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Numerical Chromosome Variation and Mitotic Segregation Defects in the Adult Brain of Teleost Fish

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ABSTRACT: **Teleost fish are distinguished by their enormous potential for the generation of new cells in both the intact and the injured adult brain. Here, we present evidence that these cells are a genetic mosaic caused by somatic genomic alteration. Metaphase chromosome spreads from whole brains of the teleost *Apteronotus leptorhynchus* revealed an euploid complement of 22 chromosomes in only 22% of the cells examined. The rate of aneuploidy is substantially higher in brain cells than in liver cells, as shown by both metaphase chromosome spreads and flow cytometric analysis. Among the aneuploid cells in the brain, approximately 84% had fewer, and the remaining 16% more, than 22 chromosomes. Typically, multiple chromosomes were lost or gained. The aneuploidy is putatively caused by segregation defects during mitotic division. Labeling of condensed chromosomes of M-phase cells by phosphorylated histone-H3 revealed laggards, anaphase bridges, and micronuclei, all three of which indicate displaced mitotic chromosomes. Quantitative analysis has shown that in the entire brain on average 14% of all phosphorylated histone-H3-labeled cells exhibit such signs of segregation defects. Together with the recent discovery of aneuploidy in the adult mammalian brain, the results of the present investigation suggest that the loss or gain of chromosomes might provide a mechanism to regulate gene expression during development of new cells in the adult vertebrate brain.** © 2007 Wiley Periodicals, Inc. Develop Neurobiol 67: 1334–1347, 2007

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INTRODUCTION

Adult neurogenesis, the generation and subsequent development of neurons during adulthood, is a widespread, and probably even universal, phenomenon of vertebrate brains (for review, see Kempermann, 2005). In the adult mammalian brain, constitutive neurogenesis occurs in two areas (for reviews, see Doetsch and Scharff, 2001; Temple, 2001; Gage, 2002; Rakic, 2002; Taupin and Gage, 2002): the anterior part of the subventricular zone of the lateral ventricle, from where the immature neurons migrate via the so-called rostral migratory stream into the olfactory bulb (Altman, 1969; Luskin, 1993; Lois and Alvarez-Buylla, 1994; Lois et al., 1996; Pencea et al., 2001), and the subgranular zone of the dentate gyrus, from where the new cells migrate a short distance into the granule cell layer of the hippocampus

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(Altman and Das, 1965; Kaplan and Bell, 1984; Eriksson et al., 1998; Gould et al., 1999; Kornack and Rakic, 1999; Seri et al., 2001).

Like in the adult mammalian brain, neurogenesis occurs in the adult brain of teleost fish. However, in contrast to mammals, dozens of neurogenic areas, including the olfactory bulb and a region homologous to the mammalian hippocampus, have been identified in the teleostean brain (Kranz and Richter, 1970a,b; Richter and Kranz, 1970a,b; Zupanc and Horschke, 1995; Zikopoulos et al., 2000; Ekström et al., 2001; Zupanc et al., 2005). Moreover, the rate of cell proliferation is at least one, if not two, orders of magnitude higher in the adult teleost fish brain than in the adult mammalian brain (Zupanc and Zupanc, 2006).

Although the sites of cell proliferation, the patterns of migration and differentiation, and the fate of the new cells are well characterized, little is known about the cellular mechanisms that regulate the development of these cells in the adult brain. However, elucidation of these mechanisms is highly desirable, as their cellular identification and the understanding of their developmental function might enable investigators to increase the rate of neurogenesis in the adult mammalian brain in response to injury or neurodegenerative disease. Such a replacement therapy could lead to a structural regeneration of whole neurons, combined with a regain of function—similar as seen in teleost fish after application of lesions to the spinal cord (Anderson et al., 1983, 1987), the retina (Hitchcock et al., 1992; Cameron, 2000), and the cerebellum (Zupanc et al., 1998; Zupanc and Ott, 1999) (for reviews, see Waxman and Anderson, 1986; Zupanc, 1999, 2001, 2006; Otteson and Hitchcock, 2003; Hitchcock et al., 2004; Zupanc and Zupanc, 2006).

Traditionally, it was thought that developmental regulation of cell proliferation and differentiation operates on a constant genome and is restricted to alterations at transcriptional and posttranslational levels (Schmucker et al., 2000; Savage et al., 2003; Zhu and Rosenfeld, 2004; Guillemot, 2005). However, recent studies examining the embryonic and adult mammalian central nervous system have provided evidence for somatic genomic alterations, resulting in a genetic mosaic of newly generated cells (Rehen et al., 2001). These genomic alterations, manifested as aneuploidy, appear to form the basis for a novel mechanism to regulate gene expression during development (Kaushal et al., 2003). By studying the brown ghost knifefish (*Apteronotus leptorhynchus*), we demonstrate in the present paper for the first time that chromosomal variation also occurs in the adult brain of a nonmammalian species, and that the portion of aneuploid cells among the newly generated cells is actually higher than the fraction of cells that have an euploid complement of chromosomes. Together with the enormous potential for the production of new cells, this finding will make it particularly attractive to study, in future investigations, the functional significance of chromosomal variation in relation to adult neurogenesis in the adult teleost fish brain.

MATERIALS AND METHODS

Animals

A total of 42 brown ghost knifefish (*Apteronotus leptorhynchus*; Gymnotiformes, Teleostei), purchased from a tropical fish importer (Aquarium Glaser), were used. The fish were kept in 40–300 L aquaria under a 12-h light/12-h dark photoperiod at a temperature of approximately 26°C, pH values around 7, and a water conductivity of approximately 150 μS/cm. The fish were fed daily on frozen mosquito larvae.

The size of the fish used in the present study ranged from 104 to 179 mm total length (mean: 131 mm; median: 125 mm) and from 2.2 to 10.6 g body weight (mean: 4.0 g; median: 3.7 g). Thus, all individuals were well into adulthood. Nineteen of the fish were male and 22 female, as determined by postmortem gonadal inspection. One individual could not be sexed. The relative gonadal weight (=fresh weight of gonads divided by body weight) ranged between 0.0003 and 0.0146 in males (mean: 0.0034; median: 0.0021) and between 0.0034 and 0.1209 in females (mean: 0.0300; median: 0.0156). These values indicate that a large variety of individuals of this seasonally breeding species, ranging from sexually immature to mature fish, were included in this study.

All experiments were carried out in accordance with the relevant German law, the *Deutsche Tierschutzgesetz* of 1998.

