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Biochemistry

Ankyrin is fatty acid acylated in erythrocytes

(erythroid development/membrane-skeleton/plasma membrane)

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ABSTRACT **Ankyrin is a peripheral membrane protein that mediates the attachment of the erythrocyte membrane skeleton to the plasma membrane. We show that [^{3}H]palmitic acid is incorporated into ankyrin *in vivo*. The majority of the ^{3}H-labeled fatty acid is covalently bound to the polypeptide, as it cannot be removed by strong detergents or by chloroform/methanol extraction but is labile to alkaline hydrolysis. The binding of fatty acid occurs predominantly after the assembly of ankyrin onto the membrane skeleton, since it continues when protein synthesis is inhibited with emetine. Fatty acid acylation of ankyrin is constitutive in erythroid cells throughout chicken embryo development. It also occurs in mature avian and mammalian erythrocytes suggesting that the fatty acid bound to ankyrin turns over more rapidly than the polypeptide. Fatty acid acylation of assembled ankyrin may modulate the interaction of ankyrin with the plasma membrane. It may also provide a mechanism by which the membrane skeleton influences the organization of the lipid bilayer.**

The cytoplasmic surface of the erythrocyte plasma membrane is lined by a protein network that is thought to influence the shape, elasticity, and stability of the erythrocyte. This network, known as the membrane skeleton, is based on the interaction of two principal components, spectrin tetramers that bind to and crosslink actin oligomers. (The membrane skeleton or cytoskeleton is the insoluble residue left after exposure of cells to a buffer containing nonionic detergent.) The interaction of spectrin and actin is strongly enhanced by a protein known as protein 4.1. The spectrin–actin network is attached to the plasma membrane by the protein ankyrin that binds simultaneously to spectrin and to the cytoplasmic domain of the transmembrane anion transporter. Spectrin is additionally linked to the membrane through the binding of protein 4.1 to glycophorin (for reviews see refs. 1–3). These linkages between the plasma membrane and the membrane skeleton are based on the relatively well-characterized interactions among their constituent proteins. Evidence exists that cytoskeletal proteins may also interact directly with membrane lipids. Protein 4.1 binds to proteolyzed vesicles and phosphatidylserine-containing liposomes, and its association with glycophorin occurs only when the latter has phosphatidylinositol 4,5-bisphosphate associated with it (3). Protein 4.1 also appears to contain tightly bound fatty acid (4). In addition several other unidentified membrane proteins of the erythrocyte are fatty acid acylated (5), a property that may facilitate their interaction with membrane lipids (for reviews on fatty acid acylation see refs. 6 and 7).

We report here that ankyrin is the major [^{3}H]palmitic acid-labeled protein of the erythrocyte membrane skeleton. The acylation with fatty acid occurs predominantly at the plasma membrane after assembly of ankyrin onto the membrane skeleton. Palmitic acid is incorporated into ankyrin at all stages of avian erythroid development examined and is also attached to ankyrin in adult avian and mammalian erythrocytes. This suggests that the ankyrin bound fatty acid turns over more rapidly than the polypeptide. Reversible attachment of fatty acid to ankyrin may be a means for a dynamic interaction of this protein with the plasma membrane.

