JOURNAL OF BACTERIOLOGY, Aug. 1984, p. 808–810
0021-9193/84/080808-03$02.00/0

Vol. 159, No. 2

Conidia Induce the Formation of Protoperithecia in *Neurospora crassa*: Further Characterization of White Collar Mutants

F. DEGLI-INNOCENTI, J. A. A. CHAMBERS, AND V. E. A. RUSSO*

Max-Planck-Institut für Molekulare Genetik, Abteilung Trautner, D-1000 Berlin 33, Federal Republic of Germany

Received 3 January 1984/Accepted 23 May 1984

The treatment of undifferentiated mycelia with heavy suspensions of their own conidia triggers protoperithecial development. This effect was also observed with white collar (*wc*) mutants and suggests that the *wc* genes are not structural genes necessary for morphogenesis of protoperithecia but that they are probably involved in regulation.

The development of protoperithecia is a very important process in the life cycle of *Neurospora crassa* (1, 15). It involves a great number of genes (8, 12, 14, 17) which can act only under proper environmental conditions. Important factors are growth temperature (13), the amount of nitrogen in the growth medium (7), oxygen and carbon dioxide (18), and blue light (4). Formation of protoperithecia can be obtained by means of extracts of mated and unmated *Neurospora* cultures (9, 10).

In this paper, we present another system for induction of protoperithecia formation: the treatment of undifferentiated mycelia with heavy, suspensions of their own conidia (macroconidia). It has been shown that white collar (*wc*) mutations affect the formation of protoperithecia (5). To clarify the role of *wc* genes in the protoperithecial development, we have determined the response of the mutants to this stimulus.

Strains and experimental conditions. We used strains described elsewhere (5) and the following *wc* strains of *A* mating type (mt) obtained with the set of crosses previously described (5): R95, *wc-1* (P829); R99, *wc-2* (ER33); R102, *wc-1* (ER57); and R161, *wc-2* (234W). Growth conditions and media were as previously described (5). To remove accumulated conidia, the edges of the plates were cleaned with wet tissues 3, 4, 7 days after inoculation. No obvious differences in the production of conidia of the wild-type (WT) and *wc* mutants were found.

When studying the induction of perithecia formation, each mycelium was kept strictly in the dark only until day 5 and was fertilized on day 7 with conidia of the corresponding strain having the opposite mating type. Drops (5 μl) of suitable dilutions (1×10^6 conidia per ml) of conidial suspensions were applied on the surface of the mycelia in areas having a few or no protoperithecia. After ca. 3 weeks, the perithecia in the fertilized areas and the protoperithecia in the surrounding unfertilized areas were counted.

When we tested the induction of protoperithecia formation, mycelia were always kept in the dark or under red safety light (Philips red TL40W/15). The stock conidial suspensions were centrifuged, the supernatants were collected, and the conidia were washed twice and then suspended in sterile double-distilled water. On day 5, drops (5 μl) of the washed conidial suspension (containing ca. 2×10^7 conidia per ml) and supernatant were applied for each strain on the surface of the corresponding mycelium. After 3 days, the developed protoperithecia were counted. In other experiments, nonviable conidia were used as a control instead of the supernatants. The conidial suspensions used by us as stock were obtained as previously described (3) and kept frozen until use.

Induction of perithecia. Perithecia are usually obtained from *N. crassa* by coinoculating spores of the two mt or by fertilizing developed protoperithecia with conidia of the opposite mt (1). We have observed that application of heavy suspension of conidia on mycelia of opposite mt. devoid of protoperithecia will cause a local and intense production of protoperithecia, which then develop in fertile perithecia (Fig. 1a and c). The number of protoperithecia in the surrounding mycelium remains very low. On the other hand, the fertilization of mycelia which have already developed many protoperithecia, such as WT grown in the light, causes a lower production of perithecia (Fig. 1b and d; Table 1). It appears that conidia cause a massive local production of new protoperithecia which then develop into perithecia only when few or no protoperithecia are present at the moment of

