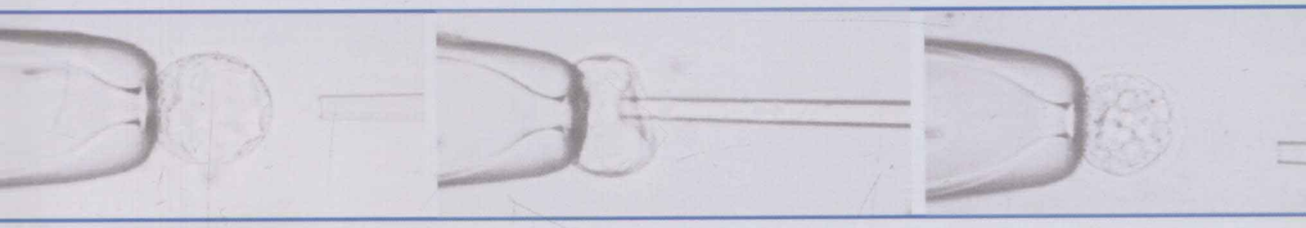
海洋生命科学“十二五规划”教材



CYTOTECHNOLOGY
细胞工程技术

郭华荣 主编

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Fig. 2.1. Laminar flow hoods for aseptic procedures. (By Van Staaveren bv, Rijsenhout, The Netherlands)

Figure 2.2. A walk-in controlled environment room for incubation of *in vitro* cultures. (By SBW, Rijsenhout Department, The Netherlands)

Figure 2.3. Shakers for the aeration of liquid cultures. (By P+S PLANTLAB b.v., The Netherlands)

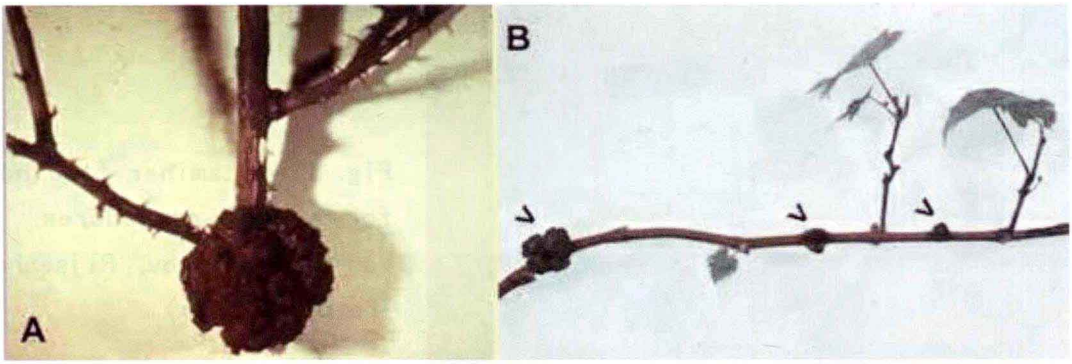

Figure 16.1. Crown Gall (*Agrobacterium tumefaciens*) in grapes.

