J. Genet., Vol. 73, Number 1, April 1994, pp. 33–41. © Printed in India.

The *Neurospora crassa erg3* gene encodes a protein with sequence homology to both yeast sterol C-14 reductase and chicken lamin B receptor

K. G. PAPAVINASASUNDARAM and D. P. KASBEKAR*
Centre for Cellular and Molecular Biology, Hyderabad 500 007, India

MS received 4 April 1994

Abstract. The *erg3* gene of *Neurospora crassa* was sequenced (EMBL accession no. X77955) and found to encode a protein of 490 amino acid residues with significant homology to the yeast sterol biosynthetic enzyme C-14 reductase (39% identity) and also to the C-terminal region in the sequence reported for the chicken lamin B receptor (41% identity). The possibility that a single protein may possess both lamin B receptor and sterol C-14 reductase functions might account for non-sterol-biosynthetic effects of mutations in sterol biosynthesis genes and of inhibitors of sterol biosynthetic enzymes.

Keywords. Sterol C-14 reductase; *Neurospora erg3* gene; lamin B receptor.

1. Introduction

We have earlier shown that alteration of membrane sterol composition in *Dictyostelium discoideum* and *Neurospora crassa*, either by mutations or with the sterol biosynthesis inhibitor 15-azasterol, conferred sensitivity to the pea phytoalexin pisatin (Kasbekar and Papavinasasundaram 1992; Papavinasasundaram and Kasbekar 1993; Kasbekar, submitted). The pisatin sensitivity of the *Neurospora crassa erg1* and *erg3* sterol mutants allowed development of sib selection procedures (Vollmer and Yanofsky 1986) using which it became possible to identify, from an ordered genomic library, cosmid clones that complemented each of these mutations (Papavinasasundaram and Kasbekar 1993).

Ellis *et al.* (1991) had shown that the *erg3* mutant was blocked in a key step in sterol biosynthesis, namely reduction of the double bond between carbons 14 and 15 of the sterol C ring, on the basis of which they suggested that the *erg3* gene encodes the C-14 reductase enzyme. Only one C-14 reductase polypeptide sequence (deduced from the cognate DNA sequence) has been reported thus far, that of the yeast *Saccharomyces cerevisiae* (Lorenz and Parks 1992). Availability of a cloned gene from another species would permit sequence comparison and identification of evolutionarily conserved stretches of amino acids. That, in turn, would facilitate study of enzyme structure–function relationships and allow rational construction of PCR primers for isolation of homologues from other species, in particular from pathogenic fungi in which this enzyme is the primary target of morpholine and piperidine fungicides (Baloch and Mercer 1987).

In this paper, we report subcloning of the *Neurospora* C-14 reductase gene from

*For correspondence

the cosmid that complemented the *erg3* mutation and determination of its nucleotide sequence. As anticipated, the deduced protein is highly homologous to the yeast C-14 reductase.

Additionally, and quite unexpectedly, the yeast and *Neurospora* C-14 reductases were also found to have extensive homology with the protein sequence reported by Worman *et al.* (1990) for the lamin B receptor of chicken. The lamin B receptor is an inner nuclear membrane protein whose proposed function is to anchor the B-lamins in a meshwork of nucleoplasmic intermediate filament proteins that constitutes the nuclear lamina (Worman *et al.* 1988). Our finding raises the possibility that at least some effects of mutations in sterol biosynthesis genes and of inhibitors of sterol biosynthetic enzymes might in fact represent non-sterol-biosynthetic functions of these enzymes.

2. Materials and methods

2.1 *Strains and growth conditions*

Neurospora crassa strain 2725 (*erg3a*), originally isolated by Grindle (1974), was obtained from the Fungal Genetics Stocks Center, USA, and also from Dr Morris Grindle, University of Sheffield, UK. Vogel's minimal medium (Davis and de Serres 1970) supplemented with 1·5% sucrose was used for routine growth and maintenance.

