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Nuclear Residual Proteins from Goose Erythroid Cells and Liver*

K. R. Shelton† and J. M. Neelin

ABSTRACT: A method has been devised for extraction of nuclear residual proteins in phenol as two successive fractions at pH 8.4 and 9.4. Residual proteins from goose liver, mature erythrocytes, and regenerating erythroid cells, differing widely in synthetic activities, were compared by gel electrophoresis in sodium dodecyl sulfate. Both fractions from avian liver are heterogeneous, resolving, respectively, into at least 20 bands each, while the fractions from erythroid cells appeared to be somewhat less heterogeneous. Besides significant quantitative differences in many bands, some proteins appeared to be qualitatively tissue specific. Differences between mature and regenerating erythroid cells were limited to relatively minor components and to variable amounts of coextracted hemoglobin.

Biological functions have been ascribed to only a few nonhistone nuclear proteins, but relatively more residual protein[1] is found in metabolically active tissues with greater contents of chromosomal RNA, but with constant amounts of histone (Dingman and Sporn, 1964; Marushige and Ozaki, 1967). The level of template capacity (Seligy and Neelin, 1970) and the specificity of transcription *in vitro* (Gilmour and Paul, 1969) are affected by residual proteins on the chromatin. Regions of disperse chromatin, relatively rich in nonhistone proteins (Frenster, 1965; Arbuzova *et al.*, 1968), synthesize RNA more actively than condensed chromatin. These studies imply that residual protein may play a role in genetic activity.

Furthermore, nonhistone chromosomal proteins are generally more active than histones in incorporation of amino acids (Daly *et al.*, 1952; Stellwagen and Cole, 1969) and phosphate (Langen, 1968; Gershey and Kleinsmith, 1969), and the level of such metabolism appears to be related to nuclear activity (Turkington and Riddle, 1969; Kleinsmith *et al.*, 1966). Synthesis of specific residual proteins has been shown to respond to gene-activating stimuli (Teng and Hamilton, 1969; Shelton and Allfrey, 1970).

In view of these suggestive observations, it was of interest to apply new methods of residual protein solubilization and resolution (Shelton and Allfrey, 1970) to a selection of cell populations in order to permit an assessment of protein heterogeneity and specificity, and to open the way for studies of the metabolism of specific components. The cells chosen were those of goose liver, normal blood, and regenerating blood. Liver is a mixed population of cells, predominantly hepatocytes, most of which are synthesizing protein, RNA, and DNA. Almost all of the cells of normal avian blood after removal of the "buffy coat" are mature, nucleated erythrocytes which do not divide or make DNA, and which synthesize little protein or RNA (Cameron and Prescott, 1963; Scherrer *et al.*, 1966). The regenerating blood used herein usually contains 10–30% erythroblasts and some reticulocytes as well as mature cells and ghosts (G. H. M. Adams and J. M. Neelin, unpublished observations); longer intervals after injection of phenylhydrazine (Adams and Neelin, 1970) diminish erythroblasts and increase reticulocytes. Such cells show a range of activities as reflected by cell and nuclear volumes (Mathias *et al.*, 1969), transport functions (d'Amelio and

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† Present address: Department of Biochemistry, Medical College of Virginia, Health Sciences Center, Virginia Commonwealth University, Richmond, Va. 23219; a National Research Council of Canada Postdoctoral Fellow, 1969–1970.

[1] The venerable term, "residual protein," is used herein to define the nonhistone nuclear proteins which are not extracted by isotonic saline and dilute acid.

Constantino-Ceccarini, 1969), energy utilization (Lucas and Jamroz, 1961), RNA synthesis (Scherrer *et al.*, 1966; Attardi *et al.*, 1966), hemoglobin accumulation, and histone synthesis (Hammel and Bessman, 1964; Freedman *et al.*, 1966).

We prepared acid-washed nucleoprotein from nuclei varying in potential from considerable activity to virtually none. The residual protein was solubilized fractionally in phenol and purified by precipitation with acetic acid. The precipitate was then dissolved in SDS[2] for electrophoretic separation in polyacrylamide gel.

Methods

Preparation of Acid-Washed Nucleoprotein. Domestic ganders (1- to 2-years old) of mixed Chinese and Pilgrim varieties were used for all cell types. To obtain regenerating blood, anemia was induced by three injections, one every third day, each consisting of 16 mg of phenylhydrazine/kg body weight. Blood enriched in erythroblasts (G. H. M. Adams and J. M. Neelin, unpublished observations) was collected 1 day after the last injection. Normal or regenerating blood was collected into sodium citrate and the buffy coat was removed by aspiration (Seligy and Neelin, 1970); erythrocytes were washed by repeated centrifugation and suspension in 0.9% sodium chloride solution, and then lysed with saponin (Seligy and Neelin, 1970; Purkayastha and Neelin, 1966). The nuclear pellet was washed by repeated centrifugation and suspension in 0.9% NaCl, until the supernatant fluid was clear, but Nomarski interference-contrast microscopy revealed membranes still attached to most nuceli; we could not remove these by shear forces (Dingman and Sporn, 1964).

