Comparison of gene expression profiling during postnatal development of mouse dentate gyrus and cerebellum

SAKAЕ SAITO,1,2 RYO MATOBA,1,2 NORIKO UENO,1 KENICHI MATSUBARA,1,2 AND KIKUYA KATO1,2
1Taisho Laboratory of Functional Genomics, Nara Institute of Science and Technology; and 2Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, 8916-5 Takayama, Ikoma, Nara, 630-0101, Japan

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Whether this global change in gene expression is a common event has yet to be determined. The cerebellar cortex and the dentate gyrus have several common features. Both tissues consist mainly of granule cells. Development proceeds similarly (1, 2, 15); cerebellar granule cells transplanted into the dentate gyrus differentiate into dentate gyrus-type cells, suggesting that they share a similar repertoire of expressed genes (18). Finally, neuroD, a basic helix-loop-helix (bHLH) transcription factor, is essential for differentiation of both types of granule cells (13). To assess whether the molecular mechanisms of development are truly similar in the two tissues, we compared the gene expression profiles during postnatal development in the cerebellum and dentate gyrus. Physiologically, the cerebellum controls posture and movement, whereas the hippocampus is critical in the formation of declarative memory. This underlying functional difference may be reflected in their respective mechanisms of development. We measured the expression levels of 1,937 genes during the postnatal development of the dentate gyrus by adaptor-tagged competitive PCR (ATAC-PCR) (8, 11). The resulting expression profile was compared with that obtained from cerebellar tissue undergoing the same postnatal developmental stages (12). Contrary to expectation, the gene expression profile of the dentate gyrus was quite different from that of the cerebellum and is poorly correlated with function.

MATERIALS AND METHODS

ATAC-PCR. Mouse brain coronal sections were excised manually at the interaural 3 and 0 mm indicated in the atlas by Franklin and Paxinos (5). For smaller samples (2- and 4-day-old animals), a cryostat was used. The dentate gyrus was excised from these sections under a stereomicroscope, avoiding contamination with regions CA1–CA3. Total RNA was purified using Trizol (Invitrogen).

Genes for the ATAC-PCR assay were selected from our in-house EST database, the Brain EST Database (BED, accessible from http://love2.aist-nara.ac.jp). BED is based on
a collection of 3’-end sequences from cDNA libraries made from several developmental stages of mouse cerebellum (10) hippocampus, and other brain regions (Matoba et al., unpublished observations). Genes were selected in order of abundance, with priority given to known genes. More than 2,500 genes were selected, and gene-specific primers were designed.

The experimental procedures and data processing of ATAC-PCR were essentially the same as those used for previous experiments with mouse cerebellum (11, 12). RNA preparations from each sample were converted into cDNA, digested by a restriction enzyme, and then ligated to an adaptor. We routinely used multiple adaptors, each having a common sequence in the “outer” region that lies next to an “inner” spacer of variable size. After mixing the ligated samples together, PCR amplification was performed using an adaptor primer and a gene-specific primer. The products were separated by polyacrylamide gel electrophoresis. Products from different samples can be discriminated by the size of their inner spacer region. The abundance of each fragment reflects the amount of original template, and relative expression levels in each sample can be deduced from their signal intensities.

Usually, six different cDNA samples attached to different adaptors were used, three of which were assigned to different amounts of control cDNA samples. For the dentate gyrus experiment, cDNA derived from the adult cerebrum was used as the control; 10, 3, and 1 portions of cDNA with different adaptors were included in each PCR reaction. Similar to the cerebellar samples, one portion each of three out of six dentate gyrus samples was included in the reaction. PCR amplification was performed with a carboxyfluorescein (FAM)-labeled adaptor primer, corresponding to the common adaptor region, and a gene-specific primer. Products were separated by polyacrylamide gel electrophoresis. For each PCR reaction, a calibration curve was made using three control samples. Thus accurate quantitation of the three dentate gyrus samples can be achieved. Sequences of oligonucleotides used for adaptors and primers are described previously (12).

