Site-specific regulation of cell cycle and DNA repair in post-mitotic GABA cells in schizophrenic versus bipolars

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GABA cell dysfunction in both schizophrenia (SZ) and bipolar disorder (BD) involves decreased GAD_{67} expression, although this change involves fundamentally different networks of genes in the 2 disorders. One gene that is common to these 2 networks is cyclin D2, a key component of cell cycle regulation that shows increased expression in SZ, but decreased expression in BD. Because of the importance of cell cycle regulation in maintaining functional differentiation and DNA repair, the current study has examined the genes involved in the G_1 and G_2 checkpoints to generate new hypotheses regarding the regulation of the GABA cell phenotype in the hippocampus of SZ and BD. The results have demonstrated significant changes in cell cycle regulation in both SZ and BD and these changes include the transcriptional complex (TC) that controls the expression of E2F/DP-1 target genes critical for progression to G_2/M. The methyl-CpG binding domain protein (MBD4) that is pivotal for DNA repair, is significantly up-regulated in the stratum oriens (SO) of CA3/2 and CA1 in SZs and BDs. However, other genes associated with the TC, and the G_1 and G_2 checkpoints, show complex changes in expression in the SO of CA3/2 and CA1 of both SZs and BDS. Overall, the patterns of expression observed have suggested that the regulation of functional differentiation and/or genomic integrity of hippocampal GABA cells varies according to diagnosis and their location within the trisynaptic pathway.

cyclin D2 | DNA polymerase | G_2 checkpoint | nicotinic receptors | p53

Schizophrenia (SZ) and bipolar disorder (BD) involve striking decreases in GAD_{67} expression (see Table S1) in the hippocampus, particularly in stratum oriens of sector CA3/2 (1), where preferential abnormalities have also been found in other postmortem studies (2). Network association analyses have suggested that GAD_{67} expression may be linked to cyclin D2 expression and has raised the possibility that the regulation of cell differentiation (1) and DNA damage (3) may play a role in GABA cell dysfunction in SZ and BD. Consistent with this idea, functional clusters of genes (refer to Table S2) showing the most robust changes in gene expression include neurogenesis, cell cycle regulation and the DNA damage response, particularly in stratum oriens of CA3/2 and CA1 (4). To learn more about the regulation of cell cycle in hippocampal GABA cells in SZ and BD, a post hoc analysis has assessed whether there is evidence for changes in the expression of genes involved in the G_1 and G_2 checkpoints, critical elements in the maintenance of terminal differentiation and DNA repair in postmitotic neurons (5). It was quite surprising to see that there were so many genes involved in the regulation of cell proliferation and cell identity or loss, showing significant changes in what are assumed to be terminally differentiated neurons in the adult hippocampus of both SZs and BDs.

The molecular events related to cell cycle regulation vary according to established canonical pathways that include not only cyclin D2, but also cyclin E; both form complexes with the cyclin dependent kinases, CDK4/6 and CDK2, respectively. The cyclin E/CDK2 complex and CDC42 (6) are particularly important for cell cycle progression because they can cause G_1 arrest to fail and allow a cell to move into the (S) phase where DNA replication is stimulated (7). The S phase then progresses toward the G_2 checkpoint where 3 possible outcomes are possible: (*i*) the formation of the cyclin B/CDC2 complex and entry into a mitotic division; (*ii*) the successful repair of damaged DNA and the survival of a functionally differentiated cell; or (*iii*) the inability to repair an over-whelming amount of DNA damage and entry into the apoptotic cascade (8). The G_2 checkpoint, arguably the most critical stage, is mediated by p53, a sequence-specific transcription factor that can promote genomic integrity or cell death, depending on the degree to which DNA has been damaged (9). When p53 forms a complex with the replication protein (RPA3), DNA repair can occur (10) in a threshold-dependent manner (11). Other genes, such as BRCA1, a tumor suppressor that activates the G_2 checkpoint genes (12) and a variety of DNA polymerases (see Table S3), play central roles in the regulation of the DNA repair response.

