Determination of the Inactivating Alterations in Two Mutant Alleles of the Neurospora crassa Cross-Pathway Control Gene cpc-1

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ABSTRACT

cpc-1 is the locus specifying what is believed to be the major trans-activating transcription factor that regulates expression of amino acid biosynthetic genes subject to cross-pathway control in Neurospora crassa. Mutants altered at this locus are incapable of the global increase in gene expression normally seen in response to amino acid starvation. Using polymerase chain reaction methodology we have cloned and sequenced the inactive mutant allele, cpc-1 (CD15). The cpc-1 (CD15) mutation was found to be a single base pair deletion in codon 93 of the cpc-1 structural gene. A second, presumed lethal, allele, cpc-1 (j-5), also was investigated. Northern analyses with strains carrying the cpc-1 (j-5) allele revealed that no cpc-1 mRNA is produced. Southern and genetic analyses established that the cpc-1 (j-5) mutation involved a chromosomal rearrangement in which a break occurred within the cpc-1 locus, normally resident on linkage group VI; a small fragment from the left arm of linkage group VI, containing the cpc-1 promoter region and ylo-1, was translocated to the right arm of linkage group I. Other studies indicate that the cpc-1 locus itself is not essential for viability. Lethality previously attributed to the cpc-1 (j-5) mutation is due instead to the production of progeny that are deficient for essential genes in an adjoining segment of linkage group VI. Molecular characterization of cpc-1 (j-5) × ylo-1 pan-2 duplication progeny indicated that cpc-1 is normally transcribed towards the linkage group VI centromere.

STARVATION for individual amino acids in Saccharomyces cerevisiae, Aspergillus nidulans and Neurospora crassa results in increased expression of genes encoding several, if not most, of the amino acid biosynthetic pathway enzymes (CARSIOTIS, JONES and WESSELING 1974; CARSIOTIS and JONES 1974; Piotrowska 1980; Goc and Węglenski 1988; Hinnebusch 1988). This global response is due to increased rates of transcription of the affected genes. Extensive genetic analyses of this regulatory mechanism in yeast, termed general control of amino acid biosynthesis, have identified a hierarchy of fifteen genes involved in this complex regulatory process. The product of one of these genes, GCN4, has been studied extensively; GCN4 is a DNA-binding protein and the transcriptional activator of amino acid biosynthetic genes (HOPE and STRUHL 1985, 1987; ARNDT and FINK 1986; Hinnebusch 1988).

Only a few genetic loci have been implicated so far in the similar regulatory response in Neurospora, referred to as cross-pathway control (DAVIS 1979; BARTHELMESS 1982, 1984; D. KRÜGER, J. KOCH and I. B. BARTHELMESS, manuscript in preparation). The majority of mutants that are defective in cross-pathway control have alterations at one locus, cpc-1. Mutations in this gene, like those in GCN4 in yeast, abolish the increase in expression of amino acid biosynthetic genes normally seen in amino acid auxotrophs grown on limiting amounts of the required amino acids. cpc-1 has been cloned and sequenced (PALUH et al. 1988). The deduced amino acid sequence of its polypeptide product, CPC1, shows homology to yeast GCN4 in regions known to be critical for transcription activation, and for DNA-binding (PALUH et al. 1988).

No alterations mapped to the cpc-1 locus have previously been characterized at the molecular level. In this paper we identify the alterations in two cpc-1 mutant alleles: cpc-1 (CD15) and cpc-1 (j-5). Strains carrying the cpc-1 (CD15) allele do not increase transcription of amino acid biosynthetic genes under conditions of amino acid limitation. However, transcription of cpc-1 (CD15) itself is increased under these conditions (PALUH et al. 1988). We have found that the cpc-1 (CD15) allele has a single base pair deletion in codon 93 of the cpc-1 structural gene. The second allele analyzed, cpc-1 (j-5), was originally characterized as being lethal in the absence of an unlinked rescuing mutation, slo, that results in a “slow growth” phenotype (BARTHELMESS 1984). Recently, however,
a deletion derivative of cpc-I, constructed by transforming Neurospora with disrupted cpc-1 DNA, was shown to be viable (M. PLAMANN unpublished results). The biochemical and genetic analyses described in this paper indicate that the cpc-1 (j-5) alteration involved a chromosomal rearrangement and that cpc-1 is not essential for viability.