2.2 *Transformation of erg3 mutant protoplasts*

Published protocols were followed for extraction of pisatin from germinated pea (*Pisum sativum* L.) seeds (Sweigard and VanEtten 1987), and preparation of *Neurospora* protoplasts (Vollmer and Yanofsky 1986) and their transformation with DNA (Sellitrennikoff and Sachs 1991). Since *Neurospora* protoplasts can readily take up linear DNA fragments and integrate them into chromosomes by nonhomologous recombination, transformation with restriction-digested DNA and selection for pisatin-resistant transformants was used to identify restriction enzymes (New England Biolabs) that do not cut within the *erg3* gene cloned on the cosmid G18A10 (Papavinasasundaram and Kasbekar 1993). The restriction enzymes *Pst*I and *Kpn*I were chosen on this basis. Individual *Pst*I restriction fragments were isolated from a low-melting-point-agarose gel and used for transformations, which identified a 3·2-kb fragment that effected the complementation (figure 1). In these transformation experiments, we also used the helper plasmid pMP6 (kindly provided by M. Orbach, University of Arizona, USA), which carries a gene for bacterial hygromycin B phosphotransferase, and employed a double selection for hygromycin and pisatin resistance. The double selection reduced the background growth of untransformed protoplasts. The selection conditions used Vogel's medium supplemented with 20 μg/ml pisatin in both top and bottom agar and 200 μg/ml hygromycin (Sigma, USA) only in the bottom agar. Control transformations with pMP6 alone did not confer resistance to pisatin.

2.3 *Subcloning and sequencing strategies*

The 3·2-kb *Pst*I fragment was subcloned into a pBluescript KS plasmid to construct

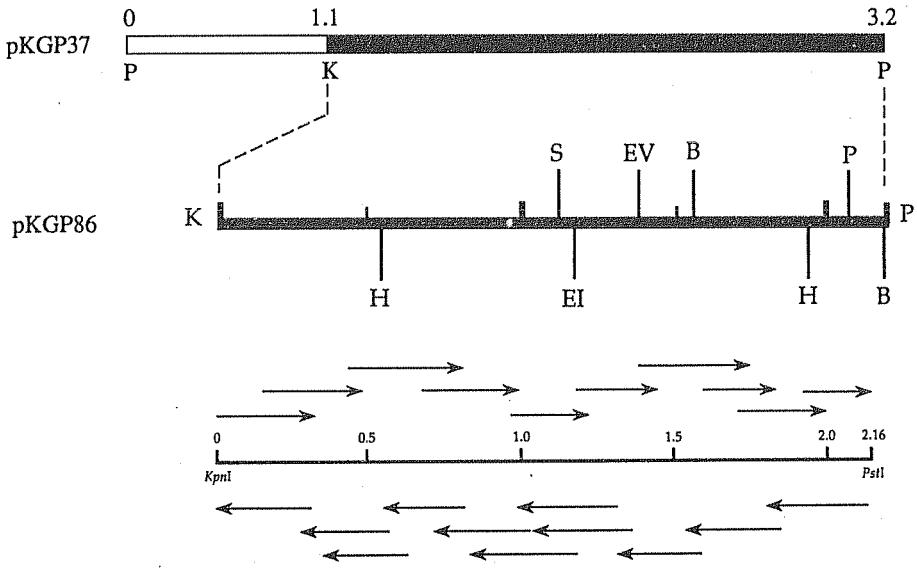

Figure 1. Subcloning of the *N. crassa erg3* gene and the strategy used to determine its nucleotide sequence. The plasmid pKGP37 was constructed by subcloning into a pBluescript KS plasmid a 3·2-kb *Pst*I fragment of the cosmid G18A10 which complemented the *N. crassa* erg3 mutant phenotype. The plasmid pKGP86 was obtained following religation of *Kpn*I-digested pKGP37. Some of the restriction sites present in the 2·1-kb *Kpn*I–*Pst*I insert are indicated (B, *Bam*HI; EI, *Eco*RI; EV, *Eco*RV; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; S, *Sac*I). The protocol given by Henikoff (1984) for exonuclease III-mediated deletion was followed to generate a series of overlapping deletions starting from the *Kpn*I site of the insert using the plasmid pKGP37 and from the *Pst*I end of the insert using the plasmid pKGP86. The nucleotide sequence of the 2·1-kb *Kpn*I–*Pst*I insert was determined on both DNA strands. The horizontal arrows indicate the direction and extent of sequence determined from the plasmid pKGP86 and the two sets of deletion clones.

the plasmid pKGP37. When digested with *Kpn*I (which cuts once in the insert and once in the multiple cloning site adjacent to one end of the insert) and then religated, this plasmid still retained the ability to complement the erg3 mutant phenotype, indicating that *erg3* was present within a 2·1-kb *Kpn*I–*Pst*I fragment, as shown in figure 1. The plasmid obtained from the religation was designated pKGP86.

