been extended to 56 electrons by A. Rosén, D. Ellis, B. Fricke, and T. Morović [in *Abstracts, Second International Conference on Inner-Shell Ionization Phenomena*, W. Mehlhorn, Ed. (University of Freiburg, Freiburg, Germany, 1976), p. 18].
16. M. Barat, in *The Physics of Electronic and Atomic Collisions*, B. C. Cobic and M. V. Kurepa, Eds. (Institute of Physics, Belgrade, Yugoslavia, 1973), p. 43; R. McCarroll, in *ibid.*, p. 71.
17. R. D. Evans, *The Atomic Nucleus* (McGraw-Hill, New York, 1955), p. 845.
18. W. Bambynek, B. Crasemann, R. W. Fink, H. V. Freund, H. Mark, R. E. Price, P. V. Rao, *Rev. Mod. Phys.* **44**, 716 (1972).
19. C. K. Davis and J. S. Greenberg, *Phys. Rev. Lett.* **32**, 1215 (1974); T. K. Saylor, personal communication.
20. W. E. Meyerhof, T. K. Saylor, S. M. Lazarus, A. Little, B. B. Triplett, L. F. Chase, Jr., R. Anholt, *Phys. Rev. Lett.* **32**, 1279 (1974).
21. H. D. Betz, F. Bell, H. Panke, W. Stehling, E. Spindler, M. Kleber, *ibid.* **34**, 1256 (1975).
22. K. Smith, B. Müller, W. Greiner, *J. Phys. B* **8**, 75 (1975).
23. B. Müller, in *The Physics of Electronic and Atomic Collisions*, J. S. Risley and R. Geballe, Eds. (Univ. of Washington Press, Seattle, 1975), p. 481.
24. W. E. Meyerhof, *Phys. Rev. Lett.* **31**, 1341 (1973); K. Taulbjerg, J. Vaaben, B. Fastrup, *Phys. Rev. A* **12**, 2325 (1975).
25. W. R. Thorson and S. H. Choi, in *Abstracts, 9th International Conference on the Physics of Electronic and Atomic Collisions*, J. S. Risley and R. Geballe, Eds. (Univ. of Washington Press, Seattle, 1975), p. 298; personal communication.
26. K. Smith and W. Greiner, *J. Phys. B*, in press.
27. J. S. Briggs, *ibid.* **7**, 47 (1974).
28. J. H. Macek and J. S. Briggs, *ibid.*, p. 1312.
29. W. Lichten, *Phys. Rev. A* **9**, 1458 (1974).
30. J. S. Briggs, *J. Phys. B*, in press.
31. R. Anholt, unpublished results.
32. D. H. Jakubassa and M. Kleber, *Z. Phys. A* **273**, 29 (1975).
33. K. Alder, A. Bohr, T. Huus, B. Mottelson, A. Winther, *Rev. Mod. Phys.* **28**, 432 (1956); J. Reinhardt, G. Soff, W. Greiner, *Z. Phys. A* **276**, 285 (1976); H. P. Trautvetter, J. S. Greenberg, P. Vincent, *Phys. Rev. Lett.* **37**, 202 (1976).
34. F. Bell, H. D. Betz, H. Panke, E. Spindler, W. Stehling, M. Kleber, *ibid.* **35**, 841 (1975).
35. W. E. Meyerhof, T. K. Saylor, R. Anholt, *Phys. Rev. A* **12**, 2641 (1975).
36. P. H. Mokler, P. Armbruster, F. Folkman, S. Hagmann, G. Kraft, H. J. Stein, in *The Physics of Electronic and Atomic Collisions*, J. S. Risley and R. Geballe, Eds. (Univ. of Washington Press, Seattle, 1975), p. 501; F. Folkmann, P. Armbruster, S. Hagmann, G. Kraft, P. H. Mokler, H. J. Stein, *Z. Phys. A* **276**, 15 (1976).
37. B. Müller, R. K. Smith, W. Greiner, *Phys. Lett. B* **49**, 219 (1974); B. Müller and W. Greiner, *Phys. Rev. Lett.* **33**, 469 (1974); M. Gros, B. Müller, W. Greiner, *J. Phys. B*, in press.
38. J. S. Greenberg, C. K. Davis, P. Vincent, *Phys. Rev. Lett.* **33**, 473 (1974).
39. W. Woelfli, C. Stoller, G. Bonani, M. Suter, M. Stoeckli, *Lett. Nuovo Cimento* **14**, 577 (1975); W. Woelfli, C. Stoller, G. Bonani, M. Stoeckli, M. Suter, *Phys. Rev. Lett.* **36**, 309 (1976).
40. R. K. Smith, paper presented at the Fourth International Seminar on Ion-Atom Collisions, Stanford University, 1975; _____ and W. Greiner, in preparation.
41. F. Folkmann, C. Gaarde, T. Huus, K. Kemp, *Nucl. Instrum. Methods* **117**, 487 (1974).
42. Recent publications, in which earlier references can be found, are: A. R. Sohval, J. P. Delvaille, K. Kalata, K. Kirby-Docken, H. W. Schnopper, *J. Phys. B* **9**, L25 (1976); *ibid.*, p. 47; H. D. Betz, M. Kleber, E. Spindler, F. Bell, H. Panke, W. Stehling, in *The Physics of Electronic and Atomic Collisions*, J. S. Risley and R. Geballe, Eds. (Univ. of Washington Press, Seattle, 1975), p. 520.
43. P. Gippner, *Joint Inst. Nucl. Res. Dubna Publ. No. E-78843* (1975).
44. K. H. Kaun, in *Proceedings of the Second International Conference on Inner-Shell Ionization Phenomena*, W. Mehlhorn, Ed. (University of Freiburg, Freiburg, Germany, in press).
45. K. H. Heinig, H. U. Jäger, H. Richter, H. Woittennek, *Phys. Lett. B* **60**, 249 (1976).
46. W. R. Stott and J. C. Waddington, *Bull. Am. Phys. Soc.* **20**, 638 (1975); R. Anholt and T. K. Saylor, *Lawrence Berkeley Lab. Rep. No. LBL-4066* (1975).
47. W. E. Meyerhof, T. K. Saylor, S. M. Lazarus, A. Little, B. B. Triplett, L. F. Chase, Jr., R. Anholt, unpublished results.
48. W. E. Meyerhof, *Phys. Rev. A* **10**, 1005 (1974).
49. J. S. Greenberg, personal communication.
50. B. Fricke and G. Soff, *Ges. Schwerionenforsch. Rep. No. GSI-T1-74* (1974); B. Fricke, *Ges. Schwerionenforsch. Rep. No. GSI-73-11* (1973), p. 88.
51. G. Bissinger and L. Feldman, *Phys. Rev. A* **8**, 1624 (1973); *Phys. Rev. Lett.* **33**, 1 (1974); J. A. Cairns and L. C. Feldman, in *New Uses of Low Energy Accelerators*, J. F. Ziegler, Ed. (Plenum, New York, in press).
52. R. K. Smith, B. Müller, W. Greiner, J. S. Greenberg, C. K. Davis, *Phys. Rev. Lett.* **34**, 134 (1975). The experimental effect appears to be spurious. See W. Woelfli, C. Stoller, G. Bonani, M. Suter, M. Stoeckli, *ibid.* **35**, 656 (1975).
53. D. Burch, W. B. Ingalls, H. Wieman, R. Vandenbosch, in *Abstracts, 9th International Conference on the Physics of Electronic and Atomic Collisions*, J. S. Risley and R. Geballe, Eds. (Univ. of Washington Press, Seattle, 1975), p. 306; R. Schule, H. Schmidt-Böcking, I. Tserruya, G. Gaukler, K. Bethge, in *ibid.*, p. 304; I. Tserruya, H. Schmidt-Böcking, R. Schule, K. Bethge, R. Schuch, H. J. Specht, *Phys. Rev. Lett.*, in press; F. Jundt, G. Guillaume, P. Fintz, K. U. Jones, *Phys. Rev. A* **13**, 563 (1976).
54. B. Müller, J. Rafelski, W. Greiner, *Phys. Lett. B* **47**, 5 (1973).
55. B. Müller and W. Greiner, *Z. Naturforsch. Teil A* **31**, 1 (1976).
56. S. S. Gershtein and V. S. Popov, *Lett. Nuovo Cimento* **6**, 593 (1973); H. Peitz, B. Müller, J. Rafelski, W. Greiner, *ibid.* **8**, 37 (1973).
57. K. Smith, H. Peitz, B. Müller, W. Greiner, *Phys. Rev. Lett.* **32**, 554 (1974).
58. D. Burch, W. B. Ingalls, H. Wieman, R. Vandenbosch, *Phys. Rev. A* **10**, 1245 (1974).
59. C. Foster, T. Hoogkamer, P. Worlee, F. W. Saris, in *Abstracts, 9th International Conference on the Physics of Electronic and Atomic Collisions*, J. S. Risley and R. Geballe, Eds. (Univ. of Washington Press, Seattle, 1975), p. 511; *J. Phys. B*, in press.
60. J. Rafelski, B. Müller, R. Anholt, personal communications; W. Betz, G. Soff, B. Müller, W. Greiner, in preparation.
61. Systematics established in W. E. Meyerhof, R. Anholt, T. K. Saylor, P. D. Bond [*Phys. Rev. A* **11**, 1083 (1975)] lead to $\sigma_K \approx 3 \times 10^{-25}$ cm^2. From (*18*), $\tau_K = 7 \times 10^{-18}$ sec. For 1600-Mev U, $v_1 = 4 \times 10^9$ cm/sec, and for U, $n = 4.7 \times 10^{22}$ cm^{-3}.
62. V. Oberacker, G. Soff, W. Greiner, *Nucl. Phys. A* **259**, 324 (1976).
63. P. Armbruster, in *Proceedings of the Second International Conference on Inner-Shell Ionization Phenomena*, W. Mehlhorn, Ed. (University of Freiburg, Freiburg, Germany, in press).
64. Enlightening discussions with R. Anholt are gratefully acknowledged. This work was partly supported by the National Science Foundation.

