W. F. Anderson, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6362 (1981).
32. A. L. Beaudet, T.-S. Su, W. E. O'Brien, P. D'Eustachio, P. E. Barker, F. H. Ruddle, *Cell* **30**, 287 (1982); S. P. Daiger, R. S. Wilden, T.-S. Su, *Nature (London)* **298**, 682 (1982).
33. U. Francke, B. deMartinville, P. D'Eustachio, F. H. Ruddle, *Cytogenet. Cell Genet.* **33**, 267 (1982); B. Blomberg, A. Traunecker, H. N. Eisen, S. Tonegawa, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3765 (1981).
34. D. Swan, P. D'Eustachio, L. Leinwand, J. Seidman, D. Keithley, F. H. Ruddle, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2735 (1979).
35. P. D'Eustachio, D. Pravtcheva, K. Marcu, F. H. Ruddle, *J. Exp. Med.* **151**, 1545 (1980).
36. D. Owerbach, W. J. Rutter, T. B. Shows, P. Gray, D. V. Goeddel, R. M. Lawn, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3123 (1981); D. L. Slate *et al.*, *J. Exp. Med.* **155**, 1019 (1982); T. B. Shows, A. Y. Sakaguchi, S. L. Naylor, D. V. Goeddel, R. M. Lawn, *Science* **218**, 373 (1982).
37. P. Gupta, J. M. Rosen, P. D'Eustachio, F. H. Ruddle, *J. Cell Biol.* **93**, 199 (1982).
38. P. D'Eustachio, R. S. Ingram, S. M. Tilghman, F. H. Ruddle, *Somat. Cell Genet.* **7**, 289 (1981).
39. D. H. Margulies, G. A. Evans, L. Flaherty, J. G. Seidman, *Nature (London)* **295**, 168 (1982).
40. M. Steinmetz, A. Winoto, K. Minard, L. Hood, *Cell* **28**, 489 (1982).
41. A. Shimizu, N. Takahashi, Y. Yaoita, T. Honjo, *Cell* **28**, 499 (1982); A. L. M. Bothwell, M. Paskind, M. Reth, T. Imanishi-Kari, K. Rajewsky, D. Baltimore, *ibid.* **24**, 625 (1981); M. Steinmetz, K. Minard, S. Horvath, J. McNicholas, J. Frelinger, C. Wake, E. Long, B. Mach, L. Hood, *Nature (London)* **300**, 35 (1982); R. Goodenow, M. McMillan, M. Nicholson, B. T. Sher, K. Eakle, N. Davidson, L. Hood, *ibid.*, p. 231.
42. S. P. Goff, E. Gilboa, O. Witte, D. Baltimore, *Cell* **22**, 777 (1980).
43. M. P. Calos and J. Miller, *ibid.* **20**, 579 (1980).
44. C. D. Wild, C. E. Crowther, T. P. Cripe, M. G.-S. Lee, N. Cowan, *Nature (London)* **297**, 83 (1982); N. Cowan, personal communication.
45. L. Bernstein, S. M. Mount, A. M. Weiner, *Cell* **32**, 461 (1983).
46. Two α-globin pseudogenes have been described in the mouse. One, α3, has a retrovirus-like structure (*42*). The other, α4, has retained the introns found in the functional α-globin gene and thus appears not to be cDNA-like. The one dispersed immunoglobulin pseudogene described to date, a human λ-chain pseudogene, has a cDNA-like structure. It corresponds, however, not to a λ-chain mRNA, but to an anomalous immunoglobulin RNA species found at low levels in some lymphoid cells. Its transcription in these cells is thought to be mediated by an aberrant promoter. It is difficult to exclude the notion that this promoter could likewise be active, rarely and at low levels, in germ cells [G. F. Hollis, P. A. Hieter, O. W. McBride, D. Swan, P. Leder, *Nature (London)* **296**, 321 (1982); R. P. Perry *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1937 (1980)].
47. H. Czosnek, U. Nudel, M. Shani, P. Barker, F. H. Ruddle, D. Yaffe, *EMBO (Eur. Mol. Biol. Org.) J.*, in press.
48. T. D. Petes and H. L. Klein, in *The Recombination of Genetic Material*, K. B. Low, Ed. (Academic Press, New York, in press).
49. O. W. McBride, P. A. Hieter, G. F. Hollis, D. Swan, M. C. Otey, P. Leder, *J. Exp. Med.* **155**, 1480 (1982).
50. D. Owerbach *et al.*, in (*2*), p. 306; D. Owerbach, W. J. Rutter, N. E. Cooke, J. A. Martial, T. B. Shows, *Science* **212**, 815 (1981).
51. C. Junien *et al.*, *Am. J. Hum. Genet.* **34**, 381 (1982).
52. D. Owerbach, G. I. Bell, W. J. Rutter, T. B. Shows, *Nature (London)* **286**, 82 (1980); B. deMartinville, J. Leary, A. Ullrich, U. Francke, in (*2*), p. 265; G. I. Bell, M. J. Selby, W. J. Rutter, *Nature (London)* **295**, 31 (1982).
53. J. Gusella, A. Varsanyi-Breiner, F.-T. Kao, C. Jones, T. T. Puck, C. Keys, S. Orkin, D. Housman, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5239 (1979); S. Orkin *et al.*, *Nature (London)* **296**, 627 (1982).
54. R. Barg *et al.*, in (*2*), p. 252.
55. A. Y. Sakaguchi, S. L. Naylor, C. Quinto, W. J. Rutter, T. B. Shows, in (*2*), p. 313.
56. D. Owerbach, W. J. Rutter, J. A. Martial, J. D. Baxter, T. B. Shows, *Science* **209**, 289 (1980).
57. C. Huerre *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6627 (1982).
58. K. L. Bennett, P. A. Lalley, R. K. Barth, N. D. Hastie, *ibid.*, p. 1220; K. Krauter, L. Leinwand, P. D'Eustachio, F. H. Ruddle, J. E. Darnell, *J. Cell Biol.* **94**, 414 (1982).
59. R. Elliott, *Mouse Newsl. No. 64* (1981).
60. M. Yagi, P. D'Eustachio, F. H. Ruddle, M. Koshland, *J. Exp. Med.* **155**, 647 (1982).
61. S. Malcolm, P. Barton, D. L. Bentley, M. A. Ferguson-Smith, C. S. Murphy, T. H. Rabbitts, in (*2*), p. 296.
62. M. E. Chandler, L. H. Kedes, R. H. Cohn, J. J. Yunis, *Science* **205**, 908 (1979).
63. M. E. Harper, A. Ullrich, G. F. Saunders, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4458 (1981).
64. D. S. Gerhard, E. S. Kawasaki, F. C. Bancroft, P. Szabo, *ibid.*, p. 3755.
65. M. E. Harper, H. A. Barrera-Saldana, G. F. Saunders, *Am. J. Hum. Genet.* **34**, 227 (1982).
66. J. M. Traut, S. Olson, R. M. Lawn, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7809 (1982).
67. E. Herbert and M. Uhler, *Cell* **30**, 1 (1982); M. Uhler, E. Herbert, P. D'Eustachio, F. H. Ruddle, *J. Biol. Chem.*, in press.
68. Supported by NIH grant GM-09966 (F.H.R.), an ACS institutional award (P.D.), and by the Leukemia Society of America, Inc. We thank Drs. Peter Barker and Chester Partridge for their suggestions.

