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Abstract

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Segregation patterns of Neurospora chromosome ends: mapping chromosome tips.

I have recently reported the isolation of the chromosomal telomere from linkage group VR (Schechtman, M. 1987. Mol. Cell. Biol. 7:3168-3177). Further work has established that the DNA sequence repeat found at the ends of Neurospora chromosomes is (TTAGGG)n, and that blocks of this repeating sequence are found only at chromosome ends (Schechtman, in preparation). Accordingly, the oligonucleotide (TTAGGG)a has been used as a hybridization probe to determine the segregation pattern for each chromosomal telomere in the genetically marked progeny of the cross multicent-2 a (FGSC 4488) x Mauriceville-lc A (FGSC 2225) (Metzenberg et al. 1984. Neurospora Newsl. 31:35-39; Metzenberg and Grotelueschen 1988. Fungal Genetics Newsl. 35:30-35). Figure 1A shows an autoradiogram displaying a portion of this analysis. Genomic DNA from parental strains and each of the progeny was digested with BamH1, subjected to electrophoresis on an agarose gel, and then to "unblot" hybridization with 32P-(TTAGGG)4 probe (Wallace and Miyada 1987. Meth. Enzymol. 152:432-442). It can be seen that the probe hybridizes to the expected number of bands, fourteen, in each parental lane. Figure 1B is a cartoon representation of the numbered telomere-derived restriction fragments seen in hybridization to the parental DNAs in lanes 1 and 2. Because of the number of bands involved, not all DNA fragments are sufficiently well resolved to permit definitive segregation assignments for each. Only those assignments that can be made with a reasonable degree of certainty are reported here. Table 1 provides the segregation data for seventeen fragments. Four pairs of these fragments are allelic: that is, one band from the Mauriceville parent was found in the progeny as an exclusive alternative of a designated Oak Ridge derived band. Five segregation patterns can be tentatively assigned to particular chromosome ends based on cosegregations with corresponding distal markers. The ends thus mapped are at IIL (R.L Metzenberg, personal communication), IIR,IVR, IIIR, and VR. The end at IVL has also been cloned and shown, by RFLP cosegregation in a separate informative cross, to correspond to the band labelled 010 (Schechtman, Gene, submitted; R.L. Metzenberg and C. Grotelueschen, personal communication). In addition, a list of unassigned segregation patterns is also reported in the table. It should also be noted that in one progeny strain, B7, a new restriction fragment approximately 5.8 kb in length appears, that is present in neither parent. This fragment may have arisen either by reciprocal recombination between two chromosomal tips or by a recombinational repair process such as has been postulated for the spread of repeated subtelomeric X and Y' elements in yeast (Dunn et al. 1984. Cell 39:191-201; Horowitz and Haber 1985. Mol. Cell. Biol. 5:2369-2380).

Figure 1. A. DNA from various Neurospora strains was purified, digested with BamH1 and electrophoresed as described (Schechtman 1987, op. cit.), and the resulting agarose gel dried and hybridized in situ with ^32 P-labeled oligomer (TTAGGG)4 as described (Wallace and Miyada 1987, op. cit.). Lane 1, Mauriceville-1c A DNA; lane 2, multicent-2 a DNA; lanes 3-10 DNAs from progeny strains, respectively, A1, A4, B6, B7, C1, C4, D5, D7 (Metzenberg et al. 1984, op. cit.). B. Cartoon representation of the numbered telomere-derived restriction fragments seen in hybridization to the parental DNAs in lanes 1 and 2

Table 1. SEGREGATION PATTERNS THAT CAN BE ASSIGNED A CHROMOSOME END

End	Gel Fragment	A1	A4	B6	B7	C1	C4	D5	D7	E1	E3	E5	E7	F1	F3	G1	G4	H5	H7	I6	I8	J1	J4	K1	K4	L1	L4	M5	M8	N2	N3	O2	O4	P1	P4	Q2	Q4	R1	R4
		PROGENY STRAIN																																					
IIL	O14, M5	M	M	O	M	M	O	O	O	M	O	M	-	O	M	M	O	M	O	M	O	O	M	O	M	O	M	O	M	O	M	M	M	M	O	O	M	O	M
IIR	O5	O	M	M	O	M	O	O	M	M	O	M	O	O	M	O	M	M	O	O	O	O	M	M	O	M	M	M	O	M	M	O	O	O	M	O	M	O	M
IIIR	O7, M13	M	O	M	O	O	M	O	M	O	M	O	-	-	M	O	M	O	O	M	O	M	O	O	M	O	O	O	M	O	O	M	M	M	O	O	M	M	O
IVL	O10	O	M	O	O	M	O	M	O	O	M	M	O	M	O	M	O	O	O	O	M	M	M	O	M	M	O	M	O	M	O	O	O	O	O	M	M	O	M
IVR	M3	M	O	M	O	M	O	O	M	M	O	O	M	O	M	O	M	O	O	O	M	O	O	O	M	O	M	O	M	M	O	M	O	O	M	M	M	M	O
VR	M14	O	M	O	M	M	O	O	M	O	M	O	M	M	M	M	M	M	O	O	M	M	M	M	M	O	M	O	M	M	O	M	O	M	O	M	M	M	O
		UNASSIGNED SEGREGATION PATTERNS OF OTHER ENDS																																					
	O9, M9	M	O	M	M	M	O	M	O	O	M	O	M	O	O	O	M	M	O	M	O	O	M	O	M	M	M	M	O	M	O	O	M	O	O	M	O	O	O
	O11, M10	M	O	O	M	M	O	O	M	M	O	O	M	O	M	M	M	O	M	O	M	O	M	O	M	O	M	O	O	O	M	O	O	O	M	M	O	M	O
	O13	M	M	O	M	M	M	O	O	M	O	M	-	O	M	M	M	M	O	M	O	O	M	O	M	O	M	O	M	O	M	O	M	M	O	O	O	O	O
	M6	M	M	M	O	M	M	M	M	O	M	O	M	M	M	O	M	M	M	M	M	M	O	O	M	O	M	O	M	O	M	M	O	O	O	M	O	O	M
	M7	M	M	M	O	O	O	O	M	O	M	M	O	O	M	M	M	O	M	O	M	O	O	O	O	M	M	M	O	O	M	O	M	M	O	M	O	M	M
	M11	M	O	O	M	M	O	O	M	O	M	O	-	O	M	M	O	O	O	M	O	O	O	O	M	O	M	O	M	O	M	M	O	M	O	M	O	O	M

Note: Ml4 has been determined to be allelic with 012 even though 012 cannot be resolved from Ml2 on these gels, because 012 derives from the previously characterized VR telomere (Schechtman, 1987, op. cit.)

Note added in proof: C. Myers and R. Metzenberg (pers. comm.; Fungal Genet. Newsl. 36:pp-pp) have determined that the unassigned segregation pattern listed in the table as "09, M9" derives from the telomere at VIIL. Supported by NSF grant DCB-8415000. --- Department of Biology, Syracuse University, Syracuse, NY 13244. (Present address: USDA, APHIS, 6505 Belcrest Road, Hyattsville, MD 20782).