Metaphase Chromosome Spreads

Chromosome spreads were obtained from whole brain using a modification of a protocol published previously (Barch et al., 1997). Briefly, adult fish were killed by immersion into a 1.5% solution of 3-aminobenzoic acid ethyl ester (Sigma) in aquarium water. Their brains were rapidly removed from the skull, dissected into approximately 10 pieces, gently triturated with a 1000 μL Eppendorf pipette, and incubated in DMEM/F12 medium supplemented with 50 ng/mL of fibroblast growth factor-2 and 1 μg/mL colcemid (all from Gibco/BRL, except fibroblast growth factor-2, which was from PeproTech) at 26°C for 6–7 h to cause metaphase arrest of mitotic cells. After trypsinization with trypsin/EDTA [0.5% trypsin (1:250)/5.3 m*M* EDTA·4 Na; Gibco/BRL], the single cells were incubated in a hypotonic

solution of 75 m*M* KCl in ddH_2O at 37°C for 20 min. The cells were fixed three times in methanol/acetic acid (3:1) and spread onto slides. Chromosomes were stained with either 4′,6-diamidino-2-phenylindoledihydrochloride (DAPI; Sigma) or 3,8-diamino-5-[3-(diethylmethylammonio)-propyl]-6-phenylphenanthridinium diiodide (propidium iodide; Sigma), embedded in polyvinyl alcohol, average molecular weight 30,000–70,000, containing *n*-propyl gallate (for methodological details, see Zupanc, 1998), and coverslipped.

Flow Cytometry

For flow cytometric analysis, adult fish were killed by immersion into a 1.5% solution of 3-aminobenzoic acid ethyl ester in aquarium water, brain and liver were rapidly removed, dissected into several pieces, gently triturated with a 1000 μL Eppendorf pipette, and dissociated with 1 mL each of the same trypsin/EDTA solution as used for the metaphase chromosome spreads. The trypsinization was stopped by the addition of 500 μL 20% fetal bovine serum/DMEM/F12, and the suspension passed through a 40 μm mesh. Cells were centrifuged at 520*g* for 10 min. The resulting pellet was resuspended in 300 μL phosphate-buffered saline (PBS)/700 μL 70% ice-cold ethanol and kept overnight at −20°C. The next day, after washing the cells twice with PBS, they were resuspended in PBS containing 50 μg/mL propidium iodide (Sigma), 0.1% Triton X-100 (Sigma), and 0.5 mg/mL RNase A (Sigma) and incubated in this solution at room temperature for 30 min in the dark. Cell cycle analysis was performed on a LSR Flow Cytometer (Becton Dickinson). At least 50,000 cells per sample were analyzed by flow cytometry. Cell cycle histograms were generated using the software CellQuest version 3.3. (Becton Dickinson).

Intraperitoneal Injection of 5-Bromo-2′-deoxyuridine

For labeling of S-phase cells, the fish were anesthetized in 2% urethane (Sigma) in aquarium water and injected intraperitoneally with approximately 50 μL/g body weight of labeling reagent [=aqueous solution of 5-bromo-2′-deoxyuridine (BrdU; 3 mg/mL) and 5-fluoro-2′-deoxyuridine (0.3 mg/mL)], as supplied with the cell proliferation kit (Amersham). After a recovery period of approximately 10 min in oxygenated aquarium water, the fish were transferred into isolation tanks for a post-BrdU administration survival period of 2 h.

Intracardial Perfusion and Cryosectioning

Fish were killed by immersion into a 1.5% solution of 3-aminobenzoic acid ethyl ester (''MS-222''; Sigma) and intracardially perfused with a flush solution containing 0.1 *M* phosphate buffer (PB; pH 7.4), 0.9% NaCl, 5 mg/100 mL heparin sodium salt (research grade, 150,000 IU/g; AppliChem), and 1 mL/100 mL 2% lidocaine. After all blood had been washed out, the perfusion was continued with 2% paraformaldehyde (AppliChem) in 0.1 *M* PB. The brains were removed from the skull, postfixed in the same fixative solution for 1–8 h at 4°C, and cryoprotected in 1 *M* sucrose in PBS overnight at 4°C. After embedding the brains in a 1:1 mixture of AquaMount (Lerner Laboratories) and Tissue Tek O.C.T. Compound (Sakura), 16-μm thick transverse sections were cut on a cryostat and thaw-mounted onto gelatin/chrome-alum-coated slides.

Phosphorylated Histone-H3 Immunohistochemistry

Cryosections of brains of untreated fish were dried in a desiccator for 90 min at room temperature and rehydrated through three changes of 0.1 *M* Tris-buffered saline (TBS), pH 7.5, containing 0.9% NaCl. To permeabilize the tissue and block unspecific binding sites, the sections were treated for 1 h with 3% normal sheep serum (Sigma), 1% bovine serum albumin (AppliChem), 1% teleostean gelatin (Sigma), and 0.3% Triton X-100 (AppliChem) in TBS. Sections were incubated overnight at 4°C with rabbit polyclonal antiphosphorylated histone-H3 antiserum (Upstate Biotechnology) diluted 1:100 in TBS* (TBS supplemented with 1% bovine serum albumin, 1% teleostean gelatin, and 0.3% Triton X-100). Unbound primary antibody was removed by three rinses for 10 min each in TBS. Antigenic sites were visualized by incubating the sections at 4°C for 90 min with a 1:1000 dilution in TBS* of the secondary antibody, a Cy3-conjugated goat-anti-rabbit IgG (H+L) (Jackson ImmunoResearch). Following three washes for 10 min each with TBS and two washes for 3 min each with PBS, the sections were counterstained by incubation with 2 μg/mL in PBS of DAPI for 3 min at room temperature. Finally, the sections were washed three times for 3 min each in PBS and once for 3 min in PB, embedded in polyvinyl alcohol, average molecular weight 30,000–70,000, containing *n*-propyl gallate (for methodological details, see Zupanc, 1998), and coverslipped.

Omission of the primary antibody resulted in abolition of labeling (not shown).

BrdU/Phosphorylated Histone-H3 Double Labeling

For immunohistochemical detection of BrdU-labeled cells, combined with antiphosphorylated histone-H3 immunostaining, a protocol similar to the one for phosphorylated histone-H3 alone (see ''Materials and Methods, Phosphorylated histone-H3 immunohistochemistry'', above) was employed. Cells that had incorporated BrdU into DNA during the S-phase of mitosis were detected by overnight incubation at 4°C of cryosections of BrdU-treated fish with a monoclonal rat-anti-BrdU primary antibody (Biozol) at a dilution of 1:200 in TBS*. This was followed by overnight incubation at 4°C with a Cy3-conjugated donkey-anti-rat

IgG (H+L) secondary antibody (Dianova) at a dilution of 1:500 in TBS*. Phosphorylated histone-H3-expressing cells were immunolabeled using polyclonal rabbit-anti-phosphorylated histone-H3 antiserum, diluted 1:100 in TBS*, and an Alexafluor-488-conjugated goat-anti-rabbit IgG (H+L) secondary antibody (Molecular Probes), diluted 1:100 in TBS*.

Omission of the anti-BrdU antibody resulted in abolition of fluorescent signals in the emission range of Cy3 (not shown).

Microscopic Analysis

For fluorescence microscopy, sections were examined under a Zeiss Axioskop microscope (Carl Zeiss) equipped with epifluorescence and 10× (NA, 0.30), 20× (NA, 0.50), 40× (NA, 0.75), and 100× (NA, 1.30) Zeiss objectives. Microphotographs were taken using an AxioCam MRc5 digital photocamera (Carl Zeiss) and AxioVision software (release 4.2; Carl Zeiss) on personal computers.