RESEARCH ARTICLE

Genetic Expression in the Developing Brain

Nirupa Chaudhari and William E. Hahn

Abstract. *The adult mouse brain contains complex populations of polyadenylated [poly(A)$^+$] and nonpolyadenylated [poly(A)$^-$] messenger RNA's (mRNA's). These mRNA's are separate sequence populations, similar in complexity, and in combination are equivalent to ~ 150,000 different mRNA sequences, of average length. Essentially all of the "adult" poly(A)$^+$ mRNA's are present in the brain at birth. In contrast, most of the poly(A)$^-$ mRNA's are absent. Brain poly(A)$^-$ mRNA's begin to appear soon after birth, but the full adult complement is not reached until young adulthood. This suggests that these poly(A)$^-$ mRNA's specify proteins required for the biological capabilities of the brain that emerge during the course of postnatal development.*

A major fraction of the DNA that is thought to code for protein (*1*) is expressed as messenger RNA (mRNA) in the mammalian (rodent) brain (*2, 3*). It seems likely that as many, or perhaps more, structural genes are required for the formation and function of the brain than are necessary for all other tissues and organs combined. The complexity of whole brain polysomal RNA is equivalent to about 150,000 different sequences, 1500 nucleotides in length (*2, 3*). The diversity of mRNA molecules in the brain is biologically consistent with the extensive cellular heterogeneity and microdifferentiation of the various cell types that occur in this organ. In addition, the greater complexity of brain RNA might be due to the inherent complexity of neurons. Studies on tumor cells of neural origin and nuclear RNA prepared from the neuronal fraction indicate that neurons contain RNA of higher sequence complexity than do other types of mammalian cells (*4*).

Measurements of the complexity of brain polysomal RNA have shown that nonhomologous populations of polyadenylated [poly(A)$^+$] and nonpolyadenylated [poly(A)$^-$] mRNA are present. The sequence complexity of these mRNA populations is about the same (*2, 3*). Also, in *Drosophila* a complex poly(A)$^-$ mRNA population is present, many species of which are present in the head of the fly (*5*).

Although poly(A)$^-$ mRNA's have been identified in various systems (*6*), a highly complex population of poly(A)$^-$ mRNA's appears to be characteristic of the brain since, in other mammalian cells, tissues, and organs that have been examined, essentially all of the sequence complexity is present in the poly(A)$^+$ RNA fraction of polysomal RNA (*7*). Most of the rare, complex class mRNA species in the brain are present at much less than one copy per cell (*3*), and, although little is known regarding anatomical localization of specific mRNA's, we can suppose that many of the rare copy species are restricted to given cell types, or subpopulations of cells, where they are present in relatively high copy frequency (*8*). The translational efficiency in cell free systems of brain poly(A)$^-$ mRNA's,

Nirupa Chaudhari is a postdoctoral fellow and William E. Hahn is a professor in the Department of Anatomy, University of Colorado, School of Medicine, Denver 80262.

both rare class and abundant species, is similar to that of brain poly(A)$^+$ mRNA's and purified prolactin mRNA (*9*). The biogenesis of poly(A)$^-$ mRNA is not defined except that respective precursors in the nuclear RNA lack poly(A) (*9*). Brain poly(A)$^+$ mRNA's are derived from high molecular weight poly(A)$^+$ hnRNA (heterogeneous-nuclear) molecules (*10*) that are encoded, at least in many instances, by genes containing intervening sequences (*11*), as has been shown for several specific eukaryotic mRNA's (*12*).