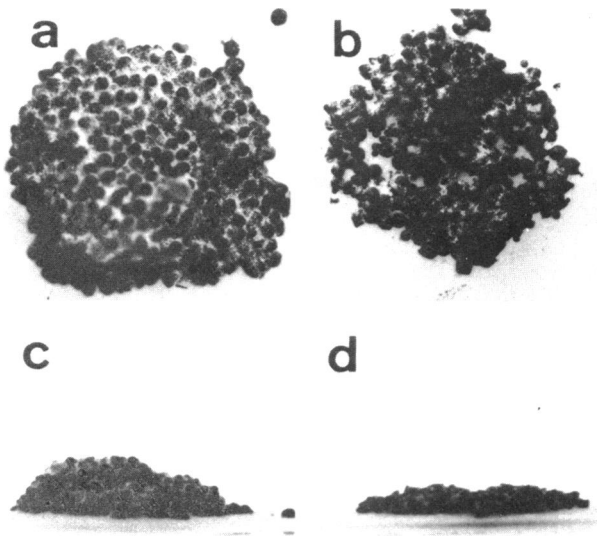

FIG. 1. Perithecia inducd by fertilizing WT mycelia. a (top) and c (side), Mycelium grown in the dark (few protoperithecia developed); b (top) and d (side), mycelium grown in the light (many protoperithecia developed). Magnification, ×3.

* Corresponding author.

TABLE 1. Induction of perithecia in WT (R2), *wc-1* (R97), and *wc-2* (R100) strains[a]

Female parent (mt, *a*)	Male parent (mt, *A*)	Experimental conditions[b]	Protoperithecia/cm²[c]	Perithecia/cm²[d]	Protoperithecia/cm²[e]
R2	R1	Light	748 ± 267	365 ± 17	642 ± 118
R2	R1	Dark	30	>400	15 ± 4
R97	R95	Light	0	>400	3.1 ± 2.1
R97	R95	Dark	0	>400	7.6 ± 3.8
R100	R99	Light	0	>400	27 ± 6
R100	R99	Dark	0	>400	16 ± 4

[a] Data shown are the average of two to four crosses ± standard error of the mean.
[b] Mycelia were grown as described in the text (Light) or strictly in the dark (Dark). In this case, a red safety light was used during the spotting.
[c] Number of protoperithecia per square centimeter present in the area of mycelium spotted with conidia at the moment of spotting.
[d] Number of perithecia per square centimeter developed in the spotted area after 3 weeks from spotting.
[e] Number of protoperithecia per square centimeter present after 3 weeks from spotting in four fields of vision in the unfertilized mycelium around the spotted area.

spotting. In *Neurospora tetrasperma*, the fertilization of protoperithecia is associated with a marked suppression of the production of additional protoperithecia (2). All *wc* strains, when treated with conidia of the same *wc* allele but opposite mt, reacted like the two *wc* strains (Table 1), although strains containing the *wc-2* mutation 234W (R162 and R161) produced few perithecia (data not shown). Our results are in agreement with an observation of Dodge, although there are species-specific differences (6).

Induction of protoperithecia. We asked whether it was also possible to induce the formation of protoperithecia with suspensions of conidia of the same mating type. In all strains except R162, the treated areas yielded a significantly higher number of protoperithecia than the control, though with a rather high variability. Although the number of conidia-induced protoperithecia varies from strain to strain, the treated/control ratio is, in most cases, similar to that for the WT (Table 2). The induction kinetics can indicate whether such differences are due to various rates of development. The induction of perithecia (see above) suggests that this probably is the case since the strains (except R162) produce approximately equal amounts of perithecia after 3 weeks of incubation. Table 2 indicates the following. (i) The induction of sexual structures is independent of mt. When mycelium and conidia have the same mt, protoperithecia do not develop into perithecia because only one mt factor is present. (ii) The induction is not due to the carotenoids present in the conidia since albino (*al*) conidia act as inducers. (iii) The *wc* mutants develop protoperithecia by treatment with their own conidia. This indicates that the *wc* mutations do not block the protoperithecial morphogenesis. A discussion about the regulatory processes impaired by the *wc* mutations has been already presented (5).

TABLE 2. Induction of protoperithecia with conidia[a]

Strain	No. of expts	No. of protoperithecia/cm² ± SEM with		Ratio (A/B)
		Conidia (A)	Control (B)	
WT	5	656 ± 173	92 ± 27	7.1
R97 (*wc-1*)	6	64 ± 14	4.3 ± 1.2	14.8
R103 (*wc-1*)	4	821 ± 60	40 ± 7.5	20.5
R110 (*wc-1*)	6	36.8 ± 8.4	2.3 ± 0.8	16
R122 (*wc-1*)	4	106 ± 42	15 ± 6	7
R126 (*wc-1*)	4	9.6 ± 3.4	0.9 ± 0.4	10.6
R131 (*wc-1*)	4	101 ± 22	7.8 ± 1.5	12.9
R145 (*wc-1*)	3	204 ± 68	16.4 ± 8.8	12.4
R89 (*wc-2 ts*)	6	242 ± 40	44 ± 13	5.5
R100 (*wc-2*)	6	56 ± 10	15 ± 5.7	3.7
R162 (*wc-2*)	3	3.7 ± 1.1	1.8 ± 0.5	2
R165 (*ws-2 ts*)	6	125 ± 26	29 ± 6	4.3
R184 (*al-2*)	2	72 ± 25	14.7 ± 4.8	4.8
R196 (*al-2*)	2	272 ± 97	22 ± 5.5	12.3
R200 (*al-1*)	2	599 ± 42	44 ± 26	13.6

[a] Mycelia of each strain were spotted (A) with a suspension of conidia of the same strain and (B) with the supernatant of the same centrifuged suspension. Each experiment had four replicates.

