DECLINE IN HISTONE H5 PHOSPHORYLATION DURING ERYTHROID SENESCENCE IN CHICK EMBRYOS

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SUMMARY

Previous studies have implicated histone H5 dephosphorylation as a causal factor in genetic inactivation and chromatin condensation during erythroid senescence in adult chickens. We show that histone H5 phosphorylation declines in two stages as various cohorts of erythroid cells senesce in chick embryos. The first decline occurs between 5 and 6 days and coincides with the senescence of primitive erythrocytes. The second decline in H5 phosphorylation occurs between 17 and 19 days of chicken development, when the definitive erythrocytes undergo senescence and chromatin condensation. These results point to a role for histone dephosphorylation during the programed senescence of erythroid cells.

INTRODUCTION

During differentiation and senescence, the avian erythroid cell loses its capacity for division and transcription. This loss of genetic activity is generally attributed to chromatin condensation, which decreases the accessibility of the DNA to transcription factors [1—4]. At the molecular level, genetic inactivation and chromatin condensation are apparently facilitated by increases in the cell's complement of histone H5 [1,3,5], an avian-specific linker histone which shares structural and functional homologies with the H1 family of mammalian histones [6,7]. The H1 family of

linker-specific histones is involved in condensing chromatin into the 30-nm fiber [8]. Like its mammalian cognate H1°, synthesis of H5 is not replication dependent but rather increases postmitotically [2,9,10]. As a result, H5 accumulates in senescent terminally differentiated erythrocytes [1].

The accumulation of H5 with its high lysine content could account for the genetic inactivation of the erythroid nucleus by condensing chromatin and rendering the DNA less accessible to regulatory proteins. However, histone H5 is also present at significant levels in young dividing erythroblasts, so that the 3- to 5-fold increase in H5 concentration observed during senescence may not be sufficient to fully account for the erythroid-specific chromatin condensation [1,3]. Therefore, Sung [3] has postulated that post-translational phosphorylation and subsequent dephosphorylation of H5 play a significant role in inducing chromatin condensation and repressing genetic activity during senescence. Presumably, dephosphorylation of H5 increases the charge attraction between histones and DNA by reducing the negative charge on H5. Support for this hypothesis has come from data showing that H5 becomes progressively less phosphorylated as erythroid cells senesce in adult chickens made anemic by phenylhydrazine [2,3].

In the adult chicken, erythroid senescence occurs asynchronously from precursor cells in the bone marrow. To date, investigations of H5 phosphorylation have been carried out using adult chickens in which erythroid senescence is synchronized by treatment with phenylhydrazine to lyse mature erythrocytes [2—4]. By contrast, chick embryos provide a natural, drug-free system in which to study synchronized erythroid senescence. Primitive erythroid cells senesce at 5 to 6 days of development and are replaced by definitive erythroid cells, which senesce between 17 and 19 days of embryonic development. In this report, we have used the chick primitive and definitive cohorts to investigate stage-specific H5 dephosphorylation during programed embryonic erythroid senescence. We report significant changes in the levels of H5 phosphorylation at the two stages of erythroid senescence, suggesting that H5 phosphorylation plays a role in the senescence of both the primitive and definitive cohorts of erythroid cells.

MATERIALS AND METHODS

Preparation of red blood cells

Fertile chicken eggs were incubated at 39°C until the desired time of development, at which time the eggs were opened. Blood was collected with a pasteur pipette and dispensed into ice-cold 0.14 M NaCl, 2.7 mM NaCl, 1.5 mM KH_2PO_4 and the erythroid cells were pelleted at 1500 × g for 5 min. Nuclei were isolated by resuspending the cells in 1 ml lysis buffer (10 mM Tris (pH 7.4), 10 mM NaCl, 3 mM $MgCl_2$, 0.3 M sucrose, 40% glycerol v/v, 0.4% nonidet P-40 v/v, 1 mM p-chloromercuriphenyl-sulfonic acid) and pelleting in a microfuge for 3 min.

Histone extraction

Histones were prepared by extracting the nuclei in 0.2 M H_2SO_4 at 4°C for 4 h, pelleting in the microfuge for 15 min at 4°C to remove nuclear debris, and precipitating the histones with three volumes of ethanol (−20°C, 12 h). Total histone concentration was determined by measuring the absorbance at 230 nm. Histones were resuspended in 1 mM HCl at 1 mg/ml.

