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No evidence for intra-allelic complementation at the osmotic-1 locus of Neurospora crassa

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Abstract

The *Neurospora crassa osmotic-1* locus (*os-1*) encodes a protein with homology to two component histidine kinase sensors. We formed forced heterokaryons between each of ten *os-1* alleles in all pair-wise combinations and found, in contrast to a previous report, no evidence for intra-allelic complementation.

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No evidence for intra-allelic complementation at the *osmotic-1* locus of *Neurospora crassa*

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The *Neurospora crassa osmotic-1* locus (*os-1*) encodes a protein with homology to two component histidine kinase sensors. We formed forced heterokaryons between each of ten *os-1* alleles in all pair-wise combinations and found, in contrast to a previous report, no evidence for intra-allelic complementation.

The *osmotic-1* (*os-1*) locus of *Neurospora crassa* encodes a protein with homology to bacterial and plant two-component histidine kinases. The *os-1* protein appears to be an osmo-sensor and is the first step in a MAP kinase cascade that regulates intra-cellular osmolarity and cell-wall synthesis (Alex L, Borkovich K, Simon MI. Hyphal development in *Neurospora crassa*: involvement of a two-component histidine kinase Proc Natl Acad Sci U S A. 93:3416-21. 1996; Schumacher M, Enderlin C, Selitrennikoff CP. The *osmotic-1* locus of *Neurospora crassa* encodes a putative histidine kinase similar to osmosensors of bacteria and yeast. Curr Microbiol. 34:340-7. 1997). Mutants defective at the *os-1* locus are sensitive to a number of hyper-osmotic conditions, including 4% NaCl (Mehadevan P. and Tatum E. Relationship of the major constituents of the *Neurospora crassa* cell wall to wild-type and colonial morphology. J. Bacteriol. 90:1073-1081. 1965). In other organisms, *e.g.,* prokaryotes, similar histidine kinases exist as homodimers (C. Tomomori C, Tanaka T, Dutta R, *et al.* Solution structure of the homodimeric core domain of *Escherichia coli* histidine kinase EnvZ Nat. Struct. Biol. 6:729. 1999) in which an extracellular signal induces the autophosphorylation of a histidyl residue of one member of the dimer. The phosphoryl group is subsequently transferred to an aspartyl residue of the other dimer pair, triggering a regulatory kinase cascade.

A number of years ago, Dr. N. Mishra reported that the *os-1* locus was comprised of two complementation groups (Mishra N. Characterization of the new osmotic mutants (*os*) which originate during genetic transformation in *Neurospora crassa*. Genet. Res. Camb. 29:9-19. 1977), based on complementation tests between strain 171-4 and three other *os-1* alleles. This indicated intra-allelic complementation, consistent with the idea that the *os-1*-encoded sensor exists *in situ* as an oligomer. We (and another group) have determined the molecular basis of each of ten *os-1* mutants currently deposited in the Fungal Genetics Stock Center (Miller T., S. Renault, and C. P. Selitrennikoff. Molecular Dissection of Alleles of the *osmotic-1* Locus of *Neurospora crassa*, Fungal Genet. Biol. 35: 147-155. 2002). In an effort to understand which of the various regions of the *os-1*-encoded protein are essential for activity, we formed pair-wise forced heterokaryons using each of these ten *os-1* mutants (unfortunately, none of the strains described by Dr. Mishra are deposited in the FGSC and, thus we were not able to use the original strains). Given the encouraging data showing intra-allelic complementation of os-1 mutants, we had predicted that an oligomer in which each partner was defective in a different portion of the *os-1* protein might have activity and hence complement, *e.g.,* grow in the presence of 4% NaCl.

Each of the *os-1* mutants indicated in Table 1 was crossed to *nic-2* and to *arg-10* and *os-1* auxotrophic progeny of each mating type isolated. Forced heterokaryons between each of the 10 *os-1* mutants (and *os-1*$^{+}$) were formed in all pair-wise combinations by co-plating macroconidia of each strain onto minimal medium to induce forced-heterokaryons. Small numbers of macroconidia from each stable heterokaryon were used to inoculate the center of petri plates containing Vogel's medium N, 1.5 % (w/v) sucrose, and 4% (w/v) NaCl. The growth rate of each heterokaryon was determined by measuring the position of hyphal fronts at various times of incubation and comparing that to the growth rate of each heterokaryon grown in the absence of NaCl. Each *os-1/os-1* heterokaryon grew in the absence of NaCl at a growth rate similar to each *os-1/os-1*$^{+}$ control (results not shown).

We found that each *os-1* mutant was recessive to *os-1*$^{+}$ and in no case did we observe that any of the *os-1/os-1* heterokaryons were able to grow in the presence of 4% NaCl (results not shown). These data indicated that none of the ten mutants could complement another mutant (i.e., we found no intra-allelic complementation). This was surprising given that the mutants localize to different regions of the *os-1*-encoded protein and the previous report of intra-allelic complementation between *os-1* mutants. We cannot offer a definitive explanation for these conflicting data.

Table 1: Strains used in this study

Strain	Allele number	FGSC number
os-1	B135	810
os-1	E11200	34
os-1	M16	813
os-1	M155-1	824
os-1	NM204(t)	2273
os-1	NM233t	4494
os-1	P668	973
os-1	P5590	2432
os-1	P6549	2584
os-1	Y256M209	3625
arg-10	B317	4091 / 4092
nic-2	43002	4006 / 4007

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