

Caveolar Microdomains of the Sarcolemma Compartmentation of Signaling Molecules Comes of Age

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For more than two decades, investigators who have been studying signal transduction in the heart (and other tissues) have struggled to explain data implicating the existence of “compartmentation.” The data have included evidence for selective effects of certain types of hormonal and neurotransmitter agonists on “downstream” events, in spite of their utilization of common “upstream” second messenger pathways.^{1,2} The concept of compartmentation arose, at least partially, as an explanation for such phenomena. Yet progress in defining the physical nature of these compartments has, until recently, been rather slow. One problem has been that the biochemical paradigm, which typically involves destroying cells, isolating subcellular fractions, and assaying enzymatic or other activity, is not particularly well suited for precise identification of signaling domains responsible for compartmentation. Consider the folly of this type of approach if one were to use it to identify each of the parts of an appliance, such as a television set. One would smash the appliance, breaking it down into smaller pieces and then attempt to assemble these “fractions” in order to try and learn the function of each component.

Nevertheless, progress has recently accelerated in this area, in part because of evolving notions regarding the existence of specialized regions of the sarcolemmal membrane. Early work in a variety of cell types focused on the role of clathrin-coated pit regions that were involved in receptor-mediated endocytosis, in particular of transport proteins and certain receptors.³ More recently, such coated pit regions have been implicated in receptor-promoted internalization of G protein-coupled receptors (GPCRs).^{4,5}

Another specialized region of the plasma membrane that has attracted increasing attention is the caveola, a specialized membrane invagination (“little cave”), whose morphology was originally described in the 1950s. These structures were first identified in endothelial cells and were described as having an endocytotic role (potocytosis and transcytosis) akin to that of clathrin-coated pits (albeit with different transported substrates and via different mechanisms). Caveolar domains are enriched in certain lipid moieties, including cholesterol

and sphingolipids. The discovery of a marker protein for these structures, caveolin, has facilitated purification and biochemical analysis of the components of caveolae. Three different caveolins have been identified, with caveolin-3, also termed M-caveolin, a muscle-specific caveolin that may be mutated in limb-girdle muscular dystrophy.^{6,7} The surprising result from studies of caveolar-rich fractions is that many proteins involved in signal transduction are enriched in these fractions. Recently, a domain near the N-terminal end of caveolin-1 has been identified as a caveolin scaffolding domain, in part based on its interaction with binding motifs that are proposed to exist in numerous signaling proteins.⁸ Thus, an emerging notion is that caveolae are structures that may organize signal transduction molecules and serve both to integrate multiple signals as well as to optimize the efficiency and fidelity of these signaling pathways. Recent reviews by Okamoto et al,⁸ Schlegel et al,⁹ and Anderson¹⁰ highlight many of the key findings. The study in this issue of *Circulation Research* presented by Rybin et al¹¹ contributes important new information regarding a possible role for cardiomyocyte caveolae in signal transduction.

The present renaissance in thinking of caveolae as organizing centers for signal transduction traces back to 1974 when Popescu et al¹² proposed that these structures were the sites of excitation-contraction coupling in smooth muscle. Subsequent evidence suggested that caveolae could function as sites for calcium entry and storage and that Ca²⁺ ATPase, IP₃ receptors, as well as calmodulin, each critical molecules involved in calcium regulation and contraction of muscle, were enriched in caveolin-containing fractions.^{8,10} The studies by Rybin et al¹¹ establish that certain isoforms of protein kinase C (PKC) can be recruited to caveolae (defined biochemically as caveolin-rich membrane fractions) by activation with phorbol ester or the hormone endothelin. In addition, Rybin et al¹¹ show the colocalization of mitogen-activated protein (MAP) kinases and caveolin as well as the colocalization of extracellular signal-regulated protein kinase (ERK), MAP kinase kinase (MEK), and certain Rafs—all components of the ERK cascade. It is well documented that activated PKC can translocate to membrane fractions of a wide variety of cell types and tissues, or more accurately, can adhere to membrane binding sites as opposed to its predominant localization in the cytoplasm of resting cells. Therefore, the finding by Rybin et al¹¹ that upon activation, specific PKC isoforms translocate (adhere) to caveolar domains provides a unification of previous work regarding PKC signaling and other studies related to caveolae. These findings make sense from a teleological perspective: activated signaling molecules are brought to a site where their targets are organized so that signals are transmitted in a highly specific and efficient