Chromosomal Subunits in Active Genes Have an Altered Conformation

Globin genes are digested by deoxyribonuclease I in red blood cell nuclei but not in fibroblast nuclei.

Harold Weintraub and Mark Groudine

Knowledge of the structure of DNA has provided many insights into its biological function (*1*). In higher cells, a detailed understanding of the structure of chromatin will probably provide analogous insights into how genes are regulated. Already, there are a number of important observations demonstrating a relation between the structure of chromatin and its biological activity (*2*, *3*).

The packaging of most of the nuclear DNA is now thought to be based on repeating units of about 180 to 200 base pairs of DNA associated with specific complexes of histones (*4*, *5*), possibly two self-complementary tetramers each containing one of the four major histones (*6*). These two tetramers could define the twofold axis of symmetry within the nucleosome. These complexes interact through 70 to 90 amino acid residues at their carboxyl terminal end to produce a tight, trypsin-resistant core (*7*). The positively charged histone amino terminal residues extend outward from this core and define what may prove to be a "kinked" or "coiled" pathway for the DNA (*5*, *8*) about the histone complexes. These so-called "particles-on-a-string" or "nu" bodies constitute the primary level of folding for the bulk of the chromosome. Through their mutual interactions higher levels of DNA packaging can be achieved, although details of this organization are not known. At present there is no proof that nu bodies are homo-

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geneous (*9*) either in composition or in conformation. Also lacking is concrete evidence that nu bodies are associated with active genes (*10*) and, if so, whether they are in the same conformation as those nu bodies associated with inactive genes. The important finding that *Escherichia coli* RNA polymerase preferentially transcribes globin genes from reticulocyte chromatin but not from liver or brain chromatin (*11*) reflects the fact that some structural aspect of an activated gene is different in different tissues. However, whether this difference occurs only at the 5′ end of the gene, perhaps at a promotor region, or throughout the entire length of the gene is not known. Similarly, it is not clear whether the tissue-specific differences in accessibility revealed by RNA polymerase arise from differences in the basic nu body configuration or in the way some or all of the nu bodies in a transcription unit are packaged into higher levels of organization.

In this article, we show that active genes are likely to be packaged by histones but that these histones are in an altered conformation, one that renders the associated DNA extremely sensitive to digestion by pancreatic deoxyribonuclease I.

Digestion of Chicken Erythrocyte Nuclei by Deoxyribonuclease I

The kinetics of digestion of chicken erythrocyte nuclei by pancreatic deoxyribonuclease I (*12*) are shown in Fig. 1. The bottom insets show the corresponding patterns of resistant single-stranded DNA fragments displayed on a denaturing acrylamide gel. The kinetics reveal a rapid initial digestion of about 15 percent of the DNA followed by a slower digestion that levels off at about 50 percent, and an even slower process that leads to digestion beyond 50 percent. The characteristic pattern of resistant products (between 20 and 160 bases in our standard gels) appears very early in digestion and persists through 50 percent digestion with no apparent increase or decrease in the intensity of any one particular band. Beyond 50 percent digestion, the larger bands disappear before the smaller bands do. This disappearance is accompanied by an accumulation of DNA between the usually well defined DNA peaks. We interpret this to mean that the enzyme is sequentially digesting a base or two at a time when digestion proceeds beyond 50 percent, and that this phase of the digestion process probably represents "nibbling" (digestion of a base at a time) that occurs in DNA regions that are intimately protected by proteins, presumably histones. In contrast, the repeating pattern of denatured DNA fragments between 20 and 160 bases probably represents the cutting at very accessible regions between and within individual nu bodies. It is not clear to us why the larger fragments fail to break down into the smaller ones before digestion reaches 50 percent and nibbling ensues. The conversion of larger fragments into smaller ones would have been predicted if the fragments arose from the statistical cleavage of a number of accessible sites within a homogeneous population of nu bodies.

Tissue-Specific, Preferential Digestion of Active Genes

Staphylococcal nuclease shows no preferential digestion of specific nuclear DNA sequences, in particular sequences coding for active genes (*13*). Since it is clear that pancreatic deoxyribonuclease has a much higher affinity for sites within nu bodies, and hence might be expected to differentiate between similar nu bodies, we decided to investigate whether pancreatic deoxyribonuclease preferentially digested the DNA coding for active genes. There are a number of indications that this might be occurring during the digestion of nuclei with this enzyme. We have shown that ribosomal DNA in nuclei is especially sensitive to deoxyribonuclease (*14*); in addition, Billing and Bonner (*15*) have shown that RNA labeled for a short period is rapidly released on mild digestion of nuclei with either deoxyribonuclease I or II. Finally, Berkowitz and Doty (*16*) have shown that a putative active fraction of sheared chromatin (isolated as a slow-sedimenting fraction on sucrose gradients) is much more sensitive to deoxyribonuclease I than is bulk chromatin.

To investigate this question further, we have prepared a complementary DNA (cDNA) probe to globin messenger RNA (mRNA) isolated from the reticulocytes of the adult chicken. The details of the purification and analysis and the characteristics of the cDNA have previously been described (*17*); in particular it has been demonstrated that the cDNA made to adult globin mRNA can be used to detect globin mRNA coding for embryon-

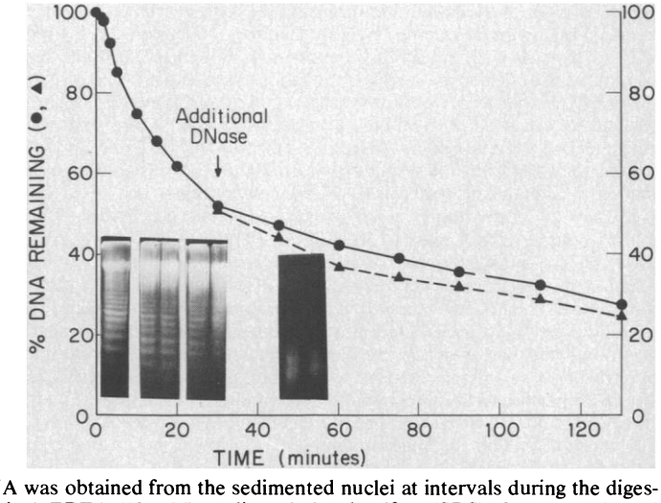

Fig. 1. Digestion of chick erythrocyte nuclei with pancreatic deoxyribonuclease I. (Curves) Avian red blood cells were isolated as described (*17*). The cells were washed twice in phosphate-buffered saline (PBS) (Grand Island), and the nuclei were isolated by suspension in reticulocyte standard buffer (RSB) (0.01*M* tris-HCl, *p*H 7.4; 0.01*M* NaCl; 3 m*M* $MgCl_2$) containing 0.5 percent NP-40 (British Drug House). The nuclei were washed several times in RSB and then digested at a DNA concentration of 1 mg/ml at 37°C with pancreatic deoxyribonuclease I (Sigma) (20 μg/ml) for increasing periods of time. The percentage of DNA remaining (●——●) was determined either by precipitation with cold 7 percent perchloric acid and subsequent measurement of the absorbancy at 260 nm of the acid soluble and insoluble fraction or by sedimenting the nuclei at low speed and measuring the release of material absorbing at 260 nm. Either method gave essentially the same results. Addition of fresh deoxyribonuclease (20 μg/ml) when the digestion begins to level off (at about 50 percent) does not affect the course of subsequent digestion. (▲——▲) The time course of digestion and the pattern of resistant fragments (see below) is the same in chromatin isolated either by sonication or by mild treatment with nuclease. Substitution of $CaCl_2$ for $MgCl_2$ also did not affect the course of digestion or pattern of resistant fragments. (Electrophoretic patterns) The deoxyribonuclease-resistant DNA was obtained from the sedimented nuclei at intervals during the digestion. The following were added to the nuclear pellet (final concentration) EDTA (10 m*M*), sodium dodecyl sulfate (SDS) (0.1 percent), and protease (Aldrich) (500 μg/ml). The mixture was incubated for 60 minutes at 37°C, boiled for 5 minutes, added directly to sample buffer (50 percent glycerol, 0.01 percent bromphenol blue), and loaded onto a 6 percent acrylamide slab gel containing 98 percent formamide and 20 m*M* sodium phosphate at *p*H 7.0. Electrophoresis was conducted for 5 hours at 160 volts in a running buffer containing 20 m*M* sodium phosphate, *p*H 7.0. The gels were stained with ethidium bromide (2 μg/ml) and the denatured DNA bands (bottom insets) were photographed through a red filter after illumination with ultraviolet light. *DNase*, deoxyribonuclease.

ic globin polypeptide chains (*17*). Thus, embryonic red cell RNA saturates about 70 percent of the cDNA probe prepared from adult red cell globin mRNA, while adult red cell RNA saturates the labeled probe at about 95 percent (*17*). The cross reaction of the adult probe with embryonic RNA is qualitatively attributable to the fact that the adult line of red cells synthesize three main types of globin chains, two of which are identical to chains synthesized by the embryonic line of red cells and the third bears about a 50 percent homology by tryptic peptide analysis (*18*) to two other embryonic globin chains.

