境污染和生态破坏等一系列重大问题。

回顾生命科学的发展历程，实验技术一直起着非常重要的促进作用。如17世纪Leeuwenhoek等人发明并应用显微镜技术，直接催生了“细胞学说”的建立和发展；1973年Cohn和Boyer完成了DNA体外重组实验，标志着基因工程的肇始；1988年Kary Mullis发明的PCR技术甚至使生命科学产生了飞跃性的发展。可以说，生命科学无时无刻离不开实验，实验是开启神奇的生命王国大门的钥匙。没有实验技术的不断进步，也就没有生命科学今天的巨大发展；同时，生命科学的发展又对实验技术提出了更高的要求，进一步刺激了后者的不断进步。生命科学正是在“实验催生和验证着基础理论，理论指导和发展了实验技术”的不断循环中从必然王国走向自由王国。

工欲善其事，必先利其器。为了有助于生命科学工作者更多地了解相关实验技术和仪器设备，更好地设计实验方案，更有效地开展实验过程，更合理地处理实验结果，化工出版社组织出版了“生物实验室系列图书”。系列图书在整体规划的基础上，本着“经典、前沿、实用，理论与技术并重”的原则组织编写，分批出版。

在题材上，系列图书涵盖综合实验技术和单项实验技术两个方面。其中综合实验技术既有以实验目的为题，如“蛋白质化学分析技术”，内容纵向覆盖多项实验技术；也有以某一生命学科领域的综合实验技术为题，如“发酵工程实验技术”、“生物化学实验技术”等。

而单项实验技术则以深入介绍某一专项技术及其应用为主，在阐述其基本原理的基础上，横向介绍该项技术在多个领域的应用，如“双向电泳技术”、“流式细胞术”等。

在内容上，系列图书主要有以下两个显著特点。一是强调先进性——除了系统介绍常用和经典实验技术以外，特别突出了当前该领域实验手段的新理论、新技术、新发展，为国内专业人员起到借鉴和引导作用。二是强调可操作性——对于每一项实验技术，系统介绍其原理方法、设备仪器和实验过程，让读者明了实验的目的、方案设计以及具体步骤和结果处理，以期起到实验指南的作用。

本系列图书坚持质量为先，开拓国内和国际两个出版资源。一方面，约请国内相关领域兼具理论造诣和丰富实验室工作经验的专家学者编著；另一方面，时刻关注国际生命科学前沿领域和先进技术的进展，及时引进（翻译或影印）国外知名出版社的权威力作。

“生物实验室系列图书”的读者对象设定为国内从事生命科学及生物技术和相关领域（如医学、药学、农学）的专业研究人员，企业或公司的生产、研发、管理技术人员，以及高校相关专业的教师、研究生等。

我们殷切希望“生物实验室系列图书”的出版能够服务于我国生命科学的发展需要，同时热忱欢迎从事和关心生命科学的广大科技人员不仅对已出版图书提供宝贵意见和建议，也能对系列图书的后续题目设计贡献良策或推荐作者，以便我们能够集思广益，将这一系列图书沿着可持续发展的方向不断丰富品种，推陈出新。

谨向所有关心和热爱生命科学，为生命科学的发展孜孜以求的科学工作者致以崇高的敬意！

祝愿我国的科技事业如生命之树根深叶茂，欣欣向荣！

化学工业出版社

现代生物技术与医药科技出版中心

Preface

Arabidopsis thaliana has emerged as the model organism of choice for a wide range of basic and applied research into plant biology. This is largely due to the strength of *Arabidopsis* for genetic approaches, and, with the completion of its genome sequence, for genomic approaches as well. Our intention was to provide a simple guide to the application of *Arabidopsis* genetics to studies of plant biology. We hope that this volume will be a useful laboratory manual for those new to *Arabidopsis* genetics, as well as a reference for more established investigators when they require a commonly used protocol with which they are not familiar.

We have organized the manual in the order that a typical project proceeds. The early chapters cover growing *Arabidopsis*, identification of mutants, and basic genetic analyses. Later chapters cover phenotypic analyses, isolation of genes identified by mutations, and methods for studying gene expression. Appendix 1 provides Internet resources for the *Arabidopsis* community. We have concentrated on methods that are widely used in the community, in an attempt to provide a useful, yet affordable reference. We have not provided protocols for a large number of basic molecular biology methods that are not specific to *Arabidopsis* research. Readers who are not familiar with such methods should consult one of the many excellent molecular biology manuals.

