A Pleiotropic Mutation Affecting Protoperithecium Formation and Ascospore Outgrowth in *Podospira anserina*

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A mutation designated *modD*, which was selected on the basis of the suppression of self-lysis, produced a decrease in the density of protoperithecia and a deficiency in ascospore outgrowth at 26 °C. Strains carrying the mutation were wild-type at 32 °C. Incubation of mutant ascospores revealed two abnormalities. First, the induction of ascospore outgrowth occurred only under a restricted range of conditions. Outgrowth of wild-type spores was normal down to 8 °C in a standard germination medium and could also be initiated in a non-germinating medium by the addition of actinomycin D or 5-fluorouracil. Mutant ascospores did not exhibit outgrowth in the presence of these two drugs or in a standard germination medium below 20 °C. The second defect was the death of 80% of mutant ascospores when they were incubated in germination medium at 26 °C. This killing was suppressed by β -phenylpyruvic acid, a protease inhibitor, and by a mutation (*modC*) which suppresses the lytic proteases associated with protoplasmic incompatibility and self-lysis. The killing of mutant spores was also suppressed by cycloheximide but was unaffected by actinomycin D and 5-fluorouracil.

INTRODUCTION

Mutations affecting the female sexual cycle in fungi have been described previously, especially in the ascomycete *Neurospora crassa* (Dodge, 1935; Horowitz *et al.*, 1960; Johnson, 1978; Mylik & Threlkeld, 1974; Srb *et al.*, 1973; Tan & Ho, 1970). Mutations inhibiting protoperithecium formation have also been reported in *Podospira anserina* (Esser, 1968; Belcour, 1971).

We have investigated mutations in *P. anserina* which were selected on the basis of the suppression of self-lysis and which also restrict female organ formation (Boucherie & Bernet, 1974). These mutations inhibit the formation of the specific proteases associated with cell destruction (Boucherie *et al.*, 1976a; Labarère & Bernet, 1977, 1978). In the present paper, we describe a thermosensitive mutation, selected on the same physiological basis, which affects both protoperithecium formation and ascospore germination.

METHODS

Organism. *Podospira anserina* is a heterothallic ascomycete whose life cycle closely resembles that of *Neurospora crassa*. Asci contain four binucleate spores that include the two products of a half tetrad. As a result of post-reduction these spores provide the heterokaryotic strains used for dominance and complementation tests. In about 5% of asci, one binucleate spore is replaced by two uninucleate spores. These give rise to the homokaryotic self-sterile (+ cr –) strains used to perform appropriate crosses and for F1 genetic analysis. For details, see Esser (1974).

Origin of the *modD* mutation. *Mod* mutations are selected by growth restoration of 'self-lysing' strains. In self-lysing strains, growth stops early in development and cells lyse as a result of either non-allelic incompatibility genes (for instance *C* and *E* or *V* and *R*) or a recessive mutation in the genes *lysA* or *lysB* (Bernet, 1965;

Delettre & Bernet, 1976). Mutations of the incompatibility genes and of *modA*, *modB* and *modC* genes have been selected from the self-lysing strains *CE*, *VR* and *lysA(I)* on the basis of growth restoration (Bernet & Belcour, 1967; Belcour & Bernet, 1969; Bernet, 1971; Boucherie & Bernet, 1974; Labarère & Bernet, 1977).

To select mutations in novel *mod* genes, we used an initial self-lysing strain of genotype *CE modA(I) modC(I)* (Labarère & Bernet, 1977). In this strain the cessation of growth is simply due to self-lysis and is not accompanied, as in *CE* and *VR* strains (Labarère *et al.*, 1974), by a defect in RNA synthesis. Consequently the *CE modA(I) modC(I)* strain (but not the *CE* strain) exhibits continuous growth on a medium supplemented with β -phenylpyruvic acid (1.5 mg ml^{-1}), a protease inhibitor. As previously shown (Labarère & Bernet, 1977), reversion to continuous growth in the *CE modA(I) modC(I)* strain may be obtained by mutation of the *C* or *E* genes, by reversion of *modC(I)* or by a dominant mutation in the gene *modB*. Mutations of the incompatibility genes produce revertant strains exhibiting the phenotype of the *modC(I)* mutation. Reversions of *modC(I)* and *modB* mutations result in revertant strains displaying a typical phenotype designated 'modified self-lysing' (Belcour & Bernet, 1969; Delettre & Bernet, 1976). Because of the dominance of these latter two mutations, the revertant strains were able to suppress the initial self-lysing phenotype when original and revertant nuclei were associated in a heterokaryon, employing the complementing auxotrophic mutations *ribA(I)* and *ribB(I)* (riboflavin-deficient). This property was used to identify further mutations of these two previously recognized types. The initial self-lysing strain carried the mutation *ribA(I)* and all the revertant strains were tested for the ability to form a freely growing prototrophic heterokaryon with a *CE modA(I) modC(I)* strain carrying the *ribB(I)* mutation.

