From Flavins to Hemes in Cytochromes P450 and Nitric Oxide Synthases During a Lifetime in Science

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Dr. Bettie Sue Masters will discuss her lifelong interest in mammalian flavoproteins and their structure-function relationships. She contributed to the discovery that NADPH-cytochrome P-450 reductase (CYPOR) was absolutely responsible for electron transfer to cytochromes P450 in the mammalian endoplasmic reticulum; there are 50 such P450's in humans. These studies led her into the study of cytochromes P450 responsible for the production of ? -hydroxylated products of long-chain saturated and unsaturated fatty acids, including arachidonic acid. The ? -hydroxylated product of arachidonic acid, 20-hydroxyeicosatetraenoic acid (20-HETE), has been shown to produce vasoconstriction in certain blood vessels and her laboratory's studies on the gestational age-dependent appearance and disappearance of a cytochrome P450 (CYP4A4) that metabolizes both prostaglandins and arachidonic acid suggested a role of this enzyme in the onset of parturition. Her laboratory, in collaboration with that of Dr. Jung-Ja P. Kim at the Medical College of Wisconsin, published the first structure of CYPOR, which serves as the paradigm for mammalian flavoproteins containing both FAD and FMN as prosthetic groups. Upon the discovery by Dr. Solomon Snyder's group that the 641-C-terminal residues of neuronal nitric oxide synthase (NOS) bear 58% sequence similarity to CYPOR, Dr. Masters' laboratory entered the NOS field to study the structure-function relationships in the NOS enzymes. These studies led to the discovery of heme as the oxygenating prosthetic group and Zn ion at the interface of the heme domain dimer of these enzymes, in addition to the flavins and tetrahydrobiopterin that had already been reported. The crystallographic determination of the structure of the heme domain dimer of endothelial NOS was the first for the constitutive isoforms, endothelial and neuronal NOSs. Recent work has focused on the regulation of the NOS enzymes by both intrinsic and extrinsic factors. The discovery of large inserts in the sequences of constitutive NOSs led to the hypothesis that these inserts served as controlling elements upon their activation by Ca^{+2}/calmodulin and the removal of these sequences (~45 residues) by genetic engineering proved this hypothesis. The C-termini were shown to affect the rates of NOS electron transfer negatively and their removal from all three isoforms resulted in a very large (5-20-fold) stimulation of flavoprotein-mediated electron transport activities and ~20% stimulation (iNOS) or ~45% and ~33% inhibition of nNOS and eNOS, respectively, of the formation of NO. A concerted mechanism of action among these intrinsic elements has been proposed and increased interaction between the FAD- and FMN-binding domains was shown to facilitate and activate the requisite electron transfer activities required for NO formation. X-ray crystallography indicates that large movements of the flavin-binding domains are required for these interactions. The quest for knowledge of the mechanisms of action of the NOS enzymes is required for the design of appropriate <u>selective</u> inhibitors of the production of NO, the excess of which can produce endotoxic shock and death during infection. Recent studies of the human mutations in CYPOR, which have been demonstrated by Dr. Walter L. Miller's laboratory to be responsible for defects in steroidogenesis resulting in sexual dimorphisms with or without Antley-Bixler syndrome (exhibiting craniofacial defects), have led Dr. Masters' laboratory to demonstrate that both FAD- and FMN-binding defects result from several of these mutations. Enzymatic studies have shown that rescue of these mutations in the purified enzymes and in membrane-bound CYPOR can be effected by the re-addition of FAD or FMN. Other mutations that may affect interactions of CYPOR with either cytochromes P450 or heme oxygenase-1 have also been examined, and X-ray structures of these mutants have been obtained.