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Brain Metabolic DNA Is Reverse Transcribed in Cytoplasm: Evidence by Immunofluorescence Analysis

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Abstract

In a previous study (Mol Neurobiol 55:7476–7486, 2017), newly synthesized brain metabolic DNA (BMD) from rat subcellular fractions has been shown to behave as a DNA-RNA hybrid when analyzed in cesium gradients at early [^{3}H] thymidine incorporation times but to assume the double-stranded configuration at later times. Conversely, BMD from purified nuclei displayed the dsDNA configuration even at early incorporation times. The results were interpreted to support the BMD origin by reverse transcription in the cytoplasm and its later acquisition of the double-stranded configuration before the partial transfer to the nuclei. This interpretation has now been confirmed by immunofluorescence analyses of newly synthesized BrdU-labeled BMD from the mouse brain that demonstrates its cytoplasmic localization and colocalization with DNA-RNA hybrids. In addition, BrdU-labeled BMD has been shown to colocalize with astroglial anti-GFAP antibodies and with presynaptic anti-synaptophysin antibodies.

Keywords Brain metabolic DNA (BMD) · DNA synthesis · Reverse transcription · Astroglia · Synaptosomes

Introduction

At variance with the widely held opinion that DNA synthesis only occurs before cell proliferation and during DNA repair, the DNA of the adult rodent brain has been shown to be continuously synthesized [1] and to be modulated by learning [2–8], post-trial sleep [9, 10], and circadian oscillations [11–13; for a review, see 14]. The lack of involvement of this DNA fraction in cell division and DNA repair and its dependence on brain activity suggested its similarity with the DNA synthesized in a wide range of non-neural cells likewise undergoing enhance cell activity [15–18; for reviews, 19–21]. Since that DNA was referred to as metabolic DNA, the brain DNA fraction sharing comparable properties was called brain metabolic DNA (BMD).

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Data obtained in the seventies but only recently recovered [22] have shown that newly synthesized BMD from adult rats is present in all subcellular fractions including the nuclear, mitochondrial and microsomal fractions, and also in purified nuclei. In addition, cesium density analyses demonstrated that newly synthesized BMD present in subcellular fractions was distributed over the entire gradient when examined after short incorporation times (30 min) but it progressively acquired the expected dsDNA localization after longer incorporation times (1–2 h). Conversely, newly synthesized BMD from highly purified nuclei behaved as dsDNA even after shorter incorporation periods. The results were interpreted to indicate that BMD was initially synthesized as DNA-RNA hybrid by cytoplasmic reverse transcription, and that it eventually acquired the dsDNA configuration before being partially transferred to nuclei [23]. The interpretation was in full agreement with in vitro data indicating that in learning rats, BMD was synthesized by an RNA-dependent DNA polymerase [24].

We are now presenting immunofluorescence data that confirm the exclusive cytoplasmic localization of newly synthesized BMD and demonstrate its colocalization with DNA-RNA hybrids. Additional immunofluorescence data also show that newly synthesized BrdU-labeled BMD colocalizes with astroglial GFAP and with presynaptic synaptophysin. A preliminary note of these results has appeared [25].

Materials and Methods

Rat

Experiments were initially made with adult male rats of the Wistar strain. Following their CO_2 anesthesia, the animals were decapitated with a guillotine, and the dissected brains kept over ice were cleaned of major vessels before being immersed in cold isotonic medium (0.32 M sucrose, 10-mM Tris-Cl pH 7.4; homogenizing medium or HM). Slices of 300-μm thickness prepared from the cerebral hemispheres using a tissue slicer equipped with a vibrating blade were placed in Petri dishes containing 2 ml HM (2 slices per Petri dish) and 5 μM bromodeoxyuridine (BrdU; Merck). Following incubation at 30° for 2 h with occasional shaking, the slices were washed with HM before being homogenized with 2 ml HM.

Mouse

Preparation of BrdU-Labeled BMD and Sub-Cellular Fractionation of the Cerebral Hemispheres

A male 2.5-month-old CD1 mouse was subcutaneously injected with 150 μl 5 mM BrdU, 0.9% NaCl at about 9.30 a.m. and was kept awake by mild stimulation during the following 2 h. The mouse was then dislocated and decapitated with a guillotine. The brain was cleared of large vessels at ice temperature, and cerebral hemispheres were homogenized in a Dounce homogenizer with 15-ml cold HM. One ml aliquots of the homogenate were centrifuged in an Eppendorf table centrifuge to sediment the nuclear fraction (4°, 1000 g, 2 min) and the resulting supernatant fractions were centrifuged at higher speed to sediment the mitochondrial cytoplasmic fraction (4°, 10,000 g, 20 min). The sediments resuspended in 1-ml HM aliquots and the 1-ml aliquots of the homogenate were incubated with equal volumes of HM containing paraformaldehyde 8% (room temperature, 20 min), and the resulting fixed material was collected by centrifugation (5000 g, 2 min), washed three times with 2 ml HM, and resuspended in 1 ml HM. Samples were stored at 4°.

