Protein Kinases and Membrane Protein Phosphorylation in Normal and Abnormal Human Erythrocytes: Variation Related to Mean Cell Age

By Grant Fairbanks, Jiri Palek, Judith E. Dino, and P. Aileen Liu

Protein kinase activities and membrane autophosphorylation reactions of normal and abnormal human erythrocytes were analyzed. Erythrocytes from patients with high reticulocytosis due to sickle cell anemia and other disorders (n = 13) exhibited elevated activities of total and membrane-bound cAMP-independent casein kinase and cAMP-stimulated histone kinase. Relative to normal controls (n = 10), the average total activities in these abnormal cells were increased 50% and 81%, respectively. The casein and histone kinase activities of normal and abnormal erythrocytes declined significantly with increasing age and buoyant density in Stractan density gradients. Casein kinase activity was highly correlated (r = 0.88; n = 23) with the percentage of reticulocytes in the fraction, consistent with either a progressive loss of activity in mature erythrocytes or an abrupt decline during reticulocyte maturation.

PHOSPHORYLATION of human erythrocyte membrane proteins has been studied in several laboratories for more than a decade,1,2 but its physiologic significance is still among the important unsolved problems in the field. In sickle cell disease, where repeated sickling and continuous exposure to the abnormal hemoglobin are associated with accelerated deterioration of cation transport functions3,7 and permanent distortion of the submembrane skeleton,3,7 membrane damage could, in principle, result from an induced abnormality in membrane protein phosphorylation. There are several reports that sickle cell membranes exhibit reduced phosphorylation of spectrin8,10 and increased phosphorylation of a polypeptide in the band 4.5 zone.9,10 Because of their potential significance in sickle cell pathophysiologic processes merit further consideration.

The experiments described in this article extend the description of red cell membrane protein phosphorylation in sickle cell anemia and other hematologic disorders. We have measured red cell protein kinase activities and membrane autophosphorylation reactions in unfractionated samples and in subpopulations varying in age. The results indicate that some activities of red cells from the patients are significantly increased because the cells are much younger, on average. At this level of analysis, red cell age-related variation in phosphorylation appears to predominate over changes that might be associated with specific disorders. Some of these observations have been reported elsewhere in preliminary form.11

MATERIALS AND METHODS

Cell and Membrane Preparation

Blood samples were obtained from clinic outpatients with homozygous sickle cell disease and from patients with comparable degrees of reticulocytosis due to autoimmune hemolytic anemia, paroxysmal nocturnal hemoglobinuria, acquired hemolytic anemia of unknown etiology, and vitamin B-12 deficiency responding to vitamin replacement therapy. Normal controls were volunteers from healthy laboratory and office personnel. All gave informed consent, and the procedures used were approved by the Human Research Subjects Committee of St. Elizabeth's Hospital.

Venous blood samples were collected in heparinized Vacutainers, chilled, and further processed within 2 hr. The red cells were washed with 5 mM sodium phosphate (pH 8)/0.15 M sodium chloride/0.25 mM dithioerythritol, as described previously,12 except that aspiration of the buffy coat was conservative to spare the subpopulation of young, buoyant cells. Hemolysis was performed by rapid dilution of washed, packed red cells into 29 vol of 5 mM sodium phosphate (pH 8)/1 mM EDTA/0.25 mM dithioerythritol at 37°C. Subsequent steps were performed at 0–4°C. The ghosts were washed once or twice with 5 mM sodium phosphate (pH 8), washed once with 20 mM sodium N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES, pH 7.5), and finally resuspended in 20 mM HEPES (pH 7.5) to twice the volume of packed cells hemolyzed.

Warm EDTA lysis has been reported to produce permanently leaky ghosts,13 and the complete (>90%) accessibility of membrane-bound glyceraldehyde-3-phosphate dehydrogenase was verified by
enzyme assay of representative ghost preparations from unfractionated red cells and cell subpopulations from Stractan density gradients.

