

Sequence and analysis of genomic sequences upstream of mei-3.

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Abstract

The *Neurospora crassa* mei-3 mutation causes sensitivity to various DNA damaging agents (Newmeyer and Galeazzi 1977 *Genetics* 85:461-487). We have recently cloned and mapped a genomic fragment capable of transforming mei-3 spheroplasts to wild type (Cheng et al. 1993 *Mut. Res.* 294:223-234). These experiments predicted the putative coding sequence of mei-3 or at least the region encompassing the mei-3 mutation. It was determined by homology that Mei-3 belongs to the RecA-like group of proteins (Bishop et al. 1992 *Cell* 69:439-456) which are intimately involved in the recombination process and had been previously identified only in prokaryotes. Since the identification of RecA-like proteins in *Saccharomyces* (Shinohara et al. 1992 *Cell* 69:457-470) and in *Neurospora*, our hypothesis that this important group of proteins may be highly conserved in other eukaryotes has been substantiated with the cloning of several Rec-A like proteins from mouse, chicken, lily, and human (Shinohara et al. 1993 *Nat. Genet.* 4:239-243). Using these data there is evidence that additional homology, upstream of our putative start site, to these other proteins exists (an additional 67% over 39 amino acids between Mei-3 and mouse Rad51: Figure 1). However, this region of homology lacks a start site or obvious splice sites. This might suggest the presence of an unidentified upstream start sequence and another exon without obvious splice sequences. In an attempt to address this possibility, we sequenced both strands of the previously unpublished genomic sequence, from -2519 to -271 bp upstream of the putative 5' end of mei-3.

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Sequence and analysis of genomic sequences upstream of mei-3.

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The *Neurospora crassa* mei-3 mutation causes sensitivity to various DNA damaging agents (Newmeyer and Galeazzi 1977 Genetics 85:461-487). We have recently cloned and mapped a genomic fragment capable of transforming mei-3 spheroplasts to wild type (Cheng et al. 1993 Mut. Res. 294:223-234). These experiments predicted the putative coding sequence of mei-3 or at least the region encompassing the mei-3 mutation. It was determined by homology that Mei-3 belongs to the RecA-like group of proteins (Bishop et al. 1992 Cell 69:439-456) which are intimately involved in the recombination process and had been previously identified only in prokaryotes. Since the identification of RecA-like proteins in *Saccharomyces* (Shinohara et al. 1992 Cell 69:457-470) and in *Neurospora*, our hypothesis that this important group of proteins may be highly conserved in other eukaryotes has been substantiated with the cloning of several Rec-A like proteins from mouse, chicken, lily, and human (Shinohara et al. 1993 Nat. Genet. 4:239-243). Using these data there is evidence that additional homology, upstream of our putative start site, to these other proteins exists (an additional 67% over 39 amino acids between Mei-3 and mouse Rad51: Figure 1). However, this region of homology lacks a start site or obvious splice sites. This might suggest the presence of an unidentified upstream start sequence and another exon without obvious splice sequences. In an attempt to address this possibility, we sequenced both strands of the previously unpublished genomic sequence, from -2519 to -271 bp upstream of the putative 5' end of mei-3.

The sequencing was accomplished by sequencing pBRC4 plasmid DNA, using custom made primers, and the Taq cycle sequencing system (United States Biochemical, Cleveland OH). Sequence analysis was performed using the Genetics Computer Group (GCG) programs. The codon preference table used in the analysis was provided by Dr. Mary Anne Nelson, Department of Biology, University of New Mexico.

The 2248 bp of DNA sequence from -2519 to -271 upstream of the putative start site of mei-3 on pBRC4 has been added to the Genbank depository under accession number L02428 (March 1994). Comparisons made between the upstream region with yeast rad51, rad57, dmc1, with the mouse rad51 homolog, with the chicken rad51 homolog, and with the lily rad51 homolog did not reveal additional regions of homology between our upstream sequence and these proteins. Analysis of the upstream sequence did not reveal obvious consensus splice sites to tie any upstream open reading frames, or any regions with high coding preference, to the open reading frame we had originally identified as mei-3.

By homology we were unable to determine the location or existence of the putative upstream start site or exon possibly because the amino terminus ends of the eukaryotic RecA-like proteins tend to be divergent and/or mei-3 contains unusual splice sequences. Additional work is required to conclusively determine the 5' end of mei-3.

N. crassa Mei-3 a.a.#-39

GFNTVESVAYTPRRVLEOIKGISEOKAGKILAEASKLVPM*GFTTATEMEQRRSE Murine
Rad51 a.a.#42

DVKKLEEAGYHTVEAVAYAPKKELINIKGISEAKADKILTEAAKLVPM*GFTTATEFHQ
RRSE Chicken Rad51

DVKKLEEAGYHTVESVAHAPKKELLNIKGISEAKADKILAEAAKLVPM*GFTTATEFHQ
RRSE

Figure 1. Partial alignment of Mei-3 with other eukaryotic RecA-like proteins. The previously predicted start site of Mei-3 is denoted by *. The first 33 aas of Mei-3 correspond to bp -188 to -90 of mei-3.