The Role of the Nuclear Protein Matrix During Development of Rabbit Granulocytes

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A proteinaceous nuclear substructure (nuclear protein matrix or nuclear pore complex-lamina) has been described in a number of cells and may be a universal feature of cell nuclei. We have investigated the nuclear protein matrix (NPM) in the rabbit blood granulocyte and its precursor cells to determine (A) whether the NPM composition is similar to other cells that have been studied and (B) to determine whether the dramatic morphological changes that the granulocyte nucleus undergoes during cell maturation are related to changes in the composition or structure of the NPM. NPM preparations from rabbit granulocytes were similar but not identical to those found in HeLa cells or rat liver nuclei. The NPM structure of more mature cells retained more DNA during the isolation procedures than did immature cell NPMs, and the DNA was less accessible to DNase in the mature cell nuclei. Scanning and transmission electron microscopy revealed a continuous outer covering of the NPM preparation and a lattice-like internal structure. Recognizable nuclear form persisted although the preparations represented less than 20% of the original nuclear protein. NPM preparations from mature cells were similar in overall dimensions and internal structure to NPM from immature cells, suggesting reexpansion during isolation of a previously compacted NPM structure in segmented neutrophils. NPM proteins are synthesized primarily in early stages of cell development. The NPM appears to play a major, but passive, structural role in the nuclear changes observed during maturation of granulocytes.

As the neutrophil leukocyte (granulocyte) differentiates in bone marrow from its primitive precursor, a number of striking morphological and biochemical changes take place. While there is a progressive loss of mitochondria, ribosomes, and Golgi complex, there are increasing numbers of enzyme-laden granules and glycogen particles. In addition, the large nucleolated nucleus with a fine euchromatin pattern gives way ultimately to a densely pyknotic nucleus composed of several segments connected by thin strands of nuclear material. The mechanisms by which these striking nuclear changes take place are unknown, but are believed to be intimately associated with a progressive diminution of gene expression and therefore pertinent to the understanding of the process of control of gene expression required for normal granulocyte differentiation.

The structure of the granulocyte nucleus and its changes during development have not been studied extensively. The structural foundation for nuclear form may be a fibrous protein skeleton, which has been described for several cell types. In 1947 Mirsky and Ris first reported such a proteinaceous "residual chromosome." Aaronson and Blobel later described the somewhat similar structure they call the nuclear pore complex-lamina. Berezney and Coffey isolated a residual structure from rat liver nuclei that they termed the nuclear protein matrix (NPM). Similar structures have been found in avian erythrocytes, HeLa cells, and in Tetrahymena macronuclei. Analyses of the polypeptide composition of these nuclear skeletons demonstrate that at least some polypeptides appear to be common to all of them. Although the molecular weights of the polypeptides may differ, the predominant species are often found in the 60-80,000 molecular weight range. Analysis of the residual nonhistone protein fraction in rabbit granulocytes also demonstrated the existence of similar proteins. For the granulocyte, it would be of interest to know whether this distinct cell has a similar NPM, and further, it would be valuable to study the NPM during the process of cellular differentiation to determine whether alterations in NPM contributed to the marked morphological changes that take place in the developing granulocytes.

Mechanisms by which a NPM may take part in the pyknosis and segmentation of the nucleus could include (A) progressively increasing crosslinking of matrix strands, (B) increasing synthesis of a more dense matrix, or (C) some form of contraction of the existing matrix during nuclear maturation. In this study we have isolated NPM preparations from purified nuclei of granulocytes from fractions enriched in cells of various stages of maturation and have performed biochemical and ultrastructural analyses of these residual structures.

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Supported in part by NIH Grants CA 08482-14, CA 15923-04, and AI 13486.

Presented in part at the American Association for Cancer Research May 18, 1979.

Submitted March 17, 1980; accepted December 8, 1980.