Cell Fractionation in Density Gradients

After conservative aspiration of overlying white cells, the first red cell pellet was resuspended and washed 3 times with 5 mM sodium phosphate (pH 7.4)/0.15 M sodium chloride. These preparations were fractionated by the method of Corash et al. using discontinuous Stractan density gradients containing 10 mM HEPES plus sodium chloride added to maintain isotonicity. The conditions for resolving irreversibly sickled cells from the majority fraction of more buoyant, reticulocyte-enriched red cells from sickle cell patients were described previously. In several experiments, the top 5%-10% fraction of normal and abnormal cells was banded on Stractan layers of specific gravity 1.06–1.08. An upper zone of specific gravity 1.047 was used to float, and remove, white cells.

Bands of red cells recovered from the gradients by aspiration were diluted with buffered saline and pelleted. Following a single washing with phosphate-buffered saline (pH 8) containing 0.25 mM dithioerythritol, the packed cell volumes were measured, and ghosts were prepared as described above. The relative amount of each gradient fraction was expressed as the ratio of its hemoglobin content to the total hemoglobin recovered from the gradient.

Protein Kinase Assays

Aliquots of hemolysates, hemolysate supernatants, and resuspended ghosts were diluted into 3 vol of a storage buffer consisting of 10 mM sodium phosphate (pH 7.5), 0.15 mM magnesium chloride, and 1.4 mg/ml bovine serum albumin in 0.3 M sucrose. These samples were frozen. They were normally assayed within the first week after their preparation, but in frozen form retained activity for more than 1 yr.

The protein kinase assay was briefly described previously. In these experiments, 0.025 ml of sample (diluted 1/120 relative to the original packed red cells) was mixed with an equal volume of 0.2% saponin (Matheson, Coleman and Bell, Norwood, OH, no. SX79 CB1089) in a chilled tube. The assays were initiated by adding 0.05 ml of reaction mixture concentrate containing 0.05–0.2 μCi of γ-32P-ATP (New England Nuclear, Boston, MA, no. NEG-002X), 0.5 mg casein (Miles Laboratories, Kankakee, IL, Pentex no. 96-005-1), or histone (Sigma Chemical Co., St. Louis, MO, no. H-9250), 30 mM hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES), pH 7.5, 10 or 20 mM magnesium acetate, and 0.1 or 0.5 mM unlabeled ATP (Sigma A-3127). Casein kinase assay mixtures contained 0.35 or 0.5 mM sodium chloride; histone kinase reaction mixtures contained 5 μM cAMP without added salt (final concentrations). During the investigation, assay conditions were adjusted to optimize protein kinase activities and recoveries: increasing the Mg2+ concentration in all assays and reducing the amount of salt added with casein did not significantly alter the activity patterns or differences between values for samples prepared and assayed simultaneously.

The 32P-nucleotide specific activities were determined by counting aliquots of the reaction mixtures. Protein kinase activities were then calculated in units of picomoles 32P incorporation per hour and normalized to the hemoglobin contents (mg) of the equivalent volumes of the corresponding hemolysates. Nearly all values reported are the means of duplicates that differed from each other, on average, by 9% and 12% for casein and histone kinase activities, respectively.

Fig. 1. Protein kinase activities in normal control red cells, sickle cells, and red cells from other patients with reticulocytosis. (A) Total hemolysate cAMP-independent casein kinase. (B) Total hemolysate cAMP-dependent histone kinase. (C) Membrane-associated casein kinase. (D) Membrane-associated histone kinase. Triangles—values from assays with 5 mM Mg2+, 0.05 mM ATP, 500 mM NaCl; circles—5, 0.05, 350 mM; squares—10, 0.25, 350 mM. Diff—differences (patient minus normal control) of paired values for samples prepared and assayed simultaneously.

In a control experiment, white cell contamination was reduced by cellulose filtration and augmented by selection of the buffy-coat-enriched top fraction of sedimented red cells. Comparison of the protein kinase activities in these preparations revealed no consistent differences, and it is unlikely that white cells contributed significantly to the values reported here. A sham Stractan fractionation was also performed to demonstrate that exposure to the density gradient medium at various concentrations does not alter the activities or partitioning of the red cell protein kinases.

