20. T. L. Simpson and C. A. Vaccaro, *J. Ultrastruct. Res.* **47**, 296 (1974).
21. D. G. Dunkelberger and N. Watanabe, *Tissue Cell* **6**, 573 (1974).
22. T. C. Moore, *Geol. Soc. Am. Bull.* **80**, 2103 (1969).
23. Extrapolation of a plot of the pore volume data in Fig. 1 against skeletal density as obtained from "heavy" liquid separation techniques (5) yields a value of about 2.1 g/cm³ at zero pore volume. It is possible that failure to obtain higher density values was partly due to the existence of closed pores in the nonporous core of the skeletons.
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Distribution of Transmembrane Polypeptides in Freeze Fracture

Abstract. *Human erythrocytes have been freeze-fractured, and the polypeptides associated with the separate halves of the membrane bilayer have been analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The transmembrane proteins were differentially separated by the fracture process. Although sialoglycoproteins associated with the outer half of the membrane, the anion transport protein (band 3) mainly remained with the inner half of the membrane. Well-defined fragments of the sialoglycoproteins were produced by the freeze-fracture procedure, indicating that selected covalent bonds of these transmembrane proteins were broken.*

The lipid bilayer of biological membranes is readily split during freeze fracture (*1*). Until recently, however, there has been no technique for differentially analyzing the two halves of a membrane biochemically. This difficult task of physically isolating sufficient quantities of half-membranes has recently been overcome by freezing a monolayer of erythrocytes between a polylysine-coated cover slip and a copper plate (*2*). When this sandwich is fractured at liquid nitrogen temperature by separating the glass from the copper, the portion of the membrane's external lipid layer that is in direct contact with the polylysine-coated cover slip remains associated with the cover slip, while the remainder of the structure, including the inside half of the fractured membrane, remains with the copper plate (*2*).

The distribution of membrane proteins in the freeze-fracture process has not been satisfactorily explained. The "bumps" produced in the process have been assumed to be the transmembrane proteins (*3*, *4*) that extend through the lipid bilayer, and the disposition of the bumps with respect to each other is taken to reflect the organization of proteins within the plane of the membrane. We investigated the distribution of membrane polypeptides associated with the two halves of the membrane bilayer, employing the technique of Fisher (*2*). Since most, if not all, of the major proteins exposed on the outside surface of the human erythrocyte membrane have been shown to be transmembrane (*5*), the erythrocyte is an especially good membrane for this study of the organization of plasma membrane proteins.

Preparations of cells, cover slips, and copper plates were similar to those used by Fisher (*2*). Number 1 cover slips measuring 24 by 50 mm were soaked overnight in chromic acid, rinsed with distilled water, dried with nitrogen, covered on one side with 0.1 ml of 5 m*M* polylysine (molecular weight, 3400; Sigma), rinsed with distilled water, and again dried with nitrogen. A flat, cold-rolled copper sheet 0.51 mm thick was cut into 76 by 32 mm plates. Nonflat plates were culled, and the rest were polished and then etched with a 1 : 1 mixture of concentrated nitric acid and water for 15 seconds, rinsed five times in distilled water, and then nitrogen-dried. The cover slips and copper plates were prepared no more than 1 day before the experiment and were kept in a covered container until used.

Erythrocytes from freshly drawn human blood (acid citrate dextrose anticoagulant) were washed three times in isotonic saline and then once in isotonic phosphate buffer, *p*H 7.4. Cells were radioactively labeled by either lactoperoxidase-catalyzed ^{125}I iodination (*5*) or periodate treatment followed by reduction with tritiated sodium borohydride (*6*, *7*). After rinsing and centrifuging several times in phosphate-buffered saline (PBS) to remove unincorporated label, the cells were centrifuged for 10 minutes at 1000*g* in a graduated test tube, decanted, and resuspended in a volume of PBS equal to 0.4 of their own volume. This results in a cell density of 6×10^9 to 7×10^9 erythrocytes per milliliter.