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0006-4971/81/5704-0019$02.00/0
MATERIALS AND METHODS

Preparation and Separation of Granulocyte Precursors

Bone marrow suspensions were obtained from male white New Zealand rabbits as previously described. Briefly, marrow was suspended in cold buffered balanced salt solution (BBSS) containing 6 mM EDTA and 15% fetal calf serum. Erythrocytes and their precursors were removed according to the method of Fallon et al. Granulocyte precursors were then separated on continuous Ficoll-Hypaque density gradients according to differences in their cellular buoyant density. Young cells are less dense and are found at the top of the gradient, while the more mature cells are found primarily at the bottom of the gradient. After centrifugation, the gradient was fractionated using an Isco 185 Density Gradient Fractionator. Then equal fractions were collected and pooled to give three final samples; the top, middle, and bottom.

Preparation of a Peritoneal Exudate

Peritoneal exudate cells were prepared as previously described. Male white New Zealand rabbits were given a sterile intraperitoneal injection of 300-500 ml of 0.1% glycogen in 0.9% sterile saline. After 18-20 hr later, the exudate was clarified by pouring through sterile gauze and the cells washed and suspended in BBSS. Cells were counted in a Fisher Autocytometer.

Preparation of Nuclei

Purified nuclei were obtained from both the gradient fractions and the peritoneal exudate cells by a modification of the method of Stein and Borun. Cells were suspended in 1% Triton solution containing 80 mM NaCl and 20 mM EDTA (pH 7.4). The cell suspension was then homogenized using a “no-clearance” homogenizer. Nuclei were then washed once with the Triton solution and once with BBSS. Nuclei were examined for cytoplasmic contamination both by phase-contrast and electron microscopy.

Nuclear Protein Matrix Preparations

Nuclear matrix preparations were made according to the method of Dodge et al. with slight modification. Triton-cleaned nuclei were suspended by pipetting in high salt buffer (HSB) containing 0.01 M Tris-HCl (pH 7.4), 0.5 M NaCl, and 0.05 M MgCl2 at a concentration of 2 x 10⁶ nuclei/ml. This mixture was digested with 50 μg/ml of DNase I (Workington Biochemicals) for 30 min at 37°C. The NPM preparations were then collected by centrifugation at 600 g for 10 min at 4°C and then resuspended by pipetting in 0.01 M Tris-HCl (pH 7.4) containing 0.01 M mercaptoethanol, 0.01 M NaCl, and 0.01 M EDTA (NEB). The suspension was then incubated at 37°C for 30 min. The NPM preparations were centrifuged as before and suspended in 2 ml of 0.01 M Tris-HCl (pH 7.4) containing 1 mM EDTA (TE) and sedimented through a discontinuous sucrose gradient onto a 60% sucrose cushion.

Nuclear matrix preparations were also obtained according to the method of Berenzon and Coffey with modifications. Purified nuclei were resuspended in 50 mM Tris-HCl buffer (TM) (pH 7.4) at a concentration of 10⁶ nuclei/ml at room temperature for 15 min, then centrifuged at 600 g for 10 min. The resulting pellet was then suspended in a low Mg (0.2 mM MgCl2) buffer (pH 7.4) to a concentration of 2 x 10⁶ nuclei/ml. After incubation on ice for 10 min, the suspension was centrifuged at 600 g for 10 min. The pellet obtained was resuspended in a high salt buffer containing 2 M NaCl, 0.2 mM MgCl2, and 10 mM Tris-HCl (pH 7.4) at a concentration of 1 x 10⁷ nuclei/ml. After 10 min on ice, the suspension was centrifuged at before. The resulting pellet was then suspended in TM buffer to a concentration of 2 x 10⁷ nuclei/ml and Triton X-100 in TM buffer added to a final concentration of 1%. The suspension was kept on ice for 10 min and centrifuged as before. This pellet was then resuspended in TM buffer to a concentration of 2 x 10⁷ nuclei/ml and DNase and RNase (200 μg/ml) added. The sample was digested at room temperature for 60 min and then centrifuged as before.

35S-Methionine Incorporation

Synthesis of matrix protein was measured by 35S-methionine incorporation. Whole cells from the three gradient fractions and the exudate sample were suspended in an amino acid mixture lacking methionine. 35S-methionine was added at a concentration of 3 μCi/ml with a specific activity of 562.21 Ci/m mole and a cell concentration of 5 x 10⁶ cells/ml. Preliminary experiments indicated that this concentration was adequate for optimum protein synthesis. After incubation the samples were washed one time with cold medium and one time with BBSS prior to use in matrix preparations.