In comparison with most other organs, postnatal development of the brain is extensive and elaborate. Therefore we decided to determine whether the complex mRNA population characteristic of the adult brain is present at birth and, if not, to quantify genetic expression during the course of postnatal development. For convenience, and with regard to background information, we used the mouse in our studies, but our results are probably relevant to the postnatal development of the brains of other mammals including man.

Most Brain Poly(A)$^+$ mRNA's Are Present at Birth

Most, if not all, of the poly(A)$^+$ mRNA species characteristically present in the young adult mouse brain are present in the brain at birth. This was shown by combining two measurements. First, we measured the sequence complexity of brain poly(A)$^+$ mRNA from adult and newborn mice by saturation hybridization of genomic single-copy DNA (scDNA) (*13*). From the percentage of DNA that was resistant to S1 nuclease after hybridization (*14*), the sequence complexity was determined to be $\sim 1.2 \times 10^5$ and $\sim 1.0 \times 10^5$ kilobases (kb) for adult and newborn brain, respectively. That these poly(A)$^+$ mRNA populations, of similar complexity, were composed of the same sequences (that is, are homologous) was shown with a complementary DNA (cDNA) probe representing the complex, rare copy class of poly(A)$^+$ mRNA from adult brain. Since reverse transcription is efficient to the extent that the sequence complexity of the cDNA is essentially equal to that of the template mRNA, and a quantitative yield of cDNA can be obtained which is within 80 to 95 percent of the theoretical maximum (*15*), this probe is suitable for making such a measurement. As shown in Fig. 1, cDNA complementary to the complex class of poly(A)$^+$ mRNA from adult brain was hybridized to completion when polysomal RNA from newborn mouse brain was used as driver, indicating that most, if not all, of the adult poly(A)$^+$ mRNA's are present in the brain at birth. A similar result was obtained with purified poly(A)$^+$ mRNA from newborn mouse brain to hybridize the cDNA (data not shown).

Table 1. Hybridization of cDNA representing rare copy (complex) class of brain poly(A)$^+$ mRNA to liver and kidney polysomal RNA. The cDNA was prepared as described in Fig. 1; the percentage of cDNA hybridized was normalized by setting the homologous system (brain mRNA) at 100 percent. The actual maximum observed hybridization with brain mRNA was 95 percent as determined by resistance to S1 nuclease (*14*).

RNA	cDNA hybridized (%)
Brain poly(A)$^+$ mRNA	100
Brain polysomal	100
Liver polysomal	32
Kidney polysomal	35

Many of the poly(A)$^+$ mRNA species present in newborn and adult brain are absent from the brain polysomes of the 17-day-old fetus (gestation is 21 days) since only about 80 percent of the cDNA was hybridized at termination when fetal polysomal RNA was used as driver (Fig. 1). Also, we observed that nuclear RNA of the 17-day fetus hybridized the probe to the same extent as did polysomal RNA (data not shown), indicating that genes specifying mRNA's which are absent in the 17-day fetal brain are not transcriptionally active. We estimate from these results that the equivalent of $\sim$ 15,000 different poly(A)$^+$ mRNA's of average size (1.5 kb) (*16*) appear during the last 3 to 4 days of gestation.

Putatively Brain-Specific Poly(A)$^+$ mRNA's

For perspective on tissue-specific differentiation, it is necessary to estimate the extent to which brain poly(A)$^+$ mRNA's are specific to the brain. Since brain poly(A)$^+$ mRNA is about twice as complex as that from liver or kidney (*7, 15, 16*), the presence of a large number of brain-specific poly(A)$^+$ mRNA's was suggested. We made sequence overlap measurements by hybridizing the cDNA complementary to the rare copy brain poly(A)$^+$ mRNA, to polysomal RNA from liver and kidney. By this assay we estimate that $\sim$ 35 percent of the poly(A)$^+$ mRNA species in the brain is also present in either the liver or the kidney (Table 1). This finding is not surprising since a large number of commonly shared mRNA's thought to code for proteins required for general "housekeeping" in all cells have been described by several investigators (*17*). Of more interest is the $\sim$ 65 percent of the cDNA that did not hybridize, which indicates the absence of respective "brain" mRNA species in the liver or kidney polysomal RNA. Although it is premature to refer to these poly(A)$^+$ mRNA's as being strictly brain-specific until comparisons are made with more organs and tissues, these results suggest that this is the case.

We considered that poly(A)$^+$ mRNA's appearing in the brain late in fetal development (see above and Fig. 1) might be mostly brain-specific relative to liver and kidney. Therefore, we prepared a complex cDNA probe from adult poly(A)$^+$ mRNA, from which most of the sequences complementary to 17-day fetal poly(A)$^+$ mRNA had been removed by saturation hybridization with fetal poly-

