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Developmental and Regional Variations in Ribonucleic Acid Synthesis on Cerebral Chromatin

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1. Chromatin was prepared from purified nuclei isolated from liver and cerebral regions of the rat. 2. The capacity of these preparations to promote RNA synthesis in the presence of bacterial RNA polymerase was determined. 3. The rate of RNA synthesis on chromatin was normally 12–21% of the rate observed with native DNA, but was markedly stimulated on addition of 200mM-ammonium sulphate. 4. At physiological concentrations (80μg./ml.), the brain-specific S-100 protein inhibited RNA synthesis on DNA and chromatin. 5. Cerebral chromatin from foetal and newborn animals was more active in RNA synthesis than were the analogous preparations from liver. 6. Cerebellar chromatin maintained a high rate of RNA synthesis during brain maturation. In contrast, RNA synthesis on chromatin from other brain regions and liver declined with age of the rat. 7. RNA synthesized on chromatin stimulated amino acid incorporation in an *Escherichia coli* ribosomal system and hybridized with homologous DNA. 8. RNA synthesized on chromatin from adult cortex or hindbrain hybridized with DNA to a greater extent than that synthesized on cerebellar chromatin. 9. The proportion of RNA formed on cerebral-cortical chromatin that hybridized with DNA increased with age of the rat. 10. The results indicate that the total amount and the types of RNA synthesized on cerebral chromatin vary regionally and during development.

Cellular specificity in heterokaryocytic organisms is thought to derive from nuclear chromatin. DNA and histones, the principal components of chromatin, appear to be identical in all tissues of the same organism (McCarthy & Hoyer, 1964; Neidle & Waelsch, 1964). However, only a small fraction of the DNA in chromatin is available as a template for RNA synthesis in any cell. Presumably this fraction differs in different cell types because selective blocking of specific sites on the DNA by the basic histones or other proteins results in the repression of certain genes, whereas other sites remain free for transcription. The organ-specific character of chromatin has been demonstrated in studies of the hybridization properties of RNA formed *in vitro* in the presence of DNA-dependent RNA polymerase (Paul & Gilmour, 1966*a*, *b*, 1968; Bonner *et al.* 1968).

The unique structural and functional characteristics of the brain may be presumed to be related to distinctive patterns of RNA and protein synthesis, which are ultimately derived from the specific properties of cerebral chromatin. The present paper deals with the composition and properties of cerebral chromatin isolated from different regions of rat brain at various stages of development. The relative capacities of these preparations to serve as templates for RNA synthesis in the presence of bacterial RNA polymerase were investigated. The results suggest that significant regional and developmental variations occur in the synthesis of cerebral RNA, including messenger RNA.

EXPERIMENTAL

Preparation of chromatin. Rats of various ages from an inbred Sprague–Dawley strain were used in these investigations. The adult animals were maintained, after weaning, on Purina laboratory chow *ad libitum* until they reached the age of 6 weeks. Newborn and foetal rats were killed by decapitation. All other animals were killed by exsanguination from the abdominal aorta under light anaesthesia with sodium pentobarbital (Nembutal). Brains and sometimes livers were rapidly removed and placed in cold 0·32M-sucrose. All subsequent procedures were carried out at 0–4°. In most instances the brain was separated into several major regions. Tissues were then homogenized by hand with a Teflon pestle in a glass homogenizing tube containing 12vol. of cold 0·32M-sucrose. The filtrate obtained after the homogenate had been passed through four layers of cheesecloth was centrifuged at 750*g* for 10min. The resulting pellet was vigorously shaken with 2·47vol. of 2·4M-sucrose–3mM-$CaCl_2$–50mM-tris–HCl medium, pH7·2 (final sucrose concentration 1·8M). This suspension was then centrifuged

for 30min. at 35000*g* in the Sorvall RC-2 centrifuge. This procedure yielded a white pellet that, when examined by phase-contrast microscopy, appeared to consist entirely of morphologically intact nuclei.

Chromatin was isolated from the nuclear preparation by the method of Zubay & Doty (1959). The pellet was suspended in medium composed of 75mM-NaCl–24mM-EDTA, pH 8·0; the volume of medium was equivalent to 1–2 times the original weight of tissue. This suspension was centrifuged at 1000*g* for 10min. The pellet was treated twice more in this manner and then three times with the same volume of 0·7mM-sodium phosphate buffer, pH 8·0. In the latter instances, centrifugation was carried out for 10min. at 4500*g*. The final pellet was treated ultrasonically for 1min. at 20kcyc./sec. with a Branson Sonifier, model S-75, equipped with a micro-tip (Sonnenberg & Zubay, 1965). The ultrasonically-treated suspension was then centrifuged at 12000*g* for 10min. The supernatant, which contained the chromatin, was retained. The characteristics of this preparation were not altered after storage at 0° for 3 weeks.