Further experiments with strains R162 (the least reactive) and R103 (the most reactive) have been done to determine the role played by conidia and mycelium in causing strain-specific responses and to answer the question, what is responsible for the lack of protoperithecium induction in strain R162? Mycelia of the two strains have been treated with conidia of other strains. Table 3 shows that R103 mycelia are induced whatever the inducer strain; induction with R162 conidia is very marked. R162 mycelia are induced by conidia of other strains much more than by its own conidia. The results indicate that R103 mycelia can supply the deficiency of R162 conidia, and conidia of other strains can supply the deficiency of R162 mycelia. Strain R162 is not isogenic with the WT. Heated or UV-killed conidia do not induce protoperithecia (Table 3).

Genotype of conidia-induced protoperithecia. Do the inducing conidia act only as a stimulus for the mycelium, or do they participate directly in the morphogenesis? In this case, part or all of the protoperithecia should have the genotype of the conidia (11). We used *wc-1* and *wc-2* mutations as markers of the mycelium and conidia in crosses. Protoperithecia induced in *wc-1* mycelia with *wc-2* conidia were themselves fertilized with conidia of the opposite mt (either *wc-1* or *wc-2* as indicated). The phenotype of random ascospores was checked. Table 4 indicates that nuclei of the inducing conidia become incorporated into the meiocytes. This suggests that the induction occurs through the formation of heterokaryon composed of nuclei of the conidia and mycelium.

Concluding remarks. Further experiments will be necessary to determine the role played by conidia in the mechanism of induction. However, it is interesting to note that conidia are differentiated cells which have very particular biochemical and physiological characteristics (16). In the mycelium, the biosynthesis of carotenoids as well as the formation of protoperithecia are induced by blue light. On the contrary, the conidia are able to bypass the photoregulation and produce carotenoids constitutively also in the dark. The *wc* genes are not necessary for this constitutive production. The fact that with conidia it is possible to induce the formation of protoperithecia in the dark both in WT and *wc*

TABLE 3. Induction of protoperithecia with conidia[a]

Mycelium	Conidia	No. of protoperithecia/cm² ± SEM induced with:				Ratio (A/B)
		Viable conidia (A)	Heated conidia[b]	UV-treated conidia[c]	No treatment[d] (B)	
R103 (*wc-1*)	R103 (*wc-1*)	487 ± 83	31 ± 12	8.9 ± 5.2	17 ± 2	28
R103 (*wc-1*)	R162 (*wc-2*)	1,209 ± 229	34 ± 9		19 ± 3	63
R103 (*wc-1*)	R126 (*wc-1*)	283 ± 109	31 ± 6		14 ± 3	20
R103 (*wc-1*)	R100 (*wc-2*)	1,191 ± 255	25 ± 5		13 ± 3	91
R103 (*wc-1*)	R2 (WT)	970 ± 184	53 ± 8		33 ± 7	29
R162 (*wc-2*)	R162 (*wc-2*)	26 ± 8	5 ± 2		7 ± 2	3.7
R162 (*wc-2*)	R103 (*wc-1*)	409 ± 105	9 ± 6	5.1 ± 2	7 ± 2	58
R162 (*wc-2*)	R126 (*wc-1*)	96 ± 20	12 ± 5		7 ± 1	13
R162 (*wc-2*)	R100 (*wc-2*)	320 ± 107	6 ± 3		3 ± 1	106
R162 (*wc-2*)	R2 (WT)	244 ± 89	10 ± 4		6 ± 2	40

[a] Mycelia were spotted with suspensions of viable or killed conidia of various strains. Data shown are the average of two to three experiments with four replicates ± standard error of the mean unless otherwise specified.
[b] Conidia heated for 20 min at 60°C. Survival: ca. 2×10^{-7} or less.
[c] Conidia irradiated with UV light from a germicidal lamp. Survival: 4×10^{-7}. Data are the average of one experiment with four replicates ± standard error of the mean.
[d] The number of protoperithecia in four fields of vision around the spotted areas were counted.