The progression of G_1 toward the G_2 checkpoint depends on many other genes, particularly those associated with a transcriptional complex that regulates the expression of cyclin E and other critical genes. The latter include E2F, a positive regulator of transcription (13) and EP300, a histone acetyltransferase that releases promoter activity via chromatin remodeling (14). Another key element is the retinoblastoma protein (Rb), which, by forming a complex with the tyrosine kinase ABL (15), suppresses the transcriptional complex (16). When E2F forms a protranscriptional complex with DP-1 (17) and Rb is phosphorylated by a CDK, the transcriptional complex can be switched to the "ON." state This switch promotes the expression of key genes, such as E2F and cyclin E, that can further promote cell cycle progression. The transcriptional repressor activation of methyl-CpG binding protein 4 (MBD4) depends on histone deacetylase (HDAC) (18). When DNA is damaged, MBD4 forms a complex with the DNA mismatch-repair protein (19) and has the ability to repress the expression of E2F and other target genes. The response to DNA damage is determined by the expression of many different DNA polymerase genes (see Table S3).

Additionally, several different growth factors, such as FGF, drive the proliferation of neuronal precursor cells during development (20). For example, TGFβ inhibits this progression via the repressive effect of SMAD3 on CDC25A (21) and neuregulin I plays a role in the growth and differentiation of neurons (22) and glia (23). Finally, nicotinic receptors have also been associated with both the survival of differentiated neurons or

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cytotoxic changes in undifferentiated cells (24). Interestingly, nicotine treatment is associated with a failure of G_1 arrest that involves a dysregulation of Rb and E2F activity (25) and over-expression of the alpha 7 nicotinic subunit has been found to prevent G_1 arrest (26).

This was undertaken to generate hypotheses regarding the regulation of cell cycle regulation and the DNA damage response in SZ and BD. As discussed below, a remarkable number of the genes involved in cell division and cell identity showed significant expression changes in cells that are assumed to be terminally differentiated neurons in the adult hippocampus of both SZs and BDs. These findings suggest that cell cycle regulation in GABA cells may be circuitry-based and vary according to their location and integration within the trisynaptic pathway (see Fig. S1). Additionally, the results suggest that there may be fundamental differences in the status of cell differentiation and genomic integrity in GABAergic interneurons in SZs versus BDs.

Results

Samples of the strata oriens (SO), pyramidale (SP) and radiatum (SR) of sectors CA3/2 and CA1 of the hippocampus were obtained from normal controls (CONs), SZs and BDs (Table S4). The samples were collected by laser microdissection (LMD) of cresyl violet-stained cryostat sections as described in ref. 1. Although the percentage of present calls in the SP and SR were similar to those obtained for the SO, the data for these layers were not included in this report because there were relatively few individual genes that showed significant changes in expression and the composite probability, P_c, for the functional clusters of genes study did not reach the level ($P_c \leq 10^{-10}$) needed for inclusion in the post hoc analyses (27) (refer to Table S2). For these reasons, the data reported here are exclusively from the SO where the most significant changes in gene expression were observed.

Consistent with conventional microscopic criteria (28), interneurons in the SO showed a distinctive cresyl violet staining of their cytoplasm and light-staining, diffuse chromatin material in their nuclei. Glial cells typically showed no cytoplasmic staining, but large amounts of heterochromatic material in their nuclei. This fundamental difference between neurons and glia suggests that the concentrations of cytoplasmic RNA in interneurons is much higher than in glial cells. Additionally, in situ hybridization demonstrated that the antisense RNA associated with a broad array of genes, including, but not limited to GAD_{67}, GAD_{65}, HDAC1, DAXX, PAX5, and Runx2 (see Fig. S2 for HDAC1, DAXX, and PAX5), was abundantly localized over neuronal cell bodies in the SO, whereas little or no autoradiographic signals were detected over glia.

Functional Clusters of Genes. The results of the GenMapp analyses demonstrated that there were 3 clusters of genes with particularly significant changes in expression and these included: neurogenesis, cell cycle, and the DNA damage response (Table S2 and Table S5).

