Gene Expression Patterns 9 (2009) 114–121

Contents lists available at ScienceDirect

Gene Expression Patterns

journal homepage: www.elsevier.com/locate/gep

PBRL, a putative peripheral benzodiazepine receptor, in primitive erythropoiesis

Fumie Nakazawa [a], Cantas Alev [a,b], Masahiro Shin [a], Yukiko Nakaya [a], Lars M. Jakt [c], Guojun Sheng [a,*]

[a] Laboratory for Early Embryogenesis, RIKEN Center for Developmental Biology, Kobe, Hyogo 650-0047, Japan
[b] Laboratory for Stem Cell Translational Research, Institute for Biomedical Research and Innovation, Kobe, Hyogo 650-0047, Japan
[c] Laboratory for Stem Cell Biology, RIKEN Center for Developmental Biology, Kobe, Hyogo 650-0047, Japan

ARTICLE INFO

Article history:
Received 13 September 2008
Received in revised form 17 September 2008
Accepted 17 September 2008
Available online 25 September 2008

Keywords:
Peripheral benzodiazepine receptor
PBR
PBRL
Chicken
Chick
Erythropoiesis
Hematopoiesis
Hemoglobin
Globin
Heme
Ferrochelatase
Coproporphyrinogen decarboxylase
Blood
Erythrocyte
Mitochondria
Proteomics
Shot-gun
Mass spectrometry

ABSTRACT

Benzodiazepines are a class of psychoactive drugs widely used for their anxiolytic, anticonvulsant, muscle relaxant and hypnotic properties. Although the benzodiazepine receptor in the central nervous system has been well studied, the role of peripheral type benzodiazepine receptor, PBR, remains elusive. Here, we show that there are two PBR homologous genes in amniotes, *PBR* and *PBRL*, based on phylogenetic analysis. In chickens, *PBRL* is exclusively expressed during early development in differentiating primitive erythrocytes and this expression is tightly correlated with that of hemoglobin genes. *PBR* is not expressed in hematopoietic system during this period and is weakly expressed in developing central nervous system. Because one of PBRs' known functions is to regulate heme transport between the mitochondria and cytoplasm, we investigated expression profiles of heme biosynthesis genes. Seven of the eight enzymes involved in heme biosynthesis, with the exception of *protoporphyrinogen oxidase*, are present in chicken genome. Five of them, *δ-aminolevulinate synthase*, *δ-aminolevulinic acid dehydrogenase*, *porphobilinogen deaminase*, *coproporphyrinogen decarboxylase* and *ferrochelatase*, show stage-specific increase in gene expression correlated with primitive hematopoiesis, but not with primitive erythrocyte differentiation. PBRL protein is localized to the mitochondria in culture cells, and pharmacological inhibition of PBRL activity results in a decrease in globin protein levels during primitive erythropoiesis. Our data suggest a developmental role of PBRs in erythropoiesis in chickens, possibly via the regulation of heme availability for the assembly of functional hemoglobins.

1. Results and discussion

1.1. Expression profiles of heme biosynthesis enzymes during primitive erythropoiesis in chickens

Primitive erythrocytes are generated from the ventral mesoderm during early vertebrate development. Their terminal differentiation, marked by the production of embryonic globins, can be separated from earlier lineage specification marked by transcriptional regulators such as Gata1, Scl and Lmo2. In chickens, hemoglobin expression in blood islands starts at stage HH7 (0–1 somite), whereas hematopoietic lineage markers are expressed in extraembryonic-fated regions as early as HH4 (Nakazawa et al., 2006). Erythrocyte terminal differentiation requires concurrent synthesis of heme. In order to understand how hemoglobin expression is coupled with heme biosynthesis during primitive erythropoiesis, we carried out a screen for genes involved in heme production that would exhibit a similar pattern of stage-specific regulation as embryonic hemoglobins. Extraembryonic or extraembryonic-fated tissues were dissected out from stages HH4 to HH9 embryos (Fig. 1A). Triplicate samples were independently prepared for RNA isolation, followed by Affymetrix chicken genome array analysis without amplification. Among eight enzymes involved in heme biosynthesis (Ponka, 1999) (Fig. 1B), we were able to find seven orthologous genes in chickens. No chicken protoporphyrinogen oxidase *(PPO)* can be found. *PPO* is present in fishes, amphibians and mammals, but not in *Anolis*, the only reptile species with genome sequenced. This suggests an evolutionary loss of *PPO* in bird/reptile lineage. Among the remaining seven genes, *UROS* showed no prominent expression, *UROD* showed no prominent change of expression, and *ALAS2*, *ALAD*, *PBGD*, *CPO* and *FCH*

* Corresponding author. Tel.: +81 78 306 3158; fax: +81 78 306 3146.
E-mail address: sheng@cdb.riken.jp (G. Sheng).