Statistical analysis. Cluster analysis was performed using ClustanGraphics4, developed by Wishart (20). The data matrix was at first standardized to z-score, and cluster analysis was performed using Ward’s method (20). Several clustering methods were evaluated using data from postnatal cerebellar development (12) and breast cancer (6). In both cases, Ward’s method was found to give the most reasonable classifications correlating with gene functions or clinical parameters.

Statistical tests to select functional categories enriched in specific clusters or groups were based on the binomial distribution.
distribution. The probability that $x$ out of $n$ genes of a functional category, belonging to a specific group or cluster, is given by the following equation
\[
f(x) = \binom{n}{x} p^x (1 - p)^{n-x}
\]
where $p = \frac{\text{number of genes belonging to a functional category}}{\text{total gene number}}$.

RESULTS

Anatomy of the developing dentate gyrus. The development of the dentate gyrus has been analyzed in detail in both rat (1) and mouse (15). Granule cells proliferate somewhat during the prenatal period, but more than 80% of the cell mass develops during the postnatal period, mainly in the tertiary germinal matrix of the hilus in the dentate gyrus. The tertiary germinal matrix is a rump of immature cells, not a layered structure such as the external germinal layer of the cerebellar cortex. Proliferation is most active in the first postnatal week (~81% of the cells are actively proliferating for 1–4 days) (15) and decreases thereafter. The differentiated cells migrate to the granule cell layer. At 17–20 days, ~11% still have mitotic activity. At the third week, the dentate gyrus is morphologically fully mature. In contrast to the cerebellar cortex, where no cell proliferation remains in the adult, mitotic cells still remain in the subgranular zone of the dentate gyrus in the young adult, a neurodevelopmental phenomenon seen only in dentate granule cells and interneurons of the olfactory bulb (3).

The temporal pattern of cell proliferation is similar in the dentate gyrus and cerebellum of mice. In both tissues, mitotic activity peaks in the first week, and development appears morphologically complete in the third week (15). In our previous study on mouse cerebellar cortex, we chose 2, 4, 8, and 12 days and 3 and 6 wk as relevant time points for evaluation (12). Here, with the dentate gyrus, we chose the same time points for gene expression analysis. Although the developmental timing is not exactly the same for both tissues, general trends, such as the predominance of proliferation at early stages and active neural function at later points, appear to be conserved.

Gene expression profiling of postnatal developing dentate gyrus. Relative levels of gene expression against the control (mouse adult cerebrum) were assayed at six time points by ATAC-PCR. A total of 1,937 genes were assayed, and the data were subjected to cluster analysis after z-score standardization. A schematic representation of the results is shown in Fig. 1. The clustering was truncated at the 12-cluster level. Most genes belonged either to group A, characterized by elevated expression at earlier stages of development, or to group B, characterized by elevated expression at later stages of development. Less than 10% of the genes exhibited a pattern characterized by elevated expression at 2 and 12 days (group C). The results clearly demonstrate organized changes in gene expression during the developmental process.

To correlate gene expression patterns with their respective gene functions, the distribution of keywords representing functional categories was examined (12). Keywords exhibiting localization to a particular cluster or group were identified. Statistical analysis of the twelve clusters revealed that only "proteasome" was localized significantly to clusters A1 and A2. Expression patterns of 12 cluster levels of each tissue are not adequate for comparison, because expression patterns of the same cluster number from cerebellum and dentate gyrus were not necessarily the same. We therefore compared the expression-function correlation of the two tissues at the three-group level. In Fig. 2, functional categories stati-

<table>
<thead>
<tr>
<th>Mouse Cerebellum</th>
<th>Mouse Dentate Gyrus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A group</strong></td>
<td><strong>B group</strong></td>
</tr>
<tr>
<td>cancer-related</td>
<td>10</td>
</tr>
<tr>
<td>ribosomal protein</td>
<td>35</td>
</tr>
<tr>
<td>RNA synthesis</td>
<td>0</td>
</tr>
<tr>
<td>carbohydrate metabolism</td>
<td>3</td>
</tr>
<tr>
<td>brain</td>
<td>33</td>
</tr>
<tr>
<td>ion channel &amp; transporter</td>
<td>2</td>
</tr>
<tr>
<td>synapse component</td>
<td>1</td>
</tr>
<tr>
<td>neurotransmitter receptor</td>
<td>1</td>
</tr>
<tr>
<td>oligodendroglia</td>
<td>0</td>
</tr>
<tr>
<td>cerebellar-dominant</td>
<td>8</td>
</tr>
<tr>
<td>intracellular ion transporter</td>
<td>1</td>
</tr>
<tr>
<td>proteasome</td>
<td>7</td>
</tr>
<tr>
<td>growth control</td>
<td>6</td>
</tr>
<tr>
<td>secretary component</td>
<td>4</td>
</tr>
<tr>
<td><strong>total gene number</strong></td>
<td><strong>584</strong></td>
</tr>
</tbody>
</table>

