Cloning and Expression of a Novel K⁺ Channel Regulatory Protein, KChAP*

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Voltage-gated K⁺ (Kv) channels are important in the physiology of both excitable and nonexcitable cells. The diversity in Kv currents is reflected in multiple Kv channel genes whose products may assemble as multisubunit heteromeric complexes. Given the fundamental importance and diversity of Kv channels, surprisingly little is known regarding the cellular mechanisms regulating their synthesis, assembly, and metabolism. To begin to dissect these processes, we have used the yeast two-hybrid system to identify cytoplasmic regulatory molecules that interact with Kv channel proteins. Here we report the cloning of a novel gene encoding a Kv channel binding protein (KChAP, for K⁺ channel-associated protein), which modulates the expression of Kv2 channels in heterologous expression system assays. KChAP interacts with the N termini of Kv2 subunits, as well as the N termini of Kvα1 and the C termini of Kvβ subunits. Kv2.1 and KChAP were coimmunoprecipitated from in vitro translation reactions supporting a direct interaction between the two proteins. The amplitudes of Kv2.1 and Kv2.2 currents are enhanced dramatically in Xenopus oocytes coexpressing KChAP, but channel kinetics and gating are unaffected. Although KChAP binds to Kv1.5, it has no effect on Kv1.5 currents. We suggest that KChAP may act as a novel type of chaperone protein to facilitate the cell surface expression of Kv2 channels.

The electrical properties of excitable cells are determined in large part by the voltage-gated K⁺ channels (Kv) they possess. Multiple Kv channels control the falling phase of the action potential in excitable cells. Kv channels are also important in many nonexcitable cells, where they may contribute to diverse processes such as volume regulation, hormone secretion, and activation by mitogens. The extensive diversity in Kv currents is matched by the multiplicity of genes encoding the pore-forming or α-subunit of Kv channels. About 20 mammalian Kvα genes have been cloned, and most have been assigned to one of four major subfamilies based on sequence similarities: Kv1, Kv2, Kv3, and Kv4 (1). Each K⁺ channel gene encodes a single subunit, and functional channels are formed by the tetrameric association of individual subunits apparently mediated by specific binding between the N-terminal domains of subunits within individual subfamilies (2, 3). With multiple Kv channel genes whose products may assemble as multisubunit heteromeric complexes (4–6), there may be hundreds of functionally distinct K⁺ channels. Given the great diversity and fundamental importance of K⁺ channels, the cellular mechanisms regulating their synthesis, assembly, and metabolism are of prime interest but remain largely unknown.

The identification and characterization of accessory or modulatory subunits for Kv channels is a new and rapidly expanding area of research. One family of modulatory proteins that interact with Kvα1 channels, Kvβ subunits, has been cloned and characterized in the past several years. Kvβ subunit genes, cloned from heart (7–10) and brain (11–13), encode cytoplasmic proteins that form stable complexes with Kvα1 subunits and exert multiple effects on Kvα1 currents. The three Kvβ isoforms and Kvβ3 introduce inactivation into Kvα1 subunit currents but with variable potency (12–14). A second effect of Kvβ subunits is to increase the surface expression of certain Kvα1 channels. This has been demonstrated both as an increase in the number of dendrotoxin-binding sites (for Kv1.2 transient expression) (15), as well as an increase in the number of functional channels (16). Complexes between Kvα1 and Kvβ subunits have been found to form in the endoplasmic reticulum (15, 17), suggesting that Kvβ subunits assist in the folding and assembly of at least some Kvα1 subunits. The association of Kv1.2 with Kvβ subunits produces more efficient glycosylation of Kv1.2, increases the stability of Kv1.2 through Kv1.2/Kvβ complex formation and results in an increase in cell surface expression (15).

