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

T. A. Belozerskaya
S S. Burikhanov
E. K. Chernyshova
M. S. Kritsky
N. P. Lvov

Follow this and additional works at: http://newprairiepress.org/fgr

Recommended Citation

This Research Note is brought to you for free and open access by New Prairie Press. It has been accepted for inclusion in Fungal Genetics Reports by an authorized administrator of New Prairie Press. For more information, please contact cads@k-state.edu.
Does nitrate reductase play a key role in photoinduction of carotenoid synthesis in Neurospora crassa?

Abstract
Does nitrate reductase play a key role in photoinduction of carotenoid synthesis in Neurospora crassa?

Creative Commons License
This work is licensed under a Creative Commons Attribution-Share Alike 4.0 License.

This research note is available in Fungal Genetics Reports: http://newprairiepress.org/fgr/vol29/iss1/1
Belozerskaya, T. A., S. S. Burikhanov, E. K. Chernyshova; M. S. Kritsky and N. P. Lvov

Does nitrate reductase play a key role in photoduction of carotenoid synthesis in Neurospora crassa?

Nitrate reductase of _N. crassa_ consists of two polypeptide subunits and a low molecular weight molybdenum containing cofactor. When NADPH 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 NADPH 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 NADPH, 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 - photoduction 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 photoduction does not differ from that of wild-type _N. crassa_ (STA4, FGSC #262). For photoduction experiments mycelium 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 arbitrary units as a differential of OD₄₇₅ before and after photoduction (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-1, 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 NADPH/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 NADH (Kritsky and Chernyshova, 1980 Doklady Akademii Nauk USSR 255: 228).
Thus, nitrate reductase can hardly be regarded as a key element of the photoreceptor system. The photoregulation mechanism may be associated with some NADH-dependent redox processes.

TABLE

<table>
<thead>
<tr>
<th>Mutant</th>
<th>uninduced</th>
<th>NO3⁻ induced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nitrate reductase, sp. act.</td>
<td>nitrate reductase, sp. act.</td>
</tr>
<tr>
<td></td>
<td>r Car/time</td>
<td>r Car/time</td>
</tr>
<tr>
<td></td>
<td>NADPH MVH</td>
<td>NADPH MVH</td>
</tr>
<tr>
<td>nada</td>
<td>0.33 0.12 0.90±0.05</td>
<td>3.05 2.83 0.99±0.11</td>
</tr>
<tr>
<td>nit-1</td>
<td>0.30 0.10 0.80±0.09</td>
<td>0.38 0.15 0.80±0.10</td>
</tr>
<tr>
<td>nit-2</td>
<td>0.0 0.0 0.85±0.09</td>
<td>0.0 0.0 0.83±0.10</td>
</tr>
<tr>
<td>nit-3</td>
<td>0.0 0.0 0.96±0.14</td>
<td>0.0 15.70 0.83±0.09</td>
</tr>
</tbody>
</table>

Specific activity of nitrate reductase is expressed in nmol N03⁻.min⁻¹.mg⁻¹ protein; the rate of carotenoid accumulation in the light—r Car/time, is expressed in arbitrary units.

Specific activity of nitrate reductase is expressed in nmol N03⁻.min⁻¹.mg⁻¹ protein; the rate of carotenoid accumulation in the light—r Car/time, is expressed in arbitrary units.

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

Charlanq, G. W. and B. Ng
Ornithine synthesis by an ornithine-deficient triple mutant.

Siderophores, small molecules that function in iron transport are produced by many microorganisms. Although there is much structural variability, most siderophores are either hydroxamates or phenolates-catecholates. Both major siderophores produced by Neurospora belong to the hydroxamate group and contain ornithine. Coprogen is secreted into the medium to scavenge for iron. Ferricrocin is an intracellular siderophore, whose probable function is iron storage.

Siderophore synthesis is regulated by a feedback system and is repressed if iron is present in the growth medium. This repression is not total, however, since we can detect both siderophores when Neurospora is grown in Vogel's minimal medium (MM) containing the usual amount of iron.

A different picture has emerged from our studies, however. Using our very sensitive bioassay for siderophores (Horowitz et al. 1976 J. Bacteriol. 127: 135), we have found that even after four transfers on agar medium that contains no ornithine (Vogel's N-free salts, asparagine and glycerol; with arginine and putrescine (both filter sterilized) and ascorbic acid (300 µg/ml) added to the autoclaved medium), the conidia of the triple mutant still contain ferricrocin at approximately 5% of wild-type level. Since the normal amount of iron is present, the siderophore production system is not derepressed.

When stressed for iron, i.e., growing in medium without added iron (Kappner et al. 1977 Arch. Microbiol. 115: 232) the triple mutant produces a respectable amount of siderophores, both coprogen and ferricrocin (Table 1). Dry weights of siderophores in the Table are post-XAD-2 column chromatography. At this stage media siderophores are 25.70% pure, while intracellular ones are still less than 10% pure.