MATERIALS AND METHODS

Strains: Neurospora crassa strains used as sources of DNA for polymerase chain reaction (PCR) analyses, southern and northern analyses were obtained from the Fungal Genetics Stock Center (University of Kansas Medical Center): cpc-I (CD15) (FGSC #4264), cpc-1 (j-5) (FGSC #4433 and #4434), and 74-OR23-1A (FGSC #8015). Strains used for genetic analyses, described in the text, were from the collection of David Perkins.

Plasmids: pCPC1-2, which contains cpc-I on a 5.5 kb genetic fragment in pUC12, was described previously (PALUH et al. 1988). pUC118, used in DNA sequence analyses, was provided by J. VIEIRA, Waksman Institute, Piscataway, N.J. pPT7C2, used for in vitro transcription/translation experiments, contains a RsaI to EcoRI 1.2-kb cDNA fragment from pM031 (PALUH et al. 1988) cloned into plbluescript (Stratagene) at the HincII and EcoRI sites, then recloned into p17-7 (TABOR and RICHARDSON 1985) at the XbaI and SalI sites. The DNA sequence between positions 1176 and 1180 (ACGGC) was modified by site-directed mutagenesis (BAUER et al. 1985; KENKEL 1985) to resemble an E. coli ribosome binding site. A RsaI-BamHI fragment from pPCD1541 (described in the text), containing the cpc-1 (CD15) mutational change, was incorporated into pPT7C2 in place of native cpc-1 sequences. The resulting plasmid was designated pPT7CD15. The genomic clone of the cpc-I structural gene, pM061, has been described (PALUH et al. 1988).

PCR analysis and DNA sequencing: Genomic DNAs for PCR (MULLIS and FALOONA 1987) and southern analyses were isolated as described (METZENBERG and BAISCH 1981) with suggested modifications (STEVENS and METZENBERG 1989). Samples containing 3 to 5 µg undigested genomic DNA from 74A (wild type), cpc-1 (CD15), cpc-I (j-5) or 0.1 µg of Salt linearized, CsCl-purified pCPC1-2 control DNA, were incubated with 0.2 to 5.0 µg each of two nonpurified oligonucleotide primers and amplified for a total of 40 cycles using an Epicomp temperature cycler. Cycle conditions were as follows: [94°C 1 min, 37°C 2 min, 74°C 2 min] for 15 cycles, [74°C 1 min] for 5 cycles; repeat. The reaction buffer and taq polymerase conditions were according to the recommendations of Perkin-Elmer/Cetus. PCR products were examined by electrophoresis on 0.8% agarose gels run in 0.5X TBE buffer. TBE is 90 mM Tris-Cl (pH 8.3), 90 mM boric acid and 2.5 mM EDTA. DNA sequencing was performed by the dyeoxy chain termination method (SANGER, NICKLEN and COULSON 1977), using Sequenase (United States Biochemical, Cleveland, Ohio).

In vitro transcription/translation: In vitro transcription was performed as recommended (United States Biochemical). Transcription was carried out using 0.5 to 3.0 µl of a 50 µl in vitro transcription mixture and 50 µCi [α-32P]UTP-methionine (800 Ci/mmol; Amersham) in a 25 µl reaction volume. Rabbit reticulocyte lysates were used as described (Promega Biotec, Madison, Wisconsin).

Northern analysis: Total cellular RNA was prepared as described (KINNAIRD et al. 1982). RNA species were separated by electrophoresis on 1.4% agarose/6% formaldehyde gels (LEHRACH et al. 1977), and transferred to nitrocellulose (MANIATIS, FRITSCH and SAMBROOK 1982). For hybridization, DNA probes were prepared by incorporating [α-32P]dCTP using the "Random Primed DNA Labeling Kit" (Boehringer) to a specific activity of at least 0.5–2 x 106 cpm/µg DNA using 0.1–0.5 µg DNA in a total volume of 20 µl.

Southern analyses: DNAs were fractionated on 1.0% agarose/Tris-borate-EDTA gels, transferred to NEN Research Products, Boston, Massachusetts) and probed with [α-32P]dCTP random-hexamer-labeled cpc-I fragments (FEINBERG and VOGELSTEIN 1988) as described (MANIATIS, FRITSCH and SAMBROOK 1982).

Crosses with cpc-I (j-5): Standard procedures were employed (DAVIS and DE SERRES 1970).

RESULTS

PCR analyses of cpc-I (CD15) and cpc-I (j-5) alleles. Pairs of oligonucleotides were chosen for PCR analyses that would prime the synthesis of fragments of cpc-I 0.2–1.8 kb in length. Undigested genomic DNA isolated from 74A (wild type) or linearized pCPC1-2 DNA containing the entire cpc-I transcription unit were used as control DNAs for adjusting annealing temperatures and extension times (MATERIALS AND METHODS).