This cell suspension (0.2 ml) was distributed over the surface of the polylysine-coated cover slip. Cells not bound

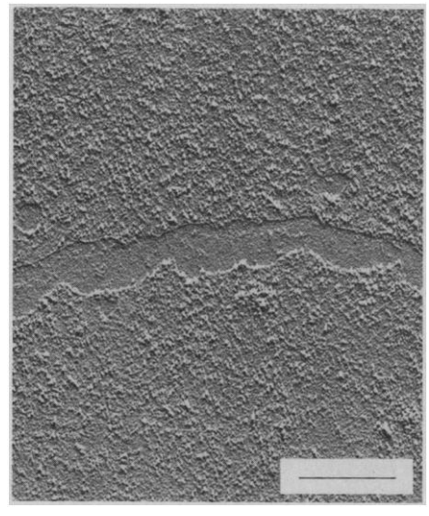

Fig. 1 (left). Replica of the E faces of freeze-fractured human erythrocytes that had been bound to a polylysine-coated cover slip. Shown are portions from two cells with the intervening gap. The marker is 0.5 μm long.

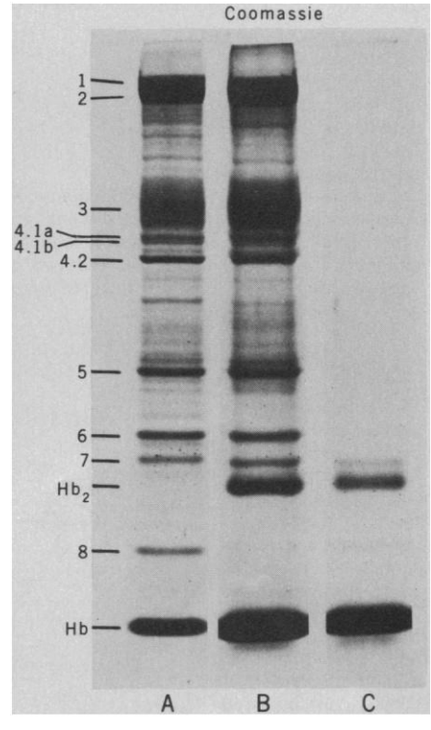

Fig. 2 (right). Coomassie blue profile of erythrocyte membrane polypeptides separated by electrophoresis on 7.5 to 15 percent linear acrylamide gradient gels, using the SDS discontinuous buffer system of Laemmli (*6*, *22*). Lane *A* contains polypeptides from the unfractured membranes of $120 \pm 10 \times 10^6$ erythrocytes. The membrane proteins recovered from the freeze-fracture sample are shown in lane *B* (fractured cells from the copper plate plus an undetermined number of unfractured cells) and lane *C* (cover slip sample, equivalent to the complete E faces of $40 \pm 10 \times 10^6$ cells). The latter value assumes 20×10^6 fracture cells per cover slip with an average of one-third of each cell's membrane being fractured (*2*). Stroma proteins were numbered according to Fairbanks *et al.* (*12*). Abbreviations: *Hb*, hemoblogin; Hb_2, apparent hemoglobin dimer.

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to the cover slip were removed by three successive 25-second rinses in PBS. After the last rinse, excess buffer was allowed to drain, and the back of the slide was wiped clean with filter paper. The cover slip was carefully placed face down on the copper plate to minimize air bubbles and was immediately submerged in liquid Freon 22 at −160°C. The copper-cell-glass sandwich was then transferred to liquid nitrogen and fractured with a stainless steel single-edge razor blade. Frozen blood cells were lysed and rinsed off of the copper plate by immersion in a hypotonic sodium phosphate solution (7 m*M*, *p*H 7.4), and the resulting membranes were isolated by centrifugation at 27,000*g* for 10 minutes. Membranes were solubilized by boiling for 5 minutes in 0.5 ml of a solution of 2 percent sodium dodecyl sulfate (SDS), 2 percent β-mercaptoethanol, 8*M* urea, and 10 m*M* sodium phosphate, *p*H 7.4.

The cover slip was mostly colorless, indicating that the bulk of the cytoplasm, and hence the hemoglobin, remained with the copper plate. However, the edges nearly always had a pink hue, and there was an occasional pink spot in the middle of the glass. To remove this unfractured material, 2 to 3 mm of glass was broken off around the edges of the cover slip. Any other pink spots were absorbed on filter paper immediately on thawing. The cover slip was then refrozen and crushed at subzero temperatures. Boiling 0.1 percent SDS (30 ml) was added to the crushed cover slips (about 25 cover slips) and boiled for 5 minutes to solubilize the membrane components. The SDS solution was decanted from the cover glass and lyophilized. The freeze-dried membrane material was resuspended with a small volume of distilled water or hypotonic phosphate buffer (typically 0.1 to 0.3 ml) for analysis by SDS polyacrylamide gel electrophoresis.

