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# Glypicans are differentially expressed during patterning and neurogenesis of early mouse brain

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**Abstract**

Glypicans are essential modulators of cell signalling during embryogenesis. Little is known about their functions in brain development. We show here that mouse *glypicans* (*gpc-1* to *gpc-6*) are differentially expressed in embryonic brains during key morphogenetic events. In gastrulating embryos, *gpc-4* is the only *glypican* expressed in anterior visceral endoderm. During neural tube closure, *gpc-4* transcripts are restricted to the anterior neural ridge and telencephalon. At this stage, *gpc-1* expression shifts from trunk and head mesenchyme to neural tube. *Gpc-3 mRNA* appears across the ventral neural tube, then in the lamina terminalis and hypothalamus. *Gpc-2* and *gpc-6* transcripts are in all brain compartments. *Gpc-5* is found in ventral brains as neurogenesis starts. Onset of neurogenesis also coincides with differential expression of *glypican* genes either in neural progenitors or in differentiating neurons. The novel expression sites of *glypicans* shown here contribute to the identification of signalling molecules involved in brain patterning.


*Keywords:* Glypicans; HSPGs; Cell signalling; Signalling modulators; Morphogens; Brain development; Anterior visceral endoderm; Neural progenitors

Glypicans are heparan sulphate proteoglycans (HSPGs) that are linked to the cell surface through a glycosyl-phosphatidylinositol (GPI) anchor (reviewed in [1,2]). To date, six *glypican* genes have been identified in mammals (*gpc-1* to *gpc-6*). In addition to the GPI modification, mammalian glypicans share a significantly conserved amino acid sequence and contain two to four consensus sites for the attachment of heparan sulphate glycosaminoglycan (HS-GAG) chains [2]. Biochemical and genetic studies have shown that Glypicans play important roles in modulating cell–cell signalling interactions. For example, they regulate the action of extracellular signals such as fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), Wnts, Hedgehog (HHs) and HGF [1,3–5]. It has been proposed that Glypicans function by promoting and/or stabilizing the interaction of these molecules with their cognate high affinity receptors [2] or by shaping their activity gradients [6]. Consistent with this, mutations in *Drosophila*, zebrafish and mouse *glypican* genes generate phenotypes reminiscent of loss-of-function mutations in Wnts, BMPs and HH [4,7,8].

*Glypicans* are predominantly expressed during morphogenesis of different embryonic structures such as the mammalian brain [2]. For example, neural precursors in the ventricular zone of the telencephalic vesicles express the *gpc-1* [9], *gpc-4* (this study, [3,10] and *gpc-6* genes [11]. Neural precursors of the striatum primordium express *gpc-5* [12]. *Gpc-1* and *gpc-5* transcripts are also found in post-mitotic neurons during differentiation and in adult brains. Post-mitotic neurons also express *gpc-2*, but the timing of its expression correlates with that of neuronal migration and axonal growth [13,14]. These studies show the expression of most

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*glypican* genes during brain neurogenesis (i.e., beyond embryonic day 11). Ybot-Gonzalez et al. have shown that *gpc-4* exhibits a distinct and dynamic expression pattern in the anterior forebrain neuroepithelium and adjacent tissues at embryonic days (E) 7–10.5, and they suggested its potential involvement in one or more signalling pathways regulating forebrain development [15]. However, it remains to be established whether most *glypicans* are expressed during earlier brain patterning events, which are known to be controlled by their potential interactors [16].

In *Xenopus laevis gpc-4* is highly expressed during neurulation by anterior neural plate cells of the presumptive forebrain [17]. In agreement with this, Gpc-4 acts as positive modulator of FGF signalling during *Xenopus* dorso-ventral forebrain patterning [17]. Here we investigate a possible glypican-signalling requirement during mouse gastrulation and early brain morphogenesis by characterizing and comparing spatial and temporal expression patterns of known *glypican* genes.

## Materials and methods

*Embryo collection.* E6.5 and E7.25 embryos were from (C57BL6×CBA) F1 matings kept under inverted day–night cycles. E8.0 to E10.0 embryos were generated from CD1 mice. Otx-2 heterozygous mutants on C57BL6/CBA background were intercrossed to obtain homozygous. Embryonic days were counted considering midday post-coitum as E0.5. Research was performed according to institutional review body and ethics committee guidelines.

