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Identification of Proteins Interacting with C-Terminal Region of Human Ankyrin-G

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Ankyrins are a ubiquitously expressed family of intracellular adaptor proteins involved in targeting diverse proteins to specialized membrane domains in both the plasma membrane and the endoplasmic reticulum. Recently, the studies with C-terminus of ankyrins have identified that ankyrin-B is capable of interacting with Hsp40 and sAnk1 is capable of interacting with obscurin and titin, but the function of C-terminal domain of ankyrin-G remains unknown. To identify proteins interacting C-terminus of ankyrin-G, we used the C-terminus of ankyrin-G as a bait for a yeast two-hybrid screen of brain cDNA library. Approximately 1.33×10^6 transformants were screened, of which 13 positive clones were obtained as determined by activation of HIS3, ADE2 and MEL1 reporter genes. Sequence analyses of these 13 plasmids revealed that cDNA inserts of 13 colonies showed highly homologous to 11 genes, including 5 known (i.e., Na^+/K^+ ATPase β1, SERBP1, UTF2, cytochrome C oxidase and collagen IV α2) and 6 unknown genes. The evaluation of the proteins that emerge from these experiments provides a rational approach to investigate the those proteins significant in interaction with ankyrin-G.

Key Words: Ankyrin-G, Yeast two-hybrid system

INTRODUCTION

Ankyrins are a family of proteins involved in the organization of specific membrane domains by mediating the interactions between proteins of the plasma membrane and the subplasma membrane cytoskeleton[3]. Vertebrate ankyrin polypeptides fall into three classes, each containing multiple alternatively spliced variants: ankyrins-R (R for restricted distribution, and the prototypic ankyrin first characterized in erythrocytes; also expressed in a subset of neurons and striated muscle) encoded by Ank1 on human chromosome 8p11; ankyrins-B (B for broadly expressed; first characterrized in brain, but now recognized in most cell types) encoded by Ank2 on human chromosome 4q25~27; and ankyrins-G (G for giant size and general expression, first characterized as a 480 kDa polypeptide in the nervous system; expressed in most cell types) encoded by Ank3, on human chromosome 10q21. Canonical ankyrins are 190~220 kDa proteins expressed in most tissues and cell types and comprise a membrane-binding domain (MBD) of 24 ANK repeats, a spectrin-binding domain, a death domain and a C-terminal domain. Whereas death domains in other proteins may function in activation of NF-κB, caspase proteases and cell death, this domain has no known role within ankyrins[13].

Current views of ankyrin function are based on co-localization and biochemical interactions of ankyrin with other proteins. Ankyrin associates with a variety of membrane proteins including ion channels (Na^+/K^+ ATPase, H^+/K^+ ATPase, anion exchangers AE 1~3, voltage-sensitive Na^+ channels, Na^+/Ca^{2+} exchanger), calcium-release channels [ryanodine receptor, inositol (1,4,5)-trisphosphate receptor], cell adhesion molecules [CD44, L1CAMs (L1, NgCAM, neurofascin, LAD-1, NrCAM, neuroglian)], as well as cytoplasmic proteins, including clathrin and tubulin[3]. Many of these interactions are mediated by ANK repeats

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within the MBD, although the Na^+/K^+ ATPase and H^+/K^+ ATPase associate at least in part with the spectrin-binding domain[13].

Members of the ankyrin family have a high degree of sequence similarity, share common structural domains, and are often co-expressed in the same cells[10,15]. The studies of ankyrin gene-specific knockouts in mice demonstrate that ankyrin isoforms have divergent and non-overlapping functions. For example, mutant mice with a cerebellar specific knockout of ankyrin-G that still show normal expression of ankyrin-B and ankyrin-R in the cerebellum display a loss of voltage-gated Na channels and neurofascin from axon initial segments, a decreased ability of Purkinje neurons to fire action potentials, and a progressive ataxia[20]. Similarly, mice deficient in ankyrin-B exhibit down-regulation and missorting of calcium-release channels in cardiac and skeletal muscle and cardiac arrhythmias, despite the fact that ankyrin-R and ankyrin-G isoforms are normally expressed in both cardiac and skeletal muscle[9,10,15,18,20]. Therefore, unlike a number of other protein families that can compensate for lack of specific isoform expression, ankyrin gene functions appear to be specific and are not compensated by other similar gene products. An important but currently unanswered question regarding these similar molecules is the basis for specificity in the unique functions of ankyrins[16]. But rescue studies with ankyrin-B/G chimeras demonstrate that the C-terminal domain of ankyrin-B has identified the C-terminal domain of ankyrin-B as the defining domain in specifying ankyrin-B activity and is critical for the normal localization of ankyrin-B. And ankyrin isoforms appear to have similar but non-overlapping roles in the organization of protein complexes and the divergent roles of ankyrins are dependent on their C-terminal domains that are highly divergent across the ankyrin family[14]. A working hypothesis to explain the cellular basis for these phenotypes is that ankyrins play roles as chaperones or guides that direct vesicle transport of a variety of ion channels to sites in the plasma membrane as well as the endoplasmic reticulum[12].