A replica of a portion of a cover slip from a fracture sample is shown in Fig. 1. Portions of half-membranes, the E faces, from two cells are shown. Membrane edges are clearly defined and the region between the two cells is relatively free of artifactual bumps, which can form on a cold surface that is moved from atmospheric pressure to a vacuum evaporator and kept for many minutes before replicating. These were minimized by keeping the cover slip under liquid nitrogen until it had been placed inside the vacuum evaporator and shielded with a brass plate cooled to liquid nitrogen temperature. After the vacuum was less than 10^{-5} mm, the glass was warmed to about −80°C, the shield was removed, and the cover slip was shadowed with platinum at an angle of 18° ± 2° (uncertainty is due to the relatively large size of the cover slip). This was followed by a layer of carbon, which was evaporated from a source normal to the cover slip.

The bump density of these E faces was found to be 815 ± 140 per square micrometer. Although it may not be obvious what cell suspension medium, if any, is comparable to freezing on a polylysine cover slip, this value is within the range found for fracturing in some commonly used cell suspension media. Pricam *et al.* (*8*) reported densities of 262 to 1040 μm^{-2} for human erythrocytes and Engstrom (*9*) reported 700 to 1200 μm^{-2} for rabbit erythrocytes. In both cases, the highest value was obtained for unfixed cells in isotonic saline; cryoprotective agents, fixation, and reduced ionic strength all reduced the bump density of the E face.

Three samples from each experiment were analyzed by SDS electrophoresis: (i) stroma proteins made from a portion of unfractured and unfrozen cells, (ii) stroma proteins recovered from cells attached to the copper plates after fracturing, and (iii) proteins removed from the cover slips.

Since most of the Coomassie-stained proteins are present at the cytoplasmic aspect of the membrane (*10*), the cover slip sample should differ markedly from either the copper plate sample or the control sample of unfractured cells. Figure 2 shows that the copper plate and

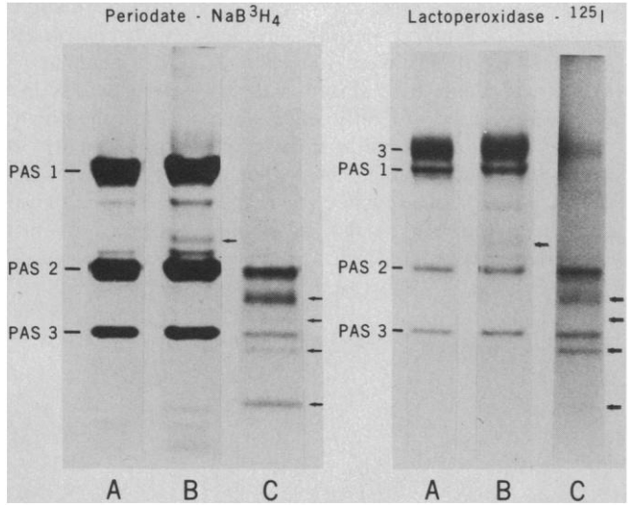

Fig. 3. Distribution of erythrocyte membrane polypeptides after freeze fracture of intact cells that had been labeled by either periodate oxidation and NaB^{3}H$_4$ reduction or lactoperoxidase-catalyzed iodination. After electrophoresis, labeled components were detected by fluorography (*23*). Labeled components of stroma isolated from unfractured red cells are in lane *A*; polypeptides derived from fractured membranes on the copper plates are in lane *B*; and labeled components recovered from the cover slip samples are in lane *C*. Arrows indicate additional labeled bands not observed in the unfractured control membrane samples. The protein in each sample is the same as in Fig. 2. To make comparison easier. the exposure times were varied for the two isotopes. The cover slip samples containing ^{3}H were exposed four times longer than the control of intact stroma, and the ^{125}I cover slip samples were exposed nine times longer.

