ResearchGate

See discussions, stats, and author profiles for this publication at:
https://www.researchgate.net/publication/22673918

Complexity of polyadenylated RNA in mouse whole brain and cortex

ARTICLE *in* FEBS LETTERS · AUGUST 1979

Impact Factor: 3.17 · DOI: 10.1016/0014-5793(79)81267-3 · Source: PubMed

CITATIONS
11

READS
24

4 AUTHORS, INCLUDING:

Armando Felsani
Italian National Research Council
89 PUBLICATIONS **1,656** CITATIONS

SEE PROFILE

Available from: Armando Felsani
Retrieved on: 26 January 2016

COMPLEXITY OF POLYSOMAL POLYADENYLATED RNA IN MOUSE WHOLE BRAIN AND CORTEX

Bernard CROIZAT, Francis BERTHELOT, Armando FELSANI[+] and François GROS

Laboratoire de Biochimie Cellulaire, Collège de France, 11, Place Marcelin Berthelot, 75231 Paris Cedex 05, France and *[+]Laboratorio di Embriologia Molecolare del CNR, Via Toiano 2, I-80072 Arco Felice, Italy*

Received 6 April 1979

1. Introduction

Data concerning the eukaryotic messenger sequence complexities broadly differ according to the techniques of measurement. The analysis of hybridization kinetics of cDNA to mRNA template tends to underestimate the complexity as compared to saturation hybridization of single copy DNA. This discrepancy has been considerably emphasized in the case of mouse brain poly(A)RNA. The data range from ~ 10 000 sequences [1,10] to 100 000 [12]. Causes for this discrepancy are not totally explained, however, by the difference in the technical approaches, since important variations also appear in the results reported by authors using the same technique. For instance, using cDNA, ~19 000 sequences were found [2] as compared to 11 600 [1]. In many cases, these variations may be explained, at least partly, by differences in the experimental protocols or (and) by the use of two different analytical methods (computer analysis or linear plot) for obtaining best fit to the data.

Independently of the technical approach, and its obvious impact on the quantitative conclusions, a higher sequence complexity has been claimed [2–5] for mouse brain than for other tissues. These authors generally interpret the high level of poly(A)RNA sequence complexity as reflecting the high heterogeneity of cell types constituting the brain.

The possibility has not been totally excluded, however, that a neuronal cell per se, perhaps because of its very large potential in establishing homotypic and heterotypic contacts, would express more genes than cells from any other tissues.

Here we have made a particular attempt to:

(i) Determine the level of messenger RNA complexity in mouse brain, by cDNA measurement, in following exactly the same experimental protocol and linear plot method for analysing the data, as we did for the neuroblastoma, a clone deriving from mouse nervous system;

(ii) Compare the level of complexity of whole brain to that of a major area, such as the cortex, in order to explore whether they exhibit differential modulations in the level of gene expression.

The present data indicates that the total complexity level of adult mouse brain tissue does not significantly differ from that of an homogeneous neuronal population such as mouse neuroblastoma, although exhibiting a somewhat higher value. This result renders unlikely that cells from a neuronal lineage display a higher register of gene expression during their ontogenic development than other somatic cells from the same individual. Interesting quantitative variations in the distribution of messenger sequences are observed when comparing cortex to the total brain tissue.

2. Materials and methods

2.1. *Animals*

Brains and cortex were obtained from week 7–8 male mice (BALB c). They were rinsed in Tris/NaCl/Mg buffer (10 mM Tris–HCl, 0.25 M NaCl, 10 mM $MgCl_2$) containing 250 μg/ml heparin and 500 μg/ml emetin (rinsing buffer), before storage at –80°C.

2.2. *Extraction of polysomal poly(A)-containing RNA*

The whole procedure was performed at 4°C. Brains (generally 100) were ground with mortar under liquid nitrogen. The powder was homogenised in a Dounce homogenizer with 2 vol. rinsing buffer containing 25 μg/ml spermine and 0.5% NP40. Nuclei were intact after homogenisation as checked by phase contrast microscopy. The homogenate was centrifuged 10 min at 12 000 × *g* and the supernatant spun on a double sucrose cushion (rinsing buffer) in the SW-27 rotor at 23 000 rev./min for 17 h. The pellets were resuspended in 50 mM Tris–HCl (pH 7.5)/100 mM NaCl/5 mM $MgCl_2$ (1.5 ml/tube) and the RNA was extracted by CsCl buoyant density centrifugation [6,7]. All solutions and vessels were sterilised by autoclaving. Plastic materials were rinsed with 50 mM NaOH.

