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Protein Interaction of Nucleosome Assembly Protein-1 and Casein Kinase 2 During Desiccation Response in the Insect-Killing Nematode *Steinernema Feltiae* IS-6

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ABSTRACT: The change in gene expression induced by desiccation in the semiarid, entomopathogenic nematode *Steinernema feltiae* IS-6, includes induction of transcription of a nucleosome assembly protein, NAP1 homolog, and of casein kinase 2 (CK2) genes. Therefore, one of the events during the dehydration response of *S. feltiae* IS-6 may be transcriptional activation by *S. feltiae* IS-6 NAP1 homolog (*Sf*-Nap1), which is regulated by *S. feltiae* IS-6 CK2 (*Sf*-CK2). This regulation necessitates physical interaction between the *Sf*-Nap1 and *Sf*-CK2 proteins. In the present study we used yeast 2-hybrid analysis to demonstrate physical interaction between the 2 proteins, thus confirming the involvement of a protein interaction-based step in the desiccation response mechanism of *S. feltiae* IS-6.

Insect-killing (entomopathogenic) nematodes in the dauer stage (infective juvenile) infect and kill a wide range of insect species. They are being used as biological control agents in the field, although their sensitivity to desiccation and other environmental stresses reduces their field efficacy (Kaya and Gaugler, 1993). The infective juvenile life stage is tolerant of desiccation and has adapted to remaining in the soil while searching for a new host (Kaya and Gaugler, 1993; Patel et al., 1997; Glazer, 2002). The semiarid entomopathogenic nematode *Steinernema feltiae* IS-6 can survive in relative humidity as low as 75% by entering quiescent anhydrobiosis (Solomon et al., 1999); the physiological changes elicited in it by desiccation include decreased glycogen concentrations (Gal et al., 2001) and increased trehalose (Solomon et al., 1999).

Recently, in adopting a comprehensive approach to the study of the molecular mechanisms of stress tolerance in *S. feltiae* IS-6 (Gal et al., 2003), we identified several classes of genes that are differentially expressed during its stress response; they include known stress-related genes, both those that are homologous to putative proteins in *Caenorhabditis elegans* and novel genes (Gal et al., 2003). The expression pattern of these genes suggested that regulation of transcription and production of stress protectants are involved in the nematode stress response (Gal et al., 2003).

One of the highest increments in gene expression, following 24 hr of desiccation, was observed for a *S. feltiae* IS-6 expressed sequence tag, which putatively encodes a nucleosome-assembly protein (i.e., a NAP1 homolog; GenBank BQ579831). NAP1 is widely conserved in eukaryotes, and it influences the activities of a wide range of transcription factors by physically interacting with coactivators (Shikama et al., 2000, Miyaji-Yamaguchi et al., 2003). It was suggested, therefore, that Nap1 is a nucleocytoplasmic shuttling protein that serves as integrator between transcriptional coactivators and chromatin (Shikama et al., 2000; Mosammaparast et al., 2002; Miyaji-Yamaguchi et al., 2003). Genetics studies performed on yeast showed that NAP1 is necessary for proper structure of the nucleosome during in vitro transcription, and that during replication it is required for the execution of mitotic events, as well as for the reorganization of the cytoskeleton during the switch from polarized to isotropic growth (Kellogg and Murray, 1995; Ohkuni et al., 2003).

In *Drosophila* sp., casein kinase 2 (CK2) binds and phosphorylates NAP1 and thus regulates NAP1 degradation and translocation between the cytoplasm and the nucleus (Li et al., 1999). CK2 is a ubiquitous second message-independent, serine/threonine-specific kinase (Allende and Allende, 1995; Lim et al., 2004). The evolutionary conservation of CK2 suggests it may play an essential role in fundamental cellular processes, including multiple activities related to cell growth and differentiation (Allende and Allende, 1995; Cavin et al., 2003; Kulartz et al., 2004; Lim et al., 2004). CK2 (GenBank BQ563207) was identified in the desiccation-specific *S. feltiae* IS-6 subtraction library (Gal et al., 2003). Thus, a plausible hypothesis suggests that one of the late events during the *S. feltiae* IS-6 dehydration response is transcriptional activation by the *S. feltiae* IS-6 NAP1 homolog (*Sf*-Nap1), whereas *S. feltiae* IS-6 CK2 (*Sf*-CK2) regulates NAP1 homolog degradation and localization.