The protocol given by Henikoff (1984) for exonuclease III-mediated deletion was followed to generate a series of overlapping deletion clones from both ends of the 2·1-kb *Kpn*I–*Pst*I insert (figure 1) using the Erase-a-base System (Promega, USA). Unidirectional deletions from the *Pst*I end of the insert were made in pKGP86 and from the *Kpn*I site of the insert in pKGP37. The plasmid pKGP86 was digested initially with *Xba*I (which cuts only at the multiple cloning site) and the T7 promoter-primer binding site in the template was protected from exonuclease III digestion by using α-phosphorothioate dNTPs (Erase-a-base kit) and Klenow large fragment to fill in the 3′ recessed ends. Then a blunt end susceptible to exonuclease III digestion was created near the *Pst*I end of the insert by digesting the linearized plasmid with *Sma*I (which cuts only at the multiple cloning site in pKGP86).

Similarly, for the other set of deletions, the plasmid pKGP37 was cut with *Sal*I and the ends were filled in with α-phosphorothioate dNTPs using Klenow large fragment to protect the site in the template for T3 promoter-primer binding. The linearized plasmid was then digested with *Cla*I to create an exonuclease III-sensitive 5′ overhang end. To generate progressive deletions, exonuclease III digestions were carried out at 34°C. Samples were withdrawn at 30-sec intervals. For all the subsequent steps, involving treatment with S1 nuclease, ligation and transformations, the protocol recommended in the Erase-a-base kit was followed.

The dideoxy chain termination method (Sanger *et al.* 1977) was used to determine the nucleotide sequence on both DNA strands of the 2·1-kb *Kpn*I–*Pst*I insert using a TaqTrack sequencing kit (Promega, USA) and denatured double-stranded DNA of the deletion clones and the plasmid pKGP86 as template. Standard protocols were followed for all procedures involving DNA manipulations (Sambrook *et al.* 1989).

2.4 *Sequence analysis*

DNA sequence analysis was performed using the PCGENE program (Intelligenetics, Inc., USA) and the search for homologous proteins in the SWISSPROT databank was carried out using the FASTA program (Lipman and Pearson 1988). Sequence alignments and similarities were determined using the GAP program of the GCG package (Devereux *et al.* 1984). Default parameters of the GAP program (gap weight 3·0 and length weight 0·1) were used for alignment of the deduced protein sequences. A comparison value of $\geq 0{\cdot}5$ as given in the amino acid symbol comparison table of the GCG package was used in the GAP program to identify similar amino acids.

3. Results and discussion

3.1 *Nucleotide sequence of erg3*

Selection for complementation of pisatin sensitivity following transformation of *erg3* mutant protoplasts with different restriction digests of the G18A10 cosmid revealed that a 2·1-kb *Kpn*I–*Pst*I fragment was sufficient for complementation. This fragment, which we presume contains the wild-type *erg3* allele, was subcloned into a pBluescript plasmid and the nucleotide sequence of the 2159-bp insert was determined on both DNA strands (figure 2).

