Title

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Journal

Journal of Biological Chemistry, 269(18)

ISSN

0021-9258

Author

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Publication Date

1994-05-01

DOI

10.1016/s0021-9258(17)36794-7

Peer reviewed

Minireview

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 269, No. 18, Issue of May 6, pp. 13057–13060, 1994

Diversity of Group Types, Regulation, and Function of Phospholipase A_2*

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Phospholipase A_2 (PLA_2)[1] catalyzes the hydrolysis of the *sn*-2 fatty acyl bond of phospholipids to liberate free fatty acids and lysophospholipids (for review see Ref. 1). PLA_2 plays a central role in diverse cellular processes including phospholipid digestion and metabolism, host defense, and signal transduction (for review see Ref. 2). PLA_2 also provides precursors for eicosanoid generation when the cleaved fatty acid is arachidonic acid or for platelet-activating factor formation when the *sn*-1 position of the phospholipid contains an alkyl ether linkage (for review see Ref. 3).

PLA_2s are a diverse class of enzymes with regard to function, localization, regulation, mechanism, sequence, structure, and role of divalent metal ions. This review will describe several of the best studied PLA_2s as a means of defining the various types of PLA_2. The classification of PLA_2s is discussed in the next section, followed by discussions of each type of enzyme and ending with a general discussion of PLA_2 function. While the human enzymes are naturally of greatest interest, the bulk of our knowledge of PLA_2s has been obtained by studying enzymes from non-human sources. However, since most of the human PLA_2s have essentially identical counterparts, the knowledge gained from the non-human enzymes is equally applicable to the human forms.

Groups

Phospholipase activity was first studied in pancreatic juice and cobra venom in the early 1900s (for review see Ref. 4). Subsequently, PLA_2s obtained from various snake and bee venoms and from mammalian pancreas (for review see Refs. 1, 5, and 6) have been well characterized, mechanistically elucidated, and structurally defined. Traditionally, these extracellular enzymes have been divided into three main groups and several subgroups based on their amino acid sequences (7–9) as summarized in Table I. In the last few years, new PLA_2s have been described that do not fit these traditional groupings. The terms "secreted" and "cytosolic" are often used in referring to these enzymes. However, these designations are often insufficient to distinguish between these enzymes. In this review the traditional group designations have been employed and have been extended to cover one of the better characterized new enzymes. Additional group and subgroup designations may be required in the future as new enzymes are found and as existing enzymes are better characterized and sequenced.

All of the traditional Group I, II, and III PLA_2s have been isolated as extracellular enzymes, have high disulfide bond content, low molecular mass, and require Ca^{2+} for catalysis. These characteristics form the defining features of these groups. Sequence homology is the chief criteria for assigning an enzyme to one of these groups (for definitions, see Ref. 9). While the majority of the Group I, II, and III enzymes that have been studied are extracellular non-human enzymes, a growing number of Group I and II enzymes have been found in human tissues. The best characterized is the Group II PLA_2 originally isolated from human synovial fluid (for review see Ref. 10). Group II enzymes have also been found in locations where they are probably not secreted, *i.e.* the enzyme found in rat liver mitochondria (11).

The Group IV PLA_2 is a distinctly different enzyme. Group IV includes the Ca^{2+}-dependent high molecular weight intracellular PLA_2 that is specific for arachidonic acid. This enzyme has been identified in a variety of cells (12, 13); its purification, sequence determination, and cloning from human U937 cells by Clark *et al.* (14, 15) and Kramer *et al.* (16, 17) established that it has a molecular mass of 85 kDa, an apparent submicromolar Ca^{2+} requirement, and that while it is isolated from the cytosol it can translocate to membranes (for a summary see Ref. 18).

Gross and co-workers (19, 20) have identified and purified from canine myocardium an intracellular cytosolic PLA_2 that does not require Ca^{2+} but does prefer arachidonyl-containing phospholipids. Its amino acid sequence has not been reported, but it is reported to be a M_r 40,000 enzyme. This enzyme seems to be activated by ATP and associates with a large oligomeric protein thought to be phosphofructokinase (PFK). A similar enzyme has been found in $P388D_1$ cells (21). The Ca^{2+}-independent intracellular PLA_2s appear to be distinct from the Group IV PLA_2. If their sequences prove to be uniquely different, these PLA_2s should be placed in a separate group.