Samples of the chromatin preparation were taken for the measurement of DNA, RNA, histone protein and non-histone protein. DNA was assayed by the method of Burton (1956). For the determination of RNA, the chromatin was first hydrolysed for 2hr. in 0·3M-KOH at 37° (Santen & Agranoff, 1963). This solution was then neutralized with $HClO_4$. Protein, DNA and $KClO_4$ were removed by precipitation at 0°. The supernatant was assayed for RNA by the orcinol method of Ceriotti (1955), with brain ribosomal RNA as a standard (Bondy & Roberts, 1967). Histone and non-histone protein were extracted from chromatin and separated on the basis of their differential solubility in cold 0·1M-H_2SO_4. The protein content of each fraction was measured by the method of Lowry, Rosebrough, Farr & Randall (1951). Calf thymus histone (Calbiochem, Los Angeles, Calif., U.S.A.) and bovine serum albumin (Sigma Chemical Co., St Louis, Mo., U.S.A.) were used as standards for histone and non-histone protein respectively.

Preparation of DNA. Cerebral DNA was prepared by a method based on the procedure of Massie & Zimm (1965) as described by Bondy & Roberts (1968).

Preparation of RNA polymerase. RNA polymerase was prepared from cells of *Escherichia coli* B in mid-exponential phase (Miles Laboratories Inc., Elkhart, Ind., U.S.A.). Purification was carried out to the stage of fraction 3 in the method of Chamberlin & Berg (1962). This fraction was then successively treated with 20%, 30% and 40% saturated $(NH_4)_2SO_4$. After each treatment, the extracts were brought to a final concentration of 50% saturated $(NH_4)_2SO_4$ to precipitate RNA polymerase. The final combined precipitate was stored at −60°. The properties of the enzyme preparation were very similar to those described by Chamberlin & Berg (1962). RNA synthesis was dependent on the presence of DNA. The four nucleotide triphosphates were essential for optimum activity (Table 1).

Template activity of chromatin. The capacity of chromatin preparations to activate RNA synthesis was assayed by the method of Marushige & Bonner (1966). Spermidine phosphate did not appear to potentiate the reaction and was therefore omitted from the incubation mixture. Incubation was carried out for 40min. at 37°. The reaction was stopped by addition of 0·25ml. of 1M-K_2HPO_4, followed by cold 5% (w/v) trichloroacetic acid. Acid-insoluble material was collected by filtration through a Millipore filter (25mm. diam., 0·8μm. pore size). The precipitate was washed three times with 15ml. of cold 5% (w/v) trichloroacetic acid and twice with 15ml. of cold chloroform–ethanol (1:1, v/v). The filter containing the air-dried precipitate was then placed in 5ml. of a toluene scintillator system composed of 0·5% 5-(biphenyl-2-yl)-2-phenyl-1-oxa-3,4-diazole and 0·01% 1,4-bis-(5-phenyloxazol-2-yl)benzene. Radioactivity was measured in the Packard Tri-Carb scintillation spectrometer at an efficiency of 24% for ^3H.

RNA–DNA hybridization. Radioactive RNA was prepared on a chromatin template with 96μg. of DNA as chromatin and 900μg. of RNA polymerase in a total volume of 2ml. Incubation was carried out for 60min. at 37°. The reaction was stopped by addition of 2ml. of 1% sodium dodecyl sulphate in 3mM-EDTA–10mM-sodium acetate buffer, pH 5·2. RNA was then extracted in the presence of hot phenol and purified by procedures described previously for the preparation of nuclear RNA (Bondy & Roberts, 1967). The recovery of radioactivity incorporated into RNA after extraction with phenol and purification was in the range 54–68%. The capacity of this RNA to hybridize with homologous DNA immobilized on a nitrocellulose membrane was measured essentially as described by Bondy & Roberts (1968). However, the incubation procedures were carried out in the presence of 30% (v/v) formamide (Bonner, Kung & Bekhor, 1967). This modification permitted hybridization to be carried out at room temperature, which decreased the loss of DNA from the filters.

