Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 3292–3296, June 1984
Biochemistry

Goblin (ankyrin) in striated muscle: Identification of the potential membrane receptor for erythroid spectrin in muscle cells

(erythrocytes/plasma membrane/cytoskeleton/immunoautoradiography/immunofluorescence)

W. JAMES NELSON AND ELIAS LAZARIDES

Division of Biology, California Institute of Technology, Pasadena, CA 91125

Communicated by Norman H. Horowitz, February 1, 1984

ABSTRACT **Goblin, a high molecular weight (M_r, 260,000) polypeptide of avian erythrocyte plasma membranes characterized by hormone-dependent phosphorylation, is shown by a variety of criteria to be the avian equivalent of ankyrin, the membrane attachment protein for spectrin; a polyclonal monospecific goblin antiserum reacts specifically with ankyrin from mammalian erythrocyte ghosts; goblin and ankyrin have highly homologous, although distinct, two-dimensional peptide maps; and, in reconstitution experiments, goblin binds to spectrin and band 3 in approximately the same molar ratio as ankyrin. Immunoautoradiography and immunofluorescence with goblin antiserum reveal that a serologically related polypeptide (M_r, 235,000) is present in highly purified membrane fractions of mammalian myocardium and in whole extracts of adult chicken cardiac and skeletal muscle-nonerythroid tissues which express predominantly the erythroid ($\alpha\beta$-) spectrin phenotype. Erythroid spectrin and goblin (ankyrin) are codistributed in skeletal muscle at the sarcolemma as discrete foci adjacent to the Z lines and, in pectoral muscle, also at the periphery of the Z discs. These spatial relationships indicate that goblin and spectrin in muscle cells form a structural framework that serves as the attachment site for the myofiber at the level of the Z line on the sarcolemma.**

Goblin is a prominent high molecular weight polypeptide of avian erythrocyte plasma membranes characterized by hormone-dependent phosphorylation (1) by at least three endogenous protein kinases (2). Phosphorylation of goblin, after hormone-induced increase of intracellular cAMP, is correlated quantitatively with a concomitant increase in Na^+-K^+ cotransport (3). Although goblin is tightly bound to the plasma membrane (4), it does not appear to be an integral membrane protein (2, 4), which raises the question of the nature of the molecular mechanism involved in maintaining a specific association between goblin in the subcortical cytoplasm and integral membrane proteins that perform specific catalytic functions. In the mammalian erythrocyte, the association of the transmembrane anion transporter (band 3) with polypeptides in the subcortical cytoplasm has been characterized extensively (for reviews see refs. 5 and 6). The anion transporter is linked to a phosphorylated protein in the subcortical cytoplasm termed ankyrin (7–9). Ankyrin, in turn, is the high-affinity protein binding site on the plasma membrane for spectrin [specifically the β-subunit of the spectrin tetramer (10–12)]. Spectrin binds to and crosslinks F-actin in the subcortical cytoplasm where, together with several other proteins (for references see ref. 5), it forms a dense filamentous network that covers the entire cytoplasmic face of the plasma membrane. These specific protein interactions have particular significance for understanding how the molecular continuum between integral membrane proteins and structural and regulatory proteins in the subcortical cytoplasm of other cell types is established and maintained, since it is now known that polypeptides functionally analogous and structurally related to spectrin have a widespread distribution in nonerythroid cells (for a review see ref. 13; see also ref. 14).

In this study, we show that goblin and ankyrin are serologically related, have highly homologous although distinct peptide maps, and that goblin interacts with spectrin and the anion transporter *in vitro*. These results indicate that goblin is the structural and functional analogue of ankyrin in avian erythrocytes. That goblin (ankyrin) is also the potential membrane binding site for the erythroid form of spectrin in nonerythroid cells is shown by the identification of a polypeptide serologically related to goblin in adult chicken cardiac and skeletal muscle, two nonerythroid tissues that express predominantly the erythroid ($\alpha\beta$-) spectrin phenotype (15). Goblin and erythroid spectrin appear to be colocalized at the sarcolemma of skeletal muscle cells as discrete foci adjacent to the Z line, and, in pectoral muscle, also in the interior of the myofiber at the periphery of the Z discs.