Analysis of Membrane Autophosphorylation Reactions

The membrane autophosphorylation reactions were studied by incubating freshly prepared ghosts with γ-32P-ATP (New England...

![Image](https://example.com/image.png)
Nuclear NEG-002X) for 20 or 60 sec at 37°C in a medium containing 5 mM magnesium acetate with 20 mM HEPES (pH 7.5), and with ATP at 5, 50, or 100 M, as indicated. In two experiments, autophosphorylation during 60-sec incubations with 50 μM γ-32P-GTP (New England Nuclear NEG-004) was also analyzed.

The membranes were preincubated for 2 min at 37°C in the assay medium in the presence or absence of 10 μM cAMP prior to addition of the labeled nucleotide. The volume of the reaction mixtures was approximately 0.6 OD x mm. Rates of 32P labeling were calculated in picomoles 32P per hour. The spectrin phosphorylation was normalized to spectrin stain intensity, while other phosphorylations were expressed relative to stain in band J plus band 4.

For higher resolution analysis of the autophosphorylation patterns, the 32P-labeled proteins were fractionated in gradient slab gels using the Maizel/Laemmli discontinuous buffer system. Autoradiography of dried slices was performed as described previously.

### Other Methods

Reticulocytes were counted as percentages of total cells in stained smears of washed cells resuspended to 50% hematocrit. Unstained smears were examined for white cell contamination. Hemoglobin concentrations in hemolysates were determined by measuring absorbances at 540 nm after conversion to cyanmethemoglobin.

In statistical analysis of the protein kinase data, activity values were grouped by donor type—normal controls, sickle cell patients, other patients, all patients (sickle plus others). The significance of differences in the means of these samples was estimated using the two-sample t test. Data from experiments in which fractions from other patients, all patients (sickle plus others) were assayed at the same time were also subjected to the one-sample t test using differences.

### RESULTS

#### Protein Kinase Activities of Unfractionated Red Cells

The protein kinase activities of total hemolysates and ghosts of unfractionated red cells are plotted in Fig. 1. Most samples from sickle cell patients and other patients with extreme reticulocytosis exhibited elevated activities, but the mean values for the two patient samples were grouped by donor type—normal controls, sickle cell patients, other patients, all patients (sickle plus others). The significance of differences in the means of these samples was estimated using the two-sample t test. Data from experiments in which fractions from other patients, all patients (sickle plus others) were assayed at the same time were also subjected to the one-sample t test using differences.

### Table 1. Total and Membrane-Bound Protein Kinase Activities

<table>
<thead>
<tr>
<th>Sample</th>
<th>Activity (nmole/hr x mg Hb ± SD)</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B &gt; C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SED</td>
</tr>
<tr>
<td>Cassein Kinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membranes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A) Normal controls</td>
<td>10</td>
<td>2.0 ± 0.63</td>
</tr>
<tr>
<td>(B) Patients (HbSS)</td>
<td>8</td>
<td>3.3 ± 1.0</td>
</tr>
<tr>
<td>(C) Patients (Other)</td>
<td>5</td>
<td>4.2 ± 1.7</td>
</tr>
<tr>
<td>(D) Patients (All)</td>
<td>13</td>
<td>3.7 ± 1.3</td>
</tr>
<tr>
<td>Membranes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A) Normal controls</td>
<td>10</td>
<td>0.98 ± 0.50</td>
</tr>
<tr>
<td>(B) Patients (HbSS)</td>
<td>8</td>
<td>1.7 ± 0.97</td>
</tr>
<tr>
<td>(C) Patients (Other)</td>
<td>5</td>
<td>2.5 ± 0.73</td>
</tr>
<tr>
<td>(D) Patients (All)</td>
<td>13</td>
<td>2.0 ± 0.94</td>
</tr>
</tbody>
</table>

Means ± standard deviations (SD) for values plotted in Fig. 1 are tabulated. Significance of differences between sickle cell and other patients, and between all patients and normal controls, was evaluated by the two-sample t test; standard errors of differences (SED) and p values are given.
subpopulations do not differ significantly (Table 1). Analysis of the pooled data for all patients, in relation to normal controls, demonstrated a 50% elevation in total red cell cAMP-independent casein kinase activity and an 81% elevation in cAMP-dependent histone kinase activity. These increments are statistically significant. The percentage increases in membrane-associated activities are comparable (within experimental error), suggesting that the additional enzyme in red cells from patients behaved normally with respect to its partitioning between membranes and cytoplasm.