Livers were removed immediately after bleeding, minced in ice-cold 0.025 M citric acid, and homogenized at high speed in a Sorvall Omni-Mixer in 8–10 volumes of 0.025 M citric acid (Paul and Gilmour, 1968; Shelton and Allfrey, 1970). The homogenate had a final pH of 4.1–4.4. The nuclei were collected by centrifugation at 2000 rpm for 5 min, suspended in 8–10 volumes of 0.01 M citric acid (pH of suspension was 3.3–3.4), and centrifuged again. The pellet was suspended in four liver volumes of 2.4 M sucrose–0.001 M $Mg(Cl)_2$–0.01 M Tris-HCl (pH 7.0) by homogenization for 2 min at 30 V in the Omni-Mixer (Chauveau *et al.*, 1956; Pogo *et al.*, 1966), and the nuclear pellet was collected by centrifugation for 1.5 hr at 25,000 rpm in the Spinco 30 rotor. Washing with 0.9% NaCl was repeated twice. The final nuclear preparation was judged by phase-contrast and interference-contrast microscopy to be quite pure with no visible cytoplasmic tags and little debris.

To extract histones the nuclei were homogenized in 0.20 M HCl, the suspension was titrated to pH 1.0, and the "acid-washed nucleoprotein" was collected by centrifugation in the cold at 9000*g* for 15–30 min (Adams and Neelin, 1969).

Extraction of the Nuclear Residual Protein. Residual proteins were freed of nucleic acid by partitioning acid-washed nucleoprotein between aqueous buffers and phenol (Viñuela *et al.*, 1967; Shelton and Allfrey, 1970) with modifications permitting rapid handling of large quantities of material and preliminary enrichment of major bands. Nucleoprotein containing 50–100 mg of DNA from liver, or 100–400 mg of DNA from blood cells, was suspended in 100 ml of TEM by homogenization in a ground-glass hand homogenizer (Dounce type A, Kontes Glass, Vineland, N. J.). This suspension was heated to 60° for 3 min and blended by a brief, full-speed burst in the Omni-Mixer. After weighing, aliquots of the suspension were taken for protein and DNA assays. Then one-third volume of TEM-saturated phenol was added and the mixture was shaken on a Burrel wrist-action shaker for 30 min at room temperature. The phases were separated by centrifugation for 20–30 min at 9000 rpm in a Sorvall SS-1 centrifuge and the lower phase was collected by aspiration. The aqueous phase and interface were extracted again with phenol, which was combined with the first extract. The second aqueous and interface layers were then titrated to pH 9.4 with concentrated NaOH, the two phenol extractions were repeated, and these extracts combined (above pH 9.5 phenol dissolves, precipitating most of the DNA and protein).

The protein was recovered from each phenol extract by dissolving the phenol in 0.10 M acetic acid, 1% 2-mercaptoethanol, and sedimenting the precipitated protein in Corex tubes at 8000 rpm for 10–20 min. The final protein pellet was taken up in 0.01 M sodium phosphate (pH 7.2), 1% 2-mercaptoethanol, 3% SDS, to a concentration of 1–3 mg/ml. After heating for 1 min in a boiling-water bath, the solution was cooled, dialyzed overnight in 0.25-in. Visking tubing against 2 l. of 0.01 M phosphate (pH 7.2), 1% 2-mercaptoethanol, 1% SDS, and clarified by a brief centrifugation.

Electrophoresis. The proteins were examined by electrophoresis in polyacrylamide gels containing SDS (Shapiro *et al.*, 1967) as described by Fromageot and Zinder (1968). Unless otherwise indicated, the stock gel solution was 10% acrylamide (Canalco Corp., Md.), 0.2% *N,N*-methylenebisacrylamide in 0.10 M sodium phosphate (pH 7.2), 0.1% SDS (running buffer). Polymerization was catalyzed by the addition of 0.075% (v/v) TEMED and 0.075% (w/v) ammonium persulfate; gels were cast in 100 × 6 mm glass tubes and prerun to eliminate excess catalyst.

Before electrophoresis, samples were adjusted to approximately 1 mg of protein/ml, to which was added one-tenth volume of saturated sucrose. Fifty microliters of sample was applied under the running buffer on the top of each gel. Electrophoresis was conducted at a constant current of 8 mA/tube for 4.5 hr in 10% gels, and 2.5 hr in 5% gels. The gels were fixed overnight in 20% sulfosalicylic acid, washed for 3 hr in 7% acetic acid, stained overnight in 0.5% Amido Black (Harleco) in 7% acetic acid, and destained by shaking gently in 7% acetic acid. Stained gels were scanned at 570 nm on a Gilford 240 spectrophotometer.