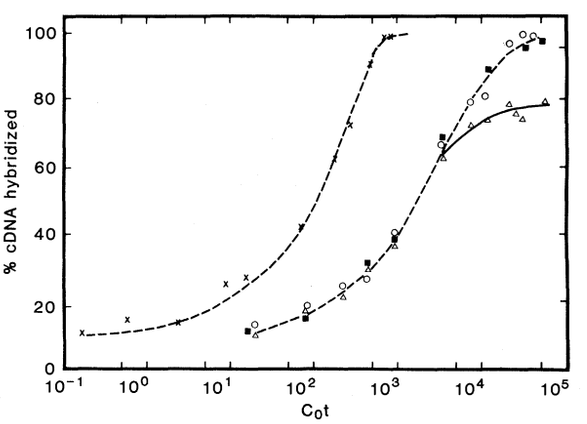

Fig. 1. Hybridization of cDNA representing rare (complex) class poly(A)$^+$ mRNA's from adult Swiss Webster mouse brain with template poly(A)$^+$ mRNA (X), or with total polysomal RNA from 17-day fetus (△), newborn (■), or adult (○) brain. C_0t is the molar concentration of the RNA multiplied by time in seconds (mole nucleotides per liter per second) corrected for sodium ion concentration above standard conditions (*35*). Percent hybridization is normalized relative to the maximum obtained with adult poly(A)$^+$ mRNA taken as 100 percent (actual level was 91 percent). Complementary DNA obtained by reverse transcription (*15*) of adult poly(A)$^+$ mRNA was hybridized to C_0t of 5 under conditions of continuous mixing (*35*). After hybridization, cDNA mostly representing rare, or complex, class mRNA was isolated by hydroxyapatite chromatography as the unbound fraction. Hybridized cDNA was determined by the S1 nuclease–DEAE filter method (*14*).

somal RNA. This "fetal null" probe hybridized to liver and kidney polysomal RNA to the same extent (~ 40 percent) as observed for the total cDNA probe. Therefore the poly(A)$^+$ mRNA population appearing late in fetal development of the brain includes both "shared" and putatively brain-specific mRNA's.

Genes Encoding Brain Poly(A)$^-$ mRNA's Activated Postnatally

Since most of the poly(A)$^-$ mRNA's appear to be specific to the brain (see below), and considering the extensive postnatal development of the brain, it was of interest to determine when these mRNA's appear. We first examined the sequence complexity of polysomal RNA from newborn brain and found it to be half that of the adult (Table 2). In contrast to the adult brain, most of the complexity of the newborn polysomal RNA is contained in the poly(A)$^+$ fraction. Thus, most, if not all, of the measurable "adult" poly(A)$^-$ mRNA's are absent at birth.

Measurements of the sequence complexity of brain nuclear RNA from the newborn suggested that the genes specifying most of the poly(A)$^-$ mRNA's are not transcriptionally active at birth. We found that the sequence complexity of total nuclear RNA from neonatal brain was ~ 1.4×10^5 kb less than in the adult (Table 2). The greater sequence complexity of adult nuclear RNA is consistent with the presence of the precursors of poly(A)$^-$ mRNA's [complexity ~ 1.1×10^5 kb (*2*)] in the nuclear RNA. Most of the sequence complexity of total nuclear RNA in the newborn, unlike that in the adult, is due to the polyadenylated fraction [poly(A)$^+$ hnRNA]. Also, the sequence complexity of adult and newborn poly(A)$^+$ hnRNA is the same, as is the case for respective poly(A)$^+$ mRNA populations. Since qualitatively most of the different brain poly(A)$^+$ hnRNA's are precursors of poly(A)$^+$ mRNA (*10*), this observation was expected.

By day 21 of postnatal development the complexity of total nuclear RNA is nearly equal to that of the adult, the rise in complexity being attributable to poly(A)$^-$ nuclear RNA. We have found that poly(A)$^-$ nuclear, but not poly(A)$^+$ hnRNA, hybridizes with cDNA transcripts from poly(A)$^-$ mRNA at the expected rates (*9*). These observations, along with direct measurements presented below, show that the change in sequence complexity of nuclear RNA during postnatal development of the brain is largely due to the expression of genes specifying poly(A)$^-$ mRNA's. The marked increase in the sequence complexity of RNA occurring postnatally might be a characteristic feature of the brain associated with the delayed development of this organ, since such an increase was not observed in the liver (*18*).

Most of the ribosomal RNA (rRNA) (~ 98 percent) can be removed from poly(A)$^-$ polysomal RNA by benzoylated-cellulose chromatography (*2, 19*). The remaining partially purified poly(A)$^-$ mRNA can be primed for reverse transcription by addition of random oligonucleotides (*15, 20*). Therefore, to further examine genetic expression which occurs during postnatal development of the brain, we prepared cDNA complementary to rare (complex) class poly(A)$^-$ mRNA of the adult brain, and used it to directly probe nuclear and polysomal RNA from the brains of mice of various postnatal ages. In the newborn ~ 10 percent of the "adult" poly(A)$^-$ mRNA sequences are present in the polysomal RNA and 20 percent in the nuclear RNA (Fig. 2). The appearance of poly(A)$^-$ mRNA's in the polysomal fraction is delayed relative to the appearance of these sequences in the nuclear RNA. After 24 days, when all poly(A)$^-$ mRNA sequences are present in the nuclear RNA, only ~ 30 percent of the sequences are present in the polysomal RNA. The adult complement of these mRNA's is apparently reached in the polysomal RNA sometime during 35 to 45 days of postnatal growth and development (Fig. 2). Surprisingly, about 20 percent of the adult poly(A)$^-$ mRNA complexity (~ 18,000 kb) is absent from the polysomes even after 35 days of postnatal development, suggesting the appearance of ~ 10,000 new proteins in the brain during the period of development from "adolescent" to young adult (35 to 45 days).

