this point the sequences diverge. The high degree of conservation of the nucleotide sequence of mature M1 RNA is consistent with the observation that another set of stable RNA's, tRNA's, also has almost perfect sequence conservation between *S. typhimurium* and *E. coli* (*15*). However, there is considerable drift in the *trp* operon messenger RNA (although it is mostly in the third position of degenerate codons) and in nearby spacer sequences when this operon is compared in the two organisms (*16*).

The M1 RNA gene in *E. coli* contains 3.5 repeats of a 113-bp sequence (*13*). The repeated DNA begins with the last 23 bp of the mature M1 RNA coding region. The *S. typhimurium* DNA also contains repeated sequences. The first repeat begins at nucleotide 353, 23 bp from the 3′ end of the mature M1 coding sequence (Fig. 2), and ends at nucleotide 460 just before the beginning of the second repeat 108 bp downstream. Analysis of regions downstream from the *S. typhimurium* M1 gene revealed the presence of at least three more repeats of this sequence (Fig. 2).

Our results provide further evidence that the catalytic subunit of the enzyme ribonuclease P is an RNA molecule. The catalytic RNA subunit and the protein cofactor of ribonuclease P from *E. coli* and *S. typhimurium* are able to form a functional, heterologous complex, as are the subunits from *E. coli* and *B. subtilis* (*1*). In comparing the gene structures for M1 RNA from *E. coli* and *S. typhimurium*, we found conservation of the transcriptional start and stop signals but no common primary sequence adjacent to the 3′ terminal site, where processing is needed for the formation of mature M1 RNA from its precursor molecule. Nevertheless, M1 RNA synthesis directed by pSa17 in an *E. coli* host is efficient. Therefore, some aspect of M1 RNA secondary or tertiary structure (or both) must be important for processing of the M1 RNA precursor molecule, and this signal must be recognizable by the appropriate enzymes from both *E. coli* and *S. typhimurium*.

Of the six differences between the mature M1 RNA's from *E. coli* and *S. typhimurium*, two are clustered near position 40 and three near position 160. Recent data indicate that the structural features of M1 RNA (*17*) near the differences may be important for M1 RNA function, but further comparative sequence information is needed before more can be said about the relation between sequence and function. The conserved organization of the genome in the flanking regions of M1 RNA indicates that the repeated sequences play some role in expression of the genome in both *E. coli* and *S. typhimurium*.

Note added in proof: There may exist conformations of *E. coli* M1 RNA other than the one that is most consistent with the data from digestion with nucleases (*17*). In one such additional conformation, four of the six differences in the nucleotide sequence of M1 RNA from *S. typhimurium* can be explained as compensatory changes that preserve secondary structure. For example, deletions at positions 37 and 45 remove a GC base pair in a hairpin structure in which C-20 pairs with G-59 at the base of the stem. Furthermore, the changes A-153 and T(U)-160 preserve a base pair in a hairpin structure in which G-148 pairs with C-166 at the base of the stem. Cimino *et al.* (*18*) have recently found evidence in studies of the intramolecular cross-linking of *E. coli* M1 RNA that a region of the molecule from about nucleotides 140 to 170 can be in a structure different from that proposed (*17*).

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11. We observed the tetraphosphate, pppGp, in ribonuclease T_2 digests of both M1 RNA's after chromatography on PEI plates in 0.75*M* phosphate buffer (*p*H 3.5).
12. A complete open reading frame begins with ATG in the third repeat at nucleotide 510 and ends with TGA in the fourth repeat at nucleotide 627. This result, together with the data from *E. coli*, suggests that the first open reading frame must end in the gap in the nucleotide sequence and that the second one must end with the TGA at nucleotide 511.
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30 November 1984; accepted 16 January 1985