For confocal microscopy, specimens were analyzed on a Zeiss LSM 510 META laser scanning microscope equipped with a Zeiss Axiovert 200M compound microscope and 20× (NA, 0.60), 40× (NA, 0.75), and 63× (NA, 1.40) Zeiss objectives. Optical sections were taken at a resolution of 1024 × 1024 pixels using LSM 5 software (version 3.2; Carl Zeiss).

Deconvolution microscopy was performed using a DeltaVision imaging station (Applied Precision), an Olympus IX-70 inverted fluorescence microscope with 60× (NA, 1.4) and 100× (NA, 1.3) Olympus oil-immersion objectives, and a CH350 camera (Photometrics). The data were deconvoluted using softWoRx (version 3.3.5) software (Applied Precision) on a Linux-based workstation.

Images were processed to improve contrast and brightness using the software programs Adobe Photoshop 6.0 (Adobe Systems) and Corel Draw, version 11 (Corel).

For the determination of the relative frequency in different brain regions of cells with chromosome segregation defects among the phosphorylated histone-H3-labeled cells, the respective brain sections were drawn with a camera lucida using the Zeiss Axioskop microscope and the 10× lens. The cells were examined at 40×, and their location indicated in the camera lucida drawings.

RESULTS

Aneuploidy Among Mitotic Cells

Examination of metaphase chromosome spreads from whole brains of adult fish revealed an euploid complement of chromosomes, assumed to be 22 (see ''Numerical chromosomal variation,'' under ''Discussion,'' later), on average in only 22% of a total of 1147 cells analyzed [Figs. 1(A) and 2]. The remaining cells showed numerical chromosomal abnormal-

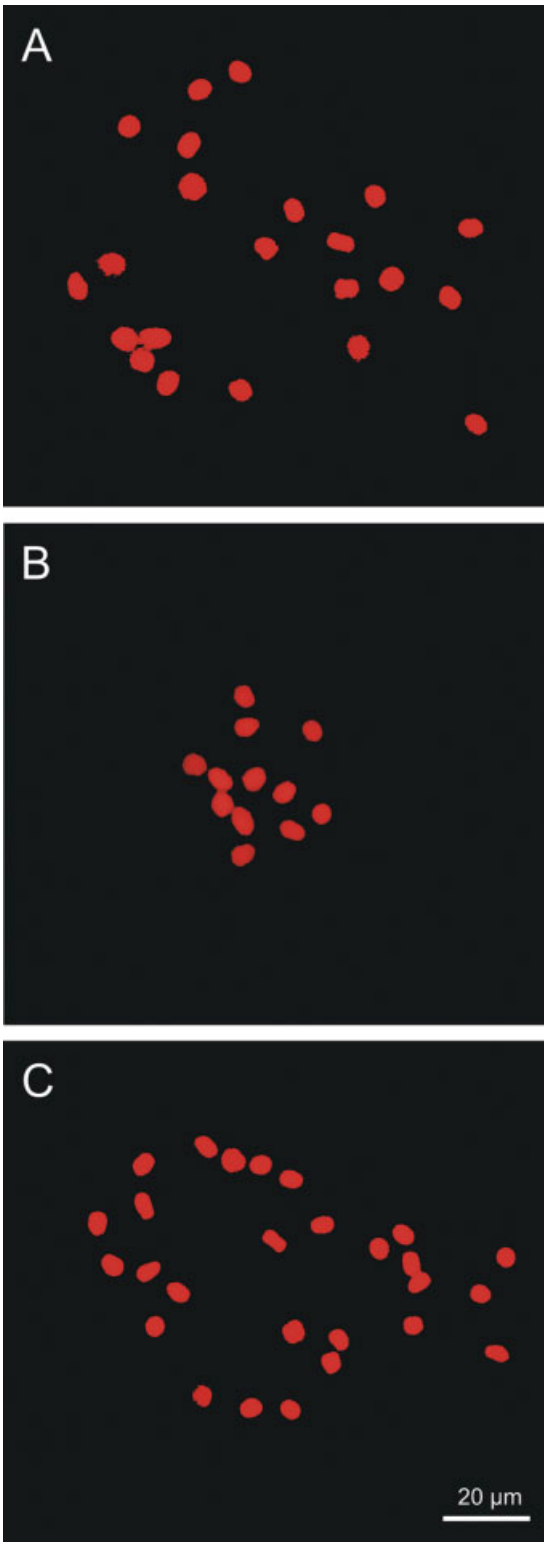

Figure 1 Euploid and aneuploid cells in the adult brain of *Apteronotus leptorhynchus*. The metaphase chromosome spreads were stained with propidium iodide. The cell in A has 22 chromosomes, the cell in B has 12, and the cell in C has 27.

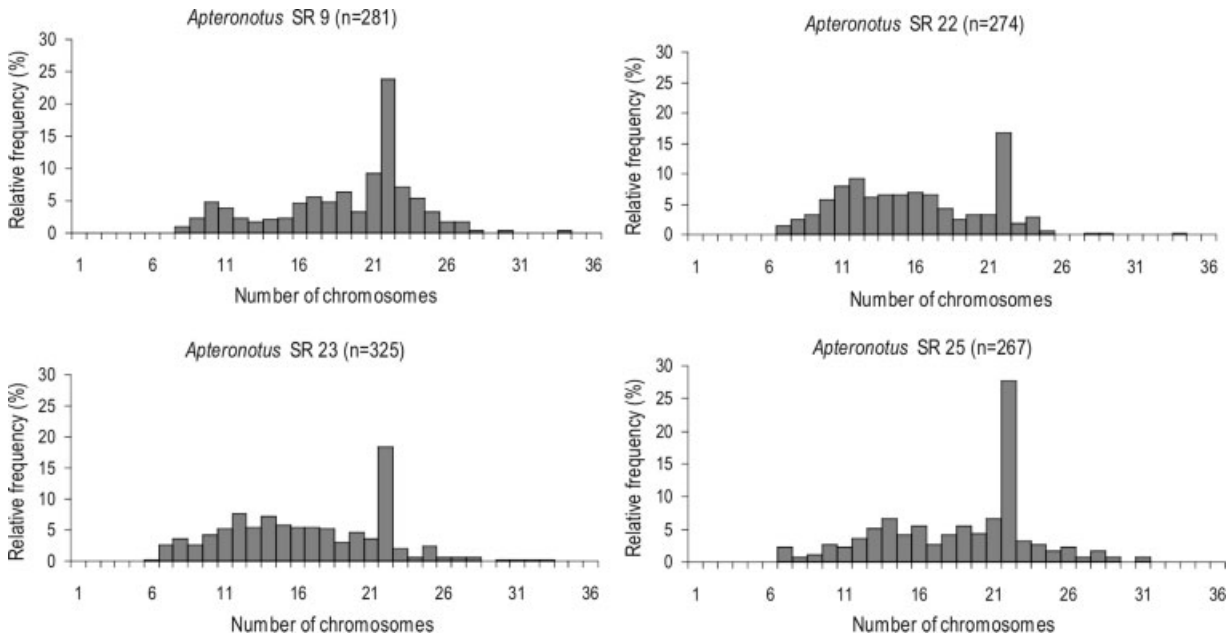

Figure 2 Chromosome number histograms based on the analysis of metaphase chromosome spreads of cells from the adult brain of four individual fish of *Apteronotus leptorhynchus*. Between 17 and 28% of the cells are euploid, having 22 chromosomes. Among the aneuploid cells, the majority lost chromosomes, whereas a rather minor portion gained chromosomes. *n*, number of cells analyzed.

ities. Among these aneuploid cells, the majority ($\sim$84%) had fewer than 22 chromosomes [Fig. 1(B)]. Typically, multiple chromosomes, up to 16, were lost (Fig. 2). Chromosome gain was observed in approximately 16% of the aneuploid cells [Figs. 1(C) and 2]. In the most extreme case, a total of 34 chromosomes was counted. However, in contrast to the preferential loss of multiple chromosomes, there was a clear tendency towards the gain of only one or a very few additional chromosomes.

