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Thomas J. Schmidhauser
The University of Southwestern Louisiana

Dan Chen
The University of Southwestern Louisiana

Yihong Wan
University of Colorado

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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.

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.

Thomas J. Schmidhauser, Dan Chen and Yihong Wan* - Department of Biology, The University of Southwestern Louisiana, Lafayette, LA and *University of Colorado Health Sciences Center, Denver, Colorado

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 LGVIL. 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).

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1  CGTTATTGAG  TATGTCAAGT  CCAAGGGACT  TGAGGTTGCG  TTCTCCTCCG  AGGATTCCTT
   V I E Y V K S K G L E V R P S S E D S F
61  CCGCTCCGAT  CTCGTGCGATC  TCCTTTCCCT  TTACCGCGCT  GTTGACAAGG  TCGGCGTCCA
   R S D L V D L L S L Y R A V D K V G V H
101 CCGTGTCCGT  ATCGCCGATA  CGTFCGGCTG  CGTTCTCCCC  CGCCAGGTCT  ATGACCTCGT
   R V G I A D T V G C A S P R Q V Y D L V
181 CCGTACCCTT  CGCGGCGTCG  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  GCCAACCGCT  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 CGTTCTCCGT  ATCGGCGAGC  GTAACGGTAT  CACCCCTCTC  GGTGGCTTGA  TGGCTCGCAT
   V L C I G E R N G Y 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  GTTGCAGGAG  CTGTTGAGAT  CAACACCCCC  TTCAACAACC  CCATCACTGG
   E D L V A E A V E I N T P P N N P I T G
481 TTTCTGCGCC  TTCACCCACA  AGGCTGGCAT  CCAOGCCAAG  GCCATCCTCA  ACAACCCAG
   F C A F T H K A G I H A K A I L N N P S
541 CACCTATGAA  ATTCTCAACC  CTGCCGACTT  CGGTCTCACC  CGCTACGTCC  ACTTCGCTTC
   T Y E I L N P A D P G L T R Y V H F A S
601 GCBCTTGACT  GGCTGGAACG  CCGTCAAGAC  CCGTGTCCGC  CAGCTTGGTC  TCGAGATGAC
   R L T G W N A V K T R V G Q L G L E M T
661 CGACGACCAG  GTCAAGGAT  GTACCCGCAA  GATCAAGGCC  CTGCGCGACG  TCGGCCAAT
   D E Q V K E C T A K I K A L A D V R P I
721 CGCCATCGAC  GACGCCGATT  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 CAAGGTCCAG  CCTCCCGCTG  TTGTGAGAA  CTAAGCGGAA  GCAGAGCGTT  CGACCAACGG
   K V Q P P A V V E N *
841 AGTTGTCTT  TAGCATGAAG  GGAATATAC  CAGGATTTT  ACGAGGAGAG  ATGCGGGCAT
901 CAFGACGATT  TTCTTTTAC  TTGTGTTGG  GGTCATTTT  CACACATCCA  CCGGAGTTCT
961 TTGAGTACTA  TATCTCCCT  GTTTGGGGAG  CAAAAAGGG  GTTGATTGGG  TTAAGTGGG
1021 ATGACTGAGC  AGGCCAATAT  TGCCGACTGT  GTTCCTAATC  AGGGGAATG  CTCGTCGAAA
1081 AATGAGCATG  AGATAGACAA  AATCAACGGG  AGACGAAAGT  AACAACGTCA  CCTGATTGTC
1141 CTTCAAAAAA  AAAAAAAA  AA

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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 *EcoRI* 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.

<i>Penicillium c.</i> HC Synthase	181	IEVIEFVKSK	GIEIRFSSD		
		+	+ +		
<i>Neurospora</i> Lys-5		VIEYVKSK	GLEVRFSSD		
Pc HC	SFRSDLVDLL	SIYSAVDKVG	VNRVGIADTV	GCASPRQVYE	LVRVLRGVVG
		+		+	+ +
Lys-5	SFRSDLVDLL	SLYRAVDKVG	VHRVGIADTV	GCASPRQVYD	LVRTLGRVVS
Pc HC	<u>CDIETHFHND</u>	TGCAIANAFD	ALEAGATHID	TSVLGIGERN	GITPLGGLMA
	+	+			
Lys-5	<u>CDIETHFHDD</u>	TGCAIANAYC	ALEAGAYHID	TSVLGIGERN	GITPLGGLMA
Pc HC	RMMVADREYV	KSKYKLEKLEK	EIEDLVAAEAV	EVNIPFNYYI	TGFCAFTHKA
	+	+		+	
Lys-5	RMIVTSPDYV	KSKYKLHKLK	ELEDLVAAEAV	EINTPFNNPI	TGFCAFTHKA
Pc HC	GIHAKAILNN	PSTYEIINPA	DFGMSRYVHF	ASRLTGWNAI	KSRAQQLKLE
		+	+ +	+	+ +
Lys-5	GIHAKAILNN	PSTYEILNPA	DFGLTRYVHP	ASRLTGWNAV	KTRVQQLGLE
Pc HC	MTDTQYKECT	AKIKAMADIR	PIAVDDADSI	IRAYHRNLKS	GENKPLLDLT
		+ +	+	+ +	+ + + +
Lys-5	MTDDQVKECT	AKIKALADVR	PIAIDDADSI	IRTFHLGLHE	QNKVQPPAVV
Pc HC	AEEQAFAFAK	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.