Development/Plasticity/Repair

Alteration of Gene Expression by Chromosome Loss in the Postnatal Mouse Brain

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Frequent chromosomal aneuploidy has recently been discovered in normal neurons of the developing and mature murine CNS. Toward a more detailed understanding of aneuploidy and its effects on normal CNS cells, we examined the genomes of cells in the postnatal subventricular zone (SVZ), an area that harbors a large number of neural stem and progenitor cells (NPCs), which give rise to neurons and glia. Here we show that NPCs, neurons, and glia from the SVZ are frequently aneuploid. Karyotyping revealed that ~33% of mitotic SVZ cells lost or gained chromosomes in vivo, whereas interphase fluorescence in situ hybridization demonstrated aneuploidy in postnatal-born cells in the olfactory bulb (OB) in vivo, along with neurons, glia, and NPCs in vitro. One possible consequence of aneuploidy is altered gene expression through loss of heterozygosity (LOH). This was examined in a model of LOH: loss of transgene expression in mice hemizygous for a ubiquitously expressed enhanced green fluorescent protein (eGFP) transgene on chromosome 15. Concurrent examination of eGFP expression, transgene abundance, and chromosome 15 copy number demonstrated that a preponderance of living SVZ and OB cells not expressing eGFP lost one copy of chromosome 15; the eGFP transgene was lost in these cells as well. Although gene expression profiling revealed changes in expression levels of several genes relative to GFP-expressing controls, cells with LOH at chromosome 15 were morphologically normal and proliferated or underwent apoptosis at rates similar to those of euploid cells in vitro. These findings support the view that NPCs and postnatal-born neurons and glia can be aneuploid in vivo and functional gene expression can be permanently altered in living neural cells by chromosomal aneuploidy.

Key words: stem cells; aneuploidy; loss of heterozygosity; mosaicism; olfactory bulb; gene expression profiling

Introduction

Rapid division of stem and progenitor cells during CNS development gives rise to the neurons and glia that populate the adult brain. Faithful transmission of genetic information during these divisions is thought to be essential for normal brain development, but until recently the fidelity of transmission of genetic information during CNS development had not been measured.

We recently examined the genomes of developing and mature neurons of the mouse cerebral cortex and made the surprising discovery that 33% of proliferating cortical stem and progenitor cells became aneuploid (i.e., lost or gained one or more whole chromosomes), in part through chromosome mis-segregation during mitosis (Rehen et al., 2001). Many, but not all, aneuploid cells appeared to survive as neurons in the adult cerebral cortex.

Is the genesis of aneuploid cells restricted to the cerebral cortex? Does aneuploidy alter gene expression, survival, or proliferation of neural cells? To address these questions, we studied aneuploidy and its effects on gene expression and function in normal neural cells from the subventricular zone (SVZ) of the postnatal mouse brain. The SVZ is one of two neurogenic areas in the mammalian CNS that remains active throughout life (Gage, 2002) via proliferation of a pool of neural progenitor cells (NPCs) (Alvarez-Buylla and Garcia-Verdugo, 2002). The cells of the SVZ have been well characterized, with an eye toward their potential therapeutic uses (Aboody et al., 2000). We used the SVZ as a model because of its accessibility and relative homogeneity (Doetsch et al., 1997), which allowed us to (1) characterize the extent of aneuploidy and identify aneuploid cells, and (2) directly examine the effects of aneuploidy on gene expression, proliferation, and survival.

Materials and Methods

Metaphase chromosome spreads. Chromosome spreads were obtained from mitotic SVZ or embryonic liver cells using standard protocols (Barch et al., 1997; Rehen et al., 2001). Briefly, postnatal day (P) 5 to P10 SVZs were dissected and held intact for 3–5 hr in Opti-Mem supple-
mented with 10 ng/ml FGF-2 and 100 ng/ml colcemid (Invitrogen, San Diego, CA) to arrest cells in metaphase. Individual cells were then dissociated and incubated in 75 mM KCl for 12–15 min before fixation in 3:1 methanol:acetic acid. Embryonic day (E) 13 livers were dissected from contaminating mesoderm and gut before arrest and fixation as described above.