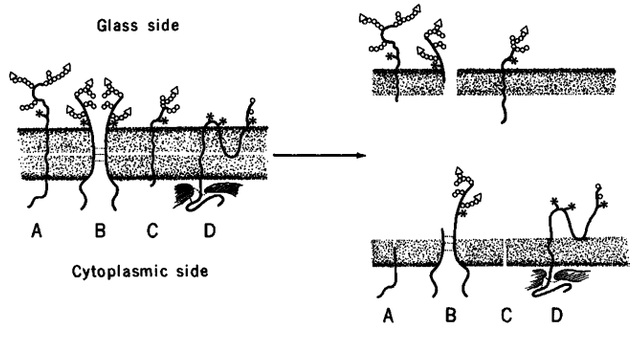

Fig. 4. Schematic of a portion of an erythrocyte membrane before and after freeze fracture. Monomeric forms of the sialoglycoproteins are represented by *A* and *C*, while *B* is a dimeric form (such as PAS 1). Molecule *D*, like protein 3, is bound to cytoplasmic proteins. (This is not intended to imply the actual state of aggregation within the membrane, but is used only for illustration.) Although the lipid bilayer readily splits during fracture, transmembrane proteins may be pulled intact to one side or the other of the membrane (*C* and *D*) or covalent bonds may be broken (*A* and *B*). Although it is not illustrated here, we do not exclude the possibility that lipid molecules remain attached to proteins that are pulled through the membrane. Dimeric or multimeric molecular aggregates of transmembrane proteins (*B*) may have some members split and others left intact. The triangles represent ^{3}H incorporated into sialic acid residues of sialoglycoproteins and the asterisks represent ^{125}I incorporated into the peptide by lactoperoxidase-catalyzed iodination.

control samples have qualitatively similar Coomassie-staining profiles, and the cover slip sample is different. Although the presence of hemoglobin in the cover slip sample indicates some contamination of the preparation with unfractured cells, little or none of the other cytoplasmic membrane proteins can be detected, indicating that protein contamination by that surface of the membrane was slight. The major proteins conclusively shown to be present at the external surface of red cells are protein 3 and the sialoglycoproteins (*5*, *10*, *11*). Only a small amount of band 3 is observed in the cover glass sample, and the sialoglycoproteins would not be detected, since they stain poorly with Coomassie blue (*12*).

The effect of freeze fracture on transmembrane proteins was studied by using radioactive isotopes. The external surface of intact cells was labeled by either lactoperoxidase-catalyzed iodination (*5*) or periodate oxidation followed by NaB^3H_4 reduction (*6*, *7*). The results are illustrated in Fig. 3. Membrane sialoglycoproteins are labeled by both methods, but band 3, the major membrane protein, is well labeled only by the lactoperoxidase method. The cover slip sample does contain labeled peptides, and the profile differs significantly from those of the unfractured control membrane and the copper plate sample. Sialoglycoproteins in the PAS 2 and PAS 3 regions are the only components clearly present in all three preparations.

The dimeric form of the major sialoglycoprotein, PAS 1, is absent from the cover slip, although the momeric form, PAS 2, is present. This may reflect the fact that in these experiments the sample was very dilute, favoring dissociation to the monomeric form (*13*). Band 3 is present in the cover slip sample in greatly reduced amounts relative to PAS 2 and PAS 3. Whether band 3 is actually contained in the outer fracture face or is a contaminant of the small amount of unfractured cells present on the cover slip is not clear. There is no doubt, however, that quantitatively, band 3 distributes disproportionately with the cytoplasmic surface of the membrane.

One of the most striking results with this sample was the detection of several new bands that are not found in the unfractured membrane. In the cover slip sample, two new bands are found between PAS 2 and PAS 3 and two additional bands are seen below PAS 3. The bands are detected by both lactoperoxidase iodination and periodate-NaB^3H_4 labeling, indicating that they are externally disposed portions of the sialoglycoproteins. It would thus appear that these new bands are generated by breakage of the peptide backbone during the freeze-fracture process as depicted in Fig. 4A.

A new band is also seen in the copper plate sample in the region between PAS 1 and PAS 2, as shown by the arrow in Fig. 3B. Since it is also labeled by both lactoperoxidase iodination and periodate-NaB^3H_4, it must be an altered product of the major sialoglycoprotein (*6*). That is, as shown in Fig. 4B, at least one of the two molecules that normally form the PAS 1 dimer has been ruptured by the freeze-fracture process, but the remaining aggregate still has a greater molecular weight than PAS 2. Although the break is shown to have occurred within the membrane in Fig. 4, it could also occur immediately to one side or the other of the membrane.

