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Abstract

The map position of the calmodulin gene (*cmd*) was determined by RFLP (Restriction Fragment Length Polymorphism) mapping in *Neurospora crassa*. The *cmd* gene was mapped on chromosome V, between *al*-3 and *inl*.

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RFLP mapping of the calmodulin gene of *Neurospora crassa*

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The map position of the calmodulin gene (*cmd*) was determined by RFLP (Restriction Fragment Length Polymorphism) mapping in *Neurospora crassa*. The *cmd* gene was mapped on chromosome V, between *al*-3 and *inl*.

The calmodulin gene of *N. crassa* was isolated by Capelli et al. (1993 FEBS lett. **321**:63-68) and Melnick et al. (1993 Biochim. Biophys. Acta **1171**: 334-336). However, neither group determined the map position of this gene. We mapped the calmodulin gene by analysis of Restriction Fragment Length Polymorphism (RFLP) as follows.

Cloning of calmodulin cDNA by PCR. A cDNA library was constructed with a Marathon cDNA Amplification Kit (Clontech, CA, USA) with mRNA isolated from wild-type strain 74A as template. cDNA for calmodulin was isolated by the 5'-RACE technique using primer AP1 (supplied with the kit) and a 24-mer primer, CAACACTGCCACAATCATCCTGCA, namely, an antisense sequence primer based on the sequence from nucleotide (nt) 603 to nt 582 of the calmodulin gene reported by Capelli et al. (1993). Calmodulin cDNA was amplified by shuttle PCR (30 cycles of incubation at 94oC for 30 sec and at 68 oC for 5 min) with ExTaq polymerase (Takara, Shiga, Japan). The PCR product was cloned into the pGEM-T vector (Promega, WI, USA) and was sequenced to confirm that it encodes calmodulin.

Isolation of genomic calmodulin DNA. Genomic DNA encoding calmodulin was isolated by dot-blot hybridization of genomic DNA with part of the calmodulin cDNA probe by a sib-selection procedure. A cosmid library of genomic DNA was constructed in the cosmid vector pDC107 (Nakashima and Ishiura, unpublished). The library contained fragments of about 40 kbp from a partial *Sau*3AI digest of genomic DNA of a *N. crassa acr-2* strain. The library was divided into forty 96-well microtiter plates. The *Pst*I fragment of calmodulin cDNA, which contained the entire open reading frame of the gene for calmodulin, was labeled by the BioPrime DNA labeling system (Life Technologies, MD, USA). The colony dot-blot was allowed to hybridize with the probe, and detection was performed with the PHOTOGENE Nucleic Acid Detection System (Life Technologies). Genomic DNA encoding calmodulin was identified in cosmid 4:G2.

RFLP mapping of the calmodulin gene (cmd). RFLP mapping was performed as described by Metzenberg et al. (1984 Neurospora Newsl. **31**: 35-39). The cosmid 4:G2 was digested by *Bgl*II. We concluded that a resultant fragment of about ~9 kbp contained the calmodulin gene by Southern hybridization with calmodulin cDNA probe and then this fragment was used as a probe for RFLP mapping. Labeling and detection were performed as described above. DNA isolated from the Mauriceville-1c-A strain (FGSC #4416; represented by "M") and from Oak-Ridge-derived parent multicent-2a strain (FGSC #4488; represented by "O") were digested by various restriction enzymes, and the DNA fragments were allowed to hybridize with the probe. Among the various digests, the digest generated by *Bgl*II revealed polymorphism (Figure 1a). DNA

isolated from 38 progeny strains (FGSC #4451 - #4487), which had been obtained by crossing the "M" and "O" strains (Metzenberg and Grotelueschen, 1993 Fungal Genet. Newsl. **40**: 130-138), were digested by *Bgl*II and then allowed to hybridize with the probe. The 38 progeny exhibited polymorphism (Figure 1b). The profile in lane 19 was not obtained because we failed to prepare sufficient DNA for hybridization. The profile of RFLPs was compared with other RFLPs obtained from various genes in linkage group V (Figure 2a). The results reveal that gene for calmodulin is located on chromosome V, between *al*-3 and *inl* (Figure 2b).

Figure 1. RFLP of the calmodulin gene in the Mauriceville-1c-A strain (M), the Oak Ridge-derived parent multicent-2a strain (O), and in 38 progeny. a) The profile of RFLPs of the calmodulin gene in "M" and "O" strains. DNA was digested by various restriction enzymes as indicated above each lane. The type of strain is indicated below each lane. Polymorphism was observed only when DNA was digested by *Bgl*II. b) The profile of RFLPs of the calmodulin gene in the 38 progeny (see text). The DNA isolated from these progeny was digested by *Bgl*II. The phenotype of the inheritance of each progeny is indicated below each lane. The phenotype of the strain that corresponded to lane 19 was not determined (see text).

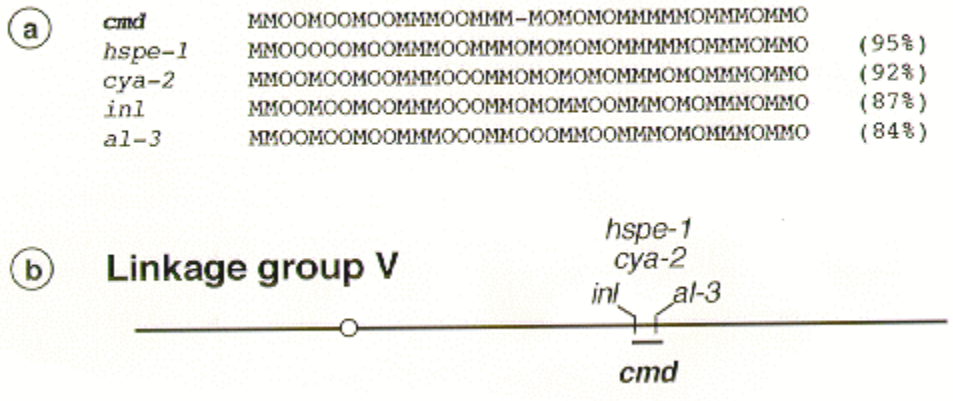

Figure 2. Mapping the calmodulin gene. a) Comparison of RFLPs of the calmodulin gene with several genes that have been mapped to linkage group V. The extent of identity of the RFLP to that of the calmodulin gene is indicated on the right in each case. b) The linkage map of the calmodulin gene (*cmd*). A partial map of linkage group V is shown, and the calmodulin gene (*cmd*) is located between *al*-3 and *inl*.

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