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Abstract

Does nitrate reductase play a key role in photoinduction of caroteniod synthesis in *Neurospora crassa?*

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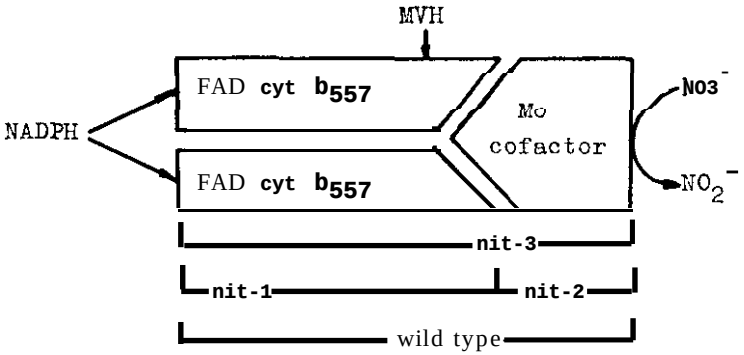
The blue light dependent photoreceptor system of *Neurospora crassa* controls carotenoid synthesis as well as the formation of conidia. The functioning of the system is related to photochemical activity of flavin molecules (Harding and Shropshire 1980 Ann. Rev. Plant Physiol. 31: 213; Senger and Briggs 1981 Photachem. Photobiol. Revs. 6: 1). It has been postulated that a flavoenzyme nitrate reductase may play a key role in the photoregulation mechanism (Klemn and Ninnemann 1979 Photochem. Photobiol. 29: 629).

Nitrate reductase of *N. crassa* consists of two polypeptide subunits and a low molecular weight molybdenum containing cofactor. When NADP·H is used as a reducing agent, the electron flow inside the enzyme proceeds according to the diagram. Nitrate can be reduced to nitrite by the enzyme *in vitro* with an artificial electron donor - reduced methyl viologen (MVH); in this case only the terminal part of the molecule is operating (Pan and Nason 1978 Biochim. Biophys. Acta 523: 297).



Mutation of a gene, *nit-1* deprives the strain of the molybdenum cofactor. Mutation of the regulatory gene, *nit-2,* completely blocks the information of the polypeptide moiety but not synthesis of the cofactor. Each of these two mutations brings about complete loss of both the NADP.H and methyl viologen-dependent nitrate reductase activities. Cells with a mutant *nit-3* gene contain a normal cofactor and a damaged polypeptide: the enzyme can reduce nitrate to nitrite only using methyl viologen, but not NADP·H, as a source of electrons (Amy and Garrett 1979 Analyt. Biochem. 95: 97).

We have used *nit* mutants to investigate the possible role of the enzyme nitrate reductase in a mechanism of blue light response - photoinduction of carotenoid formation: *nit-1* (34547); *nit-2* (K31); *nit-3* (14789). The mutant nada (100) was used as a control with nonmutant nitrate reductase. Its ability for photoinduction does not differ from that of wild-type *N.crassa* (STA4, FGSC #262). For photoinduction experiments mvcelium was suspended in the distilled water (1g/20 ml) and illuminated by fluorescent light (2,000 erg·cm^{-2}.sec^{-1} in the range 300 - 500 nm). Carotenoids were extracted by absolute ethanol and their concentration was estimated by optical density at 475 nm. The rate of carotenoid accumulation was calculated in arbitary units as a differential of OD_{475} before and after photoinduction (Afanasieva *et.al.*1980 Applied Biochem. and Microbiol. (Russian) 16: 156).*

It can be seen from the Table that neither a damage or a complete loss of the palypeptide moiety, nor an absence of the molybdenum cofactor, influences the ability of cells to respond to light by accumulating carotenoid pigments. That is, in spite of the different levels of nitrate reductase activities, cells with normal and mutant genes *nit-l, nit-2* and *nit-3* show practically the same rate of photoinduced carotenogenesis

It can be concluded, therefore, that structural and functional damage of nitrate reductase does not influence the photoreceptor mechanism controlling induction of carotenoid synthesis. In addition, no significant changes are observed in the redox state of the NADP.H/NADP$^+$ system, a physiological source of electrons for nitrate reduction in N. crassa cells. In contrast, light has been shown to stimulate the flavin dependent oxidation of NAD.H$^-$(Kritsky and Chernyshova, 1980 Doklady Akademii Nauk USSR 255: 228).

TABLE

Mutant	uninduced			NO_3^- induced		
	nitrate reductase, sp.act. NADPH	MVH	r Car/time	nitrate reductase, sp. act. NADPH	MVH	r Car/time
nada	0.33	0.12	0.90±.05	3.05	2.83	0.99±.11
nit-l	0.30	0.10	0.80±.09	0.38	0.15	0.80±.10
nit-2	0.0	0.0	0.85±.09	0.0	0.0	0.83±.10
nit-3	0.0	0.0	0.96±.14	0.0	15.70	0.83±.09

Specific activity of nitrate reductase is expressed in nmole $NO_2^-.min^{-1}.mg$ protein-I; the rate of carotenoid accumulation in the light- r Car/time, is expressed in arbitary units.

Thus, nitrate reductase can hardly be regarded as a key element of the photoreceptor system. The photoregulation mechanism may be associated with some NAD·H-dependent redox processes.

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