Such a regulatory network necessitates physical interaction between *Sf*-Nap1 and *Sf*-CK2 (Li et al., 1999). In the present study we used yeast 2-hybrid analysis to demonstrate the occurrence of physical interaction between the 2 proteins, and so revealed an upstream, protein-interaction-based step in the desiccation response mechanism of *S. feltiae* IS-6.

The yeast 2-hybrid system has proved to be a powerful and invaluable tool in molecular biology; it offers many advantages such as facilitating the analysis of the interactions of proteins that occur in vivo in yeast cells (Walhout et al., 2000; Fang et al., 2002). To determine a possible protein interaction, *Sf*-CK2 and *Sf*-Nap1 proteins were cloned into the yeast 2-hybrid vector system. *Sf*-Nap1 fragments including a CK2 phosphorylated site and an *Sf*-CK2 fragment that included a serine/threonine protein kinase-active site (Li et al., 1999) were amplified from the subtracted desiccation library (Gal et al., 2003) using the following primers: *Sf*-CK2 forward, 5′-CACCGCTCTTATATTCGAGTATGTC AAC-3′*Sf*-CK2 reverse, 5′-CTACAAACCCCAATCAATCAACCTC-3′*Sf*-Nap1 forward, 5′-CACCGAGTTCCACCTCTCTCCTAAC-3′*Sf*-Nap1 reverse, 5′-CTAGATGATTTGCCCSSGAGAAAAG-3′

The amplified fragments were cloned into the pENTER/D Topo cloning kit (Invitrogen, Carlsbad, California) according to the manufacturer's instructions. The 2 gene fragments were transferred from the entry clone into the destination vector with Gateway LR clonase enzyme mix (Invitrogen) according to the manufacturer's instructions, to make the expression clone. The Proquest 2-hybrid system kit (Invitrogen) was used to clone *Sf-Nap1* into the DNA binding domain (DB) of the Gateway Destination Vector (pDEST™32), whereas *Sf-CK2* was cloned into the activation domain (AD) of the Gateway Destination Vector (pDEST™22). DB-*Sf*-Nap1 and AD-*Sf*-CK2 were transformed into *Escherichia coli* DH5α-competent cells (Invitrogen), extracted with the high-purity plasmid isolation kit (Roche, Mannheim, Germany), and sequenced to ensure accuracy of the sequence of cloned fragments (ABI, Columbia, Maryland).

To test for the interaction between the 2 proteins, DB-*Sf*-Nap1 and AD-*Sf*-CK2 were cotransformed into MaV203-competent yeast cells by the lithium acetate/single-strand DNA/polyethylene glycol procedure (Invitrogen). To test for self activation (which does not result from a genuine 2-protein interaction), DB-*Sf*-Nap1 and pEXP-AD502 or AD-*Sf*-CK2 and pDBLeu were cotransformed into MaV203-competent yeast cells (Invitrogen). Yeast control strains included A strain, which contains no interacting proteins; B strain, which contains weakly interacting proteins; C strain, which includes moderately interacting proteins; D strain, which contains strongly interacting proteins; and E strain, which contains very strongly interacting proteins (Invitrogen). The transformed yeast cells and the A–E group controls were transferred onto a master plate. The master plate contained SC medium (Kaiser et al., 1994) lacking leucine and tryptophan (SC-Leu-Trp), to select for colonies that can grow on this media (i.e., they contain both DB [pDEST™32] Vector and AD [pDEST™22] Vector, or the appropriate kit controls [described above]). pDEST™32 included the *LEU2* gene and thus allowed growth on a medium lacking leucine; pDEST™22 included the *TRP1* gene and thus allowed growth on a medium lacking tryptophan.

Interaction between DB-Sf-Nap1 and AD-Sf-CK2 proteins was detected through the expression of reporter genes on 2 selective media.