The lower-molecular-weight labeled bands could also have been generated by proteolytic degradation. However, care was taken to be sure that the fragmentation was not attributable to proteolytic enzymes in the preparations. White cell–derived proteases often contaminate erythrocyte stroma preparations, even when the buffy coat is carefully removed during red cell washing (*12*, *14*). To be certain that white cell contamination was minimized, fresh whole blood was passed over microcrystalline cellulose columns, which efficiently remove white cells (*15*). Cells prepared by this procedure still produced the new bands described above. In addition, inclusion of protease inhibitors such as iodoacetate (*16*) or phenylmethylsulfonylfluoride (*17*) had no effect on the results. Care was also taken to keep the samples frozen as much as possible. Cover slips were stripped of membranes by adding boiling SDS to the frozen slip and continuing the boiling for 5 minutes.

Two experiments were done to see if binding of poly-L-lysine to the erythrocyte membrane could be responsible for the new bands in the electrophoretic gel pattern: (i) polylysine was added to a suspension of erythrocytes, and (ii) erythrocytes were sandwiched between a polylysine-coated cover slip and a clean cover slip, frozen, but not fractured. The cells were hemolyzed in hypotonic phosphate in both cases, and the membrane proteins were isolated in the standard way (*5*) and compared to a control. No evidence of the new bands was observed in the gel patterns of these control samples.

Band 3 (*3*, *4*, *18*) and the sialoglycoproteins (*19*) are thought to be components of the intramembrane particles observed after freeze fracture of the erythrocyte membrane. The data presented here suggest that freeze fracture affects these transmembrane components in different ways. The predominant membrane protein, band 3, remains largely with the cytoplasmic half of the membrane. This may explain the higher bump density of the erythrocyte P face relative to the E face. It is also consistent with data suggesting that band 3 is associated with other proteins located at the cytoplasmic surface (*10*, *20*), which may serve to anchor band 3 in the membrane and prevent it from being "pulled through" with the external half of the membrane by fracturing. On the other hand, although there is some evidence for interaction of the sialoglycoproteins with cytoplasmic proteins (*21*), it is clear that the sialoglycoproteins do not interact with the cytoplasmic proteins as extensively as protein 3. Thus, the sialoglycoproteins may be more easily pulled through the cytoplasmic half of the lipid bilayer and remain with the outer surface of the membrane.

The fraction of sialoglycoproteins remaining with the outer surface may vary depending on the medium in which the sample is frozen. Since increased glycerol and decreased salt tend to decrease the bump density (*8*, *9*), it is possible that ionic bonds between the protein's sialic acid residues and the suspension medium may be important in determining how the sialoglycoproteins distribute between the membrane halves during freeze fracture.

The appearance of four smaller polypeptide fragments in the external half-membrane sample shows that sialoglycoproteins were fragmented and that freeze fracture can break covalent bonds. From these results, we conclude that the fragmentation of the sialoglycoproteins resulting from the freeze-fracture procedure is not confined to a single susceptible covalent bond but is due to cleavage at a number of points. The fact that fragments with well-defined molecular weights are generated indicates that the cleavage is not random, but at selected points. These cleavage points may be specific primary sequence sites, but they may also be points of greatest strain in the peptide chain and can therefore be varied by the protein conformation. It will be interesting and important to characterize these parts of the peptide chain.