For comparison, we prepared metaphase chromosome spreads from liver tissue of two adult fish. On the basis of these spreads, a complement of 22 chromosomes was found in 52% ($n = 42$ nuclei) and 72% ($n = 29$ nuclei) of the cells analyzed.

The notion that there is a difference in the degree of aneuploidy between brain cells and liver cells is supported by the results of a flow cytometric analysis (Fig. 3). Such an analysis of the DNA content revealed in brain tissue, in addition to cells with 2C, 4C, and 8C DNA content (C = quantity of DNA in a single haploid genome), consistently a population of cells with 6C DNA content. Such a cellular population was virtually absent in liver tissue. Relative to the total number of cells in the different phases of the cell cycle, on average, 3.83% of the brain cells corresponded with the 6C peak, when compared to only 1.10% of the liver cells ($n = 3$ fish with 2 brain samples and 1 liver sample each).

Chromosome Displacement

A possible mechanism proposed to contribute to aneuploidy in the developing central nervous system is chromosome displacement during mitosis (Rehen et al., 2001). To examine chromosome segregation, brain sections were immunostained for phosphorylated histone-H3, which labels the condensed chromosomes of cells in M-phase. Combination with anti-BrdU immunohistochemistry at post-BrdU survival times of 2 h demonstrated that the distribution of phosphorylated histone-H3-labeled cells is restricted to zones of high mitotic activity in the adult brain [Fig. 4(A,B)]. At this time point, the vast majority of BrdU-labeled cells expressed phosphorylated histone-H3 [Fig. 4(C)].

Analysis of the phosphorylated histone-H3-expressing cells revealed, in addition to normal profiles [Fig. 5(A,C)], the existence of lagging chromosomes [Figs. 4(B,C: insets), 5(D–F), and 6(A,B)]. These so-called laggards are displaced mitotic chromosomes that frequently become encapsulated in a micronucleus and are excluded from the daughter

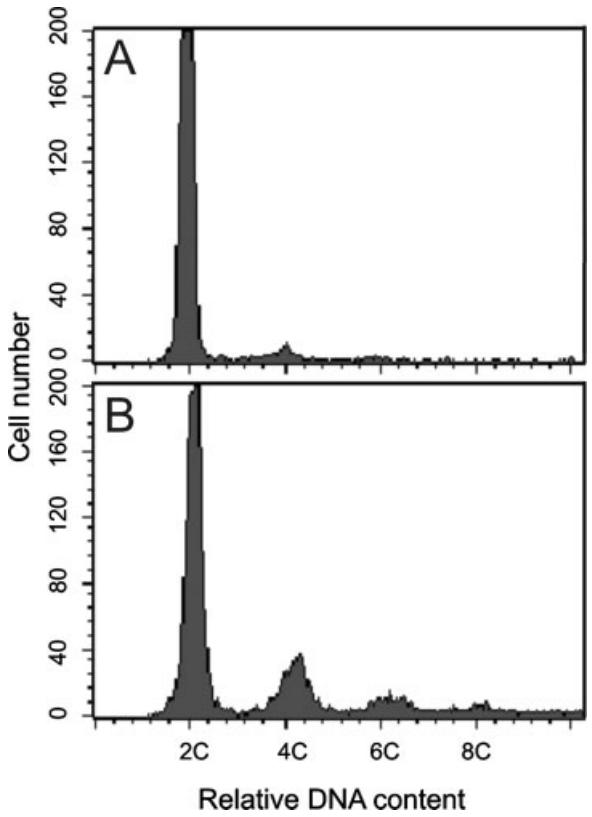

Figure 3 DNA content of liver tissue (A) and brain tissue (B) of *Apteronotus leptorhynchus*, as measured by flow cytometry. In contrast to the liver tissue, in brain tissue there is a significant fraction of cells that have a 6C DNA content.

nuclei as mitosis ends. In brain sections of *Apteronotus leptorhynchus*, in addition to single lagging chromosomes [Fig. 5(D–F)], cells with multiple laggards, both in metaphase [Fig. 6(A)] and in anaphase [Fig. 6(B)], were observed.

In addition to lagging chromosomes, anaphase bridges were observed as a second type of segregational defect during mitotic division. Two such cells in which chromatin formed continuous bridges connecting the two sets of separating chromosomes during the anaphase are shown in Figure 7. Anaphase bridges were evident in high-power confocal images