Among ankyrin genes, Ank3 shows a broad tissue expression, including kidney and the nervous system, where it was first identified. Tissue-specific alternative processing of Ank3 transcripts results in distinct ankyrin-G isoforms with presumably related but distinct functions. The largest 480/270 kDa ankyrin-G isoforms are specifically expressed in neurons, where they are targeted to the nodes of Ranvier and initial axonal segments. These isoforms contain extended "tail" sequences between the spectrin-binding and C-terminal domains. Ankyrins-G expressed in tissues other than brain lack the tail domain, and their molecular masses range from 100 to 220 kDa[5]. The 480 kDa isoform is expressed early in neural development and accumulates at the initial segment of growing axons in culture, whereas the 270 kDa polypeptide is expressed later than the 480 kDa form and is therefore likely to have a distinct. Ankyrin-G at nodal and initial axon segments therefore defines specialized domains within the spectrin-actin network[9].

Recently, the studies with C-terminus of ankyrins have identified that the C-terminus of ankyrin-B interacts with Hsp40[12] and the C-terminus of sAnk1 (one of ankyrin-R isoforms) interacts with obscurin[1,8] and titin[7]. But The functions of the C-termius of ankyrin-G are, however, not yet known. Based on current studies, we have searched for cellular proteins interacting with ankyrin-G C-terminal domain in human brain cDNA library by employing the yeast two-hybrid system.

MATERIALS AND METHODS

1. Materials

The strains and plasmids used in this study are listed in Table 1. The *S. cerevisiae* AH109 strain (Clontech, Matchmaker System III) were used to perform the yeast two-hybrid system. For the system, plasmids pAS2-1 and pACT2 (Clontech, Matchmaker System II) were used as sources of DNA binding and activation domain, respectively. The *E. coli* strain HB101 was used for prey plasmid recovery, selecting on plates lacking leucine. *E. coli* strain DH5α was used for bait plasmid recovery (Table 1). Human Brain Matchmaker cDNA library (HL4004AH), YPD medium, minimal SD base and DO supplement medium, Advatage-HF 2 PCR Kit were purchased from Clontech. Mini- and Megapreps plasmid kits were obtained from Qiagen. Restriction endonucleases and T4 DNA ligase were purchased from Promega. Thermo Sequenase Cycle Sequencing Kit were from USB.

2. Construction of a bait plasmid

To make the bait plasmid for this study, the primers containing EcoRI/BamHI were designed from the human

Table 1. Strain and plasmid for yeast two-hybrid

Strain	Relevant characteristics
S. cerevisiae AH109	MATa, *trp*1-901, *leu*2-3, 112, *ura*3-52, *his*3-200, *gal*4Δ, *gal*80Δ, LYS2: : $GAL1_{UAS}$-$GAL1_{TATA}$-HIS3, $GAL2_{UAS}$-$GAL2_{TATA}$-ADE2, URA3: : $MEL1_{UAS}$-$MEL1_{TATA}$-*lac*Z
E. coli HB101	*sup*E44 Δ*lac*U1691 (ϕ80*lac*Z Δ M15) *hsd*R17 *rec*A1 *end*A1 *gyr*A96 *thi*-1 *rel*A1
E. coli DH5	*sup*E44 *hsd*S20 ($r^-_B m^-_B$) *rec*A13 *ara*-14 *pro*A2 *lac*Y1 *gal*K2 *rps*L20 *xyl*-5 *mtl*-1
Plasmid	
pAS2-1	Cloning vector; used to generate fusions of the bait protein with the GAL4DNA-BD.
pACT2	Cloning vector; used to generate fusions of a known protein (or a collection of random, unknown proteins) with the GAL4 AD.
pVA3-1	Positive control plasmid used with pTD1-1; encodes a DNA-BD/murine p53fusion protein in pAS2-1.
pTD1-1	Positive control plasmid used with pVA3-1; encodes an AD/SV40 large T-antigen fusion protein in pACT2.
pCL1	Positive control plasmid; encodes the full-length, wild-type GAL4 protein.
pLAM5'-1	False-positive detection plasmid; encodes a DNA-BD/human lamin C fusion protein in pAS2-1.