To gain more information about the synthesis, assembly, and metabolism of K⁺ channels, we have used the yeast two-hybrid system to identify novel cytoplasmic molecules that interact with Kv subunits. Using Kvβ1.2 as bait, we screened a rat brain cDNA library in the GAL4 activation domain vector and isolated a novel gene that encodes a K⁺ channel-binding protein that we have termed KChAP (for K⁺ channel-associated protein). In addition to Kvβ subunits, KChAP also binds to the N termini of Kvα1 and Kvα2 subunits. Coexpression of KChAP with Kvα2 subunits results in a dramatic enhancement of both total Kv2.1 protein and surface expression of functional Kv2 channels. The unique sequence and properties of KChAP suggest that it may belong to a novel class of proteins with “chaperone-like” properties.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Library Screen—The entire Kvβ1.2 coding sequence (amino acids 1–408) was subcloned in frame into the GAL4 DNA binding domain vector, pGBT9 (CLONTECH) after polymerase chain

* This work was supported by National Institutes of Health Grants HL-57146 (to B. A. W.), NS-23877, HL-36930, and HL-55404 (to A. M. B.) and a grant from the American Heart Association, Northeast Ohio Affiliate (to B. A. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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reaction-mediated addition of a 5′ EcoRI site and a 3′ SalI site and used to screen a pGAD10 rat brain cDNA library (CLONTECH). Transformants in the yeast Y190 strain were plated on synthetic medium lacking tryptophan, leucine, and histidine but containing 3-amino-triazole (25 mm). After incubation for 8 days at 30 °C, His+ colonies were screened on a histidinyl activating strip (CLONTECH). Only the hybridizing colonies contained an insert of 3.2 kb with a single open reading frame of 574 amino acids. KChAP cDNA without 5′-untranslated sequences for subcloning in frame into the yeast two-hybrid vector pGBT9 was prepared by polymerase chain reaction to the EcoRI site and a 3′ SalI site.

**Analysis of Protein-Protein Interactions by the Yeast Two-hybrid System**—Protein-protein interactions were monitored with the yeast Matchmaker two-hybrid system (CLONTECH). The following fragments were tested for interaction with KChAP. Kvβ1.2 (amino acids 1–408), Kvβ1.2 N terminus (amino acids 1–79), Kvβ1C (carboxyl-terminal 329 amino acids of the Kvβ1 subfamily), Kvβ2 (amino acids 1–367), Kvβ1.1 N terminus (amino acids 1–168), Kvβ1.2 N terminus (amino acids 1–168), Kv1.1 N terminus (amino acids 62–654), Kv1.5 N terminus (amino acids 1–248), Kv2.1 N terminus (amino acids 1–168), Kv2.2 N terminus (amino acids 1–185), Kv6.1 N terminus (amino acids 1–209), Kir2.2 N terminus (amino acids 1–86), and HERG N terminus (amino acids 1–396). Human Gu-binding protein (GBP) cDNA encoding the region 1–248), Kv2.1 N terminus (amino acids 1–168), Kv2.2 N terminus (amino acids 1–367), Kv1.1 N terminus (amino acids 1–168), Kv1.2 N terminus (amino acids 1–408), Kv1.2 N terminus (amino acids 1–79), Kv1.3 N terminus (amino acids 1–168), and Kir2.2 N terminus (amino acids 1–86) were cloned into the yeast two-hybrid vector pGBT9 and used in each 25-μl reaction. To monitor the ability of the two proteins to associate specifically, the oocyte sections were incubated at 4 °C overnight at 68 °C in NorthernMax hybridization buffer (Ambion) following linearization of the construct with XhoI. CKAP cRNA was prepared with T7 polymerase. cRNAs were mixed and incubated with primary antibodies either a monoclonal Kv2.1 antibody (Upstate Biotechnology, Inc; 1:1000) or monoclonal anti-c-myc antibody (1:400), for 1 h at room temperature. The blots were then incubated with secondary antibody (anti-mouse HRP conjugate, Amersham Pharmacia Biotech; 1:3000) and developed with the ECL-Plus detection system (Amersham Pharmacia Biotech).