Table II compares the basic characteristics of each of these groups. The characteristics of the Group I, II, and III enzymes are so similar that they are listed together. These three groups are distinguished from one another by sequence and structural homologies. The group IV and the Ca^{2+}-independent enzymes can be distinguished solely by the properties listed in Table II.

There are numerous other Ca^{2+}-dependent and -independent intracellular PLA_2 activities described in the literature. These include a phosphatidylserine-specific Ca^{2+}-dependent PLA_2 (22) and a 30-kDa dimeric PLA_2 purified from sheep platelets whose sequence is homologous to one of a family of brain proteins that have been termed 14–3–3 proteins (23, 24). Other Ca^{2+}-independent PLA_2 activities have also been reported such as a M_r 97,000 ectoenzyme found in guinea pig intestinal brush-border membranes that also exhibits lipase activities (25, 26). These enzymes have not been characterized sufficiently to allow their assignment to a particular group. What is known about them indicates that the PLA_2s may be a much more diverse group of enzymes than had been previously believed and that the group designations will undoubtedly need to be adjusted. The variety of mammalian PLA_2s have been recently summarized (for review see Ref. 27).

Structure and Mechanism of Groups I, II, and III Phospholipase A_2

Group I, II, and III PLA_2s are the smallest and evolutionarily (9) perhaps the most elementary of the PLA_2s. Mechanistically, they currently constitute the simplest and most well understood enzymes of lipid metabolism and perhaps more generally of all enzymes that act on membranes. Their amino acid sequences and high disulfide bond content (seven) are conserved among all Group I and II species. The enzymes whose x-ray crystal structures have been determined all contain about 50% α-helix and share a small region of anti-parallel pleated sheet (termed the "beta wing"). The α-chain backbones are shown in Fig. 1 for PLA_2s from Group IA Indian cobra (28), Group IIA rattlesnake (29), Group IB bovine pancreas (30), and a Group IB mutant porcine pancreas (31), which was engineered without the "pancreatic loop" characteristic of all Group IB PLA_2s. The human Group IIA PLA_2 (32) appears similar to the rattlesnake PLA_2 α-chain backbone at this resolution. The Group III bee venom enzyme has distinct and rearranged sequence homologies (9), but x-ray crystallography has revealed identical catalytic residues (33) (see below).

Mechanistic studies by numerous laboratories (for review see Refs. 1, 5, and 6) have shown that the secreted PLA_2s do not form

* This minireview will be reprinted in the Minireview Compendium, which will be available in December, 1994. This work was supported by National Institutes of Health Research Grants HD 26,171 and GM 20,501.

[1] The abbreviations used are: PLA_2, phospholipase A_2; LPL, lysophospholipase; PFK, phosphofructokinase.

TABLE I
Phospholipase A_2 groups

Groups I, II, and III and subgroups are defined by sequence differences (9), and Group IV has a distinct sequence (15, 17, 18).

Source	Location	Size	Ca^{2+}
		kDa	
Group I			
A. Cobras and kraits	Secreted	13–15	mM
B. Porcine/human pancreas			
Group II			
A. Rattlesnakes and vipers; human synovial/platelets	Secreted	13–15	mM
B. Gaboon viper			
Group III			
Bee/lizard	Secreted	16–18	mM
Group IV			
Raw 264.7/rat kidney; human U937/platelets	Cytosolic	85	μM
Group ?			
Canine/human myocardium	Cytosolic	40	None

TABLE II
Properties of phospholipase A_2s

Characteristics	Group		
	I/II/III	IV	?[a]
Localization	Secreted	Cytosolic	Cytosolic
Molecular mass	~14 kDa	~85 kDa	~40 kDa
Amino acids	~125	~750	
Cysteines	10–14	9	
Disulfides	5–7	0	
Dithiothreitol sensitivity	+	–	–
Arachidonate preference	–	+	+
Ca^{2+} required	~mM	~μM, not absolute[b]	None
Ca^{2+} role	Catalysis	memb. assoc.	None
Regulatory protein	–	–	PFK (?)
Regulatory cofactors	–	–	ATP
Regulatory phosphorylation	–	+	–
Lyso-PLA activity	–	High	+
PLA_1 activity	–	+	–
Transacylase activity	–	+	–
Fatty acyl-CoA hydrolase	–	–	+

[a] Data for canine myocardial PLA_2 from Refs. 20 and 60.
[b] Other metals and salts can substitute *in vitro* (51); see text.