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References and Notes

1. D. Branton, *Proc. Natl. Acad. Sci. U.S.A.* **55**, 1048 (1966); P. Pinto da Silva and D. Branton, *J. Cell Biol.* **45**, 598 (1970); T. W. Tillach and V. T. Marchesi, *ibid.*, p. 649.
2. K. A. Fisher, *Science* **190**, 983 (1975); *Proc. Natl. Acad. Sci. U.S.A.* **73**, 173 (1976).
3. J. Yu and D. Branton, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3891 (1976); J. P. Segrest, T. Gulik-Krzywichi, C. Sardet, *ibid.* **71**, 3294 (1974).
4. P. Pinto da Silva and G. L. Nicolson, *Biochim. Biophys. Acta* **363**, 311 (1974).
5. T. J. Mueller and M. Morrison, *J. Biol. Chem.* **249**, 7568 (1974).
6. T. J. Mueller, A. W. Dow, M. Morrison, *Biochem. Biophys. Res. Commun.* **72**, 94 (1976).
7. O. O. Blumenfeld, P. M. Gallop, T. H. Liao, *ibid.* **48**, 242 (1972); C. G. Gahmberg and L. C. Andersson, *J. Biol. Chem.* **252**, 5888 (1977).
8. C. Pricam, K. A. Fisher, D. S. Friend, *Anat. Rec.* **189**, 595 (1977).
9. L. H. Engstrom, thesis, University of California, Berkeley (1970).
10. T. L. Steck, *J. Cell Biol.* **62**, 1 (1974).
11. M. S. Bretscher, *J. Mol. Biol.* **58**, 775 (1971); D. R. Phillips and M. Morrison, *FEBS Lett.* **18**, 95 (1971).
12. G. Fairbanks, T. L. Steck, D. F. H. Wallach, *Biochemistry* **10**, 2606 (1971).
13. J. K. Tuech and M. Morrison, *Biochem. Biophys. Res. Commun.* **59**, 352 (1974); M. Silverberg, H. Furthmayr, V. T. Marchesi, *Biochemistry* **15**, 1448 (1976).
14. D. Kobylka *et al.*, *Arch. Biochem. Biophys.* **148**, 475 (1972).
15. E. Beutler, C. West, K. G. Blume, *J. Lab. Clin. Med.* **88**, 328 (1976).
16. R. Arnon, *Methods Enzymol.* **19**, 226 (1970); T. Nakai, P. S. Otto, T. F. Whayne, *Biochim. Biophys. Acta* **422**, 280 (1976).
17. J. R. Pringle, *Methods Cell Biol.* **12**, 149 (1975).
18. T. Bachi, K. Whiting, M. J. A. Tanner, M. N. Metaxas, D. J. Anstee, *Biochim. Biophys. Acta* **464**, 635 (1977); J. M. Wolosin, H. Ginsberg, Z. I. Cabantchik, *J. Biol. Chem.* **252**, 2419 (1977).
19. T. W. Tillack, R. E. Scott, V. T. Marchesi, *J. Exp. Med.* **135**, 1209 (1972); G. L. Nicolson, *J. Cell Biol.* **57**, 373 (1973); C. W. M. Grant and H. M. McConnell, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4653 (1974); T. J. Triche, T. W. Tillack, S. Kornfeld, *Biochim. Biophys. Acta* **394**, 540 (1975).
20. A. Elgsaeter, D. M. Shotton, D. Branton, *Biochim. Biophys. Acta* **426**, 101 (1976); S. C. Liu, G. Fairbanks, J. Palek, *Biochemistry* **16**, 4066 (1977).
21. G. L. Nicolson and R. G. Painter, *J. Cell Biol.* **59**, 395 (1973).
22. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
23. W. M. Bonner and R. A. Laskey, *Eur. J. Biochem.* **46**, 83 (1974).

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Amino Acid Sequence Homology Between Histone H5 and Murine Leukemia Virus Phosphoprotein p12

Abstract. *The amino terminal acid sequences of several mouse leukemia virus phosphoproteins (p12) show definite homology with the amino terminal conserved region of H5 histones, the phosphorylated nuclear proteins of nucleated erythrocytes. Differences in the amino acid compositions of the two groups of proteins seem to rule out the possibility that they evolved from a single common ancestral gene. The finding of sequence homology between viral p12's and cellular histones, however, is consistent with evolution of retrovirus structural proteins by a process of differentiation from preexisting cellular genes. The conserved primary and secondary structure at the amino terminal region, common to both groups of proteins, may be related to their common function of nucleic acid binding modulated by phosphorylation.*

Histone H5 is a phosphorylated nuclear protein found only in nucleated erythrocytes (*1*). Partial primary structure studies of several H5 proteins isolated from different avian sources indicate extensive amino acid sequence homology (*1-3*). In addition, H5 histones show definite homology with H1 histones isolated from a wide variety of sources (*4*). During erythropoiesis, H1 is replaced by H5 in the nucleus of the developing nucleated erythrocyte (*5*). Through the early stages of erythropoiesis, H5 is highly phosphorylated and becomes dephosphorylated as the erythrocyte matures. Dephosphorylation of H5 appears to coincide with chromatin condensation and genomic inactivation (*6, 7*). Although the precise function of H5 is unknown, it is probably involved in genomic regulation and may serve to maintain a highly repressed state of avian erythrocyte chromatin (*1, 7*).