The Stability of DNA in Human Cerebellar Neurons

Abstract. *Human tissues have carbon-isotope ratios ($^{13}C/^{12}C$) that reflect dietary ratios. This observation has been used to determine the extent of metabolic turnover of DNA in cells of the adult human cerebellum (90 percent of which are neuronal). If adult human neuronal DNA were metabolically stable, its $^{13}C/^{12}C$ would reflect that in the maternal diet during fetal development as nearly all neurons are formed during maturation of the fetal brain and do not undergo cell division thereafter. The $^{13}C/^{12}C$ ratios in the food chains and body tissues of Europeans differ from corresponding American ratios by about 50 parts per million on the average. Therefore, turnover was studied by comparing $^{13}C/^{12}C$ ratios in cerebellar DNA of American-born Americans, European-born Americans, and European-born Europeans. The $^{13}C/^{12}C$ ratios in cerebellar DNA from European-born Americans were closer to $^{13}C/^{12}C$ ratios in cerebellar DNA from European-born Europeans than from American-born Americans, indicating that there was little or no turnover of neuronal DNA.*

Stable carbon-isotope ratios, $^{13}C/^{12}C$, are slightly but consistently greater in North Americans than in Europeans. The difference is attributed to the relative proportions of two photosynthetic pathways in the food chains of these populations (*1–4*). Human migration from Europe to North America provides a basis for a natural stable carbon-isotope tracer experiment that can detect turnover of long-lived body constituents such as neuronal DNA (*5*) without exposure to radioactive tracers.

Tritium-labeled mouse brain DNA has an average biological half-life of 1 to 4 years (*6, 7*). A substantial proportion of mammalian whole brain DNA is in glial and endothelial cells which undergo mi-

tosis. Although mature neurons contain DNA polymerase (*8*), may incorporate small amounts of exogenous thymidine (*9*), and can be induced to divide in vitro (*10*), they do not undergo mitosis in vivo (*11, 12*). Thus, the average biological half-life of tritium-labeled mouse neuronal DNA is probably as long as or longer than the median life-span of mice (1 to 2 years). Consequently, most DNA in mouse brain neurons is considered to be metabolically stable in vivo.

Human senescence is often associated with impairment of central nervous system function. If human neuronal DNA were metabolically stable, then neurological senescence might be due to progressive accumulation of abnormalities such as chemical adducts in neuronal DNA. This speculation led us to study the stability of human neuronal DNA.

About 90 percent of cell nuclei in the human cerebellum are in small neurons of the cortical granular layer (*13–15*). A significant difference in $^{13}C/^{12}C$ between the whole cerebellar DNA of European-born Americans and European-born Eu-

Table 1. Stable carbon-isotope ratios in cerebellar DNA and white matter from American-born Americans (A), European-born immigrants to the United States (I), and European-born Europeans (E). The mean of n measurements of $\delta^{13}C$ and the standard deviation from the mean (σ_n) are listed for each cerebellum; S.E.M., standard error of the mean.