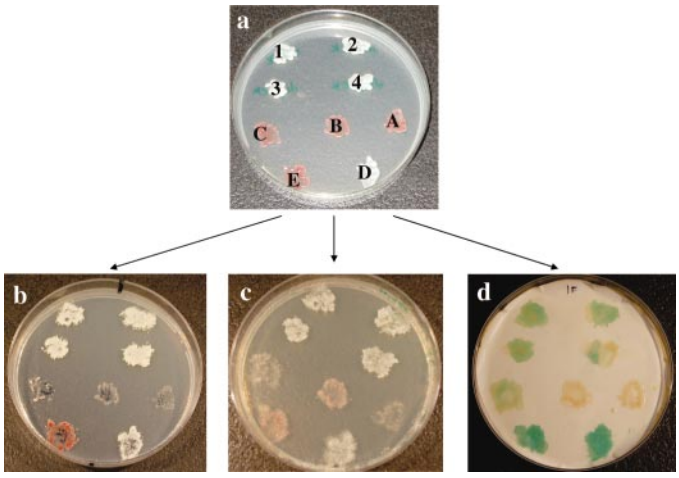

FIGURE 1. Detection of protein interactions in yeast colonies by selection and detection of reporter gene expression. Yeast colonies on plates: (1–4) 4 different yeast colonies, each cotransformed with both DB-*Sf*-Nap1 and AD-*Sf*-CK2 to test for protein interactions. **A–E**. The kit control yeast strains. (**a**) Sc-Leu-Trp master plate, (**b**) replica on SC-Leu-Trp-Ura selective media, (**c**) replica on SC-Leu-Trp-His+3AT (50 mM) selective media (pink colonies reflect shortage of adenine in the media), (**d**) replica on a nylon membrane for X-Gal assay; colonies in which proteins interacted are stained blue.

For the first of these, following titration of 3AT to determine the lowest concentration of 3AT that would inhibit *HIS3* expression (and thus growth on histidine) to some extent (not shown), yeast colonies from the master plate were replica-plated into selective media that lacked histidine as well as leucine and tryptophan (SC-Leu-Trp-His), and that contained 50 mM 3AT. They were then incubated for 18 hr at 30 C. In a second test, yeast colonies from the master plate were replicated into selective media that lacked uracil as well as leucine and tryptophan (SC-Leu-Trp-Ura), and were incubated for 18 hr at 30 C.

As an additional test for an interaction between the DB-Sf-Nap1 and AD-Sf-CK2 proteins, the expression of X-Gal reporter gene was examined. SC-Leu-Trp-containing colonies from the master plate were patched directly onto a nylon membrane that had been placed on the surface of a YPAD agar plate, which was then incubated for 18 to 24 hr at 30 C. Following incubation, the membrane was removed from the plate, immersed in liquid nitrogen for 20–30 sec for yeast cells to break, and placed, with the colonies side up, on top of a Whatman filter paper soaked in X-gal solution (Invitrogen). The membrane was covered and incubated at room temperature for 2–24 hr until blue staining of replicated colonies appeared.

Four different colonies (1–4), each cotransformed with both DB-*Sf*-Nap1 and AD-*Sf*-CK2 (to test for protein interactions) and yeast control strains A–E were replica-plated on the selective media and treated for X-Gal staining (Fig. 1). Yeast colonies that were cotransformed with both the DB-*Sf*-Nap1 and AD-*Sf*-CK2 proteins and the kit controls that contained strongly interacting proteins grew on the selective media (Fig. 1b, c), and were stained blue in the X-Gal expression test (Fig. 1d). Notably, the partially stained colony ''4'' may result from partial breakage of yeast cells (Fig. 1d). Because the only yeast colonies that expressed the reporter genes (i.e., they grew on the selective media and were stained blue) were those in which cloned proteins interacted, we suggest that DB-*Sf*-Nap1 and AD-*Sf*-CK2 proteins interact in the yeast 2-hybrid system.

Neither colony growth nor X-Gal staining was observed for the kit controls that contained no interacting or weakly interacting proteins (Fig. 1), or for colonies that were cotransformed with DB-*Sf*-Nap1 and pEXP-AD502 or with AD-*Sf*-CK2 and pDBLeu (for testing of self activation; data not shown). Together, these results suggest that the interaction between DB-*Sf*-Nap1 and AD-*Sf*-CK2 was a genuine protein-protein interaction.

Our previous findings suggested that these 2 proteins were specifically expressed during desiccation stress (Gal et al., 2003). NAP1 activates the transcription of numerous genes (Shikama et al., 2000; Mosammaparast et al., 2002; Miyaji-Yamaguchi et al., 2003; Ohkuni et al., 2003), whereas CK2 regulates NAP1 degradation and localization by its phosphorylation (Li et al., 1999). Therefore, we suggest that the interaction between the *Sf*-Nap1 and *Sf*-CK2 proteins is a step in the signal transduction pathway and that it is activated during desiccation stress in *S. feltiae* IS6. This protein-protein interaction step may lead to pleotrophic effects of gene expression that form part of the response of the semiarid entomopathogenic *S. feltiae* IS6 nematode to desiccation stress. A better understanding of the mechanism of nematode response to desiccation stress is expected to promote the development of nematodes that are tolerant of field conditions, perhaps via their genetic improvement for stress tolerance, thus expanding their use as biopesticides. ?3

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