In situ hybridization. Hybridizations of metaphase chromosome spreads were performed using mouse Spectral Karyotyping (SKY) paints (Applied Spectral Imaging, Carlsbad, CA) according to manufacturer instructions. Hybridizations of interphase nuclei or cultured cells with mouse whole chromosome paints (Applied Spectral Imaging) were performed as described previously (Rehen et al., 2001). Error rates for SKY and interphase fluorescence in situ hybridization (FISH) karyotypes were estimated to be <2% in previous studies in which rates of chromosome loss measured by interphase FISH or SKY were compared with rates estimated from direct measurement of DNA content (Rehen et al., 2001).

Cell culture. Culture of SVZ cells for FISH was performed by the methods of Lim and Alvarez-Buylla (1999) or Reynolds et al. (1992) with the following modifications: cells were cultured in Opti-Mem (Invitrogen) supplemented with 2.5% fetal calf serum and/or 10 ng/ml FGF-2 and 20 ng/ml epidermal growth factor (EGF). For FISH analysis, cells were dispersed onto poly-lysine-coated dishes (Sigma, St. Louis, MO) or slides after brief incubation and mild trituration in PBS with 2 mM EDTA. For flow-sorting and gene expression profiling or antibody staining, SVZ glia and glial-restricted NPCs were expanded in Opti-Mem containing 2.5% serum, EGF, and FGF-2 (Levison and Goldman, 1997) for 10 d before fluorescence-activated cell sorting (FACS). For antibody staining after flow-sorting, cells were harvested by centrifugation for 10 min at 300 × g, resuspended in 50–100 µl of Opti-Mem, and plated onto 12-mm coverslips (Fisher Scientific, Pittsburgh, PA) coated with cell-tak (Collaborative Research, Bedford, MA) or poly-lysine (Sigma) as described previously (Fukushima et al., 1998).

Flow sorting. Cells from green fluorescent protein (GFP) transgenic mice were sorted by standard protocols (Kawakami et al., 1999) in PBS supplemented with 2% bovine serum albumin (Sigma). GFP+ and GFP− cells were isolated first by establishing suitable gates for live cells on the basis of forward and side light scatter, GFP peak width, and exclusion of propidium iodide (Sigma), and then sorted into GFP+ and GFP− bins on the basis of GFP intensity. After sorting, cells were either cultured for 12–24 hr and stained with antibodies or harvested for isolation of genomic DNA or total RNA.

PCR amplification of DNA was isolated by standard protocols with the exception that in the FACS-sorted cells (because of low genomic DNA quantities), 10 µg of yeast tRNA was used as a carrier to precipitate the DNA. The amount of genomic DNA (gDNA) in the samples was estimated on the basis of cell counts from FACS. PCR reactions of 20 µl consisted of 1 PCR buffer (Invitrogen), 2 mM MgSO4, 0.25 mM each dNTP, 0.5 mM each primer, and 0.5 U HiFi Taq (Invitrogen). Tubes were cycled 35 times (95°C for 30 sec; 56°C for 30 sec; 72°C for 30 sec; 5 min for 30 sec). Primer pairs used for sliP3 were edg3a (5′-CGGATGATCTTTTATTTGGGCAA-3′) and edg3c (5′-GGTTTCATAGGCGGAGTGAG-3′), and for GFP, eGFPP2 (5′-GGCAAGCTACCTGTTT-3′) and eGFPR3 (5′-GGGCCTCCTGTTTGGG-3′). Expected PCR product sizes were 528 bp (eGFPP2–eGFPR3) and 627 bp (edg3a–edg3c). Products were separated and visualized by electrophoresis on 1.2% agarose gels containing 0.5 µg/ml ethidium bromide. Gels were also Southern blotted and probed with fragments of the corresponding gene. Products were quantitated using a densitometer.