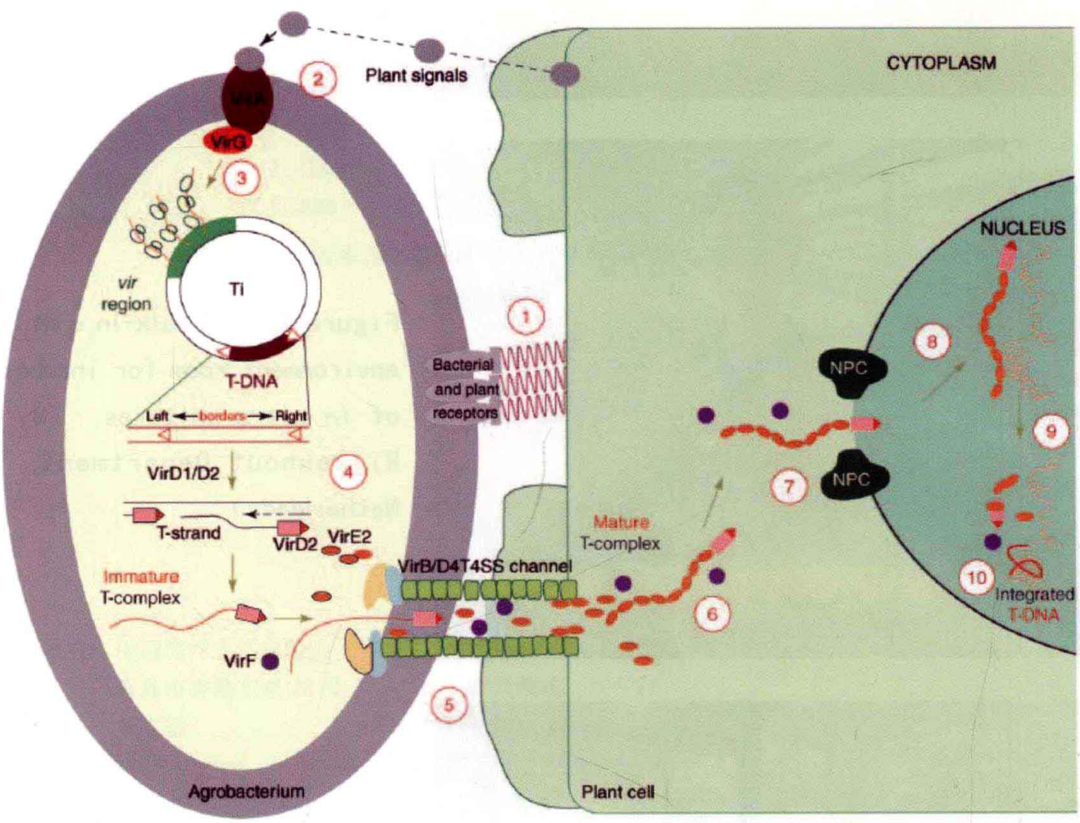

Fig. 16.3. A model for Agrobacterium-mediated transformation. The transformation process comprises 10 major steps and begins with recognition and attachment of the Agrobacterium to the host cells ① and the sensing of specific signals by the Agrobacterium VirA/VirG two-component-signal-transduction system ②. Following activation of the vir gene region ③, a mobile copy of the T-DNA is generated by the VirD1/VirD2 protein complex ④ and delivered as a VirD2-DNA complex (immature T-complex), together with several other vir proteins, into the host cell cytoplasm ⑤. Following the association of the virE2 with the T-strand, the mature T-complex forms, travels through the host cell cytoplasm ⑥ and actively imported into the host cell nucleus ⑦. Once inside the nucleus, the T-DNA is recruited to the point of integration ⑧, stripped of its escorting proteins ⑨ and integrated into the host genome ⑩.