For mutagenesis, the initial self-lysing strain *CE modA(I) modC(I) ribA(I)* was grown for 2 d on cellophane pads deposited on NG medium supplemented with β -phenylpyruvic acid (1.5 mg ml^{-1}) and riboflavin ($10 \mu\text{g ml}^{-1}$). These cellophane pads were placed in a solution containing $50 \mu\text{g N}$ -methyl- N' -nitro- N -nitrosoguanidine ml^{-1} for 10 min and then transferred directly to NG medium lacking β -phenylpyruvic acid, in which growth stopped immediately and the cells lysed. Of more than 500 revertant strains, 67 exhibited the 'modified self-lysing' phenotype. These strains were tested on minimal medium with the *CE modA(I) modC(I) ribB(I)* reference strain for the formation of a prototrophic heterokaryon. Only one revertant 'modified self-lysing' strain was unable to form such a heterokaryon and hence carried a recessive mutation in a gene different from *modA*, *modB* or *modC*. We have called this gene *modD*.

Media and temperature conditions. For growth and to perform crosses, two media which did not support the germination of ascospores (non-germinating media, NG) were used. These were a natural medium (basically corn meal extract) and a synthetic medium whose carbon source was urea (Esser, 1974). In these media, ascospore outgrowth generally did not exceed 2%. Ammonium, for which ammonium acetate (4 mg ml^{-1}) was the best source, was used as germinant and was added to both NG media to give standard germination (G) media. On G media ascospore germination could exceed 98%, but after mycelial growth no protoperithecia developed. The complete life cycle of *P. anserina* may be obtained at temperatures between 10 and 32 °C. The standard temperature for spore germination, mycelial growth, protoperithecium formation and perithecial development was 26 °C.

Drugs. β -Phenylpyruvic acid, a competitive inhibitor for certain proteolytic enzymes (Barnett, 1967; Geratz, 1965) suppressed cell destruction in the self-lysing strains and inhibited the proteolytic enzymes found in the cell extracts of normal strains as well as the lytic proteases specific to self-lysing strains (Delettre *et al.*, 1978). At the concentration used (1.5 mg ml^{-1}) the drug has no effect on the growth of normal strains. β -Phenylpyruvic acid, cycloheximide, 5-fluorouracil and actinomycin D (ref. A7890) were obtained from Sigma.

RESULTS

Effect of the modD mutation on protoperithecium formation and ascospore outgrowth

The revertant strain produced by the *modD* mutation from the self-lysing strain *CE modA(I) modC(I)* was crossed to a reference normal strain used as the female parent. About 40% of the uninucleate spores isolated in the hybrid fruiting bodies did not germinate when incubated under standard conditions for ascospore germination (26 °C, G medium). About 3% of the F1 progeny strains produced by those ascospores which did germinate exhibited a special phenotype characterized by reduced female fertility under conditions (26 °C, NG medium) that support the formation of protoperithecia of wild-type strains. In these partially female-sterile strains, while most of the mycelium was flat and deprived of female organs, rare protoperithecia developed in association with a few aerial hyphae. Analysis of the progeny of crosses involving these F1 strains as the female parent and the initial self-lysing

Table 1. *Protoperithecial density, perithecial density and ascospore outgrowth in modD and wild-type strains at 26 or 32 °C*

Strain	No. of protoperithecia mm ⁻²		No. of perithecia cm ⁻²		Percentage of germinated ascospores*	
	26 °C	32 °C	26 °C	32 °C	26 °C	32 °C
Wild-type	22.3 ± 3	18.1 ± 2.5	53.0 ± 5.1	55.3 ± 6.1	97.5	96
<i>modD</i>	3.1 ± 0.8	17.6 ± 2.7	6.5 ± 2.2	49.4 ± 5.2	23.5	94

* 200 ascospores were incubated.

