

Supplemental Methods

Preparation of RNA and cRNA targets for microarray analyses

Embryos were dissected from the uterus obtained from pregnant female rats under deep anesthesia. To prepare RNA of distinct brain regions, the rostral part of WT and *Pax6* homozygous (*rSey*²/*rSey*²) embryos were first cut off at the upper forelimb level, and then subdivided into two parts including the forebrain (FB) and the hindbrain (HB), respectively, by cutting at the isthmus. Thus, the FB tissue also included the eye and nose primordia and the midbrain, and the HB tissue included pharyngeal arches as well. All the following procedures were according to the manufacturer's protocols. Total RNA from the two regions was prepared by TRIzol Reagent (Invitrogen). mRNA was purified from 250 μ g of total RNA by OligotexTM-dT30 <Super> mRNA Purification Kit (Takara). Approximately 2 μ g of mRNA was reverse transcribed by Superscript II reverse transcriptase (Invitrogen) with T7- (dT)₂₄ primer containing a T7 RNA polymerase promoter sequence. Then, double strand DNA (dsDNA) was synthesized by SuperScriptTMChoice System (Invitrogen), and used to generate biotinylated antisense cRNA by *in vitro* transcription reaction with BioArrayTMHighYieldTMRNA Transcript Labeling Kit (ENZO Diagnostics). Finally, 20 μ g of biotinylated antisense cRNA targets were fragmented for microarray experiments according to the manufacturer's protocol.

Microarray procedures

The detailed protocol for the microarray processing was available from Expression Analysis Technical Manual (Affymetrix). 15 μ g of fragmented cRNA targets were hybridized to Rat U34 A Array (Affymetrix) containing 8,799 rat cDNA sequences (5,382 known genes and 3,417 EST clusters) for 16 hours at 45°C with constant rotation at 60 rpm (hybridization oven 640). After hybridization, the microarray was washed and stained on an Affymetrix fluidics station using the fluidics station protocol (EukGE-WS2), and fluorescent intensities for individual genes were scanned by HP GeneArray Scanner. Hybridization experiments were conducted in duplicate.

Data analyses

To quantify gene expression levels of transcribed, the average difference value (Adv), Presence (P), Absence (A) and Marginal (M) calls were calculated from intensities against probe set by global methods of normalization using Microarray Suite ver.4.0 software (MAS4) (Affymetrix). The ratio of 3'/5'Adv for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was analyzed to assess the quality of cRNA targets. The quality and reproducibility of the duplicated microarray analyses were summarized in Supplemental Table 1A. Commonly up-or down-regulated genes in the duplicate analyses were listed after normalization by MAS4. For up-regulated genes, the Absent (A) genes in *rSey*²/*rSey*² samples were omitted, while for down-regulated genes, the Absent (A) genes in WT samples were omitted. The numbers of reproduced genes were

summarized in Supplemental Table 1B. Furthermore, the comparative expression analyses between WT and $rSey^2/rSey^2$ rat samples, each average difference value (Adv) was normalized by per-chip normalization methods with the default percentile (50th) to the distribution of all genes using GeneSpring 4.2 Gene Expression Analysis Software (Silicon Genetics). The normalized average difference value (nAdv) in duplicate experiments was used to draw a scattered plot (Supplemental Figure 1). Genes that fulfill the following criteria were selected as down- or up- regulated genes in $rSey^2/rSey^2$ FB or HB regions: (1) the $nAdv > 0.02$, (2) the fold change >2.0 or <-2.0 (Table 1, Supplemental Table 2A and B). Additionally, the summary of microarray data including the quality and reproducibility of each microarray was shown in Supplemental Table 1.