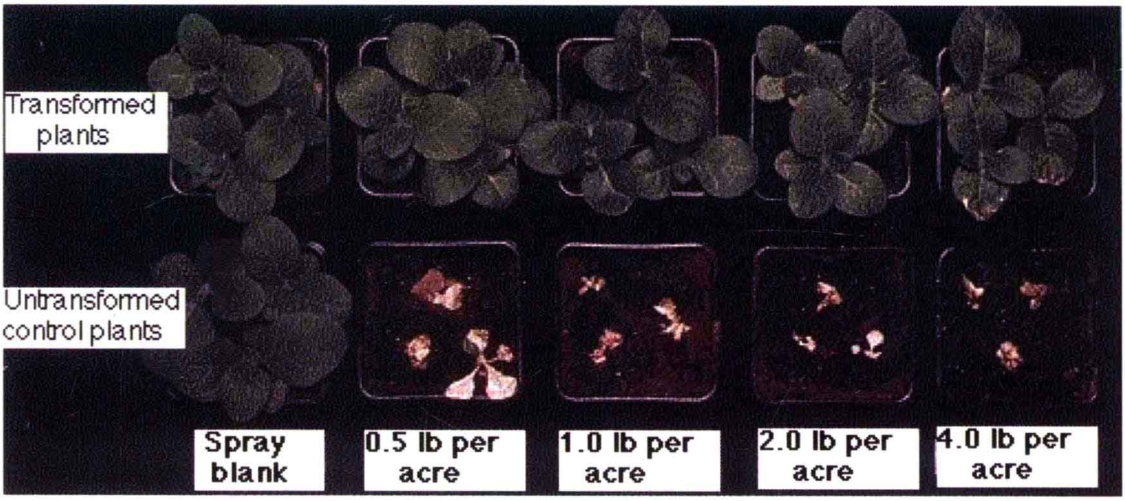

Figure 16.5. Effect of the herbicide bromoxynil (溴苯腈) on tobacco plants transformed with a bacterial gene whose product breaks down bromoxynil (top row) and control plants (bottom row). "Spray blank" plants were treated with the same spray mixture as the others except the bromoxynil was left out. (By Calgene, Davis, CA.)

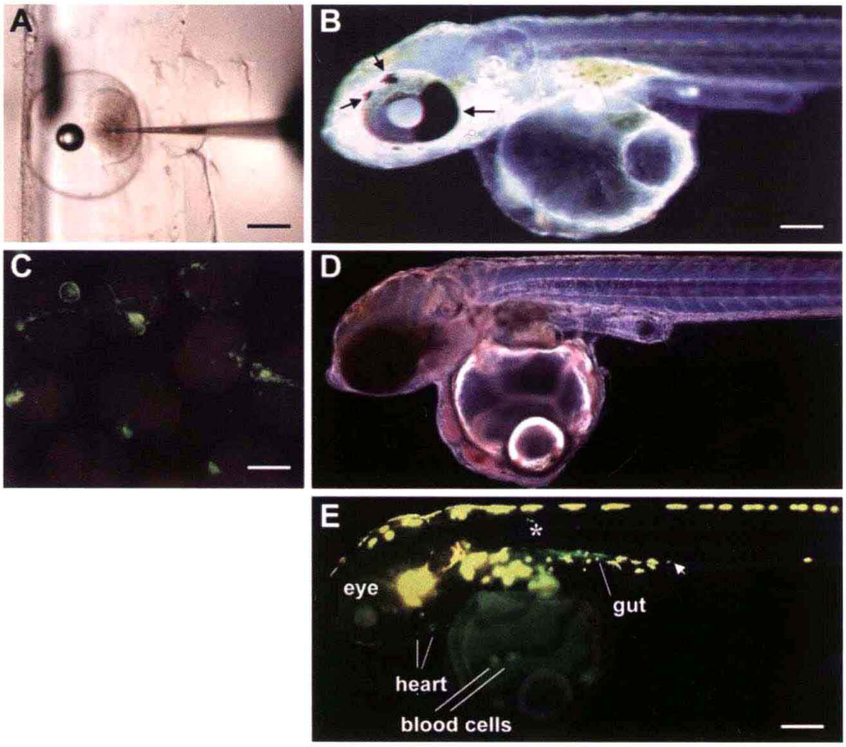

Fig. 31.8. Fish chimera. **A.** Injection of SaBE-1c cells into a recipient embryo (bar = 250 2m). **B.** Pigmented chimeric fry showing the contribution of MES1 derivatives to melanocytes (black areas, arrows) in the developing eye (bar = 200 μm). **C.** Group of GFP positive chimeras 24 hrs post fertilization (bar =600 μm). **D, E.** Chimeric fry showing the contribution of GFP expressing MES1 derivatives to the trunk (star), somite (arrow) and other cell types. Dark-field **D.** and merge (E) between dark field and fluorescent optics (bar = 200 μm). (By M. C. Alvarez et al., 2007)