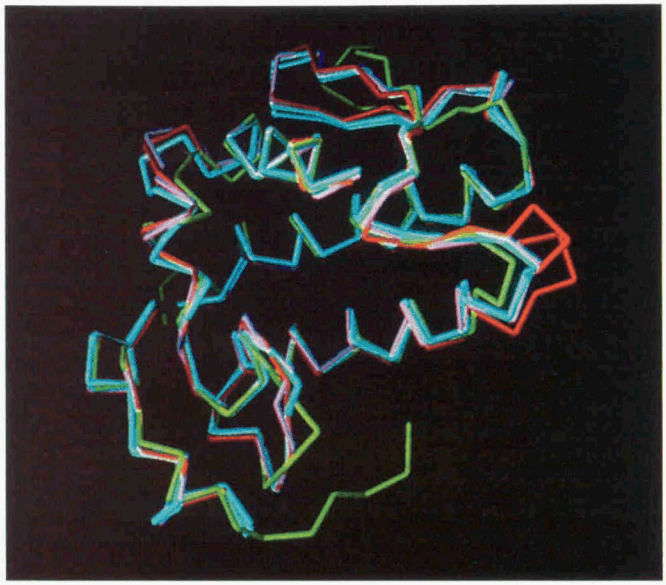

FIG. 1. **X-ray crystal structures of the α-chain backbones of several secreted PLA_2s, including *Naja naja naja* (*cyan*), *Crotalus atrox* (*green*), bovine pancreas (*red*), and mutant porcine pancreas (*purple*).**

a classical acyl enzyme intermediate characteristic of serine proteases. Rather they utilize the catalytic site His, assisted by an Asp, to polarize a bound H_2O, which then attacks the carbonyl group.

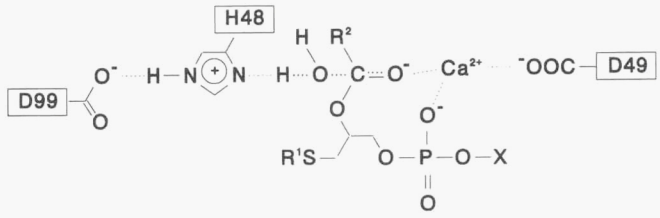

FIG. 2. **Interactions of the catalytic residues of secreted PLA_2s and Ca^{2+} with the tetrahedral transition state arising from the addition of a water molecule to a substrate carbonyl group (from Ref. 41).**

The required Ca^{2+} ion, bound in the conserved Ca^{2+} binding loop, stabilizes the transition state as illustrated in Fig. 2.

The more vexing problem in understanding PLA_2 action is the role of the lipid-water interface. The secretory enzyme acts optimally when the phospholipid substrate is part of an interface such as a micelle or membrane. Based on "surface dilution kinetics," we (34) proposed that the water-soluble enzyme (E) first binds to phospholipid substrate (S), constrained in an interface, to form ES and then binds a phospholipid substrate (S) in its catalytic site to form the Michaelis complex (ESS) as shown in Equation 1.

$$E + S \rightleftarrows ES \overset{+S}{\rightleftarrows} ESS \rightarrow ESP \rightleftarrows ES + P \quad \text{(Eq. 1)}$$

The interfacial substrate concentration should be expressed in mole fraction units. Verger *et al.* (35) proposed a more general binding to the interface as the first step. In this model the enzyme (E) first binds to the interface itself but not to individual phospholipid molecules. This produces an interfacially activated enzyme (E*), which subsequently binds a phospholipid substrate (S) as shown in Equation 2.

$$E \rightleftarrows E^* \overset{+S}{\rightleftarrows} E^*S \rightarrow E^*P \rightleftarrows E^* + P \quad \text{(Eq. 2)}$$

These two models produce mathematically equivalent kinetics, and both expressions serve equally well to describe the necessary association of PLA_2 with interfacial phospholipid to achieve optimal catalysis. Work of Jain and Berg (for review see Ref. 36) with bilayer vesicle substrates has focused on the question of whether the PLA_2 stays associated with the interface while it hydrolyzes successive phospholipid substrate molecules ("scooting mode") or whether it dissociates from the interface after each catalytic event ("hopping mode"). Use of anionic vesicle substrates forces the equilibrium toward the scooting mode and has allowed Jain and coworkers (37) to develop equations to analyze the individual rate constants of the reaction.