The elevation in protein kinase activities of red cells from patients was also found to be significant by applying the one-sample t test using differences of paired values (Fig. 1) obtained in nine experiments in which patient and control samples were assayed at the same time. This established that the differences in the means of the distributions in Fig. 1 cannot be attributed to differences in the number of assays performed under each condition.

**Age Dependence of Protein Kinase Activities**

The foregoing observations indicated that red cell protein kinase activities are elevated under various conditions in which reticulocytes and young mature red cells predominate in the circulation, suggesting that the protein kinases, like other red cell enzymes, might suffer loss in activity or be partially eliminated during reticulocyte maturation and in vivo aging. This possibility was investigated by analyzing red cell subpopulations obtained by centrifugation of blood samples on Stractan density gradients. As illustrated in Fig. 2, the most buoyant, reticulocyte-enriched fractions from the tops of the gradients exhibited the highest activities, whereas the activities in the densest, reticulocyte-depleted, bottom fractions were significantly lower. The cAMP-independent casein kinase activity usually decreased in a monotonic fashion with increasing age and buoyant density, and, in 14 trials in which cells from patients and controls were resolved on gradients under various conditions, the average decrease in total cellular activity from top to bottom was 25%. Parallel decreases in the membrane-bound activity were observed (Fig. 2).

Age-dependent change in the cAMP-dependent histone kinase activity was also demonstrable, but the results were more variable and, in some cases, the histone kinase activity was lower in intermediate fractions than in the bottom fraction (data not shown).

The fractions from six Stractan gradients were analyzed for their content of reticulocytes as well as for protein kinase activities. As shown in Fig. 3, the cAMP-independent casein kinase activity of these samples increased more-or-less linearly with the percentage of reticulocytes in the range 5%-90% reticulocytes (coefficient of correlation, \( r = 0.88 \) for total activity, \( r = 0.80 \) for membrane-associated activity; \( n = 23 \)). This suggests that, if the reticulocytes have 2–3-fold higher levels of casein kinase activity than mature red cells, variation in reticulocyte content might be largely responsible for both the higher activities of unfractionated samples from patients and the decline in activity with increasing buoyant density.

**Autophosphorylation Reactions**

The initial rates of protein phosphorylation in membranes from red cell fractions of 12 Stractan density gradients were measured. As illustrated in Fig. 4, the

![Fig. 2. Casein kinase activities in hemolysates and isolated membranes from red cells fractionated on Stractan gradients. Hemolysates: (A) normal, (C) sickle cell, (E) treated B-12 deficiency. Membranes: (B) normal, (D) sickle cell, (F) treated B-12 deficiency. Numbers in parentheses in (A) and (C) are percentages of reticulocytes in the fractions.](image-url)
major phosphopeptides resolved by autoradiography of gradient slab gels are bands 2, 2.1 (and subfragments of 2.1), 3, 4.1, 4.8, and 4.9; and all exhibited reduced labeling with increasing red cell age and buoyant density. The decline of phosphorylation was monotonic in the majority of gradients (8 of 12 for spectrin, 6 of 12 for the band 4.5 zone). The autophosphorylation activity of membranes from the top 5%-10% always exceeded that of the bottom 5%-10%, and for spectrin phosphorylation, the average change from top to bottom was ~36%.