PCR fragments were generated using genomic DNA isolated from strains carrying the cpc-I (CD15) or cpc-I (j-5) alleles. A series of overlapping DNA fragments which encompassed the entire cpc-I transcriptional unit, were produced. One tenth of each PCR reaction mixture was electrophoresed on agarose gels, transferred to Genescreen, and probed with cpc-I DNA fragments to identify specific cpc-I PCR DNA products.

No discrepancies in predicted fragment lengths were observed between PCR DNA fragments generated from cpc-I (CD15) DNA or from control DNAs. PCR DNA fragments generated from cpc-I (j-5) DNA were as predicted for the cpc-I structural gene and for part of the cpc-I mRNA leader region, but no PCR DNA fragments were obtainable that included the entire region encoding the 5′ segment of the cpc-I transcript (data not shown).

Sequence analysis and cloning of cpc-I (CD15). PCR-generated DNA fragments, that contained overlapping segments of the cpc-I (CD15) structural gene were isolated from agarose gels and sequenced directly and/or cloned into pUC118, and then sequenced. Sequencing of several cpc-I (CD15) PCR DNA fragments identified a single base pair deletion removing a G of codon 93 of the cpc-I structural gene (Figure 1). No other changes were detected. This was confirmed by sequencing several isolates of cpc-I (CD15) PCR products cloned into pUC118.

For in vitro synthesis experiments, the intact cpc-I (CD15) structural gene was cloned from genomic DNA using PCR methods (PCR analyses and mate-
A 1.15-kb DNA fragment was generated which contained the entire cpc-1 (CD15) structural gene, including 130 bp of upstream and downstream sequences. DNA fragments from several PCR reactions were isolated and cloned into the HincII site of pBluescript and sequenced to confirm the single base pair change. One of these isolates was designated pJPCD1541.

Analysis of cpc-1 (CD15)-directed in vitro translation product: pJPT7C2, pMO61 and pJPT7CD15 contain the cDNA copy of the cpc-1 structural gene, the genomic copy of cpc-1, or cpc-1 of the mutant cpc-1 (CD15), respectively, inserted downstream from a T7 promoter located within the vector (MATERIALS AND METHODS). mRNAs synthesized from these constructs using T7 RNA polymerase were translated in vitro using rabbit reticulocyte lysate.

cpc-1 encodes a polypeptide 268 amino acids in length, with a predicted molecular mass of 30 kDa. The CPC1 polypeptide has an aberrant mobility (42 kDa) which presumably is due to the highly charged carboxyl end of the protein (PALUH et al. 1988).

In vitro translation of mRNA derived from the genomic copy of the cpc-1 structural gene, which contains an in-frame intron that encodes 17 additional amino acids, yields a polypeptide 285 amino acids in length. This polypeptide migrates on SDS-polyacrylamide gels with a mobility of 45 kDa (PALUH et al. 1988).

The position of the frameshift mutation in cpc-1 (CD15) leads to the prediction that its specified polypeptide should be 220 amino acids in length, and should have a molecular mass of 25 kDa. Since the last 127 amino acids of this polypeptide would be translated from a shifted reading frame, the aberrant mobility observed with native CPC1 would not be expected.

The expected translation products were detected when pJPT7C2 and pMO61 mRNAs were used as templates but no discrete products were observed with cpc-1 (CD15) mRNA prepared using the pJPT7CD15 template (data not shown).

Northern analyses of cpc-1 (j-5): 74A(wild type) and a strain carrying the cpc-1 (j-5) allele were grown on minimal medium (DAVIS and DE SERRES 1970) or under conditions of amino acid starvation [in the presence of the inhibitor of histidine biosynthesis, 3-amino-1,2,4-triazole (3AT)]. Total cellular RNA was isolated from 150 mg lyophilized mycelial samples and the RNA species were separated electrophoretically (MATERIALS AND METHODS). To verify equal loading and transfer of RNA samples, filters were first probed with a 32P-labeled pBT6 plasmid DNA containing the β-tubulin structural gene (ORBACH, PORRO and YANOFSKY 1986). The filters were then stripped and reprobed with a labeled DNA fragment from pCPC1-2 (Figure 2).

The cpc-1 mRNA level was low when total RNA was isolated from wild-type cultures grown in minimal medium, but was clearly induced 30 min after 3AT treatment. By contrast, cpc-1 (j-5) total RNA did not contain detectable levels of cpc-1 mRNA even following treatment with 3AT for up to 240 min.