For counting, 50-μl aliquots of samples were plated on copper planchets and duplicate samples counted in a low background gas flow counter.

Electron Microscopy of the NPM

Samples were prepared for transmission electron microscopy by fixation in 3% glutaraldehyde in 0.01 M TE buffer and postfixed in 1% OsO4 in 0.1 M Tris buffer (pH 7.4). Samples were embedded in resin according to the method of Spurr. Ultrathin sections were stained with uranyl acetate and lead citrate. Samples were observed and photographed with a Hitachi HU-12 electron microscope.

Samples were prepared for scanning electron microscopy by fixation and postfixation as described for transmission electron microscopy. Samples were resuspended in 100% acetone and placed on a cover glass and coated using an Eiko Engineering IB-2 ion coater. Samples were observed and photographed with a Hitachi S-500 Scanning Electron Microscope.

Electrophoresis of Matrix Protein

Matrix proteins were electrophoresed in slab gels containing SDS according to the method of McGuire et al. Samples were dissolved in 1% SDS, 10% glycerol, 1% pyronin Y, 1% mercaptoethanol, and 0.05 M Tris-HCl (pH 6.8) and electrophoresed at 125 V until the dye band migrated to the bottom of the gel. Gels were stained for 30 min in 0.5% Coomassie Blue R, 25% isopropanol, and 10% acetic acid. Destaining was accomplished using a 10% acetic acid, 10% methanol solution. Slab gels were scanned at 550 nm in a Corning Microdensitometer.

Fluorography Treatment of Slab Gels

Slab gels containing radioactive methionine were prepared for fluorography by the method of Bonner and Laskey. Gels were treated with dimethyl sulfoxide for 30 min, the solution removed, and the procedure repeated once more. Gels were then treated with 22% PPO in DMSO for 1 hr and then soaked in 10% HAc, 5% MeOH for 1 hr. The treated gel was then dried using a Bio Rad drying apparatus. The dried gel was then placed on Royal X-O Mat film and placed at −70°C for varying periods of time depending on the amount of radioactivity in the sample. After the film was developed, it was scanned with a Corning microdensitometer.

Biochemical Determinations

Protein was determined by the method of Lowry et al. using bovine serum albumin standards and making appropriate correc-
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DNA and protein analysis. The results of this type of extraction are presented in Table 2. With this method, the extraction of nuclear protein was more complete, resulting in NPM preparations consisting of 12%–15% of the original nuclear protein. Although the lowest figures for recovered protein were found in the less mature granulocyte precursors, these differences were not statistically significant.

The residual DNA content, however, was higher than that reported in similar preparations from HeLa cell or rat liver nuclei 1,2 and higher than that found in the Berezney and Coffey-type preparations of granulocytes (Table 1). In the granulocyte precursors, the residual DNA content was 26.0% in the least mature granulocyte precursors, and progressively increased to 65.4% in mature granulocytes. When these preparations were subjected to a fourfold increase in the concentration of DNase I, the amount of residual DNA in the NPM preparations decreased in all cases, but not to the low levels found in other cell types. At the same time, there was no change in residual protein concentration. It is evident that as the granulocyte nucleus matures and becomes more condensed and inactive, the DNA most closely associated with the nuclear protein matrix becomes less and less accessible to the exogenous nuclease.

Electron Microscopy of the Nuclear Protein Matrix

The NPM preparations isolated according to the method of Hodge et al. contain much residual DNA

<table>
<thead>
<tr>
<th>Sample</th>
<th>DNA (Low DNase Concentration)*</th>
<th>DNA (High DNase Concentration)*</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top gradient fraction</td>
<td>26.0 ± 10.2 ±</td>
<td>20.1 ± 5.9</td>
<td>12.1 ± 2.5</td>
</tr>
<tr>
<td>Middle gradient fraction</td>
<td>34.0 ± 13.5</td>
<td>30.7 ± 4.7</td>
<td>13.4 ± 3.8</td>
</tr>
<tr>
<td>Bottom gradient fraction</td>
<td>44.4 ± 18.1</td>
<td>23.8 ± 2.3</td>
<td>14.2 ± 3.1</td>
</tr>
<tr>
<td>Peritoneal exudate cells</td>
<td>65.4 ± 16.1</td>
<td>16.4 ± 3.3</td>
<td>15.0 ± 2.0</td>
</tr>
</tbody>
</table>