Figure 2. The nucleotide sequence of the *Kpn*I–*Pst*I insert containing the *N. crassa erg3* gene and its flanking regions (EMBL accession number X77955). The nucleotide sequence is numbered from the *Kpn*I site. The putative CAAT and TATA motifs are underlined. The deduced amino acid sequence starting with the first methionine is displayed below the middle base of the corresponding codon. The 86-bp intron sequence (nt 466–552) is shown in lower case letters, and conserved splice-regulatory sites (donor site, lariat structure and the acceptor site) are given in bold letters. The stop codon is indicated by an asterisk.

```
GGTACCGACTGGAGGACATCGAGTGAGCAGGAGTACTTACTTTGAGCATAGACAGCGAGT   60
AGTACTTAGACGCCAAGGAAGGTTGCCAAGACTGGAGATCCCAGCCGGAAAGCAGAGATC  120
CGTCTGGCACGACAGGTCGATTGGCTCCACTCTCCCAGCCGGCAACCTAATTGAAGCAGT  180
CAATTGGCCCCCCAATTGGCCCATCGAAGCTCCCGTCCTTTGGCTCCTCTTCGTCCGAAC  240
CGGGGACCGTCACTGTTCCGGTTGTCTGGTTGGCTGCCGGCTGAGTTGTCCCACGACCAA  300
CATCGGGGAGCTGAACATTTTAAAAAGGCAGCTTGATCCTCTCCCCGCTCTCCGTGTTGT  360
CGTCTACAGCATCACAGCCAGCATCACCATGGCCGGGAAGCAAAACCAAGCTGCCCCAAA  420
                              M  A  G  K  Q  N  Q  A  A  P  K
GAAGGCGGTTGCGCCGCGCCAGCAGCACTACGAGTTTGGCGGCCCgtaagtcaaccaaaa  480
  K  A  V  A  P  R  Q  Q  H  Y  E  F  G  G  P
acagaaccccgactccatctcgttcgtgtccagaccacgcccgctaacaagcttgaca    540
acgatcaaacagCATCGGCGCCTTTGGCATCACCTTTGGCCTCCCCATTCTCGTCCACGT  600
                  I  G  A  F  G  I  T  F  G  L  P  I  L  V  H  V
CTTCAACCTTTTCTGCAACGACATCTCGGGCTGCCCCGCGCCCTCGCTCCTCCACCCCAA  660
  F  N  L  F  C  N  D  I  S  G  C  P  A  P  S  L  L  H  P  K
GTCTCTCGACCTCGCCCAGCTCAAGCGCGAAATCGGCTGGCCTGACAATGGCGTCTTCGG  720
  S  L  D  L  A  Q  L  K  R  E  I  G  W  P  D  N  G  V  F  G
CCTCTTCTCGTGGTCCGCCACGCTGTGGACTTTGGGCTACTACGCTCTCTCGCTCGTTCA  780
  L  F  S  W  S  A  T  L  W  T  L  G  Y  Y  A  L  S  L  V  Q
ATACCGCTTTCTCCCCGGCCACCACGTCGAGGGAACCGAGTTGAGCACCGGCGGCCGGCT  840
  Y  R  F  L  P  G  H  H  V  E  G  T  E  L  S  T  G  G  R  L
CAAGTACAAGCTGAACGCTTTCAACTCGGCCATGTGCACCTTGGCCATCCTGGCTGCCGG  900
  K  Y  K  L  N  A  F  N  S  A  M  C  T  L  A  I  L  A  A  G
CACCATCGCTCAGGGCGCCGAGTTCCCCGTTTGGACTTTCATTTCCGACAACTTTGCCCA  960
  T  I  A  Q  G  A  E  F  P  V  W  T  F  I  S  D  N  F  A  Q
GATCATCAGCGCCAATATCCTCTTCGCTTTTGCCCTCGCCATCTTCGTCTACGTTCGCAG 1020
  I  I  S  A  N  I  L  F  A  F  A  L  A  I  F  V  Y  V  R  S
CTTTGACGTGAAGCCGGGCAACAAGGACATGCGCCAGCTAGCAGCAGGCGGTGTCACCGG 1080
  F  D  V  K  P  G  N  K  D  M  R  Q  L  A  A  G  G  V  T  G
CTCCCTCATCTACGATTTCTACATTGGTCGCGAGCTCAATCCTCGCATCACTCTGCCCCT 1140
  S  L  I  Y  D  F  Y  I  G  R  E  L  N  P  R  I  T  L  P  L
CATCGGCCAAGTAGACATCAAGGAATTCATGGAAATGCGCCCTGGTCTTCTGGGCTGGAT 1200
  I  G  Q  V  D  I  K  E  F  M  E  M  R  P  G  L  L  G  W  I
CATTCTGAACTGCGCCTTCATCGCCAAGCAATACCGCCTTTACGGTTACGTCACCGATTC 1260
  I  L  N  C  A  F  I  A  K  Q  Y  R  L  Y  G  Y  V  T  D  S

AATTCTCTTCATCACTGCCATCCAGGCTTTCTATGTCTTTGACGGAATCTACATGGAGCC 1320
  I  L  F  I  T  A  I  Q  A  F  Y  V  F  D  G  I  Y  M  E  P
GGCCGTCTTGACTACGATGGATATCACTACCGACGGCTTCGGGTTCATGCTGAGTTTCGG 1380
  A  V  L  T  T  M  D  I  T  T  D  G  F  G  F  M  L  S  F  G
AGATGTCGTTTGGGTGCCCTTCATGTACTCGACTCAGACTCGCTACCTTTCCGTCCATCC 1440
  D  V  V  W  V  P  F  M  Y  S  T  Q  T  R  Y  L  S  V  H  P
TCAGCAGCTGGGCGCTTTCGGACTGATTGCTGTTGGTGCTGTCCTGGCTGCTGGTTATAG 1500
  Q  Q  L  G  A  F  G  L  I  A  V  G  A  V  L  A  A  G  Y  S
CATCTTCCGCCTGTCCAACTCGCAGAAGAACAACTTTCGCACGAACCCCGAGGATCCTAG 1560
  I  F  R  L  S  N  S  Q  K  N  N  F  R  T  N  P  E  D  P  S
TGTTAAGCACCTTACCTACCTCCAGACCAAGACTGGATCGCGACTCATTACCTCGGGATG 1620
  V  K  H  L  T  Y  L  Q  T  K  T  G  S  R  L  I  T  S  G  W
GTGGGGCATCGCTCGCCACATCAATTATCTCGGTGACTGGCTTCAGAGCTGGCCCTATTC 1680
  W  G  I  A  R  H  I  N  Y  L  G  D  W  L  Q  S  W  P  Y  S
TCTCCCGACCGGCATTGCTGGCTACCAGATCTTGAGCGCCGGCAGCAACGCCCCTGGTGC 1740
  L  P  T  G  I  A  G  Y  Q  I  L  S  A  G  S  N  A  P  G  A
AATTACCATGCTTGACGGGCGCGAGGTTGTCCAGGGCGAGGCGAGAGGCTGGGGTATCGT 1800
  I  T  M  L  D  G  R  E  V  V  Q  G  E  A  R  G  W  G  I  V
TTTCACTTACTTTTACATCTTGTACTTTGCTATTCTCTTGATCCACCGCGACCTCAGAGA 1860
  F  T  Y  F  Y  I  L  Y  F  A  I  L  L  I  H  R  D  L  R  D
TGACGAGAAGTGCTCCAAGAAGTATGGTGACGATTGGGAAAAGTACAAGAAGCTTGTCAA 1920
  D  E  K  C  S  K  K  Y  G  D  D  W  E  K  Y  K  K  L  V  K
GTGGAGAATTGTCCCTGGCATCTACTAGCCCGATGGATAATGTTCTGTGGTTATGTGGTT 1980
  W  R  I  V  P  G  I  Y  *
GTGGTTTTGGATACCCGGCGGAGCTTCCTCTGTTATCTGCAGCGTTGCAAAGTATCAGCA 2040
GTGTGGTGGAACCGGCTATACCGGGTGCACATCCTGCGCTGTGAGTGTCCTCCTGTTTAA 2100
TGGTCGAGTTGAGGGTTGGCTTGCTAATGTGACTTTATCTAGTCCGGATCCACCTGCAG  2159
```