TABLE 4. Genetic origin of ascogonia[a]

Mycelium[b]	Conidia[c]	Male parent[d]	WT ascospores	Total ascospores	WT ascospores (%)
R103 (*wc-1*)	R103 (*wc-1*)	R102 (*wc-1*)	0	400	0
R103 (*wc-1*)	R162 (*wc-2*)	R102 (*wc-1*)	52	426	12.2
R103 (*wc-1*)	R162 (*wc-2*)	R161 (*wc-2*)	64	288	22.2
R103 (*wc-1*)	R100 (*wc-2*)	R102 (*wc-1*)	44	351	12.5
R103 (*wc-1*)	R100 (*wc-2*)	R99 (*wc-2*)	45	390	11.5

[a] Phenotypic analysis of progeny resulting from fertilization of the protoperithecia induced with conidia. Data shown have been obtained by two sets of crosses. Only mycelia from single ascospores were considered.
[b] Mycelium treated with conidia (mt, *a*).
[c] Strain used as inducer (mt, *a*).
[d] Strain used for fertilization (mt, *A*).

mutants leads to the interesting supposition that in conidia an inducer capable of mimicking or bypassing the action of blue light is present.

We thank C. Ernsting and N. Götz for technical assistance and R. Bachman for secretarial assistance.

This work was partially supported by the Deutsche Forschungsgemeinschaft.

LITERATURE CITED

1. **Beadle, G. W.** 1945. Genetics and metabolism in *Neurospora*. Physiol. Rev. **25**:643–663.
2. **Bennett, S. N., and H. B. Howe, Jr.** 1980. A quantitative study of protoperithecial and perithecial development in *Neurospora tetrasperma*. Trans. Br. Mycol. Soc. **74**:51–59.
3. **Davis, R. H., and F. J. De Serres.** 1970. Genetic and microbiological research techniques for *Neurospora crassa*. Methods Enzymol. **17A**:79–143.
4. **Degli-Innocenti, F., U. Pohl, and V. E. A. Russo.** 1983. Photoinduction of protoperithecia in *Neurospora crassa* by blue light. Photochem. Photobiol. **37**:49–51.
5. **Degli-Innocenti, F., and V. E. A. Russo.** 1984. Isolation of new white collar mutants of *Neurospora crassa* and studies on their behavior in the blue light-induced formation of protoperithecia. J. Bacteriol. **159**:757–761.
6. **Dodge, B. O.** 1946. Self-sterility in "bisexual" heterocaryons of *Neurospora*. Bull. Torrey Bot. Club **73**:410–416.
7. **Hirsch, H. M.** 1954. Environmental factors influencing the differentiation of protoperithecia and their relation to tyrosinase and melanin formation in *Neurospora crassa*. Physiol. Plant. **7**:72–97.
8. **Ho, C. C.** 1972. Mutations blocking development of the protoperithecium in *Neurospora*. Neurospora Newsl. **19**:15–16.
9. **Islam, M. S., and J. Weijer.** 1969. Sex hormones in *Neurospora crassa*. Neurospora Newsl. **15**:24–25.
10. **Islam, M. S., and J. Weijer.** 1972. Development of fertile fruit bodies in a single-strain culture of *Neurospora crassa*. Folia Microbiol. **17**:316–319.
11. **Johnson, T. E.** 1976. Analysis of pattern formation in *Neurospora* perithecial development using genetic mosaics. Dev. Biol. **54**:23–36.
12. **Johnson, T. E.** 1978. Isolation and characterization of perithecial mutants in *Neurospora*. Genetics **88**:27–47.
13. **McNelly-Ingle, C. A., and L. C. Frost.** 1965. The effect of temperature on the production of perithecia by *Neurospora crassa*. J. Gen. Microbiol. **39**:33–42.
14. **Mylyk, O. M., and S. F. H. Threlkeld.** 1974. A genetic study of female sterility in *Neurospora crassa*. Genet. Res. **24**:91–102.
15. **Rotschild, H., and S. R. Suskind.** 1966. Protoperithecia in *Neurospora crassa*: technique for studying their development. Science **154**:1356–1357.
16. **Schmit, J. C., and S. Brody.** 1976. Biochemical genetics of *Neurospora crassa* conidial germination. Bacteriol. Rev. **40**:1–41.
17. **Tan, S. T., and C. C. Ho.** 1970. A gene controlling the early development of protoperithecium in *Neurospora crassa*. Mol. Gen. Genet. **107**:158–161.
18. **Viswanath-Reddy, M., and G. Turian.** 1975. Physiological changes during protoperithecial differentiation in *Neurospora tetrasperma*. Physiol. Plant. **35**:166–174.