A central question is how does the enzyme bind to a phospholipid molecule that is part of an interface. Unfortunately, it is very difficult to obtain crystals of PLA_2 in the presence of interfacial phospholipid. Thus far, it has only been possible to obtain high resolution x-ray crystal structures with monomeric substrate analogues containing short alkyl chains. Structures of the mutant porcine pancreatic PLA_2 with an amide analogue by Thunnissen *et al.* (31) and of Group IA cobra, Group IIA human, and Group III bee venom PLA_2s by Sigler and co-workers (33, 38, 39) with a phosphonate transition state analogue have revealed their mode of binding in the catalytic site consistent with the mechanism (40) shown in Fig. 2. Non-crystallographic experiments are also consistent with this mechanism. Kinetic studies on the pH dependence of inhibition by related amide and phosphonate inhibitors suggest that a hydrogen bond is formed between the unprotonated catalytic site His and the nitrogen of the amide inhibitors at high pH. At low pH, the protonated His hydrogen bonds to the oxygen of the phosphonate inhibitors (41). Transferred nuclear Overhauser effect studies of the solution conformation of the inhibitor-enzyme complex also support this mechanism (42).

Using the x-ray crystal structure of the native cobra venom enzyme and a space-filling model for the phospholipid substrate, Fig. 3 illustrates how the substrate might interact with the active site of PLA_2. Only about 9–10 carbons of the *sn*-2 acyl chain interact with the enzyme (43). The remainder of the chains presumably remain buried in the interface, and it is not possible to precisely

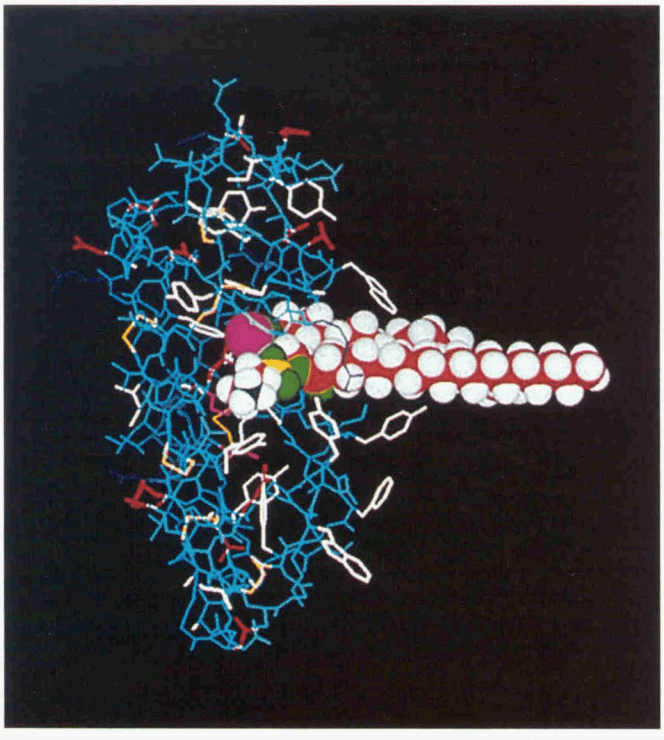

FIG. 3. **X-ray crystal structure of cobra venom (*Naja naja naja*) PLA_2 with bound Ca^{2+} (*magenta*) showing a space-filling model of dimyristoyl phosphatidylethanolamine bound in the catalytic site.** The ends of the fatty acid chains stick out of the enzyme and are presumably associated with the micelle or membrane. Normal phospholipid fatty acid chains would be 4–6 carbons longer.

picture or model this interface at this time. Fig. 3 also illustrates why the extracellular PLA_2s fail to show specificity for the particular fatty acid in the *sn*-2 position (*e.g.* arachidonic acid).