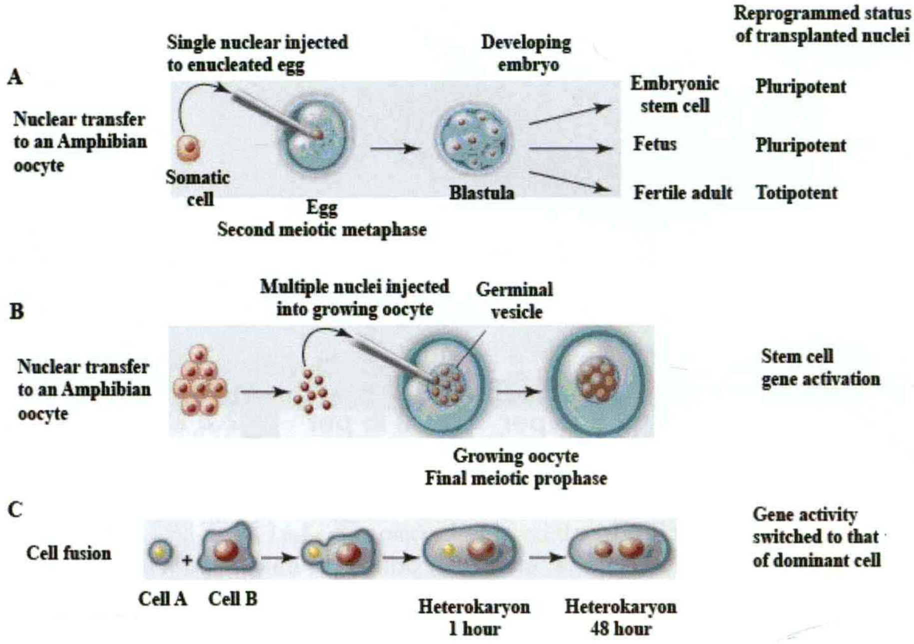

Fig. 32.1. Designs of nuclear transfer experiments. **A.** to unfertilized eggs (second meiotic metaphase) of frogs or mammals or **B.** to first meiotic frog oocytes. **A.** and **B.** show the transfer of somatic cell nuclei. **C.** Design of cell fusion experiments. (Modified from J. B. Gurdon and D. A. Melton, 2008)

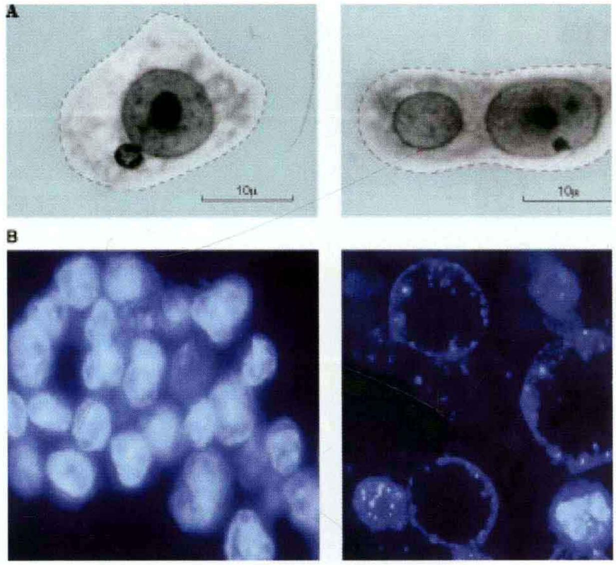

Fig. 32.2. Nuclei enlarge and chromatin decondenses（去致密化）during nuclear reprogramming. **A.** A chick erythrocyte 1 hour (left) and 2 days (right) after fusion to a human HeLa cell. The dotted lines indicate the outside of the fused hybrid cells. The smaller nucleus is that of the chick erythrocyte. **B.** Mouse ES cell nuclei immediately (left) and 2 days (right) after injection into an amphibian oocyte germinal vesicle. The injected nuclei have enlarged about 30 times in volume. (By J. B. Gurdon1 and D. A. Melton, 2008)