Case	Life-span (years)	Birthplace	Year of birth (±1 year)	Sex	Cerebellar DNA			Cerebellar white matter		
					$\delta^{13}C$	n	σ_n	$\delta^{13}C$	n	σ_n
A1	49	United States	1930	M	−18.0	2	1.6	−17.2	2	0.2
A2	51	United States	1926	M	−14.5	3	0.3	−14.5	2	0.4
A3	56	United States	1922	F	−15.8	1				
A4	56	United States	1923	M	−17.0	3	0.5	−16.5	2	0.2
A5	58	United States	1920	M	−16.4	1				
A6	59	United States	1919	M	−18.3	2	1.3	−17.1	2	0.2
A7	60	United States	1918	M	−15.2	1		−17.4	3	0.6
A8	65	United States	1912	F	−13.2	3	2.2			
A9	70	United States	1909	M	−15.4	3	0.8	−17.7	2	0.2
A10	74	United States	1903	M	−14.2	1				
A11	77	United States	1901	M	−16.0	1				
A12	79	United States	1898	M	−15.7	1				
A13	79	United States	1900	M	−16.4	1		−17.7	2	0.1
A14	79	United States	1900	M	−17.5	2	0.2	−17.5	2	0.2
A15	84	United States	1893	M	−14.3	5	1.2	−16.7	3	0.2
A16	85	United States	1892	F	−14.8	5	0.7	−18.2	3	0.2
A17	85	United States	1894	M	−17.4	2	0.3	−17.5	2	0.2
A18	86	United States	1893	M	−17.9	3	0.9	−18.8	2	0.1
A19	88	United States	1890	M	−16.9	1				
			(Mean_A ± S.E.M.)		(−16.05 ± 0.33)			(−17.23 ± 0.31)		
I1	52	Germany	1927	M	−21.5	1		−17.3	2	0.4
I2	57	France	1923	M	−19.9	4	0.9	−17.1	2	0.2
I3	57	Germany	1922	M	−21.3	3	2.0	−16.9	2	0.5
I4	58	Germany	1921	M	−20.3	3	0.7	−17.4	2	0.0
I5	65	Germany	1914	M	−20.7	4	0.9	−17.4	2	0.2
I6	66	Italy	1913	M	−16.9	2	0.8	−17.1	2	0.1
I7	67	Scotland	1911	M	−17.0	2	1.0	−16.0	2	0.7
I8	70	Malta	1908	M	−17.6	7	1.6	−16.0	3	1.4
I9	73	Czechoslovakia	1905	M	−19.4	2	0.8			
I10	75	Ireland	1903	F	−19.1	4	1.2	−16.6	2	0.3
I11	76	Germany	1903	M	−23.0	2	1.2	−17.9	4	0.3
I12	79	Ireland	1900	M	−19.4	2	0.7	−18.8	2	0.2
I13	81	Spain	1898	M	−18.6	4	2.3	−18.4	3	0.1
I14	83	Italy	1895	M	−18.0	1		−18.9	2	0.3
I15	84	Italy	1894	F	−20.0	2	0.0			
I16	84	Italy	1894	M	−19.3	2	0.2			
I17	85	Italy	1895	M	−19.6	1				
I18	91	Lithuania	1888	F	−20.3	1		−17.9	2	0.3
			(Mean_I ± S.E.M.)		(−19.55 ± 0.37)			(−17.41 ± 0.24)		
E1	36	Switzerland	1942	M	−19.5	2	0.6	−21.6	1	
E2	44	Norway	1934	M	−19.9	1		−20.8	1	
E3	61	Switzerland	1917	M	−21.8	1		−21.4	1	
E4	63	Norway	1915	M	−20.9	1		−22.0	1	
E5	64	England	1914	F	−19.1	2	0.2	−21.1	1	
E6	65	Norway	1913	M	−22.4	1				
E7	68	Norway	1910	F	−20.2	1				
E8	69	Norway	1909	M	−18.8	1		−21.7	1	
E9	72	Norway	1906	M	−20.6	1		−20.5	1	
E10	75	Norway	1903	F	−19.9	2	0.5	−21.2	1	
E11	77	Norway	1901	F	−19.0	1				
E12	78	Norway	1900	M	−21.7	1				
E13	78	Norway	1900	F	−19.4	1		−21.2	1	
E14	78	England	1900	M	−19.5	1		−20.6	1	
E15	78	Switzerland	1900	M	−22.9	2	0.3	−21.8	1	
E16	81	Switzerland	1897	M	−21.5	2	0.6	−21.8	1	
E17	83	England	1895	F	−20.5	2	0.4	−21.8	1	
E18	93	England	1885	F	−18.5	1		−21.1	1	
			(Mean_E ± S.E.M.)		(−20.34 ± 0.30)			(−21.33 ± 0.13)		

ropeans would be attributed to turnover of a fraction of neuronal DNA in the mature cerebellum. We now report stable carbon-isotope ratios in DNA isolated (*5, 16, 17*) from 55 American and European postmortem cerebellar tissue specimens (Table 1). The $^{13}C/^{12}C$ ratios are expressed in del units ($\delta^{13}C$), which are the deviation (in parts per thousand) from the $^{13}C/^{12}C$ ratio in the PDB standard (derived from a fossilized *Belemnitella americana* from the Peedee formation in South Carolina) (*3*). For European-born Americans (I), American-born Americans (A), and European-born Europeans (E), the mean ± standard error of the mean of cerebellar DNA ($\delta^{13}C_A - \delta^{13}C_I$) and ($\delta^{13}C_A - \delta^{13}C_E$) were 3.50 ± 0.50 and 4.29 ± 0.45, respectively (Table 1). Comparison of these differences in $\delta^{13}C$ suggests the similarity of neuronal DNA in Europeans and in European-born Americans. The $\delta^{13}C_I$ for cerebellar DNA includes an estimated 10 percent contribution from DNA of non-neuronal cells such as glial and endothelial cells which divide infrequently and are expected to reflect $\delta^{13}C_A$. Thus the value of ($\delta^{13}C_A - \delta^{13}C_I$) is reduced by ~10 percent from that expected for values based exclusively on DNA from neuronal cells. This reduction brings the above comparison into closer agreement with the conclusion that the carbon of neuronal DNA is isotopically similar in Europeans and in European-born immigrants.