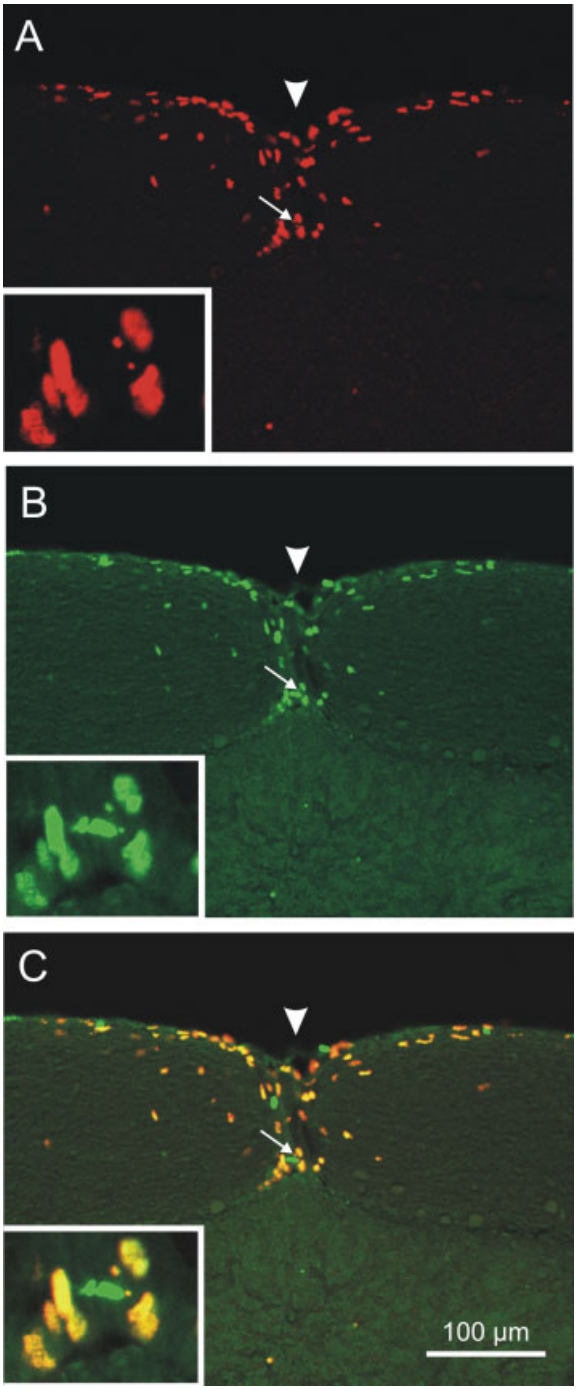

Figure 4 Chromosome displacement in mitotic cells of the proliferation zone of the corpus cerebelli. S-phase cells were labeled with BrdU by intraperitoneal injection of this thymidine analogue, followed by a post-BrdU survival time of 2 h. The confocal image of the 16-μm transverse section through the corpus cerebelli shows a large number of BrdU-labeled cells at the midline (arrowhead) and in an area stretching approximately 200 μm laterally from the midline in the dorsal molecular layer, immediately beneath the pial surface, in each hemisphere (A). Two BrdU-labeled cells are shown in the area of the midline in the granule cell layer, near the bottom of the picture. The same section as shown in (A) immunostained for phosphorylated histone-H3 to label condensed chromosomes (B). A large number of the BrdU-labeled cells express phosphorylated histone-H3, as evident from the yellow color in the overlay (C). The insets show high-power confocal images of chromosomes with laggards from the area indicated by arrow in A–C.

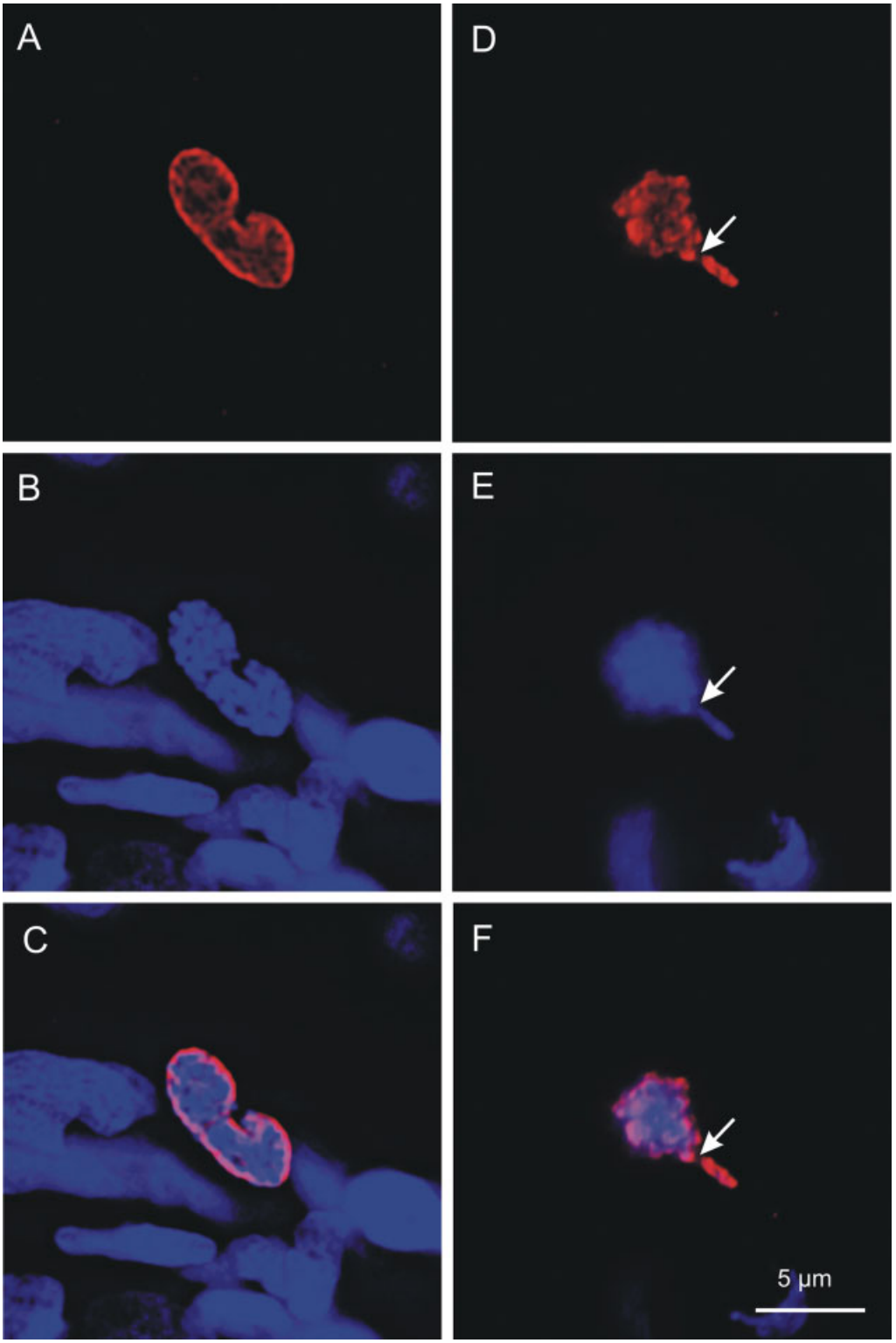

Figure 5 High-power images obtained through deconvolution microscopy of phosphorylated histone-H3-labeled cells (red) in the molecular layer of the corpus cerebelli. The nuclei were counterstained with DAPI (blue). A–C: Morphologically normal metaphase profile. D–F: Prometaphase/metaphase profile with single laggard. Note the clear gap between the laggard and the daughter nucleus (arrow). In addition to the phosphorylated histone-H3 label (A,D) and the DAPI counterstain (B,E), the overlay (C,F) is shown.