MATERIALS AND METHODS

Production of Goblin Antibodies. Adult chicken erythrocyte plasma membranes were prepared as described (16), and their proteins were separated by $NaDodSO_4$/12.5% polyacrylamide gel electrophoresis based on the system of Laemmli (17) as modified and described (18). The band corresponding to goblin (apparent M_r, 260,000; see ref. 16) was excised from the gel, homogenized in $NaDodSO_4$ sample buffer (18), and reapplied to a $NaDodSO_4$/12.5% polyacrylamide gel. After re-electrophoresis, the goblin band was excised from the gel and equilibrated in 25 mM sodium phosphate buffer, pH 7.5/120 mM NaCl. The gel slices (20–30 μg of total protein) were homogenized and then emulsified with Freund's complete adjuvant for the immunization of a New Zealand White rabbit. Booster injections containing Freund's incomplete adjuvant were administered 44 days and 78 days after the initial immunization. Blood was collected 6 days after the second injection, and 6 days and 14 days after the third injection. The IgG fraction was partially purified by precipitation with ammonium sulfate at 50% saturation at 4°C. Antiserum from the first bleed after the third injection was used in this study; preimmune serum was obtained from the same rabbit prior to the first injection of immunogen.

Polyclonal antisera specific for the α- (4) and β- (15) subunits of chicken erythrocyte spectrin, and for human erythrocyte ankyrin (19) have been characterized in detail elsewhere.

Immunological Methods. The immunoprecipitation of polypeptides from chicken erythrocyte membranes was performed as described (20), using method B. For immunoautoradiography, mixtures of proteins were separated on $NaDodSO_4$/12.5% polyacrylamide gels and then electrophoretically transferred onto a nitrocellulose sheet for 5 hr at 0.25 A essentially according to the method of Towbin *et al.* (21), except that the electrophoresis buffer contained 10 mM

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Tris·HCl/20 mM Hepes, pH 7.56/0.1% $NaDodSO_4$/0.1 mM dithiothreitol/20% (vol/vol) ethanol. The nitrocellulose sheet was incubated in 0.1% (wt/vol) gelatin/120 mM NaCl/20 mM Tris·HCl, pH 7.5/10 mM NaN_3/0.05% (vol/vol) Tween 20 (buffer A) overnight at 37°C, and then at room temperature in a 1:1000 dilution of goblin or ankyrin antisera in buffer A for 3 hr, followed by six changes of buffer A. The nitrocellulose sheet was incubated with ^{125}I-labeled protein A (specific activity, 8–9 μCi/μg; 1 Ci = 37 GBq) for 3 hr, followed by six changes of buffer A, and finally dried and exposed to Kodak XAR-5 x-ray film with an intensifying screen for various periods of time at −80°C.

Immunofluorescence on frozen sections (8 μm) of adult chicken skeletal muscle with goblin and β-spectrin antisera was performed as described (4, 15).

Preparation of Membrane Fractions. Chicken erythrocyte plasma membranes were prepared as described (16). Rabbit and human erythrocyte ghosts were prepared by hypotonic lysis of intact cells in 10 mM Tris·HCl, pH 7.5/5 mM EDTA/0.1 mM dithiothreitol/0.5 mM phenylmethylsulfonyl fluoride. Spectrin-depleted inside-out rabbit erythrocyte vesicles were prepared as described (22). Sarcolemma and sarcoplasmic reticulum membrane vesicle fractions were prepared from mammalian cardiac microsomes as described in detail elsewhere (23).

Interaction of Goblin with Spectrin and Band 3 *in Vitro*. Purified adult chicken erythrocyte plasma membranes (30 μl packed volume) were solubilized in 700 μl of 9 M urea/10 mM Tris·HCl, pH 7.5/5 mM EDTA/1 mM dithiothreitol/0.5% (wt/vol) Triton X-100 at 15°C. Insoluble material was removed by centrifugation at 12,000 × g for 5 min. Aliquots (50 μl) of the supernatant were dialyzed at 4°C against 2 liters of 20 mM Tris·HCl, pH 7.5/120 mM NaCl/5 mM EDTA/2 mM EGTA/0.1 mM dithiothreitol/1% (wt/vol) Triton X-100 (buffer B). Aliquots were removed after 1, 2, 4, 9, and 20 hr of dialysis, diluted 1:10 with buffer B, and incubated with 5 μl of goblin antiserum or preimmune serum at 4°C for 1 hr. Twenty-five microliters of a 10% (vol/vol) suspension of formalin-fixed *Staphylococcus aureus* (Pansorbin, Calbiochem-Behring), which had been washed extensively in buffer B, was added and the sample was then turned end over end for 1 hr. The sample was centrifuged through a 1 M sucrose cushion (100 μl) in buffer B at 12,000 × g for 5 min in an Eppendorf minicentrifuge. The pellet was resuspended in 600 μl of buffer B by Vortex mixing and again centrifuged through a sucrose cushion as described above; this procedure was repeated twice more. The final pellet was resuspended in 100 μl of $NaDodSO_4$ sample buffer (18), boiled for 5 min, and centrifuged; the polypeptides coimmunoprecipitated with goblin were analyzed by $NaDodSO_4$/12.5% polyacrylamide gel electrophoresis, followed by densitometric analysis with a Quick-Scan (Helena Laboratories, Beaumont, TX) and determination of the area under each peak.