*Low DNase concentrations (50 μg/ml), high DNase concentration (200 μg/ml).
†All figures indicate mean percent of original nuclear DNA or protein ± SE (n = 4).
but only 12%–15% of the original nuclear protein. If the remaining small portion of protein is mostly structural protein, then electron micrographs should demonstrate some form of structure resembling an intact nucleus. This assumption was tested by both transmission and scanning electron microscopy of similar NPM preparations.

Scanning electron micrographs of nuclei that had been exposed to Triton, DNase, and high salt treatments are shown in Fig. 1. It is evident that many nuclei are still completely intact spherical or “segmented” structures. Figure 1A shows preparations from the lower portion of the density gradient containing mostly band and segmented forms of nuclei. Figure 1B is from a top fraction of the density gradient, including mostly immature forms (promyelocytes and myelocytes). It also appears that there is a continuous nuclear surface. No cytoplasmic contamination can be recognized. No fibrillar structure is evident in views of the surface of these preparations.

In an effort to determine whether some Triton-insoluble nuclear membrane lipids accounted for the apparently continuous surface, nuclear preparations were treated additionally with the ionic detergent, sodium deoxycholate, and further scanning electron micrographs taken. As shown in Fig. 1C and D there is little appreciable difference in the apparently continuous surface features of the nuclear protein matrix preparation with (Fig. 1D) and without (Fig. 1C) additional detergent treatment.

To investigate the internal structure of these nuclear preparations, transmission electron microscopy was performed. These results appear in Fig. 2. In Fig. 2A the cross-section of a typical nucleus of immature granulocyte precursors from the top gradient fraction is seen. Inside the intact “shell” there is a meshwork of internal structure. It appears that this lattice-work is ruptured in places leaving expanded holes. This apparent rupture varied in different nuclei. The residual DNA is most likely represented by the electron-dense material associated with these membraneous structures.

In the mature granulocyte nuclei, an identical internal reticulum structure was seen. Figure 2B represents a portion of the nucleus of a nearly mature granulocyte. Many such nuclei were observed, and they all had the same network of intranuclear reticulum as seen in the younger cells. There was no evident difference in the size of the filaments or in the degree of branching of the matrix in the mature cell nuclei compared to the immature cell nuclei. In addition, although mature nuclei showed evidence of segmentation (Fig. 1), the largest diameter of the segmented (mature) nuclei was not appreciably different than that of the immature cells, whose original unextracted nuclei are larger. This suggests that the isolation procedure expanded a greatly concentrated nuclear form that is composed of a similar volume of nuclear protein matrix material regardless of the stage of maturation.

**Electrophoresis of Nuclear Protein Matrix**

Proteins were separated by electrophoresis to determine whether the expected 60–80,000 molecular weight group of NPM proteins was present and what other residual proteins might be present. Electrophoresis was performed in SDS-acrylamide gels as described in Materials and Methods.

Densitometer tracings of typical electrophoretic separations are shown in Fig. 3. These samples represent NPM preparations of young granulocytes (top gradient fractions), intermediate granulocytes (middle gradient fractions), nearly mature cells (bottom), and mature polys from the peritoneal exudate cells.

While a variety of polypeptides is present in each fraction, there are no significant differences in the number of prominent species during cell maturation. However, there is a striking change in the relative distribution of matrix polypeptides. This is especially obvious in the lower molecular weight region of the gel (less than 25,000 daltons).

In the top fraction there are several large peaks. The most prominent peak exhibits a molecular weight of about 18,000 daltons (Fig. 3, band 1). As cell maturation progresses, there is a continuous decline in the relative proportion of these peptides concomitant with the contraction of the granulocyte nucleus. It is of interest to note that Wonderlich and Herlan also detected a prominent protein of approximately the same molecular weight in the expanded nuclear matrix of Tetrahymena macronuclei, which is present only in minimal amounts in Tetrahymena under contraction conditions.

