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The erythrocyte skeletons of β-adducin deficient mice have altered levels of tropomyosin, tropomodulin and EcapZ

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Abstract The erythrocyte membrane cytoskeleton is organized as a polygonal spectrin network linked to short actin filaments that are capped by adducin at the barbed ends. We have constructed a mouse strain deficient in β-adducin having abnormal erythrocytes. We show here that the levels of several skeletal proteins from β-adducin mutant erythrocytes are altered. In fact, CapZ, the main muscle actin-capping protein of the barbed ends that in the erythrocytes is cytoplasmic, is 9-fold upregulated in mutant skeletons of erythrocytes suggesting a compensatory mechanism. We also detected upregulation of tropomodulin and downregulation of α-tropomyosin and actin. In addition, purified adducin can be re-incorporated into adducin-deficient ghosts.

1. Introduction

The erythrocyte membrane skeleton is a dynamic network of proteins associated with the plasma membrane. The main components are spectrin and actin that are organized as a polygonal spectrin network linked to short actin filaments. The spectrin–actin junctions contain a group of proteins that promote and modulate spectrin–actin interactions, form membrane associations, and regulate the actin filament length. This group includes adducin, tropomodulin (Tmod), tropomyosin (TM), protein 4.1 and dematin [1]. The barbed ends of actin filaments are capped by adducin in erythrocytes [2]. The pointed ends are capped by Tmod [3], while TM stabilizes actin filaments [3–7].

In the muscle, the control of actin filament growth at the barbed ends is achieved by the actin capping protein (CapZ) [8,9] and erythrocytes contain a cytosolic form of CapZ (EcapZ) [10]. Surprisingly, adducin is the erythrocyte actin capping protein despite having a 20–100-fold lower affinity than ECapZ ($K_{\text{cap Add}} \sim 100$ nM vs $K_{\text{cap EcapZ}} \sim 1$–5 nM) [2,10].

The adducin protein family is composed of 3 members encoded by closely related genes: α-, β- and γ-adducin [11,12]. Adducin is present in human erythrocytes as a mixture of α/β heterodimers and heterotetramers [13], whereas the γ-subunit is also found at low levels in mouse red cells [14,15] and combinations of α/β and α/γ oligomers are found in other cells [11,13]. Adducin function is modulated by phosphorylation of several kinases (Rho kinase, PKA, PKC and casein kinase II (CKII)) in different sites [16–22]. Mice bearing a targeted mutation in the β-adducin gene develop mild spherocytic hereditary elliptocytosis (SphHE) [14,15] in addition to hypertension [23].

SphHE is a phenotypical hybrid of mild hereditary elliptocytosis and hereditary spherocytosis: red cells are osmotically fragile, elliptocytes are less pronounced and somewhat rounded, but no poikilocytes or fragmented forms are present [24]. Some patients presented mutations in β-spectrin [25] and protein 4.1 deficiency [26,27], but the molecular basis is poorly understood. Therefore, further dissection of the SphHE molecular pathology was needed and the presence of red cell abnormalities in the β-adducin deficient mice similar to those found in SphHE patients highlights the importance of adducin in the maintenance of the shape and mechanical properties of the erythrocyte membrane skeleton. On the other hand, it raises the possibility that compensatory mechanisms might be activated by the absence of the β-adducin subunit, preventing the manifestation of a more severe phenotype in the KO mice.

In this report, we have analyzed the molecular changes present in the membrane CK of erythrocytes triggered by the absence of β-adducin in mice.

2. Materials and methods

2.1. Mice and antibodies

β-Adducin deficient mice having C57Bl/6 genetic background and the anti α- and β-adducin antibodies have been described previously [15]. To transfer the β-adducin gene mutation to a C3H genetic background, the mutant mice were backcrossed with C3H mice (Harlan, Italy) for six additional generations. Monoclonal antibodies anti-CapZ-α and -β (5B12.3 and 3F2.3, respectively) and anti-α-TM (CH1) were from Developmental Studies Hybridoma Bank at the University of Iowa. The CH1 monoclonal antibody was generated using adult heart TM (mainly α-TM isoform) as antigen [28]. Anti protein 4.1 was a gift from Dirk Hofer, Anatom. Institut, Wurzburg, Germany, anti-p55 (sc-13603) and anti-Tmod (sc-19206) were from Santa Cruz Biotechnology, anti-dematin (D77620) was from BD Transduction Laboratories, and anti-actin (A2066) was from Sigma.

