

Research article

Open Access

## Developmental expression profile of the *yy2* gene in mice

David Drews<sup>1,3</sup>, Martin Klar<sup>1</sup>, Christof Dame<sup>\*†1</sup> and Anja U Bräuer<sup>†2</sup>

Address: <sup>1</sup>Department of Neonatology, Charité – Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany, <sup>2</sup>Institute of Cell Biology and Neurobiology, Charité – Universitätsmedizin Berlin, Philippstraße 12, 10115 Berlin, Germany and <sup>3</sup>Department of Biology, Chemistry and Pharmacy, Freie Universität Berlin, Takustraße 3, 14195 Berlin, Germany

Email: David Drews - david.drews@charite.de; Martin Klar - martin.klar@charite.de; Christof Dame\* - christof.dame@charite.de; Anja U Bräuer - anja.braeuer@charite.de

\* Corresponding author †Equal contributors

Published: 28 July 2009

Received: 18 March 2009

BMC Developmental Biology 2009, 9:45 doi:10.1186/1471-213X-9-45

Accepted: 28 July 2009

This article is available from: <http://www.biomedcentral.com/1471-213X/9/45>

© 2009 Drews et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Abstract

**Background:** The transcription factor Yin Yang 2 (YY2) shares a structural and functional highly homologue DNA-binding domain with the ubiquitously expressed YY1 protein, which has been implicated in regulating fundamental biological processes. However, the biological relevance of YY2 has not been identified yet.

**Results:** Towards the understanding of YY2 biology, we analyzed in detail the expression pattern of *yy2* in various organs during embryonic and postnatal mouse development till adulthood. Thereby, a constant *yy2* level was detected in heart and lung tissue, whereas in different brain regions *yy2* expression was dynamically regulated. Interestingly, in any analyzed tissue neither the homologue *yy1* nor the *mbtps2* gene showed changes in mRNA expression levels like *yy2*, although the intronless *yy2* gene is located within the *mbtps2* locus.

Furthermore, we detected *yy1*, *yy2*, and *mbtps2* mRNA in primary mouse neurons, microglia cells, and astrocytes. In comparison to *yy2* and *mbtps2*, *yy1* revealed the highest expression level in all cell types. Again, only *yy2* showed significantly altered gene expression levels among the cell types. Higher *yy2* expression levels were detected in microglia cells and astrocytes than in primary neurons.

**Conclusion:** *Yy2* expression in the heart and lung is constitutively expressed during embryogenesis and in adult mice. For the first time, developmental changes of *yy2* transcription became obvious in various areas of the brain. This suggests that *yy2* is involved in developmental gene regulation.

### Background

The biology of the transcription factor Yin Yang 2 (YY2) is not well characterized yet, but may be of particular interest due to its similarities to the ubiquitously expressed YY1, which has been implicated in fundamental biological processes such as DNA-replication, cell cycle regulation and organogenesis [1-5]. YY2 is a zinc finger protein

that shares 56% sequence homology with YY1 [6]. Especially, the C-terminal DNA-binding zinc finger is highly conserved [6-8] and mediates a similar binding specificity to the 5'-(A/c/g)(A/t)NATG(G/a/t)(C/a)(G/c/t)-3' DNA consensus motif [8,9], suggesting synergistic or competitive functions, if both factors act within the same cell [10]. The biological significance of *yy1* is highlighted by early

embryonic lethality of mice with homozygous  $\gamma 1$  deletion. In addition, mice with heterozygous  $\gamma 1$  ablation show growth retardation and defects in neurulation [3].

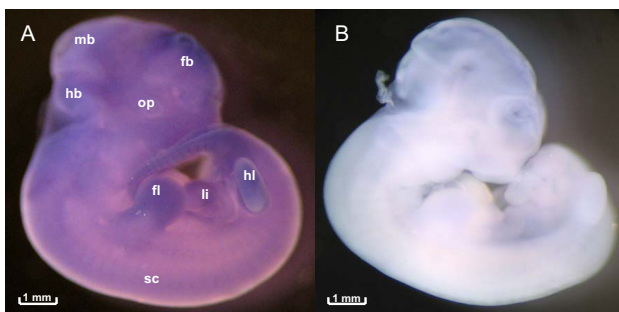
Analysis of  $\gamma 2$  ablation has not been reported, yet. The intron lacking  $\gamma 2$  gene is extraordinarily positioned between exons 5 and 6 of another X-chromosomally located gene encoding *mbtps2* (membrane bound transcription factor protease site 2). First expression analyses by *in situ* hybridization in testis, ovaries and brain from adult mice suggested a shared control of  $\gamma 2$  and *mbtps2* gene activities [7]. However, our data indicate that the upstream region of the human *YY2* gene mediates significant promoter activity independently from *MBTPS2* [11].