The production by arg-5, ota, aga (FGSC #2744) lacks enzymes in all known pathways leading to ornithine. G. Winkelmann reported that indeed, this mutant is unable to produce siderophores (1973 Arch. Microbiol. 88: 49).

A different picture has emerged from our studies, however. Using our very sensitive bioassay for siderophores (Horowitz et al. 1976 J. Bacteriol. 127: 135), we have found that even after four transfers on agar medium that contains no ornithine (Vogel's N-free salts, asparagine and glycerol; with arginine and putrescine (both filter sterilized) and ascorbic acid (300 µg/ml) added to the autoclaved medium), the conidia of the triple mutant still contain ferricrocin at approximately 5% of wild-type level. Since the normal amount of iron is present, the siderophore production system is not derepressed.

When stressed for iron, i.e., growing in medium without added iron (Kappner et al. 1977 Arch. Microbiol. 115: 232) the triple mutant produces a respectable amount of siderophores, both coprogen and ferricrocin (Table 1). Dry weights of siderophores in the Table are post-XAD-2 column chromatography. At this stage media siderophores are 25.70% pure, while intracellular ones are still less than 10% pure.

The production by arg-5, ota, aga (FGSC #2744) lacks enzymes in all known pathways leading to ornithine. G. Winkelmann reported that indeed, this mutant is unable to produce siderophores (1973 Arch. Microbiol. 88: 49).

A different picture has emerged from our studies, however. Using our very sensitive bioassay for siderophores (Horowitz et al. 1976 J. Bacteriol. 127: 135), we have found that even after four transfers on agar medium that contains no ornithine (Vogel's N-free salts, asparagine and glycerol; with arginine and putrescine (both filter sterilized) and ascorbic acid (300 µg/ml) added to the autoclaved medium), the conidia of the triple mutant still contain ferricrocin at approximately 5% of wild-type level. Since the normal amount of iron is present, the siderophore production system is not derepressed.

When stressed for iron, i.e., growing in medium without added iron (Kappner et al. 1977 Arch. Microbiol. 115: 232) the triple mutant produces a respectable amount of siderophores, both coprogen and ferricrocin (Table 1). Dry weights of siderophores in the Table are post-XAD-2 column chromatography. At this stage media siderophores are 25.70% pure, while intracellular ones are still less than 10% pure.

The production by arg-5, ota, aga (FGSC #2744) lacks enzymes in all known pathways leading to ornithine. G. Winkelmann reported that indeed, this mutant is unable to produce siderophores (1973 Arch. Microbiol. 88: 49).

A different picture has emerged from our studies, however. Using our very sensitive bioassay for siderophores (Horowitz et al. 1976 J. Bacteriol. 127: 135), we have found that even after four transfers on agar medium that contains no ornithine (Vogel's N-free salts, asparagine and glycerol; with arginine and putrescine (both filter sterilized) and ascorbic acid (300 µg/ml) added to the autoclaved medium), the conidia of the triple mutant still contain ferricrocin at approximately 5% of wild-type level. Since the normal amount of iron is present, the siderophore production system is not derepressed.

When stressed for iron, i.e., growing in medium without added iron (Kappner et al. 1977 Arch. Microbiol. 115: 232) the triple mutant produces a respectable amount of siderophores, both coprogen and ferricrocin (Table 1). Dry weights of siderophores in the Table are post-XAD-2 column chromatography. At this stage media siderophores are 25.70% pure, while intracellular ones are still less than 10% pure.

The production by arg-5, ota, aga (FGSC #2744) lacks enzymes in all known pathways leading to ornithine. G. Winkelmann reported that indeed, this mutant is unable to produce siderophores (1973 Arch. Microbiol. 88: 49).

A different picture has emerged from our studies, however. Using our very sensitive bioassay for siderophores (Horowitz et al. 1976 J. Bacteriol. 127: 135), we have found that even after four transfers on agar medium that contains no ornithine (Vogel's N-free salts, asparagine and glycerol; with arginine and putrescine (both filter sterilized) and ascorbic acid (300 µg/ml) added to the autoclaved medium), the conidia of the triple mutant still contain ferricrocin at approximately 5% of wild-type level. Since the normal amount of iron is present, the siderophore production system is not derepressed.

When stressed for iron, i.e., growing in medium without added iron (Kappner et al. 1977 Arch. Microbiol. 115: 232) the triple mutant produces a respectable amount of siderophores, both coprogen and ferricrocin (Table 1). Dry weights of siderophores in the Table are post-XAD-2 column chromatography. At this stage media siderophores are 25.70% pure, while intracellular ones are still less than 10% pure.

The production by arg-5, ota, aga (FGSC #2744) lacks enzymes in all known pathways leading to ornithine. G. Winkelmann reported that indeed, this mutant is unable to produce siderophores (1973 Arch. Microbiol. 88: 49).