Amino Acid Analyses. Proteins were precipitated by 15–20% trichloroacetic acid, washed in 5% trichloroacetic acid, heated at 90° for 15 min in 5% trichloroacetic acid, and dissolved in 6 M HCl. Each sample was evacuated in the hydrolysis vial for 10 min before sealing, and maintained at 110° for 21 hr. Acid was removed by rotary evaporation below 40° and rinsing with water. Amino acids were resolved and measured on a Beckman 120C amino acid analyzer with 6 × 55 cm columns for basic and nonbasic amino acids, respectively.

Protein and DNA Determinations. Protein was assayed by micro-Kjeldahl nitrogen determination with the assumption that nitrogen accounted for 16% of the protein mass. Nucleoprotein was assayed for total N before and after two extractions with perchloric acid at 70° for 20 min. The perchloric acid extracts were assayed for DNA by the Burton diphenylamine procedure (Burton, 1956) with Worthington calf thymus DNA as the standard. The hot acid-soluble nitrogen was all DNA. Hot acid-insoluble nitrogen was taken as a measure of protein because of inconsistencies with colorimetric methods

[2] Abbreviations used are: SDS, sodium dodecyl sulfate; TEM, 0.10 M Tris-HCl (pH 8.4)–0.01 M EDTA–1% 2-mercaptoethanol; TEMED, *N,N,N',N'*-tetramethylethylenediamine.

TABLE I: Effectiveness of Partitioning Protein and DNA from Acid-Washed Nucleoprotein between Phenol and Aqueous Buffers.[a]

Cells	Expt	Protein/DNA Original	Protein/DNA Final	DNA Recov[b] (%)	Protein Recov[c] (%)
Liver	1	2.2	0.33	100	81
	2	2.5	0.27	105	72
	3	2.5	0.43	111	91
RBC	1	0.62	0.20	71	76
	2	0.72	0.07	80	59
	3	0.84	0.14	96	
IRBC	1	0.91	0.08	93	
	2	0.94	0.07	84	92
	3	1.4	0.08	92	92

[a] Acid-washed nucleoprotein was dispersed in 0.10 M Tris (pH 8.4), 0.01 M EDTA, and 1% 2-mercaptoethanol. Weighed aliquots were removed, precipitated with 15–20% cold trichloroacetic acid, washed in trichloroacetic acid, and analyzed for hot perchloric acid soluble and insoluble nitrogen by the Kjeldahl procedure. The hot acid-soluble solution was further analyzed for DNA by the Burton procedure. After the phenol extractions (see Methods) this analysis was repeated on the combined aqueous and interface material. [b] DNA recovered in the aqueous and interface material. [c] Protein recovered in the four fractions described in Table II.

TABLE II: Distribution of Protein Recovered after Phenol Extraction of Residual Protein.[a]

Tissue	Acetic Acid Insoluble: Phenol Soluble, pH 8.4	Acetic Acid Insoluble: Phenol Soluble, pH 9.4	Acetic Acid Soluble Phenol Soluble, Combined	Phenol Insoluble
Liver[b]	52	20	9	16
RBC[c]	40	10	27	23
IRBC[c]	60	12	20	7

[a] Per cent of total protein recovered in all four fractions. Protein was determined as Kjeldahl nitrogen in trichloroacetic acid precipitable material. In the case of the phenol-insoluble protein, the precipitate was first extracted with hot perchloric acid. [b] Averages of three experiments. [c] Averages of two experiments.

arising from variable color responses (Seligy and Neelin, 1970), poor solubility of some proteins in alkaline color reagents, and colored contaminants.

Results

Phenol Extraction of Nuclear Residual Protein. Table I demonstrates the effectiveness of phenol in separating acidic proteins from DNA. The protein to DNA ratios of acid-washed nucleoprotein, before and after phenol extractions, and the recoveries of protein and DNA in all fractions, are compared in the three cell populations. Liver chromatin has a much higher ratio of protein to DNA than red blood cell chromatin (Daly *et al.*, 1952; Seligy and Miyagi, 1969), and it is confirmed that this difference arises chiefly from residual protein (Dingman and Sporn, 1964; Seligy and Neelin, 1970).

The rather high levels of residual protein in our preparations may arise from the mode of nuclear isolation (Elgin and Bonner, 1970), incomplete extraction of basic proteins (Sonnenbichler and Nobis, 1970), and contamination by adhering nucleoplasmic proteins. For example, the high protein:DNA ratios from regenerating erythrocytes (Table I) included substantial and variable amounts of hemoglobin, which resisted removal with isotonic saline washes from nuclei prepared shortly after phenylhydrazine injection. This globin was solubilized by phenol at pH 8.4 (Figure 5). The proportion of phenol-insoluble, residual protein from liver (Table I) was consistently several times more than that of red cells.