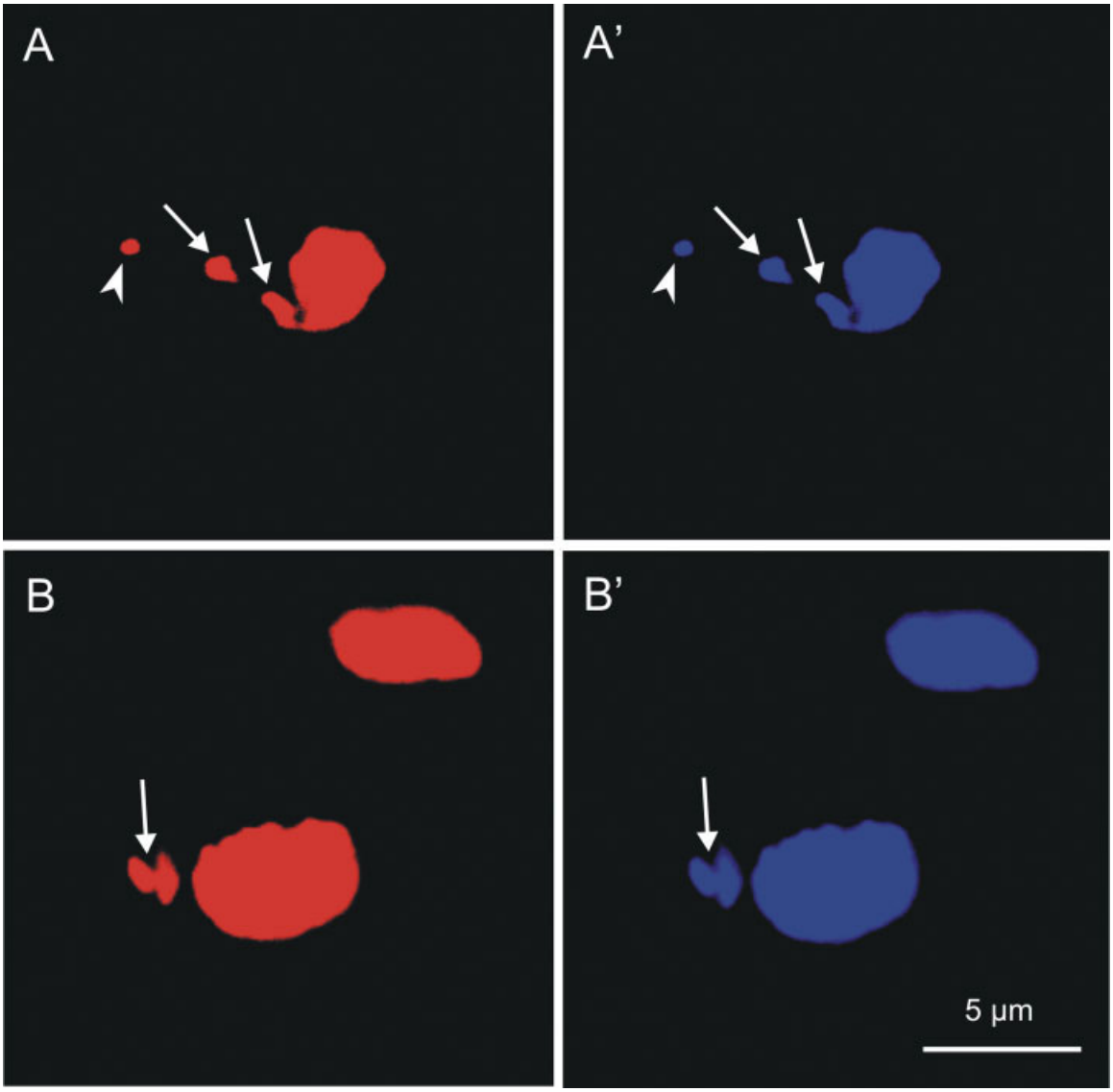

Figure 6 Confocal images of phosphorylated histone-H3-labeled nuclei with multiple laggards. A: Metaphase cell in the molecular layer of the valvula cerebelli pars medialis. B: Anaphase cell with the two daughter nuclei in the nucleus tuberis lateralis pars posterior. The lagging chromosomes are indicated by arrows. The arrowhead indicates a putative micronucleus. A′, B′: DAPI counterstain.

both after immunolabeling against phosphorylated histone-H3 [Fig. 7(A,B)] and after DAPI staining [Fig. 7(A′,B′)]. For comparison, a normal anaphase profile is shown in Figures 7(C,C′).

The relative frequency of laggards was quantitatively analyzed in the entire brain. In each of four fish used, at least half out of the total set of complete brain sections were analyzed. A cell was considered to contain laggard(s) if (i) both the daughter nucleus and the laggard(s) were DAPI positive, and (ii) the gap between the daughter nucleus and the laggard(s) was not wider than 2.5 μm. Out of 5529 phosphorylated histone-H3-labeled cells found in the entire brain of the four fish, 793 (=14.3%) exhibited chromosome segregation defects. Out of the latter, 48.8% were metaphase cells with single laggards, 39.6% were metaphase cells with multiple laggards, 9.7% were anaphase cells with lagging chromosomes, and 1.9% were cells with anaphase bridges. The relative frequency of phosphorylated histone-H3-labeled cells with chromosome segregation defects was very similar in different brain regions (Fig. 8), thus demonstrating that such defects are not restricted to certain parts of the brain or to specific types of neurons.

