Fundación Juan March

Workshop on Pathogenesis-Related Proteins in Plants

Organized by
V. Conejero and L. C. Van Loon

L. C. Van Loon
R. Fraser
J. F. Antoniw
M. Legrand
Y. Ohashi
F. Meins
T. Boller
V. Conejero
C. A. Ryan
D. F. Klessig
J. F. Bol
A. Leyva
F. García-Olmedo
The works summarized in this publication were presented by their authors at a Workshop held on 23rd to 25th October 1989 at the Parador Nacional “Luis Vives”, Valencia (Spain).

Impresión: Ediciones Peninsular. Tomelloso, 27. 28026 Madrid

GENERAL PROGRAMME OF THE
INTRODUCTION. V. Conejer
FIRST SESSION
AN INTRODUCTION TO PROTEINS. L.C. Van Loon.
PLANT-PATHOGEN INTERACTIONS-
PATHOGENESIS-RELATED RESISTANCE. J.F. Anto
ORAL PRESENTATIONS
SOME SALICYLATE-INDUCED EVENTS IN POTATO LEAVES. W.S. F.
BIOCHEMICAL AND IMMUNOLOGICAL STUDIES OF THE PRODUCTION OF PAT-PR-B PROTEINS IN P. TOMATOICUS BY CHALARA ELEGANS.
APOLASTIC PATHOGENESIS FROM THE INTERACTION (syn. Fulvia fulva) - Joosten
SECOND SESSION
CHARACTERIZATION OF POTATO LEAVES. Y. Ohashi.
HORMONAL REGULATION AFFECTING THE BASIC ISOMORPHIC GLUCANASE AND CHITINASE.
ORAL PRESENTATIONS
MAIZE PATHOGENESIS-RELATED CHARACTERIZATION AND CELL WALL METABOLISM. G. Burkard
CHITINASE AND B-1,3-GLUCANASE ACTIVITY IN TABACO BY INFECTED TABACO AND PHYTOPHthora NICOTIANAE. P. Ahl.
chitinase and α-1,3-glucanase in the observation that the two walls. More recently, such an strated directly. For example, of the saprophytic fungi, actions as small as 100 nM, appear to lack a similar

tions as small as 100 nM. inhibit the inhibitory activity lysis of the hyphal tips.

of different fungi have shown an antifungal activity only in -1,3-glucanase alone inhibited t fungi examined. In contrast, 3-glucanase have been found to with chitin-glucan cell walls, that the inhibitory activity

of fungal activity only in α-1,3-glucanases plant from outside, it may be ases and α-1,3-glucanases form iso be involved in the release citons) that act as chemical s. The vacuolar chitinases and line of defense, the vacuolar vading fungus only if there is all. This is the case when the ive response. It remains to be ung activity of combinations observed in vitro operates in a

ions in vivo.

VIRUS-INDUCED TOMATO PATHOGENESIS-RELATED (PR) PROTEINS AS COMPONENTS OF A GENERAL MECHANISM OF RESPONSE TO AFFlicting AGENTS

V. Conejero, J. M. Bélles, F. García-Breijo, R. Garro, J. Hernández-Yago, I. Rodrigo and P. Vera

Departamento de Biotecnología, E.T.S.I. Agrónomos, Universidad Politécnica de Valencia, Spain.

The finding that the polypeptides associated with viroid infection are not specified by the viroid but result from a disease-induced alteration of host metabolism prompted the search for specific proteins possibly implicated in the pathological response, later on termed pathogenesis-related (PR) proteins (1). Since the non-denaturing electrophoretic system normally used to detect tobacco PRs excluded basic proteins, a system with the capability of separating cationic proteins was tested. This approach led us to the detection of 10 PR proteins (C1 to C10) in tomato plants infected with CEV (2). These tomato PR proteins shared with those of tobacco the characteristics of being preferentially extracted at low pH and being relatively resistant to proteinase digestion. Tomato PR proteins were found to accumulate also as a consequence of silver and ethephon treatments in association with viroid-like pathogenic reactions (3). These results suggested that PRs and developmental aberrations are reflection at different levels of a general response mediated by ethylene.

The discovery of these proteins posed a number of challenging questions, among which the most important are their biochemical function and biological role, not only in viroid pathogenesis but in a more general system for transducing and responding to pathogenic or stressing signals. First findings in this respect have been made recently: characterization of P69 as an alkaline cysteine proteinase and P32 and P34 (respectively C7 and C6) as chitinases. Also, a β-1,3 glucanase has been assigned to a CEV-induced PR protein in tomato.

Critical to the unraveling of the biological role of these proteins is their in vivo localization. Immuno-gold-EM technique applied to study P1(p14) and P69 led to the discovery of two main locations for these proteins: the vacuole, in association with inclusion bodies, a newly described location, and the intercellular spaces of CEV-infected tomato leaves.

An interesting feature of PR proteins is their reputed resistance to degradation by endogenous proteases. Consistently, they have long half-lives. Nevertheless, they need to be turned-over. The fact that the intercellular space seems to be the compartment where they finally accumulate suggests that their degradation must be carried out there. To this respect, it has been found that some of the tomato PR
proteins are selectively degraded by an extracellular 37-kDa constitutive aspartyl protease that could be implicated in a regulatory mechanism for the biological action of PR proteins. The relevance of this enzyme in the PR metabolism is reinforced by the fact that we have found an analogous enzyme in intercellular washing fluids of tobacco plants.

Although the biological role of most of these proteins is as yet unknown the following must be stressed: i) chitinases and β-1,3 glucanases could be implicated in the defense against pathogens containing chitin or β-1,3 glucans as a component of their structure (bacteria and fungi); ii) the role of P88 protease either in symptom production or as a defense tool remains to be elucidated. iii) Although P1(p14), the most abundant tomato PR protein, was the first PR proteins whose entire sequence was determined (4), no biochemical function could be assigned to this protein. Nevertheless, P1(p14) has been found in leaves from healthy (non-infected) plants and always associated with cell material under disorganization. This suggested the idea that P1(p14) is involved in cell degeneration, either naturally activated as a normal event of the biological cycle of the plant (i.e. lysigenous development of intercellular spaces by cell ageing) or exogenously provoked by afflicting agents. The possible involvement of P1(p14) with the resistance induced in the systemic reaction of the host or with some other biological role is not discarded. The biological significance of P1(p14) is also sustained by the fact that this protein is synthesized as a pre-protein, then targeted to the vacuole and to the apoplast in association with F88. The vacuolar and apoplastic localization of chitinases have also been reported (5); iv) the reported evidence to date indicates that ethylene is an intermediary step (second messenger) in the coordinate activation of PR synthesis as part of the response to viroid infection.

All this suggests that the viroid-induced PR proteins, although with different biochemical activities, might have the common biological role of being part of an adaptive response against potential aggressions coming from the environment.

One could hypothesize that these coordinately interconnected defense tools have been surely built up in two steps: i) specific and individual development of each tool by coevolution with a given pathogen (in our case those containing chitin or β-1,3 glucan as part of their structure) or with certain aggressive environmental conditions; ii) interlinking of the individual specific responses. Thus, what it appears to be a non specific response results from evolutionary integration of specific individual components. With this strategy, the battery of defensive tools would be progressively enriched with new experiences. A prediction of this model would be the possible existence or future development of proteinases and nucleases against viruses and viroids.

REFERENCES