Stimulation of protein synthesis on ribosomes by RNA prepared on chromatin. RNA was synthesized on a chromatin template and purified as described above. The capacity of this RNA to stimulate incorporation of [^{14}C]phenylalanine into the cell-free S-30 fraction of *E. coli* was determined as described by Bondy & Roberts (1967). The reaction was stopped by addition of 0·25ml. of 1M-K_2HPO_4 and 5ml. of cold 5% (w/v) trichloroacetic acid. Incorporation of labelled amino acid into polypeptide was assayed by a nitrocellulose-filter technique (Bondy & Roberts, 1967).

Protein synthesis on ribosomes, supported by chromatin-dependent RNA synthesis. Stimulation of amino acid incorporation in the *E. coli* ribosomal system was also measured during RNA synthesis on a chromatin template.

Table 1. *Properties of RNA polymerase*

The complete reaction mixture (0·25ml.) contained 10μmoles of tris–HCl at pH 8·0, 1·0μmole of $MgCl_2$, 0.25 μmole of $MnCl_2$, 3·0μmoles of β-mercaptoethanol, 30μg. of RNA polymerase (*E. coli* B), 50μg. of salmon sperm DNA (Calbiochem) and 0·1μmole each of ATP, CTP, GTP and UTP. The UTP was present as 0·1μc of [U-^3H]UTP (Schwarz BioResearch Inc., Orangeburg, N.Y., U.S.A.). Incubation was carried out for 10min. at 37°.

	UMP incorporated (pmoles)
Complete system	1640
DNA omitted	22
RNA polymerase omitted	16
CTP omitted	370
CTP, ATP and GTP omitted	195

The incubation mixture (0·25ml.) contained 12·5μmoles of tris–HCl, pH8·0, 2μmoles of KCl, 1·5μmoles of β-mercaptoethanol, 1·0μmole of ATP, 0·1μmole each of UTP, CTP and GTP, 12μg. of DNA as chromatin, 0·2μc of L-[U-^{14}C]phenylalanine (409mc/m-mole), 35μg. of RNA polymerase and 120μg. of S-30 protein from an *E. coli* cell-free system (Bondy & Roberts, 1967). The reaction was stopped and amino acid incorporation was measured as described above.

Physical methods. 'Melting' profiles of DNA and chromatin dissolved in 0·15M-NaCl–1·5mM-sodium citrate were measured with a Gilford spectrophotometer equipped with simultaneous temperature read-out.

All experiments reported were repeated at least three times.

RESULTS

Physical characteristics of chromatin. Chromatin preparations from liver and from different regions of the brain at various ages in the rat all revealed the same characteristic extinction profile (see e.g. Fig. 1). A peak of extinction in the u.v. was observed at 260nm. and a trough at 240nm.; the E_{260}/E_{240} ratio was about 1·63. The extinction at 320nm. due to random scatter was very low (less than 2% of the maximum). This profile suggested that the chromatin preparations were relatively free of material causing non-specific absorption. These results were similar to those reported for chromatin from chicken tissues (Dingman & Sporn, 1964) and rat liver (Marushige & Bonner, 1966).

The 'melting' profiles for cerebral DNA and chromatin revealed striking differences (Fig. 2). The temperature of 'half-melting' was 59° for native DNA and 77° for the chromatin complex. The 'melting' curve for chromatin prepared from brain of newborn rats was indistinguishable from that of chromatin from adult brain. Thermal denaturation proceeded much more gradually for chromatin than for DNA. This finding suggested that the 'melting' process was non-co-operative in the case of chromatin (Goel & Montroll, 1968).

Template activity of chromatin. The capacity of chromatin from rat brain and liver to support RNA synthesis was examined. These preparations had little endogenous activity in the absence of added RNA polymerase (Table 1). Marushige & Bonner (1966) also found that hepatic chromatin required exogenous RNA polymerase for optimum RNA synthesis. In contrast, Dingman & Sporn (1964) reported that chromatin prepared from embryonic chick brain possessed considerable RNA-polymerase activity. In the present experiments RNA synthesis on a chromatin template derived from adult rat brain continued actively for at least 60min. when 30μg. of RNA polymerase was added (Fig. 3). At this concentration of enzyme, the K_m of the reaction was equivalent to 1·7μg. of DNA as chromatin/0·25ml. of incubation medium (Fig. 4). The reaction rate was proportional to the amount of RNA polymerase added over the range 13–130μg. of enzyme/0·25ml. of reaction mixture (Fig. 5).