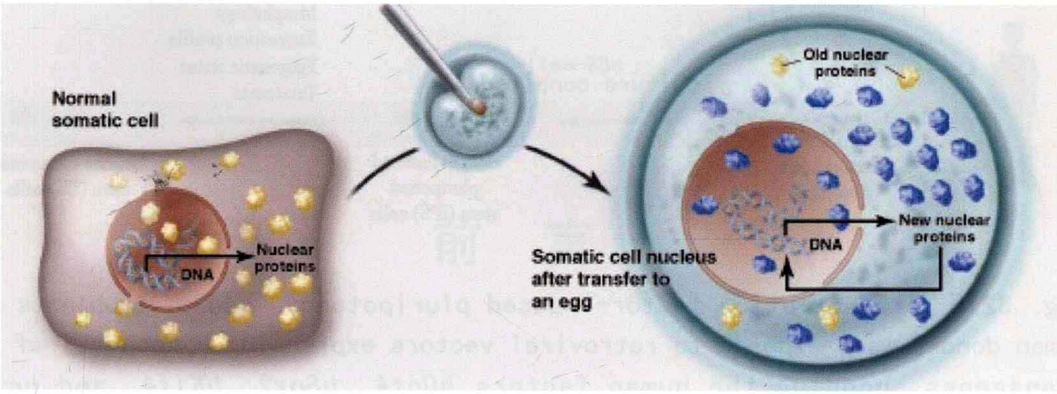

Fig. 32.3. Chromosomal protein exchange in a normal cell (left) or after nuclear transfer to an egg or oocyte (right). Yellow indicates donor-cell nuclear proteins that maintain gene expression. Blue indicates egg nuclear proteins that replace somatic proteins lost by dilution and that induce new gene expression. (By J. B. Gurdon1 and D. A. Melton, 2008)

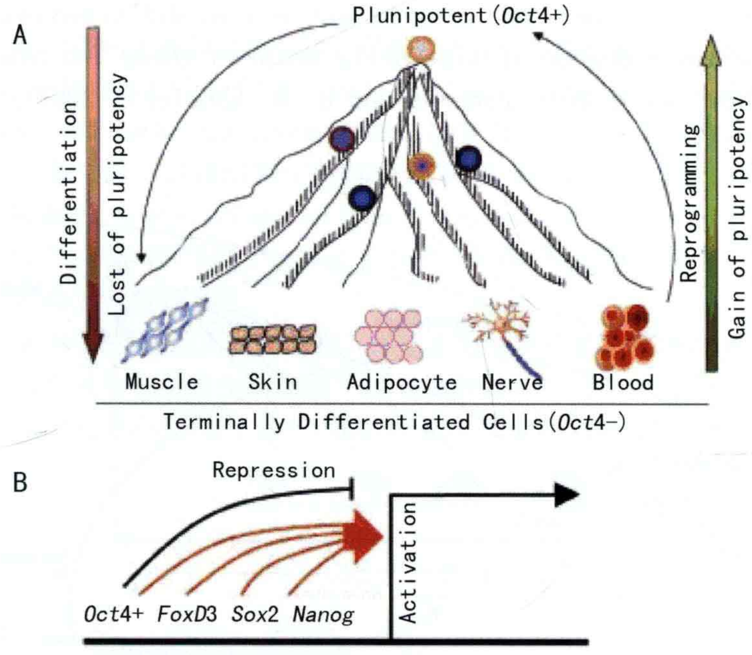

Fig. 32.4. Stem cell pluripotency. **A.** the three emphases of stem cell biology, self-renewal, differentiation, and reprogramming, are depicted here to show their relationship to pluripotency. The pluripotent stem cells may be viewed as being positioned at the top, ready to differentiate spontaneously into various cell types of tissues and organs (bottom). The pluripotent state is maintained by self-renewal at the top bymaintaining the expression of *Oct4* at the optimal level. Pluripotent stem cells differentiate by losing pluripotency into a specific lineage accompanied by the gradual loss of expression for pluripotent genes and the activation of differentiation genes. Differentiated somatic cells regain pluripotency by reprogramming back to the pluripotent state (on the right). **B.** multiple factors are involved in the maintenance of *Oct4* expression in ES cells. (Modified from D. Pei, 2009))