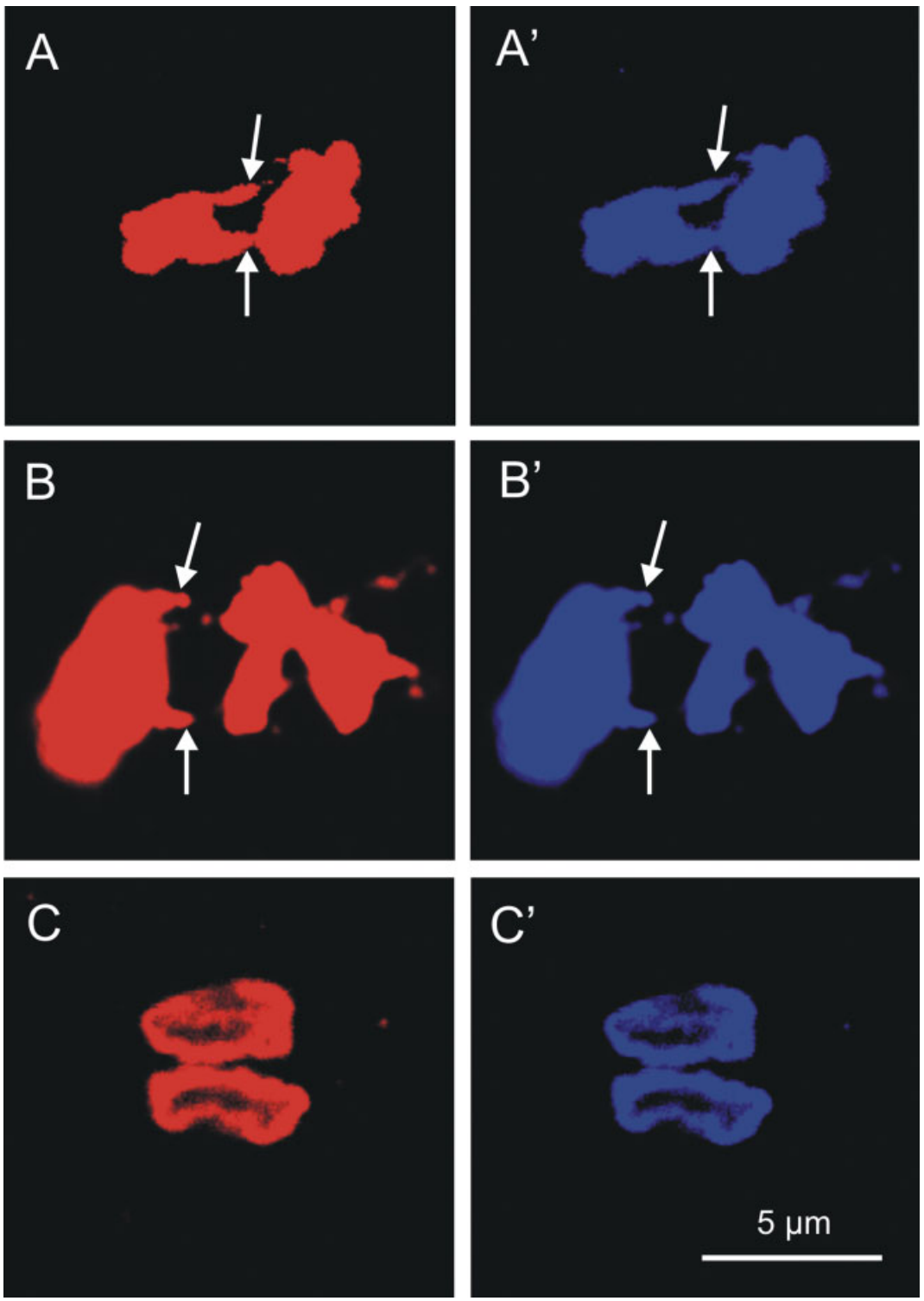

Figure 7 Anaphase bridges in cells of the adult brain of *Apteronotus leptorhynchus*. In both the phosphorylated histone-H3 immunolabeled sections (A,B) and the DAPI counterstain (A′,B′) of the two cells, analysis of confocal images confirmed the existence of chromatin extrusions between the two daughter nuclei (arrows). The cells were located at the boundary between the ventral molecular layer of the corpus cerebelli and the molecular layer of the valvula cerebelli pars medialis (A,A′) and in the dorsal molecular layer of the corpus cerebelli (B, B′), respectively. For comparison, a normal anaphase profile found in the dorsal molecular layer of the corpus cerebelli is shown (C, phosphorylated histone-H3 immunostaining; C′, DAPI counterstain).

DISCUSSION

Numerical Chromosomal Variation

This study provides for the first time evidence that the adult brain of a teleost fish is a genetic mosaic: the majority of metaphase cells were aneuploid, whereas only a minor fraction was euploid. Among the aneuploid cells, a great numerical chromosomal variability was observed. Up to 16 chromosomes were lost and up to 12 chromosomes gained, relative to the euploid number of chromosomes.

Since the most frequent number of chromosomes in each of the four fish analyzed quantitatively was 22, we assume this number to represent the euploid complement. This notion is supported by analysis of chromosome spreads prepared from adult liver tissue, which revealed 22 chromosomes in 52 and 72%, respectively, of the cells analyzed in 2 fish. In the closely related species *Apteronotus albifrons*, the diploid set of chromosomes has also been proposed to be 22, based on the examination of blood cells or sperms, in one study (Hinegardner and Rosen, 1972). However, using regenerating fin epithelium and kidney tissue, respectively, in two other studies, the diploid complement was determined to consist of 24 chromosomes (Howell, 1972; de Almeida Toledo et al., 1981). The reasons for this discrepancy are unknown.

We furthermore assume that the 22 chromosomes in *Apteronotus leptorhynchus* represent the diploid set of chromosomes. Although polyploidy is a frequent phenomenon in fishes (for review, Leggatt and Iwama, 2003), and spontaneously occurring cases of triploidy have been reported in the gymnotiform species *Eigenmannia* sp. (de Almeida Toledo et al., 1985) and *Gymnotus carapo* (Fernandes-Matioli et al., 1998), karyotyping of the chromosomes in *Apteronotus albifrons* clearly supports the notion of a diploid complement (Howell, 1972; de Almeida Toledo et al., 1981).

The markedly higher rate of aneuploidy in the adult brain compared to adult liver in *Apteronotus leptorhynchus*, as revealed by metaphase chromosome spreads is interesting and deserves further examination. A similar phenomenon was found in mice through karyotyping of embryonic (E13) liver cells and postnatal (P5-P10) subventricular zone cells (Kaushal et al., 2003). In *Apteronotus leptorhynchus*, the observation of a higher rate of aneuploidy in brain cells, when compared to liver cells, is supported by the results of the flow cytometric analysis, which showed a higher relative number of cells with greater than 2C DNA content in brain than in liver. The occurrence of cellular populations that fall not into the 2C, 4C, and 8C peaks has also been observed in

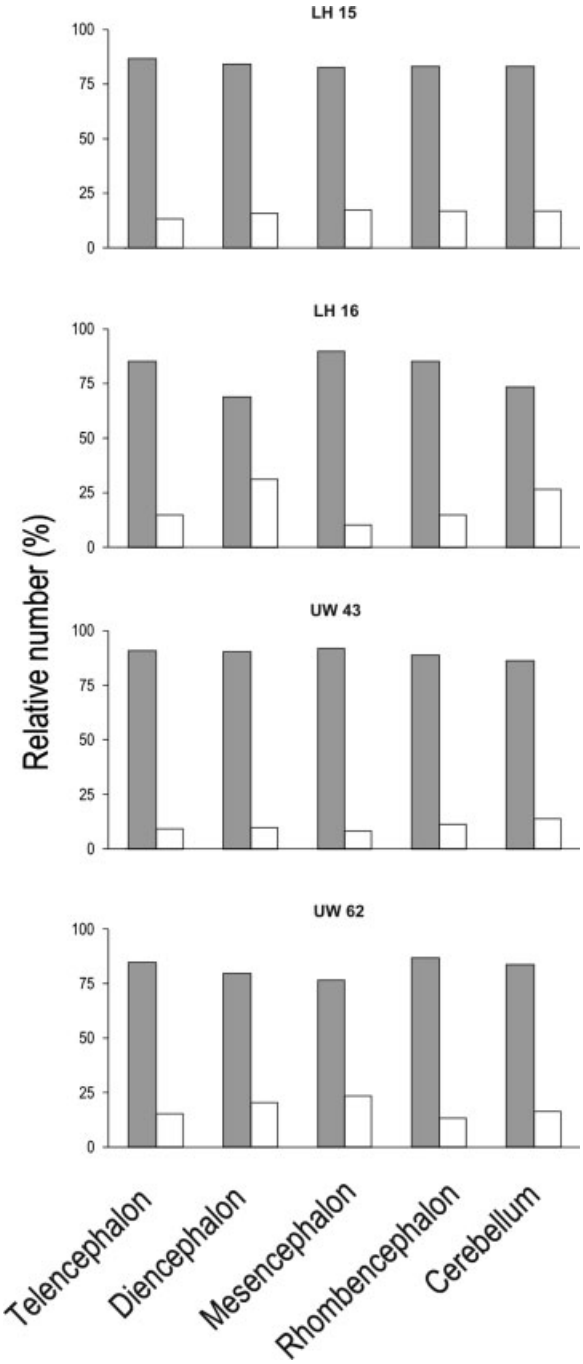

Figure 8 Proportion of cells with normal nuclei (gray) and nuclei exhibiting laggards (white) in the entire brain of four individuals of *Apteronotus leptorhynchus*. A total of 5529 phosphorylated histone-H3-labeled nuclei were analyzed. The fraction of cells with laggards among the phosphorylated histone-H3-labeled nuclei is similar among the five major subdivisions (telencephalon, diencephalon, mesencephalon, rhombencephalon, and cerebellum) of the brain analyzed.

hepatocytes after transient expression of cyclin D1 (which promotes progression through the G_1 phase of the cell cycle, but can also act as an oncogene), and has been interpreted to reflect cells with grossly abnormal chromosome content (Nelsen et al., 2005). We hypothesize that the occurrence of a cellular population with 6C DNA content in the brain of *Apteronotus leptorhynchus* corresponds to cells in the latter category.