Why many of these mRNA sequences are present in the nucleus well in advance of their appearance in the polysomal fraction is puzzling. We considered that these gene products might be released from the nucleus but were blocked from association with ribosomes. Therefore we prepared RNA from the nonpolysomal cytoplasmic fraction of brains from 24- and 35-day-old mice. Using the complex class cDNA probe we found that about the same percentage of the adult poly(A)$^-$ mRNA sequences was present in this fraction as in the polysomal fraction. Therefore it appears that a substantial number of genes encoding poly(A)$^-$ mRNA's are activated well in advance of the release of respective mRNA's to the cytoplasm, implying some nuclear mechanism of

Table 2. Sequence complexity of mouse brain RNA in relation to development. Various RNA's were isolated as described (*2, 16, 35*) and used to hybridize scDNA (*13*). Hybridized DNA was quantified by the S1 nuclease–DEAE filter method (*14*). Standard deviations are based on five or more values for the amount of scDNA which was hybridized. Sequence complexity was estimated on the basis of a complexity of 3.2×10^9 nucleotides for the scDNA of the haploid genome.

RNA	scDNA hybridized (%)	Sequence complexity (kb)
Newborn		
Total polysomal	3.6 ± 0.3	1.2×10^5
Poly(A)$^+$ mRNA	3.3 ± 0.2	1.0×10^5
Adult		
Total polysomal	7.4 ± 0.5	2.3×10^5
Poly(A)$^+$ mRNA	3.6 ± 0.3	1.2×10^5
Newborn		
Total nuclear	13.3 ± 0.8	4.2×10^5
Poly(A)$^+$ hnRNA	12.4 ± 0.1	4.0×10^5
21-day		
Total nuclear	16.4 ± 0.6	5.2×10^5
60 day (adult)		
Total nuclear	17.8 ± 0.7	5.6×10^5
Poly(A)$^+$ hnRNA	12.5 ± 0.3	4.0×10^5

Table 3. Apparent restriction of poly(A)$^-$ mRNA of high sequence complexity to brain. Sequence complexity estimates of liver and kidney polysomal and poly(A)$^+$ mRNA are based on saturation hybridization of scDNA (see Table 2). Values obtained by the cDNA method, not shown, were similar. Complementary DNA complementary to the complex class of brain poly(A)$^-$ mRNA (see legend, Fig. 2) was used to probe for the presence of these mRNA's in the liver and kidney polysomal RNA. Hybridization only slightly above a background of 2 percent was observed. Percentages are normalized against 100 percent for the homologous system (brain). Actual hybridization with brain polysomal RNA was 94 percent as assayed by resistance to S1 nuclease (*14*).

RNA	Sequence complexity (kb)	Brain cDNA hybridized (%)
Brain polysomal		100
Kidney total polysomal	6.5×10^4	6
Kidney poly(A)$^+$ mRNA	6.5×10^4	
Liver total polysomal	6.0×10^4	5
Liver poly(A)$^+$ mRNA	6.0×10^4	
Liver nuclear		8
Kidney nuclear		9

posttranscriptional control. In the developing sea urchin some abundant mRNA's, and a large number of rare mRNA's, are apparently also regulated by posttranscriptional mechanisms (*21*).

Poly(A)$^-$ mRNA's Appearing Postnatally Are Specific to Brain

Complementary DNA complementary to complex class brain poly(A)$^-$ mRNA was used to probe polysomal and nuclear RNA of the liver and kidney (Table 3). Little hybridization was observed above background, indicating that most of the different poly(A)$^-$ mRNA species from brain polysomes are specific to the brain, at least relative to these two complex organs. The slight hybridization of the cDNA with liver and kidney nuclear RNA suggests that most of the genes specifying brain poly(A)$^-$ mRNA's are regulated transcriptionally, at least relative to tissue type. To establish rigorously whether or not a complex population of poly(A)$^-$ mRNA's exists only in the brain will require further investigation of more cells, tissues, and organs, but our data suggest that this is probably the case.

Comparisons of total polysomal RNA and poly(A)$^+$ mRNA from the liver and kidney also show that virtually all of the sequence complexity is contained in poly(A)$^+$ mRNA (Table 3). Similar studies on polysomal RNA's from primary cell cultures (*22*) and other cells and organs (*7*) also indicated the absence of a complex population of poly(A)$^-$ mRNA's, separate from the poly(A)$^+$ population. Thus the presence of a complex population of poly(A)$^-$ mRNA's may be a characteristic of the brain.