Table 2. *Effect of temperature at different stages of the female sexual cycle on the germination of modD ascospores*

Growth and protoperithecial formation temp. (°C)	Perithecial development and ascospore formation temp. (°C)	Ascospore incubation temp. (°C)	Percentage of germinated ascospores*
26	26	26	23.5
26	26	32	91.0
26	32	26	20.5
26	32	32	89.5
32	26	26	20.5
32	26	32	86.5
32	32	26	19.5
32	32	32	83.5

* 200 ascospores were incubated.

strain as the male parent showed that the partially female-sterile F1 strains carried the *modD* mutation responsible for the reversion of the initial self-lysing phenotype.

We investigated 32 F1 strains exhibiting reduced female fertility. In crosses involving each of these strains and a reference normal strain, ungerminated ascospores were found. When the F1 *modD* strains were grown at 32 °C, the mycelia were covered with aerial hyphae and protoperithecia were uniformly distributed at nearly wild-type density (20 mm⁻²) (Table 1). Following fertilization, the perithecial density and the outgrowth rate of *modD* ascospores were also practically normal at 32 °C (Table 1). The co-segregation of the mutant defects in protoperithecial formation and ascospore outgrowth and their coupled thermosensitivity suggested that they were the consequence of a single mutation. Germination depended only on the temperature during incubation of the ascospores and not on the temperature of protoperithecial and perithecial development or ascospore formation (Table 2).

Microscopic examination of *modD* mycelia grown at 26 °C prior to their fertilization did not reveal aborted protoperithecia. Furthermore *modD* perithecia produced at 26 °C were phenotypically normal and contained wild-type numbers of asci. Ungerminated *modD* spores examined after 2 d incubation under germination conditions showed no external sign that germination had started. Thus, the *modD* genetic block acts only in the early stages of protoperithecium formation and in ascospore outgrowth.

A killing process occurs during incubation of modD ascospores

Non-germinated *modD* ascospores were isolated after 12 h incubation in a germination (G) medium at the non-permissive temperature (26 °C). These spores did not exhibit outgrowth when submitted to a second incubation at the permissive temperature (32 °C) (Table 3, expt A). In contrast, when *modD* ascospores were kept on a non-germinating (NG) medium for 36 h at 26 °C and then incubated in permissive conditions, nearly all of them

Table 3. *Effect of extrinsic and intrinsic factors on modD and wild-type (+) ascospore outgrowth*

Expt	Ascospore genotype	First incubation (at 26 °C) No. of ascospores*			Second incubation (at 32 °C) No. of ascospores*		
		T	G	NG	T	G	NG
		G medium			G medium		
A	<i>modD</i>	100	21	79	79	1	78
	+	100	98	2	2	0	2
B		NG medium			G medium		
	<i>modD</i>	100	0	100	95	91	4
	+	100	3	97	92	90	2
C		G medium + β PPA†			G medium		
	<i>modD</i>	100	96	4	3	0	3
	+	100	93	7	5	0	4
D1‡		G medium			G medium		
	+	32	32	0			
	+	28	26	2	2	0	2
	<i>modD</i> +	28	6	22	22	0	22
	<i>modD modC</i>	32	26	6	6	0	6
D2	<i>modD</i> + <i>modD modC</i>	300	{ 38 110 }	152	150	{ 2 3 }	145
E		G medium			G medium		
	+	56	55	1	1	0	1
	<i>modD</i> / +	88	86	2	2	0	2
	<i>modD</i> / <i>modD</i>	56	9	47	47	1	46

* T, Total number of spores incubated in the conditions indicated; G and NG, numbers of germinated and non-germinated spores, respectively. Non-germinated spores were submitted to a second incubation.

† β PPA, β -Phenylpyruvic acid at 1.5 mg ml⁻¹.

‡ In the cross + + $\times$ *modD modC*, uninucleate spores were isolated from asci including two uninucleate spores in each half ascus. The genotype of non-germinated spores was deduced from tetrad analysis and from the genotype of germinated ascospores.

exhibited outgrowth (Table 3, expt B). These two experiments indicated that *modD* ascospores were killed during incubation in a G medium at the non-permissive temperature: 3 h incubation at 26 °C was sufficient for spore killing. When *modD* spores were transferred to 32 °C after only 3 h at 26 °C on G medium, ascospore germination was identical to that of ascospores directly incubated at 26 °C (about 20%). The killing of *modD* spores at 26 °C was suppressed by the addition of β -phenylpyruvic acid to the G medium (Table 3, expt C). Phenylalanine and tyrosine, which have biochemical structures related to that of β -phenylpyruvic acid, did not protect *modD* spores against killing, indicating that the suppression of ascospore killing by β -phenylpyruvic acid was a result of its acting as a protease inhibitor. It appears, therefore, that the death of the ascospores is due to the presence or an excess of proteolytic activity.

