THE MOLECULAR AND HORMONAL BASIS OF PLANT-GROWTH REGULATION

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BY

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Introduction

ALBERT EINSTEIN once said: "I believe in perfect laws in a world of existing things, in so far as they are real, which I try to understand with wild speculation." In this account of the molecular and hormonal basis of plant-growth regulation, the author has attempted to describe the existing knowledge and to distinguish clearly laws and speculation.

In the first part of his book the author describes the building stones that form the basis of present-day biology. The author inquires into the chemical and physical nature of the nucleic acids, considers how these can be a source of information which determine the character and, in part, the activities of the cell, and fit these into general biological theory.

The second part of the book deals with the modern aspects of hormone action introducing the reader to the growth-regulatory hormones existing in most higher plants. The molecular aspects of hormonal control have interested scientists for a relatively long time, but only recently with the development of modern technology has serious and accurate work been possible. The author being himself actively engaged in this field could critically and intelligently review this interesting field of work.

The briefness of this book, without loss of precision and of high scientific standard, makes it valuable for young scholars and biology teachers and provides them with a bird's-eye view of an interesting field. The bibliography after each chapter, arranged in a fashion that tells the reader the aim and scope of each quoted paper, will stimulate and permit him to widen the scope of his interest.

Although not being a classical textbook it is of value for all biologists, teachers and students who seek to widen their general knowledge and it is hoped that it will introduce the young plant physiologists into a field of great future.

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PART ONE

CHAPTER 1

Molecular Horizons

THE recent achievements in the understanding of basic biological processes which have paved the road towards a more thorough understanding of Life Sciences have been aided by the concept that all biological entities are intrinsically similar and obey the same basic laws as regards differentiation and growth. The biochemical approach, figuratively speaking, has pulled the carpet from under the feet of classical genetics and has produced a new science designated "Molecular Biology" which in its turn has produced tenets of belief based on the "Central Dogma":

However, with the unravelling of mode of replication of certain RNA viruses and phages a corollary has been added to the Dogma: the sequence of the nucleic acids in the above statement may be reversed, or RNA may completely replace DNA.

The combination of biological and physical scientific disciplines enabled Watson, Crick and Wilkins in 1953 to determine the structure of DNA and this consequently led to the formulation of the genetic code by Nirenberg, Matthei, Crick and Gamow in the early sixties. In 1957 Kornberg isolated an enzyme–DNA polymerase (now termed polymerase I) which was reported to participate in DNA replication. This later led to the discovery of RNA polymerase participating in transcription (see Chapter 4) in 1960. Jacob and Monod in 1961 presented their classic model of regulation of genetic information which now, with certain important modifications, provides a unifying concept for the understanding of the genetic processes in at least some of the biological Phyla. These are but a few of the several advances made by

molecular biology and in their wake practical applications in medicine, animal and plant sciences have followed even though many problems have yet to be solved.

To give examples of practical applications of molecular biology we may mention the attempt to transfer genetic information from one individual to another by means of transferring given and genetically mapped chromosomal fragments by what is termed DNA transduction. This is achieved by attaching these fragments to certain viruses which essentially are also genetic material and thereupon transferring the "enriched" virus to a new individual in which the whole particle is integrated into its DNA. By certain means the virus may subsequently be freed from the host DNA while leaving the chromosomal fragment of the donor with the recipient (this process will be discussed in detail in Chapter 9). Biochemical techniques are now sophisticated enough to enable the laboratory production of polynucleotides with base sequences corresponding to those of natural genes and Khorana's group in Wisconsin has achieved the *in vitro* synthesis of the gene for the alanine tRNA molecule. This is still a far call from the synthesis of a genetic entity enabling production of a complete protein or of a complete chromosome which may contain millions of nucleotide sequences, but nevertheless is an important step forwards towards therapy of genetic disorders caused by the inability to produce certain biologically active proteins.

Instances have been reported whereby not DNA but its transcriptional product, RNA, has been able to transfer genetic information. Several of the cases reported pertain to RNA mediated informational transfer from hormone-treated tissues; for instance, as induced by auxins in plants or by testosterone or oestrogens in mammals. RNA which was extracted from hormone-treated organs caused typical hormone-induced effects in non-hormone treated control organs.[3] Other RNA mediated effects concern transfer of information for protein enzyme synthesis, anti-body production and with certain reserve induction of memory. This subject will be elaborated upon in a later chapter. These techniques of DNA or RNA informational transfer present future possibilities of "genetic engineering" and while its applied utilization is still in its infancy it is probable that in the not too distant future some practical use may be made in this respect.