Fig. 2. Functional categories enriched in specific groups of gene expression patterns during mouse cerebellar or dentate gyrus development. Each value represents the number of genes belonging to each group and each functional category. Dark shading indicates statistically significant enrichment. Light shading indicates statistically significant rare cases.
cally enriched in any one of the groups are shown for the cerebellar and dentate gyrus experiments. “Ribosomal protein” is less represented in the group B in both tissues, suggesting the protein synthesis is not active at later stages of development. For the most part, however, these two tissues exhibited clearly contrasting enrichment patterns: nine functional categories were enriched in group B of the cerebellum, whereas none was enriched in the corresponding group from the dentate gyrus. Functional categories enriched in cerebellar group B were those related to neural functions such as receptors, neurotransmitters, and synaptic components, suggesting neuronal differentiation of the cerebellar granule cells had occurred. In contrast, the results of Fig. 2 suggest that no such organized changes occur in the dentate gyrus, suggesting differentiation of dentate gyrus granule cells is more modest than that of cerebellar cells.

Identification of genes with expression patterns common to both tissues. We then tried to identify genes whose expression patterns were similar in the cerebellar cortex and the dentate gyrus and examined their characteristics. A total of 1,412 genes were expressed both in the cerebellum and the dentate gyrus. As variables, all data points from both the cerebellar cortex and the dentate gyrus were used. Each set of data points, either from the cerebellum or the dentate gyrus, was standardized to z-score. Figure 3 shows the results of cluster analysis. To assess the similarity in expression patterns between the two tissues, the following squared distance \( d \) was used

\[
d = \sum_{i=1}^{n} (x_{\text{cb}} - x_{\text{dg}})^2
\]

where \( n \) is 6, the number of time points; \( i \) represents the time points themselves, which are 2, 4, 8, and 12 days and 3 and 6 wk; \( x_{\text{cb}} \) is the gene expression level in the cerebellum at the \( i \) time point; and \( x_{\text{dg}} \) is the gene

Fig. 3. Left: cluster analysis of combined gene expression patterns. A total of 1,412 genes are vertically aligned. The data matrices of cerebellum and dentate gyrus are individually standardized to z-score, as in Fig. 1, and schematically shown. Columns represent the following time points: 2 days after birth (column 1), 4 days (column 2), 8 days (column 3), 12 days (column 4), 3 wk (column 5), and 6 wk (column 6). The other features are the same as those in Fig. 1. Right: graphic representation of the \( d \) value, representing similarity of the two gene expression patterns. Note that genes numbered from 1050 to 1135 have the most similar gene expression patterns.

Fig. 4. Gene expression of members of functional categories. The data are schematically shown as in Figs. 1 and 3. Cells in the left frames represent expression levels during cerebellar development, and those in the right frames represent levels during dentate gyrus development. The time points of each expression patterns are, from left to right: 2 days, 4 days, 8 days, 12 days, 3 wk, and 6 wk. The color scale is the same as in Fig. 1.
**Expression Profiling during Dentate Gyrus Development**