Stratum Oriens of CA2/3. ***Schizophrenia.*** In SZs (Table 1 and Fig. 1), TGFβ2, neuregulin I, FGF2, VEGF, the nicotinic α4 receptor subunit (Table S6), and cyclin D2 were all up-regulated, suggesting that the phase of this GABA cell population may be arrested in early G_1. For the transcriptional complex, MBD4, HDAC1 and its corepressor DAXX were also up-regulated, changes that could shut-down the transcription complex (29) associated with cell cycle progression. E2F showed decreased expression, providing further support for the possibility that the transcriptional complex is modulated in the "OFF" state and could result in reduced expression of target genes, such as cyclins A and E, which drive G_1 toward the G_2 checkpoint (30). Cyclin D2 showed a significant increase of expression and a similar finding has been observed in a recent postmortem study of the cingulate region in SZs (31, 32). Very few changes in the expression of genes specific for the G_2/M phase were observed. p53 and CHK2 show normal expression in this group, which suggests that the G_2 checkpoint is capable of inhibiting progression toward mitosis in SZs (12). Additionally, some DNA repair may be occurring at this locus I SZs, because BRCA1 and DNA polymerase iota (POLI) (see Table S3) were both up-regulated. The latter change could be associated with inaccurate copying of templates that result in point mutations on chromosomes of GABA cells in CA3/2-SO in SZ. In contrast, a decrease in the expression of POLD (Table 1 and Table S3), was also observed in the SZs (33).

Bipolar disorder. In BDs, the regulation of the G_1 and G_2 checkpoint mechanisms in CA3/2 seemed to be quite different from that seen in SZs (Table 1 and Fig. 1). TGFβ1, neuregulin I, FGF3 and 9, neurotrophin 3, VEGF, cyclin D2, CDK9 and HDAC3 were all down-regulated. Four different nicotinic receptor subunits, including the alpha 7 isoform, showed a significant decrease in expression at this locus in BDs (Table S6). Significant decreases in the expression of ANAPC1 (APC1), the anaphase promoting complex 1 (34) were also observed in BDs. A decrease of APC1 activity can potentially contribute to a failure of G_1 arrest and functional differentiation (35). However, another isoform ANAPC5 (APC5) was up-regulated at this locus in BDs, suggesting that these 2 changes could compensate one another, if both changes are present in the same GABA cells. The increased expression of SMAD3 (1), a key component of the TGFβ signaling pathway, could help to promote neuronal differentiation (36) and further suppress the down-regulated cyclin D2/CDK9 complex.

As in the SZ group, the expression of MBD4 was also significantly increased at this locus in BDs. In the setting of normal levels of the expression of E2F and the phosphorylation of Rb by CDK2, the transcriptional complex may be capable of increasing the expression of E2F/DP-1 target genes and could theoretically promote progression toward the G_2 checkpoint. However, another key gene, CDC42, was also down-regulated.

Other G_2 checkpoint genes, such as p53, CHK2 and BRCA1 were also down-regulated. Of these, p53 may be particularly important at the CA3/2-SO locus in BDs, because it is capable of promoting excisional repair (37). Accordingly, the decrease of p53 expression observed at this locus in BDs may contribute to the repair of single nucleotide mutations (9). Consistent with this, DNA polymerase lambda (POLL) and gamma (POLG) (refer to Table S3) both showed decreased expression at this locus in BDs (refer to Table 1). The replication protein (RPA3), which interacts directly with p53 in the setting of DNA damage (11), was also significantly decreased in expression.

SO-CA1. ***Schizophrenia.*** In the stratum oriens of CA1, there was an increased expression of MBD4 and ABL1, but a decreased expression of E2F, suggesting that the transcriptional complex at this locus, like its counterpart in CA3/2, may also be modulated in the "OFF" state in SZs (Table 1 and Fig. 1). Unlike CA3/2, however, TGFβ2 and VEGF both showed decreased expression, without any associated change in the expression of cyclin D2. Noteworthy was the finding of a significant increase in the expression of cyclin E and the RPA3 replication protein. Although these latter changes are consistent with the possibility that G_1 arrest might have been failing in SZs, there appears to be a dissociation between these latter changes and those in the transcriptional complex, which may be modulated in the "OFF" state. POLD, another DNA polymerase (refer to Table S3), and BRCA1, were both decreased in expression, whereas CHK2 and p53 both showed

Table 1. Target Genes in Neurogenesis, Cell Cycle and DNA Damage Response of Schizophrenics and Bipolars