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MATERIALS AND METHODS

Preparation and Labeling of Cells. Chicken embryo erythroid cells were isolated from fertilized eggs at different stages of development. Blood was collected from the eggs in minimal essential medium (MEM, GIBCO) after puncturing a main yolk vein with a needle. Adult erythrocytes were isolated from blood drawn from a wing vein (chickens) or ear vein (rabbits) into MEM supplemented with 0.1% heparin (Sigma). The diluted blood was filtered through glass wool, and the cells were collected by centrifugation in a clinical centrifuge. They were washed five times in MEM and any buffy coat was removed. Cells were labeled separately with fatty acids or amino acids at 38°C for 4 hr. About 30 μl of packed cells were labeled in 2 ml of MEM supplemented with 5 mM sodium pyruvate, nonessential amino acids (GIBCO), 0.1 mM of all of the L-amino acids, and 3–10 mCi of [9,10-^{3}H]palmitic acid (specific activity 30 Ci/mmol; 1 Ci = 37 GBq, New England Nuclear). For labeling with amino acids cells were incubated in Earles balanced salt solution (GIBCO) supplemented with 5 mM sodium pyruvate, 1% of MEM, 1% of nonessential amino acids and 25–150 μCi of an L-^{14}C-labeled amino acid mixture (specific activity 55 mCi/mol atom carbon; (New England Nuclear). After labeling, cells were washed once with cold MEM and fractionated. In some experiments cells were labeled in the presence of a protein synthesis inhibitor. These cells were preincubated in MEM supplemented with 50 μM emetine for 15 min at 38°C. They were then pelleted at room temperature, labeled in either fatty acid or amino acid labeling medium containing 50 μM emetine, mixed during the washing step after labeling, and fractionated together. Under the conditions used incorporation of radioactive amino acids was inhibited to the same extent (by more than 90%) in both labeling media (data not shown).

Cell Fractionation. Chicken erythrocytes were resuspended in 20 vol of 10 mM Hepes, pH 7.3, 5 mM $MgCl_2$, 5 mM EGTA, 137 mM NaCl, 1% Triton X-100, 1 mM aprotinin (Boehringer Mannheim), 0.1 mM leupeptin (Boehringer Mannheim), 1 mM phenylmethylsulfonyl fluoride (PMSF), incubated for 3 min on ice, and resulting structures were pelleted in a clinical centrifuge for 3 min in the cold. They were washed once in 20 vol of the same buffer, and the supernatants were combined. The pellets were extracted with 10 vol of 10 M urea in one-half vol of the above buffer (containing 1 mM aprotinin, 1 mM PMSF, and 0.1 M leupeptin) for 15 min at 4°C. The suspension was centrifuged for 5 min in an

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Eppendorf centrifuge in the cold, and the supernatant containing the cytoskeletal proteins was removed. Under these conditions extraction of cytoskeletal protein is quantitative. Rabbit erythrocytes were hypotonically lysed in 20 vol of 5 mM sodium phosphate, pH 7.4, 1 mM EDTA, 1 mM aprotinin, 1 mM PMSF, 0.1 mM leupeptin for 3 min on ice, and the resulting ghosts were pelleted in a clinical centrifuge for 5 min in the cold. They were washed three times with the same buffer. The ghosts were dissolved in 10 vol of $NaDodSO_4$ sample buffer (125 mM Tris·HCl, pH 6.8, 1% $NaDodSO_4$, 25 mM dithiothreitol).

Immunoprecipitation and $NaDodSO_4$ Polyacrylamide Gel Electrophoresis. For immunoprecipitation, extracts were diluted with 10–20 vol of 100 mM Tris·HCl, pH 9.0, 200 mM NaCl, 5 mM EDTA, 5 mM EGTA, 5 mM dithiothreitol, 1% Triton X-100, 0.1% $NaDodSO_4$, 1 mM aprotinin, 0.1 mM leupeptin, 1 mM PMSF. Chicken erythrocyte ankyrin (goblin) antiserum (8) was added (10 μl per 1 μl of packed cells extracted), and the immunoprecipitation was performed as described (9) using Protein A-Agarose (Boehringer Mannheim). Precipitated proteins were eluted by heating in $NaDodSO_4$ sample buffer and loaded onto 12.5% $NaDodSO_4$/polyacrylamide gels (10) after centrifugation. For direct analysis, extracts were diluted in $NaDodSO_4$ sample buffer, the $NaDodSO_4$ concentration was adjusted to 1 or 2%, and the samples were heated, centrifuged, and loaded onto the gel. After electrophoresis, gels were either stained overnight with Coomassie brilliant blue and destained, or directly immersed into dimethylsulfoxide. They were then prepared for fluorography as described by Bonner and Laskey (11).