To gain more information on the biology of  $\gamma 2$ , we established whole mount *in situ* hybridization. In parallel, real-time PCR was used to quantify  $\gamma 2$  mRNA expression in various organs of developing and adult mice more precisely. Herein, we demonstrate that the  $\gamma 2$  mRNA expression pattern differs from that of  $\gamma 1$  and *mbtps2*. Importantly,  $\gamma 2$  expression underlies significant changes during development, particularly in various areas of the brain.

## Results

### Expression of $\gamma 2$ in mouse embryos

Since there are only limited data available on the organ-specific  $\gamma 2$  expression [6,7], we established the whole mount *in situ* hybridization technique. First experiments with DIG-labeled riboprobes showed wide spread signals of  $\gamma 2$  transcripts on the entire E11.5 embryo (Figure 1A), suggesting ubiquitous expression similar as described for  $\gamma 1$  [3]. The specificity of the antisense riboprobe staining



**Figure 1**  
**Expression of  $\gamma 2$  in developing mice.** A. Whole mount *in situ* hybridization of E11.5 embryo with a DIG-labeled  $\gamma 2$  antisense riboprobe. For a better orientation different regions are indicated; forebrain (fb), midbrain (mb), hindbrain (hb), optical vesicles (op), spinal cord (sc), forelimb buds (fl), liver (li) and hindlimb buds (hl). B. Hybridization with the  $\gamma 2$  sense riboprobe served as control (magnification: 4-fold).

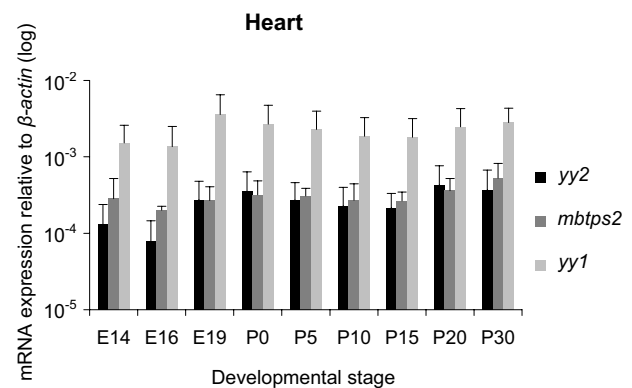
is underlined by the negative control, where the embryo was incubated with the corresponding sense riboprobe (Figure 1B). To analyze the  $\gamma 2$  expression pattern more precisely, we subsequently performed TaqMan real-time PCR in selected organs and tissue specimens of developing and adult mice.

### Quantitative analysis of $\gamma 2$ expression in heart and lung

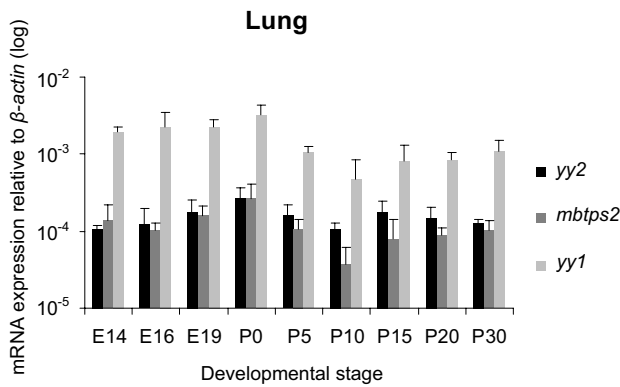
Specimens of the heart and lung, taken at several developmental stages, were dissected, and cDNAs were amplified out of isolated RNA. We analyzed samples spanning from E14 to P60, since in earlier developmental stage region-specific specimens cannot be isolated due to technical limitations. By using TaqMan real-time PCR technique with FAM-labeled probes, we tested the specimens also for  $\gamma 1$  and *mbtps2* transcripts. For all three transcripts we found almost stable expression level throughout all tested developmental stages of the heart (Figure 2) and lung (Figure 3). However, in contrast to the highly expressed  $\gamma 1$ , the amount of  $\gamma 2$  and *mbtps2* transcripts was clearly lower in all developmental stages. Interestingly,  $\gamma 2$  and *mbtps2* showed always similar expression levels. Thus, neither the  $\gamma$  genes nor *mbtps2* showed any significant changes in expression during heart and lung development.