2.2. Preparation of ghosts and skeletal proteins and Western blot analysis

Regarding the experiments described in Fig. 1, skeletal fractions were prepared as previously described in the absence of Mg^{2+} [15].

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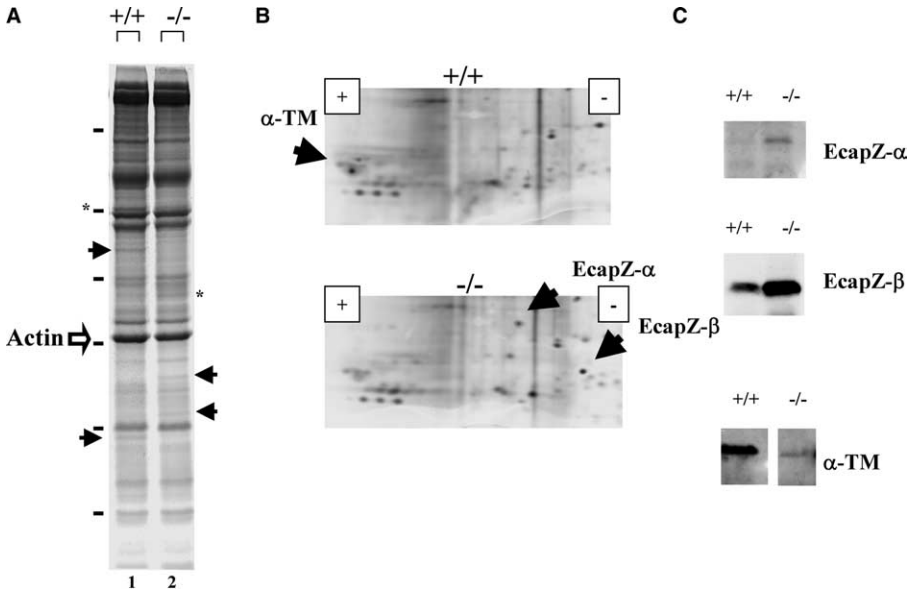

Fig. 1. Mutant erythrocytes have an increase in erythrocyte actin capping protein EcapZ-α and -β, and a decrease in α-TM levels. Panel A. Control (lane 1) and mutant (lane 2) erythrocyte membrane cytoskeleton (80 μg) were separated by SDS–PAGE (8%) and coomassie blue stained. The black arrows indicate proteins that show different levels between control and mutant preparations. The asterisks correspond to degradation products of higher molecular weight proteins. The band corresponding to actin is indicated. A 14% decrease in actin levels was observed in the extracts form mutant erythrocytes. Molecular weight markers are indicated on the left as black bars (175, 83, 62, 47.5, 32.5 and 25 kDa from top to bottom). Panel B. The same extracts from Panel A were analyzed in a silver stained 2D gels. The acidic and basic sides are indicated by the symbols "+" and "−", respectively. α-TM, EcapZ-α and EcapZ-β spots are indicated by arrows. Panel C. Western blot analyses were performed using anti α-TM, anti EcapZ-α and anti EcapZ-β antibodies. +/+ and −/− depict wild type and β-adducin deficient mice, respectively, of the same extracts utilized in Panel A. The same extracts used in Panel A were used for the Western Blot analysis and the same amount of protein was loaded for +/+ and −/− extracts.