2.3. *Preparation and titration of polysomal poly(A)-containing RNA*

Polysomal poly(A)-containing RNA was prepared as in [8]; all titrations were performed, using the same batch of poly([^{3}H]U) [8].

2.4. *cDNA synthesis*

Polysomal poly(A)-containing RNA from whole brain and cortex was copied into cDNA using avian myoblastosis virus reverse transcriptase. The reaction mixture (0.25 ml) contained: 40 mM Tris–HCl (pH 8) at 37°C, 100 mM KCl, 5 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM EDTA, 0.64 mM dATP, dGTP, dXTP (Calbiochem), 0.075 mM d[^{3}H]CTP (5200 cpm. $pmol^{-1}$) (Amersham), 200 μg/ml actinomycin D, 5.6 μg/ml oligo(dT_{12-18}), 24 μg/ml RNA poly(A) and 480 U/ml reverse transcriptase. This mixture was incubated for 15 min at 45°C and the reaction stopped by the addition of 0.45 ml H_2O, 0.2 ml 1 N NaCl/citrate, 0.05 ml 0.5 M EDTA and 0.05 ml 10% sodium dodecylsulphate. The cDNA was extracted and purified as in [8]. The specific activity of cDNA was about 4×10^6 cpm $.\mu g^{-1}$, for a total quantity of 0.5 μg.

3. Results

3.1. *Preparation and characterization of polysomal poly(A)-containing RNA*

Poly(A) RNA was prepared from a purified population of polysomes exhibiting a high polysomes/monosomes ratio. Only polysomes sedimenting faster than 100 S were used for RNA extraction. Poly(A) RNA was separated from non-poly(A) RNA by at least 2 passages on an oligo(dT)-cellulose column. The number average-size was determined by hybridization to poly([^{3}H]U) of poly(A)RNA from each fraction of a sucrose formamide gradient as in [8]. The calculated number average nucleotide length was taken as 1750 for both brain and cortex. The average-size of the poly(A) tract, obtained by centrifugation on sucrose/formamide gradients as in [8], was estimated to represent 10% of poly(A)-containing RNA.

3.2. *Polysomal poly(A)-containing RNA sequence complexity*

The conditions for RNA–DNA hybridization and detection of the hybrids were performed according to [9]. Here the RNA/DNA ratio ranged from 125–1000 for 0.6 μg cDNA/ml. The cDNA size was estimated as corresponding to ~400 nucleotides.

Knowing the hybridization kinetics of any RNA population, it is possible to determine its sequence complexity by comparison with the hybridization kinetics of a standard RNA of known complexity [10,11].

For that purpose, we performed homologous hybridization, in vast RNA excess, between poly(A)-containing RNA from whole brain and cortex and their corresponding cDNA. The hybridization kinetics are shown in fig.1 for whole brain and fig.2 for

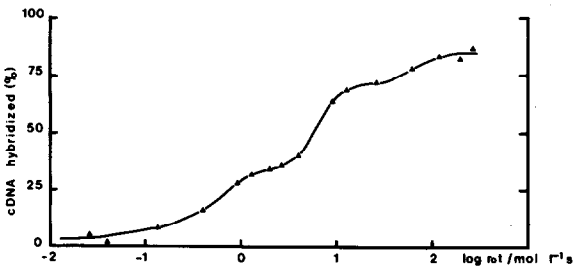

Fig.1. Homologous hybridization kinetics (whole brain). c[^{3}H]DNA from whole brain was hybridized with its poly(A)-containing RNA template. For r_0t values ≤ 0.5 mol $.l^{-1}$ .s, hybridization reaction contained 75 μg RNA/ml; for r_0t values of 0.5–15 mol $.l^{-1}$ .s, 300 μg RNA/ml; and for greater r_0t values, 650 μg RNA/ml. The extent of hybridization was determined by S_1 nuclease digestion. Each point was corrected for 0.8% background of S_1 nuclease-resistant cDNA (self-annealed fraction), obtained by digestion of cDNA after incubation with *E. coli* ribosomal RNA. r_0t is the product of RNA concentration (mol nucleotide $.l^{-1}$) and time (s).