Chloroform/Methanol Extraction and Alkaline Hydrolysis of Bound Fatty Acids. For all treatments aliquots of the extracts were lyophilized. One aliquot was extracted three times with chloroform/methanol (2:1, vol/vol) and one time each with chloroform/methanol (1:2, vol/vol), chloroform/methanol/H_2O (1:1:0.3, vol/vol), acetone (6), and then air-dried. The other aliquots were dissolved in 0.2 M NaOH in absolute methanol, 0.2 M KOH in absolute methanol, or 1 M hydroxylamine in H_2O, pH 9.0 and incubated for 16 hr at 4°C. They were then neutralized, frozen in liquid nitrogen and lyophilized. All samples were redissolved in $NaDodSO_4$ sample buffer for $NaDodSO_4$ gel electrophoresis. These treatments were also performed after separation of the polypeptides by $NaDodSO_4$ gel electrophoresis; gels were immersed directly in (*i*) chloroform/methanol (2:1, vol/vol), (*ii*) 0.2 M KOH in absolute methanol, (*iii*) 0.2 M NaOH in absolute methanol, or (*iv*) 1 M hydroxylamine in H_2O, pH 9.0. The solutions were changed after 60 min. Treatments were for 12 hr. Gels *i–iii* were washed for 30 min with H_2O and prepared for fluorography (see above), while the washing step was omitted for gel *iv*.

RESULTS

Ankyrin is Fatty Acid Acylated. To examine the possibility that proteins of the erythroid membrane skeleton may contain bound long-chain fatty acids, we incubated erythroid cells from 14-day-old chicken embryos with [^{3}H]palmitic acid. Preliminary experiments were carried out to optimize the uptake of labeled fatty acid into the cells while simultaneously minimizing its conversion to amino acids. Under these conditions [^{3}H]palmitic acid is incorporated into a number of proteins. A comparison of the urea-extracted cytoskeletal polypeptides labeled with [^{3}H]palmitic acid with those labeled with a mixture of ^{14}C-labeled amino acids reveals a striking difference in the patterns of labeled polypeptides, indicating the specificity of the fatty acid labeling (Fig. 1*A*). Most labeled polypeptides contain a low level of [^{3}H]palmitic acid (see Fig. 4 for a better visualization of these polypeptides). However, a comparably high incorporation of [^{3}H]palmitic acid is apparent in a protein of the cytoskeletal fraction that comigrates with ankyrin (Fig. 1*A*). To investigate whether this [^{3}H]palmitic acid-labeled polypeptide is indeed ankyrin, we immunoprecipitated ankyrin from solubilized cytoskeletons with ankyrin antibodies. Fig. 1*B* shows that the [^{3}H]palmitic acid-labeled polypeptide is immunoprecipitated with these antibodies. The ratio of [^{3}H]palmitic acid to ^{14}C-labeled amino acid-labeled ankyrin is the same in the immunoprecipitates and the total cytoskeletal fractions (data not shown). This further substantiates that ankyrin contributes to all or most of the radioactivity of the ^{3}H-labeled band.

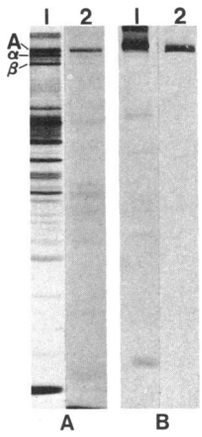

FIG. 1. Analysis of amino acid- and palmitic acid-labeled avian erythroid polypeptides. Erythroid cells from 14-day-old chicken embryos were labeled with either ^{14}C-labeled amino acids (lane 1) or [^{3}H]palmitic acid (lane 2) and urea extracts containing the cytoskeletal proteins were prepared. Polypeptides of total extracts (*A*) or polypeptides immunoprecipitated from extracts with ankyrin antiserum (*B*) were separated on 12.5% $NaDodSO_4$ polyacrylamide gels and visualized by fluorography. Lanes 2 were exposed 2.5 times longer than lanes 1. A, ankyrin; α, α-spectrin; β, β-spectrin.

