

Reversible Oxidation of a Conserved Methionine in the Nuclear Export Sequence Determines Subcellular Distribution and Activity of the Fungal Nitrate Regulator NirA.

Gallmetzer A, Silvestrini L, Schinko T, Gesslbauer B, Hortschansky P, Dattenböck C, Muro-Pastor MI, Kungl A, Brakhage AA, Scazzocchio C, Strauss J (2015) Reversible Oxidation of a Conserved Methionine in the Nuclear Export Sequence Determines Subcellular Distribution and Activity of the Fungal Nitrate Regulator NirA. *PLOS Genet* 11(7), e1005297.

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Abstract

The assimilation of nitrate, a most important soil nitrogen source, is tightly regulated in microorganisms and plants. In *Aspergillus nidulans*, during the transcriptional activation process of nitrate assimilatory genes, the interaction between the pathway-specific transcription factor NirA and the exportin KapK/CRM1 is disrupted, and this leads to rapid nuclear accumulation and transcriptional activity of NirA. In this work by mass spectrometry, we found that in the absence of nitrate, when NirA is inactive and predominantly cytosolic, methionine 169 in the nuclear export sequence (NES) is oxidized to methionine sulfoxide (Metox169). This oxidation depends on FmoB, a flavin-containing monooxygenase which in vitro uses methionine and cysteine, but not

glutathione, as oxidation substrates. The function of FmoB cannot be replaced by alternative Fmo proteins present in *A. nidulans*. Exposure of *A. nidulans* cells to nitrate led to rapid reduction of NirA-Metox169 to Met169; this reduction being independent from thioredoxin and classical methionine sulfoxide reductases. Replacement of Met169 by isoleucine, a sterically similar but not oxidizable residue, led to partial loss of NirA activity and insensitivity to FmoB-mediated nuclear export. In contrast, replacement of Met169 by alanine transformed the protein into a permanently nuclear and active transcription factor. Co-immunoprecipitation analysis of NirA-KapK interactions and subcellular localization studies of NirA mutants lacking different parts of the protein provided evidence that Met169 oxidation leads to a change in NirA conformation. Based on these results we propose that in the presence of nitrate the activation domain is exposed, but the NES is masked by a central portion of the protein (termed nitrate responsive domain, NiRD), thus restricting active NirA molecules to the nucleus. In the absence of nitrate, Met169 in the NES is oxidized by an FmoB-dependent process leading to loss of protection by the NiRD, NES exposure, and relocation of the inactive NirA to the cytosol.

Involved units

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