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Neurospora msh4 ortholog confirmed by split-marker deletion

Abstract

Although most eukaryotes have both MSH4 and MSH5 orthologs, Neurospora was initially thought to lack msh-4. We have deleted the most likely msh-4 candidate and observed a delay in the sexual cycle, disruption to meiosis and a reduction in fertility. Deletion is dominant, showing msh-4 is subject to MSUD. We conclude that Neurospora has a MSH4 ortholog and that it may have remained undetected because of an unusually high number of introns.

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Neurospora *msh4* ortholog confirmed by split-marker deletion

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Although most eukaryotes have both *MSH4* and *MSH5* orthologs, Neurospora was initially thought to lack *msh4*. We have deleted the most likely *msh4* candidate and observed a delay in the sexual cycle, disruption to meiosis and a reduction in fertility. Deletion is dominant, showing *msh4* is subject to MSUD. We conclude that Neurospora has an *MSH4* ortholog and that it may have remained undetected because of an unusually high number of introns.

MSH4, a member of the MutS protein family, is conserved in higher organisms and has a role in crossover regulation. Disruption of *MSH4* reduces the frequency of crossing over in many organisms (Ross-Macdonald and Roeder, 1994; Zalevsky *et al*., 1999) and increases the incidence of chromosomal non-disjunction, identical phenotypes to that of *MSH5* mutants (Hollingsworth *et al*., 1995; Hunter *et al*., 1997). MSH4 and MSH5 function as a heterodimer, and removal of the ATPase domain from either leads to loss of function (Alani, 1997; Haber and Walker, 1991; Pochart *et al*., 1997). All MSH4 orthologs contain MutS DNA-binding and ATPase domains. MSH4 also seems to have a role in the formation or elongation of the synaptonemal complex, a proteinaceous structure that holds homologous chromosomes in close proximity prior to the reductional division of meiosis. Chromosome synapsis is delayed and incomplete in *Saccharomyces cerevisiae MSH4* mutants (Novak *et al.*, 2001), and MSH4 localises at synapsis initiation sites in both yeast and mammalian meiotic cells (Novak *et al.*, 2001; Neyton *et al*., 2004).

Gene prediction algorithms used in the Neurospora genome project failed to identify an MSH4 ortholog. A subsequent bioinformatic analysis used tBlastn to identify a region with homology to MSH4 sequences of *S. cerevisiae*, Mouse and Human (Borkovich *et al*., 2004). Alignment of the predicted protein sequence with human and *S. cerevisiae* MSH4 protein sequences using ClustalW showed that Neurospora MSH4 shares conserved regions with the other two amino acid sequences (figure 1).

We concluded that the *msh4* gene is about 3 kb in length (figure 2), and is located on LG1R, between *met-6* and *aap-2*, on supercontig 7.2, nucleotide positions 1061633-1064675.

A 3.2 kb sequence including the potential *msh4* gene was analysed using GenScan (Burge and Karlin, 1997) to identify possible coding sequence and to suggest possible amino acid sequences by removing introns predicted by identification of eukaryotic splice sites (figure 2). Using either human or Arabidopsis parameters gave very similar results.

Analysis of the predicted 871 amino acid sequence using Motifs (GCG: Wisconsin Package^{TM}) identified a potential gene with an ATP/GTP binding site and a DNA MMR motif. Use of a CD search, which identifies conserved domains within a protein sequence by comparing the sequence with other known protein sequences (Marchler-Bauer *et al.* 2005), identified a MutS homolog with a DNA binding mismatch repair domain (MUTSd) and an ATPase domain (MUTSac; figure 3).

Thus, Msh4 appears to possess DNA-binding and ATPase domains as expected, and it seems likely that there are six introns (figure 2) within the *msh4* nucleotide sequence. Since Neurospora genes have, on average, 1.7 introns (Borkovich *et al*., 2004), the unusually large number in this *msh4* candidate might explain why it was missed by the gene prediction algorithms.

