Different Isoforms of Synapse-associated Protein, SAP97, Are Expressed in the Heart and Have Distinct Effects on the Voltage-gated K⁺ Channel Kv1.5*  

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The SAP97 isoforms differ by alternatively spliced insertion domains that regulate protein localization and oligomerization. We used reverse transcription-PCR to identify SAP97 isoforms of human and rat myocardium. In Chinese hamster ovary cells, cloned protein expression was studied using Western blot, confocal imaging of green fluorescent protein-tagged proteins, and patch clamp technique. The two main cardiac SAP97 isoforms contained both I3 and I1B inserts and differed by the I1A insert. Both isoforms co-precipitated with hKv1.5 channels. Only the isoform lacking I1A increased the current (by 215 ± 22%), whatever the level of channel expression. To examine the involvement of the proline-rich I1A insert in the effect of SAP97, a W623F mutation in the Src homology 3 domain was created, and that restored the effect of the SAP97 on current. SAP97 isoform containing an I1A and I2 domain instead of the I3 domain stimulated the current, whereas SAP97 after deletion of the Src homology 3 and guanylate kinase-like domains did not. In cells co-expressing I3(+I1A) or I3(−I1A), green fluorescent protein-tagged Kv1.5 channels were organized in plaque-like structures at the plasma membrane level, whereas intracellular aggregates of channels predominated with the I3 isoform. The two cardiac SAP97 isoforms have different effects on the hKv1.5 current, depending on their capacity to form channel clusters.

Localization of ionic channels in distinct plasma membrane domains is critical for cell function. For instance, in the myocardium, a number of ionic channels are concentrated in the intercalated disk, where they contribute to the normal propagation of the electrical influx between myocytes. The functional properties of ionic channels are generally regulated by various auxiliary proteins that compose, together with the α-subunits of the channels, large protein networks.

The mechanisms that regulate this highly specialized organization and localization of ionic channels are unclear. Important clues have come from the identification of a family of anchoring proteins named MAGUK (membrane-associated guanylate kinases) that appear to play a critical role in the formation and subcellular localization of channel complexes (1).

The MAGUK protein SAP97 (the mammalian homologue of Dlg in Drosophila) is abundantly expressed in both human and rat ventricular myocardium and is associated with potassium Kir2.2 and Kv1.5 channels (2–4). Like other MAGUK proteins, SAP97 bears multiple sites of protein-protein interactions, namely three PDZ (postsynaptic, disc large, zonula occludens) domains, an Src homology 3 (SH3) region, and a guanylate kinase-like domain (GUK). The PDZ domains are the best characterized and bind to the carboxyl-terminal peptide motif (S/T)XX(V/L) in a number of proteins, including voltage-gated and inwardly rectifying K⁺ channels (1). It has been reported that the SH3 domain interacts with PXXPR-like sequences in several proteins, whereas the partners of GUK domain are members of the GKAP/SAPAP1/DAP1 family or brain-enriched guanylate kinase-associated protein (BEGAIN) (5–7). Amino acid sequences located in the N-terminal part of the protein (the S97N region) or lying between the SH3 and GUK domains (the U5 region) have been shown to modulate the localization and cytoskeletal attachment of SAP97 (7–9). Several alternatively spliced insertions have been described for the human Dlg in the N-terminal domain of the protein (I1) and in the region between the SH3 and GUK domains (I2 and I3) (8, 10). Only the alternatively spliced insertion I3 has a characterized function, contributing to human Dlg localization at sites of cell-cell contact (10, 11).

The present study was undertaken to identify SAP97 isoforms expressed in heart and to determine how they interact with the voltage-gated potassium channel hKv1.5. These channels are responsible for one of the major repolarizing currents of cardiac myocytes. By using various electrophysiological and cytological approaches, we found that cardiac SAP97 isoforms have distinct effects on the functional properties and membrane expression of hKv1.5 channels.

MATERIALS AND METHODS

Tissue Samples—Whole hearts were excised from Wistar rats, washed in 1× PBS, frozen in liquid nitrogen, and stored at −80 °C. Specimens of human right atrial appendage were obtained, with approval from the ethics committee of our institution, from patients undergoing heart surgery. Myocardial samples were also frozen in liquid nitrogen and stored at −80 °C.