### Quantitative analysis of $\gamma 2$ expression in the brain

Previously, the expression of  $\gamma 2$ ,  $\gamma 1$  and *mbtps2* in the neocortex and cerebellum of adult mice has been shown by *in situ* hybridization, but not quantified [7]. To analyze the gene expression during development we used the quantitative real-time PCR technique. As shown in Figure

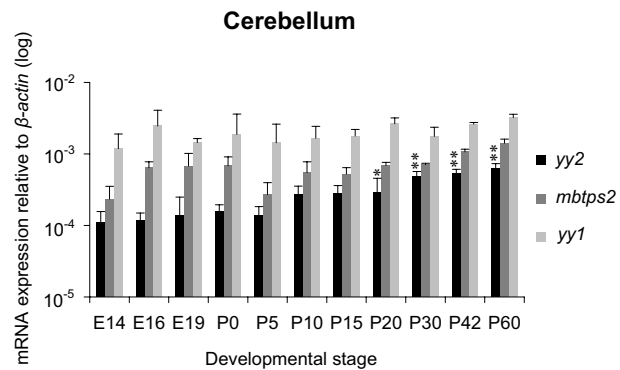


**Figure 2**  
**Quantification of  $\gamma 2$ , *mbtps2* and  $\gamma 1$  in developing heart.** Expression levels of  $\gamma 2$ , *mbtps2* and  $\gamma 1$  transcripts in the heart during development and in adult mice (E14 to P30) quantified by TaqMan real-time PCR. The expression is normalized to  $\beta$ -actin. All three gene products are constitutively expressed at constant levels throughout all stages. (n = 3 sets).

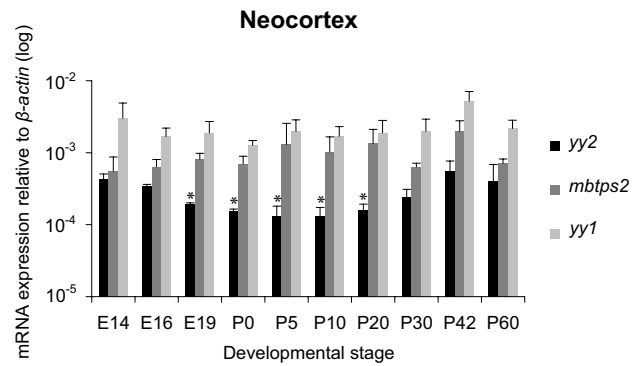


**Figure 3**  
**Quantification of *yy2*, *mbtps2* and *yy1* in developing lung.** Quantification of *yy2*, *mbtps2* and *yy1* transcripts levels in the lung during embryogenesis and adult stages (E14 to P30) by TaqMan real-time PCR. The expression data are normalized to  $\beta$ -actin. The pattern is similar to the heart analyzes. (n = 3 sets).

4 and 5, there were significant changes of *yy2* expression from day E14 to P60 by means of TaqMan real-time PCR. By contrast, the *yy1* levels remained stable. Interestingly, in cerebellum the amount of *yy2* increased up to 6-fold with maturation. In the neocortex *yy2* expression significantly decreased during intrauterine gestation, reached a minimum in the early neonatal period, before it increased again in adult mice (P60). However, the content of *yy2*

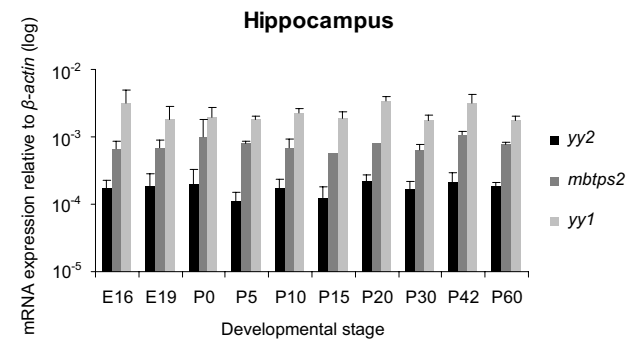


**Figure 4**  
**Quantification of *yy2*, *mbtps2* and *yy1* in developing cerebellum.** Analyzes of *yy2*, *mbtps2* and *yy1* expression in the cerebellum throughout development (E14 to P60). TaqMan real-time PCR revealed that *mbtps2* and *yy1* are constitutively expressed at almost constant levels, whereas *yy2* expression is low at E14, but increases significantly until P60. Expression levels are normalized to  $\beta$ -actin. (n = 3 sets; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; statistical significances refer to E14).



**Figure 5**  
**Quantification of *yy2*, *mbtps2* and *yy1* in developing neocortex.** Quantitative expression analyses (TaqMan real-time PCR) of *yy2*, *mbtps2* and *yy1* transcripts in the neocortex during development and in adult mice (E14 to P60). The expression of *yy2* reaches a minimum around day P5. Expression levels are normalized to  $\beta$ -actin. (n = 3 sets; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; statistical significances refer to E14).

transcripts in the hippocampus remained stable on a lower level compared to those of cerebellum and neocortex (Figure 6). In all brain regions, *mbtps2* and *yy1* were consistently higher expressed (up to 10-fold) than *yy2*. In contrast to *yy2*, transcripts of *yy1* and *mbtps2* were almost constitutively expressed during development. The combined data indicate that *yy2* expression underlies a tissue- and developmental-stage specific regulation.