The distribution of the recovered protein among the various fractions from the three cell groups (Table II) showed that a plurality of the "residual protein" was soluble in the pH 8.4 phenol extraction, and that much of the remainder was released at pH 9.4. Most of this was precipitated by acetic acid, but the combined acetic acid soluble fraction was much larger from both the normal and regenerating red blood cells than from liver. When acid extraction of histones from residual nucleoprotein was incomplete, the acetic acid step eliminated most of the phenol-soluble basic proteins. The degree of such contamination could be reduced by more exhaustive extraction at pH 1 before phenol treatment.

Amino Acid Compositions. All of the phenol-soluble, acetic acid insoluble fractions have high acidic to basic amino acid ratios (Table III), especially the proteins dissolving at the higher pH. Each type of phenol extract showed considerable similarity among the three groups of cells, differing chiefly in aspartic acid, glutamic acid, alanine, and leucine in the pH 9.4 protein from liver. The acetic acid soluble proteins tended to be more basic than other fractions, with the small variable fraction from liver resembling the copious "residual protein from natural chromatin" of calf thymus, reported recently by Sonnenbichler and Nobis (1970). The larger acetic acid soluble fractions of blood were more basic with compositions suggesting contamination by arginine-rich histone (Vidali and Neelin, 1966) and globin (Neelin, 1964).

The phenol-insoluble liver protein differed from that of the normal and regenerating erythrocytes chiefly in the content of glycine, which varied from 9 to 24% of total recovered amino acids. This suggests a spurious source, such as degraded nucleic acid (Hotchkiss, 1949), for this amino acid. Under our conditions of acid hydrolysis, each mole of adenine would produce 0.4 mole of glycine, and incomplete but variable DNA extraction prior to hydrolysis would be reflected in the apparent protein composition.

Electropherograms in SDS. Electrophoretic patterns of the phenol-soluble proteins are shown in Figure 1. The liver extracts contained more protein with molecular weights in the range 20,000–80,000 than did the red cell extracts, which had more protein of larger molecular weights. In "pH 9.4 extracts" of red cells one heavy band migrated a few millimeters into the gel, in the vicinity of γ-globulin standard (146,000 molecular weight).

The minimum number of peaks detectable in either liver or

TABLE III: Amino Acid Compositions.[a]

	Phenol pH 8.4			Phenol pH 9.4			Acetic Acid Phase			Phenol Insoluble		
	Liver[c]	RBC[d]	IRBC	Liver[d]	RBC[c]	IRBC	Liver[c]	RBC[d]	IRBC	Liver[d]	RBC[d]	IRBC
Lys	7.0	6.0	6.6	7.6	6.3	6.3	8.7	10.4	10.1	6.6	7.8	7.5
His	2.6	2.3	3.4	2.2	2.2	2.3	2.6	2.6	3.8	1.8	2.0	2.3
Arg	6.1	6.0	4.9	6.5	7.5	5.9	7.5	10.8	7.3	6.9	8.7	7.1
Asp	9.3	8.2	8.7	10.0	8.7	8.8	8.4	5.6	7.2	9.5	7.9	8.5
Thr	5.0	5.5	5.3	4.7	5.0	4.7	5.0	6.0	5.3	4.0	5.1	4.8
Ser	5.8	6.0	5.8	5.5	5.8	6.1	5.8	4.8	5.7	5.9	6.4	7.0
Glu	11.8	11.4	10.9	13.7	15.3	14.7	11.4	9.7	9.6	13.5	13.1	12.9
Pro	5.3	5.3	5.0	5.0	4.4	4.5	4.6	3.8	4.5	6.6	4.6	5.1
Gly	8.2	7.6	7.5	7.5	6.2	6.8	9.3	9.3	8.1	15.0	10.9	12.3
Ala	8.5	8.9	9.5	8.0	9.1	9.3	9.5	11.0	11.8	8.5	8.9	8.7
Cys	1.3	0.9	1.5	1.2	0.7	0.9	Trace	Trace	Trace	Trace	Trace	0.0
Val	6.4	6.6	6.9	6.0	5.9	6.8	6.6	6.6	6.9	5.2	5.8	5.5
Met	1.6	1.7	1.6	1.7	1.4	1.8	Trace	Trace	Trace	0.6	0.6	0.9
Ile	4.6	4.6	4.2	4.3	4.0	4.0	4.6	5.0	4.2	3.3	3.9	3.5
Leu	9.5	10.7	10.9	9.4	11.4	10.7	8.8	8.8	9.5	7.4	8.8	8.3
Tyr	3.2	2.9	2.6	3.1	2.5	2.6	2.9	2.8	2.2	2.3	2.7	2.0
Phe	3.8	4.1	4.8	3.6	3.5	3.9	3.4	2.4	3.8	2.8	2.8	3.0
A/B[b]	1.3	1.4	1.3	1.5	1.5	1.6	1.1	0.6	0.8	1.5	1.1	1.3