Concerning the rest of the manuscript, erythrocyte ghosts and skeletal fractions were prepared in the presence of Mg^{2+} as described [29]. Briefly, red cells were separated from freshly drawn blood anticoagulated with acid citrate-dextrose by sedimentation at 1×*g* through 4 volumes of sedimentation buffer [150 mM NaCl, 5 mM sodium phosphate, pH 7.5, and 0.75% (w/v) Dextrane 500-Pharmacia] at 4 °C. Erythrocytes were then washed three times with 4–10 volumes of PBS (10 mM sodium phosphate pH 7.5, 150 mM NaCl) and centrifuged at 6000 rpm. Pellets were resuspended in lysis buffer (5 mM sodium phosphate, pH 8, 1 mM DTT, 40 μg/μl PMSF and protease inhibitors cocktail, Roche) containing or not 2 mM $MgCl_2$ and frozen at −80 °C. The lyzed cells were frozen/thawed twice in dry-ice and cold water and centrifuged at 40 000×*g* for 10 min in a Ti60-rotor. The cytoplasmic fractions were separated and stored at −80 °C, while the pellets were washed three times with cold lysis buffer (with or without $MgCl_2$) and centrifuged at 40 000×*g*. The final ghost pellets were resuspended in the same buffer at 1/15 of starting volume, briefly sonicated (3 × 10 s) and stored at −80 °C. For skeletal preparations, the ghost fractions were resuspended with Triton X-100-containing solution (10 mM Tris–HCl, pH 8, 100 mM NaCl, 1 mM EDTA, 2 mM DTT, protease inhibitors containing or not 2 mM $MgCl_2$, and 0.5% v/v Triton-X-100) and centrifuged for 40 min at 16 000 rpm. The pellet was resuspended again in the Triton X-100-containing solution and centrifuged for 50 min at 16000 rpm. The final pellet (skeletons) was resuspended in the same solution without Triton X-100. The protein concentration of cytoplasmic and ghost fractions was measured by triplicate by using the Bradford method (Bio-Rad) and analyzed by SDS–PAGE followed by Coomassie blue staining.

Analysis of the ghosts proteins by Western blot was performed as previously described [15]. The gel images were acquired with the VersaDoc Imaging System (Bio-Rad) and bands (or spots) quantified with the help of the Quantity One or PDQUEST software (Bio-Rad).

2.3. 2D gels and mass spectrometry analysis

The protein extracts were precipitated in acetone:tributylphosphate:methanol (1:12:1) as described [30]. The pellets were resuspended in 2D buffer (7 M urea, 2 M thiourea, 2% CHAPS, 4 mM tributyiphosphine and protease inhibitor cocktail). The first dimension was performed by loading 150–300 μg of protein extract into IPG strips with different pH ranges (3–10, 3–6, 4–7 and 5–8; strips were 17 cm long). The Protean IEF (Bio-Rad) cell was used following the protocol described by the manufacturer. Gradient (8–15%) or non-gradient (12%) SDS–PAGE were used for the second dimension.

The selected bands and spots (from the SDS–PAGE and 2D gels, respectively) were extracted from the acrylamide after digestion with trypsin (Promega). The digestion products were separated by micro-high pressure liquid chromatography and analyzed by electrospray ionization mass spectrometry (Finnigan LCQ DECA, Thermo-Finningan Corp., San Jose, CA). The obtained data were analyzed with MASCOT (Matrix Science) and SWISSPROT software.

2.4. Preparation of purified adducin and complementation assay of mutant ghosts

HPLC-purified human erythrocyte adducin was prepared as previously described [13,29,31]. The binding assay of mutant ghosts with HPLC-purified human adducin was performed as described [10] with minimal modifications. Briefly, ghosts from β-adducin deficient erythrocytes (20 μg) were incubated with increasing amounts of HPLC-purified human adducin (0.7, 1.4 and 2.8 μg) for 30 min at 0 °C. Control reactions were performed by omitting ghosts and adding 20 μg of BSA or by omitting purified adducin. The soluble fraction was separated from the membrane-associated skeletons by centrifugation through a sucrose cushion. The pellets were electrophoresed on 10% SDS–PAGE and blotted onto a PVDF membrane.

3. Results

3.1. Erythrocyte skeletons of mutant mice have increased levels of EcapZ and decreased levels of α-TM

Erythrocyte cytoskeletal protein preparations of control and β-adducin deficient mice showed evident differences in at least four bands of apparent MW of 65 and 30–32 kDa observed only in control mice and 38 and 34 kDa present only in mutant mice (see Muro et al. [15] and Fig. 1A). Two-dimensional gels

followed by silver staining evidenced two spots of 38 and 34 kDa (having an approximated pI of 5.4 and 5.7, respectively) that were visible only in the preparation from mutant mice (Fig. 1B). Mass spectrometry analysis indicated that they were the EcapZ-α and EcapZ-β subunits, respectively. Another spot of approximate pI 4.6 having a relative MW of 30 kDa was visible only in the wild type 2D gel. Mass spectrometry analysis identified this spot as α-TM (Fig. 1A and B).