Cell Preparation and Transfection—Chinese hamster ovary (CHO) cells were cultured in Ham's F-12 medium (Invitrogen Nutrient Mixure) supplemented with 10% fetal calf serum at 37 °C in a 5% CO₂ incubator. Single and double transfection of CHO cells was performed with hKv1.5 and/or SAP97 isoforms cloned into tagging or bicistronic vectors, as previously described (12). Except when indicated, cells were transfected with 0.1 μg of Kv1.5 plasmid plus 1.5 μg of SAP97 plasmid and 0.4 μg of empty plasmid or with 0.1 μg of Kv1.5 plasmid plus 1.9 μg

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The abbreviations used are: SH3, Src homology 3; GUK, guanylate kinase-like; GK, guanylate kinase; GFP, green fluorescent protein; EGFP, enhanced green fluorescent protein; CHO, Chinese hamster ovary; pA/pF, picoamps/picofarads.
of open plasmid. For proteasome inhibition, growing transfected cells were treated with calpain I inhibitor (MG132 or N-acetyl-leucyl-leucyl
leucyl-7-norleucinal (ALLN)) at a final concentration of 50 μM for 24 h before confocal microscopy.

Reverse Transcriptase-PCR Assay—Total RNA was extracted from tissue by using the phenol-chloroform method (13) or Trizol® (Invitrogen) procedure and then reverse-transcribed using Maloney murine leukemia virus reverse transcriptase (Invitrogen) and oligo(dT) according to the manufacturer’s guidelines. To evaluate the relative abundance of the different isoforms, reverse-transcribed RNA (100 ng) was submitted to polymerase chain reaction amplification (PCR) in a 25-μl reaction mixture with conventional Tag (Invitrogen) (annealing temperature 55 °C, 3 cycles), using the following sense and antisense primers: for the I2–GGTGATCCCGGCG-3′ and 5′-CAGGGCAGTTGACTAGAGACACTGCCA-3′ for the I3 insertion. For human tissue, the following primers were used: 5′-TGTGACTTCAGAGACACTGCCA-3′ for the I2 versus I3 insertion. For human tissue, the following primers were used: 5′-TGTGACTTCAGAGACACTGCCA-3′ for the I2 versus I3 insertion.

Cloning Procedures—Reverse-transcribed cardiac RNA (100 ng) was submitted to PCR in a 50-μl reaction mixture using high fidelity Taq Platinum (Invitrogen) in the following conditions: 32 cycles (annealing temperature 55 °C, 30 s, elongation temperature 72 °C, 1 min). The sense and antisense primers used to investigate the I1A and I1B region of SAP97 were 5′-TCAGAGGACAGAGTCTCCTGGC-3′ and 5′-CCGTTCGATCAGTCAAACA-3′. Primers 5′-GAAACCAGCCGTC-CTCTATGTC-3′ and 5′-ATGGTCTGGCCTTCCTGCTAC-3′ were used to investigate the hook region. After verifying that the amino acid sequences of SAP97 domains were similar in rats and humans, we constructed the cardiac isoforms from a rat SAP97 sequence already available in the laboratory. The new insertions of SAP97 amplified by PCR were digested with appropriate enzymes and ligated into the previous SAP97 construct (4). pIREs2-EGFP (Clontech) or homemade pIREs2-DsRed1 expression vectors containing the coding sequence for appropriate primer sequences.

A both-modified EGFP was produced by PCR with the primers 5′-AAGGATCCAGCCGAGGAGGCTGT-3′ and 5′-AAGGATCCAGGAGGTATCCCGG-3′ and pIREs2-EGFP as matrix. The product was inserted into the BamHI restriction site of the hKv1.5 coding sequence and then inserted into the EcoRI/Sall cloning site of plasmid pCDNA3 to create an internally GFP-tagged hKv1.5. The GFP-tagged SAP97 (I3 isoform containing the I1A domain) was constructed in pEGFP-N3. All PCR products were sequenced to confirm that the inserted nucleotides sequences were correct.