Analysis of the autophosphorylation data for membranes from unfractionated red cells demonstrated that the rate of cAMP-independent spectrin phosphorylation in samples from patients was within the normal range (Table 2). This finding contrasts with the results for membrane-associated casein kinase activity presented above. The data in Table 2 also indicate that the rate of phosphorylation in the band 4.5 zone was elevated in membranes from sickle cells and from red cells of patients undergoing vitamin B-12 replacement therapy for vitamin B-12 deficiency.

In two experiments, autophosphorylation during incubation with γ-32P-GTP was analyzed to define the patterns of cAMP-independent phosphorylations in the absence of background due to basal activity of cAMP-activated protein kinase(s). The decline in activity with increasing cell age was clearly seen in the GTP-utilizing reactions (Table 3, Fig. 4). The increased band 4.5 zone phosphorylation in sickle cell membranes was attributable primarily to increased incorporation into band 4.8 (mol wt ~47,800; Fig. 4 and densitometric data not shown).

DISCUSSION

Labeling of intact cells by 32P-orthophosphate is the most valid approach to the comparative analysis of
unfractionated red cells of 7 normal control donors, 5 sickle cell patients, and 2 vitamin-B-12-deficient patients were prepared in 8 experiments.

Table 2. Autophosphorylation Reactions in Membranes of Unfractionated Red Cells

<table>
<thead>
<tr>
<th>ATP Concentration (μM)</th>
<th>Cell Sample</th>
<th>Spectrin</th>
<th>-cAMP</th>
<th>+cAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Normal controls</td>
<td>14.4 ± 4.4 (4*)</td>
<td>1.8 ± 0.5 (4*)</td>
<td>5.6 ± 1.9 (3*)</td>
</tr>
<tr>
<td>Patients (HbSS)</td>
<td>13.2 ± 3.3 (4*)</td>
<td>3.4 ± 1.3 (4*)</td>
<td>9.5 ± 1.9 (3*)</td>
<td></td>
</tr>
<tr>
<td>50, 100</td>
<td>Normal controls</td>
<td>21.8 ± 5.9 (4)</td>
<td>4.8 ± 2.9 (4)</td>
<td>12.2 ± 3.7 (4)</td>
</tr>
<tr>
<td>Patients (HbSS)</td>
<td>25.7 ± 0.3 (2)</td>
<td>11.4 ± 0.5 (2)</td>
<td>22.8 ± 1.9 (2)</td>
<td></td>
</tr>
<tr>
<td>Patients (PA + B-12)</td>
<td>20.3 ± 0.7 (2)</td>
<td>7.7 ± 2.2 (2)</td>
<td>25.1 ± 16.0 (2)</td>
<td></td>
</tr>
</tbody>
</table>

Autophosphorylation reactions were analyzed at low (5 μM) and intermediate (50 or 100 μM) concentrations of γ-32P-ATP. Ghosts from unfractionated red cells of 7 normal control donors, 5 sickle cell patients, and 2 vitamin-B-12-deficient patients were prepared in 8 experiments.

Table 3. GTP-Utilizing Autophosphorylation Reactions

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Cell Sample</th>
<th>Relative Amount</th>
<th>Spectrin 4.5 Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Normal control</td>
<td>Unfractionated</td>
<td>1.0 2.9 0.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Top</td>
<td>0.12 3.6 1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>0.68 2.2 1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>0.07 1.7 0.72</td>
<td></td>
</tr>
<tr>
<td>Patient (HbSS)</td>
<td>Unfractionated</td>
<td>1.0 3.9 2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Top</td>
<td>0.67 3.9 2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>0.16 3.9 1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>0.06 3.0 1.7</td>
<td></td>
</tr>
<tr>
<td>2 Normal control</td>
<td>Unfractionated</td>
<td>1.0 3.1 0.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Top</td>
<td>0.14 4.3 1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>0.62 3.9 1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>0.08 3.2 0.94</td>
<td></td>
</tr>
<tr>
<td>Patient (PA + B-12)</td>
<td>Unfractionated</td>
<td>1.0 4.5 1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Top</td>
<td>0.04 5.1 1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>0.69 3.9 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>0.08 3.2 1.4</td>
<td></td>
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</tbody>
</table>