**Figure 6**  
**Quantification of *yy2*, *mbtps2* and *yy1* in developing hippocampus.** Expression levels of *yy2*, *mbtps2* and *yy1* transcripts in the hippocampus during development and in adult mice (E14 to P30) quantified by TaqMan real-time PCR. The expression is normalized to  $\beta$ -actin. All three gene products are constitutively expressed at constant levels throughout all stages. (n = 3 sets).

### Quantitative analysis of *yy2* expression in neurons, astrocytes and microglia

Due to the dynamic regulation of *yy2* expression in neocortex and cerebellum, we analyzed the expression of *yy2*, *yy1* and *mbtps2* transcript levels in primary neurons, astrocytes and microglia of mice by using real-time PCR. As depicted in Figure 7, *yy1* was strongly expressed at similar levels within all three cell types, whereas *yy2* and *mbtps2* were found at lower levels. Especially neurons contained a very low amount of *yy2* transcript, if compared to astrocytes and microglia cells [see also additional file 1].

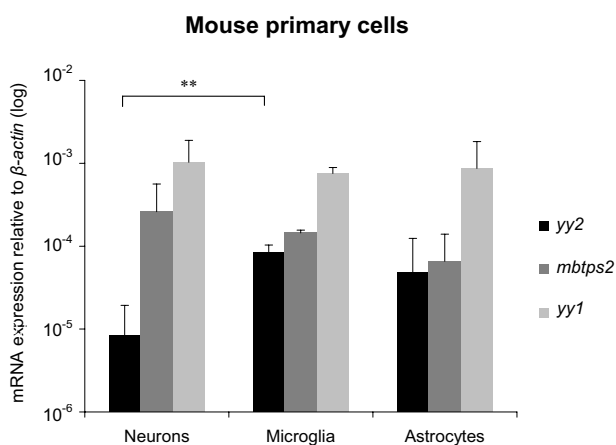
### Discussion

Herein, for the first time we provide data implying that the transcription factor *yy2* possess a functional role during development, as it has already been shown for its famous and well characterized homologue *yy1* [3,12,13]. Since, *yy2* is broadly, possibly ubiquitously expressed in embryonic mice (Figure 1), we conducted extensive real-time PCR analyses indicating a tissue-specific expression pattern that is in part developmentally regulated. Thereby, our experiments were especially focused on the cardiopulmonary and central nervous system.

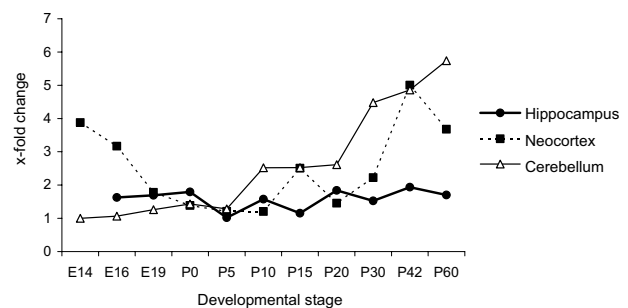
Expression of *yy1*, *yy2* and *mbtps2* could be detected in all analyzed organs, tissues and cells [see also additional file 2]. While their expression levels did not change significantly during development of heart and lung, the specimens from the brain revealed a more dynamic expression pattern. The relative high amount of *yy1* transcripts in the

cerebellum, neocortex and hippocampus underlines the broad implication of *yy1* in the brain at all stages of mice development [14]. In fact, its functional role in the nervous system has been demonstrated by analyses of heterozygous *yy1* knock-out mice [3,12] and conditional ablation of *yy1* in oligodendrocytes [15]. By contrast, the amount of *yy2* showed tissue-specific variations throughout brain development (summarized in Figure 8). In the cerebellum, *yy2* expression increased significantly from early postnatal to adult. During this time period cerebellum undergoes dramatic morphological changes, including active neurogenesis, neuronal migration and differentiation [16]. In cortical samples *yy2* expression revealed a u-shaped progression with a minimum starting at birth. At this time point neurogenesis and neuronal migration are completed in the cortex [17]. Here, it has to be noted that cerebellum and cortex have specific parallel neuronal connections between each other [18]. For example, neurons from the motor cortex project to the cerebellum. The hippocampus, however, involved in learning and memory processes, exhibited a constant low level of *yy2* transcripts throughout development. Further analyses of *yy2* protein localization at the cellular level could help to understand whether *yy2* could be involved in controlling axon formation, migration, or dendritogenesis. This issue cannot be examined so far, because currently no appropriate antibody against murine *yy2* is available.