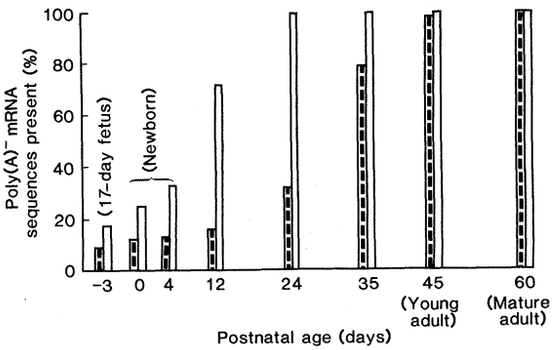

Fig. 2. Appearance of poly(A)$^-$ mRNA sequences in nuclear and polysomal RNA's during development. ^{3}H-labeled cDNA, representing the rare (complex) class of poly(A)$^-$ mRNA of adult brain, was hybridized with polysomal RNA (hatched bars) or nuclear RNA (open bars) from mice of the indicated ages. Poly(A)$^-$ mRNA was obtained from the polysomal RNA, remaining after removal of the poly(A)$^+$ mRNA (*36*), by three chromatographic cycles on columns of benzoylated cellulose (*2, 19*). This procedure removed ~ 98 percent of the rRNA to yield an RNA preparation that was 25 to 50 percent, by mass, poly(A)$^-$ mRNA. The cDNA was prepared by reverse transcription of adult poly(A)$^-$ mRNA primed by annealing with random oligonucleotides (*15, 20*). The cDNA complementary to the complex class of mRNA was isolated by hybridization with total brain polysomal RNA to C_0t of 50, and with poly(A)$^+$ mRNA to C_0t of 1000. The unhybridized fraction of the cDNA, representing about 10 percent of the original mass, was isolated by hydroxyapatite chromatography and used as a probe to measure the appearance of poly(A)$^-$ mRNA in polysomal RNA from brains of mice of various ages. Percent hybridization was normalized relative to the maximum obtained with poly(A)$^-$ mRNA from adult brain taken as 100 percent (actual level was 98 percent).

Discussion and Perspectives

Various anatomical and biochemical properties of the brain develop after birth. The mass of the mouse brain increases rapidly during the first 15 days after birth and adult levels of various neurotransmitters and enzymes are reached by ~ 30 days (*23*). Locomotor abilities are well developed by 15 to 20 days, and sexual maturity occurs by 4 to 7 weeks, depending on the strain of mouse (*24*). The mice used in our experiments are regarded as young adults by 45 days and reach adult weight by 60 days.

During postnatal development there is an increase in the number of glial cells and myelinization of neurons occurs. Neurons develop complex dendritic arrays (arborization), and synaptogenesis is a very active process in the postnatally developing brain (*23*). These events are relevant to the integration and fine tuning of neural circuitry. The possible connection between these events and the postnatal appearance of poly(A)$^-$ mRNA's can only be speculated, but it seems likely that various proteins (receptor proteins, protein components of ionic channels, transmitter peptides) would be required for these and other processes that enable the brain to receive, process, and integrate information.

The extent to which the postnatal expression of genes encoding the poly(A)$^-$ mRNA's in the brain is the result of a genetically "hardwired" developmental schedule remains to be determined. Certainly much of the prenatal developmental program is strongly predetermined as shown from the differentiation of fetal brain tissue transplanted into the anterior chamber of the eye (*25*). However, postnatal aspects of brain development are plastic and sensitive to environmental conditions. For example, the need for sensory input in postnatal development of the brain is indicated by the failure of specific cortical "barrels" to develop if certain sensory vibrissae are destroyed at birth (*26*), and by failure of the visual cortex to complete development upon omission or restriction of visual input (*27*). Also, the size and number of the dendrites of Purkinje cells in the cerebellum is sensitive to environmental conditions (*28*). It is possible that the activation of a large number of genes is dependent on environmental stimuli arriving via various sensory modalities, perhaps in a manner somewhat analogous to the activation of certain genes by hormones. Thus RNA and protein synthesis is apparently altered in neurons as a result of electrical stimulation or during learning (*29*). Signals from the environment play a role in inducing reproductive behavior in various species. In this regard, egg-laying behavior is elicited when a small family of genes encoding neuroactive peptides (egg-laying hormones) is expressed in specific neurons of the mollusc *Aplysia* (*30*).

It is unclear to what extent the mouse is a representative model for the molecular genetics of postnatal development of the mammalian brain. Guinea pigs, for example, are much more advanced at birth, as are a number of other mammals that are born singly instead of in litters. During postnatal development, the human brain increases about fourfold in mass, and behavioral capabilities take many years to emerge and become refined. The extensive genetic program that emerges during a few days of postnatal development of the mouse brain, if also the case for the human brain, may take years to complete. It is likely that various environmental inputs, including a wide array of social interactions, influence the outcome of postnatal development of the brain more in some species than in others.

As reported here, about 65 percent of the sequence complexity of poly(A)$^+$ mRNA in brain, the equivalent of ~ 50,000 1.5-kb species, is restricted to the brain at least relative to two other complex organs, the liver and kidney. A repetitive 82-base sequence has now been identified in rat which hybridizes with brain poly(A)$^+$ RNA but not with

liver and kidney RNA (*31*). At least 90,000 copies of this repeat are present per haploid genome (*32*), and, as has been proposed, this repeat might be a tissue "identity sequence" that plays a role in differential genetic expression (*31*). Interestingly, the copy number of this repeat is roughly similar to the number of brain-specific poly(A)$^+$ mRNA's in the mouse, which further suggests a possible role of this sequence in tissue-specific genetic expression.

In order to place the molecular genetics of brain development and function into the context of cellular biology, the general anatomical and cellular distribution of specific mRNA's must be determined. The fact that specific neurons or classes of neurons apparently contain "exclusive" proteins, as identified with monoclonal antibodies (*33*), implies that certain mRNA species are confined to specific neural or glial cell populations. Recent improvements in in situ hybridization applied to slices of brain have permitted the detection of viral RNA in cells of the brain at an estimated frequency of 20 copies per cell, or less (*34*). Thus, the use of cloned cDNA probes in in situ hybridization should yield information leading to a better understanding of regional specializations of the brain relative to specific proteins and protein families.