Membranes from the cell samples, prepared as described in Table 2, were incubated with 50 μM γ-32P-GTP under autophosphorylation conditions.

steady-state phosphorylation of red cell membrane proteins. However, measurements of the autophosphorylation rates in isolated membranes incubated with γ-32P-ATP or GTP are much more easily made, and if major artifacts are controlled and ancillary information obtained, the results may be useful in defining the kinetics of the reactions and in probing the membrane environment in which the enzymes and their substrates interact. In our experiments, we used leaky ghosts to ensure that the labeled substrate would be available continuously, and we measured the initial labeling rates in brief incubations to minimize interference due to depletion of substrate, dephosphorylation, or ATP-dependent membrane reorganization. We also measured the activities of the protein kinases against soluble exogenous substrates presented in excess. When this approach was applied to fractionated red cells, we found that both protein kinase activities and membrane autophosphorylation reactions were elevated in young cells, and both decreased with increasing buoyant density in Stractan gradients.

It is possible, in principle, to evaluate the contribution of reticulocytes to these patterns by direct analysis of affinity-purified reticulocyte samples from normal blood. But, because the Stractan gradient technique yields incomplete resolution and buoyant density is not perfectly correlated with red cell age, our results do not definitively exclude any of the following models: (1) elevated activities in reticulocytes with reduced, but stable, activities thereafter; (2) progressive decline in activities throughout the red cell lifespan in vivo; and (3) reduction in activities due to cellular dehydration per se or to closely associated processes not directly related to red cell aging.

In our experiments, the rate of spectrin phosphorylation in membranes from the majority population of sickle cells appeared to be normal. This is not necessarily inconsistent with the earlier reports of reduced spectrin phosphorylation, because we prepared ghosts by warm EDTA hemolysis, used brief incubations, and normalized the incorporations to spectrin staining intensity. Hosey and Tao first reported that the cAMP-dependent phosphorylation reactions in isolated
sickle cell membranes yielded significantly increased labeling in the band 4.5 zone. We have confirmed this result, with resolution of band 4.8 (mol wt ~47,800) as the preferred substrate; and Dzandu and Johnson have detected elevated steady-state labeling in the same zone. A straightforward explanation for these results is that more of the band 4.8 protein may be present in membranes from sickle cells and other populations in which very young cells predominate. An earlier report presented a comparison of the phosphopeptide labeling patterns of the whole hemolysate, hemolysate supernate, and washed ghosts from normal red cells labeled with 32P-orthophosphate. In the present context, it is suggestive that a cytoplasmic phosphopeptide migrates to the position of band 4.8, just above the membrane phosphopeptide now denoted 4.9 ('4.8'' in reference 2). Because of the problem of membrane retention of cytoplasmic proteins, the possibility that the increased labeling of band 4.8 simply reflects altered membrane–cytoplasm partitioning (which may or may not be significant physiologically) needs to be considered.

The results do not reveal any significant impairment in the spectrin phosphorylation apparatus of sickle cell membranes. When it is considered that these cells are chronologically young and endowed with relatively high casein kinase activity, spectrin phosphorylation in their membranes appears relatively inefficient. However, the finding of comparable autophosphorylation rates associated with reticulocytosis in vitamin-B-12-deficient patients suggests that the additional casein kinase activity of the ghosts may be distinct functionally from spectrin kinase or limited in its expression by nonspecific factors. In this and earlier reports, apparent membrane alterations in abnormal red cells have been ascribed to accelerated erythropoiesis and reticulocytosis induced by anemia, and it is widely recognized that this presents a serious problem in comparative studies directed at the characterization of specific membrane lesions associated with hemolytic anemias. In the case of sickle cell disease, our results suggest that, while possible alterations in membrane autophosphorylation may still be of interest, the features recognized in previous work are probably not related to the specific red cell abnormality.

ACKNOWLEDGMENT

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Protein kinases and membrane protein phosphorylation in normal and abnormal human erythrocytes: variation related to mean cell age

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