1567-133X/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.gep.2008.09.005

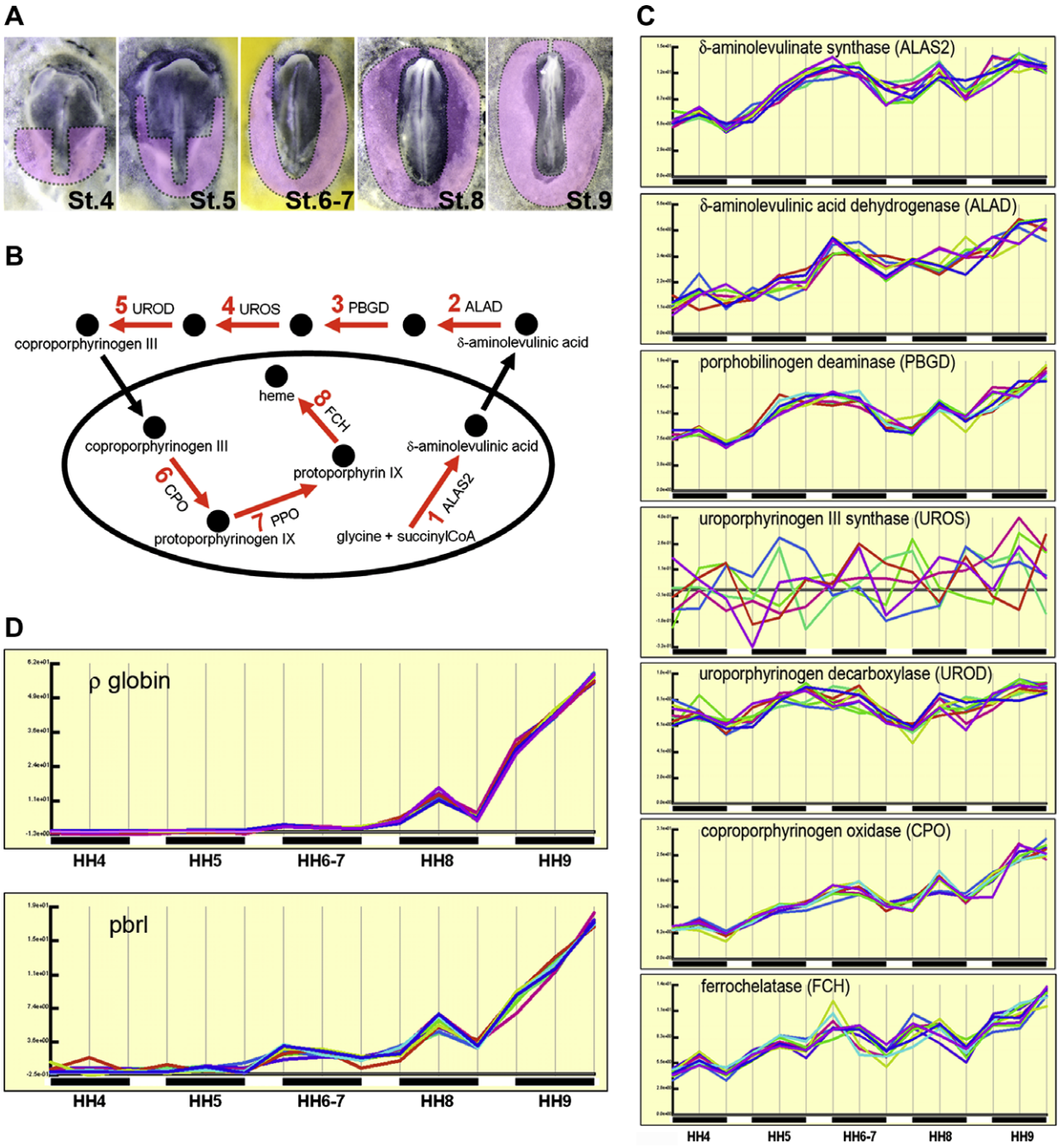

Fig. 1. Gene-array profiling of heme biosynthesis enzymes and PBRL. (A) Schematic drawing of embryonic tissues dissected for the screen. (B) Schematic drawing of biochemical steps catalyzed by heme biosynthesis enzymes and their locations. (C) Expression profiles of seven chicken orthologous heme biosynthesis enzymes. Each stage is shown with values from independent triplicate samples. (D) Expression profile of PBRL in comparison to that of ρ-globin.

showed a gradual increase from HH4 to HH9 (Fig. 1C). None of these expression profiles exhibited a tight correlation with that of hemoglobin genes. Nevertheless, the general increase of these five gene products correlates well with hematopoietic lineage markers. This was further confirmed by *in situ* analysis of *CPO* and *FCH*, two enzymes catalyzing the final steps of heme biosynthesis in mitochondria. Both genes exhibited a restrictive hematopoietic tissue expression in mesoderm cells (Fig. 2), and *CPO* is prominently expressed in addition in developing CNS at later stages (Fig. 2A). Both the onset and region of their expression suggest a correlation with hematopoietic lineage specification, but not with erythrocyte terminal differentiation.

1.2. Two PBR homologs, PBR and PBRL, are present in amniotes

We then performed an extensive profile search of our screen data, and found only one gene to be both highly expressed and with its profile tightly matching that of hemoglobin genes (Fig. 1D). Although this gene is annotated as similar to peripheral benzodiazepine receptor (*PBR*), we found another similarly annotated, but not similarly expressed, gene in chicken genome. To clarify this, we first performed sequence comparison with vertebrate PBR homologs. To avoid confusion, the new nomenclature (Papadopoulos et al., 2006) proposed for PBR as translocator protein (TSPO) is not adopted in our work. We found one homolog