For generations horticulturalists have utilized the method of vegetative propagation of plant material, a practice as yet awaiting exploitation in animal husbandry. There is at least a theoretic possibility of non-sexual propagation of animal species and it has been claimed that *Homo sapiens* in this respect does not differ from other species. However, the possibility of "cloning" humans, even if at some future date this became practicable, would doubtlessly cause such great complexity of legal problems (e.g. parentage and inheritance) that this practice will be decided upon or rejected not by biologists but rather by legal and moral administrative authorities. A more feasible use of "test-tube" culture is that of certain tissues or differentiated organs which may provide specific genetic information, which by means of transduction, DNA or RNA transfer or other methods may be applied for therapeutic purposes.

In 1959 Steward at Cornell University succeeded in producing a perfectly normal carrot plantlet possessing root, shoot and flowers from tissue cultures of single vegetative cells growing in a nutrient medium containing growth substances, minerals, etc. (see Plate 1). This approach affords widespread agricultural use and in several instances has now been adopted commercially. Orchid growing normally necessitates an extensive period (several years) for the production of nursery stock and at present several nurseries, the world over, have developed commercial techniques whereby meristematic growing tips are cultured in a suitable and sterile medium, culture conditions causing the production of thousands of plantlets from a single tip. Once the plantlet stage has been reached the flasks are opened and the plantlets may be transferred to nursery beds and continue to develop normally. This technique saves 2 to 3 years of growth and has the further advantages of production of uniform material and, moreover, in the initial stages, is most economical in space. Plants produced from meristem culture are termed *mericlones*, and besides the practical use for orchids, successful experimentation has also been conducted on other "cash crops" such as chrysanthemums, carnations and bromelias, with special reference to the pineapple.

Steward's culture was of diploid (2n) cells stemming from lineage which at least in some previous stage underwent sexual fusion. Subsequent work has demonstrated the possibility of cloning haploid (n) cells as well, as achieved in the genus *Nicotiana*: the haploid tissue in

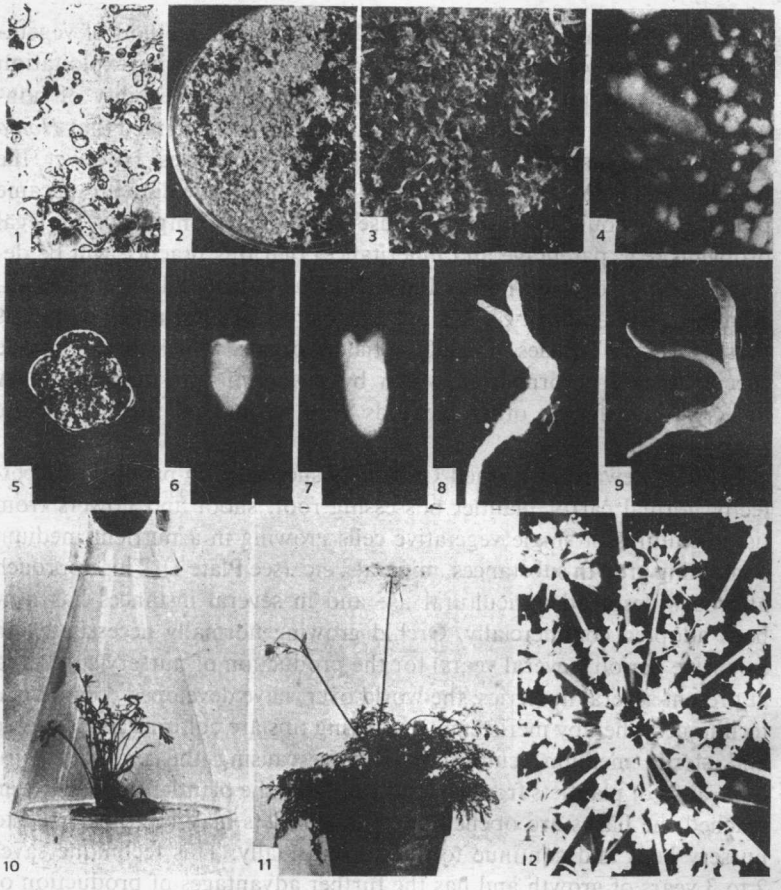

PLATE I

The development of a carrot plant from individual root cells growing on nutrient medium under culture. In F. C. Steward's experiment the following stages can be distinguished: 1. Groups of individual cortical root cells from which cultures commenced. 2–5. Subsequent stages of tissue organization. Nos. 2 and 3 indicate development of groups of cells to form embryo-like organs (embryoids) which later produce plantlets. Nos. 4 and 5 are embryoids under magnification. 6. A normal carrot embryo. 7. An embryoid markedly resembling no. 6. 8–9. Later stages of development of the embryoid and the development of a plantlet. 10. Growth of plantlet on culture medium. 11. The plantlet has been potted and continues to grow normally. 12. Production of normal flowers on the potted plant. (Plate produced with the kind permission of F. C. Steward, Cornell University, U.S.A.)