Immunofluorescence

Anti-BrdU Immunofluorescence

Thirty μl aliquots of each sample (rat and mouse) were spotted on Superfrost slides and dried (2 h, 37°). After fixation in 4% paraformaldehyde, 0.1-M phosphate buffer pH 7.4 (PB; 15 min, room temperature), permeabilization with 0.5% Triton X-100 in PB (10 min) and blocking with 5% bovine serum albumin (BSA) in PB (30 min), slides were incubated (1 h, room temperature) with mouse anti-BrdU antibody Alexa Fluor 488 conjugate (Merck Millipore) diluted 1:5 with 1% BSA in PB. Negative controls were prepared by omitting the anti-BrdU antibody. Slides were washed in PB, incubated (10 min) with 5-μg/ml Hoechst 33258 (Sigma-Aldrich), washed again, and mounted using the Aquovitrex Erba (Carlo Erba Reagents).

Fluorescence Colocalization Analysis

Immunofluorescence analyses were also made to examine the colocalization of anti-BrdU antibody with anti-DNA-RNA hybrids (anti-DR hybrids), anti-glial fibrillary acidic protein (anti-GFAP), and anti-synaptophysin antibodies in mouse samples. The slides prepared as described above were fixed in 4% paraformaldehyde in PB (15 min, room temperature), permeabilized with 0.5% Triton in PB (10 min), and blocked with 0.1% Triton, 5% BSA in PB (30 min). They were then incubated overnight at 4° C with the following primary antibodies diluted in 1% BSA in PB: mouse monoclonal anti-DR hybrids (Kerafast) 1:100; rabbit anti-GFAP (Abcam) 1:500; or rabbit anti-synaptophysin (Abcam) 1:50. The following day, they were treated with the secondary anti-mouse or anti-rabbit Alexa Fluor 594-conjugate (Abcam) antibodies (1:200 in PB) (1 h, room temperature), then with the anti-BrdU antibody Alexa Fluor 488-conjugate (Merck Millipore) 1:5 in 1% BSA in PB (1 h, room temperature), and finally with 5-μg/ml Hoechst 33258 (10 min). Negative controls were prepared by omitting primary antibodies. The slides were washed and mounted using the Aquovitrex Erba (Carlo Erba Reagents).

Fluorescent signals were analyzed with the Zeiss Axioskop microscope and images acquired with an AxioCam MRc5 camera and analyzed with AxioVision 4.7 software of the Axioskop System (Zeiss, Germany).

Dot-Blot

The mouse nuclear fraction, the mitochondrial cytoplasmic fraction, and the fraction obtained by resuspending the nuclear fraction in HM containing 0.5% Triton X-100 and centrifuging it (4°, 2000 g, 2 min) to discard nuclei were used to purify their corresponding DNA and RNA with the TRI Reagent (Sigma-Aldrich Merck) according to the manufacturer's protocol. Two μl aliquots of the purified samples were spotted on a nylon membrane that was exposed to UV light (10 min) to bind the nucleic acids. Non-specific binding sites were blocked by incubating the membrane with the Blocking Reagent (1 h; Sigma-Aldrich Merck). The membrane was then incubated with the mouse anti-DR hybrids monoclonal antibody (Kerafast) 1:1000 in the Blocking Reagent (1 h, room

temperature), and subsequently with the biotinylated secondary anti-mouse antibody (Abcam). The reaction outcome was determined by exposing samples to the highly sensitive Avidin-Biotin Complex (Thermo Scientific; 1 h, room temperature) and 0.5% 3,3′-diaminobenzidine (DAB) chromogen.