The fact that the ^{3}H-labeled fatty acid remained associated with ankyrin after Triton X-100 extraction and $NaDodSO_4$ denaturation and gel electrophoresis was indicative of a tight association between the two. To examine this further we extracted gels containing [^{3}H]palmitic acid- and ^{14}C-labeled amino acid-labeled samples of cytoskeletal fractions with $CHCl_3/CH_3OH$ to remove noncovalently bound fatty acids. As Fig. 2*B* shows, the majority of the ankyrin-bound [^{3}H]palmitic acid is resistant to extraction by $CHCl_3/CH_3OH$ suggesting a covalent attachment to ankyrin. To exclude a conversion of the palmitic acid into amino acids and their subsequent incorporation into the polypeptide backbone we attempted to release the bound fatty acid with hydroxylamine. This treatment hydrolyzes esterified fatty acids. Fig. 2*C* (lane 2) shows that most of the ^{3}H can be released from ankyrin by this treatment. The decrease in label is not due to the elution of proteins from the gel during the treatment as indicated by the retention in the gel of the ^{14}C-labeled amino acid-labeled polypeptides (Fig. 2*C*, lane 1). Similar results were obtained when the samples were treated with NH_2OH or $CHCl_3/CH_3OH$ prior to electrophoresis (data not shown). Methanolic KOH or methanolic NaOH treatment of gels or of extracts prior to electrophoresis reduced the amount of [^{3}H]palmitic acid bound to ankyrin to a lesser degree than hydroxylamine treatment (data not shown). Together these observations strongly argue that the ^{3}H associated with ankyrin is not due to conversion of the [^{3}H]palmitic acid to ^{3}H-labeled amino acids and their subsequent incorporation into the polypeptide backbone. They indicate that ankyrin contains long-chain fatty acids, the majority of which are bound covalently to the protein.

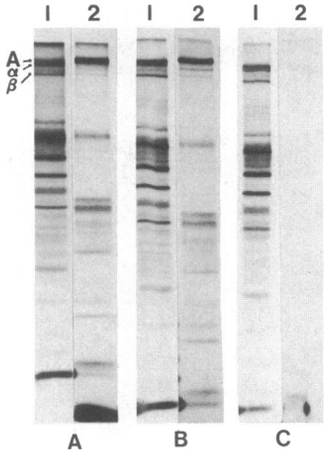

FIG. 2. Extraction properties of the palmitic acid bound to ankyrin. Urea extracts containing the cytoskeletal proteins were prepared and separated on polyacrylamide gels as described in the legend to Fig. 1. After completion of electrophoresis gels were treated with (*A*) untreated control, (*B*) chloroform/methanol, (*C*) hydroxylamine. Lanes 1, ^{14}C-labeled amino acid-labeled polypeptides; lanes 2, [^{3}H]palmitic acid-labeled polypeptides. Lanes 1 and 2 were exposed for different times. A, ankyrin; α, α-spectrin; β, β-spectrin.

Synthesis and Acylation of Ankyrin Are Not Coupled. To determine whether the acylation of ankyrin required continuous protein synthesis, one aliquot of chicken embryo erythroid cells was incubated with ^{14}C-labeled amino acids and another with [^{3}H]palmitic acid, in the presence or in the absence of the protein synthesis inhibitor emetine. Emetine was shown to be equally effective in both types of labeling media. The ^{3}H- and ^{14}C-labeled cells were mixed after labeling, lysed, and cytoskeletal fractions were prepared. Fig. 3*A* shows the electropherograms of samples labeled without emetine while Fig. 3*B* shows the corresponding samples labeled in the presence of emetine. The patterns of labeled polypeptides in the untreated samples largely reflect the incorporation of amino acids (compare Fig. 1*A*, lane 1 with Fig. 3*A*, lane 2). As can be seen in Fig. 3*B*, emetine drastically reduces labeling of most proteins. The resulting pattern of labeled polypeptides is indistinguishable from the polypeptide pattern obtained after labeling with [^{3}H]palmitic acid alone (compare Fig. 1*A*, lane 2 with Fig. 3*B*, lane 2). The most prominent polypeptide labeled is ankyrin, and the intensity of its labeling is comparable to its labeling in untreated cells. This result further argues that ankyrin is labeled with [^{3}H]palmitic acid and not label that has been converted to amino acids. It also shows that fatty acid acylation of ankyrin can occur in cells that do not synthesize and assemble this protein. We conclude that acylation of ankyrin is not necessarily coupled to its synthesis or assembly. Rather it can occur on assembled ankyrin. The identity of the other polypeptides that label with [^{3}H]palmitic acid (compare Fig. 1*A*, lane 2 and Fig. 3*B*, lane 2) has not been established.