When the DNA in nuclei from adult chick erythrocytes—this line of red blood cells contains the adult globin polypeptide chains—is digested with pancreatic deoxyribonuclease I so that 10 percent is acid soluble, approximately 75 percent of the globin cDNA probe fails to hybridize with the remaining 90 percent of the purified erythrocyte DNA (Fig. 2). Similarly, after the same amount of digestion, DNA isolated from the embryonic line of erythroblasts protects only about 50 percent of the cDNA at saturation. In contrast, undigested DNA isolated from either embryonic or adult red cells saturates the probe at more than 94 percent. These experiments show that, in the different red cell lines, specific globin sequences are particularly sensitive to digestion. We present evidence below that embryonic but not adult-specific globin sequences and digested in embryonic red cells and adult but not embryo-specific sequences are digested in adult red cells. The same preparations of digested nuclei from either the adult or embryonic line of red cells contains the DNA sequences coding for ovalbumin mRNA (Fig. 2). Most importantly, nuclei from cultured chick fibroblasts or freshly isolated chick brain, after 10 to 20 percent digestion (more extensive amounts of digestion were not tested), retain the DNA sequences coding for both adult and embryonic globin (Fig. 3). In addition, the fibroblast nuclei retain the DNA sequences coding for ovalbumin (Fig. 2). In contrast, digestion of red cell nuclei to DNA fragments of approximately the same size by a different nuclease, staphylococcal nuclease, results in no preferential digestion of any globin sequences (Fig. 3). This was first shown by Axel *et al.* (*13*) and recently extended by Lacy and Axel (*19*).

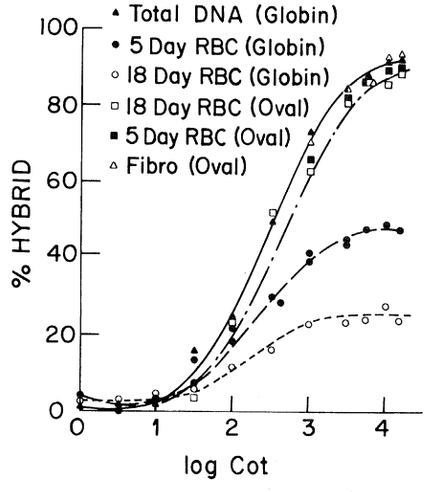

Fig. 2. Preferential digestion of active genes by pancreatic deoxyribonuclease I. Red blood cells (RBC) were obtained by vein puncture from 18-day (containing adult-type globin chains) and 5-day (containing embryo-type globin chains) chick embryos. Fibroblasts were dissected from the region of the developing breast muscle of 11-day chick embryos and grown in culture (*17*). Nuclei from 18-day embryo RBC's, 5-day embryo RBC's, and 11-day cultured chick embryo fibroblasts were isolated in RSB containing 0.5 percent NP-40 and digested with pancreatic deoxyribonuclease I until 10 to 20 percent of the DNA was soluble in acid (legend to Fig. 1). DNA was prepared as follows. Nuclei were centrifuged and suspended in 0.1 percent SDS, 100 μg of pronase per milliliter, and 5 m*M* EDTA overnight at 37°C. The sample was extracted several times with equal volumes of a mixture of phenol and chloroform (1 : 1), and several times with a mixture of chloroform and isoamyl alcohol (24 : 1). The resultant aqueous phase was made 0.1*M* with respect to NaCl, and the nucleic acid was precipitated overnight at −20°C with two volumes of 95 percent ethanol. The nucleic acid was recovered by centrifugation for 30 minutes at 10,000 rev/min (HB-4 head of a Sorvall RC-5 centrifuge), suspended in 10 m*M* NaCl, 10 m*M* tris-HCl (*p*H 7.4), and incubated for 30 minutes at 37°C with ribonuclease A (20 μg/ml) (Worthington) that had been boiled for 30 minutes. The preparation was again extracted with the phenol-chloroform and then chloroform–isoamyl alcohol mixtures and the extract was precipitated with ethanol. The DNA concentration was determined by absorbancy at 260 nm in a Zeiss spectrophotometer (DNA at 1 mg/ml gave 20 A_{260}). Total DNA was prepared directly from 18-day red cell nuclei and sonicated so that the average length corresponded to 500 nucleotides. The cDNA complementary to globin mRNA from adult reticulocytes was prepared as described (*17*). Hybridizations were conducted with an excess of DNA to cDNA (1×10^7 to 2×10^7 : 1) and analyzed (*17*). The DNA samples suspended in a mixture of 0.3*M* NaCl, 50 m*M* tris-HCl (*p*H 7.4), and 0.1 percent SDS and ranging in concentration from 1 to 20 mg/ml were denatured by heat and annealed at 65°C (at 1000 count/min per 5 μl of reaction mixture) to [^{3}H]deoxycytidine- and [^{3}H]thymidine-labeled cDNA (5×10^7 to 8×10^7 count/min per microgram). Under these conditions the calculated ratio of globin DNA to globin cDNA was 10 : 1 to 15 : 1. Polypropylene tubes overlaid with paraffin oil were used for the hybridizations. At intervals (from 5.7 minutes to 96 hours), 10-μl samples of reaction mixtures were pipetted into 400 μl of a mixture of 30 m*M* sodium acetate (*p*H 4.5), 0.15*M* NaCl, 1 m*M* $ZnSO_4$, and 10 μg of denatured DNA from salmon sperm. Half (200 μl) of the above mixture was immediately precipitated with trichloroacetic acid (TCA) and the other half was incubated with partially purified S1 nuclease at 45°C for 40 minutes. The percentage of hybridization was determined by comparison of the TCA-precipitable radioactivity remaining after S1 digestion to that precipitable in the undigested samples. The S1 background radioactivity ranged from 2 to 6 percent. The percentage of hybridized cDNA is plotted as a function of the C_0t, which represents the concentration of deoxyribonucleotide (moles) times the time of digestion (seconds) per liter (*28*). The same plateaus were obtained at concentrations of driver DNA from 2 to 20 mg/ml in a reaction volume of 50 μl, to which cDNA (5000 count/min) was added. (▲——▲) Total DNA hybridized to globin cDNA; (●——●) 5-day red cell DNA treated with deoxyribonuclease I and hybridized to globin cDNA; (○——○) 18-day red cell DNA treated with deoxyribonuclease I and hybridized to globin cDNA; (□——□) 18-day red cell DNA treated with deoxyribonuclease I and hybridized to ovalbumin cDNA; (■——■) 5-day red cell DNA treated with deoxyribonuclease I and hybridized to ovalbumin cDNA; (△——△) fibroblast DNA treated with deoxyribonuclease I and hybridized to ovalbumin cDNA.

In summary, digestion of nuclei with pancreatic deoxyribonuclease reveals that specific globin sequences are preferentially degraded in erythroid cells, but not in nonerythroid cells. Similarly, the ovalbumin gene is not preferentially digested in cells that do not produce ovalbumin.

Identification of Globin Genes Digested in Adult and Embryonic Nuclei

The failure of DNA isolated from red cell nuclei treated with deoxyribonuclease I to saturate the cDNA probe (Fig. 2) could be due (i) to the specific degradation of a unique subset of globin genes, as we have suggested, or (ii) to an overall reduction in DNA sequences complementary to all sequences present in the cDNA, resulting in a situation where the cDNA is in excess. To exclude the second possibility, we have performed several of the reannealing experiments shown in Fig. 2 with one-tenth the amount of driver DNA (that is, the DNA which determines the rate of the reaction) and the same amount of cDNA. Under these conditions, no change is observed in the level of saturation of the cDNA. This demonstrates that specific globin DNA sequences are digested by pancreatic deoxyribonuclease and also that specific sequences are resistant.

As was mentioned previously, our cDNA probe contains sequences complementary to three adult globin mRNA

molecules. The resistant DNA obtained after embryonic erythroblast nuclei are mildly treated with deoxyribonuclease I saturates the cDNA probe at about 50 percent. Our hypothesis is that only actively transcribing globin genes are digested. Since two of three adult globin genes are also active in the embryonic red cell line, we predict that two adult globin genes are digested in embryonic cells and the third is resistant. To test this, polysomal RNA from embryonic red cells was added to the hybridization mixture which also contained ^{3}H-labeled cDNA from adult globin mRNA and partially digested DNA from embryonic red cell nuclei. Under these conditions, the cDNA is fully saturated at about 98 percent (Fig. 4). Thus, embryonic red cell RNA fully complements the deficiency in globin DNA sequences resulting from the digestion. This implies that embryonic sequences are absent and the adult-specific sequences are present in the DNA from the embryonic red cell nuclei treated with deoxyribonuclease I.

