SYNOPSIS

Sterol C-14 reductase is an enzyme that catalyses the reduction of C-14,15 double bond present in the intermediates of the sterol biosynthetic pathway in plants, animals and fungi. The sterol C-14 reductase of *Neurospora crassa* is encoded by the *erg-3* gene. The *erg-3* mutants are sensitive to pisatin, a phytoalexin, and resistant to α-tomatine, a saponin. Our laboratory cloned the *erg-3* gene from *Neurospora* by complementation of the pisatin-sensitive phenotype of the *erg-3* mutant. A BLAST search using the sequenced *erg-3* gene, revealed that the sterol C-14 reductase of *Neurospora* has 41% amino acid sequence identity with the carboxyl terminal domain of human lamin B receptor.

Lamin B receptor (LBR) is an inner nuclear membrane protein of the vertebrate nuclear envelope. It binds to lamin B, a component of the nuclear lamina and helps in anchoring the lamina onto the inner nuclear membrane. Genes encoding LBR have been cloned from *Xenopus laevis*, chicken, rat and human. LBR has a basic amino terminal domain and a hydrophobic carboxyl terminal domain that has eight or nine putative transmembrane segments. Apart from lamin B, the basic amino terminal domain has been shown to bind double stranded DNA, HP-1 chromodomain proteins and to a nuclear localization sequence. LBR has been proposed to be a key player in the nuclear reassembly that occurs at the end of mitosis. Hence it was quite unexpected to find the carboxyl terminal domain of LBR bearing similarity to a sterol biosynthetic enzyme. My work was aimed at investigating the possible significance of this sequence conservation.

The *erg-3* mutant can be easily distinguished from the wild type on the basis of its altered sensitivity to pisatin and tomatine. Additionally, the UV spectrum of the sterols from the *erg-3* mutant is characteristically different from that of the wild type strain. The *erg-3* mutant phenotype is robust and has helped in assigning function to *erg-3* homologues from *Nectria haematococca*, *Septoria lycopersici* and *Ascobolus immersus*. I used this *in vivo* assay for sterol C-14 reductase activity to determine whether the carboxyl terminal domain of human lamin B receptor is a sterol C-14 reductase. I constructed three
recombinant genes that encode proteins chimeric for sterol C-14 reductase and human lamin B receptor and tested their ability to complement the erg-3 mutant phenotype. The three constructs complemented the erg-3 mutant phenotype suggesting that the encoded chimeric proteins have sterol C-14 reductase activity. The sequence of one of the chimeric proteins is almost entirely derived from LBR except for the last 12 residues, strongly suggesting that the human lamin B receptor is a sterol C-14 reductase. Contemporaneously, Silve et al. (1998) showed that the human lamin B receptor complements the erg24 (sterol C-14 reductase) defect of Saccharomyces cerevisiae. This part of the work is described in Chapter 3.

Sterol biosynthesis takes place in the endoplasmic reticulum, whereas LBR localizes in the inner nuclear membrane. Is LBR’s sterol C-14 reductase activity required for its function in the nucleus? Does sterol biosynthesis also take place within the nucleus? These possibilities could be addressed by engineering a gene replacement of LBR with a variant that has all the properties of the amino terminal domain but lacks sterol C-14 reductase activity. Knowledge of residues essential for sterol C-14 reductase activity would be required for performing the above experiment. I therefore undertook a site directed analysis of conserved residues of the sterol C-14 reductase of Neurospora.

The proposed catalytic mechanism of the sterol C-14 reductase involves the addition of a proton derived from an appropriately positioned charged or polar residue in the active site to the substrate double bond resulting in the formation of a positively charged carbonium ion and followed by the addition of hydride ion derived from NADPH to the carbonium ion, to form the reduced product. Hence I included the conserved charged and proton carrying polar residues in my study to identify residues that could act as proton donors in the proposed catalytic mechanism. Thirty-two conserved residues were replaced with neutral residues of a similar size or with alanine and the effect on the sterol C-14 reductase activity was determined using the in vivo assay for sterol C-14 reductase activity. The results were corroborated by expressing the non-functional sterol C-14 reductases in the
erg-24 mutant of *Saccharomyces cerevisiae*. This work led to the identification of four residues that are irreplaceable for sterol C-14 reductase activity. These residues could now be utilized in engineering the gene replacement of LBR. Some of the residues could act as proton donors in the proposed catalytic mechanism. This is the first study of residues required for sterol C-14 reductase activity. This part of the work is described in Chapter 4.

Are there additional sterol C-14 reductases in humans? Holmer *et al.* (1998) reported the sequence of a cDNA clone (TM7SF2), which encodes a polypeptide with 40% amino acid identity to the Neurospora sterol C-14 reductase. If this is also a sterol C-14 reductase, then the reason why LBR has conserved its sterol C-14 reductase activity would become more interesting. I tested this possibility by constructing six recombinant genes encoding proteins chimeric for Neurospora sterol C-14 reductase and the TM7SF2 encoded product and tested their ability to complement the *erg-3* mutant phenotype. Surprisingly, none of them complemented the *erg-3* mutant. Although the TM7SF2 product has as much sequence similarity with sterol C-14 reductase as does the carboxyl terminal domain of LBR, it appears to behave differently. This part of the work is described in Chapter 5.