Biochemistry and Cytology of Plant-Parasite Interaction

Edited by

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Preface

As the history of plant pathology reveals, more than three quarters of a century have elapsed since the pioneer workers began to elucidate the mechanisms of plant resistance to versatile etiological agents. It has now become clear that the key to the infection mechanism resides in the interaction between host cells and parasite.

Research efforts in the last three decades have focused mainly on physiologically active substances that are directly or indirectly involved in pathogenesis and on various metabolic alterations that occur in infected host cells or tissues. The results are best illustrated by the isolation and characterization of phytotoxins, cell-wall degrading enzymes, and phenolics, and by the elucidation of changes in respiration (or more specifically, the activity of oxidative enzymes), carbohydrate catabolism, the production of polyphenols, and the synthesis of nucleic acids and proteins. These investigations disclosed that activation of metabolic processes closely coupled with energy production is a prerequisite for the defense action of plants against parasites. On the other hand, morphological and ultrastructural studies have provided much information on the processes of cellular degeneration and symptom expression in various diseases.

One of the rewards obtained through this work is certainly the isolation of phytoalexins as chemical entities. Progress in this field cast new light on the eternal question of how plants defend themselves against penetration, intracellular growth, and the development of parasites in their tissues. Important advances have also been made in the field of host-specific toxins, providing a model for the mechanism of specificity in some plant-parasite systems. The evidence obtained serves to explain how certain pathogens establish themselves as entities different from other microorganisms.

Recent trends in studies of the host-parasite interaction indicate that the metabolic alterations detected by chemical procedures and morphological or ultrastructural changes under the microscope are a result of, and not the cause of resistance. Hence, there must be some yet undisclosed processes mediating in the interaction between host and parasite. Accordingly, by mere observation of metabolic changes or cytomorphological abnormalities, it may not be possible to discern the basis of the general principle that disease is an exception rather than the rule. It is essential then to search at depth for the trigger of such responses and to disclose the mechanism of induction of the defense action of plants, which probably involves some mutual recognition

between the host cells and parasite as an initiation event. As a consequence of this enquiry, even though much is dependent on recent progress in studies on biochemical regulation in higher organisms, the function of genes in host-parasite interactions has now become a subject of immediate concern to plant pathologists, physiologists, and biochemists.

In September, 1974, an international group of scientists interested in this particular field of plant pathology gathered in Osaka, Japan, to hold a symposium entitled "Induction Mechanism of Biochemical and Cytological Responses in Diseased Plants": current topics and future problems were discussed. This book derives directly from that symposium, although the individual contributions here have mostly been prepared in review form. It is hoped that this volume will thus provide research workers, teachers and students with a new insight into the modern, comprehensive aspects of host-parasite interactions.

We wish to express our sincere thanks to Dr. K. Ono, Director of the Institute of Agricultural Chemicals, Takeda Chemical Company, for his thoughtful local arrangement of the original symposium. Thanks are also due to all our friends and colleagues who have given us invaluable help, in one way or another, in the publication of this book. Foremost among these are Dr. W.R. Bushnell, Dr. J. E. DeVay, Dr. R. D. Durbin, Dr. J. L. Gay, Dr. S. S. Patil, Dr. R. Rohringer and Dr. R. P. Scheffer, who not only read the manuscripts but also provided expert advice in the areas of their own specialities. A special vote of thanks must also go to the staff of Kodansha for their unselfish and tireless efforts in every phase of the publication of this book.

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K. TOMIYAMA, J. M. DALY,
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CONTENTS

CHAPTER 1

Introduction

The Early Biochemical and Cytological Events in Plant Disease: A Perspective

J. M. DALY*

The subject matter of this book represents one of the most difficult in all of biology. We are concerned with understanding and interpreting the significant events of the interaction between a microorganism and a higher plant at the cellular and subcellular level. The interaction basically is antagonistic. If the microorganism is successful, disease, with all of its economic impact, is the end result. The impact of plant disease carries an increasingly higher cost as the world's supply of food and fiber comes under more and more pressure.

Unlike higher animals, plants as far as we now know, have not evolved a central coordinated mechanism to deal with an invading microorganism. There is no substantial evidence for a highly specialized system that routinely recognizes foreign entities and responds to them in a uniform and predictable way by antibody formation. Rather, the plant response is highly individualized and appears to be localized primarily in the cells directly under invasion. To use the phrase coined by a former diplomat, the confrontation is "eyeball to eyeball" between the host and the parasite and the outcome is decided at the site of invasion. The remarkable property of such interactions is their great specificity, an aspect of plant disease which received attention at the recent IAMS Congress in Tokyo.

On reviewing the contents of this book, I think it is apparent that as individual research workers we approach our problems with concepts such as those outlined in Fig. 1. Historically, plant pathologists have been concerned with the nature of disease resistance because of its obvious economic importance. As a beginning, in the early part of this century, considerable effort was spent in looking for morphological or chemical characteristics of uninfected plants which explain their resistance to certain diseases.