A similar type of analysis suggests that adult-specific sequences are preferentially digested in the adult line of red cell nuclei. Figure 2 shows that the cDNA is saturated at about 25 percent by DNA isolated from adult red cell nuclei treated with pancreatic deoxyribonuclease. Our hypothesis in this case is that only adult-specific globin genes are digested. In principle, if this is true, then none of the probe should be protected from S1 nuclease. We believe that the partial protection of the probe by DNA obtained from adult nuclei treated with deoxyribonuclease I can be explained by two embryonic globin genes that bear a marked homology to the β-globin gene in the adult (*18*). These embryo-specific globin genes (named ϵ and ρ) would be present in the deoxyribonuclease I–resistant fraction from adult red cell nuclei and, by virtue of their homology to the adult β-globin, would be expected to partially protect some of the cDNA. Since the adult β-gene codes for about 50 percent of the adult globin chains, it is not unreasonable that it constitutes about 50 percent of our cDNA population. In addition, peptide analysis has shown about a 50 percent identity in tryptic peptides between the β-globin in adults and the ϵ- and ρ-globin chains in the embryo (*18*). Thus, if our logic is correct, we predict that the digested adult nuclei should saturate about 25 percent of the cDNA probe (50 percent $\times$ 50 percent = 25 percent). To test this, embryonic red cell RNA was added to the hybridization mixture containing the cDNA and DNA from treated adult nuclei. Under these conditions, the probe is saturated at about 70 percent (Fig. 4). This is precisely the saturation achieved by pure embryonic red cell RNA alone (*17*), an indication that the deoxyribonuclease I–resistant DNA from adult red cells contains no adult-specific globin sequences (Table 1).

The sensitivity of the globin genes to deoxyribonuclease I could be related to the fact that they are extremely active in transcription and are therefore not typical of most of the genes actively being transcribed. The chicken genome contains endogenous DNA sequences complementary to those of avian tumor viruses (ATV). A cDNA probe made against a specific type of avian tumor virus, avian myeloblastosis virus RNA (AMV), hybridizes with chicken DNA with a sequence homology of about 60 percent and a log $C_0t_{1/2}$ of about 1.75. This corresponds to about eight to ten copies of endogenous ATV DNA per cell. In separate experiments the total RNA from embryonic chick erythroblasts was hybridized to the ^{3}H-labeled AMV cDNA. The kinetics of hybridization indicated that from one-tenth to two copies of endoge-

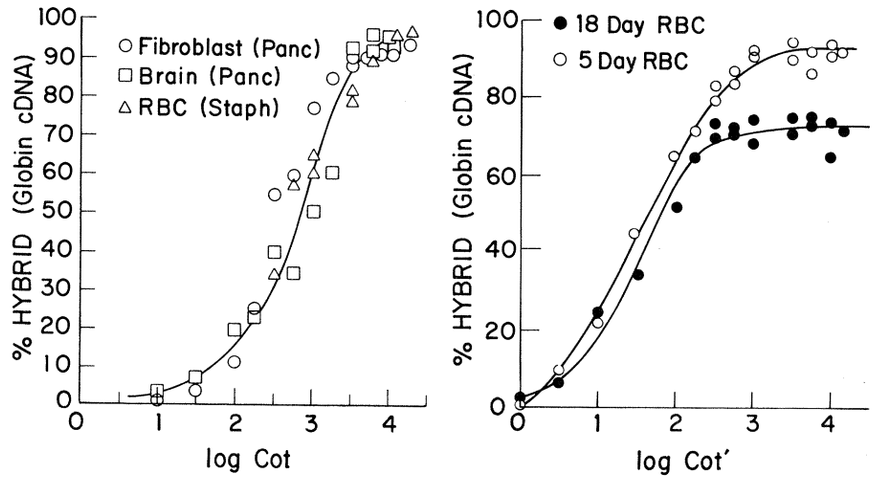

Fig. 3 (left). Retention of inactive genes after treatment of nuclei with pancreatic deoxyribonuclease. As was described (legend to Fig. 2), 18-day RBC nuclei and 11-day fibroblast nuclei were isolated from chick embryos. Brains were dissected from 11-day chick embryos, incubated for 30 minutes in PBS (Gibco) with 0.1 percent trypsin, washed repeatedly with PBS to eliminate contaminating RBC's, and lysed by homogenization in RSB with 0.5 percent NP-40. Fibroblast and brain nuclei were digested so that 20 percent of the DNA was acid soluble (legend to Fig. 1). The 18-day RBC's were digested until 50 percent of the DNA was acid soluble with staphylococcal nuclease (50 μg/ml) for 15 minutes at 37°C in RSB supplemented with $10^{-4}M$ $CaCl_2$. More than 80 percent of the acid insoluble DNA from this preparation consisted of 20 to 145 base pairs, as analyzed on 6 percent acrylamide gels. The isolation of resistant DNA from the digested nuclear preparations and the conditions and analysis of hybridization, including DNA concentrations and times of incubation, were as described (Fig. 2 legend). (○——○) Fibroblast DNA digested with deoxyribonuclease I and hybridized to globin cDNA; (□——□) brain DNA digested with deoxyribonuclease I and hybridized to globin cDNA; (△——△) 18-day red blood cell DNA digested with staphylococcal nuclease and hybridized to globin cDNA. Fig. 4 (right). Hybridization kinetics of globin cDNA and mixtures of 5-day erythroid RNA and DNA from 18-day or 5-day RBC nuclei digested with pancreatic deoxyribonuclease. Embryonic erythroid RNA was prepared from the cytoplasm of RBC's from 5-day chick embryos. The 5-day RBC's were obtained by vein puncture, washed repeatedly with autoclaved PBS, and lysed in autoclaved RSB supplemented with 0.5 percent NP-40 and mouse liver ribonuclease inhibitor (*17*). Nuclei were sedimented in a table-top centrifuge, and the supernatant was extracted several times with equal volumes of a solvent mixture containing phenol-chloroform (1 : 1) and then several times with a mixture of chloroform and isoamyl alcohol (24 : 1). The aqueous phase was made $0.1M$ with respect to NaCl, and the nucleic acid was precipitated with two volumes of 95 percent ethanol (overnight at −20°C) and recovered by centrifugation for 60 minutes at 11,000 rev/min (HB-4 head of a Sorvall RC-5 centrifuge); it was suspended in a solution of 10 mM NaCl, 10 mM tris-HCl (pH 7.4), and 5 mM $MnCl_2$, incubated with ribonuclease-free deoxyribonuclease (10 μg/ml) (Worthington) for 30 minutes at 37°C, and again extracted with the above solvent mixtures. The RNA was precipitated with ethanol and suspended in a solution of 10 mM NaCl, 10 mM tris-HCl (pH 7.4), and the amount of RNA was determined at A_{260} in a Zeiss spectrophotometer (RNA at 1 mg/ml = 24 A_{260}). The nuclei were digested with pancreatic deoxyribonuclease (10 μg/ml) (Worthington) for 30 minutes at 37°C, and again extracted with the conditions, and analysis of hybrid formation are described in Fig. 2. The ratio of DNA to RNA in the hybridization mixture was 10 : 1. The nucleic acid concentration ranged from (per milliliter) 19 mg of DNA and 1.9 mg of RNA to 1.9 mg of DNA and 0.19 mg of RNA. Because of the minor contribution of RNA to the total nucleic acid concentration, the RNA concentration was not considered in the calculation of C_0t'. (●——●) DNA from pancreatic deoxyribonuclease–treated 18-day red blood cells plus RNA from 5-day embryonic red blood cells hybridized to globin cDNA. (○——○) DNA from pancreatic deoxyribonuclease–treated 5-day red blood cells plus RNA from 5-day embryonic red blood cells hybridized to globin cDNA.

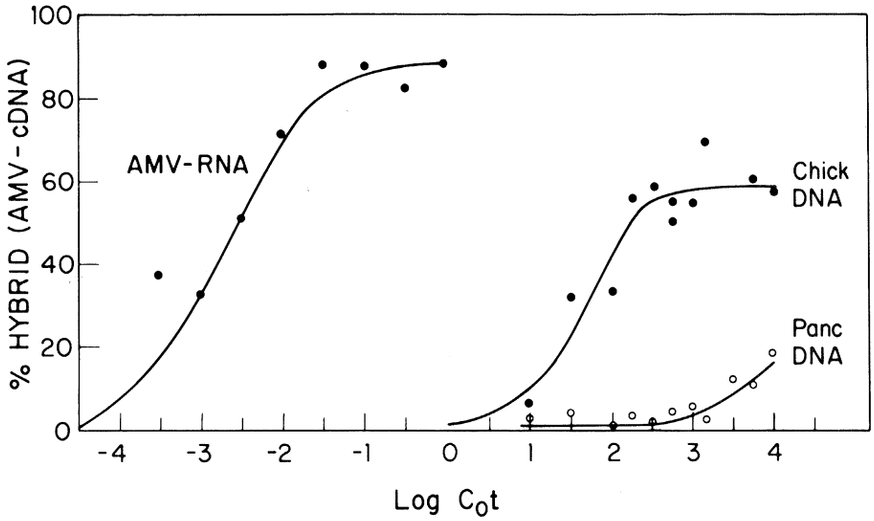

Fig. 5. Preferential digestion of endogenous avian tumor virus (ATV) genes by deoxyribonuclease I, in the embryonic line of chick erythroblasts. Preparations of DNA and procedures for digestion and hybridization were as described (Figs. 1 and 2). The AMV cDNA was prepared by the method described (*17*) for globin cDNA. The template for the reaction was the 35*S* RNA prepared from purified virus (a gift from J. Beard). The specific activity of the probe was about 4×10^7 to 6×10^7 count/min per microgram, and the probe hybridized to about 90 percent with its template, with a log $C_0t_{1/2}$ of about -2.75.

nous RNA sequences were present per cell. Similar very low but detectable levels of accumulation of RNA sequences from endogenous chicken viruses have been reported by Hanafusa *et al.* (*20*). Despite the fact that this gene has a low activity in erythroblasts, it is nevertheless sensitive to digestion by pancreatic deoxyribonuclease in isolated nuclei (Fig. 5). From the $C_0t_{1/2}$ of the reactions, it is possible to calculate that these endogenous viral sequences are about 100 times more sensitive to deoxyribonuclease I than the bulk of the nuclear DNA. Thus, two specific classes of active genes are preferentially digested by deoxyribonuclease I—the very active globin genes and the less active endogenous RNA tumor virus genes.