this case is not necessarily somatic, since pollen grains under culture have also produced haploid plants.

The above experiments illustrate the concept of "totipotency", as coined by Bonner,[1] most convincingly. Totipotency implies that each and every cell contains the total sum of the genetic information required to produce the complete organism and under favourable conditions, such as those provided by Steward and outlined above, is capable of producing a complete new organism. This assumes the presence of a given amount of identical DNA in all plant cells independent of their differentiational state and specific functions. According to this hypothesis, the quantity of DNA per cell may vary only as a function of cell ploidy. Table 1 shows results of an experiment of Bonner's on maize (*Zea mays*) in which relative amounts of DNA in various cell types were compared.

TABLE 1. RELATIVE AMOUNTS OF DNA PER NUCLEUS IN VARIOUS CELL TYPES OF MAIZE (*Zea mays*)
(According to Bonner)[1]

Cell type	Ploidy	Relative amount of DNA/nucleus
LEAF	2n	5·0
Root meristem—telophase	2n	5·0
Root meristem—prophase	4n	10·2
EMBRYO	2n	5·6
Seed—scutellum	2n	5·1
Seed—aleurone	3n	7·5
Microgamete	n	2·5

The data presented in the table indicate a clear correlation between amount of DNA and cell ploidy and that in all cells possessing similar ploidy, the amount of DNA is, within experimental error, equal. It is therefore inferred that development and differentiation of totipotential cells are dependent upon repression or depression mechanisms of genes which are defined regions of DNA. In most somatic cells of higher organisms it has been estimated that at the most only 5 per cent of the total DNA is utilized at a given period, different regions of the chromosome being turned on or off according to differentiation patterns. It is

furthermore claimed that not all the information produced by active DNA in the nucleus reaches the cytoplasm, there to be translated, thus only a small proportion indeed of the totipotency of cells is finally expressed.

Disease control and especially viral disease control is being aided by molecular biology. In 1957 Lindenmann and Isaacs reported the existence of a substance named interferon which is produced in cells upon virus invasion and induces changes in the host cell, interfering with further multiplication of the invader while not hindering normal cell multiplication (see Chapter 11). Interferon which is formed by infection with an artificial virus—double-stranded RNA—has stemmed the spread of the viral eye infection herpetic keratoconjunctivitis in fowl. Research on the same lines is being conducted with the hope of combatting the common cold, the pathological cause of which is viral.

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CHAPTER 2

The Structural Units of DNA

THE chromosome of higher plants and animals comprises a double chain of deoxyribonucleic acid (DNA). According to the model suggested by Watson, Crick and Wilkins the two chains are intertwined and form a "double helix". A gene is at present defined as a certain segment of a DNA molecule possessing specific structure which upon being active may finally translate itself into one polypeptide chain. This gene concept differs somewhat from the earlier contention made by Beadle and Tatum,[1] of "one gene, one protein". The "cistron" now is seen to be comprised of those stretches of DNA which participate in the formation of final polypeptide protein subunits. The basic structural units of DNA are:

1. The purine bases—adenine and guanine; and the pyrimidine bases—thymine and cytosine (see Fig. 1).
2. The sugar deoxyribose which is joined to the nucleic bases at serial position 1 of the sugar.
3. Phosphate groups which link up the sugar units by a 3,5-linkage forming a 3-5-phospho-diester polymer (see Fig. 2).

When one of the bases combines with the sugar ribose or deoxyribose a *nucleoside* is formed and when in addition to the two former units a phosphate group is included the product is termed a *nucleotide* (see Fig. 3). Besides the four common bases mentioned above, in certain types of DNA such as phage (bacterial virus) DNA, 5-hydroxymethylcytosine has been found, and in DNA of certain higher plants and animals respectively, 6 and 30 per cent of total nucleic base composition is methylcytosine. DNA of bacteria may contain small amounts of 6-methylaminopurine. These bases are termed minor bases and in