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**PURIFICATION, SEQUENCING, CLONING, AND EXPRESSION
OF
PHOSPHOLEMMAN:**

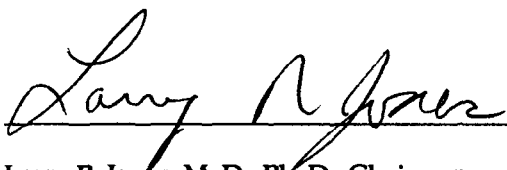
**THE MAJOR SARCOLEMMA SUBSTRATE PHOSPHORYLATED BY
cAMP-DEPENDENT PROTEIN KINASE AND PROTEIN KINASE C IN MYOCARDIUM**

Cathy Joy Palmer

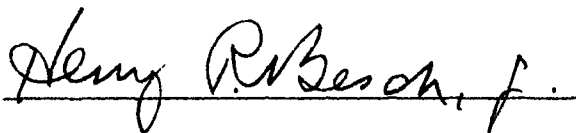
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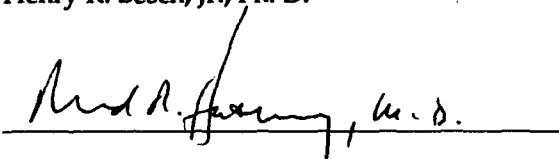
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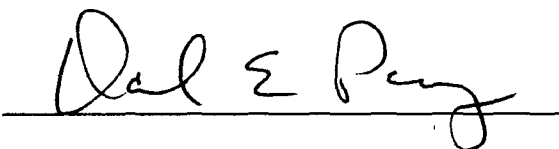
Larry R. Jones, M. D., Ph. D., Chairman



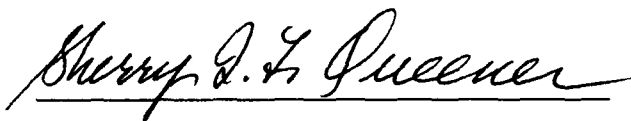
Henry R. Besch, Jr., Ph. D.



David R. Hathaway, M. D.



Daniel E. Peavy, Ph. D.



Sherry I. F. Queener, Ph. D.

April 26, 1993

ABSTRACT

The major sarcolemmal substrate phosphorylated in response to α - and β -adrenergic receptor stimulation of the heart is a unique phosphoprotein of M_r 15,000 which has been postulated to play a regulatory role in trans-sarcolemmal ion flux. This "15-kilodalton protein" is also the main protein phosphorylated by cAMP-dependent protein kinase and protein kinase C in purified cardiac sarcolemmal vesicles. However, the precise structure and function of the protein remains unknown. Therefore, the canine cardiac "15-kilodalton protein" was purified, sequenced, and cloned for complete biochemical characterization as well as for expression studies to probe for function. Sequencing and cloning revealed that the mature protein consists of only 72 amino acids ($M_r = 8409$). A single, hydrophobic, membrane-spanning segment of 19 uncharged amino acids (residues 18-36) traverses the sarcolemmal membrane such that the carboxyl-terminus of the protein faces the cytoplasm. The carboxyl-terminus (residues 37-72) is highly basic and contains three predicted phosphorylation sites for protein kinase C and one predicted site for cAMP-dependent protein kinase. The name *phospholemman* was proposed for this protein to signify a major phosphoprotein substrate localized to the plasmalemma (Palmer *et al.*, 1991). Site-specific antibodies demonstrated that phospholemman exhibits widespread tissue and species distribution (Palmer and Jones, 1993). When phospholemman cRNA was expressed in *Xenopus* oocytes, a new type of chloride channel activity, $I_{Cl(PLM)}$, was demonstrated (Moorman *et al.*, 1992). This channel activity was confirmed by incorporation of purified phospholemman into planar lipid bilayers. Correlations of phospholemman structure and function with other newly characterized proteins and activities allude to its participation in processes as basic as cellular growth and development. Phospholemman appears to be representative of a unique, newly described, and potentially important family of proteins.

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