Schizophrenia				Bipolar Disorder			
CA3/2 Stratum oriens							
	Neurogenesis						
		FC	**P**			**FC**	**P**
FGF2	Fibroblast Growth Factor 2	1.33	0.006	**FGF3**	Fibroblast GF 3	-1.62	0.013
NRG1	Neuregulin I	1.59	0.03	**FGF9**	Fibroblast GF 9	-11.33	0.0007
VEGF	Vascular Endothelial GF	2.13	0.036	**NRG1**	Neuregulin 1	-1.77	0.022
SOX11	Preserves cells in undiff. state	-3.14	0.0057	**VEGF**	Vascular Endothelial GF	-1.41	0.03
				PAX5	Paired box gene 5	-1.48	0.0267
				NTF3	Neurotrophin 3	-1.57	0.00096
	Cell Cycle Regulation						
CCD2	Cyclin D2	1.44	0.027	**CCD2**	Cyclin D2	-2.18	0.0007
E2F3	E2F Transcription Factor	-1.77	0.043	**HDAC3**	Histone Deacetylase 3	-1.36	0.022
HDAC1	Histone Deacetylase I	1.49	0.022	**CDC37L1**	Cell Division Cycle 37 Homolog	-2.34	0.0066
TGFB2	Transforming GF beta2	1.43	0.016	**TGFB1**	Transforming GF beta 1	-1.40	0.0088
BRCA2	Breast CA 2	1.35	0.042	**CDK9**	Cyclin Dependent Kinase	-1.44	0.036
				CDC42	Cell Division Cycle 42	-3.37	0.00018
				ANAPC1	Anaphase Promoting Complex	-2.39	0.0019
				ANAPC5		3.13	0.047
	DNA Damage Response						
MBD4	Methyl-CpG Binding Domain	2.91	0.016	**MBD4**	Methyl-CpG Binding Domain	1.75	0.014
POLD3	DNA-Directed Polymerase delta 3	-2.87	0.00027	**RPA3**	Replication Protein A3	1.35	0.042
POLI	DNA-Directed Polymerase iota	1.84	0.00065	**BRCA1**	Breast Cancer 1	-1.42	0.031
				CHEK2	CHK2 checkpoint homolog	-1.46	0.038
				TP53	Tumor Protein p53	-1.51	0.024
				POLG2	DNA-directed Polymerase	-1.48	0.0147
				POLL	DNA-directed Polymerase γ	-1.30	0.03
CA1-Stratum oriens							
	Neurogenesis						
VEGF	Vascular Endothelial GF	-1.47	0.032	**FGF6**	Fibroblast Growth Factor	-1.24	0.011
				EP300	E1A binding protein	3.62	0.000007
				NRP1	Neuropilin; axon guidance	1.92	0.044
				ROBO1	Roundabout axon guidance rec.	1.84	0.008
				NTRK3	Neurotrophic tyrosin kinase rec.	1.94	0.022
				STMN3	Strathmin-like 3	1.19	0.05
	Cell Cycle Regulation						
ABL1	v-abl viral leukemia factor	1.51	0.027	**ABL1**	v-abl murine leukemia virus	1.50	0.012
E2F1	claudin 7 transcription factor	-1.43	0.039	**E2F1**	claudin 7 transcription factor	-1.54	0.029
CCNE2	Cyclin E2	2.07	0.033	**E2F3**	E2 TF 3	2.02	0.019
CDC2L1	Cell Division Cycle-like 2L1	-1.42	0.036	**TP53BP2**	Tumor protein p53 binding prot.	2.45	0.046
CDC42	Cell Division Cycle 42	1.48	0.044	**CDK6**	Cyclin dependent kinase 6	-1.32	0.008
TGFA	Transforming GF alpha	-1.44	0.043	**CDC2L2**	Cell division cycle 2-like	-1.65	0.048
TGFB3	Transforming GF beta	-1.56	0.018	**CCNE2**	Cyclin E2	2.15	0.005
				ANAPC1	Anaphase promoting complex	-1.25	0.036
				ANAPC5	Anaphase promoting complex	4.37	0.041
				HDAC3	Histone Deacetylase 3	-1.50	0.0045
				TGFA	Transforming GF A	1.31	0.044
				TGFB2	Transforming GF β	3.00	0.031
	DNA Damage Response						
MBD4	Methyl CpG Binding Protein	1.77	0.04	**MBD4**	Methyl CpG domain protein	1.97	0.0002
RPA3	Replication Protein 3	1.4	0.006	**RFC5**	Replication factor	1.43	0.042
BRCA1	Breast CA	-1.92	0.01	**BRCA3**	Breast CA	-2.03	0.006
POLD3	DNA Directed Polymerase	-2.46	0.012	**POLH**	DNA directed polymerase	-1.64	0.00002
				APEX1	DNA repair enzyme	1.66	0.008