Digestion of a Specific Class of Nuclear DNA Sequences

The next question is whether after 20 percent digestion of nuclei by deoxyribonuclease I a specific 20 percent of the DNA sequences is absent from the remaining DNA. Total [^{3}H]thymidine-labeled chick DNA was hybridized with a 10,000-fold excess of driver DNA obtained after mild deoxyribonuclease treatment of embryonic red cell nuclei. Figure 6 shows that 78 percent of the total ^{3}H-labeled DNA is protected at saturation when DNA from nuclei treated with deoxyribonuclease I is used to drive the reaction. In contrast, when the driver DNA is DNA obtained from the 11*S* monomers produced by staphylococcal nuclease treatment of nuclei, 94 percent of the labeled DNA is protected from S1 nuclease. Addition of total DNA (but not deoxyribonuclease I–treated DNA) to the reaction after 78 percent saturation is achieved increases the extent of hybridization to 95 percent (Fig. 6 and Table 2A). This suggests that the smallest deoxyribonuclease-digested fragments do not inhibit the hybridization of longer fragments and that the failure to reach full saturation is due to the absence of a particular subset of sequences in the deoxyribonuclease-treated DNA. The saturation value decreases to 65 percent after 52 percent digestion (Table 2).

Whether the specific nuclear DNA sequences that are preferentially digested by deoxyribonuclease I are related to the sequences that are actively being transcribed was tested as follows. The DNA from nuclei digested to 20 percent acid solubility was hybridized to total [^{3}H]thymidine-labeled tracer DNA. After saturation had been reached (78 percent), nuclear RNA was added to the hybridization reaction. Under these conditions, the saturation value increases to 89 percent (Fig. 6 inset). The difference in saturation between the reactions that occur in the presence and absence of added nuclear RNA (78 versus 89 percent) is very reproducible and is probably due to RNA-DNA hybrid formation

Table 1. Observed and predicted saturation of globin cDNA by various nucleic acid preparations. Globin gene terminology is based on the tryptic peptide analysis of Brown and Ingram (*18*), as is the representation of globin chains in the adult (18-day) and embryonic (5-day) red blood cell (RBC) populations; (+) indicates either the presence of the DNA sequences coding for particular globin chains or the presence of the globin polypeptide chains in the respective embryonic or adult populations; (−) indicates the absence of the globin genes or the absence of the globin polypeptide chains. In the nucleic acid mixtures containing both DNA and RNA, the contribution of each nucleic acid to the protection of cDNA from S1 nuclease digestion is represented by the subscripts D and R, respectively. Saturation values are taken from Figs. 2 and 3.

Item	Globin genes						Saturation of adult cDNA (%)	
	π (α-like)	α_A	α_D	ε (β-like)	ρ (β-like)	β	Observed	Predicted
cDNA from adult RBC*		35%	15%			50%		
Active genes in adult RBC	−	+	+	−	−	+		
Active genes in embryonic RBC	+	+	+	+	+	−		
Postulated sequences remaining after treatment of:								
(A) Embryonic RBC (Fig. 2)	−	−	−	−	−	+	50	50
(B) Adult RBC (Fig. 2)	+	−	−	+	+	−	28	25†
(C) Embryonic RBC plus embryonic RBC RNA (Fig. 3)	$+_R$	$+_R$	$+_R$	$+_R$	$+_R$	$+_D$	94	100
(D) Adult RBC plus embryonic RBC RNA	$+_{D,R}$	$+_R$	$+_R$	$+_{D,R}$	$+_{D,R}$		72	75

*Complementary DNA was prepared from adult globin mRNA (*17*). The percentage representation of specific globin genes in the cDNA population is based on the analysis of Brown and Ingram (*18*), and on the assumption that a stoichiometric relation exists between the template mRNA and cDNA product. †The predicted 25 percent saturation of cDNA by DNA of deoxyribonuclease I–digested adult RBC nuclei is based on the shared tryptic peptides of the adult β-chain and the embryonic ϵ-and ρ-chains.

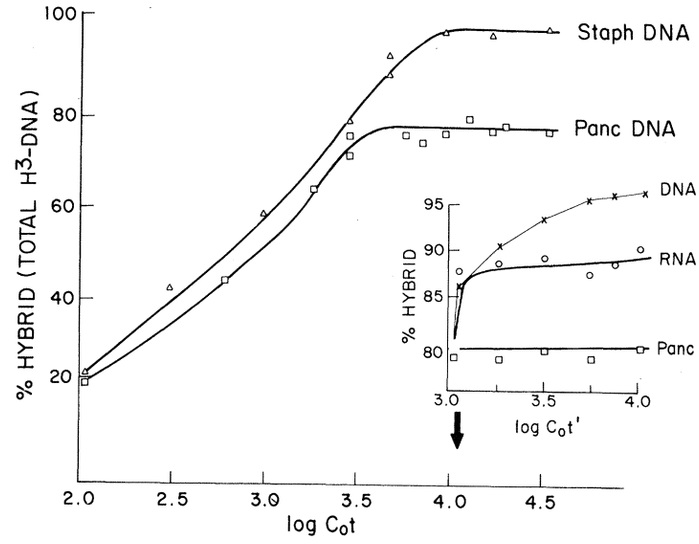

Fig. 6. Kinetics of reassociation of chick total ^{3}H-labeled DNA and DNA from nuclease-treated nuclei of 18-day RBC's. Total ^{3}H-labeled DNA was prepared by incubation of RBC's from 4-day chick embryos with [^{3}H]deoxythymidine. Cells were obtained by vein puncture, washed several times in medium F-12 (Gibco), and incubated for 5 hours in F-12 with [^{3}H]deoxythymidine (50 μc/ml; 16 c/mmole; New England Nuclear). The preparations of total DNA and of DNA from nuclei of 18-day RBC's digested with pancreatic deoxyribonuclease I were as described (Fig. 2); and the conditions of staphylococcal nuclease digestion were as described in Fig. 3 except that digestion was for 10 minutes to produce a population of fragments with a weight average molecular weight of 150 bases. Nuclear RNA was prepared from 5-day RBC's by lysis of these cells in the presence of mouse liver ribonuclease inhibitor (*18*), with an autoclaved solution of 0.5 percent NP-40 in RSB. The nuclei were washed several times in autoclaved RSB and lysed by gentle homogenization in 20 volumes of 0.15*M* NaCl, 0.05*M* sodium acetate (*p*H 5.1), and 0.3 percent SDS. The nuclear lysate was extracted three times with equal volumes of a mixture of phenol and chloroform (1 : 1) and numerous times with a mixture of chloroform and isoamyl alcohol (24 : 1). Ethanol precipitation, elimination of DNA, and determination of RNA concentration were as described (Fig. 4). Hybridization and analysis of hybrid formation were as described (Fig. 2). The concentration of labeled DNA was 10^{-4} that of driver DNA. Total ^{3}H-labeled DNA (5000 count/min per 5 μl of hybridization mixture) was annealed with DNA (20 mg/ml) from deoxyribonuclease I digestion of 18-day RBC nuclei (□——□) or DNA (20 mg/ml) from 11*S* monomers obtained from staphylococcal nuclease digestion of nuclei from 18-day RBC's (△——△). All points are plotted as DNA C_0t. (Inset) After the reaction with the digested DNA from 18-day RBC's reached saturation (log C_0t = 4.0), an equal volume of the digested DNA was added to the reaction at 20 mg/ml (□——□), or an equal volume of total DNA at 20 mg/ml (x——x), or an equal volume of nuclear RNA at 10 mg/ml (○——○). The reaction was allowed to proceed until a second saturation was achieved. The arrow shows when the additional nucleic acids were added to the original hybridization reaction.

between nuclear RNA and its ^{3}H-labeled template DNA since treatment of the hybrids with ribonuclease H (which specifically degrades RNA in DNA-RNA hybrids) makes an additional 15 percent of the hybrids sensitive to S1 nuclease (Table 2A).