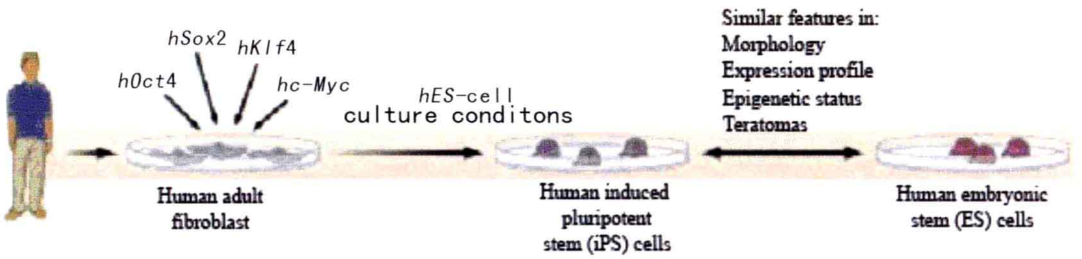

Fig. 32.5. Transcription factor-induced pluripotency. Adult fbroblasts from human donors were exposed to retroviral vectors expressing a cocktail of four transgenes encoding the human factors *hOct4*, *hSox2*, *hKlf4*, and *hc-Myc* (Takahashi et al., 2007). Thirty days after transduction and further cultivation under human ES cell growth conditions, human induced pluripotent stem (iPS) cell colonies (among others) that could be propagated and expanded further were isolated. Comparative analysis of human iPS cells and human ES cells using assays for morphology, surface-marker expression, gene expression profling, epigenetic status, and in vitro and in vivo differentiation potential revealed a remarkable degree of similarity between these two pluripotent stem cell types. (Modified from H. Zaehres and H. R. Schöler1, 2007))

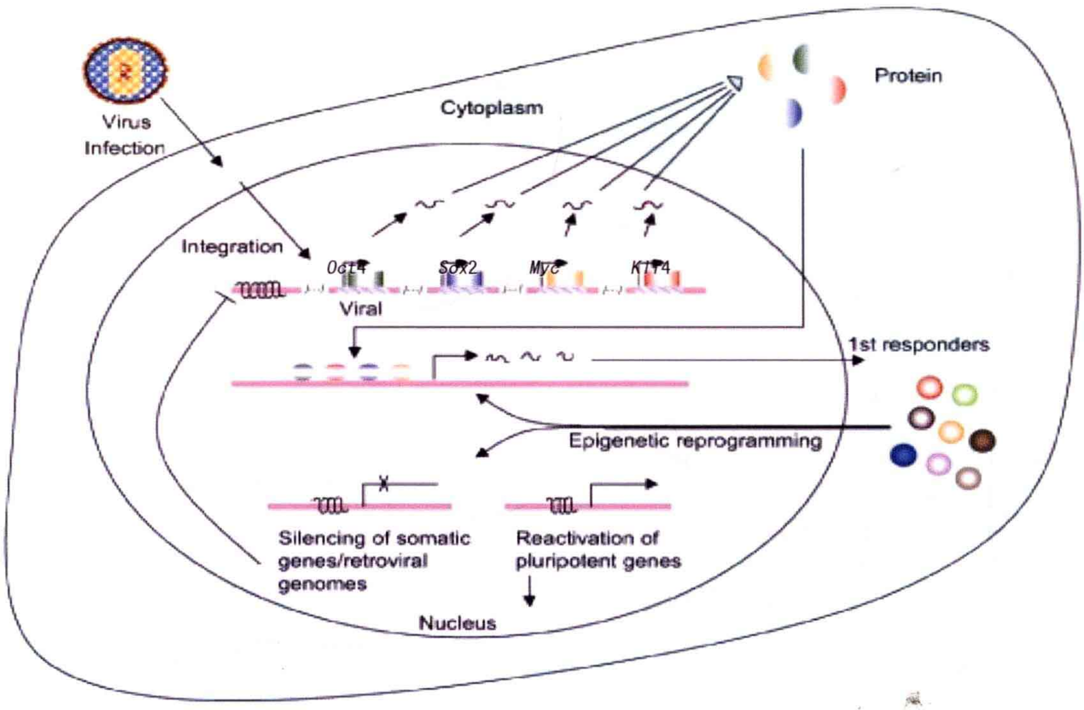

Fig. 32.6. Mechanical steps involved in the reprogramming of somatic cells into pluripotent ones by *Oct4/Sox2/Klf4/Myc*. (By D. Pei, 2009)

前　言

细胞工程是细胞水平上的生物技术，是一门既有悠久历史，又有崭新内容，不断发展更新的学科。近年来，国内已有多种版本的《细胞工程》教材出版，但尚缺乏具有相应教学内容的英文原版教材，从而阻碍了该课程双语教学内容的开展。我在10多年的教学和科研实践的基础上，经过精心筛选和提炼，从英文原版专著或公开发表的论文中，收集了细胞工程相关的教学内容并编辑成书。书中主要专业词汇均标注了汉语释义，以帮助学生理解书中内容。