[a] Moles per 100 moles of recovered amino acids, uncorrected for hydrolytic loss, or for amides of glutamic and aspartic acids. [b] A/B is the ratio of acidic to basic amino acids. [c] Average of two experiments. [d] Average of three experiments.

erythrocyte proteins could be increased by considering the resolution in both 5 and 10% gels. For example, in "pH 8.4 protein" from liver, the 9 fastest running peaks (labeled a) in the absorbance profile of 5% gels (Figure 2A) became 13–15 peaks in 10% gels (Figure 2B). Conversely 4–5 peaks of protein with molecular weights greater than about 100,000 (labeled b in Figure 2) in the 10% gels resolved into 10–13 peaks in 5% gels. Thus in phenol-soluble, acetic acid insoluble, residual proteins of goose liver, a minimum of 23 components

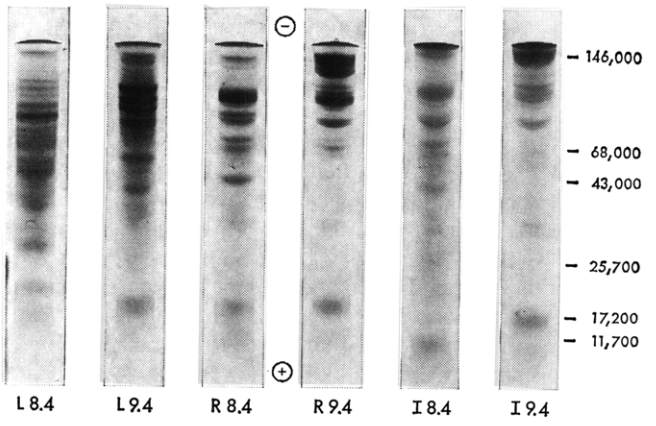

FIGURE 1: Polyacrylamide gel electrophoresis of the nuclear residual proteins of three avian cell populations (liver, L; mature erythrocytes, R; regenerating erythroid cells, I). Proteins were extracted from acid-washed nucleoprotein in phenol successively at pH 8.4 and 9.4 (as indicated). Molecular weight standards were γ-globulin (147,000), serum albumin (68,000), ovalbumin (43,000), chymotrypsinogen (25,700), myoglobin (17,200), and cytochrome *c* (11,700) (Weber and Osborn, 1969). Gels contained 10% acrylamide–0.1% SDS in 0.01 M sodium phosphate (pH 7.2). Descending migration to the anode.

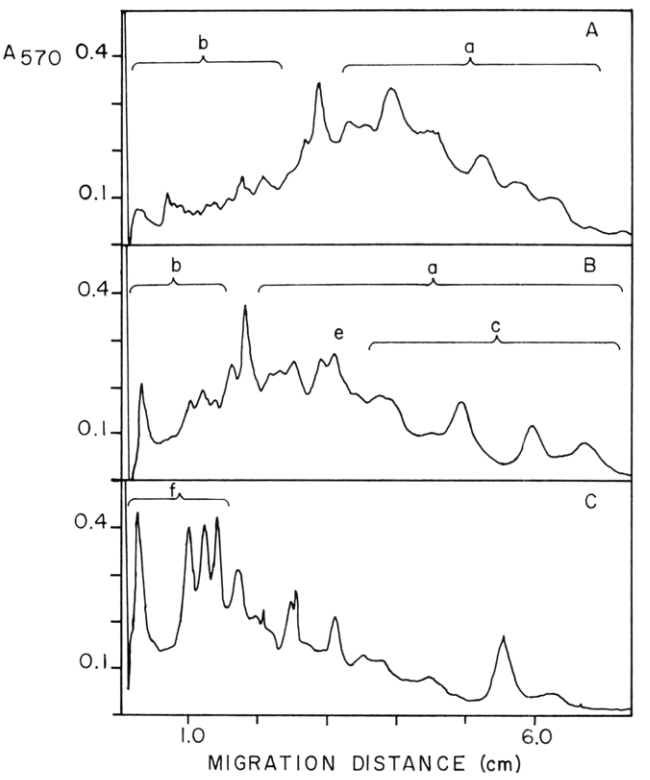

FIGURE 2: Spectrophotometric tracing of electropherograms of nuclear residual proteins from liver. (Lettered peaks are referred to in the text.) (A) pH 8.4, phenol-soluble protein on a 5% gel. (B) pH 8.4, phenol-soluble protein on a 10% gel. (C) pH 9.4, phenol-soluble protein on a 10% gel. Proteins were separated and stained as described in Methods. Descending, anionic migration is oriented toward the right for illustration; distance axes of the scanned gels do not precisely correspond because of variable speeds in the scanning apparatus.