The evidence for little or no turnover in neuronal DNA becomes clearer upon further numerical analysis of Table 1 (*18*). The average difference between $\delta^{13}C_I$ and $\delta^{13}C_E$, or between ($\delta^{13}C_A - \delta^{13}C_I$) and ($\delta^{13}C_A - \delta^{13}C_E$) reflects the turnover of an average fraction (f_D, 0.09) of immigrants' neuronal DNA during residence in the United States. Such differences also reflect an average fraction (f_W, 0.96) of immigrants' cerebellar white matter that is metabolized during residence in the United States (*19*).

Statistical variations in $\delta^{13}C$ measurements (σ_n, Table 1) were greater for cerebellar DNA than for unfractionated white matter. These variations could be due, in part, to greater diversity in the photosynthetic origins of foods in the United States and Europe during the late 19th and early 20th centuries than during more recent years. Furthermore, variation in cerebellar DNA $\delta^{13}C$ might have been introduced by the additional extraction and purification steps that preceded oxidation.

Partial life histories were obtained from 13 of the 18 immigrants. Estimates of the lower limit of duration of these immigrants' United States residence were 27 to 72 years with an average lower limit, L_{av}, of 46 years. Values of $\delta^{13}C$ in cerebellar white matter of native-born (−17.23 ± 0.31) and of European-born (−17.41 ± 0.24) Americans indicate that there was practically complete turnover of cerebellar white matter during the average duration of United States residence. The $^{13}C/^{12}C$ ratios are about one del unit less in cerebellar white matter than in cerebellar DNA in Europe and in the United States. This observation follows the general rule, in plants and in animals, that lipids are slightly depleted in ^{13}C relative to the rest of the organism (*20, 21*).

From our measurements and calculations (*22*), 95 percent of the probability distribution of f_D is less than 0.36 and 95 percent of the probability distribution of f_W is greater than 0.82. If $(f_D)_{max}$ is an estimate of the upper limit of the fraction of cerebellar neuronal DNA that was metabolized during L_{av} years after migration to the United States, an estimate of the lower limit of $T_{1/2}$, the biological half-life of neuronal DNA, is

$$T_{1/2} \geq -L_{av} \ln 2/\ln[1 - (f_D)_{max}]$$

Substitution of $(f_D)_{max} = 0.36$ and $L_{av} = 46$ years in this equation gives $T_{1/2} \geq 71$ years. We conclude that a large proportion of the DNA in small neurons of the cerebellum undergoes no metabolic turnover during the human life-span.

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16. Frozen (−70°C) cerebellar samples were selected at random for DNA extraction. Each DNA preparation was isolated (*5, 17*) with a new ultrafiltration membrane and with new cellulose and hydroxylapatite columns. Its $\delta^{13}C$ was then measured as described [D. N. Slatkin *et al.*, *Hum. Pathol.* **9**, 259 (1978)]. RNA was assayed by modifying an orcinol technique [S. Zamenhof in *Methods Enzymol.* **3**, 696 (1957)]. A dried aliquot of the DNA preparation was pretreated with 0.20 ml of 6.0*N* HCl for 20 minutes at 100°C. A freshly prepared solution of orcinol and $FeCl_3$ in 0.80 ml of 9.75*N* HCl was added to make the reaction mixture 1 percent orcinol, 0.1 percent F_eCl_3, and 9.0*N* HCl. Absorbance at 660 nm was measured within 2 hours of heating the mixture for 10 minutes at 100°C and cooling it quickly on ice. DNA preparations so tested contained less than 1 percent RNA by weight. Protein in the preparations, as assayed by fluorescamine [S. Udenfriend *et al.*, *Science* **178**, 871 (1972)], was also less than 1 percent by weight. The buoyant density of one such DNA preparation in a CsCl gradient (1 m*M* tris buffer, 1 percent sodium dodecyl sulfate, *p*H 8.0) was 1.68.
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18. Let f_D be the average fraction of cerebellar neuronal DNA that was metabolized after migration from Europe to the United States; f_n is the proportion of all cerebellar nuclei which is neuronal. If non-neuronal cerebellar DNA turned over completely in the United States, then