The data shown represent the fold changes (FC) and probability of significance (*P*) for individual genes within the neurogenesis, cell cycle regulation and DNA damage response clusters that are related to the maintenance of G_1 arrest and/or its progression toward the G_2 checkpoint in the stratum oriens of sectors CA3/2 and CA1 of subjects with schizophrenia and bipolar disorder. The red and blue fill patterns for FCs indicates increased or decreased expression, respectively, for the various genes. There are a total of 54 different genes listed, but many (42.4%) appear in both groups and/or in CA3/2 and/or CA1. For example, TGFβs, NRG1, VEGF, CCD2, ANAPC1 and ANAPC5, ABL1, E2F1 and E2F3, BRCA1, MBD4, POLD3, and RPA3 all appear in more than one locus or diagnostic group. MBD4 is unique because it showed a significant up-regulation in CA3/2 and CA1 of both SZs and BDs.

normal expression. As shown in Table 1, SOX11, which ensures the expression of neuronal traits (38), showed a robust decrease in expression (FC = −3.14; $P = 0.0057$) at this locus of SZs.

Bipolar disorder. The most striking changes in the transcriptional complex in CA1-SO of BDs was and increased expression of E2F3 (2.02-fold; $P = 0.019$) and EP300 (3.6-fold; $P = 0.000007$). These robust changes in E2F and EP300 could ensure that the transcriptional complex is regulated in the "ON" state at this locus in BDs. Consistent with this, cyclin E (2.15-fold; $P = 0.005$) also showed a significant increase of expression, a change that would further promote progression toward the G_2 checkpoint. Additionally, CDC2L2, APC1 and CDK6 were also down-regulated. The expression of p53, the replication factor (RFC5) and the DNA polymerase APEX1 (refer to Table S3) were all increased. Another polymerase,