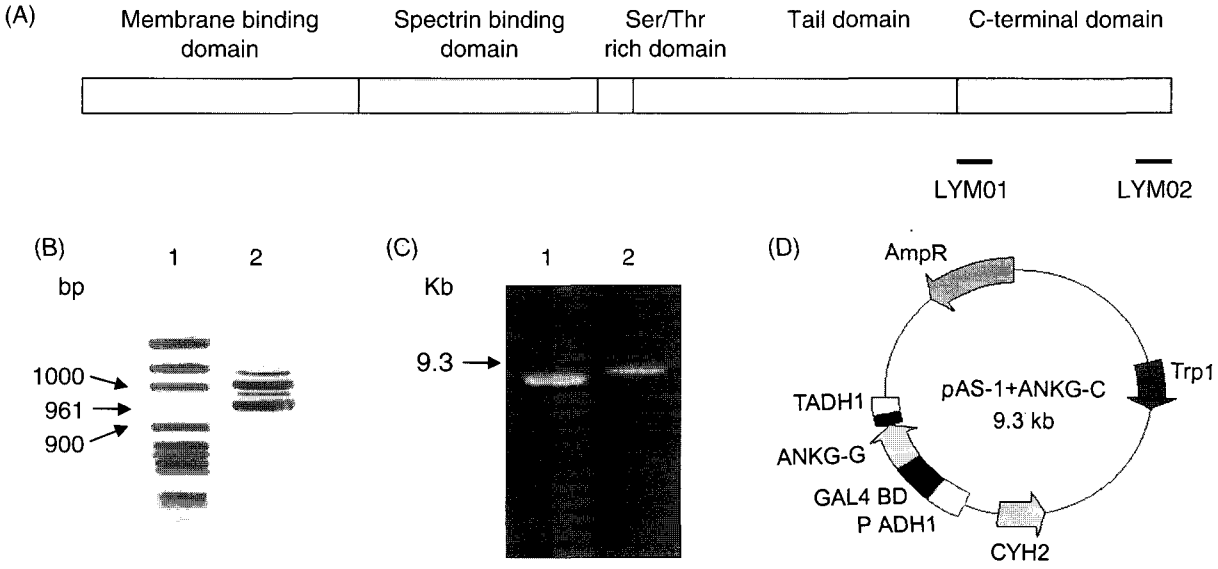

Fig. 1. Construction of a bait plasmid. **(A)** Schematic diagram of ankyrin-G showing the domain structure of human brain ankyrinG_{480} and the location of PCR primers. **(B)** PCR analysis of amplification of ankyrin-G C-terminus (lane 2) from human brain. Size maker (lane 1). **(C)** Agarose gel analysis of cloning of pAS2-1/AnkG-C (lane 2). Size maker (lane 1). **(D)** Schematic drawing of the bait plasmid, pAS2-1/AnkG-C.

ankyrin-G_{480} cDNA sequence (GenBank/EMBL/DDBJ accession number U13616) to generate a 961 bp human ankyrin-G_{480} C-terminus fragment. A 961 bp human ankyrin-G_{480} C-terminus fragment was amplified with Advantage-HF 2 PCR Kit (K191401, Clontech). The PCR product was purified and then digested by EcoRI/BamHI. The vector and the insert fragment were then ligated to yield construct of pAS2-1/AnkG-C. The ligation products were transformed into *E. coli* DH5α and then carried out DNA isolation.

