Fungal Genetics Reports

Volume 39 Article 12

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Recommended Citation

Reimer, H., and H. Ninnemann (1992) "Effect of light on conidiation of nit and lis mutants of Neurospora crassa grown on different nitrogen sources," *Fungal Genetics Reports*: Vol. 39, Article 12. https://doi.org/10.4148/1941-4765.1438

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Abstract

In addition to a recent paper (Ninnemann 1991. J. Photochem. Photobiol. 9:189-199), we are reporting further data on *Neurospora crassa* mutants supporting our hypothesis that the flavohemoenzyme nitrate reductase (NR) is involved in photoreception for light- promoted conidiation (Klemm and Ninnemann 1979. Photochem. Photobiol. 29:629-632). We looked for light-stimulated conidiation and NR activities in the NR- regulatory mutants *nit-2* (FGSC 2698), *nit-4* (2992) and *nit-5* (985) from the Fungal Genetics Stock Center, University of Kansas, as well as the four NR+ mutants *bd lis-1* (2891), *bd lis-2* (2892), *bd lis-3* (2983), and *al-2 bd*, received from Dr. S. Brody, University of California, San Diego. The three "light insensitive" *lis* mutants were isolated and described by Paietta and Sargent in 1983 (Genetics 104:11-21): the expression of their circadian rhythm was relatively insensitive to continuous light.

Effect of light on conidiation of *nit* and *lis* mutants of *Neurospora crassa* grown on different nitrogen sources

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In addition to a recent paper (Ninnemann 1991. J. Photochem. Photobiol. 9:189-199), we are reporting further data on *Neurospora crassa* mutants supporting our hypothesis that the flavohemoenzyme nitrate reductase (NR) is involved in photoreception for light- promoted conidiation (Klemm and Ninnemann 1979. Photochem. Photobiol. 29:629-632). We looked for light-stimulated conidiation and NR activities in the NR- regulatory mutants *nit-2* (FGSC 2698), *nit-4* (2992) and *nit-5* (985) from the Fungal Genetics Stock Center, University of Kansas, as well as the four NR+ mutants *bd lis-1* (2891), *bd lis-2* (2892), *bd lis-3* (2983), and *al-2 bd*, received from Dr. S. Brody, University of California, San Diego. The three "light insensitive" *lis* mutants were isolated and described by Paietta and Sargent in 1983 (Genetics 104:11-21): the expression of their circadian rhythm was relatively insensitive to continuous light.

The mutants were grown either on NR-inductive Vogel's medium (with 10 g/l glucose) modified so that 50 mM $NaNO_3$ was the only nitrogen source, and solidified with 1.2% agar, or on repressive medium (same as above with nitrate replaced by 25 mM NH_4Cl). In addition, we chose two repressive-derepressive media with either 25 mM NH_4NO_3 or with arginine (5 g/l) plus 3 mM $NaNO_3$ (plus glucose at 3 g/l for partial starvation) added to the salts of Vogel's medium (Ninnemann 1991. J. Photochem. Photobiol. 9:189-199) so that some NR activity could be induced. The albino-band strains *al-2 bd* in which we had found light-promoted conidiation long ago (Klemm and Ninnemann 1978. Photochem. Photobiol. 28:227-230) was included in all experiments as a control. Maintenance of the strains, the method of growing them in large (20 cm) Petri dishes and irradiating them with white light (5 W/m2, 0.5-24 h) as soon as the mycelia had grown one third of the diameter of the Petri dishes have been described before (Ninnemann 1991. J. Photochem. Photobiol. 9:189-199).

The effect of light on conidiation was recorded photographically (Reimer 1992. Master's Thesis, University of Tübingen). Since conidia cannot quantitatively be washed off the mycelia for a quantitative determination, the culture plates and the photographs thereof were evaluated qualitatively. This evaluation is summarized in Table 1. Indicated is the lack or appearance of conidia stimulated by light in addition to conidia eventually formed in darkness.