Electrophoretic analysis

High-resolution acetic acid-urea polyacrylamide gels were prepared according to the method of Hardison and Chalkley [11] consisting of 2.5 M urea, 0.9 M acetic acid, 15% polyacrylamide, and were cast in 32 cm plates. The gels were pre-electrophoresed in an electrode buffer of 2.5 M urea, 0.9 M acetic acid for 24 h at 100 V. Fifty micrograms of histone were loaded per lane at a concentration of 1 mg/ml in 2.5 M urea, 0.9 M acetic acid and run in 0.9 M acetic acid at 250 V for 36 h. When electrophoresis was complete, the gels were fixed in 12.5% trichloroacetic acid and stained with silver stain (Sigma). After staining, the gels were dried between cellophane for photography and densitometer scanning. When acid-extracted histones from developing chick embryos were separated on acid-urea gels and silver stained, a characteristic spread of core, H1, and H5 histones was observed (Fig. 1). Quantitation was carried out on a Hoefer densitometer and analyzed using Gel Scan computer software.

RESULTS

Incubation of adult erythrocyte in medium containing various radioactive precursors has demonstrated that histone H5 is post-translationally modified by phosphorylation but not by acetylation or methylation [2]. Radiolabeled phosphorylated H5 variants from adult erythrocytes have been successfully fractionated by electrophoresis on urea-aluminum lactate starch gels [3,4]. Although phosphorylated H5 subfractions are not well separated on shorter acid-urea gels (see Ref. 5), we have obtained good separation of phosphorylated H5 variants from embryonic erythroid histones using high-resolution electrophoresis on 32 cm acid-urea gels (see 5 day sample in Fig. 1). The slower migrating H5 subbands (P_1-P_4 in Fig. 2) have been verified as phosphorylated H5 by their sensitivity to alkaline phosphatase digestion (data not shown). P_0 and P_0', which remain after phosphatase treatment, represent known H5 variants [5].

As previously demonstrated for adult chickens [2,3], Fig. 1 shows that histone H5 becomes dephosphorylated during erythroid senescence. To better illustrate these changes, densitometer tracings of H5 variants (Fig. 2) were prepared from the gel shown in Fig. 1. Figure 2 demonstrates a significant loss of the phosphorylated variants P_1-P_4 during development at 5, 12 and 20 days, so that by 20 days nearly
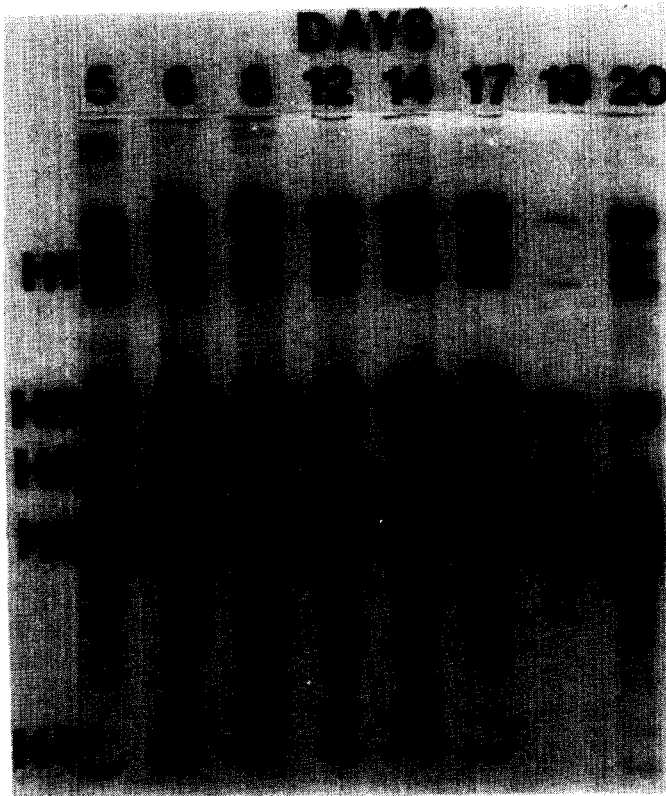
Fig. 1. Acid-urea gel showing fractionation of erythroid histones from through 20 day chick embryos. Note that histones from 19- and 20-day erythrocytes are underloaded in this gel.

all the H5 is in the dephosphorylated P_0 or P_0' form. To quantitate these changes, the areas under the two non-phosphorylated H5 peaks (P_0 and P_0') were divided by the total H5 areas for two duplicate gels. Figure 3 shows the increase during development in the fraction of the total H5 which is non-phosphorylated. Significantly, this dephosphorylation is not gradual but occurs in two bursts, first at 5—6 days and next at 17—19 days.