Protein Extraction and Immunoprecipitation—Two days after transfection, 100-mm dishes of confluent CHO cells were washed twice with phosphate-buffered saline and then harvested with ice-cold 10 mM Tris buffer, 5 mM EDTA, pH 7.4, in the presence of the following enzyme inhibitors: iodoacetamine (1 mM), 4-[(2-aminoethyl)-benzenesulfonyl fluoride (0.5 mM), aprotinin (10 μg/ml), leupeptin (10 μg/ml), pepstatin (1 μg/ml), and Na2VO4 (1 mM). Final protein extraction and immunoprecipitation experiments were carried out as previously described (4). Goat anti-mouse IgG M-450 Dynabeads (Dynal) were used for immunoprecipitation. Negative control consisted in incubating proteins with beads that were not coated with the PSD95 family or GFP mouse antibodies. Proteins were separated on 8% polyacrylamide SDS gels and transferred to polyvinylidene difluoride membranes (PerkinElmer Life Sciences). Western blots were incubated with appropriate primary antibodies, secondary antibodies and then revealed with chemiluminescence with the Amersham ECL kit. The antibodies used were as follows: rabbit anti-mouse Kv1.5 antibody (Alomone Labs), mouse anti-PSD-95 family antibody (Upstate Biotechnology, Inc., Lake Placid, NY), and rabbit or mouse anti-GFP (Torrey Pines Biolabs Inc. and Chemicon International, respectively). The specificity of the primary antibodies used was checked by probing the membrane with the secondary antibody only.

Confocal Microscopy—Unfixed transfected CHO cells were examined with a Zeiss LSM-510 confocal scanning laser microscope equipped with a 25-milliwatt argon laser and a ×63 oil objective with a numerical aperture of 1.4. Green fluorescence was observed with a 505–550-nm bandpass filter and a 515–530-nm bandpass filter under 488-nm laser illumination.

Current Measurements—Whole-cell patch clamp currents were recorded with borosilicate glass pipettes (resistance 1.5–2 MΩ) connected to the input stage of a patch clamp amplifier (Axoclamp 200A; Axon Instruments). Resistance in series was compensated to obtain the fastest capacity transient current, but the capacitive and leakage currents were not compensated. Currents were filtered at 5 kHz, digitized with a Labmaster (Lab Rac; Scientific Solutions), and stored on the hard disk of a personal computer. Data were acquired and analyzed with Acquis-1 software (G. Sadoc, CNRS, GiVYvette, France).

Solutions and Drugs—Cells were bathed in an external solution containing 137 mM NaCl, 5.4 mM KCl, 1 mM MgCl2, 10 mM Hepes, 1 mM CaCl2, pH 7.3 with NaOH. Patch pipettes were filled with an internal solution containing 115 mM potassium aspartate, 5 mM KCl, 5 mM MgATP, 5 mM sodium pyruvate, 3 mM MgCl2, 4 mM EGTA, and 10 mM Hepes, adjusted to pH 7.2 with KOH. All experiments were carried out at room temperature.

Statistical Analysis—Data are presented as means ± S.E. For comparison of current effects or for quantitative assessment of different SAP97 mutants studied, the following statistical analyses were used: (i) for each experiment, a Student’s unpaired t test was used to determine the significance of differences between control current and the SAP97 isoform tested; (ii) for comparisons of data obtained from the different experiments of the study, Dunnett’s test was used. Current density, expressed in pA/P, or normalized current (i.e. for each experiment, values were normalized with the average current density obtained in control conditions) were used for statistical analysis. p values of <0.05 were considered significant.

RESULTS

Multiple SAP97 Isoforms Exist in Human Atrial Myocardium—as illustrated in Fig. 1A, in addition to PDZ repeats and SH3 and GUK domains, SAP97 is known to contain the alternatively spliced exons I1A, I1B, I2, and I3 (8, 10). Using a semiquantitative reverse transcriptase-PCR assay, we only detected transcripts for SAP97 isoforms containing I3 and I1A domains (I3(−I1A)) or containing I3 but lacking I1A (I3(−I1A)), in both human atrial and ventricular myocardium (Fig. 1B). When a large number of PCR amplification cycles was used, the other SAP97 isoforms, including the variant containing the I2 domain, were not detected, using rat liver as positive control (Fig. 1B).