References and Notes

1. S. Ohno, *Nature (London)* **234**, 134 (1971); T. Ohta and M. Kimura, *ibid.* **233**, 118 (1971); J. O. Bishop, *Cell* **2**, 81 (1974).
2. J. Van Ness, I. H. Maxwell, W. E. Hahn, *Cell* **18**, 1341 (1979). An estimate of the number of different mRNA's requires that the number average size of the complex, rare copy class of mRNA's be known. Recent studies indicate that the size of some rare copy rat brain poly(A)$^+$ mRNA's is 5000 nucleotides (J. G. Sutcliffe, personal communication). If this is the case for most mRNA's, the estimated number of different mRNA's must be adjusted accordingly.
3. D. M. Chikaraishi, *Biochemistry* **18**, 3249 (1979).
4. B. Kaplan and C. Finch, in *Molecular Neurobiology*, I. R. Brown, Ed. (Academic Press, New York, 1982), p. 71; H. Ozawa, E. Kushiya, Y. Takahashi, *Neurosci. Lett.* **18**, 191 (1980).
5. J. L. Zimmerman, D. L. Foute, J. E. Manning, *Genetics* **95**, 673 (1980); L. S. Levy and J. E. Manning, *Dev. Biol.* **85**, 141 (1981). Suggestive evidence has also been reported for the presence of a complex class of poly(A)$^-$ mRNA's in the rodent liver [L. J. Grady, W. P. Campbell, A. B. North, *Eur. J. Biochem.* **115**, 241 (1981)]. However, evidence to the contrary has been reported [J. A. Bantle, C. L. Courchesne, M. Couch, *Biochem. Biophys. Res. Commun.* **95**, 1710 (1980)].
6. M. Nemer and L. M. Dubroff, *Cell* **6**, 171 (1975); J. R. Greenberg, *J. Mol. Biol.* **108**, 403 (1977); L. Miller, *Dev. Biol.* **64**, 118 (1978); J. V. Ruderman and M. L. Pardue, *ibid.* **60**, 48 (1977); Y. Kaufman, C. Milcarek, H. Berissi, S. Penman, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4801 (1977); B. P. Brandhorst, D. P. S. Verma, D. Fromson, *Dev. Biol.* **71**, 128 (1979); A. J. Ouellette, *J. Biol. Chem.* **255**, 2740 (1980).
7. C. Milcarek, *Eur. J. Biochem.* **102**, 467 (1979); A. J. Ouellette and C. P. Ordahl, *J. Biol. Chem.* **256**, 5104 (1981); also see Table 3 of this article.
8. In a homogeneous population of cells, or in a population largely comprised of one type of cell, mRNA is present in three very *general* abundance classes; mRNA species present in the range of about 10^3 to 10^4 copies per cell comprise the abundant class, 10^2 to 10^3 comprise the intermediate class, and 1 to 10 comprise the rare class. Most of the sequence complexity is contained in the rare copy or complex class [G. D. Birnie, E. MacPhail, B. D. Young, M. J. Getz, J. Paul, *Cell Differ.* **3**, 26 (1974); J. O. Bishop, J. G. Morton, M. Rosbash, M. Richardson, *Nature (London)* **250**, 199 (1974); J. G. Williams and S. Penman, *Cell* **6**, 197 (1975)]. Such a distribution can also be observed in organs such as liver, in which the cellular diversity is modest. Lumping mRNA's into three copy frequency classes is arbitrary and an oversimplification [T. J. Quinlan *et al.*, *Nucleic Acids Res.* **5**, 1611 (1978)]. In a highly heterogeneous cell population, such as the brain, the matter of abundance classes becomes more complex and difficult to interpret. For example, mRNA's that are highly abundant, but restricted to a specific cell type or anatomical region, would appear, in the kinetics of hybridization, as rare class species relative to total brain polysomal RNA. Hence, in considering mRNA from whole brain, the rare class is expected to be composed of cell type–specific species, as well as species that are present as few copies in most, or all, cells of the brain. Species that are present in few copies per cell, and restricted to a small percentage of the total cells, would constitute such a small portion of the total mRNA mass that they would be largely undetected by the methods we have used. Thus, it is possible that the complexity of the mRNA of the brain might be greater than our estimates and those of others.
9. N. Chaudhari and W. E. Hahn, unpublished data.
10. W. E. Hahn, J. Van Ness, I. H. Maxwell, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5544 (1978). This is the case for most poly(A)$^+$ mRNA's [J. E. Darnell, *Prog. Nucleic Acids Res.* **22**, 327 (1979)].
11. I. H. Maxwell, F. Maxwell, W. E. Hahn, *Nucleic Acids Res.* **8**, 5875 (1980).
12. R. Breathnach, J. L. Mandel, P. Chambon, *Nature (London)* **270**, 314 (1977); S. M. Tilghman, P. J. Curtis, D. C. Tiemeier, P. Leder, C. Weissmann, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1309 (1978); T. D. Sargent, J.-R. Wu, J. M. Sala-Trepat, R. B. Wallace, A. A. Reyes, J. Bonner, *ibid.* **76**, 3256 (1979); J. H. Nunberg, R. J. Kaufman, A. C. Y. Chang, S. N. Cohen, R. T. Schimke, *Cell* **19**, 355 (1980).