Western blot analysis with antibodies against both subunits of EcapZ showed a ~9-fold increase of both proteins in the skeletal fractions of mutant animals (Fig. 1C) confirming our observations in the SDS–PAGE gels, 2D gels and mass spectrometry, while we observed a 40–50% reduction in the mutant mice using an antibody against α-TM (Fig. 1C).

The 65 kDa band is probably a degradation product of β-adducin as diverse β-adducin peptides were detected by mass spectrometry. The other bands showing differences between control and mutant preparations (Fig. 1A, 55 and 83 kDa of MW, indicated by asterisks) are probably degradation products of high molecular weight proteins as mass spectrometry analysis revealed mostly the presence of α- and β-spectrin, or protein 4.1 peptides (data not shown). We were unable to identify in the 2D gels the 55, 65 and 83 kDa bands marked with the arrow and asterisks in Fig. 1A.

3.2. Altered levels in other major components of the RBC membrane cytoskeleton from β-adducin deficient mice

To determine variations in other erythrocyte skeletal proteins not evidenced by the SDS–PAGE or the 2D analysis, we performed a Western blot analysis using a set of antibodies directed against the main actin-associated proteins.

The protein extracts were prepared using a protocol that contained 2 mM Mg^{2+} that was different to the one used in Fig. 1 (without Mg^{2+}). This change was aimed to obtain a higher stability in the actin filaments (see Section 2 and [10]). In fact, the levels of some of the actin-associated proteins are reduced in ghosts prepared in the absence of Mg^{2+} in comparison to Mg^{2+}-ghosts indicating a skeleton-stabilizing role of Mg^{2+} during protein preparation [10].

In addition to the variations in EcapZ and α-TM (not shown), we observed changes in Tmod, dematin and actin levels. A mean decrease of 74% in the levels of α-adducin, a 65% decrease in α-TM levels and 9-fold increase in EcapZ-β levels in the skeleton mutant extracts preparations were observed (Fig. 2 and Table 1). We confirmed the previously reported trend of ~15% decrease in the actin levels in the cytoskeletal preparations of mutant erythrocytes [15] (Fig. 2 and Table 1). We were not able to detect in the Western blot analysis the appearance of smaller extra bands in the mutant extracts indicating that the decrease in α-TM and actin could be the consequence of increased proteolytic degradation. To verify whether the observed differences were dependent on the genetic background of the mice, we performed protein preparations from erythrocytes of control and β-adducin deficient mice having a C3H genetic background. We observed similar results to those presented in Fig. 2 and Table 1 in preparations from mice with a C3H genetic background (not shown).

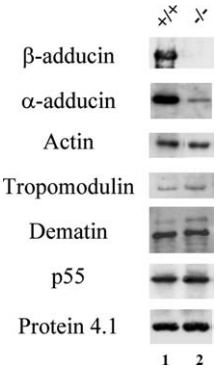

Fig. 2. Differences in the levels of other major skeletal proteins are observed in erythrocyte skeletal preparations of mutant mice. Western blot analysis of erythrocyte skeletal protein preparations of control ("+/+", lane 1) and mutant mice ("–/–", lane 2) using a set of antibodies directed against cytoskeletal proteins (indicated on the left).

Western blot analysis using an anti-Tmod antibody showed a 65% increase in Tmod levels in the mutant preparation (Fig. 2 and Table 1). Dematin showed a minor decrease in the mutant preparations of the main lower band intensity and a 79% increase in the upper band intensity (Fig. 2 and Table 1).

The levels of other cytoskeletal protein (protein 4.1 and p55) showed no significant differences between wild type and KO mice by Western blot analysis (Fig. 2 and Table 1).

No variations were observed in the cytoplasmic levels of α- and β-EcapZ (data not shown). In addition, we were neither able to detect Tmod and α-TM in cytoplasmic preparations from control animals, as reported previously by another group [10], nor from mutant preparations.