The other primer pair used to amplify the GFP gene gave a PCR product of 645 bp using eGFPP1 (5′-CGA CGT GTA AAG GCG CAA ATG TTT-3′) and eGFPR1 (5′-TGGATGATCTTTTATTTGGGCAA-3′). We also amplified sliP2 from chromosome 9 (expected product size, 415 bp; using edg4a 5′-TTA ACT CCC GTT GAG TGT TTG GCC-3′ and edg4b 5′-ACG ATG TTG ACC GTC TTG AGG A-3′).

Gene expression profiling. Three separate paired GFP+ and GFP− SVZ cell samples were sorted into RNA-Later (Ambion, Austin, TX), and RNA was isolated using a StrataPrep total RNA isolation kit (Stratagene, La Jolla, CA). RNA was quantitated with RiboGreen reagent (Molecular Probes, Eugene, OR). For each GFP+ or GFP− sample, RNA was linearly amplified by alternating rounds of cDNA synthesis and in vitro transcription using MessageAmp Kits (Ambion). RNA was labeled using BioArray high-yield RNA transcript labeling kits (Enzo, Farmingdale, NY). Synthesized RNA from each sample was hybridized to a Mouse Genome U74Av2 array (Affymetrix, Santa Clara, CA). Each sample was processed independently and hybridized to a different array. Array results were analyzed using the Microarray Suite software (version 4.0; Affymetrix). All arrays were normalized to the same average intensity on the basis of the hybridization signal of the probe sets corresponding to the 60th–90th percentile. Array data were analyzed using the Teradata relational database and algorithms (TeraGenomics; available at http://www. teragenomics.com). Criteria used to identify probe sets with signal differences between GFP+ and GFP− cells included a consistent call of increased and/or marginally increased or decreased and/or marginally decreased change >1.8-fold, an absolute difference change >50 in at least six of the nine comparisons and a present call in at least one of the comparison files. To approximate the false positive rate for this analysis, we applied the same criteria independently to comparisons between individual GFP+ or GFP− samples.

Results

Chromosome displacement and aneuploidy among mitotic SVZ cells in vivo

Chromosome displacement, postulated to contribute to CNS aneuploidy in the developing cerebral cortex (Rehen et al., 2001), was identified among proliferating cells of the postnatal SVZ. Twenty micrometer sections of P5 SVZ were immunostained for phosphorylated histone H3 (phospho-H3) to label the condensed chromosomes of cells in M-phase (Hendzel et al., 1997; Rehen et al., 2001) (Fig. 1B). In 7% of cells examined, chromosomes were displaced from the main metaphase plate (Fig. 1C), suggesting that some SVZ cells mis-segregate their chromosomes.

The predicted result of chromosome mis-segregation is aneuploidy. Rates of aneuploidy in the SVZ were determined by examination of metaphase chromosome spreads isolated from P5–P10 mice (Fig. 1D) as described previously (Barch et al., 1997; Rehen et al., 2001). Karyotyping of such spreads by staining with 4′,6′-diamidino-2-phenylindole hydrochloride (DAPI) or SKY (Lianage et al., 1996) provides an accurate estimate of chromosome number in neural cells (Rehen et al., 2001). DAPI staining (Fig. 1E,F) and SKY of 65 chromosome spreads from P5–P10 SVZ revealed frequent numerical, but not structural, chromosomal abnormalities in these cells. In contrast, karyotype abnormalities were rarely seen in stimulated peripheral lymphocytes, a standard cytogenetic control preparation (data not shown) (Rehen et al., 2001). The normal number of chromosomes in a diploid mouse cell is 40. One-third (33%) of cells from the postnatal SVZ did not have 40 chromosomes (Fig. 1G). A strong bias toward chromosome loss existed among aneuploid cells: ~25% had fewer than 40 chromosomes. Importantly, a majority of aneuploid cells was missing multiple chromosomes.