全书分为植物细胞工程和动物细胞工程两大部分。植物细胞工程部分的第1～15章内容主要来源于"Dodds J H and Roberts L W，1995. Experiments in plant tissue culture. 3rd edition，Cambridge University Press，New York，USA"。为减少篇幅，本书中省略了其中的参考文献。动物细胞工程部分的第17～30，33～35章内容主要来源于"Freshney R I，2000. Culture of animal cells — a manual of basic technique. 4th edition. Wiley-Liss Inc，New York，USA"。其他各章内容是在参考了多篇公开发表的中外学术论文和论著的基础上编撰完成的。

本书的内容力求精炼，重点阐述细胞工程技术的原理和方法，可读性好，实用性强，适合作为高等院校本科生和研究生的细胞工程双语教材，以及科研工作者的实验工具书。作者尽管已倾注了大量心血，限于水平，难免有不当之处，恳请读者批评指正。

编者

2011年8月

CONTENTS

PART Ⅰ CYTOTECHNOLOGY IN PLANTS（植物细胞工程）

PART Ⅱ CYTOTECHOLOGY IN ANIMALS（动物细胞工程）

PART Ⅰ

CYTOTECHNOLOGY IN PLANTS

（植物细胞工程）

CHAPTER 1 INTRODUCTION FOR PLANT CELL, TISSUE AND ORGAN CULTURE(植物细胞、组织和器官培养简介)

The idea of experimenting with the tissues and organs of plants in isolation under controlled laboratory conditions arose during the latter part of the nineteenth century, finding its focus in the work of the great German plant physiologist Haberlandt (1902). Haberlandt's vision was of achieving continued cell division in explanted tissues on nutrient media-that is, of establishing true, potentially perpetual (长期) tissue cultures. In this, he was himself unsuccessful, and about 35 years were to elapse(逝去)before the goal was attained-as it could be only after the discovery of the auxins(生长素). Gautheret, Nobecourt, and White were the pioneers in this second phase. The research they set in train was at first mainly concerned with establishing the conditions in which cell division and growth would take place in explants, and in exploring the nutritional and hormonal requirements of the tissues. But this quickly gave place to a period during which cultured tissues were used as a research tool, in studying more general problems of plant cell physiology and biochemistry and the complex processes of differentiation and organogenesis. The achievements were considerable; but above all, the finding that whole plants could be regenerated from undifferentiated tissues-even single cells-in culture gave the method enormous power. In an extraordinary way this has meant that at one time the entity-a plant-can be handled like a microorganism and subjected to the rigorous procedures of molecular biology, and at another called almost magically back into existence as a free-living, macroscopic organism. If genetic engineering, involving the direct manipulation of the stuff of heredity, is ever to contribute to that part of man's welfare that depends on his exploitation of plants, the procedures adopted will inevitably depend ultimately upon the recovery of "real" plants from cultured components. No wonder, then, that the technology has escaped from the confines of the university laboratory to become part of the armory(军械库)of industry and agriculture.

HISTORY

Early Attempts, 1902-1939

The concept that the individual cells of an organism are totipotent (全能性) is implicit in the statement of the cell theory. Schwann (1839) expressed the view that each living cell of a multicellular organism should be capable of independent development if provided with the proper external conditions. A totipotent cell is one that is capable of developing by regeneration into a whole organism. The basic problem of cell culture was clearly stated by White (1954): If all the cells of a given organism are essentially identical and totipotent, then the cellular differences observed within an organism must arise from responses of those cells to their microenvironment and to other cells within the organism. It should be possible to restore suppressed functions by isolating the cells from those organismal influences responsible for their suppression. If there has been a loss of certain functions so that the cells in the intact organism are no longer totipotent, then isolation would have no effect on restoring the lost activities. The use of culture techniques enables the scientist to segregate cells, tissues, and organs from the parent organism for subsequent study as isolated biological units. The attempts to reduce an organism to its constituent cells, and subsequently to study these cultured cells as elementary organisms, is therefore of fundamental importance.