Southern analysis of cpc-1 (CD15) and cpc-1 (j-5). Approximately 10 μg of genomic DNAs isolated from 74A(wild type) and from strains carrying the cpc-1 (CD15) or cpc-1 (j-5) allele were digested with restriction endonucleases and the fragments separated electrophoretically. The DNA fragments used as hybridization probes either contained the region corresponding to the 5' end of the cpc-1 transcript, or flanked this region.

Restriction patterns generated with digested cpc-1 (CD15) and 74A(wild type) genomic DNAs were found to be identical (data not shown). The probe used was a 1.4 kb HindIII-SphI fragment which contains the 5' noncoding region of the cpc-1 transcript.
and upstream sequences (Figure 3B). However, a 250-bp PstI-BglII fragment present in digested 74A(wild type) DNA was absent from cpc-1 (j-5) DNA digests; a new, 2.0-kb band, was present in the cpc-1 (j-5) digests (Figure 3A). This result indicates that there is a chromosomal break within the 250-bp PstI-BglII region of cpc-1 (j-5) (Figure 3B). An MboII digest of cpc-1 (CD15) and cpc-1 (j-5) DNAs, probed with the 1.4-kb HindIII-SphI DNA fragment, contains an 815-bp MboII fragment which partially overlaps the 250-bp PstI-BglII fragment (Figure 3, A and B). The smaller MboII fragment, located in the region of the breakpoint, was too small to be detected by southern analyses. The extent of overlap between these two fragments resolves the breakpoint of cpc-1 (j-5) to within a 170-bp MboII-BglII region (Figure 3, A and B, brackets).

Using DNA probes that flank this MboII-BglII 170 bp segment the breakpoint in the cpc-1 (j-5) allele was further localized to within a 100 bp segment, starting 40 bp downstream from the cpc-1 transcription start site. At least 5.0 kb of foreign DNA was determined to be attached to the cpc-1 locus 5' to the break point, and at least 10 kb of foreign DNA was attached to the cpc-1 locus 3' to the break point. A partial restriction map of these foreign DNA segments is shown in Figure 3C.

Crosses with the cpc-1 (j-5) mutant: Initial genetic analysis of cpc-1 (j-5) suggested that it represented a lethal allele of cpc-1 that could only be identified in a strain carrying an unlinked modifier, slo, that causes a "slow growth" phenotype (Barthelmes 1984). A cross of cpc-1 (j-5) with wild type produces three classes of progeny which were classified as do+ cpc-I+, slo cpc-I+ (barren in subsequent crosses) and slo cpc-I. The lack of do+ cpc-1 progeny was interpreted to indicate the lethal nature of the cpc-1 (j-5) allele in the absence of the slo mutation.

During the course of our work the observation was made that a cross of cpc-1 (j-5) by wild type produced
only 75% black (viable) spores. The presence of 25% defective white spores is characteristic of an insertional translocation that generates viable duplications and nonviable deficiencies (PERKINS 1974). Examination of unordered ascii from the above cross showed: 14.6% 8 black:0 white (8B:0W) spores, 64.7% 6B:2W, 19.3% 4B:4W, 0.5% 2B:6W, 0.5% 0B:8W (n = 218). The high frequency of 6B:2W ascii with approximately equal numbers of 8B:0W and 4B:4W ascii is consistent with the hypothesis that the cpe-I (j-5) strain contains a chromosomal rearrangement with one breakpoint near a centromere (at cpe-I) and the other breakpoint far from the centromere of another chromosome.

Therefore, the progeny from the above cross should consist of four classes: normal wild type, insertional translocation, viable duplication and lethal deficiency (Figure 4). It would be predicted that the duplication class would be barren (this is typical for Neurospora duplications) and the cpe-I progeny lacking the chromosome with the translocated part of linkage group VI would be present as white, viable spores (Figure 4B). The original hypothesis required the progeny containing the modifier slo to be barren when segregated with cpe-I+ but fertile when segregated with cpe-I (j-5).

If the insertional translocation hypothesis is correct then recessive markers that enter the cross with the normal sequence parent will be expressed in the duplication progeny if they map outside the translocated segment, but will be heterozygous and not expressed if they map within the translocated segment. Therefore, linkage group VI recessive markers covered by the duplication should be expressed at a 1+:2 ratio among the progeny and linkage group VI recessive markers not covered should be expressed at a 2+:1 ratio (see Figure 4B).