Two-Dimensional Peptide Mapping. Two-dimensional mapping of chymotryptic iodopeptides derived from chicken erythrocyte goblin and rabbit erythrocyte ankyrin was performed essentially according to the method of Elder *et al.* (24) as modified and described (25).

RESULTS

Characterization of Goblin Antiserum. The polypeptide of chicken erythrocyte plasma membrane designated as goblin from previous studies (4, 16) was purified by $NaDodSO_4$/polyacrylamide gel electrophoresis and used as an immunogen. The specificity of the resulting antiserum was determined by a variety of methods. First, in the presence of 0.1% $NaDodSO_4$, the antiserum was shown to immunoprecipitate a single polypeptide from $NaDodSO_4$-solubilized chicken erythrocyte plasma membranes, which had an electrophoretic mobility in $NaDodSO_4$/12.5% polyacrylamide gels similar to that of goblin (Fig. 1, lanes 1 and 2). The immunoprecipitated polypeptide had a two-dimensional chymotryptic iodopeptide map indistinguishable from that of goblin, which had been isolated from whole extracts of chicken erythrocyte plasma membranes (not shown). The goblin antiserum did not immunoprecipitate either α- or β-spectrin. Furthermore, antibodies specific for chicken erythrocyte α-spectrin (lane 3) and β/β'-spectrin (lane 4) did not immunoprecipitate goblin.

The specificity of the antiserum was determined further by immunoautoradiography. Fig. 1 (lane 5) shows the Coomassie-blue-stained $NaDodSO_4$/polyacrylamide gel of chicken erythrocyte plasma membrane proteins. Immunoautoradiography of a duplicate sample demonstrated that the goblin antiserum reacted only with the immunogen. Overexposure of the autoradiogram revealed that a second minor polypeptide with an apparent electrophoretic mobility faster than that of α-spectrin reacted also with the antiserum. Antiserum that had been affinity-purified on strips of nitrocellulose containing only goblin, using the method of Olmsted (26), reacted also with this lower molecular weight polypeptide (data not shown), demonstrating that this polypeptide was not a contaminant of the goblin sample used as the immunogen. Finally, the antiserum did not react with synemin, a high molecular weight intermediate filament polypeptide found in avian erythrocytes (16), which has a molecular weight similar to this goblin-related polypeptide (data not shown). The goblin antiserum reacted also with turkey erythrocyte goblin (data not shown).

Goblin Is Serologically Related to Mammalian Erythrocyte Ankyrin. Immunoautoradiography of total proteins of rabbit erythrocyte ghosts revealed that the goblin antiserum reacted with a single polypeptide of apparent M_r 235,000 (Fig. 1, lanes 6 and 8). Furthermore, this polypeptide could be phosphorylated *in vivo* (not shown) or *in vitro* (lane 10), as could

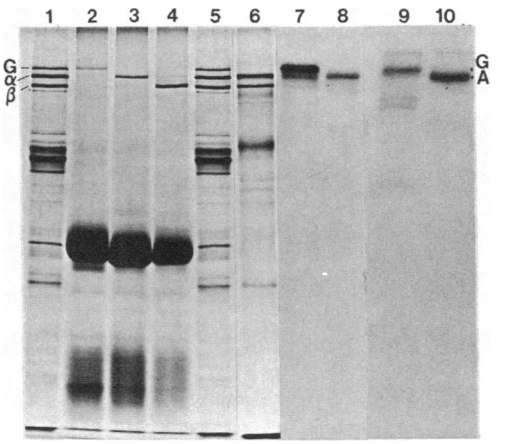

FIG. 1. Immunological characterization of the goblin antiserum. Lanes 1–4, Coomassie-blue-stained $NaDodSO_4$/12.5% polyacrylamide gel of total proteins of purified chicken erythrocyte plasma membranes (lane 1) and proteins immunoprecipitated from $NaDodSO_4$-solubilized extracts of chicken erythrocyte plasma membranes with antiserum specific for goblin (lane 2), α-spectrin (lane 3), and β-spectrin (lane 4). The small amount of goblin and α-spectrin coimmunoprecipitated with β-spectrin antibodies results from the reassociation of these polypeptides during the course of the immunoprecipitation (see ref. 15 and Fig. 4). Lanes 5–8, stained gel (lanes 5 and 6) and corresponding immunoautoradiogram with goblin antiserum (lanes 7 and 8) of chicken (lanes 5 and 7) and rabbit (lanes 6 and 8) erythrocyte ghosts (exposure time was 3 hr). Lanes 9 and 10, autoradiogram of proteins immunoprecipitated with goblin antiserum from chicken (lane 9) and rabbit (lane 10) erythrocyte ghosts incubated with [γ-^{32}P]ATP and protein kinase (exposure time was 40 min). G, goblin; A, ankyrin; α, α-spectrin; β, β-spectrin.

goblin (lane 9), by using the catalytic subunit of the cAMP dependent protein kinase. This polypeptide was identified as ankyrin by the fact that a polyclonal antiserum against human erythrocyte ankyrin (19) reacted specifically with the same polypeptide (Fig. 2, lanes 2 and 5). The difference in the electrophoretic mobility of ankyrin compared with that reported previously (see refs. 8 and 9) may be due to the fact that different electrophoretic gel systems were used. The ankyrin antiserum reacted also with goblin from chicken erythrocyte plasma membranes (lanes 1 and 4) and with a polypeptide with an apparent molecular weight lower than that of goblin (lane 4).