3. Yeast two-hybrid screening

Yeast two-hybrid screenings were performed utilizing the Matchmaker 3 of Clontech Laboratories (Palo Alto, CA). The bait, pAS2-1/AnkG-C was transfected by the lithium acetate (LiAc)-mediated method into the yeast reporter strain AH109 together with a cDNA library prepared

Table 2. Sequence analysis of positive clones interacting with ankyrin-G

GenBank No.	Protein name	Total clones	Clone No.
NM_001677	Na^+/K^+ ATPseβ1	1	H305
BC049821.1	UTF2	1	E109
NM_004176.2	SREBF1	1	F091
NM_001846.1	Collagen IV α2	1	F051
AY275537	Cyt C oxidase	1	F106
BX6406490.1	Unknown	1	E111
AK096373.1	Unknown	1	H403
AF075040.1	Unknown	3	E072, H153, H154
AC091492.2	Unknown	1	G061
AC127459.3	Unknown	1	F081
AP001710.1	Unknown	1	F141

from human brain (HL4042AH) in the prey vector pACT2. In brief, the yeast competent cells were prepared and suspended in a LiAc solution with the plasmids DNA to be transformed, along with excess carrier DNA. Polyethylene glycol (PEG) with an appropriate amount of LiAc was then added and the mixture of DNA and yeast was incubated at 30℃. After incubation, DMSO was added and the cells were heat shocked at 42℃. The cells were then plated on agar plates lacking adenine, histidine, tryptophan, and leucine to select HIS3, ADE2 positive transformants. Positive colonies appearing after 5~10 days at 30℃ were assayed for α-galactosidase activity on plate. The plasmid DNA of positive colonies was isolated with phenol and glass beads as suggested by the supplier of the system. Positive two-hybrid protein interactions were verified by transfection of the plasmids back into the reporter strain AH109 together with the original bait or with selected controls. The plasmids of positive colonies were amplified in *E. coli* DH5α and sequenced by the dideoxy chain termination method.

4. PCR

For size of positive clone 13 proteins were amplified by PCR with primers (GAD I, GAD II). The sense primer named GAD I (5' TTCGATGATGAAGATACCCCACCAAACCC3', 29mer) and the antisense primer named GAD II (5'GAACTCCCGGGGTTTTTCAGTATCTACGAT3', 30 mer) was constructed for insert of pACT2. The PCR reaction was allowed to proceed in a Perkin-Elmer 2400 thermal cycler programmed for 30 cycles (94℃, 1 min; 94℃ 10 sec; 53.6℃, 30 sec; and 68℃, 1 min) with one additional cycle for 7 min at 68℃. The PCR products was run on a 1.5% agarose gel to determine the size of the bands.

RESULTS

1. Construction of bait plasmid

The PCR primers for C-terminus of ankyrin-G for construction of bait plasmid were (i): 5'-GGAATTCCTGGTCCACAGAGTCCAT-3' (sense) containing an EcoRI restriction site and 25 bases of the 5' end of ankyrin-G gene and (ii): 5'-CGGGATCCCGCCTTGACTGACCGTTCGC-3' (antisense) containing a BamHI restriction site and 38 bases of the 3'end of ankyrin-G gene. The "bait" cDNA fragments fragment encoding the C-terminus of ankryin-G (nt 4081~4303) was inserted into the pAS2-1 bait vector at EcoRI/BamHI sites after PCR amplification with primers. The resulting plasmid was designated as pAS2-1/AnkG-C (Fig. 1). The authenticity of the construct was verified by sequence analysis. Sequencing revealed that the AnkG-C gene was fused in the correct reading frame with the coding sequence of GAL4 DNA-binding domain.

2. Identification of proteins interacting with ankyrin-G

For screening, AH109 reporter strain was simultaneous transformed with bait and prey plasmids (HL400AH) using a modified lithium acetate protocol. When screening 1.33×10^6 clones from a human brain library with pAS2-1/AnkG-C,