**Expression in Xenopus Oocytes and Electrophysiology**—For cRNA synthesis and expression in Xenopus oocytes, full-length KChAP coding sequence was subcloned into the vector, pCR3 (Invitrogen). KChAP cRNA was prepared using the T7 mMESSAGE mMACHINE kit (Ambion) following linearization of the construct with NotI. GBP cDNA was subcloned into a modified pSP64 vector (pSV1) for linearization of the template DNA for in vitro transcription with SP6 polymerase. cRNAs for Kvα1a subunits were prepared as described previously (7, 14). Rat Kv2.1 in pBluescript was linearized with NotI, and cRNA was prepared with T7 polymerase. cRNAs were mixed and injected into Xenopus oocytes as described previously (7). HERG cRNA was kindly provided by Dr. M. Keating. Kv2.2 was a gift from Drs. S. Snyder and J. Trimmer. Kir2.2 cRNA was prepared as described previously (20). We also used a Xenopus oocyte cDNA library in which the N-terminal 139 amino acids had been deleted (21).

**Measurement of Xenopus oocyte whole cell currents** was performed using the standard two-microelectrode voltage clamp technique. Bath solution contained (in mM/liter): 5 KOH, 100 NaOH, 0.5 CaCl2, 2 MgCl2, 100 methanesulfonic acid, and 10 HEPES (pH 7.4). Solution containing 50 KCl was prepared to replace an equivalent concentration of NaCl. All solutions were filtered and had a resistance of 0.3–0.6 megohms. All recordings were made at room temperature. Linear leakage and capacitive transient currents were subtracted (P4 prepulse protocol) unless specified, and data were low pass-filtered at 1 kHz. Clamp software (Axon Instruments) was used for generation of the voltage-pulse protocols and data acquisition. Data are reported as means ± S.E. Comparisons among multiple groups of oocytes were performed by one-way analysis of variance test, Student’s t test, and Student-Newman-Keuls post hoc test. Means are considered to be significantly different when p < 0.05.

**RESULTS**

**Isolation of a Novel Kvβ and Kva Subunit-Binding Protein with the Yeast Two-hybrid System**—Full-length Kvβ1.2 was used as bait to screen a rat brain cDNA library in the GAL4 activation domain vector, pGAD10. We isolated one clone that exhibited a strong positive signal in the β-galactosidase assay. pGAD10 plasmid DNA containing a 1.78-kb insert was isolated from this clone and tested for positive interaction with Kvβ1.2. Sequence analysis of the clone, which we termed KChAP-Y (for K channel-associated protein, Y refers to the fragment isolated in the yeast two-hybrid screen), revealed a novel clone with no similarity to Kvα or Kvβ subunits.

We tested the specificity of interaction of KChAP-Y with a panel of Kvβ, Kva, and other K+ channel subunit fragments.
with the yeast two-hybrid assay. As shown in Fig. 1, KChAP-Y interacted with both Kvβ1.2 and Kvβ2 subunits. KChAP-Y interacted with the conserved Kvβ1 C terminus but not the unique N terminus of Kvβ1.2, suggesting that the protein may recognize conserved sequences among Kvβ subunits. Kvβ subunits interact specifically with the N terminus of Kvα subunits, so we tested these fragments for binding to KChAP-Y as well. Surprisingly, a positive signal was observed between the N termini of Kv1.1, Kv1.2, Kv1.4, Kv1.5, and KChAP-Y. Just as with the Kvβ subunits, however, no interaction was evident between the Kv1.4 C terminus and KChAP-Y. KChAP-Y also interacted with the N termini of Kv2.1 and Kv2.2 but not with the N terminus of the electrically silent Kv2 partner, Kv6.1 (22). Further specificity for a subset of Kv channels was apparent from the lack of interaction with the N terminus of the inward rectifier K+ channel, Kir2.2, and the N terminus of the delayed rectifier K+ channel, HERG. Thus, KChAP-Y apparently interacts with both the C terminus of Kvβ subunits and the N termini of Kv1 and Kv2 α-subunits.