Comparison of the Two-Dimensional Peptide Maps of Goblin and Ankyrin. The relatedness of goblin and ankyrin was confirmed by two-dimensional peptide mapping (Fig. 3). The peptide maps of goblin and ankyrin show a high degree of homology with five prominent iodopeptides (arrowheads) in the center of the maps having identical mobilities. However, there are peptides unique to each protein. Thus, goblin and ankyrin appear to be structurally related but distinct polypeptides.

Interaction of Goblin, Spectrin, and Band 3 *in Vitro*. To determine whether goblin interacts with spectrin and band 3, and thus is functionally related to ankyrin, we attempted to reconstitute a goblin–spectrin–band 3 complex *in vitro* with components from chicken erythrocyte plasma membranes that had been solubilized in urea and Triton X-100. After dialysis of the urea-solubilized membrane proteins in a nondenaturing buffer, goblin was immunoprecipitated with goblin antiserum, and the immunoprecipitates were analyzed by $NaDodSO_4$/12.5% polyacrylamide gel electrophoresis. Fig. 4*A* shows a densitometric tracing of the proteins (M_r, >75,000) of $NaDodSO_4$ extracts of whole chicken erythrocyte plasma membranes. Integration of the area under the peaks corresponding to goblin, α- and β spectrin revealed a molar ratio of approximately 1:2:2. After solubilization of chicken erythrocyte membrane proteins with urea, and renaturation by dialysis for 1 hr, a complex of goblin, α- and β-spectrin, and band 3 was coimmunoprecipitated with goblin antiserum. The molar ratio of goblin/α-spectrin/β-spectrin was approximately 1:1:1. After 4 hr of renaturation by dialysis, the amount of goblin in the immunoprecipitate was the same as that after 1 hr; however, the relative amount of α- and β-spectrin had increased, and the molar ratio of goblin/α-spectrin/β-spectrin was now approximately 1:2:1.7 (Fig. 4*C*). This ratio did not change even after prolonged dialysis of the samples for up to 20 hr (data not shown). The ratio of goblin/band 3 was approximately 1:1 throughout the time course of the reconstitution (compare Fig. 4 *B* and *C*). A similar result was obtained if β-spectrin antiserum was used instead of the goblin antiserum (not shown). It should be noted also that urea-solubilized vimentin (not shown) and most of the anion transporter were not coimmunoprecipitated with goblin antiserum. As a further control, a sample prior to dialysis was diluted 1:10 and immunoprecipitated in the presence of 0.1% $NaDodSO_4$ and 1% Na-deoxycholate (Fig. 4*D*); in this sample, ≈4 times as much goblin antiserum was used compared to the reconstitution experiments to demonstrate unequivocally the absence of spectrin and band 3 in the goblin immunoprecipitates under these denaturing conditions, and thus the specificity of the goblin antiserum and of the interactions in the complex coimmunoprecipitated under nondenaturing conditions. The preimmune serum did not immunoprecipitate any of the solubilized erythrocyte components (data not shown).

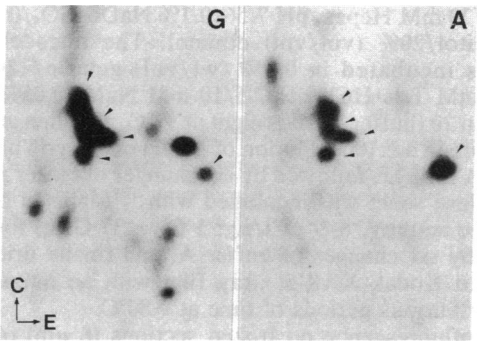

FIG. 3. Comparison of the two-dimensional chymotrypic iodopeptide maps of goblin (G) and ankyrin (A). The polypeptides were radioiodinated in solution in the presence of 1% $NaDodSO_4$. After $NaDodSO_4$/12.5% polyacrylamide gel electrophoresis, the Coomassie-blue-stained bands were excised from the gel, digested with α-chymotrypsin, and the resulting iodopeptides were separated by high-voltage electrophoresis in the first dimension (E) and ascending chromatography in the second dimension (C). Iodopeptides of goblin and ankyrin with similar mobilities are shown (arrowheads).

