A Mutation Inhibiting Protoplasmic Incompatibility in *Podospira anserina* that Suppresses an Extracellular Laccase and Protoperithecium Formation

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INTRODUCTION

In *Podospira anserina*, protoplasmic incompatibility due to non-allelic gene interaction involves the genes of nine loci (Delettre & Bernet, 1976). Biochemical investigations, which were possible on the *R/V* interaction because of its temperature dependence, have shown that cell destruction is associated with the appearance of three enzyme activities absent in normal cells: a phenoloxidase (laccase III), and two proteolytic enzymes (proteases III and IV) thought to be responsible for cell lysis (Bégueret & Bernet, 1973).

In the present paper, we show that a mutation in gene *modC*, selected for the suppression of cell destruction, resulting from the *R/V* interaction (Labarère & Bernet, 1977), also inhibited formation of the three associated enzyme activities.

METHODS

Organism, strains and culture conditions. These have been described previously (Esser, 1974). The cross ♀ *rV* × ♂ *RV1* was the source of the thermosensitive self-lysing strain *RV* combining the incompatibility genes *R* and *V*. At 32 °C, this strain grows normally, but exposure to 26 °C, at which temperature the *R/V* interaction starts, results in cell destruction (Labarère, 1973). The heterokaryotic strain *modC*⁺ + *modC* was formed from homokaryotic plus strains, forced by non-allelic riboflavin-requiring mutations and fertilized by pouring a suspension of microconidia from a strain of the opposite mating type.

Preparation of cell-free extracts, chromatographic procedures, proteolytic activity and phenoloxidase (laccase) measurements. These procedures have been described previously (Boucherie *et al.*, 1976).

Drugs. Protease inhibitors, phenylmethylsulphonyl fluoride (PMSF) and ovomucoid were purchased from Sigma.

RESULTS AND DISCUSSION

The same three proteolytic enzymes were found in cell extracts of a normal strain and of *RV* strains grown at 32 °C (Boucherie *et al.*, 1976): the first two eluted (NaCl gradient on a DEAE-cellulose column) were basic enzymes (proteases A and B), sensitive to PMSF and ovomucoid; the third (protease C) was an acidic enzyme showing complete resistance to both inhibitors. When proteases were investigated in an *RV modC(1)* strain cultured at 32 °C, the elution profile again showed that proteolytic activity was distributed between three fractions (Fig. 1). Subsequent separation on Sephadex G-100 showed that these three enzyme activities were identical to proteases A, B and C. Thus, the *modC(1)* mutation had no effect on the proteases present in normal cells.

Previously, proteolytic activity in an *RV* strain cultured at 32 °C and shifted for 10 h to 26 °C was found to be distributed between four fractions (Bégueret & Bernet, 1973): the first two proteases eluted were proteases A and B; the later two were novel acid proteolytic enzymes (proteases III and IV) that were sensitive to PMSF and ovomucoid. However, when the *RV* strain carried the *modC(1)* mutation, the proteolytic activity elution profile was not

Fig. 1. Elution profile from DEAE-cellulose of the proteolytic enzymes of strain *RV modC(1)* after 3 d culture at 32 °C (●) and after 3 d culture at 32 °C plus 15 h exposure to 26 °C (○). The fractions pooled for further chromatography on Sephadex G-100 are indicated.

significantly modified by 15 h exposure to 26 °C (Fig. 1). Investigations of the third activity eluted revealed similarities with protease C: an apparent molecular weight of 40000 to 42000, complete resistance to PMSF and ovomucoid and an identical pH dependence curve. *ModC(1)* is thus the second example of a mutation (Boucherie *et al.*, 1976) which suppresses both cell lysis and its two associated specific proteases.

The *R/V* interaction also resulted in the presence in cell extracts of laccase III (Boucherie *et al.*, 1976). This enzyme activity was not found in an *RV modC(1)* strain, either when the strain was grown continuously at 32 °C or after 24 h exposure to 26 °C. In contrast to proteases III and IV, the laccase was found in normal strains, but it was present exclusively as an extracellular enzyme activity (Boucherie & Bernet, 1977). In the mutant strain *modC(1)*, laccase III was never found, either in cell extracts or in the culture filtrates. Thus the *modC(1)* mutation suppresses laccase III both in its extracellular (or normal) and intracellular (or pathological) forms.

Preliminary investigations on three independent *modC* mutations showed that they resulted in defects in the formation of protoperithecia (Labarère & Bernet, 1977). The mutant strain *modC(1)*, for instance, was completely devoid of protoperithecia. To see if this mutant trait exhibited an autonomous expression, we examined the heterokaryotic strain *modC⁺ + modC(1)*, forced by auxotrophic mutations. To the wild-type nucleus, the incompatibility gene *e(A)* was added. Because of its interaction with the *c(s)* gene, the presence of the *e(A)* gene reduces the fertility rate in the cross ♀ *e(A)* × ♂ *c(s)* to 2×10^{-4} when compared with that of the control crosses *c(s)* × *c(s)* or *e(A)* × *e(A)*. As expected, because of the recessiveness of the *modC(1)* mutation, the heterokaryotic strain *e(A) modC⁺ + modC(1)* produced protoperithecia at a normal density (20 per mm²). When *c(s)* microconidia were poured on the heterokaryon cultures, the fertility rate was 2×10^{-4} , i.e. that found for the homokaryotic *e(A)* cultures. Since the reaction preventing ♀ *e(A)* × ♂ *c(s)* hybridizations developed between the trichogyne *e(A)* and the microconidia *c(s)* (Esser, 1959; Bernet, 1965), it can be deduced that in the heterokaryotic strain *modC⁺ + modC(1)*, no compatible *modC(1)* trichogyne (or protoperithecium) was formed. Investigations of the progeny of the rare perithecia that developed (about 100 were examined) showed that they arose from *e(A) modC⁺* oogonia. Similar results were also obtained with a second mutation *modC(2)* that resulted in the formation of abnormal protoperithecia which never developed into perithecia. In the heterokaryotic strain *modC⁺ + modC(2)*, none of the fruiting bodies derived from the female *modC(2)* nucleus. Thus, from these experiments, we deduce that *modC⁺* expression is needed for normal protoperithecium formation since the *modC* genetic block was not relieved by its presence in a common cytoplasm with the wild-type gene *modC⁺*.

In conclusion, the *modC* mutation which abolishes cell lysis induced by the *R/V* interaction also prevents the formation of its associated specific enzymes. In addition, like bacterial mutations that pleiotropically inhibit exoenzymes and specific morphogenetic processes (Ayusawa *et al.*, 1975; Kunst *et al.*, 1974), the *modC(1)* mutation *per se* not only suppresses laccase III, an extracellular activity, but also abolishes protoperithecium formation.

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