To additionally characterize the incidence of mosaic aneuploidy among normal somatic cells, we karyotyped mitotic cells from the livers of E13 mice. Among 48 mitotic liver cells karyotyped by SKY or DAPI staining, five (10.42%) were aneuploid (data not shown). This rate of aneuploidy was significantly lower than that seen among P5–P10 SVZ cells (p < 0.01; χ²) but was not significantly different from the rate seen among peripheral lymphocytes (3.4%), suggesting that the rates of mosaic aneuploidy are higher in the CNS than in other tissues.

Aneuploidy among interphase SVZ and postnatal-borne olfactory bulb cells in vivo

To confirm that many SVZ cells were aneuploid, we harvested nuclei from male P5–P10 SVZ and counted their sex chromo-
somes by interphase FISH with chromosome paints for mouse X and Y chromosomes (XY FISH) (Rehen et al., 2001). On the basis of karyotyping of mitotic SVZ cells and previous measurements of sex chromosome gain and loss among proliferating embryonic neuronal progenitors, 6–8% of SVZ nuclei were expected to have lost a sex chromosome. XY FISH measurement revealed 5% of cells examined had lost an X or Y chromosome (Table 1, postnatal SVZ), a rate near that measured for proliferating neuronal progenitor cells of the embryonic cerebral cortex (Rehen et al., 2001) and substantially above the false positive rate for this assay (<2%). XY FISH and karyotyping of metaphase chromosome spreads require different methods for sample preparation and capture cells in different stages of cell-cycle progression, yet the two techniques yielded complementary results. This demonstrated that some SVZ cells were aneuploid.

The substantial rate of aneuploidy among proliferating SVZ cells prompted us to ask whether aneuploidy persists in SVZ-born cells that migrate to the olfactory bulb. Aneuploidy was examined among OB neurons that are born in the postnatal SVZ and migrate to the granular and periglomerular layers of the OB through the rostral migratory stream (Lois and Alvarez-Buylla, 1994; Craig et al., 1999). These cells were identified by injecting 7-d-old male mice with bromodeoxyuridine (BrdU) (Fig. 2A). Eight days after injection, cell nuclei from the OB were harvested, processed for XY FISH, and immunostained for BrdU (Fig. 2A). In the OB, 5.97% of BrdU-positive cells lost or gained a sex chromosome (Fig. 2B; Table 1, postnatal-born OB), a rate slightly higher than that observed in all OB cells (4.33%) (Table 1, adult olfactory bulb). These results suggest that some of the aneuploid cells born in the SVZ are competent to migrate and survive as neurons in the OB.

Table 1. Sex chromosome loss and gain among SVZ and OB cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>XY (%)</th>
<th>XO (%)</th>
<th>OY (%)</th>
<th>XXX (%)</th>
<th>XYY (%)</th>
<th>Total abnormal (%)</th>
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<tbody>
<tr>
<td>Adult olfactory bulb</td>
<td>95.67</td>
<td>3.54</td>
<td>0.39</td>
<td>0.39</td>
<td>0.39</td>
<td>4.33</td>
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<tr>
<td>Postnatal SVZ</td>
<td>95</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Postnatal-born OB</td>
<td>94.03</td>
<td>2.24</td>
<td>0.75</td>
<td>2.24</td>
<td>0.75</td>
<td>5.97</td>
</tr>
<tr>
<td>SVZ neurons in vitro</td>
<td>91.03</td>
<td>7.62</td>
<td>0.45</td>
<td>0.9</td>
<td>0</td>
<td>8.97</td>
</tr>
<tr>
<td>SVZ glia in vitro</td>
<td>93.53</td>
<td>2.99</td>
<td>1.49</td>
<td>1</td>
<td>1</td>
<td>6.47</td>
</tr>
<tr>
<td>SVZ NPCs in vitro</td>
<td>92.24</td>
<td>3.67</td>
<td>1.63</td>
<td>1.63</td>
<td>0.82</td>
<td>7.76</td>
</tr>
</tbody>
</table>