Chemical composition and template activity of chromatin preparations from different cerebral regions and from liver were studied at various stages of development in the rat (Table 2). With increasing age, a progressive decline in RNA synthesis was observed on chromatin templates derived from whole rat brain or liver when [^{3}H]UTP was used as the radioactive precursor. Comparable results were obtained when other radioactive nucleotides were employed. Thus the apparent

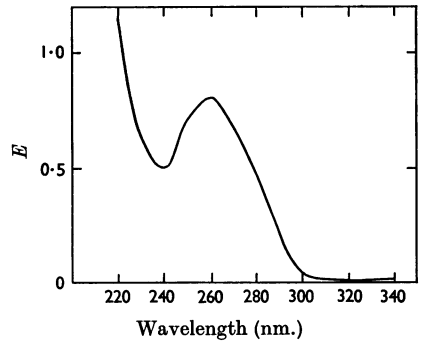

Fig. 1. Extinction spectrum of chromatin prepared from adult rat cerebellum. Chromatin was dissolved in 0·15M-NaCl–1·5mM-sodium citrate at a concentration of 40μg. of DNA/ml. The extinction was determined at a series of different wavelengths in the Beckman DU spectrophotometer.

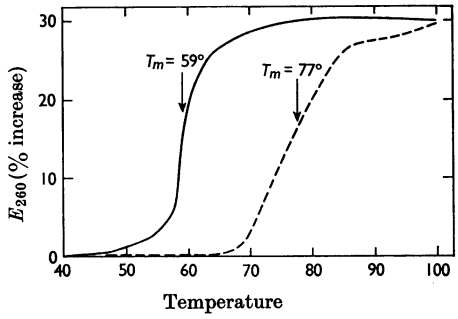

Fig. 2. Thermal-denaturation profiles of deproteinized DNA and chromatin from adult rat brain. Chromatin or DNA was dissolved in 0·15M-NaCl–1·5mM-sodium citrate at a concentration of 35μg. of DNA/ml. As the temperature of the solution was increased from 20° to 100°, E_{260} was continuously monitored in the Gilford recording spectrophotometer. ——, DNA; - - - -, chromatin.

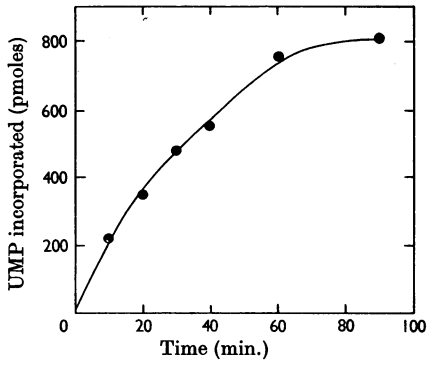

Fig. 3. Time-course for the synthesis of RNA on chromatin from adult rat brain. Each reaction mixture contained 12 μg. of DNA as chromatin, 30 μg. of RNA polymerase (*E. coli* B) and the other additives listed in Table 1, in a final volume of 0·25 ml. Incubation was carried out at 37° for various times ranging from 10 to 90 min.

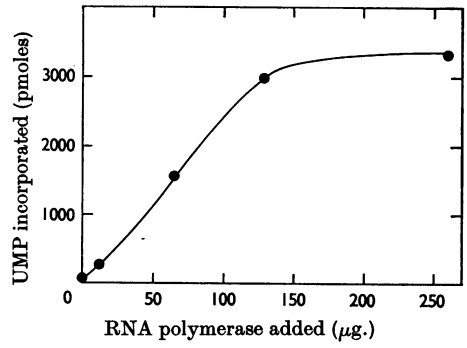

Fig. 5. Influence of RNA polymerase concentration on synthesis of RNA on chromatin isolated from adult rat brain. Each sample contained 12 μg. of DNA as chromatin and various amounts of RNA polymerase in 0·25 ml. of reaction mixture. The other additives were as listed in Table 1. Incubation was carried out at 37° for 40 min.

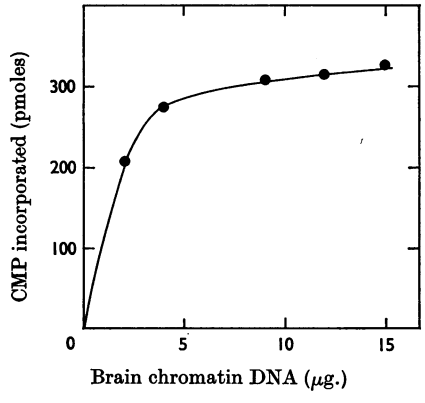

Fig. 4. Influence of concentration of chromatin prepared from adult rat brain on synthesis of RNA. Incubation conditions were as given in Table 1 except that [^{3}H]CTP was substituted for [^{3}H]UTP. Each reaction mixture (0·25 ml.) contained 30 μg. of RNA polymerase and various amounts of chromatin (expressed as μg. of DNA). Incubation was carried out at 37° for 20 min.

