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Identification of a cosmid clone containing the *Neurospora crassa* *lys-5* and *un-4* genes, isolation of a partial *lys-5* cDNA and associated chromosome walking.

Abstract

The *un-4* gene of *Neurospora crassa* was cloned to determine the limits of a chromosome walk on linkage group VI (LGVI) and to allow analysis of *un* loci on LGVI. Subsequent analysis identified the *lys-5* locus on the same cosmid clone as *un-4*. We have isolated and sequenced a partial *lys-5* cDNA clone and initiated a chromosome walk from the *lys-5*, *un-4* cosmid clone.

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The *un-4* gene of *Neurospora crassa* was cloned to determine the limits of a chromosome walk on linkage group VI (LGVI) and to allow analysis of *un* loci on LGVI. Subsequent analysis identified the *lys-5* locus on the same cosmid clone as *un-4*. We have isolated and sequenced a partial *lys-5* cDNA clone and initiated a chromosome walk from the *lys-5*, *un-4* cosmid clone.

A chromosome walk from the *cpc-1* locus has been extended 420 kb towards the left telomere of linkage group VI, (LGVII, Wan *et al.* 1997 Fungal Genet. Biol. 21:329-336). One of three heat-sensitive loci of unknown function on LGVI, *un-13*, was found in the *cpc-1* walk. The *un-4* locus maps to LGVII. Three rounds of transformation using sib-selection with cosmid DNA pools from the Orbach/Sachs *Neurospora crassa* genomic library identified an *un-4* cosmid, G13:8:G, by selection for transformants able to grow at the restrictive temperature of 34°C. A 1.2-kb cDNA isolate from a cDNA library (based on mRNA isolated from dormant conidia and kindly provided by M. Sachs), designated pYW19-2, was identified using a G13:8:G insert probe.

DNA sequence analysis of pYW19-2 identified an open reading frame encoding a deduced polypeptide with strong similarity to homocitrate synthases and isopropylmalate synthases from other organisms (Figure 1). *Neurospora lys-5* mutants lack homocitrate synthase activity. G13:8:G DNA complements *lys-5* spheroplasts allowing growth on minimal medium. *lys-5* maps 2% away from *un-4* and *un-4*, by definition, is irreparable by supplementation at the restrictive temperature. Thus, *un-4*⁺ and *lys-5*⁺ are separate loci and both are present in G13:8:G. pYW19-2 likely represents a partial *lys-5* cDNA clone. The partial deduced Lys-5 polypeptide has highest similarity to the homocitrate synthase of *Penicillium chrysogenum* with 80% identity in an optimized alignment (Figure 2).

1	CGTTATTGAG	TAIGTCAAGT	CCAAGGGACT	TGAGGTTGCG	TTCTCTCCG	AGGATTCCTT
	V I E	Y V K	S K G L	E V R	P S S	E D S F
61	CCGCTCCGAT	CTCGTCGATC	TCCTTTCCCT	TTACCGCGCT	GGTGACAAGG	TCGGCGTCCA
	R S D	L V D	L L S L	Y R A	V D K	V G V H
1	CCGTGTGGT	ATCGCCGATA	CGTGGCGGTG	CTGTTCTCCC	CGCCAGGTCT	ATGACCTCGT
	R V G	I A D	T V G C	A S P	R Q V	Y D L V
181	CCGTACCTT	CGCGCGGTG	TTTCGTGCGA	TATCGAGACC	CACTTCCACG	ACGACACTGG
	R T L	R G V	V S C D	I E T	H F H	D D T G
241	CTGCGCCATT	GCCAACGCGT	ACTGTGCTCT	CGAGGCTGGT	GCCACCCACA	TCGACACCTC
	C A I	A N A	Y C A L	E A G	A T H	I D T S
301	CGTTCTCGGT	ATCGGCGAGC	GTAACGGTAT	CACCCCTCTC	GGTGGCTTGA	TGGCTCGCAT
	V L C	I G E	R N G I	T P L	G G L	M A R M
361	GATCGTTACC	AGCCCCGACT	ACGTCAAGAG	CAAGTACAAG	CTCCACAAGC	TCAAGGAGCT
	I V T	S P D	Y V K S	K Y K	L H K	L K E L
421	CGAGGATTTG	GTTGCCGAGG	CTGTTGAGAT	CAACACCCCC	TTCAACAACC	CCATCATCTG
	E D L	V A E	A V E I	N T P	P N N	P I T G
481	TTTCTGCGCC	TTACCCACAC	AGGCTGGCAT	CCAOGCCAAG	GCCATCCTCA	ACAACCCACG
	F C A	F T H	K A G I	H A K	A I L	N N P S
541	CACCTATGAA	ATTCTCAACC	CTGCGCAGTT	GGGTCTCACC	CTGTACGTCC	ACTTCGCTTC
	T Y E	I L N	P A D F	G L T	R Y V	H F A S
601	CGGCTTGACT	GGCTGGAACG	CGTCAAGAC	CCGTGTGGGC	CAGCTTGCTC	TCGAGATGAC
	R L T	G W N	A V K T	R V G	Q L G	L E M T
661	CGAGCACCAG	GTCANAGGAT	GTAACGCCAA	GATCAAGGCC	CTTGCCGAGC	TGCGCCCAAT
	D D Q	V K E	C T A K	I K A	L A D	V R P I
721	CGCCCTGAGC	GACGCGGATT	CGATCATCCG	TACTTTCCAC	CTCGGTCTTC	ACGAGCAGAA
	A I D	D A D	S I I R	T P H	L G L	H E Q N
781	CAAGGTCGAG	CCTCCCGCTG	TTGTGAGAG	CTAAGCGGAA	GCAGAGCGTT	CGACCAACGG
	K V Q	P P A	V V E N	*		
841	AGTTGTGCTT	TAGCATGAAG	GGGAATATAC	CAGGATTTT	ACGAGGAGAG	ATGCGGGCAT
901	CAFGACGATT	TTCTTTTTC	TTGTGTTTGG	GGTCATTTT	CACACATCCA	CCGGAGTTCT
961	TTGAGTACTA	TGATTTCCCT	GTTTGGGGAG	CAAAAAGGGG	GTTGATTGGG	TTAACTGGGG
1021	ATGACTGAGC	AGGCCAATAT	TGCCGACTGT	GTTCTTAATC	AGGGGGAATG	CTCGTCGAAA
1081	AATGAGCATG	AGATAGACAA	AATCAACGGG	AGACGAAAGT	AACAACGTCA	CCTGATTGTC
1141	CTTCAAAAAA	AAAAAAAAAA	AA			