The experiments described in Fig. 6 suggest that pancreatic deoxyribonuclease preferentially digests nuclear DNA sequences active in transcription of nuclear RNA. To verify this, [^{3}H]thymidine-labeled nuclei were partially digested with staphylococcal nuclease to produce a random population of small, resistant DNA fragments of predominantly 180 and 360 base pairs. This labeled DNA was hybridized to saturation with DNA obtained from red cells treated with pancreatic deoxyribonuclease, and the unhybridized labeled DNA (about 20 percent of the total) was isolated by passing the mixture over hydroxyapatite (HAP). According to the data in Fig. 6, the single-stranded flow-through DNA from HAP should be enriched in those sequences coding for active genes. To test this, excess total nuclear RNA was hybridized with the HAP flow-through DNA. About 48 percent of the labeled DNA is protected from S1 nuclease at saturation, and in a control experiment, nuclear RNA saturated only 9.8 percent of the total ^{3}H-labeled DNA (Table 2B). Thus the HAP flow-through DNA is enriched in DNA sequences complementary to nuclear RNA.

These conclusions can be tested in another way. Nuclear RNA, which has a kinetic complexity five to ten times greater than cytoplasmic RNA (*21*), was labeled for a short period and then hybridized to an excess of total red cell DNA or to an excess of embryo red cell DNA that had been treated with deoxyribonuclease I (Table 2C). Whereas 91 percent of the labeled RNA was protected from ribonuclease A digestion after being saturated by total DNA and 86 percent was protected by the staphylococcal nuclease DNA fragments, only 16 percent of the labeled RNA was protected at saturation by the pancreatic deoxyribonuclease I–treated DNA. Since 70 to 90 percent of the nuclear RNA is predominantly one major kinetic class of sequences (*21*), these experiments also suggest that pancreatic deoxyribonuclease preferentially digests much of the DNA coding for nuclear transcripts.

Altered Histone Conformation Associated with the Globin Genes

It is possible that the preferential digestion of the globin genes by deoxyribonuclease I is related to the way the 11*S* monomers are packaged into higher order structures within the cell nucleus. In order to test this possibility, the purified 11*S* monomers were mildly digested with pancreatic deoxyribonuclease in the presence of 3 m*M* $MgCl_2$ until 15 percent of the DNA was acid soluble. When the isolated DNA was hybridized to the globin cDNA, the adult-specific globin genes were absent (Table 2D). Thus, although the 11*S* monomers contain all the globin sequences, in the appropriate ionic conditions the proteins protecting the globin genes adopt a configuration that renders the associated globin DNA sensitive to digestion by pancreatic deoxyribonuclease. If these proteins are histones, then perhaps they are modified, either by direct chemical modification or by association with nonhistone proteins.

The sensitivity of monomers to pancreatic deoxyribonuclease apparently rules out a class of explanations for the digestion of active genes by deoxyribonuclease I based on the higher order packaging of monomers within the nucleus. The following experiment also supports the view that monomer packaging is not the basis for the digestion of active genes. The 11*S* monomers isolated from 18-day red cell nuclei were treated with trypsin (*22*) to remove 20 to 30 residues from the NH_2-terminus of each of the histones (*7*). The trypsin was then inactivated by addition of soybean trypsin inhibitor, and the particles were redigested with staphylococcal nuclease. The resistant DNA (about 20 percent of the total) was isolated and hybridized to tracer amounts of total ^{3}H-labeled DNA and to globin ^{3}H-labeled cDNA. Whereas more than 80 percent of the total ^{3}H-labeled DNA hybridizes to the resistant DNA, only 25 percent of the globin cDNA forms stable hybrids. (We have made no attempt

to show that the 25 percent hybridization represents cross reaction with inactive embryo genes as we describe in Fig. 4). Thus, active globin 11*S* monomers become sensitive to staphylococcal nuclease after but not before treatment with trypsin, suggesting again that the conformation of transcriptionally "active" monomers is different from that of the inactive ones. The increased accessibility of actively transcribing genes to staphylococcal nuclease after treatment with trypsin is in good agreement with the in vivo experiments of Roberts and Kroeger (*23*), who showed

Table 2. Observed saturation of trace amounts of ^{3}H-labeled probes by vast excesses of driver nucleic acids. (A) The treatment of nuclei, isolation of DNA, and reannealing were as described (Fig. 6). Percentage digestion was determined by the absorbancy at 260 nm of the perchloric acid soluble fraction. The 11*S* monomers were prepared as described (D) below. The weight average molecular weight of the DNA fragments decreased from 200 bases at 10 percent digestion to about 80 bases at 52 percent digestion. The data for the 19 percent digestion are taken from Fig. 6. In the remaining experiments, DNA was at a concentration of 15 mg/ml and hybridization was assayed as described (Fig. 6). For ribonuclease H digestions, the hybridized mixture of total ^{3}H-labeled DNA, the DNA from nuclei treated with deoxyribonuclease to 19 percent acid solubility, and the nuclear RNA was desalted by passage over Sephadex G25 equilibrated with a solution of 40 m*M* tris-HCl (*p*H 7.7), 4 m*M* $MgCl_2$, and 1 m*M* dithiothreitol. The sample was treated with ribonuclease H (Miles; 7 units) in the presence of bovine serum albumin (30 μg/ml) and glycerol (4 percent) for 30 minutes at 37°C. This procedure was followed by digestion with S1 nuclease, as described. (B) The 4-day RBC's were incubated with [^{3}H]thymidine (Fig. 6). Monomers were prepared from these labeled cells (D, below). The DNA from ^{3}H-labeled monomers was annealed with excess DNA from RBC nuclei treated with deoxyribonuclease I (Fig. 1) as described (Fig. 2). At $\log C_0t$ of 4.25, approximately 80 percent of the ^{3}H-labeled DNA was hybridized as assayed by S1 digestion. Single-stranded DNA was isolated by HAP chromatography. Portions of the reaction mixture (100,000 count/min) were pipetted into 0.15*M* sodium phosphate (PB) (*p*H 7.0) and placed on a water-jacketed column containing 10 g of HAP (Bio-Rad-DNA grade) at 60°C. Repeated washings with 0.15*M* PB resulted in the elution of single-stranded DNA (approximately 20,000 count/min). This fraction is referred to as "HAP flow-through DNA." It is 98 percent sensitive to S1 nuclease and contains more than 90 percent single-copy sequences. The remaining double-stranded DNA (80,000 count/min) was eluted with 0.48*M* PB. The HAP flow-through DNA was desalted by passage over Sephadex G25 (equilibrated in 0.1*M* NaCl, 0.01*M* tris-HCl, *p*H 7.4) and precipitated with 100 μg of (carrier) yeast transfer RNA (tRNA) (Sigma) in two volumes of 95 percent ethanol. The HAP flow-through DNA was recovered by centrifugation for 30 minutes (11,000 rev/min, HB-4 head of a Sorvall RC5 centrifuge) and suspended in a solution of 10 m*M* NaCl, 10 m*M* tris-HCl (*p*H 7.4). Nuclear RNA (12 mg/ml) was prepared from 10-day-old chick embryo reticulocytes (Fig. 6 legend) and used to drive the reaction against total ^{3}H-labeled DNA or the HAP flow-through fraction. The hybridization conditions and analysis of hybrid formation were as described (Fig. 2). Seven percent of the total DNA and 1 percent of the HAP flow-through DNA behaved as foldback DNA, as assayed by S1 nuclease, and was subtracted from the observed saturation values to yield the figures of 9.8 percent and 48 percent, respectively. Saturation was achieved by $\log Cr_0t = 3$, although points were taken to $\log Cr_0t = 4$ (Cr_0t, moles of ribonucleotide per liter × seconds). (C) ^{3}H-Labeled nuclear RNA was prepared by incubation of 4-day chick embryo RBC's for 30 minutes in medium F-12 (Gibco) with [^{3}H]uridine (100 μc/ml) (New England Nuclear; 16 c/mmole). The isolation of nuclear RNA and the preparation of the staphylococcal nuclease limit digest were as described. Total DNA and DNA from nuclei treated with deoxyribonuclease I (isolated as described above) were annealed with tracer ^{3}H-labeled nuclear RNA (5000 count/min per 5 μl of the hybridization mixture). At intervals, a sample (10 μl) of the hybridization mixture was pipetted into 400 μl of double-strength SSC (SSC consists of 0.14*M* NaCl; 0.014*M* sodium citrate); half of this sample was immediately precipitated with trichloroacetic acid, and the other half was incubated for 30 minutes at 37°C with previously boiled ribonuclease A (20 μg/ml) (Worthington). The percentage hybridization was then determined (Fig. 2 legend). Of the labeled RNA 6 percent was resistant to ribonuclease in the absence of DNA and this was subtracted from the observed saturation values obtained at $\log Cr_0t = 4.25$. (D) Conditions and analysis of hybridization for reactions with trace amounts of ^{3}H-labeled globin cDNA and the isolation of total DNA and the DNA from digested RBC and fibroblast were as described (Fig. 2 legend). The posterior one-third of de-embryonated blastoderms (area vasculosa) were dissected from approximately 500 24-hour chick embryos, washed several times in PBS (Gibco), and gently lysed by homogenization in RSB with 0.5 percent NP-40. The resultant nuclei were then treated with pancreatic deoxyribonuclease to 20 percent acid solubility and the DNA was isolated as described (Fig. 1 legend). Monomers were prepared by incubation of 18-day RBC nuclei with staphylococcal nuclease (50 μg/ml) (Worthington) for 5 minutes at 37°C in RSB supplemented with 10^{-4}*M* $CaCl_2$. The nuclei were then centrifuged and washed in 0.075*M* NaCl, 0.02*M* EDTA, and 0.01*M* tris-HCl (*p*H 7.4). The resultant pellet was suspended in 5 m*M* sodium phosphate (*p*H 6.8), and insoluble material was removed by low speed centrifugation. Of the soluble material, 90 percent sedimented at 11*S* and 10 percent at 15*S*. Removal of contaminating 15*S* material had no effect on our results. Monomers were treated with pancreatic deoxyribonuclease in either 5 m*M* sodium phosphate (*p*H 6.8) or in RSB. The isolation of DNA was as described (Fig. 2 legend). (E) The 18-day RBC monomers were prepared as described above. Trypsinized monomers were prepared by the incubation of RBC monomers with trypsin (100 μg/ml) in RSB for 20 minutes at 37°C. After the addition of soybean trypsin inhibitor (200 μg/ml) (Worthington), the suspension was made 10^{-4}*M* with respect to $CaCl_2$ and digested with staphylococcal nuclease (50 μg/ml) for 30 minutes at 37°C. The isolation of the remaining DNA (50 to 80 bases in length) and the conditions and analysis of hybridization were as described (Fig. 2 legend). About 20 percent of the DNA was resistant after these procedures; yet, this resistant DNA saturates more than 80 percent of the labeled total DNA, an indication that the inactive regions of the chromosome are randomly covered by the trypsin-resistant histone cores.