Figure 2. For caption, see page no. 36.

3.2 *Homology of ERG3 protein and yeast sterol C-14 reductase*

Analysis of the nucleotide sequence allowed us to predict the existence of an intron interrupting the coding sequence for a polypeptide of 490 amino acids (M_r 54,722) on the basis of two criteria: (i) the sequence of the deduced polypeptide encoded by DNA on either side of the intron displayed extensive similarity (39% identity) to that of sterol C-14 reductase of *S. cerevisiae* (Lorenz and Parks 1992), and (ii) there were appropriate splicing regulatory sequences similar to those found in other *N. crassa* genes (Jon *et al.* 1993) that maintained the translation frame in the predicted mRNA sequence (figure 2). Alignment of the *Neurospora* and yeast sequences introduced a gap between residues 385 and 386 of the yeast sequence (figure 3). This suggests that there may be an additional intron in the *Neurospora* sequence that would delete residues 406–437 from the polypeptide. However, no conserved splice sites were found in this region. Thus a definitive answer on whether the *Neurospora* gene has a second intron will have to await sequencing of the cDNA. Our demonstration that the *erg3* mutation is complemented by a sterol C-14 reductase clone is consistent with the proposal by Ellis *et al.* (1991) that *erg3* is, in fact, the structural gene for this enzyme. Computer analysis of the *Neurospora* protein sequence predicted five transmembrane segments (figure 3), indicating that, like the yeast protein, the sterol C-14 reductase of *N. crassa* is an integral membrane protein.

3.3 *Homology of Neurospora and yeast sterol C-14 reductase and lamin B receptor of chicken*

A FASTA search (Lipman and Pearson 1988) of the SWISSPROT databank with the ERG3 amino acid sequence also revealed a very significant homology (41% identity) with a 460-amino-acid region at the C-terminus of the chicken lamin B receptor (Worman *et al.* 1990). The similarity noted between lamin B receptor and sterol C-14 reductase (figure 4) suggests that at least in chicken, and possibly in other species as well, both functions are subserved by the same protein. This may provide a plausible explanation for an earlier observation that sterol biosynthesis inhibitors, including inhibitors of sterol C-14 reductase, interfere with the function of the spindle apparatus in *Aspergillus nidulans* and Chinese hamster cells (Ziogas *et al.* 1990).