Besides the adult teleostean brain, aneuploidy has been studied in the mammalian brain. Spectral karyotype analysis of metaphase spreads of mouse embryonic cerebral cortical neuroblasts revealed aneuploidy in approximately 33% of the cells, with the vast majority of them being hypoploid (Rehen et al., 2001). These results have received further support by interphase fluorescence *in situ* hybridization using X and Y chromosome paints. Application of this approach to embryonic cerebral cortical cells has demonstrated that approximately 6% of the nuclei were missing an X or Y chromosome, whereas approximately 1% of nuclei had gained an X or Y chromosome. In the adult male cerebral cortex, the rate of loss or gain of an X and Y chromosome was 1 and 0.2%, respectively. Similar results have been obtained by karyotyping of mitotic cells in the murine subventricular zone (Kaushal et al., 2003), as well as by examination of cerebral cortical neurons and cerebellar Purkinje cells through the use of a nuclear transfer technique (Osada et al., 2002). Significant levels of aneuploidy have also been reported for individual chromosomes of cells in the normal human brain during embryonic stages of development and during adulthood (Rehen et al., 2005; Yurov et al., 2005). Taken together, the findings made in both teleost fish and mammals contradict the widely held assumption that the cells of the normal vertebrate brain contain a constant euploid complement of chromosomes.

Although it is tempting to speculate that the rate of aneuploidy is markedly higher in the adult teleostean brain than in the embryonic and early postnatal mammalian brain, more experiments are required to establish whether such a difference between the two taxonomic groups indeed exists. The estimates of an approximately 33% aneuploidy rate in the mammalian brain were obtained through metaphase chromosome spreads and karyotyping using SKY (Rehen et al., 2001; Kaushal et al., 2003). The latter technique is currently not available for chromosomes isolated from tissue of teleost fish. Moreover, the investigations in mammals were restricted to embryonic and early postnatal (P5-P10) stages of development, whereas the analysis in this study was based on brain tissue from adult fish. These differences in both the techniques employed and the developmental stages examined make a direct comparison of the data available, at present, impossible.

Mitotic Segregation Defects

In addition to, and consistent with, the enormous numerical variability in chromosome complement of individual brain cells, chromosome segregation defects—notably laggards, micronuclei, and anaphase bridges—were identified in various regions of the adult teleostean brain. Quantitative analysis showed that approximately 14% of all phosphorylated histone-H3 cells displayed chromosome segregation defects. It is likely that the actual rate of occurrence of laggards is even higher because (i) due to their small size laggards could easily have been missed, and (ii) we excluded any laggard from our analysis that was located more than 2.5 μm distant from the nucleus under examination, although many of them may also have originated from that nucleus.

Among phosphorylated histone H3-labeled subventricular zone cells of early postnatal mice, approximately 7% exhibit chromosome displacement (Kaushal et al., 2003). Since the protocol, including the phosphorylated histone H3 antibody, employed in these experiments was very similar to the one used in our study, these results suggest a higher rate of missegregation of chromosomes in the adult teleostean brain than in the embryonic or early postnatal mammalian brain. Such a difference is in line with the possibility that teleost fish and mammals also differ in terms of rate of aneuploidy in the brain, although further experimental support for the latter hypothesis is still required (see ''Numerical chromosomal variation'' under ''Discussion,'' above).

Lagging chromosomes and micronuclei have been identified previously in neural progenitor cells of various areas of both the embryonic and the postnatal mammalian brain (Rehen et al., 2001; Kaushal et al., 2003; Yang et al., 2003). By contrast, anaphase bridges have, to our knowledge for the first time in the present study, been reported to exist in the adult vertebrate brain.

As demonstrated by several techniques in both normal brain cells and peripheral tumor cells, the chromosome segregation defects, reflected by the existence of lagging chromosomes and anaphase bridges, can mechanistically account for aneuploidy (Yang et al., 2003; Stewénius et al., 2005; for review, Gisselsson and Höglund, 2005). For example, anaphase bridging is caused by abnormally short telomeres during mitosis and typically results in either inter-centromeric chromatin fragmentation or centromere detachment. These defects, in turn, lead to pericentromeric chro-

mosome rearrangements and loss of whole chromosomes, respectively (Stewénius et al., 2005).

As shown in cancer cells, one mechanism that leads to the formation of micronuclei involves the resolution of chromosomes in anaphase bridges by breaking into fragments at the end of mitosis (Hoffelder et al., 2004). Similarly, in neural progenitor cells, time-lapse imaging revealed lagging chromosomes, which were subsequently encapsulated so that they formed micronuclei contained in one of the daughter nuclei (Yang et al., 2003). The chromatin within these micronuclei displays greatly reduced transcriptional activity (Hoffelder et al., 2004), an observation that is particularly important from a functional point of view.

Functional Implications

A possible consequence of acquired chromosomal aneuploidy is a modification of expression levels of genes residing on the affected chromosomes. In a cell line model for prostate cancer, which at advanced primary or metastatic stages is typified by aneuploidy, it had been demonstrated that the average gene expression levels are, indeed, related to DNA gain and loss (Phillips et al., 2001). However, a gain or loss of chromosomes and chromosomal subregions does not directly translate to increases or decreases in the expression level in the same ratio identified for the genomic imbalances. Thus, this finding suggests that loss of heterozygosity may influence, but does not override, transcriptional control mechanisms that are already in place.

Alteration of gene expression by chromosome loss has also been suggested to occur in cells of the subventricular zone of the adult mouse (Kaushal et al., 2003). At least some of the aneuploid cells born in the subventricular zone are competent to migrate into their target region, the olfactory bulb, and survive after differentiation into neurons. Within the olfactory bulb, combination of retrograde tracing with fluorescent *in situ* hybridization for chromosome-specific loci and with immediate early gene immunolabeling indicated that these aneuploid neurons are functionally active and form proper anatomical connections (Kingsbury et al., 2005). Along similar lines, hybrid embryonic stem cell-derived tetraploid mice have been shown to exhibit a normal phenotype (with the exception of elevated body weight and hematocrit) in 90 morphological, physiological, and behavioral parameters, when compared to euploid controls raised from normal matings (Schwenk et al., 2003). These results demonstrate that aneuploidy does not necessarily result in death of the individual aneuploid cells and in dysfunction of the whole animal.

In *Apteronotus leptorhynchus* and in the zebrafish, approximately half of the newborn cells in the adult brain survive for long periods of time, whereas the other half undergo apoptotic cell death shortly after they have arrived at their target areas (Soutschek and Zupanc, 1995, 1996; Zupanc et al., 1996, 2005; Ott et al., 1997). It is unknown whether the death rate differs between aneuploid and euploid cells. Examination of this aspect is important, as long-term persistence of a significant portion of the aneuploid cells, a possibility suggested by the findings made in the mammalian brain (Kaushal et al., 2003; Kingsbury et al., 2005), would lead to a genetically heterogenous cellular population. Such a mosaicism could provide a molecular basis for the phenotypic plasticity commonly exhibited by cells during embryonic and postnatal development.

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