Characteristics of Nuclear Proteins During Granulocyte Development*

Christine E. Eastment, Robert B. Scott, Keith R. Shelton, and W. McLean Grogan

Departments of Medicine, Pathology, Biochemistry and the Cancer Center, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298, USA

Summary. Alterations in nuclear proteins during maturation may be responsible for gene activation and repression. Study of these proteins requires: (1) a system for separating cells into varying degrees of maturity, and (2) a procedure for...
separating the nuclear proteins. The former was accomplished using Ficoll/Hypaque density gradients to separate rabbit granulocyte precursors. Erythrocytes and their precursors were removed by hypotonic lysis. Histones were extracted from purified nuclei with sulfuric acid, and analyzed on polyacrylamide gels containing urea. Residual non-histone proteins were separated by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels.

Quantitation of nuclear proteins during development shows no change in the histones, but a significant increase in the non-histone proteins. Therefore, the ratio of non-histone to histones increases progressively during maturation.

Histone electrophoresis revealed no significant qualitative or quantitative changes in their five major classes during development. By contrast, electrophoretic analysis of the non-histone proteins revealed distinct changes which include a striking decrease in low molecular weight protein during maturation, and also certain changes in other peptide bands. These changes may reflect alterations in nuclear structure, a changing complement of the nuclear proteins involved in genetic regulation, or a combination of both.

**Key words:** Nucleus – Nuclear protein – Histone – Granulocyte – Leukocyte

The nucleus of the developing granulocyte undergoes striking morphologic changes as it progresses from the myeloblast stage to a mature polymorphonuclear leukocyte. The large nucleus with nucleoli and a fine chromatin pattern gives way ultimately to a highly contracted dense nuclear structure divided into several segments. In addition, there are distinct cytoplasmic changes during granulocyte maturation. These include a progressive loss of ribosomes, Golgi structures, and mitochondria, and an increase in lysosome-like granules and glycogen. All of these orderly changes must reflect a sequential modulation of gene expression to produce specific proteins (enzymes or structural components) necessary for the structural and chemical changes recognized during cell differentiation.

The mechanisms by which such sequential gene action is effected are poorly understood. Proteins associated with nuclear chromatin are most probably involved. Study of these nuclear protein alterations during cellular differentiation would be facilitated by a system in which cells would be separated according to their different stages of development and in which distinct morphological changes could be compared with biochemical changes. The differentiation of the granulocyte is an excellent model for such study since, in addition to morphologic changes, the granulocyte undergoes variations in cellular buoyant density which allow separation of cells in differing stages of development. The more mature granulocyte precursors are more dense than their immature counterparts and fractions enriched in cells of differing degrees of maturity can be collected from density gradients.

From these enriched fractions nuclei can be isolated and nuclear proteins extracted and analyzed. In this study histone and non-histone nuclear proteins of granulocyte nuclei have been separated from precursor cells in rabbit marrow. Characteristic protein patterns and changes in protein content during granulocyte development are described in preparations analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).