It may also explain why all pythiaceous fungi, such as *Pythium* and *Phytophthora* species, which are sterol auxotrophs, do nevertheless retain some sterol biosynthetic activity (Knights and Elliott 1976). These fungi lack squalene epoxidase and therefore cannot convert squalene to lanosterol; instead they meet their sterol requirements by absorbing sterols from the medium (Weete 1989).

However tantalizing the homology between the sterol C-14 reductase sequences and that reported for the lamin B receptor might be, it presents us with two apparent discrepancies that will need to be explained. First, the molecular weight of the yeast enzyme as deduced from its sequence is 50·6 kDa, but Georgatos *et al.* (1989) have identified a 58-kDa protein as the yeast analogue of the avian lamin B receptor. Second, the lamin B receptor has been localized exclusively to the inner nuclear membrane in chicken and the 58-kDa yeast protein also appears

```
NC 1 MAGKQNQAAPKKAVAPRQQHYEFGGPIGAFGITFGLPILVHVFNLFCNDI    50
                *: **   :**** ***:** :***::  ::* :
SC 1 ---------MVSALNPRTTEFEFGGLIGALGISIGLPVFTIILNQMIR--    39

     SGCPAPSLLHPKSLDLAQLKREIG-----WPDNGVFGLFSWSATLWTLGY    95
      ::   ::::   :*: :*  :*       : : ::: ::::        :
     PDYFIKGFFQ--NFDIVELWNGIKPLRYYLGNRELWTVYCL--------W    79

     YALSLVQYRFLPGHHVEGTELSTGGRLKYKLNAFNSAMCTLAILAAGTIA   145
     *::  *   :***: : * :*  *::* **:*::      : :**
     YGILAVLDVILPGRVMKGVQLRDGSKLSYKINGIAMSTTLVLVLAIRWKL   129

     QGAEFPVWTFISDNFAQIISANILFAFALAIFVYVRS-----FDVKPGNK   190
      ::::* : :: :*   :    *** * ** : ** *     *      *
     TDGQLPELQYLYENHVSLCIISILFSFFLATYCYVASFIPLIFKKNGNGK   179

     DMRQLAAGGVTGSLIYDFYIGRELNPRITLPLIGQVDIKEFMEMRPGLLG   240
       : ** **  * :***::******** :* :*** * *:***:*
     REKILALGGNSGNIIYDWFIGRELNPR-----LGPLDIKMFSELRPGMLL   224

     WIILNCAFIAKQYRLYGYVTDSILFITAIQAFYVFDGIYMEPAVLTTMDI   290
     *:::*   :  :*   * : * ::::  :*:**:***:  * :*** ***
     WLLINLSCLHHHYLKTGKINDALVLVNFLQGFYIFDGVLNEEGVLTMMDI   274

     TTDGFGFMLSFGDVVWVPFMYSTQTRYLSVHPQQLGAFGLIAVGAVLAAG   340
     ********* ***: :*** ** * ***** * :**   :::: *::  *
     TTDGFGFMLAFGDLSLVPFTYSLQARYLSVSPVELGWVKVVGILAIMFLG   324

     YSIFRLSNSQKNNFRTNPEDPSVKHLTYLQTKTGSRLITSGWWGIARHIN   390
     : **:  * ** :**    :  : :*  :*** * :*:  ***:   ***
     FHIFHSANKQKSEFR----QGKLENLKSIQTKRGTKLLCDGWWAKSQHIN   370

     YLGDWLQSWPYSLPTGIAGYQILSAGSNAPGAITMLDGREVVQGEARGWG   440
     *:**** *: ::*:*                                :
     YFGDWLISLSWCLAT.............................WFQ   388

     IVFTYFYILYFAILLIHRDLRDDEKCSKKYGDDWEKYKKLVKWRIVPGIY   490 NC
       :**:* **** **:**: **: **  ***::** * : * ::*:* :*
     TPLTYYYSLYFATLLLHRQQRDEHKCRLKYGENWEEYERKVPYKIIPYVY   438 SC
```