The idea of writing this manual grew out of our experiences teaching the *Arabidopsis* summer course at Cold Spring Harbor Laboratory. We thought that the manual for that course would be useful for the wider community, but should be expanded to cover a broader range of methods. Daphne Preuss was instrumental in the early stages of planning and compiling the manual, and she contributed one of the chapters as well as several protocols. Other protocols were provided by us, members of our laboratories, or colleagues. We are very grateful to all contributors, without whom this manual could not have been produced. They are credited at the beginning of each protocol. Many of these protocols have been widely used for a long time, and we certainly do not pretend to have invented them, we merely wrote them down.

We are indebted to the excellent staff of Cold Spring Harbor Press. Mark Curtis drew the figures, Siân Curtis did a terrific job as developmental editor, making our text suitable for publication, and production editor Dorothy Brown made it into a book. Finally, without the persistence, patience, and organization of Kaaren Janssen and Mary Cozza, this project would never have been completed.

We hope that investigators of *Arabidopsis* will find this manual useful, and we welcome any feedback on the improvement of the protocols or any mistakes in them.

Detlef Weigel
Jane Glazebrook

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1

How to Grow *Arabidopsis**

The plant *Arabidopsis thaliana*, like most model organisms, is convenient and inexpensive to grow. It thrives in soil-filled pots and can be maintained in greenhouses, growth chambers, custom-made growth rooms, or even on window ledges and outdoors. The plants also grow readily in defined solid or liquid media, allowing rapid screening for seedling defects, recovery of transformants, and propagation of pathogen-free tissues. Parameters to consider for each of these growth environments are discussed in this chapter, and a brief introduction to the anatomy and morphology of *Arabidopsis* is given below.

MORPHOLOGY AND ANATOMY

The major anatomical features of *Arabidopsis* and its key developmental landmarks are briefly summarized here; cited references provide a more thorough analysis.

Recently fertilized ovules contain small embryos with a suspensor (see Figure 1.1, dark gray) and the embryo proper (light gray). The subsequent developmental phase takes place over approximately 7 days and is divided into the globular, heart-shape, torpedo-shape, and cotyledonous stages (Meinke and Sussex 1979; Mansfield and Briarty 1991; Jürgens and Mayer 1993). As the embryos mature, the seed pods (siliques) become yellow and then dry completely, finally shattering and releasing the seeds. The collection and storage of seeds are discussed later in this chapter.

*Chapter contributed by Daphne Preuss (Howard Hughes Medical Institute, University of Chicago, Illinois).