Detection of a Polypeptide in Striated Muscle Serologically Related to Goblin. Previous studies from this laboratory have demonstrated the presence in striated muscle of polypeptides serologically related to the α- and β-subunits of chicken erythrocyte spectrin (4, 15, 27). To determine whether a polypeptide related to goblin coexists with the erythroid form of spectrin in these tissues, $NaDodSO_4$-solubilized extracts of adult chicken pectoral and cardiac ventricular muscle, and purified sarcolemma and sarcoplasmic reticulum from mammalian myocardium were tested by immunoautoradiography with goblin antiserum. Fig. 5 shows the Coo-

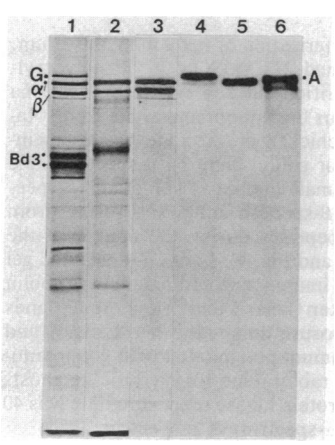

FIG. 2. Reactivity of an antiserum raised against human erythrocyte ankyrin (19) with goblin and ankyrin. Coomassie-blue-stained $NaDodSO_4$/12.5% polyacrylamide (lanes 1–3) and the corresponding immunoautoradiogram with ankyrin antiserum (lanes 4–6) (exposure time was 2 hr). Lane 1, purified chicken erythrocyte plasma membranes; lane 2, rabbit erythrocyte ghosts; lane 3, human erythrocyte ghosts. A, ankyrin; G, goblin; α, α-spectrin; β, β-spectrin; Bd 3, band 3 or the anion transporter.

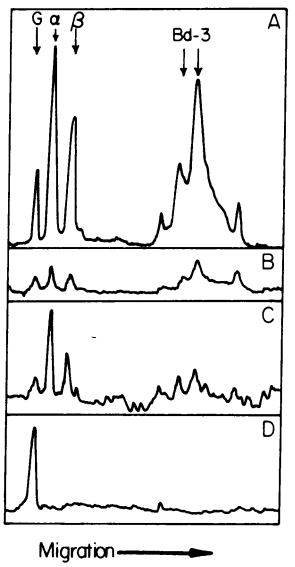

FIG. 4. Densitometric analysis of total proteins (M_r, >75,000) of chicken erythrocyte plasma membranes (*A*) (G, goblin; α, α-spectrin; β, β-spectrin; Bd 3, band 3 or the anion transporter) and proteins coimmunoprecipitated with goblin antiserum after reconstitution for various times of urea-solubilized components of chicken erythrocyte plasma membranes. (*B*) Reconstitution for 1 hr. (*C*) Reconstitution for 4 hr. (*D*) Immunoprecipitate of chicken erythrocyte membrane proteins with goblin antiserum under denaturing conditions in the presence of 0.1% $NaDodSO_4$/1% Na-deoxycholate.

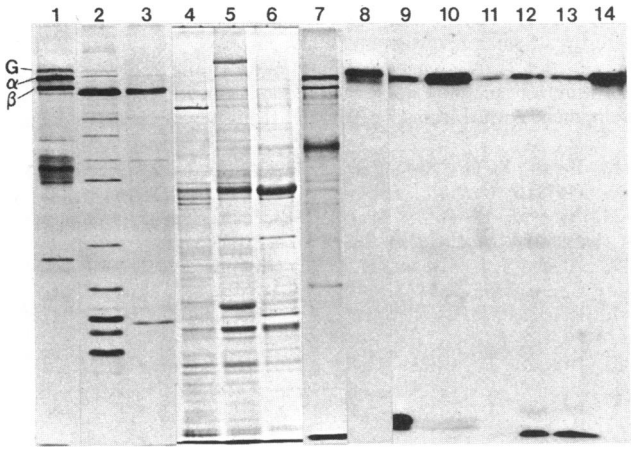

FIG. 5. Reactivity of the goblin antiserum with polypeptides of chicken and mammalian striated muscle. Coomassie-blue-stained $NaDodSO_4$/12.5% polyacrylamide gel (lanes 1–7) and the corresponding immunoautoradiogram (lanes 8–14) with goblin antiserum. Lanes 1 and 8, chicken erythrocyte plasma membrane; lanes 2 and 9, $NaDodSO_4$ extract of chicken pectoral muscle; lanes 3 and 10, $NaDodSO_4$ extract of chicken cardiac ventricular muscle; lanes 4–6 and 11–13, purified membrane fractions of mammalian myocardium: sarcolemma (lanes 4 and 11), longitudinal sarcoplasmic reticulum (lanes 5 and 12), and junctional sarcoplasmic reticulum (lanes 6 and 13); lanes 7 and 14, rabbit erythrocyte ghosts. Exposure time was 12 hr. G, goblin; α, α-spectrin; β, β-spectrin.

massie-blue-stained polyacrylamide gel (lanes 1–7) and the corresponding autoradiogram (lanes 8–14). A single polypeptide with an apparent M_r of 235,000 reacted with the goblin antiserum in all samples.