3.3. Purified adducin is incorporated into adducin-deficient ghosts

To determine whether purified adducin (as α/β heterodimer) was able to be incorporated into cytoskeletons from β-adducin-deficient mice to eventually replace EcapZ we performed a binding assay utilizing ghosts from KO mice and purified human erythrocyte adducin.

Fig. 3 shows the Western blot analysis of both β-adducin and EcapZ-β after incubation of a fixed amount of mutant ghosts with increasing amounts of purified adducin. We observed that β-adducin was incorporated into adducin-deficient ghosts in a dose-dependent manner (Fig. 3, lanes 2–4). The α-adducin subunit was incorporated in a similar manner to the β-

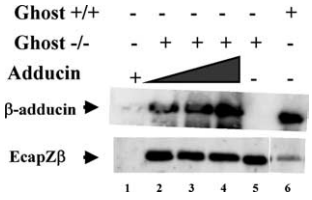

Fig. 3. Purified adducin can be incorporated into adducin-deficient ghosts in vitro. Ghosts from mutant mice were incubated with increasing amounts of purified adducin (0.7, 1.4 and 2.8 μg in lanes 2, 3 and 4, respectively) and the membrane-skeleton (pellet) was separated from the soluble fraction through a sucrose cushion. β-Adducin and EcapZ-β protein levels were detected by Western blot analysis. Lane 1 contains 2.8 μg of adducin plus 20 μg of BSA. Lanes 5 and 6 contain 20 μg of mutant and control ghosts protein preparations, respectively, that underwent the same experimental procedure as the other lanes. Quantification of the bands showed an increase of β-adducin and a decrease of EcapZ-β (relative values of β-adducin: 8, 91, 111, 131, 0 and 100 for lanes 1–6, respectively; relative values of EcapZ-β: 0, 1280, 1069, 985, 1210 and 100 for lanes 1 to 6, respectively; ratio β-adducin/EcapZ-β: 7.1, 10.5 and 13.5 for lanes 2–4, respectively).

Table 1
Altered levels of cytoskeletal proteins in RBC from mutant mice

Band #	Protein	Confirmed by mass spectrometry/WB	Levels in +/+ mice	Levels in −/− mice	*P* value
1	β-Adducin	No/Yes	100[a]	ND	NA
2	α-Adducin	No/Yes	100 ± 6	26 ± 6	⩽0.01
3	EcapZ-α	Yes/Yes	ND	100[a]	NA
4	EcapZ-β	Yes/Yes	100 ± 20	880 ± 153	⩽0.01
5	α-TM	Yes/Yes	100 ± 12	35 ± 9	⩽0.01
6	Actin	No/Yes	100 ± 2	83 ± 8	⩽0.01
7	Tropomodulin	No/Yes	100 ± 13	165 ± 5	⩽0.05
8a	Dematin 52 kDa	No/Yes	100 ± 8	179 ± 20	<0.05
8b	Dematin 48 kDa	No/Yes	100 ± 3	94 ± 4	NS
9	P55	No/Yes	100 ± 2	105 ± 7	NS
10	Protein 4.1	No/Yes	100 ± 9	91 ± 11	NS

The levels of the various cytoskeletal proteins shown in Fig. 2 were quantified as described in Section 2. The numbers indicate relative amounts of each protein in cytoskeleton extracts relative to those found in those control extract (±S.D.). "WB" stands for western blot. ND, not detected; NA, not applicable; NS, non significant.
[a] An arbitrary value of 100 was given as no signal was detected in mutant and control mice, in the case of β-adducin and EcapZ-α, respectively. *P*-value was calculated using the Student *t* test.

subunit (not shown). EcapZ-β levels decreased after the incubation of mutant ghosts with increasing amounts of adducin (Fig. 3, lanes 2–4). The control lane confirmed that the purified adducin did not auto-precipitate (Fig. 3, lane 1) as it included the maximum amount of adducin used in lane 4 (2.8 μg) plus an amount of BSA similar to that of ghosts used in the other lanes (20 μg). Quantification of the bands indicated that the ratio between adducin and EcapZ increases with the addition of higher amounts of adducin supporting the idea that the incorporation of EcapZ is a compensatory mechanism triggered by the absence of β-adducin.