Figure 3. Sequence homology between the deduced product of the *erg3* gene of *N. crassa* (NC) and the yeast sterol C-14 reductase (SC). The putative transmembrane segments of the *Neurospora* protein are indicated by bold lines above the sequence. Identical amino acids are indicated by asterisks and similar amino acids by colons. Similarity was determined using a comparison value of $\geq 0{\cdot}5$ in the GAP program of the GCG package (Devereux *et al.* 1984). Dashes indicate gaps introduced to maximize the alignment.

to be an integral nuclear membrane protein, but sterol biosynthetic activity is not confined to the inner nuclear membrane; in fact, it occurs in mitochondria and the endoplasmic reticulum as well. The yeast 58-kDa protein was identified by its strong cross-reactivity with monospecific polyclonal antibodies raised against turkey erythrocyte lamin B receptor. Upon limited proteolysis the 58-kDa yeast protein and the turkey lamin B receptor produced similar cleavage products but there were differences in the cleavage pattern upon more extensive proteolysis. The 58-kDa protein also bound avian lamin B, but with low avidity. Thus the 58-kDa protein is a good candidate for the yeast lamin B receptor. It would be important to determine whether it is affected by null mutations isolated in the yeast sterol C-14 reductase gene by Lorenz and Parks (1992) and Marcireau *et al.* (1992). If it is

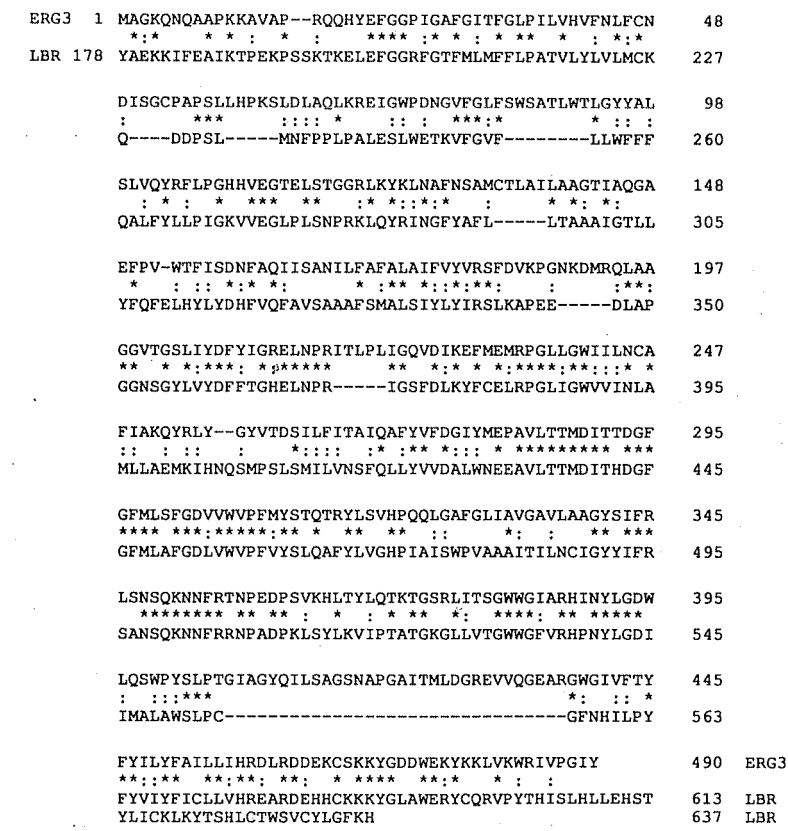

Figure 4. Sequence homology between the deduced product of the *erg3* gene of *N. crassa* (ERG3) and the 460-amino-acid region at the C-terminus of the chicken lamin B receptor (LBR). Identical amino acids are indicated by asterisks and similar amino acids by colons. Similarity was determined using a comparison value of $\geq 0{\cdot}5$ in the GAP program of the GCG package. Dashes indicate gaps introduced to maximize the alignment.

shown that in yeast the lamin B receptor and C-14 reductase are products of different genes, it would raise the question of how the two came to be associated in a single polypeptide in chicken.

Acknowledgements

We thank D. Murali, J. Gowrishankar and G. Swarup for valuable suggestions and T. Bhavani Prasanna for technical assistance. K. G. P. was supported by a postdoctoral fellowship from the Department of Biotechnology, Government of India.