Type C leukemia viruses contain a phosphoprotein (p19 in avian, p12 in murine, and p15 in viruses of primate origin) which has been shown to bind specifically to homologous viral RNA (*8-10*). A low level of phosphorylation is necessary for binding activity, but highly phosphorylated forms of the proteins do not bind to RNA (*10*). It has been postulated that these phosphoproteins may have a role in viral assembly and in regulation of transcription (*9-11*). Partial amino acid sequences have been determined for several p12 proteins isolated from murine viruses (*12, 13*). We now report that the amino acid sequences of conserved regions near the amino terminus of the leukemia virus p12 proteins show a distinct homologous relation to conserved regions near the amino terminus of the H5 histones.

In Table 1, the amino terminal amino acid sequences of H5 histones isolated from goose (*3*) and chicken (*2*), together with the amino terminal sequences of various leukemia virus p12 proteins (*12, 13*), are aligned to give a maximum number of identities. Chicken H5 is aligned with goose H5 by introducing a gap between residues 10 and 11 in the chicken sequence (*3*) (position 8 in the alignment, Table 1). The AKR mouse leukemia virus (MuLV) p12 sequence is aligned with the other p12 sequences by placing a gap in positions 8 and 9 in the AKR sequence (*12*). The H5 and p12 sequence regions of primary interest are residues 4 to 15 in the goose H5 and 1 to 12 in Moloney (Mo)-MuLV p12 (positions 1 to 12 in the alignment). These two sequences give six identities out of 12 residues without the introduction of a gap in either sequence. The introduction of a gap at position 13 in the alignment of the H5 sequences increases the number of positionally identical amino acids between

Table 1. Alignment of amino terminal amino acid sequences of type C leukemia virus p12 proteins to avian histone H5 proteins. Rauscher murine leukemia virus (R-MuLV), AKR murine leukemia virus (AKR-MuLV), and Moloney murine leukemia virus (Mo-MuLV) p12 sequences are aligned with goose and chicken histone H5 sequences with the introduction of gaps (- * -). Residues in italics are positionally identical when comparing at least one of the H5 histones to one of the p12's; residues in bold face are considered to be functionally homologous in the two groups of proteins. In the chicken H5, there are at least two allelic genes for chicken H5, giving either Arg or Gln at residue 15 (*24*), position 14 in the alignment (last line of the table).

Source	Protein	Reference	Sequence														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
R-MuLV	p12	(*12*)	*Pro*	**-Thr**	*-Leu*	**-Thr**	-Ser	-Pro	-Leu	-Asn	**-Thr**	*-Lys*	*-Pro*	**-Arg**	-Pro	*-Gln*	*-Val*
AKR-MuLV	p12	(*12*)	*Pro*	-Ala	*-Leu*	**-Thr**	*-Pro*	**-Ser**	-Leu	- *	- *	*-Lys*	*-Pro*	**-Arg**	-Pro	-Ser	**-Leu**
Mo-MuLV	p12	(*13*)	*Pro*	-Ala	*-Leu*	**-Thr**	*-Pro*	**-Ser**	-Leu	**-Gly**	*-Ala*	*-Lys*	*-Pro*	*-Lys*	-Pro	*-Gln*	*-Val*
Goose	H5	(*4*)	Thr-Asp-Ser	*-Pro*	-Ile	-Pro	**-Ala**	*-Pro*	**-Ala**	-Pro	**-Ala**	*-Ala*	*-Lys*	*-Pro*	*-Lys*	- *	**-Arg**	-Ala
Chicken	H5	(*4*)	Thr-Glu-Ser	-Leu	**-Val**	*-Leu*	**-Ser**	*-Pro*	**-Ala**	-Pro	- *	*-Ala*	*-Lys*	*-Pro*	*-Lys*	- *	*-Gln*	*-Val*

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