Proteolytic activity in normal and in self-lysing strains has been investigated previously (Bégueret & Bernet, 1973; Boucherie *et al.*, 1976a). The *modC* mutation suppresses proteases specific to self-lysing strains without affecting those from normal cells (Labarère & Bernet, 1978). We observed that the germination rate for *modD modC* spores in the progeny of the cross ♀ + + $\times$ ♂ *modD modC*, submitted to tetrad analysis, was nearly normal (Table 3, expt D1). Similar results were also obtained for the progeny of the cross ♀ *modD* + $\times$ ♂ *modD modC* (Table 3, expt D2). The near wild-type germination of the *modD modC* double mutant indicates that the *modC* mutation suppresses the *modD* mutation and provides further evidence that the ascospore killing produced by *modD* is due to proteolytic activity.

In the progeny of the cross ♀ + $\times$ ♂ *modD*, the post-reduction rate for the *modD* locus

Fig. 1. Effect of temperature on *modD* spore outgrowth. Ascospores (100) were first incubated in G medium at different temperatures. Some spores exhibited outgrowth (G). Non-germinated spores were then incubated at 32 °C in G medium. Ascospores which germinated during the second incubation were considered to be spores in which germination was not induced (GNI) under the initial incubation conditions; those which did not germinate were considered to be killed (K) spores. ○, Percentage of germinated ascospores during the first incubation (11, 16, 20, 26 or 32 °C); ●, percentage of germinated ascospores during the second incubation (32 °C).

was estimated to be 0.47 ± 0.06 . Binucleate spores were investigated to determine the recessiveness or the dominance of the *modD* mutation. The outgrowth rate for heterokaryotic spores of genotype *modD/modD*⁺ was identical to that for wild-type spores (Table 3, expt E), indicating that the *modD* mutation is recessive.

Germination of modD ascospores occurs in restrictive temperature conditions

The results presented above suggest that the killing of *modD* ascospores occurs at 26 °C under conditions normally inducing outgrowth. We investigated the effect of a wider range of temperatures from 11 to 32 °C over which wild-type ascospore outgrowth was nearly 100%. The outgrowth of *modD* ascospores was less than 3% when the temperature during incubation was lower than 20 °C (Fig. 1). Non-germinated spores from the first incubation at each temperature were exposed to a second incubation in a G medium at 32 °C, where most of them exhibited outgrowth. Thus, at low temperatures, incubation of *modD* ascospores did not result either in spore outgrowth or in self-killing. This is an additional argument supporting the idea that in *modD* ascospores self-killing and outgrowth are the alternative consequences of breaking ascospore dormancy.

These results were not affected by the temperature during peritheciium development and ascospore formation or by the presence of the *modC* mutation. Ascospores of *modD* genotype that developed at 32 °C failed to germinate at incubation temperatures below 20 °C. The addition of β -phenylpyruvic acid did not affect the germination rate of *modD* ascospores at 11 and 16 °C. Furthermore, ascospores isolated in the progeny of the cross ♀ *modD* + × ♂ *modD modC* did not exhibit a higher germination rate than *modD* spores. Thus, in addition to the killing effect, *modD* ascospores possess a defect restricting the range of temperatures that can break dormancy compared with wild-type ascospores. In contrast to self-killing, this additional defect was not suppressible by the *modC* mutation or by β -phenylpyruvic acid. Cold-sensitivity was specific to *modD* ascospores since the rates of increase in mycelial diameter of *modD* and wild-type strains were similar at temperatures between 8 and 32 °C.

Table 4. *Effect of cycloheximide on modD and wild-type ascospore outgrowth*

Expt	Ascospore genotype	First incubation (at 26 °C) No. of ascospores*			Second incubation (at 32 °C) No. of ascospores*		
		T	G	NG	T	G	NG
A		G medium + CH† (7 h)			G medium		
	<i>modD</i>	100	0	100	93	85	8
	+	100	0	100	96	93	3
B		G medium + CH† (7 h)			NG medium		
	<i>modD</i>	100	0	100	89	2	87
	+	100	0	100	93	68	25

* See footnote to Table 3.