References

Baloch R. I. and Mercer E. I. 1987 Inhibition of sterol Δ^8-Δ^7 isomerase and Δ^{14} reductase by fenpropimorph, tridemorph and fenpropidin in cell free enzyme systems from *Saccharomyces cerevisiae*. *Phytochemistry* 26: 663–668

Davis R. H. and de Serres F. J. 1970 Genetic and microbiological techniques for *Neurospora crassa*. *Methods Enzymol.* 17A: 79–143

Devereux J., Haeberli P. and Smithies O. 1984 A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12: 387–395

Ellis S. W., Rose M. E. and Grindle M. 1991 Identification of a sterol mutant of *Neurospora crassa* deficient in $\Delta^{14,15}$ reductase activity. *J. Gen. Microbiol.* 137: 2627–2630

Georgatos S. D., Maroulakou I. and Blobel G. 1989 Lamin A, Lamin B and Lamin B receptor analogues in yeast. *J. Cell Biol.* 108: 2069–2082

Grindle M. 1974 The efficacy of various mutagens and polyene antibiotics for the induction and isolation of sterol mutants of *Neurospora crassa*. *Mol. Gen. Genet.* 130: 81–90

Henikoff S. 1984 Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene* 28: 351–359

Jon J., Bruchez P., Eberle J. and Russo V. A. E. 1993 Regulatory sequences in the transcription of *Neurospora crassa* genes: CAAT box, TATA box, introns, poly(A) tail formation sequences. *Fungal Genet. Newslett.* 40: 89–96

Kasbekar D. P. Nondegradative pisatin resistance in *Dictyostelium discoideum, Neurospora crassa* and *Nectria haematococca*: Similarities and differences. Submitted

Kasbekar D. P. and Papavinasasundaram K. G. 1992 An inducible, nondegradative phytoalexin resistance mechanism in *Dictyostelium discoideum* is suppressed by mutations that alter membrane sterol composition. *Appl. Environ. Microbiol.* 58: 2071–2074

Knights B. A. and Elliott C. G. 1976 Metabolism of Δ^7 and $\Delta^{5,7}$ sterols by *Phytophthora cactorum*. *Biochim. Biophys. Acta* 441: 341–346

Lipman D. J. and Pearson W. R. 1988 Improved tools for biological sequence comparisons. *Proc. Natl. Acad. Sci. USA* 85: 2444–2448

Lorenz R. T. and Parks L. W. 1992 Cloning, sequencing and disruption of the gene encoding sterol C-14 reductase in *Saccharomyces cerevisiae*. *DNA Cell Biol.* 11: 685–692

Marcireau C., Guyonnet D. and Karst F. 1992 Construction and growth properties of a yeast strain defective in sterol 14-reductase. *Curr. Genet.* 22: 267–272

Papavinasasundaram K. G. and Kasbekar D. P. 1993 Pisatin resistance in *Dictyostelium discoideum* and *Neurospora crassa*: Comparison of mutant phynotypes. *J. Gen. Microbiol.* 139: 3035–3041

Sambrook J., Fritsch E. F. and Maniatis T. 1989 *Molecular cloning: A laboratory manual* (Cold Spring Harbor: Cold Spring Harbor Laboratory Press)

Sanger F., Nicklen S. and Coulsen A. R. 1977 DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74: 5463–5467

Sellitrennikoff C. P. and Sachs M. S. 1991 Lipofectin increases the efficiency of DNA mediated transformation of *Neurospora crassa*. *Fungal Genet. Newslett.* 38: 90–91

Sweigard J. and VanEtten H. D. 1987 Reduction in pisatin sensitivity of *Aphanomyces euteiches* by polar lipid extracts. *Phytopathology* 77: 771–775

Vollmer S. J. and Yanofsky C. 1986 Efficient cloning of genes of *Neurospora crassa*. *Proc. Natl. Acad. Sci. USA* 83: 4869–4873

Weete J. D. 1989 Structure and function of sterols in fungi. *Adv. Lipid Res.* 23: 115–167

Worman H. J., Evans C. D. and Blobel G. 1990 The lamin B receptor of the nuclear envelope inner membrane: A polytopic protein with eight potential transmembrane domains. *J. Cell Biol.* 111: 1535–1542

Worman H. J., Yuan J., Blobel G. and Georgatos S. D. 1988 A lamin B receptor in the nuclear envelope. *Proc. Natl. Acad. Sci. USA* 85: 8531–8534

Ziogas B. N., Vitoratos A. G., Sideris E. G. and Georgeopoulos S. G. 1990 Effects of sterol biosynthesis inhibitors on mitosis. *Pestic. Biochem. Physiol.* 37: 254–265