Ca^{2+} Role and Regulation by Phosphorylation of Group IV Phospholipase A_2

The Ca^{2+}-dependent PLA_2 is present in the cytosol of a variety of species and cell types including human U937 cells (14, 16, 44, 45), platelets (12, 46, 47), kidney (48), and macrophages (13, 49). Although the enzyme has been shown to be 85 kDa by sequence and cloning (15, 17), it shows an anomalously high molecular weight on SDS-polyacrylamide gel electrophoresis of about M_r 110,000 (13, 14, 16). This enzyme is translocated from the cytosol to the membrane fraction in a Ca^{2+}-dependent fashion (50). The enzyme contains a CaLB domain in the N-terminal sequence (15, 17), similar to the C-2 Ca^{2+}-binding domain in other signal transduction proteins such as protein kinase C, GTPase-activating protein, and phospholipase C. The presence of the CaLB domain and the enzyme's submicromolar Ca^{2+} dependence (14, 16, 50) support the idea that a Ca^{2+}-dependent translocation occurs in response to Ca^{2+} signals. Indeed, this PLA_2 appears to function as a membrane-bound PLA_2 rather than as a cytosolic PLA_2 (where it is isolated from), making the use of the Group nomenclature preferable.

Interestingly, the Ca^{2+} can be replaced by a large variety of other divalent metal ions including Ba^{2+}, Mn^{2+}, Sr^{2+}, and Mg^{2+} (12, 51–53). Also, high concentrations of salt, such as NaCl or Na_2SO_4, can substitute for Ca^{2+} (51–53). Indeed, in the presence of high salt concentrations, higher enzymatic activity is observed in the absence of Ca^{2+}, arguing that the metal ion or salt serves to promote association of the enzyme with membrane substrate rather than playing a role in catalysis (51).

Further support for this notion comes from analyzing the substantial lysophospholipase (LPL) activity of this enzyme, which was originally reported to be Ca^{2+}-independent (54). We (51) have found that in the presence of glycerol, which activates both the PLA_2 and LPL activity of this enzyme, the latter activity is also activated by Ca^{2+}, suggesting that Ca^{2+} binding to the CaLB domain may play a similar role for both LPL and PLA_2 activities. Thus, in contrast to the secretory PLA_2s, Ca^{2+} does not play a catalytic role for this enzyme; rather, it may physiologically aid in the association of this cytosolic enzyme with membrane phospholipid.

Studies of the LPL activity of the PLA_2 revealed the presence of a novel additional activity in the form of a CoA-independent transacylase (51). Under a variety of experimental conditions, it was found that the transacylase activity was about 7% of the LPL activity. This implies that, at least for the lysophospholipid metabolism, there is a common acyl enzyme intermediate in which the acyl enzyme acceptor can be either H_2O (LPL) or another lysophospholipid (transacylase). The presence of an acyl enzyme intermediate, the lack of a role for Ca^{2+} in catalysis, and the preference of the enzyme for arachidonate in the *sn*-2 position allow us to predict that the chemical mechanism of this PLA_2 is not the same as that of the Group I and II PLA_2s but may instead be closer to that of the serine proteases and lipases.

Recently, attention has focused on the role of phosphorylation in regulating this PLA_2 (55–59). Phosphorylation of this enzyme *in vivo* leads to enhanced arachidonate release as well as somewhat enhanced activity *in vitro*. Furthermore, treatment of enzyme isolated from activated cells with alkaline phosphatase leads to a loss of phosphate in a gel shift assay and loss of activity. Although there are many potential phosphorylation sites in the sequence, evidence (56, 59) has pointed to the phosphorylation of Ser-505 by microtubule-associated protein kinase. The demonstration of a phosphorylation cascade culminating in the microtubule-associated protein kinase phosphorylation of this PLA_2 along with the presence of the CaLB domain has enhanced the notion that this PLA_2 is implicated in signal transduction processes.

Ca^{2+}-independent Phospholipase A_2 and ATP Regulation

A Ca^{2+}-independent PLA_2, which is activated and stabilized by ATP, has been identified in canine myocardium by Wolf and Gross (19). Subsequently, a M_r 40,000 catalytic protein was purified to homogeneity. This M_r 40,000 protein was found to associate with a M_r 85,000 protein. Peptides obtained from the latter protein were found to share sequence homology with phosphofructokinase (PFK), and the protein cross-reacted with antibodies to canine muscle PFK suggesting its identity as PFK (60). Prior to separation of the M_r 40,000 and 85,000 proteins, PLA_2 activity elutes on gel chromatography as a large aggregate of about M_r 400,000. Gross and co-workers have suggested the occurrence of an active ATP-regulated oligomer consisting of one M_r 40,000 catalytic subunit and a tetramer of M_r 85,000 PFK regulatory subunits (60).