Item	Saturation* (%)
(A) Tracer ^{3}H-labeled DNA (total) annealed with driver DNA from	
11*S* monomers	94 ± 3†
18-day RBC DNA—10% digestion with deoxyribonuclease I	85 ± 4
18-day RBC DNA—19% digestion with deoxyribonuclease I	78 ± 3
18-day RBC DNA—52% digestion with deoxyribonuclease I	65 ± 5
18-day RBC DNA—19% digestion with deoxyribonuclease I	78 ± 3
plus DNA—19% digestion with deoxyribonuclease I	79 ± 4
plus total DNA	95 ± 4
plus nuclear RNA	89 ± 3
plus nuclear RNA and then ribonuclease H	74 ± 4
(B) Driver nuclear RNA annealed with tracer	
^{3}H-DNA (HAP flow-through)	48 ± 8
^{3}H-DNA (total)	9.8 ± 1
(C) Tracer ^{3}H-nuclear RNA annealed with driver	
DNA (total)	91 ± 6
18-day RBC DNA (staphylococcal nuclease, limit digest)	85 ± 5
18-day RBC DNA digestion with deoxyribonuclease I	16 ± 5
(D) Tracer ^{3}H-labeled globin cDNA annealed with driver	
DNA (total)	93 ± 3
18-day RBC DNA digestion with deoxyribonuclease I	25 ± 3
Fibroblast DNA digestion with deoxyribonuclease I	94 ± 3
24-hour chick blastoderm DNA digestion with deoxyribonuclease I	94 ± 4
18-day RBC monomers (deoxyribonuclease I–5 m*M* sodium phosphate; 0.5 m*M* $MgCl_2$)	91 ± 4
18-day RBC monomers (deoxyribonuclease I–3 m*M* MgC_2; 10 m*M* NaCl)	25 ± 4
(E) Driver DNA from 18-day RBC monomers treated with trypsin and then staphylococcal nuclease and annealed with tracer	
^{3}H-globin-labeled cDNA	25 ± 5
^{3}H-DNA (total)	83 ± 4

*Saturation refers to the plateau in percent hybridization of tracer ^{3}H-labeled probe by driver DNA or RNA. All experiments were taken out to $\log C_0t$ or $\log Cr_0t = 4.25$. The plateau was defined in all cases by 4 to 10 points. †Mean ± standard deviation of the mean.

that, after injection of trypsin into salivary glands, the puffed regions were morphologically more sensitive than the unpuffed regions and were consequently even more accessible to transcription by the endogenous RNA polymerase.

Implication for Gene Regulation

Experiments with staphylococcal nuclease and deoxyribonuclease II have led to the conclusion that active genes are probably associated with histones (*10, 13, 19*), while our experiments are best understood if the conformation of these histones is different from that of the bulk of the histones—a conformation that renders the associated DNA particularly sensitive to digestion by pancreatic deoxyribonuclease I in the proper ionic environment. While our experiments with the globin cDNA show that the nontranscribed strand of the DNA double helix is digested by deoxyribonuclease I, the experiments with nuclear RNA (Table 2) and those previously described with ribosomal RNA (*14*) show that the transcribed strand of the double helix is also digested by deoxyribonuclease I.

Much of our data involves hybridization with very small DNA fragments 20 to 250 bases in length (the weight average molecular weight is about 150 to 200 bases). Almost certainly, the smallest fragments do not participate in the hybridization reaction; consequently, our estimate of the effective DNA concentration during renaturation is likely to be slightly high. The hybrids formed between self-annealed fragments from the pancreatic deoxyribonuclease digestion of nuclei have a melting temperature (T_m) (measured by hyperchromicity and by thermal elution from HAP) of 77°C in 0.15*M* sodium phosphate buffer at *p*H 7.0 (data not shown). This is significantly lower than the T_m of 84°C that we observe for self-annealed fragments that are much longer (500 base pairs). Although we cannot offer a conclusive explanation for these results, it is likely that the difference in T_m is a consequence of the small size of the DNA fragments from digestion. Nevertheless, the difference in T_m suggests the interesting possibility that active genes may yield a subclass of deoxyribonuclease I–resistant fragments that are protected by histones, but smaller in size than that needed for stable hybrid formation at 65°C. This is probably not the case since the preferential digestion of the globin genes can still be demonstrated even after the hybridization reaction is performed under less stringent conditions at 50°C (data not shown). Given these reservations, we think our interpretation of the deoxyribonuclease digestions is probably correct since the biological controls are so striking. Thus, in adult erythrocytes the staphylococcal nuclease fragments retain globin sequences, while the DNA fragments from pancreatic deoxyribonuclease digestion, which are about the same size, do not contain hybridizable adult globin DNA. Similarly, the same-sized deoxyribonuclease I fragments from fibroblasts or brain retain the globin and ovalbumin sequences, whereas those from embryonic and adult red cells retain only the ovalbumin sequences and not their respective activated globin sequences.

It is unlikely that the described preferential digestion of active genes is a consequence of the transcription process per se since the adult globin genes are sensitive to the nuclease in mature adult erythrocytes that have stopped synthesizing RNA. This observation also demonstrates that the inactivation of the avian red cell during erythroid development is not necessarily a consequence of an altered chromosome structure imposed at this primary level of DNA folding.

Since our cDNA is complementary to the globin structural gene and since this region of the transcription unit is clearly in an altered conformation (as revealed by deoxyribonuclease I digestion) in red blood cells, but not in brain or fibroblasts, a simple model of gene activation involving only the activation of some promotor sequence at the 5′ end of the transcription unit can probably be excluded. We therefore propose that gene activation requires the assembly of an altered subunit structure throughout the entire transcription unit. The mechanism by which an altered subunit structure is propagated across the entire length of a transcription unit represents the primary conceptual problem raised by these results [see also (*24*)].

The deoxyribonuclease-resistant DNA protects less of the tracer ^{3}H-labeled total DNA as digestion of nuclei is increased (Table 2A). Similar effects are not observed with DNA fragments obtained after digestion of nuclei with staphylococcal nuclease. Interpretation of these data is complicated by the fact that the DNA fragments become progressively smaller as the digestion proceeds; nevertheless, $C_0t_{1/2}$ for the reaction of those sequences that do hybridize is not significantly different from control values, and the saturation values increase by no more than 4 to 8 percent when the reaction mixture is shifted to less stringent conditions (50°C) after reaching saturation at 65°C. These observations suggest the possibility that there may actually be a spectrum of transcribing (or potentially transcribable) chromosomal structures which can be defined by their sensitivity to deoxyribonuclease I.

The findings from these experiments with pancreatic deoxyribonuclease raise the possibility that gene activation during the development of the red cell lineage may involve the sequential assembly of a different type of chromosome structure (one that is deoxyribonuclease I sensitive) about the specific genes to be activated. To study this question, we have isolated a population of precursor erythroid cells from the developing yolk sac of 25-hour chick embryos. This population has been reported to contain more than 80 percent precursor red blood cells (*25*). When nuclei from these cells were digested with deoxyribonuclease I, the globin genes were not preferentially digested (Table 2D), and the kinetics of hybridization were essentially the same as those obtained from total DNA (Fig. 2). Thus, between 25 and 35 hours of development (when hemoglobin first appears in the chick embryo) there appears to be a new type of chromosome structure imposed on the globin genes in cells within the erythroid lineage. Since the chromosome is assembled at the time of DNA replication (*26*), it is not unreasonable that this new type of structure is actually dictated as the globin genes are replicating (*14, 27*). These observations are consistent with previous findings (*17*) that globin RNA sequences are not detectable in embryos at 25 hours of development, but begin to appear at 35 hours in coordination with the appearance of detectable hemoglobin. Thus, even though posttranscriptional controls are important in gene regulation (*21*), a major component of regulation is a transcriptional one mediated through chromosome structure.