Cloning and Sequence Analysis of Full-length KChAP—Screening of a rat brain cDNA library with the KChAP-Y coding sequence produced a 3.2-kb insert, which overlapped KChAP-Y and contained a single open reading frame of 574 amino acids. The initiating methionine was assigned as the first ATG downstream from three in frame stop codons. Hydrophathy analysis indicated no potential membrane spanning domains in KChAP, suggesting that the protein was cytoplasmic (not shown).

Search of the GenBankTM nonredundant data base revealed significant homology with the mammalian gene encoding GBP (29). GBP was isolated originally in a yeast two-hybrid screen as a protein that binds to the Gu/RNA helicase II subunit. Alignment of KChAP with GBP is presented in Fig. 2. GBP has an N-terminal extension of 55–57 amino acids compared with KChAP, but over the 574-amino acid open reading frame of KChAP, the two proteins are 50% identical. We tested the binding of both full-length KChAP (amino acids 1–574) and human GBP (amino acids 49–645) with K+ channel fragments in the yeast two-hybrid assay as was described for KChAP-Y in Fig. 1. Full-length KChAP was identical to KChAP-Y in its interaction with protein partners in the yeast two-hybrid assay, while GBP did not interact with any of the tested fragments including Kvβ and Kvα subunits (data not shown). Thus, although KChAP shares significant homology with GBP, interaction with Kvβ and Kvα subunits appears to be a unique feature of KChAP.

**Northern Blot Analysis**—The expression of KChAP mRNA was examined in a panel of rat tissues. The blot was probed with a fragment of KChAP encoding amino acids Lys8–107–Asp574, a region with minimal homology to GBP, to avoid detecting GBP transcripts as well. As shown in Fig. 3, a single band of ~3.2 kb was detected in a variety of tissues including heart and brain with especially high levels in lung and kidney.

**Functional Characterization of KChAP-Kv Interactions**—The surprising finding that KChAP associated with Kvα1 and Kvα2 subunits as well as Kvβ subunits led us to examine the functional consequences of KChAP-K+ channel interaction upon heterologous expression in Xenopus oocytes. Whole oocyte currents were recorded by two-electrode voltage clamp from eggs injected with cRNAs encoding different Kvα-subunits alone or with saturating concentrations of KChAP. Coexpression with KChAP produced a dramatic 3-fold increase in the amplitude of Kv2.1 currents (Fig. 4A). No change in Kv2.1 currents was apparent when the channel was coexpressed with GBP (data not shown). At more depolarized potentials, Kv2.1 has an opening probability of about 0.9 (24), suggesting that the increased currents recorded when KChAP was coexpressed were probably due to an increase in the number of functional channels. KChAP also interacted with the N terminus of Kv1.5 but, in contrast to Kv2.1, produced no significant change in Kv1.5 currents when coexpressed in oocytes (Fig. 4B). The experiments were done so that Kv1.5 expressed whole cell currents at +70 mV in the range of 0.5–5 μA. This greatly reduced the possibility that amplitude changes might be missed as a result of voltage clamp difficulties. Thus, while KChAP interacted with the N termini of both Kv2.1 and Kv1.5 in the yeast two-hybrid assay, KChAP only produced amplitude increases in Kv2.1 currents in oocyte expression assays.

Fig. 4C summarizes the effects of KChAP on the current amplitudes of a variety of K+ channels. In 13 batches of oocytes coinjected with both Kv2.1 and KChAP, we observed an average increase in whole oocyte currents of about 2.5-fold compared with oocytes expressing Kv2.1 alone. KChAP also produced comparable increases in Kv2.2 currents. We also examined the functional expression of a deleted Kv2.1 in which the N-terminal 139 residues were removed (21). As shown in Fig. 4C, coexpression with KChAP did not significantly alter current amplitudes in oocytes expressing Kv2.1ΔN. This suggests that binding between the Kv2.1 N terminus and KChAP is critical for current enhancement.