Results

Experiments with Rats

The first experiments were made by incubating slices of rat cerebral hemispheres with 5 μM BrdU (2 h, 30°; see "Methods") and monitoring the localization of BrdU-labeled BMD in the homogenate by the green fluorescent anti-BrdU antibody. As shown in Fig. 1, fluorescence was present in isolated and aggregated small particles (less than 2-μm size) that could only be derived from cytoplasm. This result extended to the rat brain the procedure that we had previously adopted to demonstrate the presence of BrdU-labeled BMD in squid optic lobe synaptosomes [26]. Nonetheless, in view of further investigations concerning BMD synthesized during a learning session, the in vitro method did not appear suitable. Indeed, BMD synthesis strictly depends on cell activity [14], and cell activity cannot be reproduced in vitro, notably during a training session lasting few hours (6). Analyzing BMD from brain slices prepared after training and comparing it with BMD from control slices could not provide meaningful information. Accordingly, additional experiments concerned mice receiving BrdU by subcutaneous injections (see "Methods").

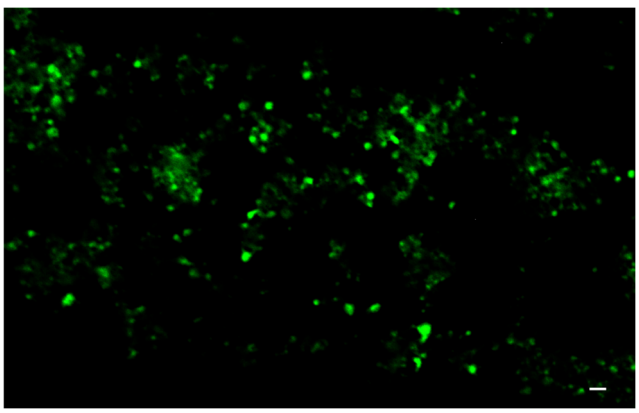

Fig. 1 BrdU-labeled BMD in the homogenate of rat brain slices incubated with 5-μM BrdU (2 h, 30°). The green fluorescent anti-BrdU antibody is present in isolated and aggregated particles of less than 2-μm size. Bar = 2 μm

Experiments with Mice

Subcellular Localization of Newly Synthesized BrdU-Labeled BMD

After the BrdU incorporation in the mouse brain for 2 hours, the homogenate of cerebral hemispheres displayed a population of subcellular particles of different shapes and sizes (down to 1–2 μm) that were often clustered in large aggregates and exhibited the green fluorescence of the anti-BrdU antibody (Fig. 2). The particles lacked the blue fluorescence of Hoechst 33258 that only identified several round-shaped nuclei. It was also noted that a few blue fluorescent nuclei also displayed the partial colocalization of the green fluorescent anti-BrdU antibody (white arrows). This observation is in agreement with the partial BMD transfer to nuclei after its acquisition of the double-stranded configuration [23]. Comparable results were observed in the nuclear fraction (not shown).

Green fluorescent particles of small size lacking the Hoechst 33258 blue fluorescence were present in the mitochondrial cytoplasmic fraction (Fig. 3). They confirmed the cytoplasmic localization of BrdU-labeled BMD also indicated by few nuclei displaying the Hoechst 33258 blue fluorescence but lacking the green fluorescence of the anti BrdU-labeled antibody.

The Colocalization of BrdU-Labeled BMD with Anti-DR Hybrids

The cytoplasmic localization of newly synthesized BrdU-labeled BMD prompted its further examination in the cortex homogenate in colocalization experiments with red fluorescent anti-DR hybrids antibody (see "Methods"). As shown in Fig. 4, several particles of a few μm sizes exhibited the colocalization of the two antibodies (arrows). In addition, a few particles only showed the green fluorescence and still fewer particles only showed the red fluorescence. The results clearly demonstrated the frequent association of BrdU-labeled BMD with DR hybrids while the less frequent occurrence of particles only exhibiting the green fluorescence was interpreted to indicate the BMD acquisition of the double-stranded configuration. Conversely, the still less frequent occurrence of red fluorescent particles was attributed to BMD hybrids present before the BrdU incorporation.

Further evidence supporting the presence of DR hybrids in brain cytoplasm was obtained by dot-blot analyses of DNA and RNA samples purified from nuclear and cytoplasmic (mitochondrial) fractions and from the Triton X-100 extract of the nuclear fraction (see "Methods"). As shown in Fig. 5, DR hybrids were conspicuously present in the nuclear and cytoplasmic fractions and, less conspicuously, in the Triton X-100 extract of the nuclear fraction. The extract contained