Indirect immunofluorescence was used to determine the distribution in chicken striated muscle of the polypeptide serologically related to goblin (Fig. 6). The antigen was shown to be localized along the cytoplasmic face of the sarcolemma as a discrete punctate pattern (Fig. 6 *A–C*), in which the periodicity of the foci of fluorescence coincided principally with the Z discs (not shown). This pattern was indistinguishable from that of erythroid spectrin in the same tissue (Fig. 6 *F* and *G*), which, as we have shown previously (15), is distributed primarily in the area of the sarcolemma adjacent to the Z lines, where it forms an intricate grid-like lattice with prominent rings around Z lines that are interconnected by lines of spectrin fluorescence in the long axis of the muscle fiber. Sections of pectoral muscle transverse to the long axis revealed also that the antigens reacting with the goblin and β-spectrin antisera were present in the interior of the muscle fiber at the periphery of the Z discs. Affinity-purified goblin antibodies, following the procedure of Olmsted (26), gave a similar fluorescence pattern as the goblin antiserum (data not shown).

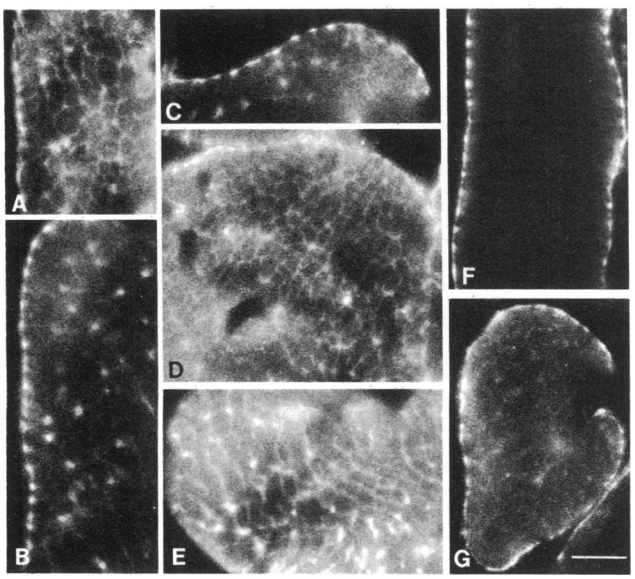

FIG. 6. Indirect immunofluorescence on frozen sections of adult chicken pectoral muscle with goblin (*A–E*) and β-spectrin (*F* and *G*) antisera. *A*, *B*, and *C* are oblique–longitudinal sections; *F* is a longitudinal section; and *D*, *E*, and *G* are transverse sections. (Bar = 8 μm.)

DISCUSSION

Recently, a study of the solubility properties of chicken erythrocyte plasma membrane proteins revealed that goblin can be selectively extracted together with the anion transporter (band 3) with high salt and nonionic detergents (4); under these conditions structural proteins such as spectrin, actin, and intermediate filaments are not extracted (4). These solubility properties of goblin are similar to those of ankyrin (M_r, 235,000), the polypeptide in mammalian erythrocytes that binds to the anion transporter and serves as the specific membrane attachment protein for spectrin (refs. 7 and 10; for reviews, see refs. 5 and 6). The results of the present study demonstrate by a variety of criteria that goblin and ankyrin are, in fact, structurally and functionally related polypeptides. First, antiserum raised against each polypeptide reacts specifically with both polypeptides. Preliminary results indicate, however, that the goblin antiserum does not react with microtubule-associated proteins and, by immunofluorescence microscopy, does not stain nuclei, unlike some antisera raised against mammalian ankyrin (28, 29). Goblin and ankyrin, although clearly distinct polypeptides with different apparent molecular weights (M_r, 260,000 and 235,000, respectively, in this gel system), have two-dimensional chymotryptic iodopeptide maps that exhibit a high degree of homology; the predominant homologous peptides may be derived from the part of the molecules that binds to the anion transporter (band 3) (30). Significantly, goblin appears to be functionally analogous to ankyrin. In reconstitution experiments, based on earlier studies on the assembly of denatured spectrin subunits (31), goblin was shown to interact with spectrin and band 3. The molar ratio of 1:2 for goblin/β-spectrin, obtained after a 4-hr reconstitution of urea-solubilized chicken erythrocyte membrane proteins, is similar to that found in whole extracts of chicken erythrocyte membranes and to the molar ratio of ankyrin/β-spectrin in mammalian erythrocytes (11, 19). The reconstitution experiments showed also that approximately equimolar amounts of goblin and band 3 were found in the goblin immunoprecipitates, indicating that goblin interacts with band 3, as has been shown also for ankyrin (8, 9).