By analyzing different primary cell types isolated from the murine brain, we found differences of *yy2* expression as well, whereas *yy1* levels showed no significant changes. In contrast to microglia cells and astrocytes, neurons express almost no *yy2*. The neuronal data suggest that *yy2* could be required for proliferation, since neurons, in contrast to



**Figure 7**  
**Quantification of *yy2*, *mbtps2* and *yy1* in primary cells.**  
 Expression levels of *yy2*, *mbtps2* and *yy1* transcripts in the different primary cells (neurons, microglia and astrocytes) quantified by TaqMan real-time PCR. While *mbtps2* and *yy1* are strongly and constantly expressed in all cell types, *yy2* is significantly reduced in neurons. Expression values are normalized to  $\beta$ -actin. (n = 3; \*\* p < 0.01).



**Figure 8**  
**Comparison of *yy2* expression in different brain regions.**  
 Comparison of *yy2* expression levels in the hippocampus, neocortex and cerebellum. E14 from cerebellum (lowest starting value) was defined as one. While expression of *yy2* is stable in the hippocampus throughout all stages, the amount of *yy2* transcripts varies significantly in cerebellum and neocortex. (n = 3 sets)

astrocytes and microglia cells, cannot divide anymore. Ongoing experiments may verify this hypothesis.

Comparing all real-time PCR results it becomes obvious that the expression of  $\gamma 2$  and *mbtps2*, respectively, is individually regulated depending upon the analyzed organ, tissue or cell type. While both genes seem to be co-regulated *via* the same promoter in heart and lung as well as in microglia and astrocytes, however, the data from primary neurons and all three brain tissues indicate an alternative transcriptional activity. Currently, two different possibilities of  $\gamma 2$  and *mbtps2* gene regulation are discussed: (a) both genes are under the control of the *mbtps2* promoter, and (b)  $\gamma 2$  transcription is mediated by its own adjacent promoter, located upstream of its coding sequence, a model that we favour according to own data on the regulation of the human *YY2* gene [7,11]. Of note, only the  $\gamma 2$  promoter shows a significant sensitivity against DNA-(de)methylation, strengthening the implication of  $\gamma 2$  in developmental processes. Our observations presented in this manuscript emphasize that in fact both promoter regions seem to be utilized in a tissue- or cell type-specific manner.

## Conclusion

The results from the real-time PCR experiments confirm that  $\gamma 2$  is differentially expressed in the lung, heart and brain, indicating a stringent spatial regulation and function. Precisely, we show a significant change of  $\gamma 2$  expression during the development and maturation of the neocortex and cerebellum. Within the central nervous system,  $\gamma 2$  is predominantly expressed in astrocytes and microglia cells.

## Methods

### Animals

C57/Bl6 mice were mated, and embryos/pups were collected at defined time points (morning of vaginal plug was considered as embryonic day E0.5). The protocol for animal use was approved by the Institutional Review Board (T 0167/08).

### Preparation of primary neurons

Serum-free preparation of mouse cortical primary cultures was performed with E18 (+/- 0.5 days) mouse embryos as previously described [19]. After removal of meninges, entire cortices were mechanically dissociated in HBSS buffer (w/o  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ), with trypsin (0.25%) for 10 min at 37°C. Trypsinization was stopped by adding minimum essential medium (Gibco-Invitrogen; Karlsruhe, Germany). Medium was supplemented with 0.6% glucose, 10% horse serum (Gibco), and penicillin and streptomycin. Further mechanical dissociation was performed by adding of DNaseI (0.06%). Cells were cultured on poly-L-lysine-coated dishes at a density of 75.000 cells/

cm<sup>2</sup>. After three hours *in vitro*, medium was changed to Neurobasal A medium (Gibco) supplemented with 2% B27 (Gibco), 0.5 mM glutamine, and penicillin and streptomycin for 12 days. Purity of neuronal cell preparation was tested by real-time PCR [see additional file 3].

### Preparation and purification of astrocytes and microglial cells

For astrocyte and microglia preparation, cerebra from mouse pups aged P1 to P3 were prepared and the meninges, including the pia mater and arachnoid, were carefully removed and the cerebrum was washed in DMEM (4.5 g/l glucose, 200 mM glutamine, pyrovate) containing 10% fetal calf serum and 100 U/ml penicillin/streptomycin. Following careful homogenization with a fire-polished Pasteur pipette, a 10 min trypsin/DNase (1.25%/2 U) incubation followed, and digestion was stopped by adding the same volume culture medium (DMEM containing 4.5 g/l glucose, 200 mM glutamine, pyrovate, 10% fetal calf serum, and 100 U/ml penicillin/streptomycin). Dissociated astrocytes were plated on poly-L-lysine (10 µg/ml)-coated dishes. Every third day, dishes were shaken for 10 min at 37°C and afterwards rinsed three times with warm PBS to remove microglial cells. They were then resuspended with a fire-polished Pasteur pipette, and replated in culture medium at low density. The suspended microglial cells were collected and replated in astroglial culture medium containing 2% fetal calf serum. Purity of both cell types was tested by real-time PCR [see additional file 3].