**Ribosomal Protein**
- M. ribosomal protein K1-4n4p13 mRNA
- Rat ribosomal protein L35 mRNA
- M. ribosomal protein S26 mRNA
- Rat ubiquitin fused rp32Ss2 mRNA
- M. ribosomal protein S11 mRNA
- Rat ribosomal protein S17 mRNA
- Rat ribosomal protein S13 mRNA
- Rat ribosomal protein L34 mRNA
- M. ribosomal protein L30 mRNA
- Rat ribosomal protein L18S mRNA
- Rat ribosomal phosphosrotein P2 mRNA
- M. ribosomal protein S12 mRNA
- Rat ubiquitin fused rpL16DCEP52 mRNA
- Rat ribosomal protein L24 mRNA
- Rat ribosomal protein S23 mRNA
- Rat ribosomal protein L32 mRNA
- Rat ribosomal protein L23 mRNA
- M. ribosomal protein S59 mRNA
- M. ribosomal protein S66 mRNA
- M. ribosomal protein L7a/surfel3 gene
- Rat ribosomal protein S19 mRNA
- M. ribosomal protein S14 mRNA
- M. ribosomal protein S5 mRNA
- M. ribosomal protein S26 mRNA
- M. ribosomal protein L7 mRNA
- Rat ribosomal protein S10 mRNA
- Rat ribosomal protein S15a mRNA
- M. ribosomal protein S4 mRNA
- M. ribosomal protein L8 mRNA
- M. heparin-binding mPr22968p15 mRNA
- M. ribosomal protein L12 mRNA
- Rat ribosomal protein S27 mRNA
- Rat ribosomal protein L15 mRNA
- Rat ribosomal protein L2 mRNA
- M. ribosomal protein L33 mRNA
- Hamster 16S ribosomal RNA gene
- Rat ribosomal protein S28 mRNA
- Rat ribosomal protein L19 mRNA
- Rat ribosomal protein L38 mRNA
- Rat ribosomal protein L36a mRNA
- Rat ribosomal protein S7 mRNA
- M. J1 protein/hpl3 mRNA

**Receptor & Synaptic Component**
- M. leucine mRNA
- M. plasma membrane protein syntaxin-4 mRNA
- M. GABA A receptor delta subunit mRNA
- Rat synaptic glycoprotein SC2 mRNA
- M. protein tyrosine phosphatase PTTP9 mRNA
- M. rab5a gene
- Rat synaptotagmin binding zyg14 mRNA
- M. neuroginin 1 mRNA
- M. Ac393phosphatase mRNA
- M. phosducin-like protein SNAP-25 mRNA
- Rat vesicle associated membrane protein VAMP-2 mRNA
- Rat Ras-related protein R6B mRNA
- M. synaptotagmin associated protein SNAP-25 mRNA
- M. AMPA receptor subunit GluR-B gene
- Rat receptor tyrosine phosphatase-lambda mRNA
- Rat neuronal pentraxin precursor mRNA
- M. GABA A receptor gamma-2 subunit mRNA
- M. synaptophysin mRNA
- Rat p65 mRNA

**Adhesion Molecule & Extracellular Matrix Protein**
- M. neural cell adhesion molecule NCAM-140 mRNA
- Mouse mRNA for OB-cadherin-1 mRNA
- M. extracellular matrix
- M. osteonectin Sparc gene
- Mouse prelactin mRNA
- M. desmin mRNA
- M. SHPS-1 mRNA
- M. platelet glycoproteinⅡb beta gene
- M. prelactin-receptor FN-1 mRNA
- M. extracellular matrix associated protein Sc1 mRNA
- Rat aggrec mRNA
- Polycystic kidney disease 1 protein Pkd1 mRNA
- M. cysin-like rich glycoprotein SPARC mRNA
- M. matrix Gla protein MGP mRNA
- Rat neurexinⅠI-alpha gene