Two K+ channels that did not exhibit N-terminal binding to KChAP, Kir2.2 and HERG, were also tested. For each channel, experiments were done with whole cell control inward currents not exceeding 10 μA at –100 mV. As shown in Fig. 4C, neither channel exhibited altered current amplitudes in the presence of KChAP.

**KChAP Increased Functional Expression of Kv2.1 without Altering Channel Kinetics or Gating**—The expression enhancement of Kv2.1 currents in the presence of KChAP could be due to an increase in the number of functional channels at the cell surface or an alteration in the kinetics or gating of individual channels. To distinguish between these mechanisms, we used both immunocytochemical and electrophysiological methods. We examined the surface expression of Kv2.1 protein in oocytes.
expressing either Kv2.1 alone or Kv2.1 plus c-myc-KChAP by
immunocytochemistry. Fig. 5 shows the whole cell currents
recorded from a single oocyte injected with Kv2.1 alone (panel
A) or Kv2.1 plus c-myc-KChAP (panel B). Currents were in-
creased about 3-fold in the c-myc-KChAP-coinjected egg. The
same two oocytes were fixed, sectioned, and co-stained with
Kv2.1 and c-myc antibodies. Kv2.1 at the cell surface was
visualized by indirect immunofluorescence with a fluorescein
isothiocyanate-conjugated secondary antibody. Fluorescence at
the oocyte surface was much brighter in the egg expressing
both Kv2.1 and KChAP (panel D) compared with the one ex-
pressing Kv2.1 alone (panel C), suggesting that the amount of
Kv2.1 protein at the cell surface was increased when the chan-
el was coexpressed with KChAP. No staining with the c-
mhc antibody was seen, suggesting that KChAP is not present at
the cell surface with Kv2.1 (panel E).

To determine whether KChAP increased the total expression
of Kv2.1 protein or only altered the subcellular distribution of
the channel, we examined membrane fractions from oocytes
injected with Kv2.1 alone or Kv2.1 plus KChAP by Western
blotting. As shown in Fig. 6A, the amount of Kv2.1 protein in
oocyte membranes was increased in oocytes coinjected with
KChAP (compare lanes 1 and 2). Densitometry of the blots
indicated about a 2.5-fold increase in Kv2.1 protein in the
presence of KChAP. Similar results were obtained when Kv2.1
was immunoprecipitated from homogenates of total oocyte pro-
tein (data not shown). This value is comparable with the in-
crease observed in Kv2.1 currents with KChAP.

Since no KChAP was detected at the cell surface of co-
expressing oocytes, we examined the cellular distribution of
KChAP in oocytes by Western blotting. We manually removed
the nuclei from pools of oocytes expressing only Kv2.1 or Kv2.1
plus c-myc-KChAP and probed the nuclear fraction as well as
the soluble and membrane fractions prepared from the enucle-
ated oocytes with an anti-c-myc antibody to detect tagged KC-
chAP. Most of the KChAP protein was present in the nuclear
fraction with smaller amounts detectable in the soluble as well
as the membrane fractions (Fig. 6B). The signal was so strong
in the nuclear material compared with the other two fractions,
However, that we were not able to estimate accurately the relative amounts in each fraction. No Kv2.1 was detected in the nuclear fraction, indicating that contamination with non-nuclear membranes was minimal (data not shown).