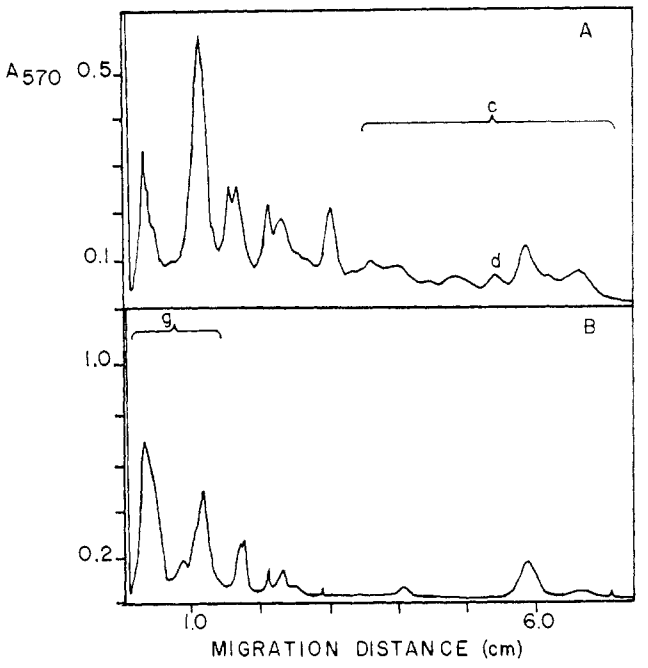

FIGURE 3: Spectrophotometric tracing of electropherograms of nuclear residual proteins from erythrocytes. (A) pH 8.4, phenol-soluble protein. (B) pH 9.4, phenol-soluble protein. 10% gels as in Figure 1.

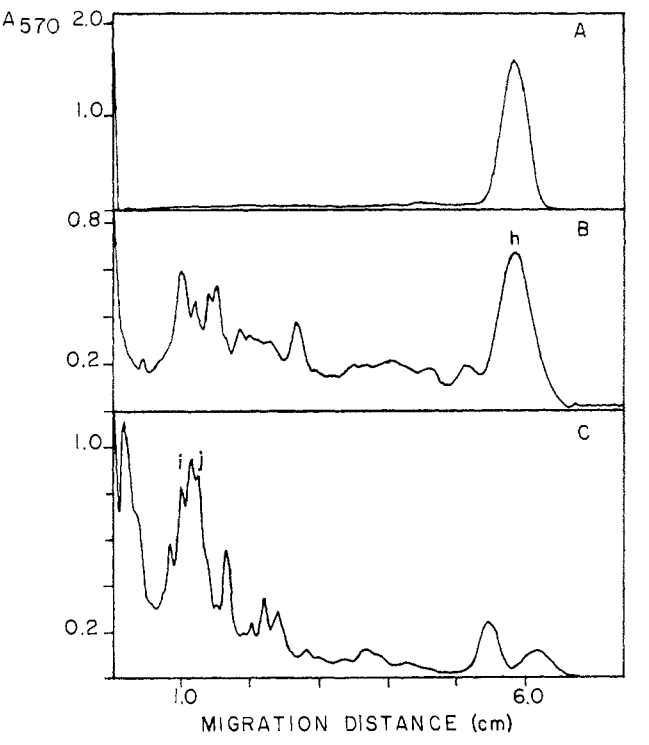

FIGURE 5: Spectrophotometric tracing of electropherograms of erythrocyte cytoplasmic protein and of regenerating erythrocyte nuclear residual proteins. (A) Cytoplasmic proteins from mature erythrocytes. (B) pH 8.4, phenol-soluble nuclear residual proteins from regenerating erythroid cells. (C) pH 9.4, phenol-soluble, nuclear residual proteins from regenerating erythroid cells.

was resolved from pH 8.4 extracts, and about 22 components from pH 9.4 extracts (Figures 1 and 2). Parallel extracts from normal erythrocytes yielded at least 17 and 13 bands for pH 8.4 and 9.4 extracts, respectively (Figures 1 and 3); extracts from regenerating blood contained slightly more (Figures 1 and 5).[3]