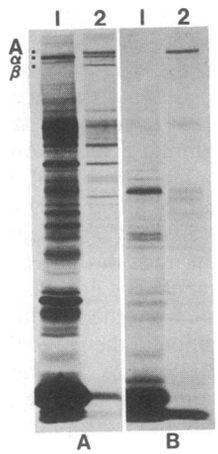

FIG. 3. Palmitic acid- and amino acid-labeled polypeptides in emetine-treated erythroid cells. Erythroid cells from 14-day-old embryos were labeled separately with [^{3}H]palmitic acid and ^{14}C-labeled amino acids in the presence or absence of emetine. Detergent and urea extracts were prepared after mixing amino acid- and palmitic acid-labeled cells. A fluorograph of a $NaDodSO_4$/polyacrylamide gel is shown containing the detergent extracts (lanes 1) and the urea (cytoskeleton) extracts (lanes 2) from untreated cells (*A*) or emetine-treated cells (*B*). *A* and *B* were exposed for different times. A, ankyrin; α, α-spectrin; β, β-spectrin.

Acylation of Ankyrin at Different Stages of Embryo Development and in Adult Chicken and Rabbit Erythrocytes. To determine whether the fatty acid acylation of ankyrin is developmentally regulated we examined the incorporation of [^{3}H]palmitic acid into ankyrin in erythroid cells from different stages of chicken embryo development. Erythroid cells from 5-day-old embryos are mostly mitotic and belong to the primitive series. Cells from 10-day-old embryos are mostly postmitotic and are a mixture of definitive erythroblasts and definitive erythrocytes, while those from 14-day-old embryos are mostly definitive erythrocytes (12). Incorporation of [^{3}H]palmitic acid into ankyrin was detectable in cytoskeletal fractions from 5-, 10- and 14-day-old cells suggesting that fatty acid acylation of this protein occurs constitutively throughout erythroid differentiation (Fig. 4, lanes 1–6). It also appears to be independent of the erythroid cell lineage. Incorporation of [^{3}H]palmitic acid into ankyrin is evident also in adult chicken (Fig. 4, lanes 7 and 8) and rabbit erythrocytes (Fig. 5) under conditions where negligible incorporation of ^{14}C-labeled amino acids into proteins is detected (Fig. 4, lane 7; Fig. 5, lane 1).

DISCUSSION

The results presented here indicate that the peripheral membrane skeleton protein ankyrin is one of the most prominent fatty acid acylated proteins after labeling of chicken or rabbit