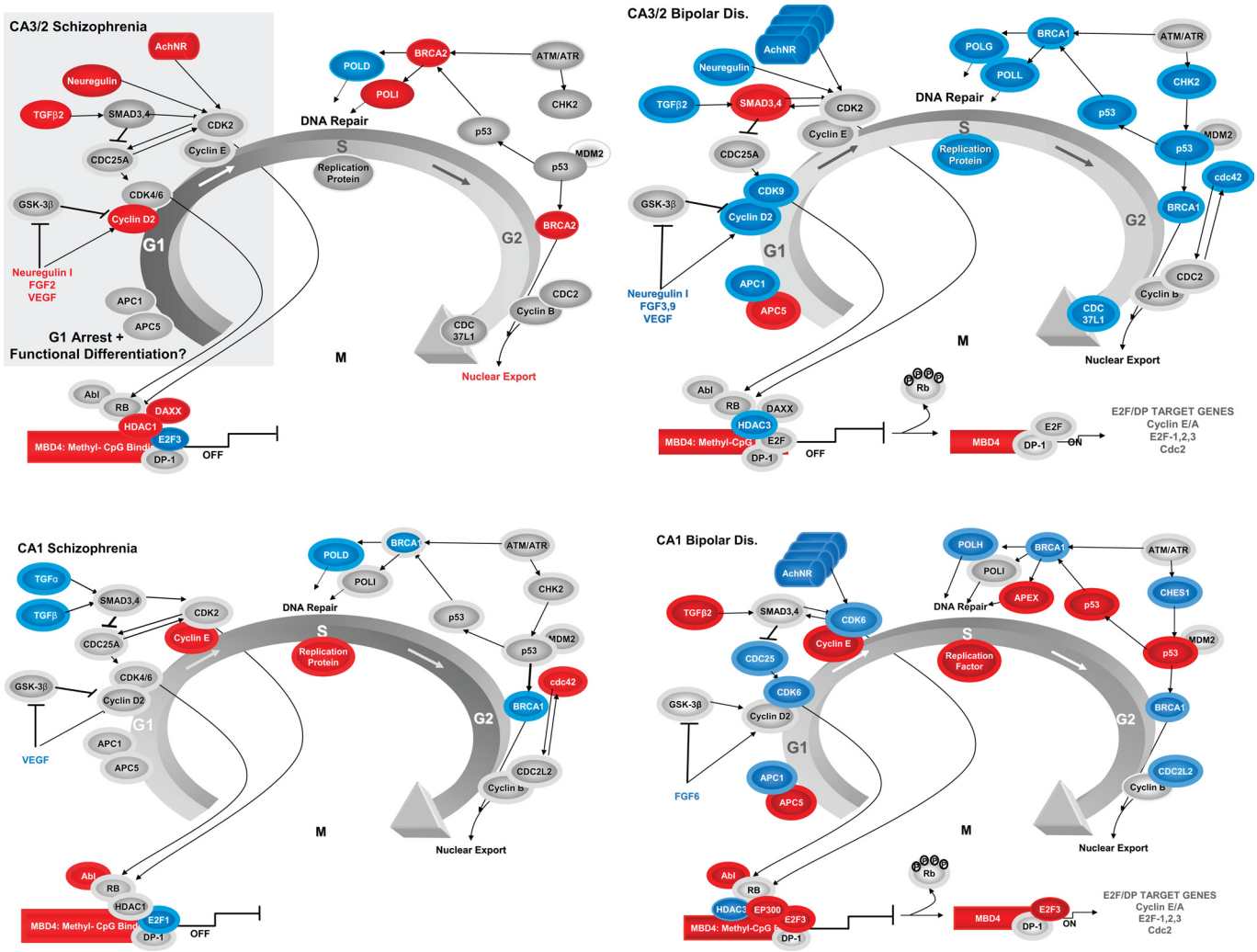

Fig. 1. The regulation of G_1/S and G_2/M phase in GABA cells of the stratum oriens (SO) in CA3/2 and CA1 of schizophrenics (SZs) (*Left*) and bipolars (BDs) (*Right*). In each of the diagrams, the G_1/S and G_2/M phases of cell cycle and their associated genes. Genes showing increased, decreased, and no change in expression are indicated in red, blue, and gray, respectively. CDKs in G_1 (arrows) are capable of phosphorylating Rb and turning the transcriptional complex ''ON.'' MBD4 is significantly up-regulated in the SO of CA3/2 and CA1 of both groups, suggesting that DNA repair may be commonly occurring in hippocampal GABA neurons at these loci in SZs and BDs. Other genes involved in the maintenance of G_1 arrest and progression to G_2 show many different expression patterns at each locus of the 2 groups. Overall, the data suggest that the transcriptional complex may be in the ''OFF'' state in SZs and in the ''ON'' state in BDs. Many different DNA polymerases (see Table S3) show significant changes in expression at the different loci of both SZs and BDs, suggesting that the cell cycle apparatus in these GABAergic interneurons may be primarily involved in the repair of damaged DNA.

POLH (Table S3), however, was down-regulated. CHES1and BRCA1 were also down-regulated. As with the SO in sector CA3/2 of BDs, 4 different nicotinic receptor subunits showed a significant decrease in expression in CA1 (see Table S6) and included the $\alpha 1$, $\alpha 2$, $\beta 3$ and γ subunits. Significant increases in the expression of neuropilin (NRP1) (39), roundabout (ROBO1) (40), and strathmin 3 (STMN3) (41), genes that are believed to play a role in the plasticity of axons and dendrites, were also observed (Table 1)

SR and SP of CA2/3 and CA1. There were very few changes in the regulation of genes associated with neurogenesis and cell cycle in the SR and SP of either sector in the SZ or BD groups.