### Whole-mount in situ expression analysis of $\gamma 2$ mRNA

For *in situ* hybridization, a  $\gamma 2$  probe was generated by cloning a  $\gamma 2$ -specific 200 bp fragment (nt 11 to 200) derived from the murine coding sequence [GenBank: EF688658] into pBluescript SK (-) plasmid (Fermentas; St. Leon-Rot, Germany). For digoxigenin (DIG)-labeling, 12 µl of linearized plasmid (EcoRI: anti-sense or BamHI: sense) were combined with 2 µl 10-fold buffer (Roche; Mannheim, Germany), 1 µl RNasin, 2 µl T3 (for anti-sense synthesis) or T7 (for sense synthesis) RNA polymerase and 2 µl labeling mix containing 7.1 µl ATP, 7.1 µl CTP, 7.1 µl GTP, 4.6 µl UTP, 20.5 µl RNase free water, and 25 µl DIG-11-UTP (Roche; Cat.-No.: 1209256) in order to conduct *in vitro* transcription for 2 h (37°C). Afterwards, the freshly prepared probe was diluted by adding 70 µl RNase free water and 10 µl 0.1 M DTT. The embryos were dissected in ice-cold PBS, followed by overnight fixation in 4% PFA, 3 washes in PBS with 1% Tween 20 (PBT) for 5 minutes, and dehydration in rising methanol concentrations. Next, specimens were rehydrated with decreasing methanol concentrations. For increasing permeability and signal detection, embryos were incubated in 6% H<sub>2</sub>O<sub>2</sub> for 1 h and subsequently treated with proteinase K for 15 min with 3 washing steps (5 min each) in PBT. Digestion was

stopped in 2 mg/ml glycine in PBT for 20 min and 2 washing steps in PBT followed by post fixation in 4% PFA (in PBT) for 20 min at room temperature. PFA was removed by 3 washing steps in PBT. After incubation in hybridization solution containing 50% deionized formamide, 5× SSC [pH 4.5], 1% SDS, 50 µg/ml tRNA and 50 µg/ml heparin at 65°C for 1 h, 1–2 µl denatured DIG-labeled probes (2 min at 90°C) were added and hybridized at 65°C overnight. Finally, embryos were washed 3 times for 1 h in solution I (50% formamide, 5 × SSC [pH 4.5], 1% SDS) at 65°C followed by 3 washing steps for 1 hour in solution II (50% formamide, 2 × SSC [pH 4.5], 1% Tween 20) at 60°C and washing 3 times in TBST for 5 min each. Before the embryos were treated with anti-DIG antibody (1:2000) in 1% sheep serum/TBST overnight at 4°C, we conducted a blocking step in 10% heat-inactivated sheep serum/TBST with 0.1% blocking reagent (Roche; Cat.-No.: 11112589001) for 1 h at room temperature. After performing post-antibody washes (3 times 10 min and 5 times 1 h in TBST), samples were hybridized in fresh prepared NTMT [pH 9.5] solution containing 100 mM NaCl, 100 mM Tris-Cl (pH 9.5), 50 mM MgCl<sub>2</sub> and 0.1% Tween 20. For signal detection, NTMT solution was removed and reaction mix (NTMT with 125 µg/ml BCIP and 250 µg/ml NBT) was added. To stop the staining process, samples were washed 3 times in PBT, followed by post-fixation in 4% PFA. The embryos were analyzed with a Leica MZ 9.5 stereo microscope (Wetzlar, Germany).

#### Preparation of cDNA

Mouse organs were immediately snap-frozen following their collection from 3 sets of 6 animals of each age. Primary mouse brain cells from 3 independent preparations of 3 pregnant animals or mouse organs were homogenized in TRIzol reagent (Invitrogen), and the total RNA was purified according to the TRIzol protocol. RNA concentrations were determined by using a UV-Visible Spectrophotometer (Biomate 3 spectrometer, Fisher Scientific, Waltham, MA, USA). A High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) was used to generate total cDNA for the real-time PCR from 5 µg total RNA from each sample as per the manufacturer's recommendations.

#### Real-time PCR (TaqMan)

TaqMan cDNA samples were prepared as described. 5'-FAM-labeled probes and appropriate primer pairs for detection of murine  $\gamma\gamma 2$  (probe: 6FAM-cagcctgttctcagctatgggatcttct-BBQ; primer: 5'-gggtgacaacagtgggagc-3' (forward) and 5'-ggatcagaaagatcaatgccaggt-3' (reverse)),  $\gamma\gamma 1$  (probe: 6FAM-agggtctgagaggtcaatgccaggt-BBQ; primer: 5'-atgaaacagtgggtgaagagcagatc-3' (forward) and 5'-caagctattgtcttgagcatcatc-3'(reverse)) and *mbtps2* (probe: 6FAM-