We therefore decided to clone the two main cardiac SAP97 isoforms, namely I3(−I1A) and I3(+I1A). Expression of the cloned proteins was first checked in transfected CHO cells, using a Western blot assay. As shown in Fig. 1C, probing membrane protein extracted from transfected CHO cells with the anti-PSD95 protein family antibody revealed a strong doublet at 140 kDa (the molecular mass of SAP97) together with a band at around 90 kDa that has been shown to correspond to a cleaved form of SAP97 (4). In control CHO cells, a weak band at 140 kDa was visible whereas the short form was absent, indicating the presence of endogenous MAGUK proteins in this cell line. It should be noted that, with both the I3(−I1A) and I3(+I1A) isoforms, the short form that cross-reacted with an anti-PDZ1–2 antibody migrated at the same molecular mass of around 90 kDa. To further discriminate between endogenous and exogenous SAP97, CHO cells were transfected with an empty pIREs2-EGFP plasmid or with a plasmid containing the sequence encoding the GFP-tagged SAP97 in CHO cells expressing GFP-tagged SAP97, in addition to the 140-kDa band, also seen in cells expressing GFP alone, the anti-PSD95 protein family antibody cross-reacted with two bands at around 170 and 120 kDa. These two 170- and 120-kDa bands were also detected by the anti-GFP antibody and thus very likely correspond to the GFP-tagged SAP97 and its short form (Fig. 1D).

SAP97 Isoforms Interact with Cloned hKv1.5 Shaker Channels—Co-immunoprecipitation assays were used to study interactions between cardiac SAP97 isoforms and hKv1.5 channels expressed in CHO cells. Both cardiac SAP97 isoforms co-immunoprecipitated with GFP-tagged hKv1.5, as indicated by the typical pattern of protein migration with a band at 140 kDa and the short form at around 90 kDa (Fig. 2A). Endogenous MAGUK proteins also co-precipitated with GFP-tagged Kv1.5 channels, but again the density of the band at around 140-kDa was lower than in transfected cells, and the cleaved form was...
absent (Fig. 2 A). It is noteworthy that in control CHO cells, beads coated with the anti-GFP antibody failed to precipitate the SAP97 (Fig. 2 A; raw 4). This result indicated that the precipitation of SAP97 obtained with the anti-PSD95 or anti-GFP antibodies in cells expressing GFP-tagged Kv1.5 channels could not be attributed to an unspecific binding of SAP97 on beads. Additional evidence of an interaction between SAP97 isoforms and GFP-tagged hKv1.5 channels was provided by the detection, in the immunoprecipitate obtained with the anti-PSD95 family antibody, of a protein probed with the anti-GFP antibody at a molecular mass of around 110 kDa, corresponding roughly to the sum of the Kv channel and GFP molecular weights (n/H11005/). These results indicated that both cardiac isoforms interact with hKv1.5 channels.

SAP97 Isoforms Have Distinct Effects on hKv1.5 Current—We then examined the functional consequences of the interaction of the two cardiac SAP97 isoforms with hKv1.5 channels for the current recorded with the patch clamp technique. Cells expressing hKv1.5, either alone (0.1 g of Kv1.5 plasmid) or together with the SAP97 isoforms, showed a typical rapidly activating voltage-dependent outward current with a threshold of activation at around -20 mV and a reversal potential of tail current at -81 ± 1 mV (Ek = -85 mV in our conditions) (Fig. 3 A). In cells co-expressing hKv1.5 and the I3(-11A) isoform, the current density was markedly increased compared with cells expressing Kv1.5 alone (215 ± 22%; n = 29, p < 0.01 and Fig. 3 B). In contrast, current density did not differ between cells expressing Kv1.5 channels alone and those also expressing the I3(+11A) isoform (126 ± 15%; n = 29, not significant and Fig. 3 B). Since the effects of SAP97 on current can vary with the level of channel expression (3), we examined the effects of the I3(+11A) and I3(-11A) isoforms in CHO cells transfected with a very small amount of Kv1.5 plasmid (0.01 g of Kv1.5 plasmid). In this experimental condition, current density was reduced (29.6 ± 3.14 pA/pF versus 13.4 ± 15.2 pA/pF with 0.1 µg and 0.01 of Kv1.5 plasmid, respectively), in keeping with the presence of fewer functional channels; in addition, the I3(+11A) isoform stimulated the current (215 ± 16%, n = 33; p < 0.01) (Fig. 3 B). These results indicated that the enhancing effect of the two cardiac SAP97 isoforms on current depends on the level of channel expression.