Aneuploidy among the major SVZ cell types in vitro

What SVZ cell types are aneuploid? The SVZ is an especially useful preparation for determining the identities of aneuploid cells on the basis of previous studies that have categorized neurons, glia, and NPCs by immunohistochemistry and ultrastructure (Doetsch et al., 1997). Each cell type can also be cultured and purified in vitro (Reynolds et al., 1992; Lim and Alvarez-Buylla, 1999). To this end, P5–P10 SVZ cells were grown in media with serum for 5–10 d and microtubule-associated protein 2 (MAP-2) immunoreactive neurons (Fig. 3C) or glial fibrillary acidic protein (GFAP) immunoreactive (Fig. 3E) glial cells were isolated by the method of Lim and Alvarez-Buylla (1999). Alternatively, SVZ cells were grown without serum and with 20 ng/mL of EGF and 10 ng/mL of FGF-2 for 5–10 d to generate cultures highly enriched for stem and progenitor cells (Reynolds et al., 1992; Craig et al., 1996). XY FISH revealed that...
6–9% of cells of each type had gained or lost a sex chromosome (Fig. 3B–F; Table 1, SVZ neurons, glia, NPCs in vitro). Similar results were obtained when cells were hybridized with whole chromosome 14 paints (data not shown). These results demonstrated that aneuploidy was common to multiple CNS cell types. Given that the majority of dividing cells in the SVZ are progenitor cells (Doetsch et al., 2002), which in turn arise from glia-like stem cells (Doetsch et al., 1999), these results strongly suggest that somatosomal changes in NPCs are propagated to neurons in vivo.

Losses of heterozygosity in aneuploid SVZ cells in vitro and in vivo

One possible functional consequence of aneuploidy is altered gene expression in cells of the SVZ and OB. Given the preponderance of chromosome loss among aneuploid cells, many SVZ and OB cells likely harbor multiple losses of heterozygosity (Lengauer et al., 1998; Serra et al., 2001; Thiagalingam et al., 2002). Although normal diploid cells have two copies of each autosome, many aneuploid cells in the SVZ and OB have only one copy of at least one autosome. In these cells, loss of heterozygosity (LOH) would abolish expression of alleles on lost chromosomes, allowing only expression of alleles present on remaining ones.

This possibility was examined in a model system that captured the essential features of LOH: loss of expression of a globally expressed hemizygous transgene. We analyzed mice hemizygous for an enhanced green fluorescent protein (eGFP) transgene driven by the chick β-actin promoter (Okabe et al., 1997) and present at a single locus on chromosome 15 (Fig. 4A and data not shown). These mice were chosen because cells possessing the transgene express it constitutively at a high level (Okabe et al., 1997), and because the transgene is expressed by >90% of CNS cells (Fig. 5A). SVZ cells from these mice were cultured and analyzed for GFP fluorescence and chromosome 15 copy number. Approximately 25% of cultured SVZ cells appeared to not express the transgene (Fig. 4B, C). Among these cells, 23% had also lost one copy of chromosome 15 (Fig. 4, box 1). In contrast, 96% of GFP+ cells had two copies of chromosome 15 (Fig. 4, box 2), suggesting that loss of one copy of chromosome 15, most likely the copy carrying the eGFP transgene, had caused loss of eGFP expression through a mechanism similar to LOH.

Chromosome loss was also identified as a source of LOH in vivo. Acutely isolated SVZ or OB cells were separated on the basis of GFP expression by FACS, resulting in purified GFP+ and GFP− cells (Figs. 5A, 6A, B). For this analysis, GFP− cells were those with a GFP fluorescence peak area that was two SDs less than the mean for all cells (i.e., the 2% of cells with the least fluorescence). FISH for chromosome 15 identified 45–50% of GFP− cells in the SVZ and OB that had lost a copy of chromosome 15 (Fig. 5B). In contrast, only 2–12% of GFP+ cells lost a copy of 15, which reflected a loss of the nontransgenic copy of chromosome 15, because these cells were derived from mice with a hemizygous eGFP genotype.