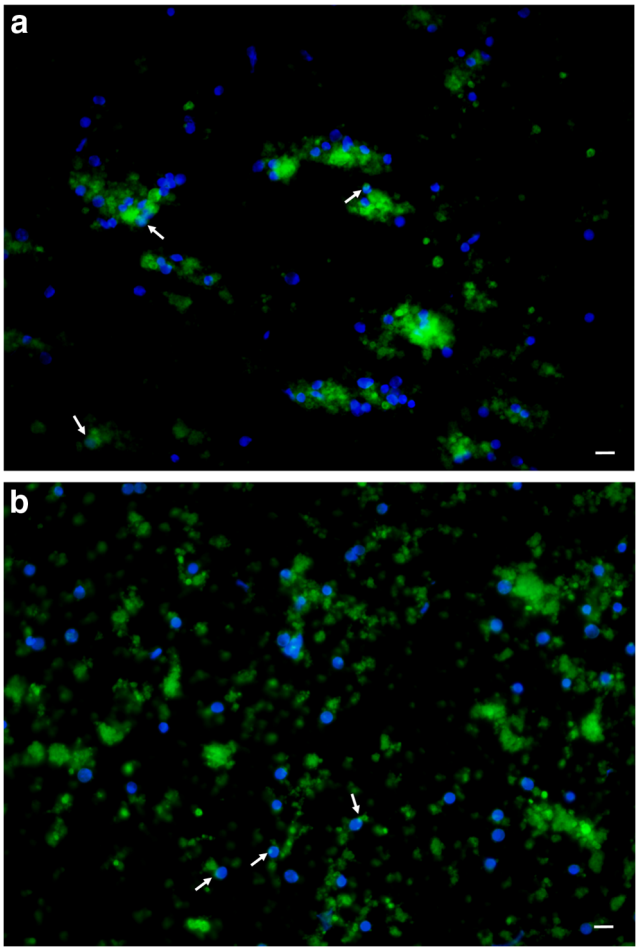

Fig. 2 BrdU-labeled BMD in the homogenate of mouse cerebral hemispheres after 2 h BrdU incorporation. **a**, **b** panels show different fields of the same homogenate. The green fluorescent anti-BrdU antibody is almost exclusively localized in isolated and clustered bodies of variable size that are clearly distinguished from the isolated round nuclei stained by Hoechst 33258 and displaying the blue fluorescence of dsDNA. The few nuclei exhibiting the partial colocalization of BrdU-labeled BMD (arrows) confirm the nuclear transfer of some cytoplasmic BMDs that have acquired the dsDNA configuration [23]. Bars = 2 μm

the cytoplasmic contaminants of the nuclear fraction, including mitochondria and synaptosomes. Nuclear DR hybrids clearly reflected the effects of nuclear DNA transcription, while cytoplasmic DR hybrids confirmed the presence of reverse transcribed cytoplasmic BMD.

Additional immunochemical analyses were made to examine the colocalization of BrdU-labeled BMD with astroglial GFAP and, in separate experiments, with presynaptic synaptophysin.

Colocalization of Newly Synthesized BrdU-Labeled BMD with the Anti-GFAP Antibody

Early autoradiographic analyses have demonstrated the presence of [^{3}H] thymidine-labeled BMD in neurons of learning

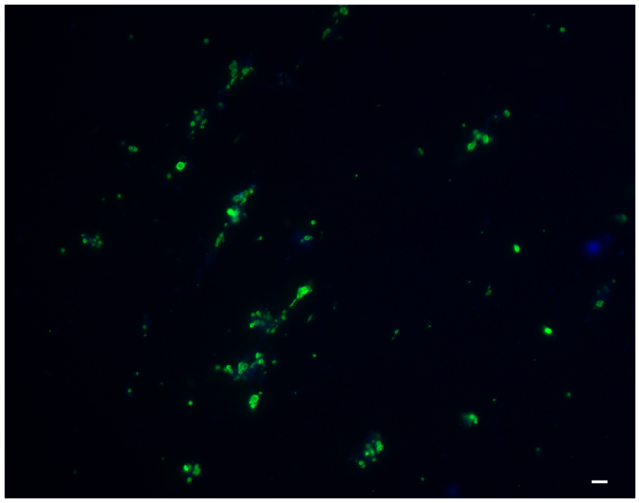

Fig. 3 BrdU-labeled BMD in the mitochondrial fraction of mouse cerebral hemispheres after 2 h BrdU incorporation. The green fluorescent anti-BrdU antibody is exclusively localized in isolated small particles. Bar = 5 μm

and control mice and its concurrent prevalence in glial cells [2]. We have further examined this result by investigating the colocalization of BrdU-labeled BMD with the glial fibrillary acidic protein (GFAP) that is a specific marker of astroglial processes. The data presented in Fig. 6 show that the green fluorescent anti-BrdU antibody colocalizes with the red fluorescent anti-GFAP antibody in most cytoplasmic particles present in the homogenate of mouse cerebral hemispheres analyzed after a BrdU incorporation lasting 2 h. The