4. Discussion

The β-adducin deficient mice showed a decrease of the α-adducin levels and an up regulation of the γ-subunit in RBC skeletons [14,15]. These adaptations seem not to be sufficient to attain normal erythrocyte function, since β-adducin KO mice have increased erythrocyte fragility and suffer from mild compensated hemolytic anemia [14,15]. We showed now that the levels of some of the actin-associated proteins in mutant mice erythrocytes are different from those found in control animals, suggesting that both the actin filament-complex and actin polymerization activity might be altered.

As previously mentioned, the main capping protein of the actin-filament barbed ends in the muscle is CapZ, while in erythrocyte membrane skeletons this function is performed by adducin [2] and EcapZ remains in the cytoplasm [10]. The affinity of adducin and EcapZ for the actin filaments has been determined in vitro by Dr. Velia Fowler's group using purified proteins [2,3,10]. The fact that adducin is the erythrocyte actin capping protein notwithstanding its lower K_{cap} respect to that of EcapZ strongly suggests that the in vivo affinity is not all and may be other proteins might cooperate in the stabilization of the binding of adducin to the actin-filaments barbed ends [10] as already observed in the case of spectrin–actin complexes [32,33]. In spite of this, we did not observe a complete displacement of EcapZ in the mutant ghosts by exogenously added adducin but increasing amounts of adducin produced a reproducible minor decrease in the EcapZ levels in the ghosts fraction (23% decrease in lane 4 of Fig. 3). Obviously, this is not an in vivo experiment and is far from what really occurs during erythropoiesis, when the cytoskeleton takes its final structure and proteins are synthesized simultaneously and have to compete for a place in the skeleton structure.

However, the increase of the EcapZ levels in the cytoskeleton of mutant erythrocytes did not fully compensate for the absence of β-adducin: variations in α-TM, Tmod and actin, and changes in α- and γ-adducin were observed. Consequently, mice have a change in the shape and mechanical properties of red cells and suffer from mild hemolytic anemia [14,15].

The binding of Tmod to the actin filaments pointed ends is stabilized by TM [34]. The increase in Tmod levels together with the decrease in α-TM and actin suggest that the in vivo interaction among actin, Tmod and TM might be also affected in mutant erythrocytes. In addition, the important decrease in the levels of α-TM in mutant preparations might suggest a new role for adducin in stabilizing α-TM within the actin filament-complex of erythrocytes. In the case of the targeted inactivation in mice of other erythrocyte protein, the anion transporter Band 3 (AE1), it was shown that the close interaction of AE1 with glycophorin A (GPA) during their biosynthesis as the AE1-deficient erythrocytes were devoid of GPA [35]. One possible hypothesis could be that, similarly to that proposed for Band 3, adducin (or other adducin partner disturbed in the erythrocyte skeletons from mutant mice) might play a "chaperon-like" role in the formation of actin filaments, affecting the levels of α-TM and Tmod, a task that seems not to be correctly performed by EcapZ. On the other hand, the absence of variations in p55 and protein 4.1 might suggest that direct or indirect interactions of adducin with p55 and protein 4.1 are limited.

We have shown here that erythrocytes skeletons from β-adducin deficient mice contain a 9–10-fold upregulation of EcapZ, a 65% decrease in α-TM and an increase in Tmod. This is neither a consequence of the protocol used to prepare the protein extract nor due to the genetic background of the mice. The described molecular defects might generate a reduction in the number, length and/or stability of the actin filaments, probably producing altered junctional complexes that could be the cause of the presence of SphHE in mutant mice [15].