Although the present study has not addressed specifically the question of how goblin (ankyrin) hyperphosphorylation may regulate transmembrane ion fluxes, it is pertinent in this respect to note that the interaction of goblin with the anion transporter (band 3) results in the localization of goblin in areas of the membrane involved in the control of ion fluxes. Furthermore, calmodulin has been shown to bind to spectrin (32), which may play a specific role in regulating the local activity of the Ca^{2+}/calmodulin-dependent protein kinase known to be involved in the phosphorylation of goblin (2).

The specific protein interactions in the erythrocyte between integral membrane proteins goblin (ankyrin) and the spectrin–actin cytoskeleton appear now to have their counterparts in a variety of nonerythroid cells. Recently, several studies have demonstrated the widespread cellular distribution of proteins functionally analogous and structurally relat-

ed to spectrin (for a review, see ref. 13). In view of the fact that striated muscle cells express predominantly, if not exclusively, the erythroid ($\alpha\beta$-) spectrin phenotype (15, 27), we sought to determine whether goblin (ankyrin) was expressed also in this particular cell type. The results of immunoautoradiography with goblin antiserum of $NaDodSO_4$ extracts of whole skeletal and cardiac tissue and highly purified membrane fractions from mammalian myocardium demonstrate unequivocally the presence of a single serologically related polypeptide in all samples that has an apparent molecular weight similar to that of rabbit erythrocyte ankyrin (M_r, 235,000). That this polypeptide, together with spectrin (data not shown), is present in samples of highly purified muscle sarcolemma and sarcoplasmic reticulum demonstrates that goblin in muscle cells is tightly bound to the plasma membrane and is not solubilized by treatment with either high or low ionic strength buffers in the absence of nonionic detergents. At present, however, it is unclear whether this goblin-related polypeptide in muscle cells accumulates as a stable processed product of a higher molecular weight polypeptide (goblin) or whether it is the product of a related but distinct gene and, hence, a member of a goblin multigene family.

Indirect immunofluorescence microscopy of adult chicken skeletal muscle with goblin and β-spectrin antisera revealed that the cross-reactive goblin antigen and erythroid β-spectrin are codistributed on the cytoplasmic surface of the sarcolemma in discrete foci adjacent to the Z lines. The spatial relationship at the membrane between goblin and spectrin indicates that, in skeletal muscle, goblin (ankyrin) may be the specific membrane attachment protein for erythroid spectrin. By analogy to the erythrocyte, the presence of goblin at the sarcolemma of muscle cells may result in the establishment of an invariant and inextensible anchorage site for spectrin and other structural proteins on the sarcolemma adjacent to the Z lines. Indeed, several studies have demonstrated the presence of structural filaments emanating from the Z lines, which appear to be attached to discrete areas of the sarcolemma directly adjacent to the Z lines (33–35). These filaments may be part of a Z-disc scaffold (36), which was shown in earlier studies from this laboratory to be composed of the intermediate filament proteins vimentin, desmin and synemin (37, 38), and which is thought to structurally integrate individual Z discs laterally to form a composite Z-line structure and thus coordinate and unify the contraction–relaxation cycles of individual sarcomeres (36). Taken together, these results indicate that each myofiber is completely enclosed in a framework of structural proteins that laterally link individual sarcomeres at the level of the Z discs and attach the myofiber to the sarcolemma; to indicate this we have termed this framework a *holomere* (from the Greek *holos*, all encompassing; and *meros*, substructure). At present, the relationship of the proposed *holomere* to sites on the sarcolemma at the level of the I band, which contain vinculin, and for which the name *costamere* has been proposed (39), is not known. We suggest that the *holomere* provides an inextensible structural support upon which tension can be developed by the muscle fiber along its lateral axis, as tension is developed by the contractile apparatus along the longitudinal axis.

We are grateful to Dr. Larry R. Jones (Indiana University School of Medicine, Indianapolis) for generously providing the purified membrane fractions of mammalian myocardium, to Dr. V. Bennett (The Johns Hopkins School of Medicine, Baltimore) for the ankyrin antiserum, to Dr. Bruce Granger for his advice on the immunoblot procedure and critical comments on the manuscript, and to Dr. R. T. Moon for preparation of rabbit erythrocyte inside-out vesicles. This work was supported by grants from the National Institutes of Health, the National Science Foundation, and the Muscular Dystrophy Association of America (to E.L.). W.J.N. was supported also by a Senior Investigator Fellowship from the American Heart Association (Los Angeles Affiliate) and a grant from the National Institutes of Health. E.L. is the recipient of a Research Career Development Award from the National Institutes of Health.