There is another cluster of polypeptides in the 30–60,000 dalton region of the gel. The more prominent bands have molecular weights of 35,000 and 43,000 (Fig. 3, bands 2 and 3, respectively). The 43,000 dalton peptide comigrates with actin, which is a prominent cytoplasmic protein in the granulocyte and which is also attached to the outer nuclear membrane in some cells.

The cluster of polypeptides in the 60–80,000 dalton region (Fig. 3, bands 4 and 5) are present in a number of nuclear matrix and pore complex-lamina preparations from a variety of cells. In the granulocyte these peptides do not constitute the major portion of the NPM as compared to the rat liver nucleus or the avian erythrocyte. However, these polypeptides are...
Fig. 1. Scanning electron micrographs of NPM preparations from both immature and mature (segmented) granulocytes. (A) NPM preparations from mature granulocytes (segmented neutrophiles). Some nuclei show persistent constricted areas suggesting nuclear segmentation. (magnification ×3400; bars in all plates indicate 1 μ). (B) NPM preparations from immature granulocyte precursors (myeloblasts, promyelocytes, myelocytes). Although some small separate nuclear fragments are seen, the majority are roughly spherical bodies with no suggestion of segmentations (magnification ×3000). (C) A higher magnification of the NPM preparation of an immature granulocyte. The surface is more or less continuous and the rough spherical shape of the nucleus is retained (magnification ×11,000). (D) A similar cell wherein the NPM preparations included in additional detergent extraction to insure complete removal of lipid. Note that the intact surface structure is retained (magnification ×11,000).
Fig. 2. Transmission electron micrographs of NPM preparations from both immature granulocyte precursors (A) and mature segmented granulocytes (B). The internal structure appears to be a laticework of protein (and residual DNA) with breaks that may have occurred during isolation. In the immature cells (A), structures resembling nuclear remnants were seen. In other respects, the NPM internal structures were similar. The bar at lower right indicates 1μ. Magnification × 27,500.
Synthesis of NPM Proteins During Granulocyte Maturation

As the cell nucleus matures, increased synthesis of a progressively more complex matrix structure might occur, leading to a more densely compacted matrix in the pyknotic mature nucleus. Whether this was the case was tested by incubating cells separated by density gradient fractionation (plus peritoneal exudate polys) in \(^{35}S\)-methionine as described in Materials and Methods. NPM preparations were then made, and the radioactivity incorporated in protein was determined for each fraction. The results are illustrated in Fig. 4. It is apparent that in fact the greatest incorporation appears in the young cells and falls to a very low level as the cells become mature.

It was also of interest to determine which specific polypeptides were incorporating the radioactive label. This was accomplished by treating slab gels containing the \(^{35}S\)-methionine-labeled matrix proteins for fluorography, as described in Materials and Methods. A representative radioautography is shown in Fig. 5.

Fig. 3. Separation of proteins of NPM preparations by SDS-acrylamide gel electrophoresis. The Densitometer tracings labeled "TOP," "MIDDLE," and "BOTTOM" refer to density gradient fractions containing granulocyte precursors of progressively increasing maturity, and the "exudate" sample is derived from mature segmented granulocytes from induced peritoneal exudates. See text for descriptions of the changes observed during cell maturation.

It should be noted that the predominance of these 60–85,000 dalton bands depends in part on the method of isolation. Cobbs and Shelton\(^{12}\) demonstrated increased amounts of these polypeptides in the HeLa cell nuclear matrix by preparing their nuclei without detergent in the initial cell homogenization steps.
Fig. 5. Autoradiogram of 35S-methionine-labeled NPM preparation proteins, separated by SDS-acrylamide gel electrophoresis. Samples 1, 2, 3 are from top, middle, and bottom fractions of a density gradient, and sample 4 is from mature cells in the peritoneal exudate. Although a number of peptides are labeled in the bone-marrow-derived fractions, the most intensely labeled bands are the presumed actin band (A) and bands in the 60,000-80,000 dalton range (bands B and C). Very little radioactivity is seen in the sample derived from mature granulocytes.