Role of the Alternatively Spliced Inserts in the Effect of SAP97 Isoforms on K+ Current—We then examined the mechanism by which the proline-rich domain I1A modulates the effect of SAP97 on K+ current. In PSD-95, W470 mediates the binding of proline-rich domain to the SH3 domain. In SAP97, this amino acid is located in position 623. We replaced it by a phenylalanine and checked that this mutant still interacted...
with hKv1.5 channels in co-immunoprecipitation experiments. In CHO co-expressing both the MAGUK and the GFP-tagged Kv1.5 channel, SAP97 protein was detected in the anti-GFP immunoprecipitate at around 140 kDa, whereas GFP-tagged Kv1.5 channels were detected in the protein precipitate obtained with the anti-PSD95 family antibody (Fig. 4, A and B). Moreover, the hKv1.5-encoded current was increased by I3(+11A)W623F with 0.1 μg of Kv1.5 cDNA: 240 ± 50%; n = 14; p < 0.01) (Fig. 5).

The I3 domain is part of a hinge region that plays a major role in the three-dimensional conformation of SAP97 and in its ability to oligomerize. Thus, we examined the effect on the K+ current of SAP97 isoforms containing an I2 domain instead of an I3 domain, with or without the proline-rich domain I1A, I2(+11A) and I2(-11A), respectively. Both I2 isoforms co-immunoprecipitated with hKv1.5 channels (Fig. 4, C and D) and enhanced the current density, by 187 ± 18%; n = 40; p < 0.01) and 188 ± 24% (n = 21; p < 0.01) with I2(+11A) and I2(-11A), respectively (Fig. 5).

To further examine the role of molecular interactions in the effects of SAP97 on Kv1.5-encoded current, a truncated SAP97 lacking the SH3 and GUK domains but containing the spliced exon I1A was created. The truncated form bound to the channel but did not significantly enhance the current density (118 ± 18%; n = 18, not significant) (Fig. 5).

Distinct Effects of SAP97 Isoforms on the Spatial Channel Organization—The role of SAP97 isoforms in the cellular localization and organization of Kv1.5 channels was studied in live cells by using GFP-tagged channels and confocal microscopy. Fig. 6A shows that in CHO cells transfected only with GFP-tagged Kv1.5 channels, the fluorescence was homogeneously distributed throughout the entire cell body (Fig. 6A). In cells co-expressing hKv1.5 channels and the I3(--11A) or I3(+11A) isoform, the fluorescence was well organized at the plasma membrane level, resembling the plaquelike clusters described in the literature and also showing a reticular distribution (Fig. 6, B and C) (14).

In cells co-expressing channels and the I2 SAP97 isoform, in addition to plaquelike clusters we observed a number of large aggregates of robust fluorescence beneath the plasma membrane (see arrow, Fig. 6D). To determine whether the large aggregates of channels co-expressed with the I2 isoform were due to aggresome of protein, in some experiments the proteasome was inhibited with 50 μM ALLN, or cells were transfected with a large amount (2 μg) of Kv1.5 plasmid (15). Incubation with ALLN of CHO co-expressing the I3(+11A) isoform and Kv channels GFP-tagged or transfected with a large amount of hKv1.5 plasmid (2 μg) resulted in the appearance of large aggregates of Kv1.5 channels that accumulated in the cytosol of more than 60% of cells studied (Fig. 6, E and F).

DISCUSSION

Previous studies have shown the existence of several isoforms of the MAGUK protein SAP97, containing different combinations of alternatively spliced insertions that regulate both the subcellular localization of SAP97 and its capacity to self-associate in multimers (8,10). In this study, we found that the two major SAP97 isoforms expressed in human atrial myocardium contain the alternatively spliced insertions I3 and I1B, whereas they differ by the presence or absence of I1A and by their effects on hKv1.5 Shaker channel-encoded current. This is the first evidence of a physiological role of SAP97 isoforms in cellular excitability.