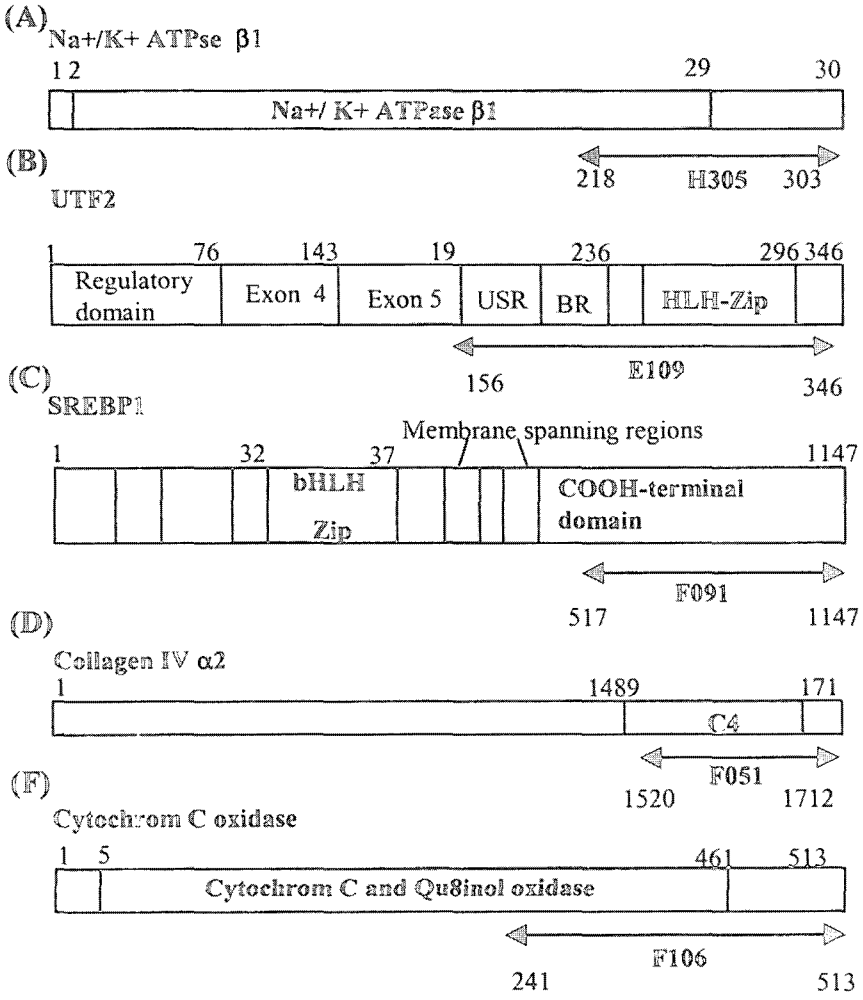

Fig. 2. Ankyrin-G binding sites. Insert sequences of the clones were analyzed by PCR.

>100 potentially interacting clones were identified. From these 40 clones with the highest levels of β-galactosidase expression were sequenced. Sequence analysis revealed that 13 clones were highly homologous to 5 known human proteins and 6 unknown human proteins (Table 2).

3. Analysis of proteins interacting with ankyrin-G

We can speculate regions of proteins interacting with ankyrin-G C-terminus by insert sequence analysis. Na^+/K^+ ATPase β1 fragment contained C-terminal domain, UTF2 fragment contained HLH-Zip domain, SERBP1 fragment contained C-terminal domain, collagen IV α2 fragment contained C4 domain and cytochrom C oxidase fragment contained COX1 (Cytochrome C oxidase polypeptide I) *(Fig. 2)*.

DISCUSSION

A yeast two-hybrid screen of 1.33×10^6 transformants from a human brain cDNA library yielded 11 independent clones that coded for genes whose products interacted with C-terminus of ankyrin-G. Of these, the Na^+/K^+ATPase β1 protein interacting with C-terminal domain of ankyrin-G is a member of Na^+/K^+ ATPase. Na^+/K^+ ATPase is integral membrane protein that plays a central role in ionic homeostasis in animals by mediating the translocation of Na^+ and K^+ ions across the plasma membrane against their electrochemical gradients. The active Na^+/K^+ATPase is a heterodimer comprised of a 100 kDa α-subunit that spans the plasma membrane and a 40~60 kDa glycoprotein β-subunit that has a short cytoplasmic N-terminal domain, a single

transmembrane domain, and a large extracellular domain. Both subunits are required for Na^+ and K^+ ion transport. The α-subunit contains the cation binding sites and the sites of ATP binding and phosphorylation, and it is therefore sometimes referred to as the catalytic subunit. The β-subunit is involved in the structural and functional maturation of the holoenzyme and transport to the plasma membrane, and it appears to influence K^+ sensitivity[2]. The smaller β subunit is a type II glycoprotein with a short cytoplasmic domain, one transmembrane segment and a large C-terminal ectodomain that comprises several *N*-glycosylation sites and three highly conserved disulfide bridges.