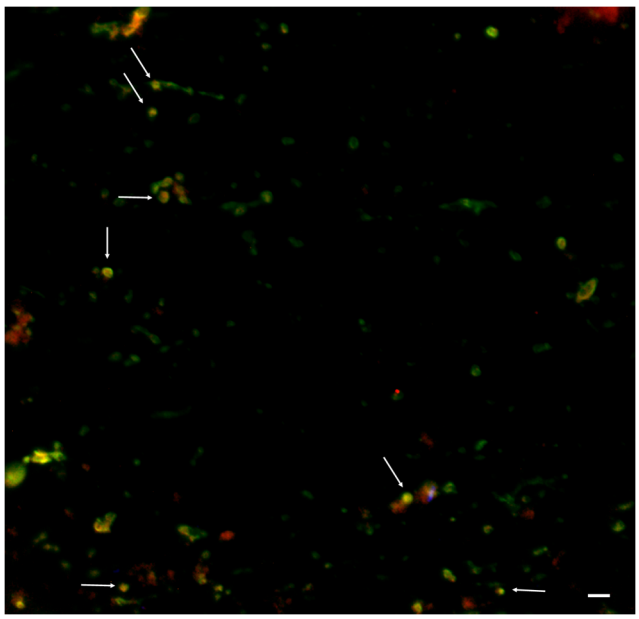

Fig. 4 Colocalization of anti-BrdU antibody Alexa Fluor 488 (green) with anti-DR-hybrids antibody Alexa Fluor 594 (red) in the homogenate of mouse cerebral hemispheres after 2 h BrdU incorporation. The fluorescence superposition is present in isolated and aggregated round particles (arrows). Bar = 5 μm

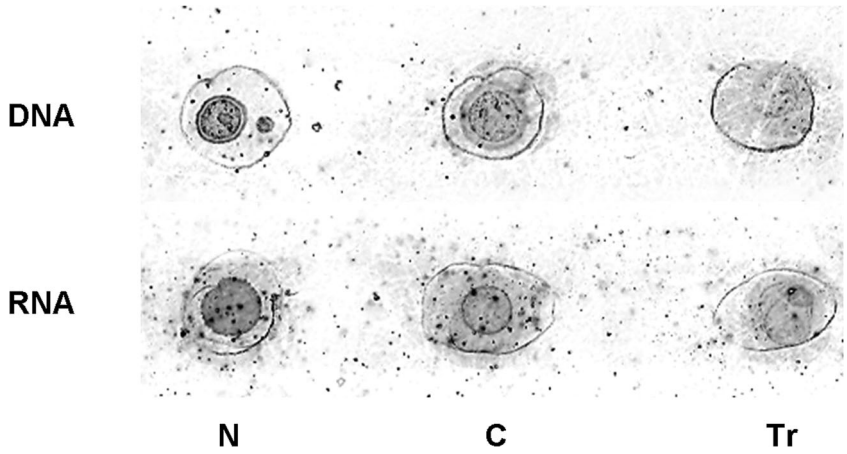

Fig. 5 Dot blot of DNA and RNA samples purified from the nuclear and cytoplasmic fraction of mouse cerebral hemispheres (respectively N and C) and from the Triton X-100 extract of the nuclear fraction (Tr). Circles outline individual samples which were treated with the anti-DR-hybrid antibody and visualized by peroxidase (see "Methods")

colocalization of the two antibodies was more frequent in the aggregates of small particles (thick arrow tails) than that in isolated particles (thin arrow tails).

Colocalization of Newly Synthesized BrdU-Labeled BMD with the Anti-Synaptophysin Antibody

Recent experiments with squid optic lobe slices incubated 2 h with BrdU have shown that BrdU-labeled DNA is localized in the large presynaptic synaptosomes derived from the nerve terminals of retinal photoreceptor neurons [26]. The results suggested that newly synthesized BMD could also be present in presynaptic mouse brain synaptosomes. Accordingly, immunofluorescence analyses were made to investigate the colocalization of BrdU-labeled BMD with synaptophysin, a specific marker of brain presynaptic synaptosomes. As shown in Fig. 7, the green fluorescent anti-BrdU antibody colocalized with the red fluorescent anti-synaptophysin antibody in a fraction of the particles present in the mouse brain homogenate prepared after a BrdU incorporation lasting 2 h. A larger number of particles only exhibited the green fluorescence of BrdU-labeled BMD or only displayed the red fluorescence of the anti-synaptophysin antibody indicating presynaptic synaptosomes.