**Transcription Factor**
- STAT
- M. NR5F1 mRNA
- M. nuclear transcriptional repressor Mnh1 mRNA
- Mus musculus BALB/c zinc-finger protein Blimp-1 mRNA
- M. DRTF-polypeptide-1-D1 mRNA
- Rat zinc finger protein mRNA
- M. protein Myc mRNA
- M. homeobox protein Meis-2d mRNA
- M. p53-inducible zinc finger protein Wtg-1 mRNA
- M. cellular nucleic acid binding protein mRNA
- M. A10 mRNA
- M. novel leucine zipper protein Minip mRNA
- Creb5 exosome regulatory element binding protein SREBP-1 mRNA
- M. zinc finger protein Requiem mRNA
- Sox-2
- M. homeobox Pex mRNA
- KROX-24
- c-Jun
- M. GT12 protein mRNA
- M. transcription factor USF2 mRNA
- M. zinc finger protein alpha-CRM1 gene mRNA
- M. neurogenic differentiation factor neuroD mRNA
- M. CIP2
- ME2 class A helix-loop-helix transcription factor ME2
- M. RING zinc finger protein Rj2 mRNA
- M. matf1 gene
- M. transcription factor ISGF3 gamma subunit gene
- M. JUN mRNA
- Rat Pex-6 mRNA
- M. CCAAT binding transcription factor CBF subunit C mRNA
- Rat TIP120 mRNA
- M. BHLH transcription factor ME1 gene
- Mouse mRNA for mouse transcription factor k4a (A7)
- Mus musculus aromatic hydrocarban receptor nuclear translocato
- Mus musculus skm-BOP2 (Bop) mRNA
- M. thymus beta protein mRNA
- M. Jun coactivator JAB1 mRNA

**Cancer-related**
- M. translationally controlled 21 kd polypeptide mRNA
- M. translationally controlled 340 mRNA
- M. c-yes mRNA
- M. eot gene
- M. insulin rib mRNA
- M. thyroid hormone receptor c-erbB alpha 2 mRNA
- M. BGLT7 mRNA
- M. adenomatous polyposis coli homolog 1B mRNA
- c-Jun
- M. FXX06-binding protein 25 homolog mPKBP25 mRNA
- M. c-sis kinase Csk mRNA

**Proteasome**
- M. proteasome beta-subunit C5 Pamb1 allele B mRNA
- M. proteasome M33 mRNA
- Rat proteasome subunit RC9 mRNA
- M. proteasome delta subunit gene
- Rat liver proteasome ATPase MSP1 mRNA
- M. 26S proteasome ATPase CIP21 mRNA
- Rat proteasome component C5 mRNA
- M. proteasome component B5 mRNA
- M. beta proteasome subunit Lmp3 mRNA
- Rat proteasome subunit B5 mRNA
- M. proteasome activator PA28 beta subunit mRNA
- M. proteasome alpha PA28 subunit mRNA
- Rat proteasome subunit R4 α mRNA
- M. proteasome Z subunit mRNA
- Rat proteasome subunit RC104mA mRNA
- M. 26S proteasomes novel ATPase subunit mRNA
- Rat proteasome subunit R-ZETA mRNA

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expression level in the dentate gyrus at the \( i \) time point.

In Fig. 3, the \( d \) value is plotted alongside the gene expression patterns in both tissues. The original data set is available as Supplementary Material\(^1\) for this article, published online at the *Physiological Genomics* web site; this material is also from our web site (http://love2.ai-st-nara.ac.jp).

Selection of genes only by a small \( d \) value is not appropriate. Reciprocal patterns with small quantitative differences could have smaller \( d \) values that are not relevant. On the other hand, similar patterns, one with marked quantitative changes and the other with small changes, could have high \( d \) values. Therefore, it is necessary to combine cluster analysis of expression patterns with the \( d \) value analysis.

Several regions were identified with a small \( d \) value; the region spanning from gene 1050 to 1135 (indexed as found in the Supplemental Material) is of particular interest. This largest group is characterized as having elevated expression through the later stages of the development. No functional characteristics were associated with genes in this region.

**Expression patterns of individual genes.** Genes belonging to several functional categories were examined in detail. Figure 4 shows only expression patterns of genes appearing in both experiments. The order of genes was determined by the results shown in Fig. 3.

"Ribosomal protein" genes exhibit characteristic expression profiles in both tissues. In the cerebellum, more than one-half of the genes peak at 4 days after birth, indicating a time of maximal protein synthesis. On the contrary, more than one-half of the genes from the dentate gyrus have their peak at either 2 or 12 days. This may suggest that dentate gyrus development may have two peaks in protein synthesis.

In cerebellum, 8 of 11 "cancer-related" genes are more active in earlier stages of development. In contrast, there is no such tendency in the dentate gyrus. This result suggests that the proliferative character of cerebellar cells, but not dentate gyrus cells, diminishes over the course of development. This may reflect the persistence of dentate gyrus cell proliferation in adulthood.