Sterol regulatory element binding proteins (SREBPs) are transcription factors that regulate the transcription of several key genes in the biosynthetic pathways of cholesterol and fatty acids. Three SREBPs are currently recognized. Two are produced from a single gene through the use of alternate promoters that produce transcripts with different first exons. The SREBPs are three-doman in proteins of 1,150 amino acids that are bound to membranes of the endoplasmic reticulum (ER) and nuclear envelope in a hairpin orientation. The NH2-terminal domain of 480 amino acids and the C-terminal domain of 590 amino acids project into the cytosol. They are anchored to membranes by a central domain of 80 amino acids that comprises two membrane-spanning sequences separated by a short 31 aa loop that projects into the lumen of the ER and nuclear envelope. The N-terminal domains of SREBPs are transcription factors of the basic-loop-helix-leucine zipper (bHLH-Zip) family. The extreme N-terminus contains a stretch of acidic amino acids that recruits transcriptional coactivators, including CBP. The SREBPs also enhance transcription of the LDL receptor, which mediates cholesterol uptake from plasma lipoproteins. Overexpression of the N-terminal nuclear domains of SREBPs also elevates mRNAs encoding many other enzymes required for lipid synthesis, including enzymes that generate acetyl CoA and reduced pyridine nucleotides[19]. These processing events require specific proteases and a regulatory protein designated SREBP-cleavage-activating protein (SCAP). In in vitro, ADD1/SREBP1c enhances the transcriptional activity of peroxisome proliferator-activated receptor (PPAR), increasing the proportion of cells undergoing adipose differentiation. It has been suggested that ADD1/SREBP1c increases PPAR activity either through the induction of enzymes responsible for the generation of its endogenous ligands and/or through increasing the transcription of PPAR1 itself. Furthermore, SREBP1 appears to mediate part of the transcriptional effects of insulin[17].

The upstream stimulatory factor (USF) is a family of proteins that belongs to the helix-loop-helix (HLH) category transcription factors as SREBPs. The transcriptional activity of USF was originally discovered because of the involvement of this transcription factor in expression of the adenovirus major late promoter. USF cDNA clones have since then been isolated from several species. Amino acid sequence comparisons have revealed a strong evolutionary conservation in the terminal portion of USF that contains the basic region-HLH domain responsible for dimerization and DNA-binding. C-terminal leucine zipper immediately adjacent to the HLH region is also conserved in all vertebrate USFs.

Type IV collagen is a major component of the basement membrane (BM) that separates epithelial cells from the underlying stroma. BM plays important roles both in biological functions such as cell adhesion, cell differentiation and tissue repair, and in pathological events such as cancer cell invasion and metastasis. Type IV collagen is the major structural component of the BM, and it consists of a family of six homologous a (IV) chains, designated a1-a6. Each chain is characterized by a long collagenous domain of 1400 residues of Gly-X-Y repeats, interrupted by 20 short noncollagenous sequences, and by a noncollagenous (NC1) domain of 230 residues at the C-terminus. Three a (IV) chains assemble into triple-helical molecules (protomers) that further associate to form supramolecular networks by dimerization at the C-terminus through NC1 domains and by formation of tetramers at the amino terminus. The chain composition, and thus the properties of the type IV collagen networks are influenced by two factors.

Here we report that ankyrin-G contains a specific domain capable to selectively interact with 11 proteins. This study will provide information to elucidate roles of ankyrins C-terminus and the cellular pathways involved in ankyrin-dependent localization of ER and plasma membrane proteins.

REFERENCES

1) Bagnato P, Barone V, Giacomello E, Rossi D and Sorrentino V (2003): Binding of an ankyrin-1 isoform to obscurin sug-

gests a molecular link between the sarcoplasmic reticulum and myofibrils in striated muscles. *J Cell Biol*, **160:** 245-253.

2) Beggah AT, Jaunin P and Geering K (1997): Role of glycosylation and disulfide bond formation in the bèta subunit in the folding and functional expression of Na, K-ATPase. *J Biol Chem*, **272:** 10318-10326.

3) Bennett V and Baines AJ (2001): Spectrin and ankyrin-based pathways: metazoan inventions for integrating cells into tissues. *Physiol Rev*, **81:** 1353-1392.

4) Colonna TE, Huynh L and Fambrough DM (1997): Subunit interactions in the Na, K-ATPase explored with the yeast two-hybrid system. *J Biol Chem*, **272:** 12366-12372.