The kinetics and gating of Kv2.1 channels were not altered in the presence of KChAP. As shown in Fig. 7A, the voltage-dependence of activation and the kinetics of activation and deactivation of Kv2.1 channels were not changed. Coexpression with KChAP did not alter the sensitivity of Kv2.1 channels to 

![FIG. 4. Effect of KChAP on K+ channel functional expression. A, averaged macroscopic currents from eight oocytes in one injection series measured on day 6 postinjection in oocytes injected with Kv2.1 cRNA (0.62 ng/μl) alone (left) or one coinjected with Kv2.1 and KChAP cRNAs (0.62 and 250 ng/μl, respectively) (right). Holding potential was −80 mV, and pulses were from −70 mV to +70 mV in 10-mV steps with 50 ms K+ in bath solution. B, averaged macroscopic currents from 10 oocytes in one injection series measured 5 days postinjection from oocytes injected with Kv1.5 cRNA (50 ng/μl) alone (left) or Kv1.5 plus KChAP cRNAs (50 and 500 ng/μl, respectively) (right). Holding potential was −80 mV, and pulses were from −70 to +70 mV in 10-mV steps with 5 mM K+ in bath solution. C, bar plot showing averaged current levels in the presence of KChAP as fractions of currents in the absence of KChAP (control current). The numbers above each bar indicate the number of batches of oocytes examined for each K+ channel. Oocytes were injected with either Kv+ channel cRNAs or K+ channel plus KChAP cRNAs, and currents were recorded from 8–12 oocytes in each batch. Whole oocyte macroscopic currents were measured 2 days after injection (Kv2.2 and Kv2.1ΔN) or 5–6 days after injection (Kv2.1, Kv2.2, Kv1.5, and HERG), and the ratio of means (Umacroscopic/Ucontrol) was calculated. For Kv2.1, Kv2.2, and Kv1.5, the holding potential was −80 mV. Steady-state currents were measured at a test potential of −70 mV (5 or 50 K+ in bath). Kir2.2 steady-state and HERG tail currents were recorded with 50 K+ in the bath at test potentials to −100 mV with a prepulse to +20 mV. Asterisks indicate that in all injection series, current amplitudes in oocytes coinjected with KChAP were significantly higher than in oocytes expressed on the oocyte surface. Macroscopic currents recorded 5 days after cRNA injection from an oocyte expressing Kv2.1 (1.25 ng/μl cRNA; panel A) or one expressing Kv2.1 (1.25 ng/μl) plus c-myc-KChAP (250 ng/μl; panel B). Recordings were obtained by stepping from a holding potential of −80 mV with 10-mV steps from −70 to +80 mV. Below each current trace is a section of the same oocyte stained by indirect immunofluorescence with anti-Kv2.1 antibody and fluorescein isothiocyanate-conjugated anti-rabbit secondary antibody (panels C and D). Note that the intensity of fluorescence at the oocyte surface is much greater in the cell expressing Kv2.1 and KChAP (panel D). The oocyte section in panel D was co-stained with anti-c-myc and a tetramethylrhodamine B isothiocyanate-conjugated secondary antibody. No tetramethylrhodamine B isothiocyanate fluorescence was visible at the cell surface, suggesting that c-myc-KChAP is not present there with Kv2.1 (panel E).
100-ms pulses were given in 10-mV steps from -50 mV to +70 mV test potential from oocytes injected with Kv2.1 alone and Kv2.1 plus KChAP. Holding potential was -80 mV. 