To confirm loss of the copy of chromosome 15 carrying the eGFP transgene among GFP− cells, genomic DNA was taken from GFP+ and GFP− cells isolated by FACS from whole P2–P4 mouse brains and analyzed by semiquantitative genomic PCR for the eGFP transgene and the s1p3/fpb3/edg3 gene, a control gene present on chromosome 13 (Ishii et al., 2001, 2002). We found that the eGFP transgene was ~90% less abundant relative to s1p3 in GFP− cells compared with GFP+ cells (Fig. 5C). These results were obtained with multiple primer sets and sample replicates (data not shown). Together with the increased rate of chromosome loss among GFP− cells, this finding demonstrates that loss of eGFP expression in some CNS cells is caused by loss of the copy of chromosome 15 carrying the eGFP transgene in vivo.

Alteration of gene expression among cells with LOH at chromosome 15

Does chromosome loss alter expression of endogenous genes? To address this question, we harvested RNA from sorted GFP+ and GFP− SVZ cells and used oligonucleotide arrays to generate gene expression profiles of sorted cells and identify differentially expressed genes. To isolate the direct effects of LOH at chromosome 15, cultures of glia and glial-restricted NPCs (Levison and Goldman, 1997) expanded for 10 d in vitro were used. Use of these cultures rather than mixed cultures of neurons and glia allowed us to isolate effects of chromosome loss in a single cell type without the complication of potential differences in chromosome 15 loss rates, gene expression programs, or flow-cytometric parameters between neurons and glia. Among GFP+ and GFP− glial cells, gene expression profiling revealed differential expression of 22 genes (Table 2), including both downregulated and upregulated genes on several different chromosomes (data not shown). These data demonstrate that endogenous gene expression programs are altered by loss of chromosome 15, and such changes are not limited to the lost chromosome.

Proliferation and survival of cells with LOH at chromosome 15

To determine whether LOH at chromosome 15 affects the proliferation or survival of glial cells, we stained sorted SVZ glial cells for a marker of proliferation, phosphorylated-vimentin (AAA; phospho-Ser55-vimentin) (Tsujimura et al., 1994; Kamei et al., 1998), or cell death (cleaved caspase-3) (Pompeiano et al., 2000). SVZ glia and glial-restricted NPCs (Levison and Goldman, 1997) were used. Use of these cultures rather than mixed cultures of neurons and glia allowed us to isolate effects of chromosome loss in a single cell type without the complication of potential differences in chromosome 15 loss rates, gene expression programs, or flow-cytometric parameters between neurons and glia. Among GFP+ and GFP− glial cells, gene expression profiling revealed differential expression of 22 genes (Table 2), including both downregulated and upregulated genes on several different chromosomes (data not shown). These data demonstrate that endogenous gene expression programs are altered by loss of chromosome 15, and such changes are not limited to the lost chromosome.
cells were found in the OB, and the rate of caspase-3 cleavage was not significantly elevated among cells with LOH on chromosome 15. These findings strongly suggest that some aneuploid cells produced in the SVZ migrate and survive within the OB as postmitotic neurons, also consistent with previous studies on the adult cerebral cortex (Rehen et al., 2001). Together, data from the SVZ and OB may be interpreted to mean that genetic diversity is generated among proliferating cells in the SVZ and persists among OB neurons.

**High rates of chromosome loss among interphase cells**

In this study, the rates of loss of both autosomes and sex chromosomes in interphase SVZ and OB cells fell in the range of 2–8% (Table 2). It is important to consider these rates in the context of metaphase karyotypes. For example, if the loss rates observed for sex chromosomes and chromosome 15 in SVZ and OB cells are representative of all chromosomes, we would predict that 100% of cells would be missing one chromosome, which is clearly not the case. This difficulty is resolved by the fact that, among metaphase SVZ cells (Fig. 1G), ~8% lost only one chromosome, whereas ~17% lost multiple chromosomes.