decrease in RNA synthesis on chromatin from adult rat brain compared with newborn rat brain was 33% for [^{3}H]UTP, 29% for [^{3}H]GTP, 18% for [^{3}H]AMP and 30% for [^{3}H]CTP. Most separated regions of the brain, including cerebral cortex and certain lower centres, showed this phenomenon. However, a high rate of RNA synthesis was maintained by cerebellar chromatin throughout development. As a consequence, the template activity of chromatin isolated from cerebellum of the adult rat was considerably greater than that of comparable preparations from cerebral cortex, thalamus–hypothalamus or hindbrain–medulla regions.

The RNA content of chromatin was 5–10% of the DNA content in most of the preparations analysed (Table 2). However, the concentration of RNA was significantly above this range in chromatin preparations from thalamus–hypothalamus and hindbrain–medulla regions of the adult rat brain. This finding may have been an indication of unusually high cytoplasmic contamination due to the relatively low yield of nuclei from these samples. Values for protein content in the histone fraction of brain were higher in newborn animals than in foetal rats. No consistent alteration could be detected at later stages of development. In general, concentrations of histone protein did not appear to vary significantly from one brain region to another throughout development. The protein content of the non-histone fraction of cerebral chromatin showed a major increase after birth and smaller variations thereafter. Hindbrain–medulla samples tended to have the highest concentration of non-histone protein as maturation of the brain proceeded. Values for non-histone protein content in rat brain chromatin were greater than those noted for liver chromatin. Dingman & Sporn (1964) reported a similar finding in the chicken.

Inhibition and activation of chromatin. The template activity of chromatin from adult rat brain was about 14% of that of the analogous preparation of deproteinized DNA (Table 3). However, in the presence of 200 mM-ammonium sulphate, the template activity of chromatin was markedly increased. Similar results have been obtained for rat liver chromatin by Chambon, Karon, Ramuz & Mandel (1968). These findings

Table 2. *Chemical composition and template activity of chromatin from rat brain and liver at various developmental stages*

Template activity was measured under the conditions described in Table 1, except that incubation was conducted for 40 min. at 37° in the presence of 12 μg. of rat liver DNA or DNA as chromatin.

	Mass ratio				
Rats used	DNA	RNA	Histone protein	Non-histone protein	UMP incorporated (pmoles)
Foetal (5 days prenatal)					
Brain	1·0	0·07	0·85	0·81	1030
Liver	1·0	0·05	0·76	0·57	847
Newborn					
Whole brain	1·0	0·08	1·08	1·46	905
Cerebral cortex	1·0	0·07	1·10	1·43	890
Cerebellum	1·0	0·08	1·12	1·32	872
Thalamus + hindbrain	1·0	0·08	1·14	1·36	926
Liver	1·0	—	—	—	770
14-day-old					
Cerebral cortex	1·0	0·06	1·11	1·25	748
Cerebellum	1·0	0·06	0·98	0·95	926
Thalamus–hypothalamus	1·0	0·07	1·05	1·28	653
Hindbrain–medulla	1·0	0·08	1·36	2·0	660
Adult (6 weeks)					
Whole brain	1·0	0·08	1·00	0·98	680
Cerebral cortex	1·0	0·105	0·98	1·15	545
Cerebellum	1·0	0·08	0·94	0·90	872
Thalamus–hypothalamus	1·0	0·18	1·10	1·25	552
Hindbrain–medulla	1·0	0·22	1·03	2·31	578
Liver	1·0	0·07	0·97	0·79	695
DNA	—	—	—	—	4860

Table 3. *Inhibition and activation of rat cerebral chromatin*

Each 0·25 ml. of the basic reaction mixture contained all of the materials listed in Table 1 except that the template was 12 μg. of rat DNA or 12 μg. of DNA as chromatin from adult whole brain. Incubation was carried out at 37° for 40 min.

Additions	UMP incorporated (pmoles)
Chromatin	
None	680
RNA polymerase omitted	31
200 mM-$(NH_4)_2SO_4$	2100
20 μg. of *E. coli* S-30 protein	950
48 μg. of *E. coli* S-30 protein	1180
20 μg. of *E. coli* S-30 protein (heated at 60° for 20 min.)	720
20 μg. of sheep S-100 protein	383
20 μg. of bovine serum albumin	642
20 μg. of sodium polyglutamate	1150
20 μg. of calf thymus histone	662
DNA	
None	4830
20 μg. of sheep S-100 protein	3280
20 μg. of bovine serum albumin	4720
20 μg. of sodium polyglutamate	4570

suggest that a high proportion of the DNA in cerebral chromatin cannot normally support RNA synthesis because it is masked by protein in a complex that readily dissociates under conditions of high ionic strength.