*Identification of glypicans cDNA and antisense digoxygenin-labelled RNA. Gpc-1*, *gpc-3* and *gpc-6* clones were identified by BLAST search of GenBank EST database using cDNA sequences of the NCBI database (*gpc-1*: Accession No. AF185613; *gpc-3*: Accession No. BC036126; *gpc-6*: Accession No. AF105268). Corresponding EST clones were obtained from RZPD Consortium (www.rzpd.de/cgi-bin/db). *Gpc-1*: RZPD clone ID-IMAGE480151; *gpc-3*: RZPD clone ID-IMAGp998O1010961; *gpc-6*: RZPD clone ID-IMAGp 998I1610907. ESTs clones were confirmed by restriction enzyme digestion and sequence analysis. Digoxygenin RNA probes were synthesized according to the manufacturer's instruction (Roche). Probes were tested by whole mount *in situ* hybridisation on embryos at later developmental stages, whose expression pattern is published. *Gpc-4* cDNA was cloned by RT-PCR of mouse embryonic kidney. Gpc-4 F(5′CTGCTTTCCATCGGGTCTCATTCTG3′) and R(5′GTCAGCTTTCTCGTTGGCACTCTTCC3′). The *gpc-2* and *gpc-5* cDNAs were cloned by RT-PCR of postnatal mouse brains and spinal cords, respectively. The following primers were used: Gpc-2 F(5′GAGCACCAGCACCCCGAGAAGAG3′) and R(5′GTAGCCCCCTTCCACTCCTCCTCA3′); Gpc-5 F(5′CATCAACACCACGGACCACATACAC3′) and R(5′CTCCACAGCCATCTTCGTCATCACAG3′). PCR products were cloned in pGEM-T-EasyVector (Promega).

*RNA in situ hybridisation.* Whole mount and paraffin sections RNA *in situ* hybridisation was performed as previously described [18]. For sectioning, early streak stage, embryos were embedded in cold glycol methacrylate (Technovit 8100) or in gelatine for cryosections. E6.7 embryos were cut transversally at 10 μm. Vibratome sections of E8.5 embryos were at 100 μm. Paraffin sections of E9.5 and E10.5 embryos were at 8 μm.

## Results

We first analysed *glypican* gene expression in gastrulating mouse embryos (E6.5–E7.25) by whole mount *in situ* hybridisation and found that only *gpc-4* was clearly expressed at this developmental stage (Fig. 1A and data not shown). In particular, *gpc-4 mRNA* becomes restricted to the region of the anterior visceral endoderm (AVE) as gastrulation proceeds (arrows in Fig. 1A). Analysis of the distribution of *gpc-4* transcripts in transverse sections of early streak stage embryos confirmed that AVE cells express *gpc-4*, while the epiblast does not (left inset in Fig. 1B). The AVE is the extra embryonic tissue that overlies the epiblast that will form the anterior CNS and surface ectoderm. It is essential for specifying the anterior neural ectoderm and initiating anterior neural plate patterning [19]. The AVE is generated by anterior displacement of distal VE cells to the prospective anterior side of gastrulating embryos [19] and *otx-2* is one transcription factor required for this process [20]. In agreement with this, AVE markers such as *hex* and *cer1* remain distal in *otx-2* deficient embryos [20]. Fig. 1B shows that *gpc-4* is also mislocalised to distal visceral endoderm in *otx-2* deficient embryos (see also right inset). Thus, *gpc-4* can be considered as a marker of the AVE at these developmental time points.

We next examined the expression of *glypicans* in mouse embryos at neural plate to two-somite stage (E7.5) and found that the genes are selectively turned on. Expression of *gpc-4* is restricted to the developing anterior embryonic structures and posteriorly from the node (Fig. 2A). *Gpc-3*

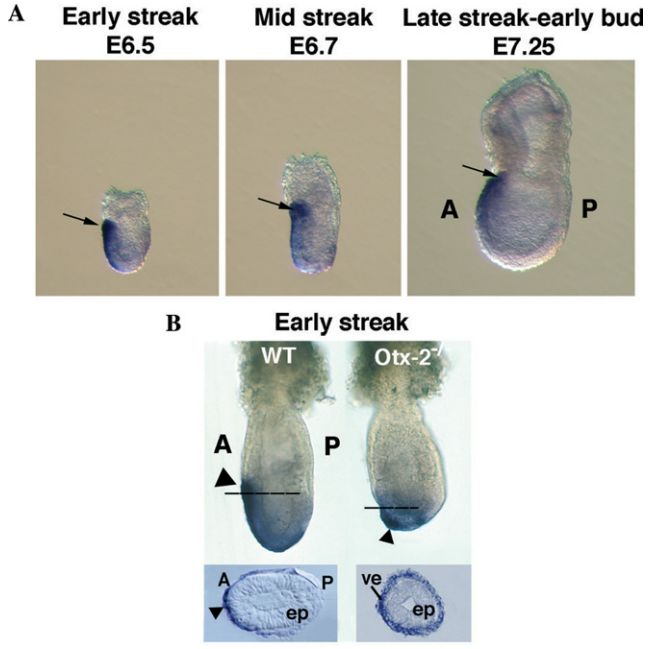


Fig. 1. *Gpc-4* is expressed in the anterior visceral endoderm (AVE). (A) Spatial and temporal distribution of *gpc-4* transcripts during early gastrulation. Arrows point to *gpc-4* in the AVE. Anterior (A) is left, posterior (P) is right. (B) Expression of *gpc-4* at advanced early streak wild type and Otx-$2^{-/-}$ embryos. The broken line indicates the level of sections in the insets of similar embryos. The left inset shows *gpc-4* expression strongest anteriorly in the AVE (arrowhead) and not in the epiblast (ep). In Otx-$2^{-/-}$ embryos the AVE has not moved anteriorly and *gpc-4* is expressed distally (compare arrowhead in WT and Otx-$2^{-/-}$). The right inset shows *gpc-4* expression in the distal visceral endoderm.