Discussion

The present data demonstrate that newly synthesized BrdU-labeled BMD from the rat and mouse brain identified by the green fluorescent anti-BrdU antibody is present in isolated and aggregated particles whose small size suggests the cytoplasmic localization (Figs. 1, and 2). Additional supporting evidence is provided by green fluorescent smaller particles present in the mitochondrial fraction of the mouse brain (Fig. 3). The complete absence of green fluorescence in the large

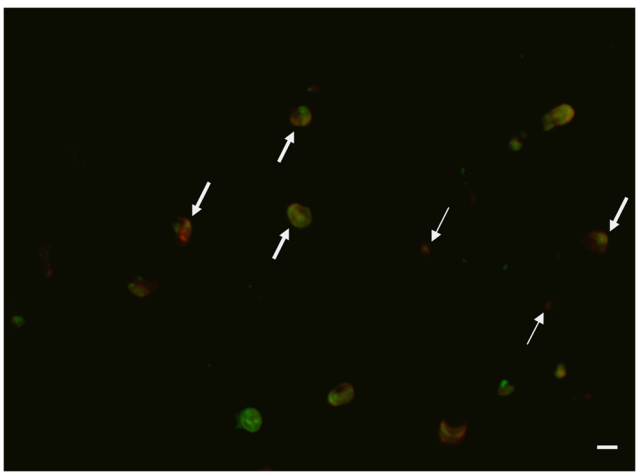

Fig. 6 Colocalization of the anti-BrdU antibody Alexa Fluor 488 (green) with the anti-GFAP antibody Alexa Fluor 594 (red) in mouse cerebral hemispheres after 2 h BrdU incorporation. The fluorescence superposition is present in isolated and aggregated round particles (arrows). Bar = 5 μm

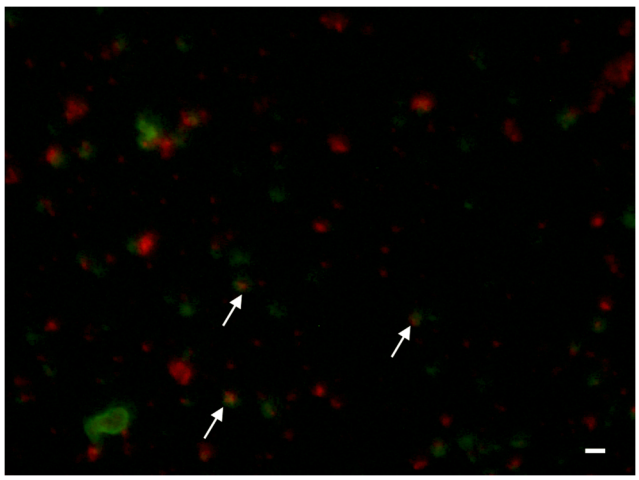

Fig. 7 Colocalization of the anti-BrdU Alexa Fluor 488 (green) with the anti-synaptophysin Alexa Fluor 594 (red) in mouse cerebral hemispheres after 2 h BrdU incorporation. The superposition of the green and red fluorescence is present in isolated and aggregated round particles (arrows). Bar = 5 μm

majority of nuclei identified by blue fluorescent Hoechst 33258 strongly confirms this conclusion in the mouse brain (Fig. 2). The very few nuclei displaying some green fluorescence on a restricted surface area reflect the transfer of a fraction of cytoplasmic BMD to nuclei, thus fully confirming the results of our previous cesium gradient analyses [22, 23]. Briefly, those data have demonstrated that after short incorporation times (30 min), newly synthesized BMD from all cytoplasmic fractions exhibits a DR hybrid behavior in cesium gradients but displays a dsDNA behavior in two classes of purified nuclei. The latter configuration became prevalent in cytoplasmic fractions only after longer incorporation times (1–2 h). The data were interpreted to indicate that newly synthesized BMD is reverse transcribed in the cytoplasm, and that a fraction of it attains the double-stranded configuration and is transferred to nuclei. The alternative view that BMD is synthesized in nuclei as dsDNA, that most of it is transferred to the cytoplasm after acquiring the DR hybrid configuration, and that it eventually reverts to the dsDNA configuration is not supported by data and may hardly be conceived.