All of the cardiac SAP97 variants co-precipitated with hKv1.5 channels, indicating that the ability of SAP97 to interact with ionic channels is not modulated by alternatively
interaction between Kv channels and the proteins SAP97 and PSD95 (2, 4, 17–19). However, the mechanisms underlying this effect are poorly understood. Changes in unitary channel conductance or open probability of the channel induced by SAP97 can be ruled out, since in both Xenopus oocytes (3) and CHO cells (4), biophysical properties of the current, such as the slope of voltage-dependent activation, are not modified by the presence of the anchoring protein. The “PDZ putative binding domain” deletion, ETDL, of Kv1.5 Shaker channels has been shown to have no effect on the percentage of channels expressed at the surface of the plasma membrane, a phenomenon that may be governed mainly by an amino acid sequence (VXXSL) in the C terminus of the channels (20). This observation does not support the possibility that the enhancing effect of SAP97 on the current is due mainly to the increase in plasma membrane channel expression. Although MAGUKs may not directly modulate the cell surface expression of Kv channels, they can stabilize them in the plasma membrane. For instance, Jugloff et al. (21) found that PSD95 prevents the internalization of Kv1.4 Shaker channels. However, this process has no effect on current density in HEK293 cells.

The stimulatory effect of SAP97 on the Kv1.5-encoded current may also be related to the capacity of the anchoring protein to cluster and to organize channels into large protein network, thereby facilitating interactions between channels and various accessory proteins (22, 23). The clustering of K+ channels, NMDA receptors, or APC protein by MAGUK proteins is due to their capacity to multimerize and to form a large protein network (9). Oligomerization of human Dlg or of its mammalian homologue SAP97 is regulated by a complex balance of inter- and intramolecular interactions that involve not only the NH2-terminal part of the protein but also SH3 and GK domain interactions (6). Molecular modeling of the SAP97 structure reveals that the protein can exist in a compact “closed” state (preventing binding of ligands to the SH3 and GK domains) or in an “open” state allowing access to the protein binding sites (7). In the present study, we found that a crypto-

**Fig. 4. Role of intramolecular interactions in the SAP97 effect on Kv1.5 channels.** A, in CHO cells co-expressing the GFP-tagged Kv1.5 channel and the SAP97 I3 variants, proteins were immunoprecipitated with the mouse anti-GFP antibody and hybridized with an anti-PSD95 family antibody. B, the same protein sample as in A was precipitated with the anti-PSD95 family antibody and probed with the rabbit anti-GFP antibody (note that a nonspecific band was also detected). C, in CHO cells co-expressing the GFP-tagged Kv1.5 channels and the SAP97 I2 isoform, proteins were immunoprecipitated with the anti-GFP antibody and hybridized with an anti-PSD95 family antibody. D, the same protein sample as in C was immunoprecipitated with an anti-PSD95 family antibody and probed with the mouse anti-GFP antibody. WB, Western blot; IP, immunoprecipitation; I3+, SAP97 I3(+11A) isoform; I3–, SAP97 I3(–11A) isoform; I3+W623F, mutated SAP97 I3(+11A); I2+, SAP97 I2(+11A) isoform; I2–, SAP97 I2(–11A) isoform; I3W623F, mutated SAP97 I3(+11A); PDZ1–3, truncated SAP97. **, p < 0.001 indicates significant difference versus control.

**Fig. 5. Effects of the various SAP97 variants on normalized current.** C, control; I3+, SAP97 I3(+11A) isoform; I3–, SAP97, I3(–11A) isoform; I2+, SAP97 I2(+11A) isoform; I2–, SAP97 I2(–11A) isoform; I3W623F, mutated SAP97 I3(+11A); PDZ1–3, truncated SAP97. **, p < 0.001 indicates significant difference versus control.

spliced insertions but rather by PDZ domains, as reported for PSD95 (1). This probably also explains why a short (90-kDa) SAP97 protein co-immunoprecipitated with hKv1.5 channels. However, the immunoprecipitation assay alone does not allow us to draw firm conclusions on the nature of the interaction or on the protein domain involved in this process. The short form probably results from MAGUK cleavage by proteases such as calpain, as observed in neurons and cardiac myocytes (4, 16). Since the molecular weight of this truncated protein is similar to some reports on the consequences on potassium currents of the