† CH, Cycloheximide at 5 µg ml⁻¹.Table 5. *Effect of actinomycin D on modD and wild-type ascospore outgrowth*

Expt	Ascospore genotype	First incubation (at 26 °C) No. of ascospores*			Second incubation (at 32 °C) No. of ascospores*		
		T	G	NG	T	G	NG
A		G medium + AcD† (7 h)			G medium		
	<i>modD</i>	100	3	97	92	2	90
	+	100	96	4	3	0	3
B		NG medium + AcD† (15 h)			G medium		
	<i>modD</i>	100	0	100	95	88	7
	+	100	91	9	7	1	6

* See footnote to Table 3.

† AcD, Actinomycin D at 10 µg ml⁻¹.

Effect of protein synthesis inhibitors on the killing effect and on the breaking of dormancy of modD ascospores

Cycloheximide. When cycloheximide (5 µg ml⁻¹) was added to the standard G medium, no outgrowth of wild-type ascospores occurred in about 15 h and nearly all spores exhibited outgrowth during a second incubation in a G medium lacking cycloheximide. Similarly when *modD* ascospores were incubated at 26 °C for 7 h in G medium supplemented with cycloheximide and then transferred to G medium at 32 °C, they nearly all exhibited outgrowth (Table 4, expt A). This experiment showed that cycloheximide prevented the killing effect. The drug also maintained spore dormancy in the *modD* ascospores since most of them did not germinate after they were transferred to NG medium at 32 °C (Table 4, expt B). In the control, wild-type ascospores germinated during the second incubation (Table 4, expt B). It seems probable that wild-type spores, in contrast to *modD* spores, initiated outgrowth on G medium despite the presence of cycloheximide.

Actinomycin D. On G medium, actinomycin D at 10 µg ml⁻¹ did not prevent the killing of *modD* spores (Table 5, expt A). Under the same conditions, wild-type spores exhibited outgrowth and subsequent development of hyphae of about 200 µm in length. Wild-type hyphae grew up to 300 µm with actinomycin D at 5 µg ml⁻¹ and formed colonial mycelia (4 mm diam.) at 2.5 µg ml⁻¹. At 25 µg ml⁻¹, wild-type spores did not show any phenotypic sign that germination had started.

Identical development was observed when wild-type ascospores were incubated on NG medium supplemented with actinomycin D (Table 5, expt B). At 2.5, 5 and 10 µg ml⁻¹, ascospore outgrowth amounted to over 90%, whereas it was only 2% in the absence of the

Table 6. Effect of 5-fluorouracil on *modD* and wild-type ascospore outgrowth

Expt	Ascospore genotype	First incubation (at 26 °C) No. of ascospores*			Second incubation (at 32 °C) No. of ascospores*		
		T	G	NG	T	G	NG
A		G medium + 5FU† (7h)			G medium		
	<i>modD</i>	100	0	100	97	1	96
	+	100	95	5	5	2	3
B		NG medium + 5FU† (7h)			G medium		
	<i>modD</i>	100	0	100	95	88	7
	+	100	85	15	13	1	12

* See footnote to Table 3.

† 5FU, 5-Fluorouracil at 75 µg ml⁻¹.

drug. With *modD* spores, no external modification suggesting that germination had started was observed, irrespective of the concentration of the drug. The *modD* ascospores did germinate when transferred to G medium at 32 °C indicating that killing had not occurred (Table 5, expt B).

5-Fluorouracil. 5-Fluorouracil gave similar results to actinomycin D. It did not prevent the killing of *modD* ascospores when added to G medium at 26 °C (Table 6, expt A) but induced wild-type ascospore germination when added to NG medium (Table 6, expt B). Subsequent development of wild-type spores depended on the concentration of 5-fluorouracil. Colonies of 5 mm diam. were formed at 25 µg ml⁻¹ and primary hyphae up to 350 µm long developed at 75 µg ml⁻¹. At 100 µg ml⁻¹ development stopped just after protoplasmic emergence. Uracil had no effect on wild-type ascospore outgrowth. When added to NG medium (10 to 200 µg uracil ml⁻¹), the wild-type ascospore germination rate did not exceed the 2% obtained in standard NG medium.