tgtcccgttactaatgtgcaagattggaa-BBQ; primer: 5'-ggagacctgtcactcatctacagga-3' (forward) and 5'-gtcgtttgtatgctctaactgggaag-3' (reverse)) were designed and synthesized by TIB MOLBIOL (Berlin, Germany).  *$\beta$ -actin* (TaqMan Gene Expression Assays; Cat.-No.: ACTB 4352664-0602004) was purchased from Applied Biosystems. For all PCR reactions, 1 µl of cDNA was added to 10 µl TaqMan 2× Universal PCR Mastermix Mix (Applied Biosystems), 1 µl of each primer [20 µM], 1 µl 5'-FAM-labeled probe [0.3 µM] and 8 µl deionized H<sub>2</sub>O. All reactions were performed in duplicates and all three transcripts (within each set of specimens) were always analyzed within the same experiment. Amplification and fluorescence detection was conducted with the i-Cycler Multicolor Real-Time PCR Detection System (Bio-Rad; Munich, Germany). The fluorescence threshold value was calculated by using the iCycle iQ Optical System Software, version 3.1.  $\gamma\gamma 2$ ,  $\gamma\gamma 1$  and *mbtps2* values were normalized against  *$\beta$ -actin*.

#### Statistical analysis

Statistical significances were determined by using the one-way analysis of variance (ANOVA). Bonferroni's multiple comparison procedure was used to discriminate, which means were different from others. A *p*-value < 0.05 was considered to be significant.

#### Authors' contributions

DD established, performed and analyzed *in situ* hybridizations, performed real-time PCR, drafted and edited the manuscript. MK and CD designed the project, supervised DD, contributed to the analysis of *in situ* hybridization as well as real-time PCR and edited the manuscript. AUB provided cDNA samples, contributed to analysis of *in situ* hybridization and real-time PCR. All authors read and approved the final version of the manuscript.

#### Additional material

##### Additional file 1

*Detection of  $\gamma\gamma 2$ , mbtps2 and  $\gamma\gamma 1$  in primary cells. Expression of  $\gamma\gamma 2$ , mbtps2 and  $\gamma\gamma 1$  in the primary cells (neurons, microglia and astrocytes) isolated from the murine brain were analyzed by conventional PCR (30 cycles per reaction;  $\gamma\gamma 2$ : 5'-accagcgtaggccaaccatcgaagta-3' (forward) and 5'-cgtcaaacacacagatccctcata-3' (reverse);  $\beta$ -actin: 5'-actgctctggctctagcac-3' (forward) and 5'-acatctgctggaaggtggac-3' (reverse); mbtps2: 5'-ggagacctgtcactcatctacagga-3' (forward) and 5'-gtcgtttgtatgctctaactgggaag-3' (reverse);  $\gamma\gamma 1$ : 5'-atgaaacagtgggtgaagagcagatc-3' (forward) and 5'-caagctattgtcttgagcatcatc-3'(reverse)). While mbtps2 and  $\gamma\gamma 1$  are strongly and constantly expressed in all cell types,  $\gamma\gamma 2$  is reduced in neurons. Expression of  $\beta$ -actin served as internal control (multiplex PCR; upper panel). n = 3*

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-213X-9-45-S1.pdf>]

### Additional file 2

**Expression of yy2, mbtps2 and yy1 in testis, liver, kidney, thymus and spleen.** Levels of yy2, mbtps2 and yy1 in the different stages of testis, liver, kidney and spleen development (E16, P10 and P30) were analyzed by conventional PCR (30 cycles per reaction; yy2: 5'-accagcgtaggccaac-catcgaagta-3' (forward) and 5'-cgtaaacacagagattccctcata-3' (reverse);  $\beta$ -actin: 5'-actgctctggctctagcac-3' (forward) and 5'-acatctctggaaggt-ggac-3' (reverse); mbtps2: 5'-ggagacctgtcactcatctacagga-3' (forward) and 5'-gtcgtttgtatgcttaactgggaag-3' (reverse); yy1: 5'-atgaacagtggtt-gaagagcagatc-3' (forward) and 5'-caagctattgtcttgagcatcatc-3' (reverse)). With the exception of thymus for yy2 and testis regarding mbtps2, both gene products are differentially regulated in all tested organs. However, yy1 expression only showed developmental changes in liver and spleen. Expression of  $\beta$ -actin served as internal control (multiplex PCR; upper panel). n = 3

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-213X-9-45-S2.pdf>]

### Additional file 3

**Purity of primary cells.** The Table shows the means of relative expression including standard deviation (SD) of specific marker genes as  $\beta$ 3-tubulin (Tuj-1) for neurons, ionized calcium-binding adapter molecule-1 (Iba-1) for microglia and glial fibrillary acidic protein (GFAP) for astrocytes related to  $\beta$ -actin (probes: Tuj-1 Cat.-No.: Mm00727586\_s1; Iba-1 Cat.-No.: Mm00479862\_g1; GFAP Cat.-No.: Mm00546086\_m1;  $\beta$ -actin: Cat.-No.: 4352933E; Applied Biosystems) determined by real-time PCR.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-213X-9-45-S3.pdf>]

## Acknowledgements

DD was supported by a fellowship from the Sonnenfeld-Stiftung, Berlin. Funding was provided by the Verein für Frühgeborene Kinder am Virchow-Klinikum e. V., Berlin. DD has cooperated with Prof. Constance Scharff (Department of Biology, Chemistry and Pharmacy, Freie Universität Berlin).