A major finding in this study is that the effect of SAP97 on the Kv1.5-encoded current depends on the presence of the alternatively spliced insertion domain 11A. There are already some reports on the consequences on potassium currents of the
also been reported that insert I3 can mask binding sites on the SH3 domain for partners interacting with SAP97 (7). Clearly, there are multiple potential mechanisms that can regulate MAGUK assembly, and our results do not allow us to draw firm conclusion on the role of inserts of the SAP97 insert I1A, I2, and I3 in this phenomenon. Nevertheless, we provide evidence that these regulatory mechanisms could have major impacts on the properties of the potassium current. Other approaches, using free fracture microscopy for instance, should help to determine the role of SAP97 oligomerization in plasma membrane channel expression and organization (26).

Because of the relatively high level of channel expression in transfected CHO cells (current density around 150 pA/pF), the stimulatory effect of SAP97 isoforms on the current may depend more on their capacity to cluster and organize channels in a large protein network than on their capacity to optimize channel surface expression. Likewise, in *Xenopus* oocytes, a relationship between the channel concentration and the magnitude of the effect of SAP97 on the Kv1.5-encoded current has been reported (3). CHO cells express endogenous MAGUKs that can regulate the functional expression of cloned Kv1.5 channels and thereby hide the full effect of exogenous SAP97 isoforms on these channels. However, it was not possible to circumvent this problem using other cell lines lacking endogenous SAP97, such as COS and HEK293 cells, since both retain SAP97-Kv channel complexes in their endoplasmic reticulum (14).

It has been shown that the I3 domain regulates the anchoring of the human Dlg/SAP97 homologue to the membrane-associated cortical skeleton through the protein 4.1/ERM and E-cadherin-catenin adhesion complex (8, 27, 28). In contrast, the I2 insert fails to address SAP97 to the membrane, leading to SAP97 accumulation in the cytosol or nucleus (10, 29). The subsarcolemmal localization of this SAP97 isoform containing the I2 insert may depend more on protein partners such as protein 4.1 (11) or Kv channels, as described for the membrane redistribution of PSD95 by Kv4.2 Shal channels (30). One consequence of the poor capacity of SAP97 lacking the I3 domain to be correctly addressed to the membrane could be also the formation of aggresomes of misfolded proteins (15, 31). This idea is supported by our observation of such cytosolic aggregates of tagged Kv1.5 channels in experimental conditions known to favor aggresome constitution (32).

In conclusion, our results confirm previous studies of the tissue specificity of alternative splicing of the SAP97 gene, showing that the isoform containing the I3 domain is the predominant cardiac isoform. The SAP97-I2 isoform appears to be restricted to neurons or to liver. It has been suggested that SAP97 bearing the proline-rich domain I1A may have a predominant signaling role, whereas isoforms lacking I1A may have a structural role (10). In the present study, we found that the I1A domain modulates the effect of SAP97 on the functional and spatial expression of Kv1.5 channels. The role of cardiac SAP97 isoforms in the electrical properties of the heart remains to be determined.

**Fig. 6. Different SAP97 isoforms lead to different cellular localizations of Kv1.5 channels.** Distribution of GFP-tagged Kv1.5 channels in live CHO cells expressing the channel alone (A) or together with the I3(+11A) (B), I3(−11A) (C), and I2(+11A) (D) SAP97 isoforms. Effects on GFP-tagged channel distribution of 50 μm protease inhibitor ALLN (E) and of an increasing concentration of expressed channels (2 μg of Kv1.5 plasmid) (F) in CHO cells co-expressing the I3(+11A) SAP97 isoform are shown. I3+, SAP97 I3(+11A) isoform; I3−, SAP97, I3(−11A) isoform; I2+, SAP97 I2(+11A) isoform. Scale bar, 20 μm.

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Different Isoforms of Synapse-associated Protein, SAP97, Are Expressed in the Heart and Have Distinct Effects on the Voltage-gated K\(^+\) Channel Kv1.5

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