Although preformed or passive barriers to infection undoubtedly exist, two other scientific developments gradually caused a shift to other lines of

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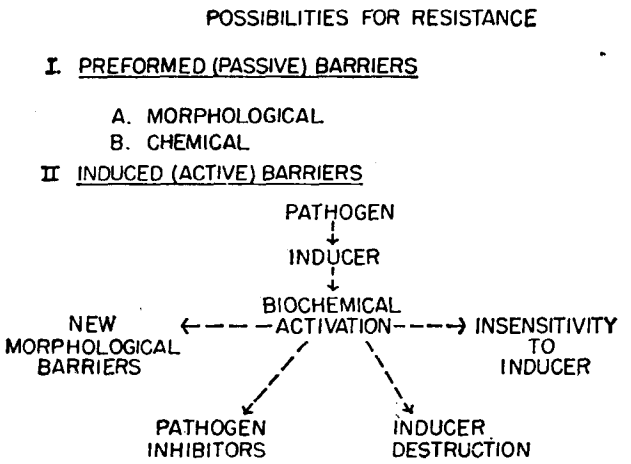

Fig. 1. Passive and active (induced) barriers to infection by plant pathogens.

thinking. The first of these was the recognition of pathogenic races within a fungal or bacterial species, races which differed only in ability to infect certain varieties of a host. The varieties themselves showed no obvious morphological or biochemical differences. We now understand somewhat better the genetic basis for host-parasite specificity from the work of Flor, Person, Ellingboe, Loegering, Moseman, etc. upon which Fig. 2 is based. With wheat stem rust, for example, there are specific dominant genes for host resistance and corresponding recessive alleles for virulence. The complete absence of genes for resistance results in a host completely susceptible to all pathogenic strains. A specific gene for resistance results in incompatibility to all races except those which have the recessive allele for virulence against the host gene.

Fig. 2 shows the expected results for a host-parasite system with three gene pairs. With 3 loci, there are 2^n phenotypes and thus 9 pathogenic races are possible; with 8 loci there could be 250 races. Some 17 loci are known for wheat stem rust alone. The fundamental problem with which we are concerned in this book is to identify the gene products controlled by either parasite or host genes and to determine their mode of action. The complexities of the genetic control of host-parasite interactions is illustrated by the system described by Uritani *et al.* (sect. 6.6). There are numerous strains of *Ceratocystis fimbriata* capable of causing black rot but each is restricted to particular species. The search for chemical "recognition factors" by Uritani *et al.* (sect. 6.6) and by Doke *et al.* (sect. 5.2) is a challenging area. Yoder (sect. 2.2) has shown how genetic reasoning may yield information on the possible existence of multiple forms of host-specific toxins. Further, he has

LOCI AND RACES (after Person)

HOST GENES FOR RESISTANCE (n=3)

PATHOGEN GENES FOR VIRULENCE (n=3)				H_1	H_2 A	H_3 B	H_4 C	H_5 A B	H_6 A C	H_7 B C	H_8 A B C
P_1				S							
P_2	a			S	S						
P_3		b		S		S					
P_4			c	S			S				
P_5	a	b		S				S			
P_6	a		c	S					S		
P_7		b	c	S						S	
P_8	a	b	c	S	S	S	S	S	S	S	S

LOCI = n PHENOTYPES = 2^n

8 LOCI = 250 RACES

9 LOCI = 500 RACES

Fig. 2. The genotype and phenotypes in a host-parasite system where single dominant resistance genes in the host are complementary for single recessive virulence genes in the parasite.

illustrated how this information might be used to define the biological potential of organisms whose virulence is a result of such factors. Genic regulation of pathogenicity is still an area worth challenging, and is dealt with by Leary and Sanchez in sect. 2.1. They demonstrate clearly that mutations resulting in loss of pathogenicity can be separated from mutations toward altered growth of the fungus.

In addition to the genetic models which aid in defining our goals, a second important concept which changed the view on host-parasite interactions came through developments in the field of biochemistry. In the 1940's and 1950's, the idea of metabolic regulation of cellular events caused a change from a search for passive barriers to infection to chemical or morphologic barriers which were induced after invasion by shifts in host metabolism. Fig. 1, lower section, presents some of the possibilities, but only some of them, which are embodied in the concept of induced barriers.

Keen (sect. 4.2), with considerable justification, has coined the word "elicitor" to describe the signals, presumably chemical signals, generated by pathogens and which bring about metabolic shifts. His recent research sug-

gests that materials with the requisite specificity are indeed produced. With his system, the elicitor or inducer causes the accumulation of toxic chemicals in an incompatible reaction but, as will be discussed in more detail later, inducers may be substances which damage tissue (toxins) or cause growth effects (hormones).