The cerebral chromatin system was markedly stimulated by addition of the S-30 preparation from *E. coli* (Table 3). This preparation was actively incorporating amino acids into protein during this process (see below). Prior heating of the S-30 preparation destroyed both the incorporating activity and the stimulatory action on RNA synthesis. These results suggested that utilization of the RNA formed on chromatin in protein synthesis facilitated synthesis of additional RNA (see below).

The brain-specific S-100 protein described by Moore & McGregor (1965), when added at a concentration of 80 μg./ml., a value comparable with that which occurs in brain tissue of adult animals (Moore & Perez, 1968), markedly inhibited the rate of RNA synthesis on chromatin (Table 3). (The purified S-100 protein from sheep brain was a gift from Dr Blake W. Moore, Washington University, St Louis, Mo., U.S.A.) At a S-100 concentration of

40 μg./ml. a lesser inhibition was found, while at 8 μg./ml. no significant effect could be detected. Since inhibition of RNA synthesis was also produced when DNA was used as the template, this effect of the S-100 protein could not have been exclusively on the binding of histone to DNA. The actions of other acidic polypeptides on RNA synthesis were also examined. Bovine serum albumin, in an amount equivalent to the inhibitory concentration of S-100 protein, had no effect on RNA synthesis in either the chromatin system or the DNA system. In contrast, the highly acidic polyglutamate strikingly stimulated template activity of chromatin, but did not alter RNA synthesis on DNA. This stimulatory effect of polyglutamate may have been effected by combination with histone and consequent de-repression of chromatin activity.

Hybridization of RNA synthesized on chromatin. Samples of radioactive RNA obtained from rat liver and various regions of the brain at different stages of development were assayed for capacity to hybridize with homologous DNA immobilized on nitrocellulose membranes (Table 4). Chromatin prepared from cerebral cortex of the adult rat formed RNA that exhibited a greater capacity for hybridization with DNA than did similar preparations from other regions of the brain in the adult animal. In addition, the hybridization capacity of RNA synthesized by cerebral-cortical chromatin from the adult rat appeared to be greater than that of cerebral chromatin from the foetal or newborn animal. However, the total output of hybridizable RNA for equivalent amounts of chromatin template appeared to be greater in the cerebral cortex of the younger animals. Thus chromatin from cerebral cortex of newborn rats was over 60% more active in total RNA synthesis than the analogous preparation from the adult rat (Table 2). The RNA produced by the former was only 25% less effective in hybridizing with homologous DNA (Table 4). The validity of these comparisons is, of course, dependent on certain assumptions. One assumption is that the purity of the chromatin preparations compared is similar. This seems to be borne out by the data on chemical composition of the different chromatins (Table 2) and the similarity of 'melting' curves (see above). In addition, the assumption must be made that the base compositions of RNA molecules synthesized on the different chromatins were grossly similar. Support for this conclusion has already been presented. The greater template activity of chromatin isolated from brain of newborn rats compared with that from adult brain was in the range 18–33% for the four different radioactive nucleotide precursors of RNA.

Stimulation of protein synthesis on ribosomes by RNA synthesized on chromatin. The ability of RNA newly synthesized on cerebral chromatin to stimulate the incorporation of radioactive amino acids into a ribosomal amino acid-incorporating system derived from *E. coli* was investigated. RNA that had been prepared on a chromatin template and then extracted with phenol and purified as described by Bondy & Roberts (1967) possessed considerable stimulatory activity (Table 5). Stimulation of amino acid incorporation could also be demonstrated under conditions in which the RNA was being formed concurrently on a chromatin template (Table 6). The presence of RNA polymerase and chromatin were both required. Endogenous RNA in chromatin did not seem to enhance protein synthesis on ribosomes in the *E. coli* system. The stimulation obtained was smaller than the eightfold enhancement of protein synthesis reported

Table 4. *Hybridization of RNA synthesized* in vitro *to homologous DNA*

Radioactive RNA was prepared on chromatin templates as described in the Experimental section. The labelled RNA (0·1–0·5 μg.) was incubated for 18hr. at 21° with 28 μg. of DNA in the presence of 30% formamide.