13. Use of saturation hybridization of scDNA and kinetics of cDNA hybridization has been discussed [W. E. Hahn, J. Van Ness, I. H. Maxwell, *Nature (London)* **283**, 601 (1980); R. B. Goldberg and W. E. Timberlake, *ibid.*, p. 601; M. Kiper, *ibid.*, p. 602]. Pitfalls, and conditions for applying these procedures, have been discussed and evaluated (*36*). Owing to recombinatorial events in genomic DNA leading to mixing of various coding sequences, the diversity of mRNA populations would be greater than measured by hybridization of short fragments of scDNA. For example, the complexity of the mRNA specifying immunoglobulins in a polyclonal population of lymphocytes would be underestimated by the procedures we have applied. For discussion of recombinatorial events of immunoglobulin genes, see J. G. Seidman and P. Leder [*Nature (London)* **276**, 790 (1978)] and M. Weigert *et al.* [*ibid.* **283**, 497 (1980)].
14. I. H. Maxwell, J. Van Ness, W. E. Hahn, *Nucleic Acids Res.* **5**, 2033 (1978).
15. J. Van Ness and W. E. Hahn, *ibid.* **8**, 4259 (1980).
16. J. A. Bantle and W. E. Hahn, *Cell* **8**, 139 (1976).
17. G. A. Galau, W. M. Klein, M. M. Davis, B. J. Wold, R. J. Britten, E. H. Davidson, *ibid.* **7**, 487 (1976); N. D. Hastie and J. O. Bishop, *ibid.* **9**, 761 (1976); B. Lewin, *Gene Expression* (Wiley-Interscience, New York, ed. 2, 1980), p. 727.
18. L. Grouse, M.-D. Chilton, B. J. McCarthy, *Biochemistry* **11**, 798 (1972).
19. W. K. Roberts, *ibid.* **13**, 3677 (1974).
20. D. L. Kacian and J. C. Myers, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3408 (1976); J. P. Dudley, J. S. Butel, S. H. Socher, J. M. Rosen, *J. Virol.* **28**, 743 (1978).
21. B. J. Wold, W. H. Hough-Evans, B. R. Britten, E. H. Davidson, *Cell* **14**, 941 (1978); G. W Shepherd and M. Nemer, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4653 (1980); J.-H. Xin, B. P. Brandhorst, R. J. Britten, E. H. Davidson, *Dev. Biol.* **89**, 527 (1982).
22. I. H. Maxwell and W. E. Hahn, unpublished data.
23. T. H. Bullock, *Introduction to Nervous System* (Freeman, San Francisco, 1979), p. 335.
24. F. H. Bronson, C. P. Dagg, G. D. Snell, in *Biology of the Laboratory Mouse*, E. L. Green, Ed. (McGraw-Hill, New York, 1966), p. 187; R. E. Wimer and J. L. Fuller, in *ibid.*, p. 629.
25. L. Olson, R. Freedman, A. Seiger, B. J. Hoffer, *Brain Res.* **119**, 87 (1977); D. J. Woodward, A. Seiger, L. Olson, B. J. Hoffer, *Exp. Neurol.* **57**, 984 (1977).
26. H. Van der Loos and T. A. Woolsey, *Science* **179**, 395 (1973); T. A. Woosley, D. Durham, R. M. Harris, D. J. Simons, K. L. Valentino, in *Development of Perception*, R. Aslin, Ed. (Academic Press, New York, 1981), vol. 1, p. 259.
27. T. N. Wiesel and D. H. Hubel, *J. Neurophysiol.* **26**, 1003 (1963); *J. Comp. Neurol.* **158**, 307 (1974); C. Blakemore, L. J. Garey, F. Vital-Durand, in *Neural Growth and Differentiation*, E. Meisami and M. A. B. Brazie, Eds. (Raven, New York, 1979), p. 311.
28. M. K. Floeter and W. T. Greenough, *Science* **206**, 227 (1979); J. J. Pysh and G. M. Weiss, *ibid.*, p. 230; C. W. Cotman, M. Nieto-Sanpedro, E. W. Harris, *Physiol. Rev.* **61**, 684 (1981).
29. V. Shashoua, *Adv. Cell. Neurobiol.* **3**, 97 (1982).
30. R. H. Scheller, J. F. Jackson, L. B. McAllister, J. H. Schwartz, E. R. Kandel, R. Axel, *Cell* **28**, 707 (1982).
31. J. G. Sutcliffe, R. J. Milner, F. E. Bloom, R. A. Lerner, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4942 (1982)
32. J. G. Sutcliffe, personal communication.
33. C. J. Barnstable, *Nature (London)* **286**, 231 (1980); B. Zipser and R. McKay, *ibid.* **289**, 249 (1981); L. A. Sternberger, L. W. Harwell, N. H. Sternberger, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1326 (1982).
34. C. Godard and J. W. Jones, *Nucleic Acids Res.* **6**, 2849 (1979); M. Brahic and A. T. Haase, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 6125 (1978).
35. J. Van Ness and W. E. Hahn, *Nucleic Acids Res.* **10**, 8061 (1982).
36. J. A. Bantle, I. H. Maxwell, W. E. Hahn, *Anal. Biochem.* **72**, 413 (1976).

37. This work was supported by grant NS10813 from the National Institutes of Health.

29 October 1982; revised 21 March 1983