Effects of Psychotropic Medications. As shown in Fig. S3*A* and Fig. S3*B* for the genes included in the cell cycle and DNA damage response clusters, respectively, the SZ subjects were broken down into those receiving low (< 500 mgs per day) or high (>500 mgs per day) dose antipsychotic medications (APDs) during the year before death. The SZs showed many more genes with significant differences in the low dose group, whereas BDs had many more significant genes in the high dose group. Assessments of the expression patterns for individual genes with GenMapp functional clusters did not show significant overlap in the 2 groups. The SZ and BD groups showed a high degree of correspondence in the medication regimens, which suggests that psychotropic agents do not explain the changes in gene expression observed, particularly because the respective profiles were fundamentally different in the 2 groups.

Discussion

This study demonstrates that genes associated with the cell cycle apparatus and the transcriptional complex that regulates it show complex expression changes that vary according to sector, layer, and psychiatric diagnosis. These results support the hypothesis that the regulation of cell cycle in hippocampal GABA cells involves a complex interplay among transcription

factors that regulate promoter activity associated with E2F/DP-1 target genes and their association with the G_1 and G_2 checkpoints. Additionally, a variety of growth factors, trophins, and nicotinic receptor subunits also help to determine whether G_1 arrest is maintained or whether it progresses toward the G_2 checkpoint. Particularly noteworthy is the significant increase in the expression of MBD4 in GABA cells in both CA3/2 and CA1 of SZs and BDs. This gene encodes a glycosylase that prefers substrates in which a G:T mismatch is present in the context of methylated or unmethylated CpG sites (42); an increase in its expression could indicate that the transcriptional complex may be suppressed. However, other genes associated with the regulation of this complex (e.g., HDAC, E2F, and ABL) probably help to determine whether the "ON" state may be achieved and whether progression from the G_1 to the G_2 checkpoint may occur in terminally differentiated GABA cells in the adult hippocampus. Generally speaking, the data presented here point to the transcriptional complex in GABA cells of the SO as playing a central role in the regulation of cell cycle progression and/or DNA repair in GABA cells of the SO in SZ and BD.

Taking together the expression changes in the transcriptional complex with those observed for cell cycle, it appears that GABA cells in the SO of CA3/2 of SZs may be more likely to suppress CpG islands of the transcriptional complex and help to maintain G_1 arrest and functional differentiation. The analogous population of interneurons in CA3/2 of BDs showed widespread decreases in the expression of genes required for progression to the G_2 checkpoint and DNA repair. The decreased expression of p53 and 2 different DNA polymerases at this locus may indicate that DNA repair is inhibited at this locus in BDs. In CA1-SO of BDs, however, the increased expression of p53, RFC5 and APEX1 suggest the opposite; GABA cells at this locus may be attempting to repair DNA damage associated with oxidative stress (43). Consistent with this possibility, GABAergic cells at this locus in BDs may be in a hypermetabolic state (see Fig. S1 *Lower*), as indicated by the very pronounced increase in the expression of genes involved in glycolysis, the Kreb cycle and the electron transport chain (1).

In summary, the results reported here suggest that genes associated with cell cycle regulation and DNA repair in hippocampal GABA cells show significant changes in expression that vary not only according to diagnosis, but also their integration within the trisynaptic pathway. As shown in Fig. S1, the patterns of connectivity are quite different within each locus of this circuit and this, in turn, may influence the regulation of gene expression in GABA cells (1). For example, in the SO of CA3/2, inputs from the basolateral amygdala and septal nuclei may help to establish gene expression changes that are unique to this locus (for a review of the connectivity, see ref. 4). The extrinsic and intrinsic afferent fiber systems that modulate the activity of GABA cells within the trisynaptic pathway probably contribute significantly to the maintenance of functional differentiation and genomic integrity in these terminally differentiated interneurons (27).

Overall, the interpretation of the expression changes reported here is based on studies of "simple" in vivo and in vitro systems in which cell proliferation or death can readily occur (44). In subjects with SZ or BD, however, the respective molecular endophenotypes within GABA cells of the adult hippocampus are probably much more complex than has heretofore been appreciated. In Alzheimer's disease, neurons that are at risk for degeneration are also at risk of reinitiating a cell cycle process that involves the expression of cell cycle proteins and DNA replication (44). Although failure of cell cycle regulation may be a root cause of several neurodegenerative disorders, it might also be a final common pathway for brain diseases, such as bipolar disorder.