Summary

Ten percent digestion of isolated nuclei by pancreatic deoxyribonuclease I preferentially removes globin DNA sequences from nuclei obtained from chick red blood cells but not from nuclei obtained from fibroblasts, from brain, or from a population of red blood cell precursors. Moreover, the nontranscribed ovalbumin sequences in nuclei isolated from red blood cells and fibroblasts are retained after mild deoxyribonuclease I digestion. This suggests that active genes

are preferentially digested by deoxyribonuclease I. In contrast, treatment of red cell nuclei with staphylococcal nuclease results in no preferential digestion of active globin genes. When the 11*S* monomers obtained after staphylococcal nuclease digestion of nuclei are then digested with deoxyribonuclease I, the active globin genes are again preferentially digested. The results indicate that active genes are probably associated with histones in a subunit conformation in which the associated DNA is particularly sensitive to digestion by deoxyribonuclease I.

References and Notes

1. J. D. Watson and F. H. C. Crick, *Nature (London)* **171**, 964 (1953).
2. M. F. Lyon, *ibid.* **190**, 372 (1961); E. B. Lewis, *Adv. Genet.* **3**, 73 (1950); W. K. Baker, *ibid.* **14**, 133 (1968); H. D. Berendes, *Int. Rev. Cytol.* **35**, 61 (1973); M. Ashburner, *Cold Spring Harbor Symp. Quant. Biol.* **37**, 655 (1973); J. H. Frenster, *Nature (London)* **206**, 680 (1965); B. J. McCarthy, J. T. Nishiura, D. Doenecke, D. S. Nasser, C. B. Johnson, *Cold Spring Harbor Symp. Quant. Biol.* **38**, 763 (1973); R. T. Simpson. *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2740 (19 → K. Marushige and J. Bonner, *ibid.* **68**, 2941 (1971).
3. J. Gottesfeld, R. F. Murphy, J. Bonner, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4404 (1975).
4. A. L. Olins and D. E. Olins, *Science* **183**, 330 (1974); C. L. F. Woodcock, *J. Cell Biol.* **59**, 3689 (1973); J. P. Baldwin, P. G. Boseley, M. Bradbury, K. Ibel, *Nature (London)* **253**, 245 (1975); R. D. Kornberg and J. O. Thomas, *Science* **184**, 865 (1974); C. G. Sahasrabuddhe and K. E. Van Holde, *J. Biol. Chem.* **249**, 152 (1974); J. A. D'Anna and I. Isenberg, *Biochemistry* **13**, 2098 (1974); S. C. R. Elgin and H. Weintraub, *Annu. Rev. Biochem.* **44**, 725 (1975); D. Hewisch and L. Burgoyne, *Biochem. Biophys. Res. Commun.* **52**, 504 (19 → M. Noll, *Nature (London)* **251**, 249 (1974); R. Axel, W. Melchior, B. Sollner-Webb, G. Felsenfeld, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4101 (1974); B. M. Honda, D. L. Baillie, E. P. M. Candido, *FEBS Lett.* **48**, 156 (1974); D. R. Oosterhof, J. C. Hozier, R. L. Rill, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 633 (19 → P. Oudet, M. Gross-Bellard, P. Chambon, *Cell* **4**, 281 (1975); H. J. Li, *Nucleic Acid Res.* **2**, 1275 (1975); V. V. Bakayev, A. A. Melnickov, V. D. Osicka, A. J. Varshavsky, *ibid.*, p. 1401; H. G. Martinson and B. J. McCarthy, *Biochemistry* **14**, 1073 (1975); J. D. Griffith, *Science* **187**, 1202 (1975).
5. J. L. Germond, B. Hirt, P. Oudet, M. Gross-Bellard, P. Chambon, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1843 (1975); R. Clark and G. Felsenfeld, *Nature (London)* **229**, 101 (1971); J. P. Langmore and J. C. Wooley, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2691 (1975).
6. H. Weintraub, K. Palter, F. Van Lente, *Cell* **6**, 85 (1975).
7. H. Weintraub and F. Van Lente, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4249 (1974).
8. F. H. C. Crick and A. Klug, *Nature (London)* **255**, 530 (1975).
9. In the strictest sense all nu bodies cannot be homogeneous since the histones themselves are not homogeneous. Thus, histones are extensively modified [A. Ruiz-Carrillo, L. Wangh, V. Allfrey, *Science* **190**, 117 (1975)] and are also genetically polymorpi → (L. H. Cohen, K. M. Newrock, A. Zweidler, *ibid.*, p. 994).
10. Recent papers by Gottesfeld *et al.* (*3*) and Lacy and Axel (*19*) as well as older experiments of Axel *et al.* (*13*) make it very likely that histones are associated with actively transcribed regions of DNA.
11. R. Axel, H. Cedar, G. Felsenfeld. *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2029 (19 → R. S. Gilmour and J. Paul, *ibid.*, p. 3 → A. W. Steggles, G. N. Wilson, J. A. Kantor, *ibid.* **71**, 1219 (19 → T. Barrett, P. Maryanka, P. Hamlyn, H. Gould, *ibid.*, p. 5057.
12. R. F. Itzhaki, *Biochem. J.* **125**, 221 (1971); A. E. Mirsky, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2945 (1971); M. Noll, *Nucleic Acid Res.* **1**, 1573 (1974); D. Oliver and R. Chalkley, *Biochemistry* **13**, 5093 (1974); T. Pederson, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2224 (1972).
13. R. Axel, H. Cedar, G. Felsenfeld, *Cold Spring Harbor Symp. Quant. Biol.* **38**, 773 (1973).
14. H. Weintraub, in *Results and Problems in Cell Differentiation*, J. Reinert and H. Holtzer, Eds. (Springer-Verlag, Berlin, 1975), vol. 7, p. 27.
15. R. J. Billing and J. Bonner, *Biochim. Biophys. Acta* **281**, 453 (1972).
16. C. Berkowitz and P. Doty, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3328 (1975).
17. M. Groudine, H. Holtzer, K. Scherrer, A. Therwath, *Cell* **3**, 243 (1974); M. Groudine and H. Weintraub, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4464 (1975).
18. J. Brown and V. Ingram, *J. Biol. Chem.* **249**, 3960 (1974).
19. E. Lacy and R. Axel, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3978 (1975).
20. H. Hanafusa, W. S. Hayward, J. H. Chen, T. Hanafusa, *Cold Spring Harbor Symp. Quant. Biol.* **39**, 1139 (1974).
21. K. Scherrer and L. Marcaud, *J. Cell. Physiol.* **72** (Suppl. 1), 181 (1968); L. Grouse, M. D. Chilton, B. J. McCarthy, *Biochemistry* **11**, 798 (1972); I. R. Brown and R. B. Church, *Dev. Biol.* **29**, 73 (1972); E. H. Davidson and R. J. Britten, *Q. Rev. Biol.* **48**, 565 (1973); M. J. Getz, G. D. Birnie, B. D. Young, E. MacPhail, J. Paul, *Cell* **4**, 121 (1975); B. R. Hough, M. J. Smith, R. J. Britten, E. H. Davidson, *ibid.* **5**, 291 (1975).
22. After treatment with trypsin, the repeating pattern of monomer, dimer, trimer (and so on), DNA fragments generated by partial digestion of nuclei with staphylococcal nuclease is largely preserved. In addition, more than 90 percent of the 11*S* monomers, after treatment with trypsin, retain a trypsin-resistant core composed of interacting histone COOH-terminal cleavage fragments (H. Weintraub, in preparation).
23. M. Roberts and H. Kroeger, *Experientia* **20**, 326 (1957).
24. K. Yammamoto and B. Alberts, *Ann. Rev. Biochem.*, in press.
25. M. Wenk, *Anat. Rec.* **169**, 453 (1971).
26. H. Weintraub, *Cold Spring Harbor Symp. Quant. Biol.* **38**, 247 (1973); R. L. Searle and R. T. Simpson, *J. Mol. Biol.* **94**, 479 (1975).
27. H. Holtzer, H. Weintraub, R. Mayne, B. Mochan, *Curr. Top. Dev. Biol.* **9**, 299 (1973).
28. R. J. Britten and D. Kohne, *Science* **161**, 529 (1968).

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City Size Effects, Trends, and Policies

The consequences as a city grows in population and recent patterns of U.S. urban growth are examined.

Irving Hoch

With increasing city size, there is an increase in wage rate for the same work, an increase that is explainable as compensation to city residents for increased costs with size. In this article I document that relation and then draw implications for prediction and policy. Rather than using the political jurisdiction as the unit of analysis, I use the economic city, which corresponds best to the census definition of urbanized area, but is measured well enough by the standard metropolitan statistical area (SMSA).

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The Wage-Cost Relationship

As a city grows in population, land values increase because more people want to locate in the city and hence bid up rents. With higher rents, land use becomes more intensive as inhabitants economize on the use of a more expensive resource. This is another way of saying that urban density will increase (*1*). With rents up, new migrants into an expanding city can be attracted only if they are paid enough to cover the now higher rents, which drives up wages. Higher rents also imply higher transportation costs, since there is a trade-off between locating near the center of town and paying higher money rents or locating at an increasing distance from the center and paying higher commuting costs (*2*). Higher rents or transport costs, or both, cause general increases in the cost of living. Food costs, for example, increase because grocery store rents are higher and because grocery store clerks receive higher wages to cover their higher residential rents. Increased size and