Although the same amount of protein was used for each sample, the greater incorporation of radioactive label in the top fraction of the gradient is evident. When the film of this radioautograph was scanned (data not shown) most of the incorporation of the radioactive label was confined to those polypeptides in the 35,000-80,000 dalton range. The most intense synthesis is localized in the presumptive nuclear structural protein region (60-80,000) and the actin band. Interestingly, there is little radioactivity in the small (~20,000 dalton) peptides. The reason for this is unknown, but it may relate to a low level of methionine in these peptides.

**DISCUSSION**

The first objective of this study was to utilize existing techniques to determine whether granulocyte precursors of the rabbit contain a nuclear matrix structure similar to that described in rat liver and HeLa cells. The second goal was to study possible changes in the NPM during cell differentiation and attempt to assess the role of NPM in the pyknosis and segmentation of the granulocyte. Preliminary experiments were performed on rabbit peritoneal exudate cells using the method of Berezney and Coffey (Table 1), and some fundamental differences were observed in the behavior of granulocyte nuclear proteins as compared to those in rat liver. In addition to the limited solubility of granulocyte nuclear proteins in the low ionic strength buffer, the level of exogenous DNase that was necessary to remove the bulk of the nuclear DNA was at least four times higher than that recommended by the authors. However, even with the inclusion of this preliminary step, the amount of residual DNA in the NPM was considerably higher for the granulocyte than for the rat liver.

When the Hodge et al. procedure was followed, the amount of residual protein was similar to that seen with rat liver or HeLa cell nuclei and did not significantly vary with the stage of maturation of the granulocytes. However, the amount of residual DNA was much greater for granulocytes (26%–65% versus 1%–5%). In addition, there was a striking increase in the residual DNA in the NPM preparation with increasing granulocyte development (Table 2). However, when a large excess of DNase was included in the extraction process, there was a significantly increased extraction of DNA, although residual DNA never fell to levels as low as that in HeLa cells similarly extracted. The increase of nuclease-resistant DNA in the extracted nuclei in maturing cells suggests an increased packing or a conformational change in a fraction of the chromatin during granulocyte maturation. It is remarkable that as much as 65% of the nuclear DNA is associated with only 15% of the total nuclear protein and suggests that the NPM plays a significant role in providing a framework on which DNA becomes progressively more tightly bound and nuclease-resistant.

Having isolated NPM, which contains only a small portion of the total nuclear protein, how then is this protein distributed? Transmission electron micrographs of matrix preparations obtained from HeLa cells and rat liver cells are quite similar to those isolated from the granulocyte. These structures possess a continuous peripheral component that is connected to certain internal elements. In contrast to the rat liver preparations, there are no identifiable nuclear pore complexes present in the granulocyte NPM. The continuity of the nuclear periphery is also evident in the scanning electron micrograph prepara-
tions of the nuclear matrix. The convoluted surface area visualized by this technique is remarkably similar to the scanning EM of nuclear ghosts isolated from HeLa cells.

Electrophoretic analysis of these matrix proteins reveals a limited group of polypeptides that are similar to those isolated from other cell systems. Interpretation of these data is complicated by the variety of isolation procedures available as well as possible inherent differences among the various cell types. However, it appears that the putative matrix polypeptides of 60–80,000 daltons are present only as a relatively minor species in the granulocyte as compared to their predominance in the rat liver or the avian erythrocyte matrix.

Recently, there has been some debate in the literature concerning the localization of these matrix polypeptides. Dwyer and Blobel have isolated a similar fraction from rat liver, which they term the nuclear pore complex-lamina. Their preparations do not possess an internal structure and yet the 60–80,000 dalton bands comprise 30%–40% of this fraction. These investigators propose that these three polypeptides occur only at the nuclear periphery. Immunofluorescent staining of these proteins in nuclei indicates their area of concentration in the nuclear periphery.