DISCUSSION

As in adult erythropoiesis, we have observed a correlation between erythroid senescence and dephosphorylation of histone H5. However, dephosphorylation in embryonic cells is not gradual but occurs in two temporally defined bursts that are coincident with senescence of primitive and definitive erythroid cells. The change between 5 and 6 days of embyronic development is concurrent with the senescence of
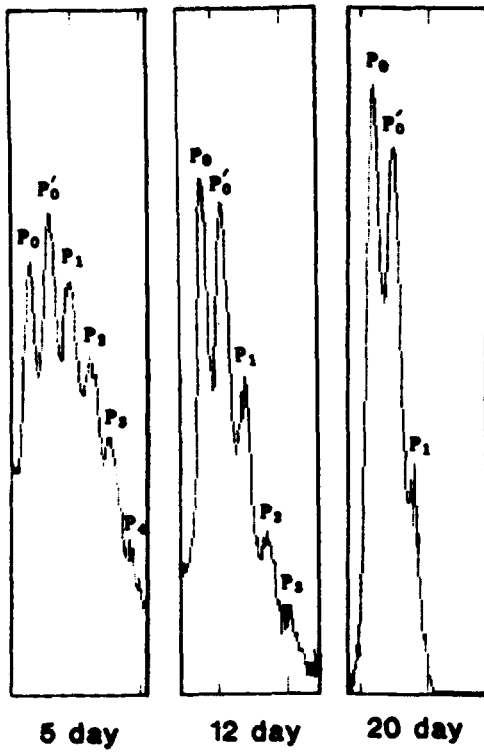
Fig. 2. Densitometer tracings of the H5 histone variants from 5-, 12- and 20-day embryos. The lanes in Fig. 1 containing histones from 5, 12, and 20 day embryos were scanned with a densitometer. The 20-day tracing has been normalized to compensate for histone underloading in this lane. P_0 and P_0' represent non-phosphorylated H5 variants while P_1—P_4 represent phosphorylated H5 variants.
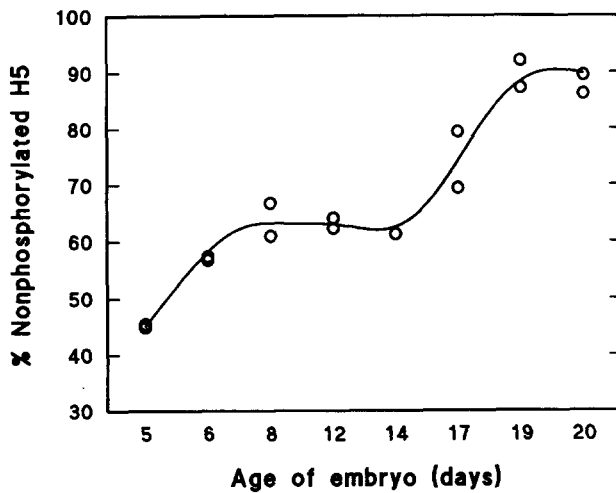

Fig. 3. Non-phosphorylated (P_0 and P_0') and phosphorylated (P_1—P_4) H1 variants as a percent of total H5. Data were obtained by averaging the densitometer values from two acid-urea gels.

the primitive cohort (expressing only embryonic globins) and with their replacement by the definitive cohort (expressing mostly adult globins-see ref. 12 and 13). The next burst of dephosphorylation occurs between 17 and 19 days at the stage when the definitive cohort becomes transcriptionally inactive as fully senescent erythrocytes [5]. Thus H5 dephosphorylation is closely associated with changes in gene expression and with senescence.

Previously reported observations are consistent with a causal role for H5 dephosphorylation in gene repression and chromatin condensation. First, histone phosphorylation makes chromatin more sensitive to DNase I digestion [14], indicating a more open chromatin structure. Thus H5 dephosphorylation should lead to tighter binding of H5 to DNA, greater DNA condensation, and less accessibility to transcription factors. Second, the decline in H5 phosphorylation between 17 and 19 days and subsequent chromatin condensation are reminiscent of protamine dephosphorylation prior to chromatin condensation during spermatogenesis [15,16]. Phosphorylated protamines largely replace the spermatocyte histones and are then progressively dephosphorylated. The highly basic, dephosphorylated protamine functions to package the chromatin tightly inside the compact sperm and at the same time inactivates transcription. In the same way, an increase in the erythroid H5 complement and its subsequent dephosphorylation could well explain the similar condensation and genetic inactivation during erythroid senescence.

As to the role of dephosphorylation in senescent cells, our results with erythrocytes indicate that nuclear dephosphorylation rates may be elevated in senescent cells. If so, other proteins besides the histones could be dephosphorylated. Support for this view comes from recent data demonstrating that the antigrowth retinoblastoma gene is dephosphorylated in senescent human fibroblasts [17]. Since phosphorylation plays a major role in regulating proteins, enhanced rates of dephosphorylation in senescence could affect many cellular processes.

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