$$R_I = f_n[f_D R_A + (1 - f_D)R_E] + (1 - f_n)R_A$$

where subscripted R values represent average $^{13}C/^{12}C$ ratios in DNA isolated from postmortem specimens of whole cerebella (subscripts I, E, and A as in Table 1). Thus,

$$f_D = 1 - \{(\delta^{13}C_A - \delta^{13}C_I)/[f_n(\delta^{13}C_A - \delta^{13}C_E)]\}$$

Substitution of $\delta^{13}C_A = -16.05$, $\delta^{13}C_I = -19.55$, and $\delta^{13}C_E = -20.34$ from Table 1 and $f_n = 0.90$ gives $f_D = 0.09$.
19. Let f_W be the average proportion of carbon in whole cerebellar white matter which was renewed after migration from Europe to the United States. Then,

$$R_I = f_W R_A + (1 - f_W)R_E$$

where subscripted R values are average $^{13}C/^{12}C$ ratios in cerebellar white matter. Thus,

$$f_W = (\delta^{13}C_I - \delta^{13}C_E)/(\delta^{13}C_A - \delta^{13}C_E)$$

Substitution of $\delta^{13}C_I = -17.41$, $\delta^{13}C_E = -21.33$, and $\delta^{13}C_A = -17.23$ from Table 1 gives $f_W = 0.96$.
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22. Oxidation and CO_2 mass-ratio measurements were performed on randomly interspersed, coded specimens from groups A, I, and E. Small systematic errors in $\delta^{13}C$ might have been introduced by deviations from ideal experimental conditions such as unequal duration of storage of cerebella at −70°C, incomplete extraction of DNA, or selective extraction of one or more types of DNA. However, such errors would have affected groups A, I, and E about equally, and therefore should not be propagated through calculations of the quotients of differences between pairs of average $\delta^{13}C$ values. In these calculations, the standard error of the quotient, x/y, of two normal variates was approximated [E. C. Fieller, *Biometrika* **24**, 428 (1932)] by

$$(\bar{x}/\bar{y})\,[(\sigma_{\bar{x}}/\bar{x})^2 + (\sigma_{\bar{y}}/\bar{y})^2]^{0.5}$$

where $\bar{x}$ and $\bar{y}$ represent the mean values of the two variates and $\sigma_{\bar{x}}$, and $\sigma_{\bar{y}}$ represent their respective standard errors. It is assumed that each set of $\delta^{13}C$ values in Table 1 is a randomly selected subset of $\delta^{13}C$ values from a normal distribution.
23. This report is dedicated to the memory of S. L. Commerford. We thank A. Benjamin, E. Brodkin, M. Dana, C. Hung, O. Laerum, J. Laissue, J. Magidson, N. Peress, Y. Robitaille, R. Rosales, E. Ross, G. Ryan, W. Sharpe, R. Singer, and I. Talbot for cerebellar specimens; G. Boykin, D. Franck, M. Loughlin, and F. Silkworth for technical assistance; R. Aronson, B. Cleveland, E. Cronkite, R. Setlow, M. Slatkin, R. Stoner, and E. Popenoe for encouragement and advice. This research was carried out at Brookhaven National Laboratory under contract DE-AC02-76CH00016 with the U.S. Department of Energy, supported by its Offices of Basic Energy Sciences and of Health and Environmental Research in the Office of Energy Research. We acknowledge grant support (NS 17822-01 RNM) from the National Institutes of Health.

13 November 1984; accepted 13 February 1985