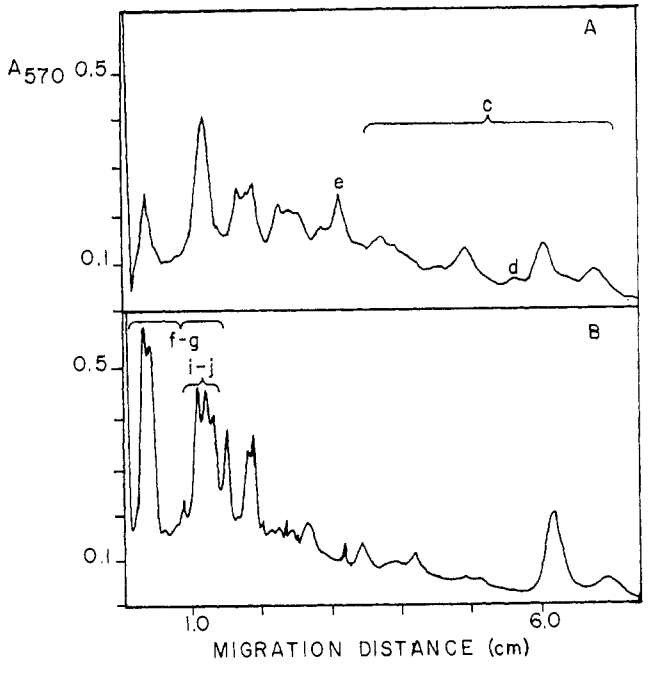

FIGURE 4: Spectrophotometric tracing of electropherograms of mixed nuclear residual proteins. (A) pH 8.4, phenol-soluble proteins from liver and mature erythrocytes. (B) pH 9.4, phenol-soluble proteins from liver and mature erythrocytes. 10% gels as in Figure 1.

Cell Specificity of Nuclear Residual Proteins. In order to minimize difficulties in comparing two gels precisely, mixtures of the proteins were run in parallel with the separate samples. The migration distances and the relative peak heights of suspected common components were then compared. The fastest migrating seven proteins in pH 8.4 extracts from liver and erythrocytes (labeled c in Figures 2–4) appeared to coincide in 5% gels, but in 10% gels a minor peak d appeared in the red cell material only (Figures 3A and 4A). Peak e in the liver material (Figures 2B and 4A) did not correspond with any erythrocyte protein. Precise comparisons were more difficult for slowly migrating proteins because of marginal resolutions and great differences in peak heights. Nevertheless it was evident that the four peaks f in the pH 9.4 proteins from liver (Figure 2C) and three peaks g in erythrocytes (Figure 3B) generated a total of six peaks when mixed (Figure 4B); thus most of the proteins in these peaks differed.

Phenol-soluble proteins from the acid-washed nucleoprotein of regenerating erythroid cells showed two clear, qualitative differences from corresponding fractions of normal erythrocytes (Figure 5 *vs.* Figure 3). The relative size of peak h (Figure 5B) was much greater in pH 8.4 proteins of regenerating cells. That this protein can be attributed to adventitious globin was demonstrated by parallel electrophoresis of supernatant protein from erythrocyte lysate (Figure 5A). In addi-

[3] The detection of approximately 30 bands in rat liver nonhistone chromosomal protein has been reported by Teng and Allfrey (*cf.* Shelton and Allfrey, 1970).

tion two small peaks i and j (Figure 5C) were found in the pH 9.4 proteins of regenerating cells, but not in the corresponding fraction of mature cells (Figure 3B). This group in regenerating cells closely resembles the triplet i–j in mixed liver and erythrocyte protein (Figure 4B) in which two peaks are contributed by liver.

Discussion

To refine the analysis of nuclear residual proteins from different cell populations, it has been necessary to adapt methods for solubilizing proteins resistant to aqueous solvents. The proportion of total nuclear protein which is insoluble in isotonic saline and dilute acid, but soluble in buffered phenol, varies widely in the three avian cell populations studied, although the difference between mature and immature erythroid cells largely reflects the degree of hemoglobin contamination. Almost no nuclear protein from avian erythrocytes remains unextracted by phenol. This phenol-insoluble fraction is difficult to compare with preparations obtained by different protocols (Steele and Busch, 1963). The high glycine content of this fraction, reminiscent of reported glycine-rich nuclear proteins (Steele and Busch, 1963; Benjamin and Gellhorn, 1968), probably arises in part from degradation of contaminating purines. Thus the phenol method provides an advantage over solubilization with salt or directly with detergent (Elgin and Bonner, 1970) in that DNA is quantitatively eliminated from the buffered phenol extracts. Furthermore the precipitation of acidic proteins with acetic acid removes a large measure of any contaminating basic protein arising from the chromatin or nucleoplasm (Sonnenbichler and Nobis, 1970; Elgin and Bonner, 1970).