Methods

Subjects. Participating in the study were 7 normal controls (CONs), 7 schizophrenic subjects, and 7 bipolar subjects (BD) from the Harvard Brain Tissue Resource Center at McLean Hospital matched for age, postmortem interval (PMI), hemisphere, gender, and tissue pH (Table S4). The procedures for retrospective diagnoses and neuropathological evaluations are described in refs. 1 and 27.

Tissue Preparation and RNA Extraction. A total of 7 frozen tissue sections (8 μm) were cut from each block on a Microm HM 560 CryoStar cryostat, mounted on LEICA Frame Slides with a PET-membrane (1.4 μm), and fixed in Streck tissue fixative (STF) (Streck Laboratories). The frame slides were mounted on a LEICA AS LMD apparatus and tissue samples from SO, SP, and SR of CA2/3 and CA1 were microdissected. Each vial into which the laser dissected specimens fell by gravity contained a small volume of a lysis/denaturing solution containing an RNase inhibitor. RNA extraction was undertaken with a Qiagen Rneasy micro kit yielding ≈20 to 30 ng of RNA. RNA quality was assessed using an Agilent 2100 bioanalyzer. Fifteen micrograms of biotinylated target RNA were fragmented and individually hybridized to the HU-133A arrays (Affymetrix). The microarrays were then stained with 2 rounds of streptavidin-phycoerythrin (Molecular Probes) and one round of biotinylated anti-streptavidin antibody (Vector Laboratories), and scanned twice. RNA quality was assessed using tissue pH, the 18S/28S ratio and the percentage of present calls for each case (see Table S4).

Data Analyses. The DNA Chip Analyzer (dChip) Version 1.3 software package (32) was used to evaluate the percentage of present calls and the significance of differences between the normal controls vs. the SZs or BDs. The variance obtained with the perfect match model of dChip ($R^2 = 0.001$) was considerably lower than that seen with any of the other models and was used throughout the first stage analysis of the microarray data. Biologically relevant clusters of genes were identified by using GenMapp algorithms (www.genmapp.org). A metric, called the composite probability, P_c, was computed for each GenMapp biopathway or cluster. The P value for inclusion of individual genes in each GenMapp cluster was set at $P \leq 0.05$. Genes meeting this criterion were multiplied by one another (i.e., P_1, $P_2 \ldots P_i$) for each pathway. This resulting number was divided by the ratio of the number of genes meeting the inclusionary criterion (n_i) and the number of genes potentially showing significant changes (n_t) in each pathway. The total number of GenMapp pathways was represented as N_p. The following equation was used:

$$P_c = [(P_1 \times P_2 \times P_3 \ldots P\mathrm{i})/(n_i/n_t)]\, N_p$$

This equation provides 2 separate corrections for multiple comparisons by multiplying by both N_p and n_t. The α-level of significance for each GenMapp biopathway or cluster is established by examining the distribution of genes that met criteria for inclusion in the analysis and the final P value is equal to the value obtained with the equation described above. GenMapp pathways with $P_c = 5 \times 10^{-10}$ or lower were considered to be significant. The latter number was based on the number of genes per biological cluster showing significance and the robustness of the P values for individual gene within the clusters.

Validation Studies. The method used for qRT-PCR described in ref. 1. As shown in Fig. S4, the genes studied covered a broad range of transmitter and receptor subunit isoforms, ion channels and transcription factors including GAD_{67}, GAD_{65}, GRIA1, GRIK1, GRIK2, HCN3, HCN4, KCNJ3, KCNJ6, HDAC1, LEF1, Runx2, and PAX5 (Fig. S4). The SO of CA3/2 and CA1 showed the most pronounced changes in expression when compared with SR and SP of these 2 sectors (see also Table S2). The target genes also showed changes in the same direction as those observed with the microarray analyses. One exception is GAD_{67} in SR of CA3/2 where expression was significantly decreased in the microarrays for the SZs, but showed no change with qRT-PCR. In addition to qRT-PCR validations, in situ hybridization was used to evaluate whether the expression of mRNA for HDAC1, DAXX, PAX5 (see Fig. S2), Runx2, GRIK1 and GRIK2 occurred in primarily interneurons versus glial cells.

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