Usually we think of inducers as relatively low mol. wt. compounds acting by diffusion at sites somewhat removed from the pathogen. The work of Doke *et al.* (sect. 5.2) and Uritani *et al.* (sect. 6.6), implicates high mol. wt. components as recognition factors early in disease but whether these factors lead to some form of altered metabolism in the host cells is not yet clear.

Fig. 1 indicates that induced barriers may be morphological. Kunoh and Ishizaki (sect. 3.5) have provided evidence for an induced accumulation of silicon in the areas surrounding lesions resulting from diverse pathogens infecting barley and suggest that it may be important in initiating incompatible reactions. The formation of papillae or equivalent structures is well known, but, as Aist and Israel (sect. 3.1) suggest, their role as barriers to penetration or spread of pathogens is not defined clearly as yet. The work of Asada and Shiraishi (sect. 3.2) has posed a question concerning the continuity of membrane around the haustorium of *Peronospora parasitica*-invaded Japanese radish root.

Two new and very interesting variations of morphological types of resistance against bacterial invasion were suggested independently by Goodman *et al.* (sect 3.3) and Horino (sect. 3.4). With *Erwinia amylovora* infections of apple, Goodman found an agglutination phenomenon which restricted bacterial movement in resistant tissues. With bacterial leaf blight of rice, Horino has described an induced resistance which apparently depends on the formation of fibrillar structures in vascular tissue and which may also function to restrict bacterial spread.

As knowledge of genetic transcription and translation becomes deeper and greater, an additional refinement of the concepts of Figs. 1 and 2 is possible. Based on the well known Jacob-Monod model of gene repression in pro-karyotic cells, it is possible to think of induced resistance in terms of derepression or promotion of synthesis of enzymes in a metabolic pathway leading to anti-microbial or anti-pathogenic chemicals (APC) (Fig. 3). This model is appealing but little direct evidence for it in plant disease or even that it operates in eukaryotic cells is available. The genetic regulation of enzyme synthesis in higher plants is little understood.

Another model (Fig. 3) can be constructed using allosteric effectors (either negative or positive) which regulate previously synthesized enzymes for pathways involved in the production of anti-pathogenic chemicals. Unlike the Jacob-Monod model, a system controlled by allosteric modulation requires little or no enzyme synthesis. A combination of both of the above

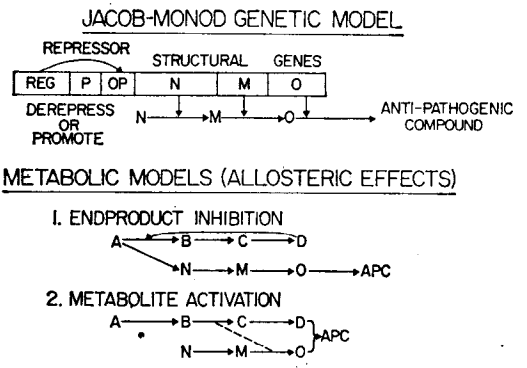

Fig. 3. A comparison of two different mechanisms for increasing enzyme activity of pathways important in plant disease.

possibilities, that is the synthesis of allosteric enzymes, would be most effective for explaining the extreme specificity of some host-parasite systems.

Several of the studies described in this book have suggested an important role for nucleic acid metabolism in diverse diseases. Shishiyama *et al.* (sect. 6.4) have shown significant changes in RNA and protein synthesis during very early stages of infection and suggested that, for obligate parasites, an early influence of infection occurred in the nucleus of host cells which were compatible with the pathogen. Shaw (*personal communication*) and his colleagues appear to have found a unique RNAse in compatible infections, unique in the sense that it is kinetically distinct from nucleases of either the healthy or the rust fungus grown in culture.

In the case of radish infected with downy mildew, Asada *et al.* (sect. 6.1) have reported early activation of host enzymes responsible for the synthesis of lignin which may act as a barrier for rapid spread of the fungus. Yamamoto and Nakao (sect. 5.5) have shown an early increase of enzymatic protein involved in phenolic synthesis during incompatible infections of potato by *Phytophthora infestans*. The effect apparently could be mimicked by application of DNA extracted from the infected tissue. The work of Uritani *et al.* (sect. 6.6) and Doke *et al.* (sect. 5.2) also supports the concept that protein biosynthesis may be a critical factor in resistance. Finally, but by no means least, the work by Howes *et al.* (sect. 4.1) points to the possibility that specific RNA molecules may impart the specificity characteristic of rust diseases.

As mentioned above, the concept of resistance mechanisms, either passive or induced, has been a central theme in all our thinking. It may be a mistake, however, to assume that this is the only way in which specificity is obtained in plant disease. Fig. 4 summarizes several different aspects that may be common to all plant diseases.