Figure 1. Nucleotide sequence of the cDNA insert of pYW19-2 and the deduced polypeptide product (GenBank AF142777). The stop codon is indicated by a *. Several isolates, including NC4A2-T7, from the Neurospora Genome Project, University of New Mexico, overlap pYW19-2 from position 549 to the polyadenylation site.

The pYW19-2 insert was used to probe a Southern blot of G13:8:G restriction digests. Results suggest that an approximately 6.3-kb *Eco*R1 fragment contains the *lys-5* gene. As cosmid G13:8:G was not identified in the *cpc-1* walk we initiated a chromosome walk from the *lys-5/un-4* region in an attempt to link up to our *cpc-1* walk. A G13:8:G based probe identified cosmid X6:6. A X6:6:F based probe identified cosmid X22:2:B. No new cosmid clones were identified with a X22:2:B based probe.

Penicillium c. HC Synthase	181	IEVIEFVKSK	GIEIRFSSD	
		+	+ +	
Neurospora Lys-5		VIEYVKSK	GLEVRFSSD	
Pc HC	SFRSDLVDLL	SIYSAVDKVG	VNRVGIADTV	GCASPRQVYE LVRVLRGVVG
		+		+ + +
Lys-5	SFRSDLVDLL	SLYRAVDKVG	VHRVGIADTV	GCASPRQVYD LVRTLGRVVS
Pc HC	CDIETHFHND	TGCAIANAF	C	ALEAGATHID TSVLGIGERN GITPLGGLMA
	+	+		
Lys-5	<u>CDIETHFHDD</u>	<u>TGCAIANAYC</u>	A	ALEAGAYHID TSVLGIGERN GITPLGGLMA
Pc HC	RMMVADREYV	KSKYKLEK	LK EIEDLVAEAV	EVNIPFNYYI TGFCATHKA
	+	+		+
Lys-5	RMIVTSPDYV	KSKYKLHK	LK ELEDLVAEAV	EINTPFNNPI TGFCATHKA
Pc HC	GIHAKAILNN	PSTYEIINPA	DFGMSRYVHF	ASRLTGWNAI KSRAQQLKE
		+	++	+ }+ +
Lys-5	GIHAKAILNN	PSTYEILNPA	DFGLTRYVHP	ASRLTGWNAV KTRVGQLGLE
Pc HC	MTDTQYKECT	AKIKAMADIR	PIAVDDADSI	IRAYHRNLKS GENKPLDLT
		+ +	+	+ + + ++
Lys-5	MTDDQVKECT	AKIKALADVR	PIAIDDADSI	IRTFHLGLHE QNKVQPPAVV
Pc HC	AEEQAFAAK	EKELLEAQAA	GLPV	
	+			
Lys-5	EN			

Figure 2. Comparison of the amino acid sequence of the homocitrate synthase of *Penicillium chrysogenum* (Gene Bank AJ223630) and Lys-5. Identical residues are indicated by a vertical line. Residues of similar chemical properties are indicated by a +. A motif conserved in all known homocitrate synthases is underlined.