Our granulocyte NPM does possess an internal structure, but the evidence presented here does not support or refute the peripheral localization of the 60–80,000 dalton proteins. It is possible that this internal nuclear matrix is composed of some of the other polypeptides that are present in the electrophoretic separations. Indeed, the presence of these 60–80,000 dalton bands in relatively constant amount in both mature and immature granulocytes may account for the homogeneity of the nuclear surface during cell maturation (as evidenced by SEM) despite dramatic changes in the overall shape and size of the granulocyte nucleus.

Interestingly, the prominent band of about 18,000 daltons, which is evident in the immature granulocytic matrix and declines with maturation and nuclear segmentation, resembles the prominent polypeptide in the expanded form of the matrix isolated from Tetrahymena macronuclei. Wonderlich and Herlan concluded that this protein was somehow involved in the reversible contraction of this expanded nuclear matrix. One could speculate that this polypeptide may be involved in a similar role in the granulocyte and thus may contribute to the segmentation and contraction of the nucleus that occurs with granulocyte maturation.

Having characterized certain features of the NPM preparation in granulocytes, what can be deduced concerning the role of this structure in the unique constriction and pyknosis the maturing nuclear granulocyte undergoes? Our original speculation was that the proteinaceous reticulum in the nucleus might be progressively increased during maturation, resulting in a more dense compact nucleus. Study of the relative rates of protein synthesis in developing cells, however, showed that the majority of the NPM preparation proteins were synthesized in young cells (Fig. 4). This lends more credence to a model in which the nuclear architecture (surface lamina and intranuclear reticulum) is synthesized early and then undergoes a contraction or progressive “collapse” to form the segmented nuclei. The notion of a collapsing structure is supported by the observation that the NPM preparations of mature, segmented nuclei are not appreciably different in overall dimensions from the NPM preparations from the most immature (an in vivo largest) nuclei.

This concept is schematically represented in Fig. 6. As illustrated, the large, presumably spherical nucleus of the immature granulocyte produces the spherical NPM preparations shown on the left. The in vivo collapsed state of the mature segmented nucleus is illustrated in the center. This then appears in vitro as a NPM preparation illustrated on the right, having reexpanded when isolated from the intact cell.

Since it appears that the NPM structures do not change appreciably during cell differentiation, what forces result in the fixed, pyknotic, and segmented structure? We have presented evidence that the DNA is more tightly packed and bound to the intranuclear reticulum as the cell matures. Yet this would hardly result in the collapse of the entire nuclear framework. Forces external to the nuclear lamina may account for...
this contraction. There are doubtless a number of possible external forces that could be invoked. A simple possibility would be that the increasing density of the cytosol results in passive collapse of the nucleus. The buoyant density of granulocyte precursors increases sharply with cell maturation, as evidenced by the ease with which these precursors can be separated according to maturity in density gradients.

A more specific mechanism might involve a contractile apparatus in the cell. Both actin and myosin\textsuperscript{25} are known to exist in blood granulocytes, and we have shown that a significant amount of a peptide comigrating with actin appears in the NPM preparations. It is possible that this actin originally was attached to the nuclear membrane, since it has been demonstrated to radiate from the nucleus in a sunburst fashion in large quantity.\textsuperscript{26,27} Although we have no direct evidence for such a mechanism, it is conceivable that actin fibers crossing the nuclear surface could contract progressively to effect the collapse of the nucleus.

The segmentation of the mature granulocyte nucleus with fine strands of nuclear material between lobes is unique to the blood granulocyte. These constrictions may represent areas of the nucleus with the least resistance to collapse due to a paucity of chromatin or nuclear reticulum. This, however, is not supported by the fact that, in the scanning electron micrographs, the segments of mature nuclei can frequently be recognized, albeit partially reexpanded. Thus, this intriguing aspect of granulocyte physiology is still unexplained.

What is evident from these studies is that the nuclear protein matrix of the granulocyte, as defined by the extraction procedures used here, plays an important, but passive, role in the nuclear pyknosis and segmentation during the maturation of the granulocyte. It appears to provide an intranuclear reticulum framework on which the majority of the nuclear DNA is arrayed in a tightly bound fashion, and the remainder of the nuclear structure (perhaps the nuclear lamina) are collapsed about it. The result, in the light microscope, is the familiar densely packed heterochromatic granulocyte nucleus.

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