5) Gagelin C, Constantin B, Deprette C, Ludosky MA, Recouvreur M, Cartaud J, Cognard C, Raymond G and Kordeli E (2002): Identification of Ank (G107), a muscle-specific ankyrin-G isoform. *J Biol Chem*, **277:** 2978-12987.

6) Hiki Y, Iyama K, Tsuruta J, Egami H, Kamio T, Suko S, Naito I, Sado Y, Ninomiya Y and Ogawa M (2002): Differential distribution of basement membrane type IV collagen α 1 (IV), α 2 (IV), α 5 (IV) and α 6 (IV) chains in colorectal epithelial tumors. *Pathol Int*, **52:** 224-233.

7) Kontrogianni-Konstantopoulos A and Bloch RJ (2003): The hydrophilic domain of small ankyrin-1 interacts with the two N-terminal immunoglobulin domains of titin. *J Biol Chem*, **278:** 3985-3991.

8) Kontrogianni-Konstantopoulos A, Jones EM, Van Rossum DB and Bloch RJ (2003): Obscurin is a ligand for small ankyrin 1 in skeletal muscle. *Mol Biol Cell*, **14:** 1138-1148.

9) Kordeli E, Lambert S and Bennett V (1995): AnkyrinG. A new ankyrin gene with neural-specific isoforms localized at the axonal initial segment and node of Ranvier. *J Biol Chem*, **270:** 2352-2359.

10) Kordeli E, Ludosky MA, Deprette C, Frappier T and Cartaud J (1998): AnkyrinG is associated with the postsynaptic membrane and the sarcoplasmic reticulum in the skeletal muscle fiber. *J Cell Sci*, **111:** 2197-2207.

11) Lin Q, Luo X and Sawadogo M (1994): Archaic structure of the gene encoding transcription factor USF. *J Biol Chem*, **269:** 23894-23903.

12) Min B-I, Ko HS and Kim C-R (2003): Ankyrin-B interacts with the C-terminal region of Hsp40. *J Biomed Lab Sci*, **9:** 105-110.

13) Mohler PJ, Gramolini AO and Bennett V (2000): Anyrins. *J Cell Sci*, **115:** 1565-1566.

14) Mohler PJ, Gramolini AO and Bennett V (2002): The ankyrin-B C-terminal domain determines activity of ankyrin-B/G chimeras in rescue of abnormal inositol 1,4,5-trisphosphateand ryanodine receptor distribution in ankyrin-B (−/−) neonatal cardiomyocytes. *J Biol Chem*, **277:** 10599-10607.

15) Peters LL, John KM, Lu FM, Eicher EM, Higgins A, Yialamas M, Turtzo LC, Otsuka AJ and Lux SE (1995): Ank3 (epithelial ankyrin), a widely distributed new member of the ankyrin gene family and the major ankyrin in kidney, is expressed in alternatively spliced forms, including forms that lack the repeat domain. *J Cell Biol*, **130:** 313-330.

16) Scotland P, Zhou D, Benveniste H and Bennett V (1998): Nervous system defects of AnkyrinB (−/−) mice suggest functional overlap between the cell adhesion molecule L1 and 440-kD AnkyrinB in premyelinated axons. *J Cell Biol*, **143:** 1305-1315.

17) Sewter C, Berger D, Considine RV, Medina G, Rochford J, Ciaraldi T, Henry R, Dohm L, Flier JS, O'Rahilly S and Vidal-Puig AJ (2002): Human obesity and type 2 diabetes are associated with alterations in SREBP1 isoform expression that are reproduced ex vivo by tumor necrosis factor-alpha. *Diabetes*, **51:** 1035-1041.

18) Wood SJ and Slater CR (1998): beta-Spectrin is colocalized with both voltage-gated sodium channels and ankyrinG at the adult rat neuromuscular junction. *J Cell Biol*, **140:** 675-684.

19) Yang YA, Morin PJ, Han WF, Chen T, Bornman DM, Gabrielson EW and Pizer ES (2003): Regulation of fatty acid synthase expression in breast cancer by sterol regulatory element binding protein-1c. *Exp Cell Res*, **282:** 132-137.

20) Zhou D, Lambert S, Malen PL, Carpenter S, Boland LM and Bennett V (1998): AnkyrinG is required for clustering of voltage-gated Na channels at axon initial segments and for normal action potential firing. *J Cell Biol*, **143:** 1295-1304.