Kv2.1 currents were measured at the end of 200-ms pulse to +70 mV from oocytes injected with Kv2.1 cRNA alone (0.62 ng/μl), filled circles (n=10); Kv2.1 plus KChAP (125 ng/μl), filled triangles (n=9). Inset, superimposition of averaged and scaled currents (at +70 mV test potential) from oocytes injected with Kv2.1 alone and Kv2.1 plus KChAP. Holding potential was -80 mV. 100-ms pulses were given in 10-mV steps from -70 mV to +80 mV. 50 mM KCl in bath solution. Recordings were performed 6 days after injection and are from one batch of oocytes. Values of τneurotransp and τneurotransp when pulsing to +70 mV and then back to -80 mV were 16.7 ± 0.2 and 5.3 ± 0.1 ms (n=10), respectively, for Kv2.1 alone and 15.8 ± 0.5 and 6.2 ± 0.2 ms (n=9), respectively, for Kv2.1 plus KChAP. B, effect of increasing amounts of KChAP cRNA on Kv2.1 expression. Current amplitudes were measured at the end of the pulse to +70 mV. In oocytes injected with Kv2.1 cRNA alone (0.62 ng/μl), the current was 10.7 ± 0.9 μA; in oocytes coinjected with KChAP cRNA, the currents were 23.2 ± 2.5, 39.5 ± 6.8, 43.7 ± 4.4, and 49.7 ± 6.2 μA at KChAP cRNA concentrations of 15, 31, 62, and 125 ng/μl, respectively; numbers of oocytes are indicated (the same batch of oocytes as in A). *, a significant difference from control Kv2.1 (p<0.05; one-way analysis of variance/Student-Newman-Keuls post hoc test); C, time dependence of KChAP effect on Kv2.1 expression in one injection series. Currents measured at the end of a 200-ms pulse to +70 mV from oocytes injected with cRNAs for Kv2.1 alone (0.62 ng/μl, filled circles) or Kv2.1 plus KChAP (125 ng/μl, filled triangles). Numbers of oocytes are indicated in parentheses above the points. Average currents in oocytes injected with Kv2.1 cRNA alone were 2.8 ± 0.7, 6.1 ± 1.9, and 5.3 ± 1.5 μA measured at 3, 6, or 9 days postinjection, respectively. Oocytes coinjected with Kv2.1 and KChAP cRNAs had average currents of 6.8 ± 2.8, 15.7 ± 4.4, and 19.9 ± 5.6 μA measured at 3, 6, or 9 days postinjection, respectively. **, a significant difference from Kv2.1 tested on the same day after injection (p<0.05; test).

In Vitro Association of KChAP and Kv2.1—The electrophysiological data suggest that a direct interaction between Kv2.1 and KChAP occurs and is responsible for the enhancement in Kv2.1 currents observed in oocytes. We used an in vitro binding assay to demonstrate the ability of the two proteins to associate. Kv2.1 and KChAP cRNAs were translated in vitro either separately or together in a rabbit reticulocyte lysate in the presence of [35S]methionine. We used a commercially available anti-Kv2.1 polyclonal antiserum to immunoprecipitate Kv2.1 and analyzed the immunoprecipitated material with SDS-polyacrylamide gel electrophoresis and fluorography to detect the presence of associated KChAP. Since an antibody to KChAP was not available, we used an epitope tag fused to the N terminus of KChAP (c-myc) to allow detection. As we had previously shown in Fig. 5, the c-myc tag did not interfere with the functional interaction of KChAP and Kv2.1. Control reactions with Kv2.1 translated alone showed that in vitro translated Kv2.1 was immunoprecipitated with anti-Kv2.1 antibody but not anti-c-myc antiserum (Fig. 8, lanes 1 and 2). Similarly, anti-c-myc antisera immunoprecipitated c-myc-KChAP but not Kv2.1 (Fig. 8, lanes 3 and 4). Kv2.1 antibody coimmunoprecipitated complexes of Kv2.1 and KChAP when the two cRNAs were cotranslated (Fig. 8, lane 5) but not when the two cRNAs were translated in separate reactions and mixed together prior to the addition of primary antibody (Fig. 8, lane 6). This result suggests that the association of KChAP with Kv2.1 occurs.
cotranslationally, since the mature proteins added after translation did not coimmunoprecipitate. All reactions involving translation of Kv2.1 cRNA shown here included canine pancreatic microsomes to allow the channel to insert into membrane as it was synthesized. When microsomes were omitted from cotranslation reactions, no coimmunoprecipitation of the two proteins was observed (data not shown).