Croses of cpe-I (j-5) with chol-2 ad-8 lys-5 un-4 pan-2 and ylo-I pan-2 strains indicated that ylo-1, but not the other linkage group VI markers, was likely included in the duplication (Table 1A and 1B). Thus, there was a low ylo-1/ylo-1+ progeny ratio (29:41) relative to the pan-2/pan-2+ progeny ratio (42:28) (Table 1B). Additional analysis of the progeny from the cpe-I (j-5) × ylo-1 pan-2 cross showed that all of the ylo-1 cpe-I+ progeny were fertile and all the ylo-1+ cpe-I+ progeny were barren as predicted by the insertional translocation hypothesis. (The original hypothesis of an unlinked modifier would have predicted that half of each class would be barren.) These observations provide strong evidence that the cpe-I (j-5) strain contains a chromosomal rearrangement which resulted from a break within the cpe-I genetic region, and that a segment of linkage group VI including ylo-1 but not un-4, has been inserted into another chromosome, far from its centromere.

The recipient chromosome was tentatively identified by the cross cpe-I (j-5) × aleo; esp-2 (method of PERKINS et al. 1969). Analysis of the progeny of this cross showed a 1:2 ratio of al-1/al-1+ which indicated that the other breakpoint was likely to reside in either the right arm of linkage group I or linkage group II. In addition, 90 of 91 al-1+ progeny were ylo-1+. This close linkage is consistent with the concept that ylo-1 is within the segment of linkage group VI that was inserted into another chromosome. A follow up cross of cpe-I (j-5) × al-1; fl (linkage group I and II markers, respectively) produced a 1:2 ratio of al-1/AL-1+ progeny but a 1:1 ratio of fl/fl+ progeny indicating that the right arm of linkage group I is likely to be the site where the segment of linkage group VI was inserted. An additional cross of cpe-I (j-5) × al-2 un-18 (two markers within the right arm of linkage group I) also produced 1:2 ratios of al-2/AL-2+ and un-18/un-18+ progeny. In addition, no al-2 cpe-I or un-18 cpe-I progeny were observed. These results confirm that linkage group I is the recipient chromosome, but the precise position of the insertion site with respect to the linkage group I markers could not be inferred from the data (data not shown).

Southern analysis of a duplication progeny from the cpe-I (j-5) × ylo-1 pan-2 cross. Southern analysis of cpe-I (j-5) established that the chromosome break which disrupted cpe-I DNA occurred in a 100 bp segment, starting 40 bp downstream from the cpe-I transcription start site (Figure 3). Restriction endonuclease generated fragments that overlap the breakpoint are not observed by southern analysis of cpe-I.
apparent recombinants in the column headed "Fertile" are bona fide crossovers, but apparent test crosses of other hybrid fragment would not be observed because the expected to identify the new fragment that extends from the parental strains. The crossovers between the parental DNA into linkage group VI sequences. The long horizontal line represents cpc-1 DNA and flanking regions, pairs of diagonal lines through this line represent DNA not shown, HindIII sites (H) are indicated below the line along with the distances between the respective sites, the bar below the line represents the approximate location of the breakpoint that occurred in cpc-1 (j-5). Above the line the transcription start site and direction of transcription is indicated (+1), the two short and one long rectangular boxes represent the two short open reading frames in the cpc-1 leader region and the cpc-1 structural gene, respectively, and the two bars above the line represent the DNA segments used as 5' and 3' probes. (B) Genomic DNAs isolated from 74A(wild type), cpc-1 (j-5) and a ylo-1 pan-2 duplication strain were digested with HindIII, separated electrophoretically and hybridized with the indicated probes. The two arrows indicate the two hybrid HindIII fragments (4.1 and 3.7 kb) found in cpc-1 (j-5) (see text). Note that only the larger fragment (4.1 kb) is found in the duplication strain and it is only observed with the 5' probe. by southern analysis using two probes that recognize cpc-1 DNA 5' or 3' to the cpc-1 (j-5) breakpoint (Figure 5A). As shown in Figure 5B, the 1910 bp HindIII fragment that overlaps the breakpoint is present using strain 74A(wild type) DNA, but is absent using cpc-1 (j-5) DNA. As expected, the use of the 5' and 3' probes together identified two new HindIII fragments (4.1 and 3.7 kb) in cpc-1 (j-5). The larger of these two fragments (4.1 kb) is also present in the duplication strain. Additional analysis showed that this hybrid fragment is observed in the duplication strain when the 5' probe is used but not when the 3' probe is used (Figure 5B). This result indicates that cpc-1 DNA 5' to the breakpoint, plus additional linkage group VI DNA, was inserted into linkage group I.