It is also worth noting that in learning rats, BMD synthesis was shown to be catalyzed by an RNA-dependent DNA polymerase, and not by a DNA-dependent DNA polymerase. The activities of these two enzymes were determined in the hippocampus soluble fraction [24], that is most likely of cytoplasmic derivation. Since RNA is the required template, and RNA is largely localized in the cytoplasm, the more likely site of BMD retro transcription is the cytoplasm, as additionally suggested by the significant modifications of cytoplasmic RNA elicited by cell activity. It may thus be concluded that the latter in vitro data [24] have been confirmed by our previous cesium data [22, 23] and by the present immunofluorescent analyses that have furthermore extended them to in vivo conditions, thereby significantly modifying our views of the BMD role.

Investigations of the expected colocalization of BrdU-labeled BMD with cytoplasmic DR hybrids have strongly confirmed this interpretation. Following the concomitant use of the green fluorescent anti-BrdU antibody and the red fluorescent anti-DR hybrid antibody, their colocalization was frequently present in cytoplasmic particles of mouse homogenate (Fig. 4). Additional support was provided by dot-blot analyses of DNA and RNA samples purified from the nuclear and cytoplasmic fractions of the mouse brain, and from the Triton X-100 extract of the nuclear fraction (Fig. 5). Treatment of the samples with anti-DR hybrid antibody and its required visualization yielded further evidence of the presence of DR hybrids in brain cytoplasm and in cytoplasmic contaminants of the nuclear fraction.

Additional data were obtained by determining the presumed colocalization of newly synthesized BrdU-labeled BMD with the GFAP and the presynaptic synaptophysin that were respectively identified by the red fluorescent anti-GFAP antibody and the anti-synaptophysin antibody. The former study was prompted by earlier data indicating the prevalence of newly synthesized BMD in glial cells [2], while the second experiment was suggested by the recent identification of newly synthesized BMD in squid presynaptic synaptosomes [26, 27].

As shown in Fig. 6, the anti-GFAP antibody colocalized with newly synthesized BMD in small isolated particles and more frequently in aggregates of comparable particles derived from astroglial processes. The data confirmed previous autoradiographic analyses [2] and further demonstrated that BMD prevalence in glial cells largely concerned astroglial processes known to be involved in synaptic activity. The latter feature raised the problem of the role played by the frequent occurrence of astroglial BMD with respect to the less frequent identification of presynaptic BMD (compare Figs. 6 and 7). The difference might be explained by keeping in mind that cytoplasmic BMD is partly delivered to nuclei soon after its synthesis [22, 23] presumably to allow a quick transcription. Accordingly, the BMD presence in astroglial processes might suggest its local synthesis and subsequent transfer to astroglial nuclei. Nonetheless, the presence of BMD in squid presynaptic synaptosomes [26, 27] and the colocalization with mouse presynaptic synaptophysin may suggest a different interpretation that is based on the much longer distance separating presynaptic regions from their cognate nuclei with respect to the much shorter distance separating astroglial processes from astroglial nuclei. On the plausible assumption that the BMD transfer to nucleus complies with a quick transcription, it appears reasonable to assume that presynaptic BMD could be transferred to astroglial processes and from them to the closer astroglial nuclei from which the resulting BMD transcripts could be more quickly delivered to the presynaptic regions by a reversed journey. This intriguing possibility would be in line with our previous demonstration that, in the squid, newly synthesized axonal and synaptic RNA derived from nearby glial cells [28, 29]. The demonstration that BMD synthesis is absent in astroglial processes would strengthen this hypothesis.

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References

1. Perrone Capano C, D'Onofrio G, Giuditta A (1982) DNA turnover in rat cerebral cortex. J Neurochem 38:52–56
2. Reinis S (1972) Autoradiographic study of 3H-thymidine incorporation into brain DNA during learning. Physiol Chem Phys 4:391–397
3. Reinis S, Lamble RW (1972) Labeling of brain DNA by ^{3}H-thymidine during learning. Physiol Chem Phys 4:335–338