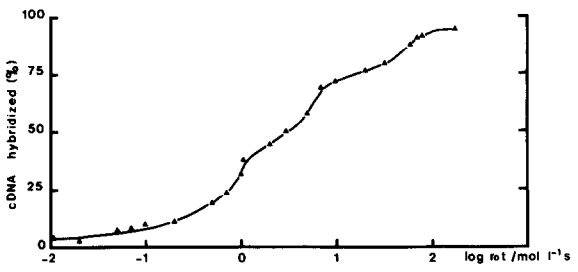

Fig.2. Homologous hybridization kinetics (cortex). c[^{3}H]-DNA from cortex was hybridized with its poly(A)-containing RNA template. For r_0t values $\leq$ 2 mol .l^{-1} .s, hybridization reaction contained 62.5 μg RNA/ml; for r_0t values of 1–70 mol .l^{-1} .s, 310 μg RNA/ml; for r_0t values > 65 mol .l^{-1} .s, 840 μg RNA/ml. The extent of hybridization was determined by S_1 nuclease digestion. Each point was corrected for 1.4% background of S_1 nuclease-resistant cDNA (self-annealed fraction), obtained by digestion of cDNA after incubation with *E. coli* ribosomal RNA. r_0t is the product of RNA concentration (mol nucleotide .l^{-1}) and time (s).

cortex. From the examination of these curves one can conclude that:

(i) By a r_0t value of 100 mol .l^{-1} .s, for brain and for cortex, the hybridization appears to be complete;

(ii) The kinetics of the hybridization reaction are heterogeneous, the curve extending over several r_0t decades.

This indicates that the hybridization reaction is a composite of several reactions each of which corresponds to an abundance class where all the different mRNA species are present in the same concentration. Table 1 for brain shows, after numerical analysis, the calculated RNA abundance classes, their $r_0t_{1/2}$ values, their base sequence complexity as determined with globin mRNA used as a complexity standard and the number of different average-size mRNAs. The analysis reveals that a good fit is obtained using RNA frequency distribution in 3 classes representing 26%, 49% and 25% of the poly(A)-containing RNA population with complexities of 1.47×10^5, 4.54×10^6 and 1.67×10^7, respectively, for the abundant, the intermediate and the rare class. The number of total different sequences is ~12 200 sequences assuming that the mRNA number average-size is 1750 nucleotides. The corresponding data for cortex are shown in table 2. The abundance classes represent 29%, 41% and 30% of the RNA population with complexities of 2.7×10^5, 2.45×10^6 and 1.86×10^7 nucleotides. The total complexity appears to be very similar to that of brain and correspond to about 12 200 sequences of the same number average-size.

Table 1
Numerical analysis of homologous hybridization kinetics: mouse brain

Class of abundance	Fraction of hybridizable cDNA (α)	$r_0t_{1/2}$		Base sequence	
		Obs.	Corr.	Complexity in nucleotides (*NT*)	No. different mRNA species
Abundant	0.26	0.31	0.08	1.47×10^5	84
Intermediate	0.49	5.1	2.5	4.54×10^6	2592
Rare	0.25	36.8	9.2	1.67×10^7	9562
Total	1.00				12 238

The analysis of brain hybridization curve was by the method in [13]. α represents the fraction of hybridizable cDNA reacting in each abundance class. $r_0t_{1/2}$ values are expressed in mol .l^{-1} .s. Corrected $r_0t_{1/2}$ is given by $r_0t_{1/2} \times \alpha$. The effect of this operation is to correct the observed $r_0t_{1/2}$ to the value it would have if the RNA of the considered abundance class were pure and comprised 100% of the total reaction. The sequence complexity of each abundance class, NT, is estimated as following:

$$NT = \frac{1200 \times (\text{corrected } r_0t_{1/2})}{6.6 \times 10^{-4}}$$

where 6.6×10^{-4} corresponds to the $r_0t_{1/2}$ of the hybridization reaction between α+β globin mRNA (sequence complexity = 1200 nucleotides) and its cDNA. The number of messenger RNA species is calculated considering an average size of 1750 nucleotides