**DISCUSSION**

We have cloned a novel gene, kchap, which encodes a K+ channel regulatory protein. All of our data point to a direct interaction between KChAP and Kv2.1 as responsible for the current enhancement observed in *Xenopus* oocytes. Yeast two-hybrid assays showed an interaction between KChAP and the N terminus of Kv2.1. When an N-terminally truncated Kv2.1 was expressed in oocytes, no expression enhancement was observed. Also, Kv2.1 and KChAP were co-immunoprecipitated from *in vitro* translations in which the two proteins were translated in the same reaction, but not when they were translated separately and mixed together before immunoprecipitation. This suggests a cotranslational association of the two proteins, which is also supported by oocyte data. In order to observe Kv2.1 current enhancement in oocytes, the KChAP and Kv2.1 cRNAs had to be mixed together and coinjected with the same pipette. No changes in Kv2.1 currents were noted when the two cRNAs were injected from two separate pipettes, suggesting that the two proteins must be translated in close proximity. However, in the oocyte, KChAP might bind only transiently to Kv2 channels and not remain stably attached to the mature channel as evidenced by the lack of KChAP staining at the oocyte surface.

In *Xenopus* oocytes, KChAP does not appear to alter the time course of Kv2.1 current expression. Without KChAP, the amplitude of Kv2.1 currents continues to increase slowly from the time of injection through about 6 days, at which time the current levels plateau and remain fairly constant through 9 days postinjection. This would suggest that transit of Kv2.1 channels to the oocyte surface is a rather slow process. KChAP does not change this profile but increases the amplitude of Kv2.1 currents recorded at each time point.

One possible explanation for the effect of KChAP on Kv2.1 expression is that KChAP may act as a chaperone to facilitate either the translation, assembly, or stability of Kv2.1 channels. An increase in the amount of Kv2.1 protein in oocyte membranes in the presence of KChAP as seen by Western blotting is consistent with this interpretation. Kvβ subunits also exhibit chaperone-like effects on Kv channels. However, Kvβ subunits (Kvβ1 and Kvβ2) form stable complexes with Kvα subunits, and although they are cytoplasmic proteins, travel to the cell surface with pore-forming Kvα subunits and remain tightly attached there as part of mature channel complexes (28–30). KChAP defines a different structural gene from Kvβ subunits. This is the same domain to which the N terminus of Kvα subunits have been shown to bind (14, 25, 26). One could envision that KChAP might modulate the association of Kvα and Kvβ subunits and, thus, indirectly affect the expression and/or kinetic properties of Kv1 currents, but this is speculative at present.

Although KChAP and Kvβ subunits share no sequence homology, KChAP is 50% identical to the human GBP (23). GBP is a novel protein that was cloned by interaction with the GuoRII-helical in the yeast two hybrid-system. GBP did not interact with either Kvα or Kvβ subunits in yeast two-hybrid assays in oocytes. GBP is a nuclear protein that has been shown to produce proteolytic cleavage of GuoRII (23). The significance of the sequence similarity between the two proteins is unclear at present. Interestingly, however, KChAP protein was primarily detected in the nuclei of injected oocytes. The relationship of the nuclear localization of KChAP with its effects on Kv2 channels is presently unclear. If a direct interaction between KChAP and Kv2.1 is required for expression enhancement, then this may occur transiently prior to KChAP moving to the nucleus. We cannot rule out, however, that KChAP may play an as yet unknown role in the nucleus, which results in enhanced Kv2.1 protein and current levels.

To summarize, we have described a novel gene product, KChAP, which binds to Kvα and Kvβ subunits. KChAP increases currents expressed by Kv2.1 and Kv2.2 but not Kv1.5, and the expression enhancement is due to an increase in total protein as well as in the number of functional Kv2 channels at the cell surface. We speculate that KChAP may act as a novel type of chaperone protein for Kv2 channels.

**Acknowledgments**—We thank Dr. M. Post for Kv2.1, Kv2.2, and Kv6.1 fragments in pG Bl9; Dr. E. Ficker for sectioning oocytes; Drs. S. Snyder and J. Trimmer for the Kv2.2 cDNA clone; Dr. M. Reating for the HERG cDNA clone; and T. Carroll and Dr. W. Dong for expert technical assistance.

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