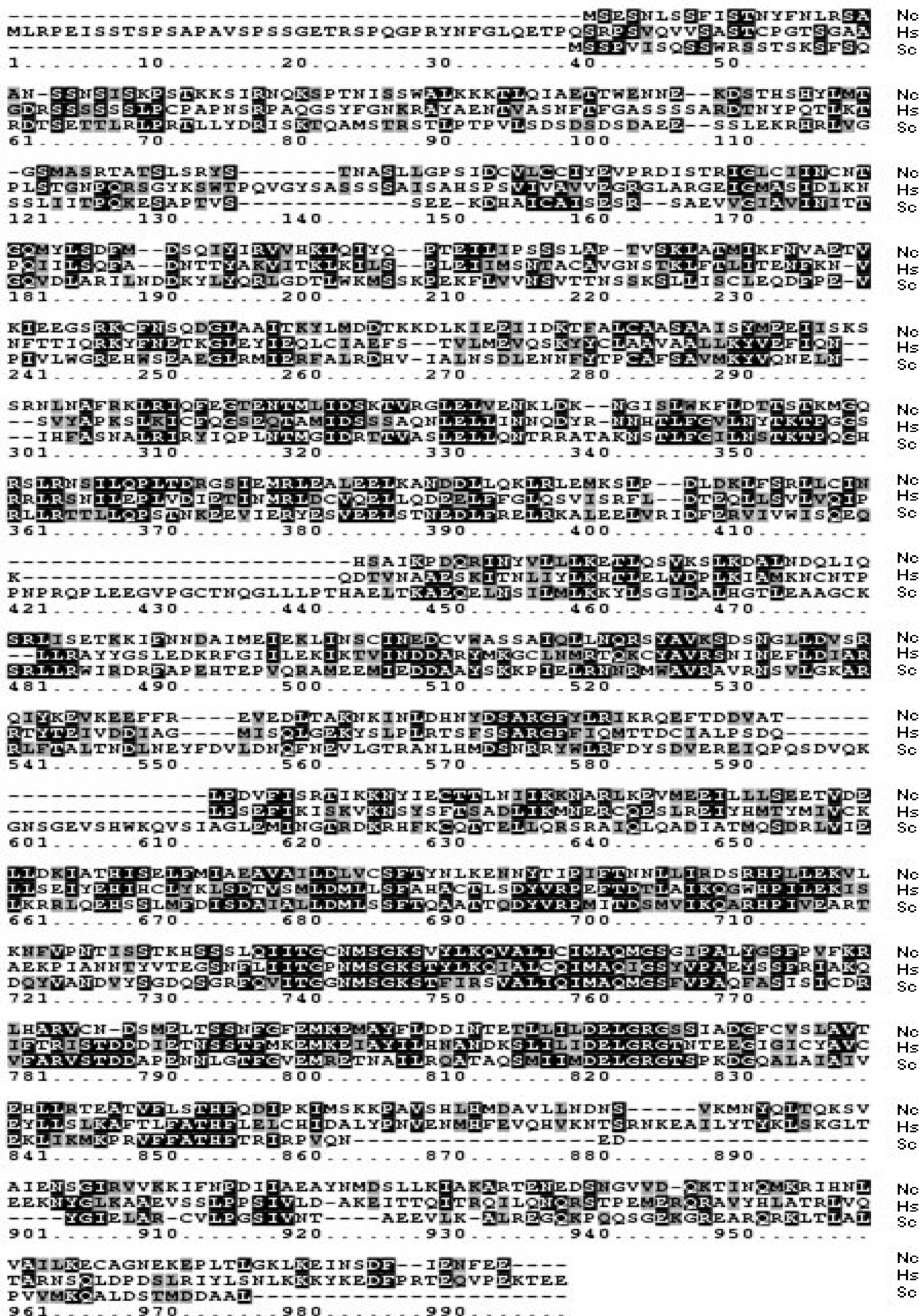

Figure 1. Alignment of the human (Hs) and Yeast (Sc) MSH4 proteins with the Neurospora (Nc) predicted 871 amino acid sequence. Dark shading represents identical and lighter shading similar residues.

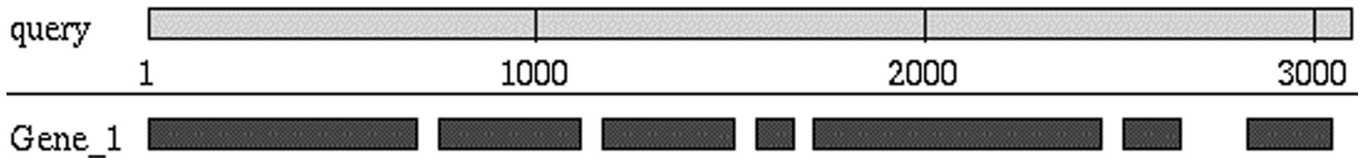

Figure 2. GenScan graphical representation of predicted coding sequences within the putative *msh4* nucleotide sequence. The gaps between blocks represent predicted introns.