We (61) have identified a Ca^{2+}-independent cytosolic PLA_2 in $P388D_1$ macrophage-like cells and recently reported the purification of the enzyme (21). It has some similar properties to the canine myocardial PLA_2 but also some unique behavior. Of particular relevance to the notion that some PLA_2s are oligomeric is the demonstration using target size analysis of radiation inactivation experiments that the active form of the crude ATP-activatable enzyme complex in macrophages is about 340 kDa (21). These experiments demonstrate the occurrence of an ATP-regulated oligomeric Ca^{2+}-independent PLA_2.

Little is known about the mechanism of action of the Ca^{2+}-independent myocardial enzyme, except that it is selectively inhibited by a bromoenol lactone, which acts as a mechanism-based inhibitor (62). Since this enzyme also has other enzymatic activities (Table II) and since it also does not require Ca^{2+}, it apparently proceeds by a different mechanism than the Group I and II PLA_2 and may well involve an acyl enzyme intermediate. The canine myocardial enzyme has been most extensively studied; however, the enzyme is also present in rabbit and human myocardium (63–65). Myocardial tissue is rich in plasmalogen-containing phospholipids. Appropriately, Gross and co-workers (19) have characterized this PLA_2 using an arachidonyl-containing plasmenylcholine substrate and suggested that the enzyme is specific for that substrate. While this substrate may be quite relevant for enzymes isolated from myocardial sarcolemma for which this is the major phospholipid species, these investigators (20) have only reported small preferences for this substrate over alkylether and acyl-containing substrates when assaying the catalytic subunit with vesicle substrates. Indeed, the macrophage PLA_2 in its ATP-activatable complex appears to hydrolyze dipalmitoyl phosphatidylcholine better than 1-alkylether,2-arachidonyl phosphatidylcholine in mixed micelles (21).

Phospholipase A_2 Function

PLA_2s play a number of important, diverse roles in cells and tissues. The pancreatic PLA_2s clearly participate in the digestion of phospholipids in the gut. The venom PLA_2s have some digestive function but in many cases are also directly involved in the toxicity of the venoms. PLA_2s must also play an important central role in the basal phospholipid metabolism of all cells. In addition to these roles in lipid metabolism, PLA_2s are increasingly being identified as participants in other physiological functions. Specific cell surface receptors for PLA_2 have been identified (66); however, their function is still unclear. The elevated expression of Group II PLA_2s in inflammatory diseases suggests a host defense function for this enzyme. PLA_2s are also generally considered to be the initial step in the eicosanoid cascade and as such a potential site for controlling eicosanoid production. The arachidonyl specificity and Ca^{2+}-dependent translocation via a CaLB domain of the Group IV PLA_2 suggest a role for that enzyme in signal transduction. However, the observation of enhanced Group II PLA_2 secretion during activation of certain cell systems and the demonstration of its contribution to prostaglandin production using antisense RNA technology (67) argue that this enzyme also plays a role in signal transduction and eicosanoid production. The Ca^{2+}-independent PLA_2s are also capable of releasing arachidonic acid for eicosanoid synthesis.

To date, numerous PLA_2s have been identified, all carry out essentially the same reaction, and all are potentially capable of participating in many cellular functions. In many cases, several of these enzymes are present in the same cell. For example, Group II, IV, and the Ca^{2+}-independent enzymes, as well as several uncharacterized PLA_2s, are present in P388D_1 macrophage-like cells (67). Group II, Group IV, and a phosphatidylserine-specific, Ca^{2+}-dependent PLA_2 are all present in rat mast cells (22). Correlating the various PLA_2 activities with the various physiological functions is a major challenge currently facing biochemists who study this important group of enzymes.

Acknowledgments—The contributions over the years of numerous excellent students, fellows, and collaborators in developing the concepts presented herein are gratefully acknowledged. Special thanks to Daved Fremont, Brent Segelke, and Yi-Ching Lio for aid in preparing Figs. 1 and 3 and Table II, respectively. Critical reading of this review by Raymond Deems, Laure Reynolds, and Elizabeth Ackermann is most appreciated.

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