Table 2
Numerical analysis of homologous hybridization kinetics: mouse cortex

Class of abundance	Fraction of hybridizable cDNA (α)	$r_0t_{1/2}$		Base sequence	
		Obs.	Corr.	Complexity in nucleotides (*NT*)	No. different mRNA species
Abundant	0.29	0.52	0.15	2.71×10^5	155
Intermediate	0.41	3.29	1.35	2.45×10^6	1400
Rare	0.30	34	10.2	1.86×10^7	10 618
Total	1.00				12 173

The analysis of cortex hybridization curve was performed as for brain hybridization kinetics (see table 1)

3.3. *Comparative distribution of whole brain and cortex poly(A)-containing RNA in frequency classes*

The experiments described thus far demonstrate that the polysomal poly(A)-containing RNA populations from both whole brain and cortex have essentially an identical complexity but a different distribution between the 3 abundance classes. Such a result can be confirmed by cross-hybridization at saturation between the cDNA transcribed from whole brain and poly(A)RNA from cortex. Figure 3 represents this heterologous hybridization reaction and, for comparison, the homologous kinetics for the cDNA participating in the cross-hybridization (solid line). It can be seen that:

(i) The cortex RNA is able to hybridize to whole brain cDNA with the same efficiency as the whole brain RNA, thus indicating that cortex RNA comprises all the sequences present in whole brain RNA;

(ii) The heterologous kinetics differs from the homologous one at a r_0t value characteristic of the intermediate abundance class.

A proportion of the intermediate class cDNA hybridizes at higher r_0t values with cortex RNA, thus showing that these mRNAs are rarer in cortex mRNA.

Then considering point (i), the best assumption is that some sequences relatively abundant in whole brain are less frequent in cortex. The rare class in cortex mRNA represents 87% of total complexity as compared to 78% for whole brain mRNA.

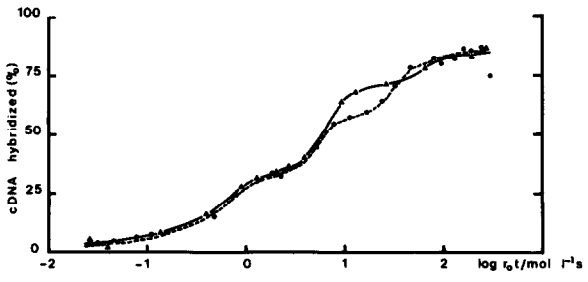

Fig.3. Heterologous hybridization kinetics. Whole brain cDNA was hybridized with cortex poly(A) RNA. For r_0t values ≤ 0.5 mol $.l^{-1}$ .s, hybridization reaction contained 64 μg RNA/ml; for r_0t values of 1–15 mol $.l^{-1}$ .s, 255 μg RNA/ml; and for greater r_0t values, 440 μg RNA/ml. The data are expressed as in fig.1, for both heterologous hybridization curve (▲——▲) and brain homologous hybridization curve (●- - -●) plotted for comparison.

4. Discussion

Here we have attempted to determine the polysomal poly(A)$^+$ mRNA sequence complexity of whole brain, using exactly the same technical procedure employed for the analysis of neuroblastoma cells. We were also interested in comparing the messenger sequence complexity of the cortex, a major area of the brain, to that of whole brain.

Our results show that mouse brain poly(A) mRNA population contains about 12 200 different sequences. According to [12] brain polyadenylated mRNA would comprise $> 10^5$ averaged-sized sequences. This very high level of complexity (1.4×10^5 kilobases) is partially explained by the technical procedure, i.e., the saturation of single copy DNA by an excess of RNA. This technique is able to detect sequences representing a very small percentage of poly(A)RNA which may not be detected by cDNA measurement. In contrast to this situation, the same values of mRNA

sequence complexity from both kinetic studies using cDNA and single copy DNA titrations were obtained for the avian liver [13], for yeast [14] and for an embryonal carcinoma cell line [15].

The discrepancy in brain mRNA sequence complexity obtained by single copy DNA titration and cDNA kinetic analysis may reflect the fact that much of the sequence diversity is contributed by many different cell types. This can be detected by single copy DNA titration because it is sensitive to diverse abundance classes representing a small mass of the mRNA. Such a class may go undetected as a reacting component in an RNA/cDNA hybridization. Single copy DNA titration can be considered as more adequately reflecting the tissue cellular heterogeneity than providing an actual index of sequence complexity for the major cellular component of the population under study.