DISCUSSION

Because of the co-segregation and the thermosensitivity of its two associated defects, it is highly probable that the *modD* mutant strain results from a single mutation. A common function is thus involved in an early stage of protoperithecium formation and in ascospore outgrowth. Especially significant was the fact that the mutation did not result in aborted or malformed protoperithecia and did not alter perithecial development or ascus and ascospore formation. The function impaired by the *modD* mutation is thus involved in two short and critical stages of the life cycle. These two stages have in common the initiation of a developmental process in a resting protoplasm; a cell at the stationary stage for protoperithecium formation and a dormant cell for ascospore outgrowth. This combination of defects is unique in fungi and can only be compared with a thermosensitive asporogenous mutant of *Bacillus cereus* which also exhibits a deficiency in spore germination (Cheng *et al.*, 1978).

Killing of *modD* ascospores occurs after 2 h incubation. It is suppressed by β-phenylpyruvic acid. No doubt the lytic protease(s) suspected of being responsible for spore death are coupled to the onset of ascospore germination. The *modD* mutant strain may be compared to the mutant strains described as self-lysing, except that in the latter protoplasmic disintegration occurs during mycelial growth (Bernet, 1965; Delettre & Bernet, 1976) instead of at the onset of ascospore germination. Additional support for this comparison is given by the demonstration that the *modC* mutation, which prevents cell destruction in a self-lysing strain (Labarère & Bernet, 1977), also suppresses *modD* ascospore killing. Thus, the lytic protease(s) whose formation in a self-lysing strain is inhibited by *modC* (Labarère & Bernet, 1978) is probably involved in the aborted outgrowth of *modD* ascospores.

The physiological basis on which the *modD* mutation was selected gives additional support to the preceding interpretation of spore killing by this mutation. The primary effect of the *modD* mutation is the suppression of self-lysis induced, in the genotype *CE modA(1) modB(1)*, by the non-allelic incompatibility gene interaction *C/E*. In contrast, when the *modD* mutation was isolated from its original genetic background it resulted in protease overproduction during ascospore incubation. Mutations exerting opposite effects on the proteases associated with protoplasmic incompatibility have been reported for the *modB* (Boucherie *et al.*, 1976*b*; Boucherie & Bernet, 1977) and *modC* genes (Labarère & Bernet, 1978). This was explained by postulating that these proteases were under the control of a multiprotein regulatory complex, of which the products of the *mod* genes and the incompatibility genes were interacting components.

Previous work, involving mutations suppressing the proteases specific to self-lysing strains, suggested that these enzymes might be involved in the early stages of protoperithecium formation (Boucherie *et al.*, 1976*a*; Boucherie & Bernet, 1974). The pleiotropic effect of the *modD* mutation now suggests that these proteases might also be normally involved in ascospore outgrowth and, thus, that the death of *modD* spores during incubation may simply result from protease overproduction.

The killing of *modD* spores was suppressed by cycloheximide suggesting that the formation of the lytic protease(s) requires protein synthesis. This synthesis is resistant to actinomycin D and 5-fluorouracil. The possibility that these two drugs did not enter *modD* ascospores can probably be rejected since they entered wild-type ascospores, and two other drugs, β -phenylpyruvic acid and cycloheximide, entered *modD* ascospores. The lack of effect of actinomycin D and 5-fluorouracil on *modD* ascospore killing may signify that the expression of the lytic protease(s) is dependent on the products of pre-existing mRNA molecules. The presence of stable messengers in fungal spores is strongly suspected (Hollomon, 1969; Brambl & Van Etten, 1970; Ramsey & Workin, 1970).

The *modD* ascospores also exhibited additional defects. The ability to respond to ammonium acetate as germinant was cold-sensitive. This modification of the external conditions required for ascospore germination suggests that the *modD* gene product has a role in breaking spore dormancy. This hypothesis is currently being tested by investigating the role, as germinants and anti-germinants, of different ions to which *modD* ascospore outgrowth is especially sensitive.

Additional support for the role of the *modD* gene in the initiation of ascospore outgrowth was given by the failure of actinomycin D and 5-fluorouracil to induce germination of *modD* spores. Wild-type ascospores exhibited outgrowth when these drugs replaced ammonium acetate, the usual germinant. This effect of actinomycin D and 5-fluorouracil suggests that *Podospira anserina* ascospores do not germinate on NG medium because of the presence, in isolated spores, of the products of a DNA-dependent synthesis. This conclusion needs further investigation. The fact that actinomycin D and 5-fluorouracil had no germinant effect on *modD* ascospores suggests that these spores have defects in the system involved in breaking dormancy. It is not known if these drugs induce spore germination in other fungal species.

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