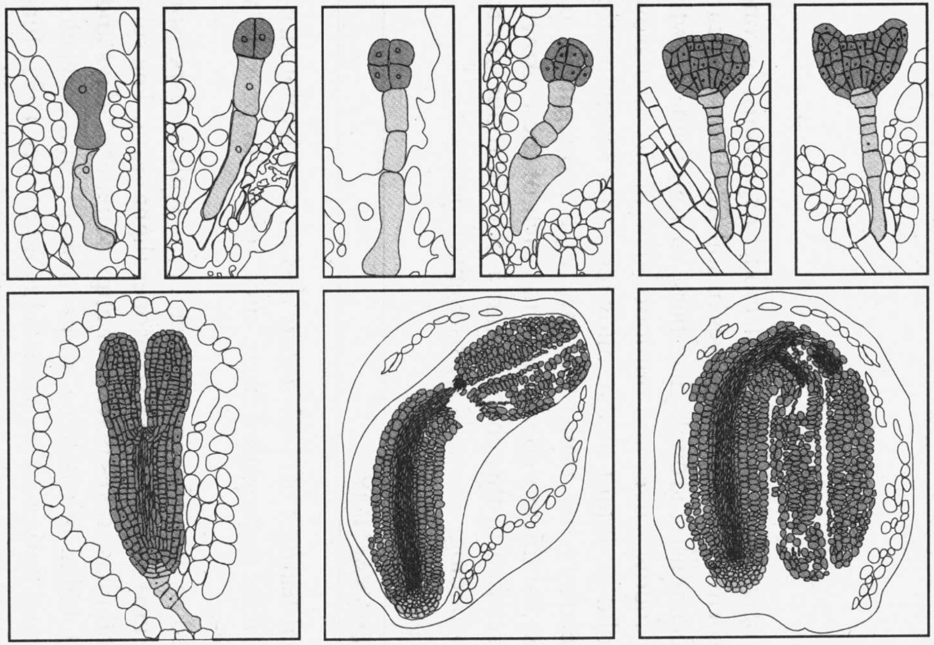

Figure 1.1.

Embryogenesis of *Arabidopsis*. The zygote divides initially into an apical cell and a basal cell. (*Light gray*) The embryo proper, which is derived from the apical cell; (*dark gray*) the descendants of the basal cell, which give rise to the suspensor and the root meristem. (*Top row from left to right*) A two-celled embryo; the octant stage, with two tiers of embryonic cells; the dermatogen stage, during which the epidermal primordium (protoderm) is set aside; and the early heart stage, during which the cotyledon primordia emerge and the root meristem develops. (*Bottom row*) The late-heart stage, early and late torpedo stages, and the bent cotyledon stage. (Adapted, with permission, from C.-M. Liu, Plant Research International, Wageningen, The Netherlands and at http://www.lse.okstate.edu/chunming/plantembryo.html)

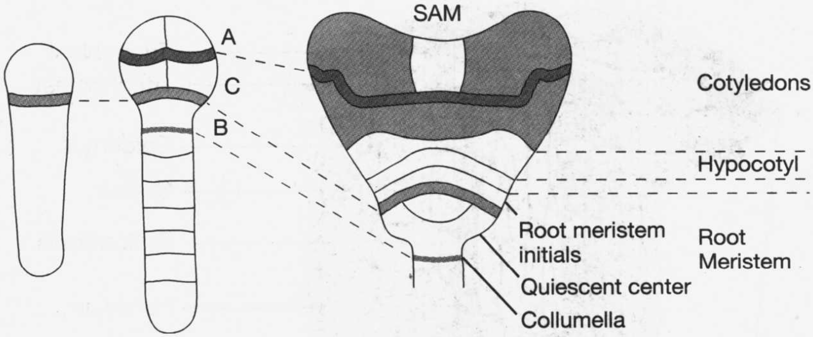

Figure 1.2.
Fate map of the early embryo. (SAM) Shoot apical meristem. (Redrawn, with permission, from Ben Scheres at http://www.bio.uu.nl/~mcbroots/fatemap.htm.)

The embryos contained within mature seeds have the necessary organs for early seedling development (see Figure 1.2). Among these are the shoot apical meristem (SAM) and cotyledons (the embryonic leaves), the hypocotyl, the radicle (the embryonic root), and the root meristem.

The key layers of the root have been established by the time the seed is mature. Root development in *Arabidopsis* follows a defined lineage (Dolan et al. 1993), with a quiescent center of meristematic cells that form the vascular bundle, pericycle, cortex, endodermis, epidermis, columella, and root cap (see Figure 1.3). Lateral roots extend from the primary root, and root hairs emerge from the epidermal cells in a regular pattern.

Shoot development continues with the formation of leaf primordia from the shoot apical meristem, which develop into rosette leaves. The basal part of the leaf, where it attaches to the stem, is called a petiole; the rest is made up of the leaf blade. The number of rosette leaves varies with growth conditions; short days favor rosette growth, whereas long days favor the initiation of reproductive development. The first two true leaves arise opposite each other and are more or less round. Subsequent leaves arise in a spiral and are more oval. Most commonly used ecotypes have leaves slightly serrated (Figure 1.4). On the leaves, hair-like projections (trichomes) serve as useful markers to distinguish juvenile and adult tissues (Kerstetter and Poethig 1998). The first few rosette leaves lack trichomes on their lower, or abaxial, surface. Subsequent leaves have trichomes on both surfaces. Leaves on the inflorescence shoot, also called cauline leaves or sometimes bracts, lack trichomes on their upper, or adaxial, surface.