4. Ashapkin VV, Romanov GA, Tushmalova NA, Vanyushin BF (1983) Selective DNA synthesis in the rat brain induced by learning. Biokhimija 48:355–362
5. Scaroni R, Ambrosini MV, Principato GB, Federici F, Ambrosi G, Giuditta A (1983) Synthesis of brain DNA during acquisition of an active avoidance task. Physiol Behav 30:577–582
6. Papa M, Pellicano MP, Cerbone A, Lamberti-D'Mello C, Menna T, Buono C, Giuditta A, Welzl H et al (1995) Immediate early genes and brain DNA remodeling in the naples high and low-excitability rat lines following exposure to a spatial novelty. Brain Res Bull 37: 111–118
7. Giuditta A et al. (1986) Synthesis of rat brain DNA during acquisition of an appetitive task. Pharmacol Biochem Behav25:651–658
8. Ivashkina OI, Zots MA, Bezriadnov DV, Anokhin KV (2012) Increased 5′-bromo-2′-deoxyuridine incorporation in various brain structures following passive avoidance training in mice. Bull Exp Biol Med 154:171–173
9. Giuditta A, Ambrosini MV, Scaroni R, Chiurulla C, Sadile A (1985) Effect of sleep on cerebral DNA synthesized during shuttle-box avoidance training. Physiol Behav 34:769–778
10. Langella M, Colarieti L, Ambrosini MV, Giuditta A (1992) The sequential hypothesis of sleep function. IV. A correlative analysis of sleep variables in learning and non learning rats. Physiol Behav 51:227–238
11. Grassi Zucconi G, Menichini E, Castigli E, Belia S, Giuditta A (1988a) Circadian oscillations of DNA synthesis in rat brain. Brain Res 447:253–261
12. Grassi Zucconi G, Carandente ME, Belia S, Giuditta A (1988b) Circadian rhythms of DNA content in brain and kidney: effects of environmental stimulation. Chronobiol 15:195–204
13. Grassi Zucconi G, Crognale MC, Bassetti MA, Giuditta (1990) Environmental stimuli modulate the circadian rhythm of (3H-methyl) thymidine incorporation into brain a DNA of male rats. Behav Brain Res 41:103–110
14. Giuditta A, Grassi-Zucconi G, Sadile A (2017) Brain metabolic DNA in memory processing and genome turnover. Rev Neurosci 28:21–30
15. Pelc SR (1964) Labelling of DNA and cell division in so called non-dividing tissues. J Cell Biol 22:21–28
16. Pelc SR (I968a) Turnover of DNA and function. Nature 219:162–l63
17. Pelc SR (1968b) Biological implications of DNA-turnover in higher organisms. Acta Histochem Suppl 8:441–452
18. Pelc SR (1972) Metabolic DNA in ciliated protozoa, salivary gland chromosomes, and mammalian cells. Int Rev Cytol 32:327–355
19. Roels H (1966) Metabolic' DNA: a cytochemical study. Int Rev Cytol 19:1–34
20. Stroun M, Charles P, Anker P, Pelc SR (1967) Metabolic DNA in heart and skeletal muscle and in the intestine of mice. Nature 216: 716–717
21. Gahan PB, Anker P, Stroun M (2008) Metabolic DNA as the origin of spontaneously released DNA? Ann N Y Acad Sci 1137:7–17
22. Rutigliano B, Giuditta A (2015) The unexpected recovery of misplaced data on brain metabolic DNA. Rend Acc Sci Fis Mat LXXXII:99–106
23. Giuditta A, Rutigliano B (2017) Brain metabolic DNA in rat cytoplasm. Mol Neurobiol 55:7476–7486. https://doi.org/10.1007/s12035-018-0932-0
24. Salganik RI, Parvez H, Tomson VP, Shumskaya IA (1983) Probable role of reverse transcription in learning: correlation between hippocampal RNA-dependent DNA synthesis and learning ability in rats. Neurosci Lett 36:317–322
25. Prisco M, Casalino J, Cefaliello C, Giuditta A(2015) DNA synthesis in mouse brain cytoplasm. Rend Acc Sc fis mat Napoli LXXXII 149-152
26. Cefaliello C, Prisco M, Crispino M, Giuditta A (2018) DNA in squid synaptosomes. Mol Neurobiol 56:56–60. https://doi.org/10.1007/s12035-018-1071-3
27. Cefaliello C, Prisco M, Crispino M, Giuditta A (2015) Newly synthesized DNA in squid nerve terminals. Rend Acc Sci fis mat LXXXII 61-64
28. Eyman M, Cefaliello C, Ferrara E, de Stefano R, Lavina ZS, Crispino M, Squillace A, van Minnen J et al (2007) Local synthesis of axonal and presynaptic RNA in squid model systems. Eur J Neurosci 25:341–350
29. Crispino M, Chun JT, Cefaliello C, Perrone Capano C, Giuditta A (2014) Local gene expression in nerve endings. Dev Neurobiol 74: 279–291