The three regulatory nit mutants (NR-) were blind for light-inducing photoconidiation on the three media tested (nit mutants cannot grow on nitrate medium), although carotenoid synthesis was induced in the irradiated mycelia. The *al-2 bd* control, however, photoconidiated on inductive nitrate medium as well as on medium with arginine and low concentration of nitrate. However, this strains photoconidiated not at all or scarcely on the repressive ammonium medium

(no NR activities present) and less profusely on the repressive-derepressive ammonium nitrate medium than on inductive nitrate medium.

Photoconidiation occurred in *bd lis-2* and *lis-3* on inductive nitrate medium; *bd lis-1* grew very poorly on nitrate medium so that no conidia were formed in darkness or in light. No, or scarcely any photoconidiation was observed on repressive ammonium. Conidia were clearly, but not profusely light-induced in the three *lis* mutants grown on the two repressive-derepressive media. Thus conidiation could be photostimulated in the three *bd lis* mutants under conditions where at least some nitrate reductase was induced: the *lis* mutations did not affect photoconidiation. At the same time, the growth of *bd lis-3* and especially of *bd lis-2* was inhibited by increasing time of irradiation (>2 h). Also, carotenoid synthesis was light-induced in these mutants.

Table 1. Light stimulated conidiation of NR+ and NR *Neurospora crassa* mutants. Indicates if light-stimulated conidiation is greater than conidiation in darkness observed in culture plates and from their photographs.

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--  no photoconidiation        +/ -  scarcely any photoconidiation
+   photoconidiation clear,    ++   photoconidiation profuse
    not profuse

Nitrate medium: Vogel's medium with nitrate (50 mM) as only
                nitrogen source; glucose 10 g/l, 1.2% agar
NH4Cl           Vogel's, NH4Cl (25 mM) as only nitrogen source;
                glucose 10 g/l, 1.2% agar
NH4NO3          Vogel's, NH4NO3  (25 mM); glucose 10 g/l, 1.2% agar
arginine        Vogel's with 5 g/l arginine + 3 mM NaNO3 as
                nitrogen sources; glucose 3 g/l, 1.2% agar
white light     4 h, 5 W/m2
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	nit-2	nit-4	nit-5	al-2 bd	bd lis-1	bd lis-2	bd lis-3
nitrate	nd	nd	nd	++	--*	++	++
NH_4Cl	--	--	--	--	+/-	+/-	--
NH_4NO_3	--	--	--	+	+	+	+
arginine	--	--	--	++	+	+	+

nd - not determined, since *nit* mutants do not metabolize nitrate *) *bd lis-1* scarcely grows with nitrate as the only nitrogen source In parallel cultures, NR activities (total = NADPH NO_3 , terminal - reduced methylviologen NO_3 , and diaphorase = NADPH cytochrome c) were determined in growth fronts of mycelia grown on solid media and also in mycelia grown in liquid media of the same composition. Independent of the nitrogen source, no total diaphorase or terminal NR activities were found in mycelia of the nit mutants grown on solid or in liquid media. In the NR+ mutants, total and partial NR activities were very much lower in mycelia from solid than from liquid media, confirming Ninnemann (1991 J. Photochem. Photobiol. 9:189-199). In the bd lis mutants grown on nitrate medium, all three NR activities were found, but none was detected in mycelia from repressive ammonium medium (except for 70- 130 nmoles cytochrome c reduced (diaphorase activity) per min and mg protein at room temp. in *bd lis-2*). Due to little material from the growth fronts (the mycelia of three parallel dishes were combined), total and terminal NR activities of the *bd lis* mutants were below threshold of detectability when mycelia were grown on the two repressive-derepressive media; diaphorase activity, however, was detected (200-500 nmoles cytochrome c reduced per min and mg protein at room temperature. Thus, photoconidiation occurred principally in strains with NR activity and

was lacking in strains without NR activity; the result on repressive-derepressive media might allow the conclusion that photoconidiation could be correlated with the presence of diaphorase activity. It had been shown (Ninnemann 1991. J. Plant Physiol. 137:677-682) that the molybdenum cofactor also appears to participate in the photoreception or signal transduction process of photoconidiation

The financial support of the Deutsche Forschungsgemeinschaft is acknowledged