Using split-marker deletion (Catlett *et al.*, 2003), we replaced the predicted *msh4* sequence with the hygromycin resistance gene (*hph*) in a mating pair of Neurospora strains, and analysed the effect through comparison of crosses that are isogenic except for the *msh4* deletion. Gross perithecial morphology seems to be unaffected by the deletion, although ejection of the first spores is delayed by ~2 days in mutant crosses. There is a 5-fold reduction in viable spores recovered from *msh4* homo- and hetero-zygotes (mutant average = 2 x 10^5 spores per cross, untransformed control = 1 x 10^6 spores per cross), and mutant crosses yield many more white spores. Sporogenesis in homozygous and heterozygous *msh4* crosses is delayed and rosettes from the *msh4* mutants contain more bubble asci (Perkins and Barry, 1977) and fewer normal size asci than the control. Asci containing less than eight spores or at least one misshapen spore are common in *msh4* crosses, regardless of whether the mutant is the male or female parent, but rarely observed in the control (figure 4).

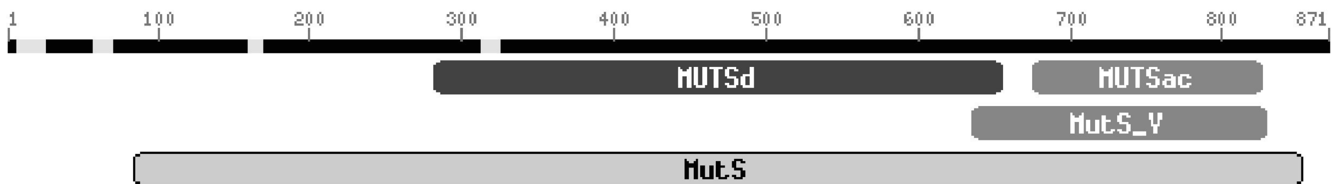

Figure 3. CD graphical representation of conserved domains within the Msh4 predicted protein sequence. MutS (MutS ATPase protein family), MutSd (DNA binding domain), MutSac (ATPase domain), MutS_V (domain V; ATPase). Gaps represent sequences with low homology to known protein domains.

A cursory analysis of chromosomal behaviour during meiosis suggests that an absence of *msh4* causes some abnormality, although at this stage we have not found evidence of non-disjunction during meiosis I.

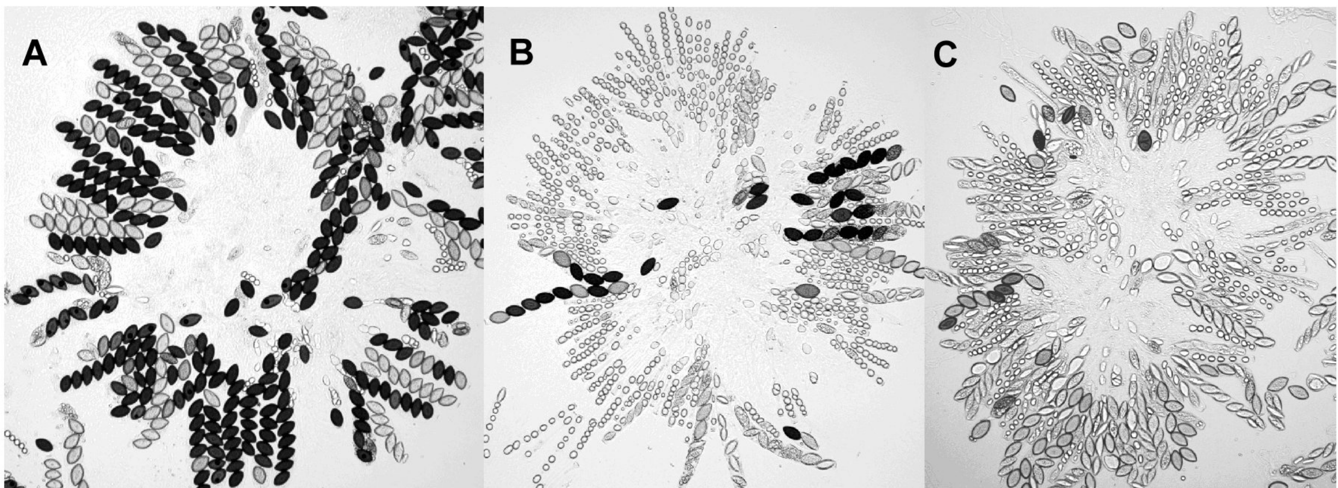

Figure 4. Examples of rosettes from control cross (A), otherwise isogenic *msh4* heterozygote (B) and *msh4* homozygote.