The authors thank Bettina Brokowski, Rike Dannenberg and Justus Goyon for preparation of cDNA samples as well as Angelika Zwirner and Jan Csupor for stimulating discussions. DD acknowledges Sandy von Salisch for guidance in TaqMan real-time PCR and Dr. Malte Cremer for supporting statistical analysis. We also thank all members of our laboratories for their support and critical discussion of the data.

## References

- Shi Y, Lee JS, Galvin KM: **Everything you have ever wanted to know about Yin Yang 1.** *Biochim Biophys Acta* 1997, **1332**:F49-66.
- Thomas MJ, Seto E: **Unlocking the mechanisms of transcription factor YY1: are chromatin modifying enzymes the key?** *Gene* 1999, **236**:197-208.
- Donohoe ME, Zhang X, McGinnis L, Biggers J, Li E, Shi Y: **Targeted disruption of mouse Yin Yang 1 transcription factor results in peri-implantation lethality.** *Mol Cell Biol* 1999, **19**:7237-44.
- Hsieh SY, Zhuang FH, Wu YT, Chen JK, Lee YL: **Profiling the proteome dynamics during the cell cycle of human hepatoma cells.** *Proteomics* 2008, **8**:2872-84.
- Obuse C, Okazaki T, Masukata H: **Interaction of transcription factor YY1 with a replication-enhancing element, REE1, in an autonomously replicating human chromosome fragment.** *Nucleic Acids Res* 1998, **26**:2392-7.
- Nguyen N, Zhang X, Olashaw N, Seto E: **Molecular cloning and functional characterization of the transcription factor YY2.** *J Biol Chem* 2004, **279**:25927-34.
- Luo C, Lu X, Stubbs L, Kim J: **Rapid evolution of a recently retroposed transcription factor YY2 in mammalian genomes.** *Genomics* 2006, **87**:348-55.
- Kim JD, Faulk C, Kim J: **Retroposition and evolution of the DNA-binding motifs of YY1, YY2 and REX1.** *Nucleic Acids Res* 2007, **35**:3442-52.
- Yant SR, Zhu W, Millinoff D, Slightom JL, Goodman M, Gumucio DL: **High affinity YY1 binding motifs: identification of two core types (ACAT and CCAT) and distribution of potential binding sites within the human beta globin cluster.** *Nucleic Acids Res* 1995, **23**:4353-62.
- Klar M, Bode J: **Enhanceosome formation over the beta interferon promoter underlies a remote-control mechanism mediated by YY1 and YY2.** *Mol Cell Biol* 2005, **25**:10159-70.
- Klar M, Drews D, Dame C: **Transcriptional activity of the novel identified human yy2 promoter is modified by DNA methylation.** *Gene* 2009, **430**:58-63.
- Affarel B, Gay F, Shi Y, Liu H, Huarte M, Wu S, Collins T, Li E: **Essential dosage-dependent functions of the transcription factor yin yang 1 in late embryonic development and cell cycle progression.** *Mol Cell Biol* 2006, **26**:3565-81.
- Ladomery M, Dellaire G: **Multifunctional zinc finger proteins in development and disease.** *Ann Hum Genet* 2002, **66**:331-42.
- He Y, Casaccia-Bonnel P: **The Yin and Yang of YY1 in the nervous system.** *J Neurochem* 2008, **106**:1493-502.
- He Y, Dupree J, Wang J, Sandoval J, Li J, Liu H, Shi Y, Nave KA, Casaccia-Bonnel P: **The transcription factor Yin Yang 1 is essential for oligodendrocyte progenitor differentiation.** *Neuron* 2007, **55**:217-30.
- Goldowitz D, Hamre K: **The cells and molecules that make a cerebellum.** *Trends Neurosci* 1998, **21**:375-82.
- Gupta A, Tsai LH, Wynshaw-Boris A: **Life is a journey: a genetic look at neocortical development.** *Nat Rev Genet* 2002, **3**:342-55.
- Ito M: **Cerebellar circuitry as a neuronal machine.** *Prog Neurobiol* 2006, **78**:272-303.
- Brewer GJ, Torricelli JR, Evege EK, Price PJ: **Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination.** *J Neurosci Res* 1993, **35**:567-76.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:  
[http://www.biomedcentral.com/info/publishing\\_adv.asp](http://www.biomedcentral.com/info/publishing_adv.asp)