1. Beam, K. G., Alper, S. L., Palade, G. E. & Greengard, P. (1979) *J. Cell Biol.* **83,** 1–15.
2. Alper, S. L., Palfry, M. C., DeRiermer, S. A. & Greengard, P. (1980) *J. Biol. Chem.* **255,** 11029–11039.
3. Alper, S. L., Beam, K. G. & Greengard, P. (1980) *J. Biol. Chem.* **255,** 4864–4871.
4. Repasky, E. A., Granger, B. L. & Lazarides, E. (1982) *Cell* **29,** 821–833.
5. Branton, D., Cohen, C. H. & Tyler, J. (1981) *Cell* **24,** 24–32.
6. Bennett, V. (1982) *J. Cell Biochem.* **18,** 49–65.
7. Bennett, V. & Stenbuck, P. J. (1979) *Nature (London)* **280,** 468–473.
8. Bennett, V. & Stenbuck, P. J. (1980) *J. Biol. Chem.* **255,** 2540–2548.
9. Hargreaves, W. R., Gledd, K. N., Verkleji, A. & Branton, D. (1980) *J. Biol. Chem.* **255,** 11965–11972.
10. Luna, E. J., Kidd, G. H. & Branton, D. (1979) *J. Biol. Chem.* **254,** 2526–2532.
11. Tyler, J., Hargreaves, W. R. & Branton, D. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5192–5196.
12. Morrow, J. S., Speicher, D. W., Knowles, W. J., Hsu, C. J. & Marchesi, V. T. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6592–6596.
13. Nelson, W. J. & Lazarides, E. (1984) in *Cell Membranes: Methods and Reviews*, eds. Elson, E. L., Frazier, W. A. & Glaser, L. (Plenum, New York), in press.
14. Glenney, J. R. & Glenney, P. (1983) *Cell* **34,** 503–512.
15. Nelson, W. J. & Lazarides, E. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 363–367.
16. Granger, B. L., Repasky, E. A. & Lazarides, E. (1982) *J. Cell Biol.* **92,** 299–312.
17. Laemmli, U. K. (1970) *Nature (London)* **277,** 680–685.
18. Hubbard, B. D. & Lazarides, E. (1979) *J. Cell Biol.* **80,** 166–182.
19. Bennett, V. & Stenbuck, P. (1979) *J. Biol. Chem.* **254,** 2533–2541.
20. Blikstad, I., Nelson, W. J., Moon, R. T. & Lazarides, E. (1983) *Cell* **32,** 1081–1091.
21. Towbin, M., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
22. Bennett, V. & Branton, D. (1977) *J. Biol. Chem.* **252,** 2753–2763.
23. Jones, L. R., Besch, M. R., Fleming, J. W., McConnaughty, M. M. & Watanabe, A. M. (1979) *J. Biol. Chem.* **254,** 530–539.
24. Elder, J. M., Pickett II, R. A., Hampton, J. & Lerner, R. A. (1977) *J. Biol. Chem.* **252,** 6510–6515.
25. Nelson, W. J., Granger, B. L. & Lazarides, E. (1983) *J. Cell Biol.* **97,** 1271–1276.
26. Olmsted, J. B. (1981) *J. Biol. Chem.* **256,** 11955–11957.
27. Nelson, W. J. & Lazarides, E. (1983) *Nature (London)* **304,** 364–368.
28. Bennett, V. (1979) *Nature (London)* **281,** 597–599.
29. Bennett, V. & Davis, J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7550–7554.
30. Weaver, D. C., Pasternak, G. R. & Marchesi, V. T. (1983) *J. Cell Biol.* **97,** 297 (abstr.).
31. Calvert, R., Bennett, P. & Gratzer, W. (1980) *Eur. J. Biochem.* **107,** 355–361.
32. Palfrey, M. C., Schieber, W. & Greengard, P. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3780–3784.
33. Pierobon-Bormioli, S. (1981) *J. Musc. Res. Cell Motil.* **2,** 401–408.
34. Chiesi, M., Ho, M. M., Luesi, G., Somlyo, A. V. & Somlyo, A. P. (1981) *J. Cell Biol.* **91,** 728–742.
35. Street, S. F. (1983) *J. Cell Physiol.* **114,** 346–364.
36. Granger, B. L. & Lazarides, E. (1978) *Cell* **15,** 1253–1268.
37. Granger, B. L. & Lazarides, E. (1979) *Cell* **18,** 1053–1063.
38. Granger, B. L. & Lazarides, E. (1980) *Cell* **22,** 727–738.
39. Pardo, J. V., Siliciano, J. D. & Craig, S. W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1008–1012.