Chromatin template	RNA hybridized (%)
Foetal, brain	11·6±0·87
Newborn, cerebral cortex	10·7±0·99
Adult, cerebral cortex	14·2±0·32
Adult, cerebellum	10·1±1·0
Adult, hindbrain–medulla	12·5±0·44
Adult, liver	12·2±0·95

Table 5. *Stimulation of amino acid incorporation on ribosomes by the RNA isolated after synthesis on chromatin from rat brain regions*

The reaction mixture (0·25ml.) contained 100mM-tris–HCl, pH 7·8, 50mM-KCl, 12mM-magnesium acetate, 1mM-sodium ATP, 0·015mM-sodium GTP, 10mM-creatine phosphate (sodium compound), 6mM-mercaptoethanol, 0·12mg. of creatine phosphokinase, 0·1mM concentrations of the 20 protein L-amino acids except phenylalanine, 0·25 μc of L-[U-^{14}C]phenylalanine (409mc/m-mole) and 0·46mg. of S-30 protein of *E. coli*. RNA prepared on chromatin was added where indicated. Incubation was carried out for 40min. at 37°.

Addition	Phenylalanine incorporated (c.p.m.)
None	1030
18 μg. of RNA prepared on cerebral-cortical chromatin	1850
18 μg. of RNA prepared on cerebellar chromatin	1920

Table 6. *Stimulation of amino acid incorporation on ribosomes by RNA during its synthesis on chromatin from the cerebral cortex of the adult rat*

The combined incubation mixture (0·25 ml.) contained 50 mM-tris–HCl, pH 8·0, 8 mM-KCl, 6 mM-mercaptoethanol, 1 mM-sodium ATP, 0·1 mM concentrations of sodium UTP, sodium CTP and sodium GTP, 0·1 mM concentrations of the 20 protein L-amino acids except phenylalanine, 0·2 μC of L-[U-^{14}C]phenylalanine (409 mC/m-mole) and 120 μg. of S-30 protein from an *E. coli* cell-free system. Where indicated 12 μg. of cerebral-cortical chromatin as DNA or 35 μg. of RNA polymerase (or both) was added. Incubation was carried out for 60 min. at 37°.

Chromatin	RNA polymerase	Phenylalanine incorporated (c.p.m.)
−	−	373
−	+	322
+	−	364
+	+	680

by Bonner, Huang & Gilden (1963) in comparable experiments with pea chromatin. However, the latter workers did not wash the protein of the ribosomes with hot trichloroacetic acid before measurement of amino acid incorporation, so that a substantial portion of the radioactivity incorporated in the presence of the chromatin system may have been associated with unhydrolysed aminoacyl-RNA.

DISCUSSION

The procedures employed in the isolation of chromatin were chosen to yield a preparation that resembled the natural combination of DNA and histone as closely as possible. A high pH during extraction is known to decrease deoxyribonuclease activity (Zubay & Doty, 1959) and contamination with non-histone protein (Dingman & Sporn, 1964). Ultrasonic treatment has been shown to sever strands of DNA without dissociating DNA from protein (Sonnenberg & Zubay, 1965) or altering chromatin template capacity (Loewus, 1968).

Chromatin obtained from whole rat brain had a protein/DNA ratio that ranged from 1·66 to 2·54. The RNA/DNA ratios were 0·07–0·08. Centrifugation of these chromatin preparations through 1·7 M-sucrose as described by Marushige & Bonner (1966) did not decrease these values. These concentrations of protein and RNA in rat cerebral chromatin were similar to those obtained for rat liver chromatin in the present studies and those noted by Marushige & Bonner (1966). In contrast, Dingman & Sporn (1964) reported significantly higher protein/DNA and RNA/DNA ratios for chromatin isolated from crude nuclei of chicken brain. However, the latter preparations also contained appreciable concentrations of RNA polymerase that were absent from chromatin preparations in the present experiments and in those of Marushige & Bonner (1966). Taken together, these results indicate that the chromatin preparations from rat brain described in this study possessed a high degree of purity.

