AGE-ASSOCIATED STUDIES ON THERMAL STABILITY AND TEMPLATE EFFECTIVENESS OF DNA AND NUCLEOPROTEINS FROM BEEF THYMUS

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Thymus DNA prepared from old cows has been reported to have a higher thermal stability than that from calves (von Hahn and Verzar, 1963). Subsequent studies indicated, however, that the age-related difference observed in T_m was due to the proteins in the DNA preparations (von Hahn, 1963). These observations suggest the possibility that there is an age-related difference in the binding of protein to DNA. In the present communication, we show that the thermal stability of beef thymus nucleoprotein increases and the template effectiveness for RNA-polymerase decreases with increasing age of the animal. These differences disappear when the nucleoprotein is prepared by a procedure which effectively diminishes the amount of RNA and nonhistone protein in the preparation. No age-related changes in DNA were observed in any characteristics studied.

MATERIALS AND METHODS: Thymus glands of calves less than two months old and cows ten years old or older were obtained from a slaughterhouse immediately after killing. The washed tissue, freed as far as possible from connective tissue membranes, was frozen in dry ice and stored frozen until used. DNA was isolated according to Colter et al (1962). The first nucleoprotein preparation followed the method of Zubay and Doty (1959). This preparation is referred to as nucleoprotein

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I. Chromatin was isolated as described by Bonner and Huang (1963). Nucleoprotein II was prepared from chromatin by the following procedure. The chromatin pellet was washed four times with a solution containing 0.25 M sucrose, 0.05 M Tris-HCl, and 0.01 M 2-mercaptoethanol. These washings substantially reduced the RNA and nonhistone protein content. Escherichia coli RNA-polymerase was purified according to Stevens and Henry (1964). The last step of their procedure, density gradient centrifugation, was omitted.

DNA was determined by a diphenylamine procedure (Dische, 1930) and RNA by mild alkaline hydrolysis (Fleck and Munro, 1962). An acidified hydrolysate with an absorbance of 34.2 at 260 mu was taken to contain 1 mg of RNA nucleotides per ml (Scott et al., 1956). Protein was determined by the method of Lowry et al (1951). A detailed description of the apparatus and the method employed in thermal denaturation experiments is given by Szybalski and Menningham (1962). The base analysis of DNA from trifluoroacetic acid hydrolyzate (Dutta et al., 1956) was carried out by paper chromatography as described by Wyatt (1951).

RESULTS AND DISCUSSION: Denaturation temperatures (T_m) of DNA and the two nucleoprotein preparations are shown in Table I. The only

Table I

The Denaturation Temperatures (T_m) of DNA and Nucleoproteins from Calf and Cow Thymus

Sample	T_m
DNA	
Calf (less than 2 months old)	60.1 ± 1.1
Cow (over 10 years old)	59.9 ± 0.8
Nucleoprotein I	
Calf (less than 2 months old)	69.5 ± 0.8
Cow (over 10 years old)	76.3 ± 0.4
Nucleoprotein II	
Calf (less than 2 months old)	78.7 ± 1.0
Cow (over 10 years old)	78.6 ± 1.1

All measurements were performed in 0.0025 M $NaNO_3$. Each value is the mean and the standard error for determinations on 4-6 animals.

preparation which showed an age-related difference for the thermal stability was nucleoprotein I. The T_m was significantly higher for the nucleoprotein from old cows than that from calves.

Nucleoprotein preparation I from both young and old animals had a high RNA content when compared with the nucleoprotein II (Table II). Probably some of the RNA was of cytoplasmic origin because the nucleoprotein was isolated directly from the tissue without previous isolation of nuclei. No significant age-related difference in the protein content of nucleoprotein I was found. This also appears to be the case for the protein content of nucleoprotein II. DNA was free of protein when assayed with the Folin-phenol reagent (Lowry et al., 1951).

Table II

The Composition of Nucleoprotein Preparations

Sample	RNA %	Protein/DNA
Nucleoprotein I		
Calf (less than 2 months old)	8.1	1.22 ± 0.13
Cow (over 10 years old)	9.3	1.34 ± 0.13
Nucleoprotein II		
Calf (less than 2 months old)	1.6	
Cow (over 10 years old)	1.5	

A striking similarity between thermal stability and template effectiveness for E. coli RNA-polymerase of these preparations was observed as shown in Table III. Only nucleoprotein I showed a difference in template effectiveness with advancing age. The nucleoprotein I from young animals resulted in more incorporation of labeled ATP than nucleoprotein I from old animals.

The age-related difference for thermal stability and template effectiveness of nucleoprotein I does not seem to be due to changes in DNA, since DNA does not show any changes for these characteristics. The base composition of DNA does not show any age-related difference either as shown in Table IV.

Table III

Template Effectiveness of DNA and Nucleoprotein Preparations for DNA-dependent RNA Synthesis by E. coli RNA-polymerase

Source	Template	muMoles of ATP incorporated
Calf (less than 2 months old)	36 ug DNA	1.61
Cow (over 10 years old)	36 ug DNA	1.50
Calf (less than 2 months old)	369 ug DNA as nucleoprotein I	2.48
Cow (over 10 years old)	369 ug DNA as nucleoprotein I	1.59
Calf (less than 2 months old)	369 ug DNA as nucleoprotein II	1.14
Cow (over 10 years old)	369 ug DNA as nucleoprotein II	1.31

The standard reaction mixture (0.25 ml) contained: 10 umoles Tris buffer, pH 7.9, 0.25 umoles $MnCl_2$, 1.0 umole $MgCl_2$, 100 umoles each of ATP, CTP, GTP, and UTP, template as indicated, 3.0 umoles 2-mercaptoethanol and enzyme. The ATP was labeled with tritium, each tube contained 13,900 cpm. After incubation at 37° for 10 minutes, the reaction mixture was chilled in ice. When DNA was used as a template, 1.2 mg of bovine serum album (0.03 ml) was added. The precipitate formed after addition of 3 ml of cold 3.5% PCA was washed twice with 2 ml portions of cold PCA and dissolved in 2 N NH_4OH, plated and the radioactivity measured. These determinations were carried out four times, each in triplicate.

Table IV

Base Composition of Calf and Cow Thymus DNA

Source of DNA	Base Composition (mole %) A	G	C	T	$\frac{A+T}{G+C}$
Calf (less than 2 mo. old)	29.4	23.3	19.8	27.5	1.32
Cow (over 10 years old)	29.6	23.4	19.8	27.2	1.32

Besides close similarities of base composition, equilibrium distribution in a Cs_2SO_4 gradient and the UV spectrum of DNA do not show any difference with advancing age (Pyhtilä, 1967; Pyhtilä and Sherman, 1967). The differences found in T_m's and template effectiveness may be due to the RNA and possibly to the nonhistone proteins present in nucleoprotein I. This difference was not observed in nucleoprotein II which contained only small amounts of these components. It is not possible to decide, on the basis of these results, whether the age-associated differences ob-

served reflect the state of chromosomes in vivo or whether the differences are due principally to the age related differences in susceptibility of the nucleoprotein to aggregation, which in turn might be due to qualitative differences in chromosomal RNA and nonhistone proteins. A closer examination of the nature and behavior of chromosomal RNA and nonhistone proteins in connection with the synthetic activity of chromosomes is needed as well as their relation to the aging process.

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