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References

[1] Bennett, V. and Gilligan, D.M. (1993) Annu. Rev. Cell Biol. 9, 27–66.
[2] Kuhlman, P.A., Hughes, C.A., Bennett, V. and Fowler, V.M. (1996) J. Biol. Chem. 271, 7986–7991.
[3] Fowler, V.M. (1996) Curr. Opin. Cell Biol. 8, 86–96.
[4] Fowler, V.M. and Bennett, V. (1984) J. Biol. Chem. 259, 5978–5989.
[5] Shen, B.W., Josephs, R. and Steck, T.L. (1986) J. Cell Biol. 102, 997–1006.
[6] Cooper, J.A. (2002) Curr. Biol. 12, R523–R525.
[7] Ono, S. and Ono, K. (2002) J. Cell Biol. 156, 1065–1076.
[8] Schafer, D.A. and Cooper, J.A. (1995) Annu. Rev. Cell Dev. Biol. 11, 497–518.
[9] Casella, J.F., Craig, S.W., Maack, D.J. and Brown, A.E. (1987) J. Cell Biol. 105, 371–379.
[10] Kuhlman, P.A. and Fowler, V.M. (1997) Biochemistry 36, 13461–13472.
[11] Dong, L., Chapline, C., Mousseau, B., Fowler, L., Ramsay, K., Stevens, J.L. and Jaken, S. (1995) J. Biol. Chem. 270, 25534–25540.
[12] Joshi, R., Gilligan, D.M., Otto, E., McLaughlin, T. and Bennett, V. (1991) J. Cell Biol. 115, 665–675.
[13] Gardner, K. and Bennett, V. (1986) J. Biol. Chem. 261, 1339–1348.
[14] Gilligan, D.M., Lozovatsky, L., Gwynn, B., Brugnara, C., Mohandas, N. and Peters, L.L. (1999) Proc. Natl. Acad. Sci. USA 96, 10717–10722.
[15] Muro, A.F., Marro, M.L., Gajovic, S., Porro, F., Luzzatto, L. and Baralle, F.E. (2000) Blood 95, 3978–3985.
[16] Shima, T., Okumura, N., Takao, T., Satomi, Y., Yagi, T., Okada, M. and Nagai, K. (2001) J. Biol. Chem. 276, 42233–42240.
[17] Matsuoka, Y., Li, X. and Bennett, V. (2000) Cell. Mol. Life Sci. 57, 884–895.
[18] Fukata, Y., Oshiro, N., Kinoshita, N., Kawano, Y., Matsuoka, Y., Bennett, V., Matsuura, Y. and Kaibuchi, K. (1999) J. Cell Biol. 145, 347–361.
[19] Matsuoka, Y., Li, X. and Bennett, V. (1998) J. Cell Biol. 142, 485–497.
[20] Matsuoka, Y., Hughes, C.A. and Bennett, V. (1996) J. Biol. Chem. 271, 25157–25166.
[21] Bianchi, G. et al. (1994) Proc. Natl. Acad. Sci. USA 91, 3999–4003.
[22] Waseem, A. and Palfrey, H.C. (1988) Eur. J. Biochem. 178, 563–573.
[23] Marro, M.L. et al. (2000) Hypertension 36, 449–453.
[24] Benz, E.J. (1994) in: The Molecular Basis of Blood Diseases (Stamatoyannopoulos, G., Nienhuis, A.W., Majerus, P. and Varmus, H., Eds.), pp. 257–292, WB Saunders Company, Philadelphia, PA.
[25] Jarolim, P., Wichterle, H., Hanspal, M., Murray, J., Rubin, H.L. and Palek, J. (1995) Br. J. Haematol. 91, 502–510.
[26] Tchernia, G., Mohandas, N. and Shohet, S.B. (1981) J. Clin. Invest. 68, 454–460.
[27] Feo, C.J., Fischer, S., Piau, J.P., Grange, M.J. and Tchernia, G. (1980) Nouv. Rev. Fr. Hematol. 22, 315–325.
[28] Lin, J.J., Chou, C.S. and Lin, J.L. (1985) Hybridoma 4, 223–242.
[29] Bennett, V. (1983) Methods Enzymol. 96, 313–324.
[30] Mastro, R. and Hall, M. (1999) Anal. Biochem. 273, 313–315.
[31] Joshi, R. and Bennett, V. (1990) J. Biol. Chem. 265, 13130–13136.
[32] Gardner, K. and Bennett, V. (1987) Nature 328, 359–362.
[33] Mische, S.M., Mooseker, M.S. and Morrow, J.S. (1987) J. Cell Biol. 105, 2837–2845.
[34] Weber, A., Pennise, C.R., Babcock, G.G. and Fowler, V.M. (1994) J. Cell Biol. 127, 1627–1635.
[35] Hassoun, H., Hanada, T., Lutchman, M., Sahr, K.E., Palek, J., Hanspal, M. and Chishti, A.H. (1998) Blood 91, 2146–2151.