In conclusion, deletion of the putative Neurospora *msh4* gene interferes with sporogenesis and possibly meiosis. The deletion appears dominant, suggesting that *msh4* is normally expressed during meiosis and subject to meiotic silencing of unpaired DNA (Shiu and Metzenberg, 2002). The *msh4* candidate we deleted contains the MutS and ATPase domains common to all MSH4 orthologs and shares considerable predicted amino acid identity with both yeast and mammalian MSH4 proteins. Since we have now demonstrated a role for this gene in the sexual phase it is likely that it is indeed the Neurospora ortholog of *MSH4*.

Acknowledgements
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References

Alani, E., S. Lee, M. F. Kane, J. Griffith and R. D. Kolodner, 1997. *Saccharomyces cerevisiae* MSH2, a mispaired base recognition protein, also recognizes Holliday junctions in DNA. J. Mol. Biol. 265: 289-301.

Borkovich, K. A., *et al*., 2004 Lessons from the Genome Sequence of *Neurospora crassa*: Tracing the Path from Genomic Blueprint to Multicellular Organism. Microbiol. & Mol. Biol. 68: 1-108.

Catlett, N. L., Bee-Na Lee, O. C. Yoder and B. Gillian Turgeon, 2003. Split-Marker Recombination for Efficient Targeted Deletion of Fungal Genes. Fungal Genet. Newslett. 50: 9-11.

Haber, L. T. and G. C. Walker, 1991. Altering the conserved nucleotide binding motif in the *Salmonella typhimurium* MutS mismatch repair protein affects both its ATPase and mismatch binding activities. EMBO J. 10: 2707-2715.

Hollingsworth, N. M., L. Ponte and C. Halsey, 1995. *MSH5*, a novel MutS homolog, facilitates meiotic reciprocal recombination between homologs in *Saccharomyces cerevisiae* but not mismatch repair. Genes Dev. 9 :1728-1739.

Hunter, N. and R. H. Borts, 1997. Mlh1 is unique among mismatch repair proteins in its ability to promote crossing over during meiosis. Genes Dev.11 :1573-1582.

Marchler-Bauer, A. and S. H. Bryant, 2004. CD-Search: protein domain annotations on the fly. Nucl. Acids Res. 32 : 327-331.

Neyton, S., F. Lespinasse, P. Moens, P. Gaudray, V. Paquis-Flucklinger and S. Santucci-Darmanin, 2004. Association between MSH4 (MutS homologue 4) and the DNA strand-exchange RAD51 and DMC1 proteins during mammalian meiosis. Mol. Human Reprod. 10: 917-924.

Novak, J. E., P. B. Ross-Macdonald and G. S Roeder, 2001. The budding yeast Msh4 protein functions in chromosome synapsis and the regulation of crossover distribution. Genetics 158: 1013-1025.

Perkins, D. D. and E. G. Barry, 1977. The cytogenetics of *Neurospora.* Adv. Genet. 19: 133-285

Pochart, P., D. Woltering and N. M. Hollingsworth, 1997. Conserved Properties between Functionally Distinct MutS Homologs in Yeast. J. Biol. Chem. 272: 30345-30349.

Ross-Macdonald, P. and G. S. Roeder, 1994. Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch correction. Cell 79: 1069-1080.

Shiu, P. K. T. and R. L. Metzenberg, 2002. Meiotic silencing by unpaired DNA: properties, regulation and suppression. Genetics 161: 1483-1495.

Zalevsky, J., A. J. MacQueen, J. B. Duffy, K. J. Kemphues and A. M. Villeneuve, 1999. Crossing over during *Caenorhabditis elegans* meiosis requires a conserved MutS-based pathway that is partially dispensable in budding yeast. Genetics 153 :1271.