Although the proteasome genes are statistically enriched in group A of the dentate gyrus, their expression patterns are varied. In contrast, expression patterns in the cerebellum are clearly divided into two groups: those expressed highest in earlier stages and those expressed highest at 3 wk.

The functional categories "receptor" and "synaptic component" are the most conspicuous indicators of neural maturation. Most of the genes belonging to these clusters show elevated expression in later stages or adult in the cerebellum. In the dentate gyrus, eight genes show similar patterns, but nine genes are expressed highest at 4 days. This peak at 4 days implies some neuronal activity at this stage. Individual neurotransmitter receptors and ion channels usually are differentially expressed members of closely related gene families, whose members have distinct physiological properties. For example, detailed analysis of the electrophysiological properties of the GABA\(_A\) receptor revealed marked differences between those expressed in the immature and mature dentate gyrus (7, 9). Those activated at 4 days may be related to specific characteristics of signal transduction at this stage.

The functional categories "adhesion molecule" and "extracellular matrix protein" comprise a group of genes exhibiting elevated expression at later stages of the development (Fig. 4). They are featured by transient high expression at 3 wk in the cerebellum and at 12 days or 3 wk in the dentate gyrus, indicating specific roles at these stages. In contrast, the expression pattern of the *reeler* gene, which is known to be involved in the development of layered structures, is not consistent. In the cerebellum, reeler exhibits elevated expression during middle stages, where cell migration is most active (Fig. 4; also, see Ref. 16). On the contrary, its expression in the dentate gyrus is most elevated in the adult. In general, expression of genes in the dentate gyrus does not appear to correlate well with their expected functions.

bHLH transcription factors are likely to be involved in cell fate determination of various neurons (14). Among them, *neuroD* is a gene responsible for terminal differentiation of cerebellar and dentate gyrus granule cells (13). bHLH activities have been confirmed by gene targeting experiments. In a *neuroD* knockout strain, both types of granule cells are depleted in adulthood. *neuroD* is expressed both in mitotic and postmitotic dentate and cerebellar granule cells (13). Dentate granule cells are affected earlier (embryonic day 18) than cerebellar granule cells (postnatal day 6) (13). This observation may be related to the expression pattern of *neuroD*: its expression reaches a peak in the dentate gyrus earlier than in the cerebellum (12).

**DISCUSSION**

As described previously, the cerebellar cortex and the dentate gyrus show similar postnatal development. In both tissues, more than 80% of all genes exhibit changes in gene expression during development, demonstrating considerable changes in mRNA repertoires. However, detailed examination of the functional categories of genes revealed marked differences. In the cerebellar cortex, granule cells at earlier developmental stages are involved in cell proliferation, whereas those in the adult have neuronal functions. During development, granule cells show changes in functional characteristics. In the dentate gyrus, the repertoire of expressed genes also changed at each developmental stage. However, few cell functions could be correlated with gene expression. Among cerebellar and hippocampal neuronal cell types, only the dentate granule cells maintain mitotic activity in adulthood (3), although
these proliferating cells decrease to about one-eighth of the maximum number during development. This discrepancy in gene expression profiles might be due to this mitotic activity; the dentate granule cell might partially retain the characteristics of an immature cell, and the transition to cellular function may not be clear.

The other possibility is that changes in phenotype may be due to changes in protein levels, either quantitative or qualitative (e.g., modification), and changes in gene expression are irrelevant or secondary in the dentate gyrus. In the experiment on mouse cerebellar development, the levels of half of the proteins did not agree with those of mRNA (11). In the dentate gyrus, changes in protein levels may be more critical than in gene expression.

The present findings on the cerebellum and the dentate gyrus demonstrate that the development of layered brain structures do not necessarily possess common features in their gene expression profiles. The principle of gene expression-function correlation established in the cerebellum cannot be generalized to other parts of the brain. Unlike experiments with the same tissue or cell line, comparison of gene expression profiles appear to have limitations due to many unknown factors. However, the present findings further elucidate mechanisms of brain development and will be helpful for designing further experiments.

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