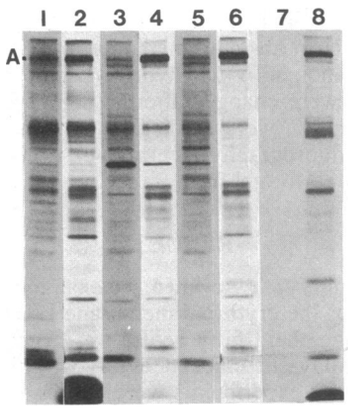

FIG. 4. Palmitic acid incorporation into chicken erythroid membrane-skeletal polypeptides during embryo development and in the adult. Erythroid cells from 5-d (lanes 1 and 2), 10-d (lanes 3 and 4), and 14-d embryos (lanes 5 and 6), erythrocytes from an adult chicken (lanes 7 and 8) were labeled with ^{14}C-labeled amino acids or [^{3}H]palmitic acid and fractionated. Urea extracts containing the membrane-skeleton material from chicken erythroid cells or erythrocytes were separated on a 12.5% $NaDodSO_4$/polyacrylamide gel and visualized by fluorography. ^{14}C-labeled amino acid-labeled polypeptides, lanes 1, 3, 5, and 7; [^{3}H]palmitic acid-labeled polypeptides, lanes 2, 4, 6, and 8. A, ankyrin.

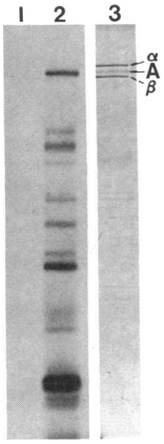

FIG. 5. Palmitic acid-labeled membrane-skeletal polypeptides of rabbit erythrocytes. Adult rabbit erythrocytes were labeled with ^{14}C-labeled amino acids or [^{3}H]palmitic acid and hypotonically lysed. The resulting ghosts were dissolved in $NaDodSO_4$ sample buffer and separated on a 10% $NaDodSO_4$/polyacrylamide gel. Under these conditions mammalian ankyrin migrates between α- and β-spectrin; it comigrates with α-spectrin in our 12.5% $NaDodSO_4$/polyacrylamide gels. Fluorographs are shown of ^{14}C-labeled amino acid-labeled ghost polypeptides (lane 1) and [^{3}H]palmitic acid-labeled ghost polypeptides (lane 2). Separated polypeptides from rabbit erythrocyte ghosts were also stained with Coomassie brilliant blue (lane 3). A, ankyrin; α, α-spectrin; β, β-spectrin.

erythrocytes with [^{3}H]palmitic acid. Palmitic acid is incorporated into ankyrin as fatty acid and not after conversion to amino acids since the label is removed by hydroxylamine treatment. Strong labeling with palmitic acid also occurs when protein synthesis is negligible; this is evident in erythroid cells after inhibition of protein synthesis with emetine, as well as in adult chicken and rabbit erythrocytes whose protein biosynthetic activity is naturally minimal. The fatty acid is most likely bound covalently to the polypeptide backbone as it is not extracted by Triton X-100, $NaDodSO_4$, and chloroform/methanol. Throughout this study we have used [^{3}H]palmitic acid for labeling; however, it remains to be investigated which fatty acid(s) is (are) actually bound to ankyrin.

We have not as yet determined the site(s) on ankyrin that become(s) fatty acid acylated. The sensitivity of the binding to hydroxylamine suggests that most of the fatty acid is esterified to a thiol group or a hydroxy amino acid as has been determined for transmembrane proteins such as vesicular stomatitis and Sindbis virus glycoproteins (13, 14) or the transferrin receptor (15). In these cases the site of the polypeptide that is fatty acid acylated is in or close to the highly hydrophobic domain that spans the lipid bilayer. However, ankyrin behaves as a typical peripheral plasma membrane protein as it is extracted from erythrocyte ghosts with high salt or at high pH, but not at low ionic strength (10, 16–18). It is unlikely that ankyrin contains a hydrophobic sequence that spans the lipid bilayer. Its acyl group(s) probably is (are) bound to less hydrophobic sequences of the polypeptide chain but this may increase the overall hydrophobicity of these domains. In this respect ankyrin is similar to the viral transforming proteins Harvey sarcoma virus p21 (19) and simian virus 40 T antigen (20) that apparently lack typical transmembrane sequences but also contain esterified fatty acids. It is possible that a small amount of the incorporated fatty acid is bound to the NH_2 terminus of ankyrin through an amide linkage as has been described for myristic acid attached to Rous sarcoma virus pp60^{src} (19), calcineurin B (21), or the cAMP-dependent protein kinase catalytic subunit (22).