The modified, two-step extraction yields most of the nuclear residual protein in two heterogeneous fractions, each resolved by acrylamide gel electrophoresis in SDS into an array of components representing a great range in molecular weights (Benjamin and Gellhorn, 1968; Shelton and Allfrey, 1970). It is difficult to compare these electrophoretic bands directly to those of Elgin and Bonner (1970) because of differences in gel conditions, as well as in the original extractions. The electropherograms of the two avian liver fractions, taken together, are reminiscent of the single fraction from rat liver (Shelton and Allfrey, 1970; Elgin and Bonner, 1970), but enrichment through fractionation has enhanced resolution. The patterns from regenerating erythroid cells differ only in detail from parallel fractions of mature erythrocytes; residual proteins from both types of cells are generally distinct from those of liver. This confirms the observations of Loeb and Creuzet (1969), using limited separation of salt-soluble proteins under different electrophoretic conditions.

The details of cell specificity of electrophoretic components can be conveniently considered in three size groups. Some protein bands of low to moderate molecular weight are common or coincident in all three avian nuclear protein fractions. If these are identical protein species, they may be involved in universal or structural functions in the nuclei. However, the amounts of these proteins relative to DNA vary among the cell types, and some appear to have spurious origins. Some of the fastest components may arise from goose hemoglobin chains or histones, which had not been completely removed by acetic acid.

It has not been possible to critically evaluate homology among the proteins of intermediate molecular weights because of the density of bands, but the stepwise extraction has facilitated comparisons of the large proteins. Like Elgin and Bonner (1970), we observed more high molecular weight protein in red blood cells than in liver. However, while they noted two cell-specific bands, one for each of these chicken tissues, we found at least five distributed between the pH 9.4 extracts of goose liver and erythrocytes. Two of the liver-specific bands appear to be shared with regenerating erythroid cells. According to the densitometric profiles, the relative amounts of these proteins are consistent with the RNA synthetic activities of the three populations of cells. It remains to be established that these interesting differences do not arise from adhering membranes, but the liver nuclear preparations should be relatively devoid of plasma and cytoplasmic membranes, while mature and regenerating erythrocytes would share "ghosts" of plasma membranes.

It is unlikely that the precise roles of nuclear proteins will be explained by methods which obliterate their tertiary structures. Nonetheless the nuclear, acidic proteins, in particular the so-called residual proteins, have been such an enigma that even an assessment of their heterogeneity is useful and their analytical resolution is essential for further experimentation. Comparisons of the protein complements of nuclei supporting different cellular functions may provide insights into the roles of these proteins, just as it has been a useful preliminary to studies on histones, another major class of nuclear proteins. The techniques described herein will permit studies of their metabolic patterns in different cell populations and environments. The use of phenol solubilization permits quantitative separation from DNA in fractional steps, with limited waste and contamination.

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Initiation of Hemoglobin Synthesis. Specific Inhibition by Antibiotics and Bacteriophage Ribonucleic Acid*

Harvey F. Lodish, David Housman, and Marion Jacobsen

ABSTRACT: In extracts of rabbit reticulocytes, both globin chains are initiated by a specific methionyl transfer RNA (Met-tRNA$_f^{Met}$). Normally, the amino-terminal methionyl residue is removed from the nascent chains. However, if the methionine amino group on the tRNA is blocked by a formyl residue (fMet-tRNA$_f^{Met}$) the modified tRNA initiates globin synthesis normally, but the amino terminal fMet residue is not removed. Hence, incorporation into globin of radioactivity from f[^{35}S]Met-tRNA$_f^{Met}$ provides a rapid assay for initiation of globin synthesis. Here we show that, at the minimum concentration necessary to completely block incorporation from fMet-tRNA$_f^{Met}$, both pactamycin and aurintricarboxylic acid specifically block initiation of globin chains, but do not affect the rate of completion of the existing nascent chains. There is incorporation of other radioactive amino acids, specific for the internal positions of globin, just sufficient to complete the existing chains, and all polyribosomes are converted to monoribosomes with release of a complete globin chain. At higher concentrations both drugs do affect some reaction in polypeptide chain elongation, as can be seen both by reduced incorporation of radioactivity into the nascent chains, and by reduced movement of ribosomes along the mRNA. RNA from bacteriophage f2 also specifically blocks initiation of globin chains. When f2 RNA is treated with formaldehyde, to relax partially its secondary structure, it becomes a considerably more potent inhibitor of globin initiation, and the treated RNA binds to reticulocyte ribosomes. By contrast, the antibiotics cycloheximide and emetine do not specifically inhibit initiation of globin chains; over a wide concentration range tested the primary inhibitory effect was on propagation of the nascent chain.

A chemical which specifically blocks initiation of protein biosynthesis in eucaryotic cells is invaluable for many types of studies on the mechanism and regulation of gene expression. Three compounds have been reported to possess this specificity: aurintricarboxylic acid (Grollman and Stewart, 1968) and the antibiotics cycloheximide (Lin *et al.*, 1966) and pactamycin (Colombo *et al.*, 1966; Cohen *et al.*, 1969; MacDonald and Goldberg, 1970). Under certain conditions, however, all

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