Comparison of the rates of chromosome loss from metaphase and interphase cells provides a mechanism for cross-checking the accuracy of these measurements. On the basis of data from metaphase spreads, the average number of chromosomes among SVZ cells is 38.75 chromosomes per cell, suggesting an average chromosome loss rate of 5.13%. For all observations of sex chromosome loss among interphase cells, the average chromosome loss rate was 4.96%, leading to an average chromosome number estimate of 39.08, in agreement with the estimates from metaphase cells. This suggests that both interphase and metaphase estimates of chromosome loss rates in the SVZ are accurate (within 0.2% of one another) and in accord with previous data that establish the accuracy of these measurements in the cerebral cortex (Rehen et al., 2001).

**Loss of heterozygosity and alteration of gene expression by chromosome loss**

The functional consequences of neural cell aneuploidy have not been examined previously. Here, we have shown that chromosome loss among normal neural cells can cause LOH and, in turn, alter gene expression profiles, in line with previous results from tumor models and yeast (Hughes et al., 2000; Phillips et al., 2001). However, we note the possibility that upregulation or downregulation of some genes may be caused indirectly by other differences between GFP+ and GFP− cells such as altered GFP expression levels.

Differentially expressed genes provide clues to the possible functional consequences of chromosome loss. For example, three
Among GFP SVZ cells, evidence for subchromosomal genomic alterations in study the effects of mosaic aneuploidy in gene networks of neuronality time (von Dassow et al., 2000) and can be adapted to robustness or fragility of networks of cells and genes over evolutionary time. Such models are useful tools for understanding the functional consequences of chromosome loss in neural cells. However, our data suggest that alterations to other chromosomes may also occur on the backdrop of mosaic aneuploidy in the CNS, as suggested by the functions of DNA modifying and/or surveillance factors during neuronal development (Gao et al., 1998; Chun and Schatz, 1999a, b; Allen et al., 2001).

Future directions
This work demonstrates that chromosome loss produces permanent genomic changes in normal neural cells of the SVZ and OB, and such changes can alter gene expression in morphologically normal cells. However, our studies have not addressed the effects of differential expression of genes known to be involved in Ca²⁺ signal transduction: annexin A1, calpain small subunit 1, and ata2 (Perlmutter et al., 1988; Gerke and Moss, 1997). Of these, ata2 expression is increased threefold (3.02 ×), possibly to compensate for decreased expression of annexin A1 (−3.2 ×) and calpain small subunit 1 (−1.89 ×).

Despite these alterations of gene expression, proliferation and survival of cells with LOH at chromosome 15 was essentially normal. This suggests that aneuploid stem and progenitor cells may produce aneuploid neurons and glia at rates near those of euploid cells. However, there may be differences in the long-term viability of differentiated aneuploid SVZ cells relative to euploid ones. Along similar lines, the functional consequences of chromosome loss may be more pronounced in differentiated or stressed cells.

The loss of a chromosome is typically lethal to organisms (Miller and Therman, 2001). However, our data suggest that although chromosome loss does lead to haploinsufficiency and altered gene expression programs in SVZ cells, such changes are not necessarily lethal. Why not? Progress toward understanding the teleological causes and effects of chromosome loss in neural cells will likely be aided by studies of computer models of gene networks. Such models are useful tools for understanding the robustness or fragility of networks of cells and genes over evolutionary time (von Dassow et al., 2000) and can be adapted to study the effects of mosaic aneuploidy in gene networks of neurobiological interest.

Evidence for subchromosomal genomic alterations in SVZ cells
Among GFP− cells, the rate of depletion of the eGFP transgene (~90%) exceeded the rate of loss of chromosome 15 carrying the transgene (~50%). This disparity may reflect a high rate of deletions (~40% among cells that lose GFP expression) that encompass the eGFP transgene but not the entire copy of chromosome 15. We speculate that similar alterations to other chromosomes may be present in many CNS cells and other genomic alterations may also occur on the backdrop of mosaic aneuploidy in the CNS, as suggested by the functions of DNA modifying and/or surveillance factors during neuronal development (Gao et al., 1998; Chun and Schatz, 1999a; b; Allen et al., 2001).