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manner. The observation of Rybin et al¹¹ that not all PKC isoforms present in the heart act in the same fashion lends even greater credibility to the idea that localization to caveolae exists to compartmentalize only specific types of signals. Previous work with cardiac myocytes and other cell types has implicated different roles and different intracellular targets for individual isoforms of PKC.^{13,14}

Studies by Page et al¹⁵⁻¹⁷ describe what may be a related role of caveolae in the heart in terms of organization of signaling molecules. This group initially speculated that caveolae were "preformed pathways" for secretion of atrial natriuretic peptide (ANP), in view of morphological studies that showed their close proximity to other secretory organelles. Subsequent studies immunolocalized ANP to caveolar structures¹⁶ and showed that the ANP type B receptor, a protein with inherent guanylyl cyclase activity, is localized in caveolae.¹⁷ These findings thus describe a signal (ANP) that is generated in a specific compartment where target proteins (ANP receptors) are specifically localized.

It is important to note that there are several potential pitfalls of the biochemical approach to studying the components localized in caveolae. In addition to the problems inherent in cell breakage and fractionation (as noted above), the work by Rybin et al¹¹ makes a further methodological point. They demonstrate that the isolation of caveolar fractions using a detergent-based method does not show evidence of PKC localization in caveolae, but that PKC isoforms do appear in these fractions when a nondetergent methodology is used. Therefore, different techniques (the detergent-free method of Song et al¹⁸ or the immunological method of Stan et al¹⁹) should be used to isolate these membrane domains. Furthermore, biochemical data ideally should be supported with morphological analysis, although detection of many signaling components, especially native molecules not coupled to epitopes or fluorescent proteins, is difficult and will require improved methods to localize them to caveolae using microscopic techniques.

Other caveats of the present work and of the role of caveolae in signaling should also be noted. First, none of the data provided¹¹ directly shows the generation of diacylglycerol (DAG) and Ca²⁺, key activators of PKC that have been found to localize in caveolae.^{20,21} Second, concentration-response studies are needed to confirm that the isoform-selective translocation on stimulation with endothelin is truly unique to specific isozymes. Third, the effects of other physiological agonists need to be investigated to form a context for the effects observed with endothelin. Lastly, it should be noted that not all investigators agree on the presence of signaling molecules in caveolae.¹⁹

As do many important studies, the report by Rybin et al¹¹ spawns more questions than it lays to rest. It is still uncertain why PKC translocates to specific plasma membrane domains on activation, especially when one considers that a number of the ultimate targets of PKC and the ERK pathway are in the nucleus. Having ERK pathway activation occur at the sarcolemmal membrane, be it in caveolae or elsewhere, seems an inefficient means to orchestrate signaling to the nucleus. Is involvement of caveolae part of a circuitous route necessary for the integration of this signal with others? Perhaps PKC

translocation to caveolae serves as a feedback mechanism given that PKC activation can mediate receptor desensitization. Moreover, other recent evidence suggests that certain GPCRs can localize to caveolae in cardiomyocytes²² and that cardiac caveolin (both types 1 and 3) can be downregulated by β -adrenergic receptor stimulation,²³ thus implying further interaction between GPCRs and caveolin in the heart. It is attractive to imagine (but as yet unproven) whether association of activated PKC with a putative signaling center may play a role in modulating responses by one or more types of GPCRs.

Moreover, the study by Rybin et al¹¹ addresses only one aspect of signaling that may be organized in caveolae. Recently, G protein-coupled receptors, G proteins, G protein-coupled receptor kinases, receptor tyrosine kinases, multiple protein kinases, and endothelial nitric oxide synthase have all been implicated as caveolae-residing proteins.^{8-10,24,25} In some cases, a direct interaction between caveolin and the signaling molecule is evident, implying that caveolin can regulate activity of these molecules. As even more components of various signal transduction pathways are localized to caveolae, we wonder how many different proteins might cram into this plasmalemmal phone booth, so as to make caveolae "crowded little caves."⁹ Perhaps part of the explanation is that the composition of signaling components residing in caveolae is specific for tissues and cells. Even so, the study by Rybin et al¹¹ plus other recent work leads to the conclusion that cardiomyocyte caveolae appear to be membrane "hot spots" for signaling. Thus, compartmentation of signaling cascades has truly come of age. Future work is likely to test whether other signaling molecules are found in caveolae, whether their activities are regulated by caveolins, and whether such interactions are altered during development, drug therapy, or disease.

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