Several plant scientists performed experiments on fragments of tissue isolated from higher plants during the latter part of the 19th century. Wound callus, formed on isolated stem fragments and root slices, was described. 'Callus' refers to a disorganized proliferated mass of actively dividing cells. Rechinger (1893) examined the "minimum limits" of divisibility of isolated fragments of buds, roots, and other plant material. He found that pieces thicker than 1.5 mm were capable of further growth on sand moistened with water, but isolated fragments thinner that 1.5 mm not, although no nutrients were used in these experiments. Rechinger reported that the presence of vessel elements appeared to stimulate growth of the fragments. Unfortunately, he did not pursue this clue, since his observations suggested the ability of cambial tissue (形成层组织) to proliferate was associated with vascular tissues(导管组织).

Haberlandt (1902) originated the concept of cell culture and was the first to attempt to cultivate isolated plant cells in vitro on an artificial medium. A tribute to Haberlandt's genius, with a translation of his paper "Experiments on the culture of isolated plant cells," has been published. Unlike Rechinger, Haberlandt believed that unlimited fragmentation would not influence cellular proliferation. The culture medium consisted mainly of Knop's solution, asparagine(天冬酰胺), peptone(蛋白胨), and sucrose. Although the cultured cells survived for several months, they were incapable of proliferation. Haberlandt's failure to obtain cell division in his cultures was, in part, due to the relatively simple nutrients and to his use of highly differentiated cells. Since Haberlandt did not use sterile techniques, it is difficult to evaluate his results because of the possible effects of bacterial contamination. As example of his genius, Haberlandt suggested the utilization of embryo sac fluids and the possibility of culturing artificial embryos from vegetative cells. In addition, he anticipated the paper-raft technique. Following his lack of success with cell cultures, Haberlandt became interested in wound healing. Experiments in this area led to the formulation of his theory of division hormones. Cell division was postulated as being regulated by two hormones: One was "leptohormone"（韧皮部激素）, which was associated with vascular tissue, particularly the phloem(韧皮部). The other was a wound hormone released by the injured cells. Subsequent research investigators verified the association of hormones with vascular tissues.

Early in the twentieth century interest shifted to the culture of meristematic tissues (分生组织) in the form of isolated root tips. These represented the first aseptic organ cultures. Robbins (1922) was the first to develop a technique for the culture of isolated roots and Kotte (1922), a student of Haberlandt's, published similar studies independently. These cultures were of limited success. Robbins and Maneval (1923), with the aid of subcultures, maintained maize(玉米) roots for 20 weeks. White (1934), experimenting with tomato roots, succeeded for the first time in demonstrating the potentially indefinite culture of isolated roots. According to White (1951), two difficulties hampered the development between 1902 and 1934 of a successful method for culturing excised plant material: (1) the problem of choosing the right plant material, and (2) the formulation of a satisfactory nutrient medium.

With the introduction of root tips as a satisfactory experimental material, the crucial problem became largely one of organic nutrition. White's early success with tomato roots can be attributed to his discovery of the importance of B vitamins, plus the fact that indefinite growth was achieved without the addition of any cell division factor to the liquid medium.

It is important at this point to make a distinction between organ culture and tissue culture. In the case of excised roots as an example of organ culture, the cultured plant material maintains its morphological identity as a root with the same basic anatomy and physiology as in the roots of the parent plant. There are some exceptions, and slight changes in anatomy and physiology may occur during the culture period. According to Street (1977), the term "tissue culture" can be applied to any multicellular culture growing on a solid medium (or attached to a substratum and nurtured with a liquid medium) that consists of many cells in protoplasmic continuity. Typically, the culture of an explant, consisting of one or more tissues, results in a callus that has no structural or functional counterpart with any tissue of the normal plant body.

The first plant tissue cultures, in the sense of long-term cultures of callus, involved explants of cambial tissues isolated from carrot and tobacco tumor tissue from the hybrid *Nicotiana glauca* × *N. langsdorffii*. The latter tumor tissue requires no exogenous cell-division factor. Results from these three laboratories, published independently, appeared almost simultaneously. Fortunately, plant physiologists working in other areas had discovered some of the hormonal characteristics of indole-3-acetic acid, IAA (吲哚乙酸), and the addition of this auxin to the culture medium was essential to the success