The 12 200 brain sequences evidenced by our analysis are in agreement with the 11 650 sequences [1] and in contrast with the 19 000 sequences [2]. Our data favour the idea of a higher level of RNA brain complexity, when compared to the 7200 sequences that we found in a neuroblastoma cell line derived from the mouse sympathetic nervous system [8].

The cortex complexity appears identical to that of brain within the limits of sensitivity of the technique. However, the sequences are not identically distributed in the abundance classes since the cortex exhibits ~1000 more rare sequences and twice as many abundant sequences than whole brain. Conversely, there are less intermediate sequences in the cortex (1400 versus 2600). This situation requires further examination. First, every sequence evidenced in the cortex should be present in whole brain since cortex is contained in whole brain. On the other hand, some sequences present in brain might be absent from the cortex. Quantitative considerations make this hypothesis improbable since homologous hybridizations reveal that cortex comprises as many sequences as whole brain. Consequently, one should be dealing with quantitative transitions between the abundance classes of sequences common to both brain and cortex. This assumption is confirmed by the examination of the heterologous hybridization of brain cDNA to cortex poly(A) RNA which provides interesting information. Practically, all the cortex sequences are present in the whole brain polysomes. Within the limits of the technical sensitivity the heterologous and homologous kinetics are apparently identical at the level of low r_0t values. The difference in the number of abundant species evidenced by homologous hybridizations corresponds to only 0.58% of total complexity. Conversely the kinetics are significantly different at the level of medium r_0t values, reflecting the absence in the cortex intermediate frequency class of sequences representing 9.8% of total complexity. These sequences have transited to the rare frequency class since the heterologous kinetics is displaced to higher r_0t values.

Using single copy DNA titration, a similar degree of complexity in whole brain and different major brain areas was claimed [16]. The authors consider that their studies do not eliminate the possibility that significant fractions of total poly(A) RNA sequences are quantitatively region-specific since their single copy DNA titration does not provide any information concerning the transition of sequences between the frequency classes. We confirm this hypothesis when showing that whole brain and cortex exhibit an identical sequence complexity accompanied by a significant quantitative variation in the distribution of some common sequences. The transition of a significant fraction of cortex sequences to the rare frequency class may be related to the very high level of heterogeneity in the neuronal population composing this area. The analysis of the sequence frequency distribution in other major brain areas is under study.

Acknowledgements

The reverse transcriptase was a gift from Dr J. Beard. We thank Dr N. Affara for helpful discussion and Denise Baron for typing the manuscript.

References

[1] Hastie, N. D. and Bishop, J. O. (1976) Cell 9, 761–764.
[2] Ryffel, G. U. and McCarthy, B. J. (1975) Biochemistry 14, 1379–1389.
[3] Hahn, E. W. and Laird, C. D. (1971) Science 173, 158–161.

[4] Brown, I. R. and Church, R. B. (1972) Dev. Biol. 29, 73–84.
[5] Grouse, L., Chilton, M. D. and McCarthy, B. J. (1972) Biochemistry 11, 798–805.
[6] Affara, N. A. and Young, B. D. (1976) MSE Application Information Sheet A 12/6/76.
[7] Young, B. D., Birnie, G. D. and Paul, J. (1976) Biochemistry 15, 2823–2829.
[8] Felsani, A., Berthelot, F., Gros, F. and Croizat, B. (1978) Eur. J. Biochem. 92, 569–577.
[9] Affara, N. A., Jacquet, M., Jakob, F., Jacob, F. and Gros, F. (1977) Cell 12, 500–520.
[10] Bishop, J. O., Morton, J. G., Rosbach, M. and Richardson, M. (1974) Nature 250, 199–204.
[11] Birnie, G. D., MacPhail, E., Young, B. D., Getz, M. J. and Paul, J. (1974) Cell Diff. 3, 221–232.
[12] Bantle, J. A. and Hahn, W. E. (1976) Cell 8, 139–150.
[13] Axel, R., Feigelson, P. and Schutz, G. (1976) Cell 7, 247–254.
[14] Hereford, L. M. and Rosbach, M. (1977) Cell 10, 453–462.
[15] Jacquet, M., Affara, N. A., Benoit, R., Jakob, H., Jacob, F. and Gros, F. (1978) Biochemistry 17, 69–79.
[16] Kaplan, B. B., Schachter, B. S., Osterburg, M. M., de Vellis, J. S. and Finch, C. E. (1978) Biochemistry 17, 5516–5523.