Figure 4. Chromosome 15 loss is reported by loss of transgene expression in vitro. A, FISH detects an eGFP transgene integrated at a single locus on chromosome 15. (Two hybridization signals are visible in this chromatid pair.) eGFP expression is driven by the chicken β-actin promoter and cytomegalovirus enhancer; a high level of expression is expected in all cells in which the transgene is present. Subsequent analyses were performed in eGFP hemizygotes. B, C, Variation in eGFP fluorescence in SVZ cells in vitro. B is a Nomarski image and C shows eGFP fluorescence (green) and chromosome 15 FISH (red). Cells in boxes 1 (GFP−) and 2 (GFP+) are enlarged in adjacent panels. Boxes 1 and 2, GFP− cells frequently have only one copy of chromosome 15 (red). Cells in box 1 are GFP− and have only one copy of chromosome 15. The cell in box 2 is GFP+ and has two copies of 15.

Figure 5. Chromosome 15 loss is a significant source of LOH in vivo. A, GFP fluorescence histogram from FACS sorting of P5 SVZ. Note that ~2% of cells have very little GFP fluorescence (expanded view below). Similar results were obtained with OB cells (data not shown). B, Quantitation of chromosome 15 loss and gain among GFP− and GFP+ sorted cells from P5 SVZ and OB. Forty-five to fifty percent of GFP− cells have one copy of chromosome 15, whereas 88–98% of GFP+ cells have two copies. GFP+ and GFP− distributions from both SVZ and OB are significantly different from one another (*p < 0.00001; χ² test). C, Ethidium bromide-stained gel showing PCR-amplified eGFP (chromosome 15) or s1p3 (chromosome 13). Known amounts of gDNA from tail tissue (left panels) were used as a standard for estimating template quantities. In the experimental samples (right panels), the template was gDNA from FACS-sorted GFP+ or GFP− brain cells. The estimated template gDNA quantities are indicated below the amplified fragments (ng). On the basis of these numbers, the percentage of eGFP loss in the samples was determined and is listed below each lane. Experimental sample determinations were repeated >8 times (also with distinct primer pairs for GFP and another control gene, s1p2), and the percentage of eGFP loss averaged ~90%.
of increased chromosomal copy number (hyperploidy) (Rehen et al., 2001), nor the effects of more complex karyotype abnormalities such as the co-existence of chromosome gain and loss in a single cell. Similarly, subtle or indirect consequences of LOH via "trans" effects (transcription and signaling factors) or other extragenic modifiers (Hamilton et al., 1997) were not addressed, although we note the possibility of such effects through LOH. LOH among neural cells could unmask recessive traits (via dominant allele loss), which could not be rescued by gene amplification or chromosome gain. Scientific understanding of the effects of mosaic aneuploidy on normal brain function and pathology will benefit from a focus on these issues in future studies.

References

Table 2. Differential expression of genes in GFP + and GFP − cells

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Fold change expression*</th>
</tr>
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<tbody>
<tr>
<td>Expresses sequence AB52726</td>
<td>3.47</td>
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<tr>
<td>Expresses sequence AI552542</td>
<td>3.1</td>
</tr>
<tr>
<td>AT1A2</td>
<td>3.02</td>
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<td>Expresses sequence A682085</td>
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<td>Hydroxyysteroid (17β) dehydrogenase 12</td>
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<tr>
<td>RIKEN cDNA 5430432P15</td>
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<td>IMAGE clone 4019917</td>
<td>1.77</td>
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<td>cDNA sequence BCO33609</td>
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<td>Gene rich cluster, C9 gene</td>
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<td>Calpain, small subunit 1</td>
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<td>Phosphoprotein enriched in astrocytes 15</td>
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<td>Dscr28 related cytoplasmic tyrosine kinase</td>
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<td>Thymus cell antigen 1, θ</td>
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<td>N-myoc downstream regulated-like</td>
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<td>Annexin A1</td>
<td>3.19</td>
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<td>Stathmin-like 2</td>
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*Expressed relative to paired GFP + samples.