**DISCUSSION**

In yeast the response to starvation for single amino acids has been shown to involve complex interactions between positive and negative-acting factors (HINNEBUSCH 1988). The end result, increased transcription of the genes of amino acid biosynthesis, requires bind-
ing of the central trans-activating regulatory protein, GCN4, to one or more copies of its target sequence, ATGACTCAT, associated with each responding gene (Hope and Struhl 1985; Arndt and Fink 1986). The cloning of the gene for GCN4 and extensive analysis of its polypeptide product, and the recent cloning and analysis of genes and proteins that modulate GCN4 expression, have provided a wealth of information on the mechanism of general control in Saccharomyces cerevisiae. A similar general coregulated response to amino acid starvation is seen in other fungi, although until recently, no genes encoding proteins similar in function to GCN4 had been cloned. Genetic and biochemical studies with Neurospora had identified one gene, cpc-1, as the principal regulatory gene responsible for cross-pathway control (Barthelmess 1982; Paluh et al. 1988). Mutations in this gene prevent the transcriptional response characteristic of this regulatory mechanism (Flint and Wilkening 1986). In agreement with these observations, the predicted amino acid sequence of CPC1 indicates that this polypeptide has residue identities with GCN4 of yeast in two functionally important regions, GCN4's transactivation and DNA binding domains. Recent in vitro analyses have also established that CPC1 is a dimeric DNA binding protein (J. L. Paluh unpublished results) that recognizes the same target sequence as GCN4 (D. Ebbolo, J. L. Paluh, M. Plamann, M. S. Sachs and C. Yanofsky, manuscript in preparation). In the studies described in this paper we determined the genetic changes in two inactive alleles of cpc-1: cpc-1 (CD15) and cpc-1 (j-5). The cpc-1 (CD15) allele was cloned from genomic DNA using PCR technology. Sequence analyses showed that the cpc-1 (CD15) mutation involved a single base pair deletion that introduces a frameshift in codon 93 of the cpc-1 coding region. This frameshift should result in the production of a polypeptide 220 amino acids in length, in which the last 127 amino acids are specified in the production of a polypeptide 220 amino acids in length, in which the last 127 amino acids are specified by a shifted reading frame. The mutant polypeptide is predicted to lack those segments that are believed to be responsible for trans-activation, DNA binding, and dimerization. Attempts to produce a discrete cpc-1 (CD15) polypeptide in vitro, using rabbit reticulocyte lysates, were unsuccessful.

The second mutant, cpc-1 (j-5) was initially described as a lethal allele whose lethality was suppressed by an unlinked second mutation, sia, resulting in a "slow growth" phenotype. Our biochemical and genetic analyses of cpc-1 (j-5) requires reinterpretation of the original results. We have established that the cpc-1 (j-5) mutation involved a chromosomal break that disrupted the cpc-1 functional unit. Southern analysis showed that the break was within a 100 bp segment, starting 40 bp downstream from the transcription start of cpc-1, and that foreign DNA now resides on either side of the break. A partial restriction map of the foreign DNAs was obtained. Genetic studies have shown that a small segment of the left arm of linkage group VI, including part of the cpc-1 functional unit and containing ylo-1, was translocated to the right arm of linkage group I. Additional southern analysis of a duplication progeny from a cpc-1 (j-5) × ylo-1 pan-2 cross provided physical evidence that it was the cpc-1 promoter region, through ylo-1 of linkage group VI, that was inserted in the right arm of linkage group I. The fact that the gene order on linkage group VI is known to be ylo-1-cpc-1-centromere (Davis 1979) allows one to conclude that cpc-1 is normally transcribed towards the linkage group VI centromere. Consistent with the genetic disruption found in cpc-1 (j-5), strains with this allele were incapable of synthesizing cpc-1 mRNA. These observations indicate that the lethality associated with the cpc-1 (j-5) mutation was not due to cpc-1 inactivation, but rather was due to a chromosomal segment deficiency in one-fourth of the progeny produced in a cross with a normal allele. The observation that a specific deletion of the entire cpc-1 gene is viable supports this interpretation (M. Plamann unpublished results). Thus, in Neurospora crassa as in Saccharomyces cerevisiae, the gene that specifies the key transcriptional activator regulating expression of amino acid biosynthetic genes is a dispensable gene.

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