The amount of non-histone protein declined somewhat in whole brain and in most cerebral regions of the rat during maturation. Only the hindbrain–medulla region appeared to exhibit a different progression, but it is likely that the nuclei and chromatin derived from this source were relatively impure, particularly in older animals, owing to the low cellularity of these samples. The decrease in protein content of rat brain chromatin with age was not as pronounced as that described by Kurtz & Sinex (1967) for mouse brain chromatin. In the latter instance, the protein/DNA ratio decreased from 4·7 at 3 days of age to 2·5 after 3 months. In spite of this striking change, the 'melting' points of mouse brain chromatin prepared from the two age-groups differed by only 1°. However, after 3 months marked alterations in 'melting' points were observed. In the present investigations no significant changes were observed in the 'melting' curves of brain chromatin preparations derived from animals of various ages. Large differences in chemical and physical properties of chromatin prepared from animal tissues at different stages of development may be partly due to variations in the purity of the nuclei or chromatin isolated from these tissues.

The general decline in the template capacity of chromatin obtained from most regions of the rat brain during development suggested that the genome gradually became more restricted with age. A prominent exception occurred with cerebellar chromatin. Loss of template activity with age has also been described for calf thymus chromatin by Pyhtilä & Sherman (1968). Moreover, Thaler & Villee (1967) reported that the template capacity of chromatin from newborn-rat liver declined by about 30% within 1 day after birth and remained at this lower value in the adult animal. Hybridization studies have suggested that the diversity of messenger RNA molecules synthesized by mouse liver may decline during development (Church & McCarthy, 1967). These various changes in the production of RNA with messenger properties may be related in part to variations in the changing hormonal milieu of the body (Dahmus & Bonner, 1965).

The ability of chromatin from rat cerebellum to maintain a high rate of RNA synthesis throughout development may be related to delayed maturation of this portion of the brain. Thus cell division appears to continue actively in rat cerebellum for at

least 35 days after birth (Balázs, Kovács, Teichgräber, Cocks & Eayrs, 1968). In contrast, cell division in cerebral cortex may be slight after 14 days. Chromatin derived from cerebellar Purkinje cells, which uniformly contain tetraploid nuclei (Lapham, 1968), may be especially active in RNA synthesis. Other cerebral regions probably have a lower proportion of cells with tetraploid nuclei (see Herman & Lapham, 1968). Chromatin derived from different cell types in the mature brain may otherwise have similar activities in overall RNA synthesis. Thus template activity of chromatin derived from regions of adult brain that were low in neuronal nuclei and high in glial content (such as the medulla) was comparable with that of chromatin from an area rich in neuronal nuclei (cerebral cortex).

RNA synthesized on chromatin from rat brain was capable of stimulating amino acid incorporation in an *E. coli* ribosomal system and of hybridizing with homologous DNA. On the basis of hybridization capacity, the proportion of RNA synthesized that was messenger-like appeared to be lower on chromatin derived from adult rat cerebellum than on chromatin from cerebral cortex, which is also rich in neurons, or on chromatin from regions containing many glia (hindbrain and medulla). However, cerebellar chromatin from the adult rat was so much more active in the overall formation of RNA *in vitro* than other regions of the adult brain that the total production of hybridizable RNA may be greatest in this region of the brain.

During maturation, chromatin derived from whole rat brain exhibited a decline in its capacity to act as a template for overall RNA synthesis. The present findings further suggest that total messenger RNA production by brain chromatin was lower in adult cerebral tissue than in the brain of newborn rats. Similar conclusions have been forthcoming from other studies of variations in the production of RNA with messenger properties in the developing brain (see Bondy & Roberts, 1968). However, chromatin from immature brain appeared to synthesize a lower proportion of messenger-like RNA. This finding may be associated with a high requirement for ribosomal RNA in rapidly dividing cells (Floyd, Okamura & Busch, 1966; Kubinski & Koch, 1966).

Several mechanisms may be proposed for the apparent repression of genetic expression in cerebral tissue during development. These include factors that are produced in the brain and those that enter it from the outside. The highly acidic S-100 protein, which is specific to nervous tissue and increases in amount in the brain with age (Moore & Perez, 1968), may be in the former category. Thus S-100 protein, but not certain other acidic proteins, was capable of depressing the template activity of cerebral chromatin. Similar amounts of S-100 protein appeared to have no significant effect on the rate of RNA synthesis by intact cerebral nuclei (Dutton & Mahler, 1968). However, the nuclear membrane may provide an effective barrier to the entry of this protein. In this regard, conflicting results have been presented on the presence of S-100 protein in cerebral nuclei (Hydén & McEwen, 1966; Dravid & Burdman, 1968). Owing to the restrictive nature of the developing blood–brain barrier, the role of systemically produced humoral agents in controlling the rate of RNA synthesis in the brain may decrease with age. Specific internal cerebral regulators, possibly including the S-100 protein, may therefore assume a highly significant role in regulating RNA synthesis in the mature nervous system.

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