Studies on the vesicular stomatitis and Sindbis virus glycoproteins indicate that the fatty acid modification occurs after completion of synthesis of the polypeptides but before the transport of these glycoproteins to the cell surface, most likely at the transitional region of the endoplasmic reticulum or in a *cis*-Golgi vesicle (6, 7). However, ankyrin, a peripheral membrane protein (23), is most likely synthesized on free polyribosomes, and there is no *a priori* reason to assume that its biogenesis resembles that of a viral transmembrane glycoprotein. From the evidence presented here it is apparent that fatty acid acylation of ankyrin is not strictly coupled to its synthesis or assembly onto the membrane skeleton. It can occur at a comparable level in cells in which both processes are minimal, namely in adult erythrocytes or in embryonic erythroid cells treated with a protein synthesis inhibitor. In these cells it is also unlikely that ankyrin is acylated at the endoplasmic reticulum–Golgi complex after an association with newly synthesized anion transporter molecules at intracellular membranes. This is particularly evident in adult erythrocytes that do not contain an extensive endoplasmic reticulum–Golgi system. Thus, ankyrin can be acylated at a site close to the plasma membrane after its assembly onto the membrane skeleton. This implies that acyltransferases forming ester on thioester linkages are active not only at the endoplasmic reticulum–Golgi system where typical transmembrane proteins are acylated but also the cytoplasmic surface of the plasma membrane. In the latter case acyltransferases may be responsible for the acylation of proteins that bind directly from the cytoplasm to the plasma membrane. In addition to ankyrin, possible examples of such proteins include the T antigen of simian virus 40 (20) and p21 of Harvey sarcoma virus (19), but also proteins which are myristilated at the NH_2 terminus [e.g., pp60^{src} of Rous sarcoma virus (19)]. Our results presented here indicate that ankyrin can be acylated at the plasma membrane. It is important to point out that they do not exclude an additional, probably minor, acylation of ankyrin during any step of its biogenesis prior to assembly.

The possible functions of the fatty acid acylation of ankyrin presently are unknown. From the arguments discussed above and the fact that the anion transporter provides the high-affinity binding site for ankyrin at the plasma membrane, we regard the possibility unlikely that fatty acid acylation drives the assembly of ankyrin onto the plasma membrane. It seems more likely that the acylation is related to a function of the assembled ankyrin molecule. In this context it is important to note that the bound fatty acid appears to turn over at a substantially higher rate than the ankyrin polypeptide backbone as judged by its rapid incorporation into this protein in adult erythrocytes and after inhibition of protein synthesis in embryonic cells. This suggests that fatty acid acylation of ankyrin is reversible and thus may serve to modulate the function of ankyrin. Alternatively, it may indicate that only a fraction of the ankyrin molecules are fatty acid acylated. A reasonable assumption is that the fatty acid attached to ankyrin allows for a direct interaction of this protein with the lipid bilayer. Thus acylation of ankyrin may induce the binding of a second site of the ankyrin molecule to the plasma membrane and thereby serve to alter the strength and the molecular geometry of the membrane cytoskeleton linkage. It is also conceivable that the direct interaction of ankyrin with the lipid bilayer may influence both the local lipid composition and organization of the bilayer. The constitutive incorporation of palmitic acid into ankyrin at all stages of chicken embryo development examined, as well as in the adult indicates that fatty acid acylation of ankyrin may be essential to the organization or functioning of the membrane skeleton irrespective of the erythroid lineage or the stage of differentiation. A more general function of the acylation of ankyrin also is indicated by the fact that this modification is not

restricted to avian erythrocytes but can be demonstrated in rabbit erythrocytes as well. Recent studies have shown that ankyrin exists in various nonerythroid cells (24, 25). Analysis of a possible fatty acid acylation of nonerythroid ankyrin may further clarify the significance of this modification for the function of ankyrin.

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