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Mechanisms of Development 92 (2000) 301–304

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Gene expression pattern

Expression of PTTG and *prc1* genes during telencephalic neurogenesis

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Received 9 September 1999; received in revised form 22 December 1999; accepted 23 December 1999

Abstract

We present the first time/space analysis using in situ hybridization for PTTG and *prc1* genes during development of the mouse telencephalon. During the stages E11.5-E13.5 PTTG and *prc1* are expressed in most tissues of the embryo. Within the telencephalon, PTTG and *prc1* are found exclusively inside of the ventricular zone (VZ). The intensity of the expression of both genes in the ventricular zone reaches its peak by E15.5. Expression starts to decrease by E18.5, it is still visible at least up to P2 and not detectable in the adult brains. Expression of the *prc1* gene, but not that of the PTTG, is also found in the mitoticaly active cells outside of the VZ within the telencephalon. Most of the cells expressing the PTTG gene were found in the lower part of the ventricular zone suggesting that the level of PTTG mRNA is regulated during different phases of the mitotic cycle.

Keywords: Ventricular zone; Differential display; Ferret; Mouse; Nervous system development

1. Results

In an attempt to discover novel genes expressed in the ventricular zone of the cerebral cortex we have used the differential display of cDNA (Liang, 1992). We have chosen the ferret as a model because of the easily distinguishable morphology of its developing brain (Jackson and Hickey, 1985).

This approach yielded several sequences expressed within the ventricular zone of the developing telencephalon. Two of these sequences were found to be homologous to the genes *prc* and PTTG. The rat PTTG gene was originally identified as a gene expressed in pituitary tumors (Pei, 1997). Human PTTG (*hpttg*) has also been cloned (Dominguez et al., 1998). Transfection of NIH 3T3 cells with *hpttg* cDNA caused in vitro transformation (Zhang et al., 1999a). *hpttg* has been shown to be expressed in some normal adult tissues as well as in several tumor cell lines (Dominguez et al., 1998; Lee et al., 1999; Zhang et al., 1999b). A transactivation capability of hPTTG has also been demonstrated (Dominguez et al., 1998). Recently this gene has been independently isolated as a vertebrate securin (vSecurin) gene on the basis of its biochemical analogy to the Pds1p protein of budding yeast (Zou et al., 1999). The vSecurin/hPTTG protein has been found to bind to a vertebrate homologue of yeast separins and is likely to be involved in sister-chromatid separation during cell cycle.

The second clone identified was found to be homologous to the human gene *prc1* (*hprc1*). Its product, nuclear protein PRC1, becomes associated with mitotic spindles during mitosis. Microinjection of anti-PRC1 antibodies into HeLa cells has been shown to block cellular cleavage but not nuclear division, indicating a functional role for PRC1 in the process of cytokinesis (Jiang et al., 1998).

The cleavage orientation of dividing cells in the developing cerebral cortex has been shown to be involved in the fate determination of daughter cells (Chenn and McConnell, 1995). Vertical cleavages produce identical daughters. In contrast, horizontally dividing cells produce young migratory neurons and cells remaining within the proliferative zone. However, the molecular details of this process are not clearly understood. PTTG and *prc* as genes involved in sister-chromatid separation and cytokinesis, respectively, could contribute into establishment of cleavage orientation which is important for cell fate specification.

Here we present a description of the expression patterns of mouse PTTG and *prc1* homologues during embryonic and postnatal development, with an emphasis on the developing telencephalon.

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0925-4773/00/$ - see front matter
PII: S0925-4773(00)00243-4

1.1. Cloning and characterization of ferret and murine PTTG and prc1 cDNA fragments

The PCR-based cDNA libraries (Lukyanov et al., 1997) were prepared from ferret embryos cortical VZ tissue, taken on defined developmental stages (E26, E32, E37, P0, P14). Profiles of gene expression, represented in those libraries, were compared by means of differential display (Liang et al., 1992). Two cDNA fragments 700 and 400 bp long, showed maximal abundance for stages E26–E37, when neurogenesis inside the VZ proceeds with the maximal intensity (Noctor et al., 1997). Each of the fragments was subsequently cloned from stage E32 VZ cDNA library and sequenced (GenBank accession numbers AF179298 and AF181855, for fPTTG and fPRC, respectively). The first sequence shared 84% identity with the rPTTG coding sequence. The second sequence open reading frame exhibited 65.2% identity with the human PRC1 protein sequence. ESTs corresponding to murine PTTG and *prc* genes were found in the GenBank dbEST database (AA674997 for PTTG and AA895686, AA915283 for *prc*). RT-PCR was used to obtain murine PTTG and *prc* cDNA fragments.

1.2. Embryonic and early postnatal pattern of expression

Whole-mount hybridization of mPTTG and *mprc1* probes to E9.5 embryos exposed a ubiquitous low level expression in all embryonic tissues (data not shown). E11.5 expression of both genes correlated with the cell density (Fig. 1A,D). As development proceeded, at the stage E13.5 (Fig. 1E,H), expression of both genes was found mostly in mitotically active tissues (as opposed to differentiating tissues). In the CNS, the expression of the genes was found within the ventricular zone (Fig. 1E,G). For the later stages, we concentrated on the analysis of PTTG and *prc1* expression in the telencephalon tissues. During the E15.5 stage, the expression of PTTG and *prc1* in the VZ of telencephalon reached its maximum (Fig. 2A,F). At this stage the expression patterns of the genes are apparently different. The expression domain of *prc1* expanded across the entire width of the VZ indicating that, the *prc1* gene was expressed by the vast majority VZ cells (Fig. 2E,H). In contrast, cells expressing the PTTG gene were unevenly distributed. Most of the cells expressing the gene were found in the lower part of the VZ (6–10 silver granules per a cell), where cells in M or late G2 phase of

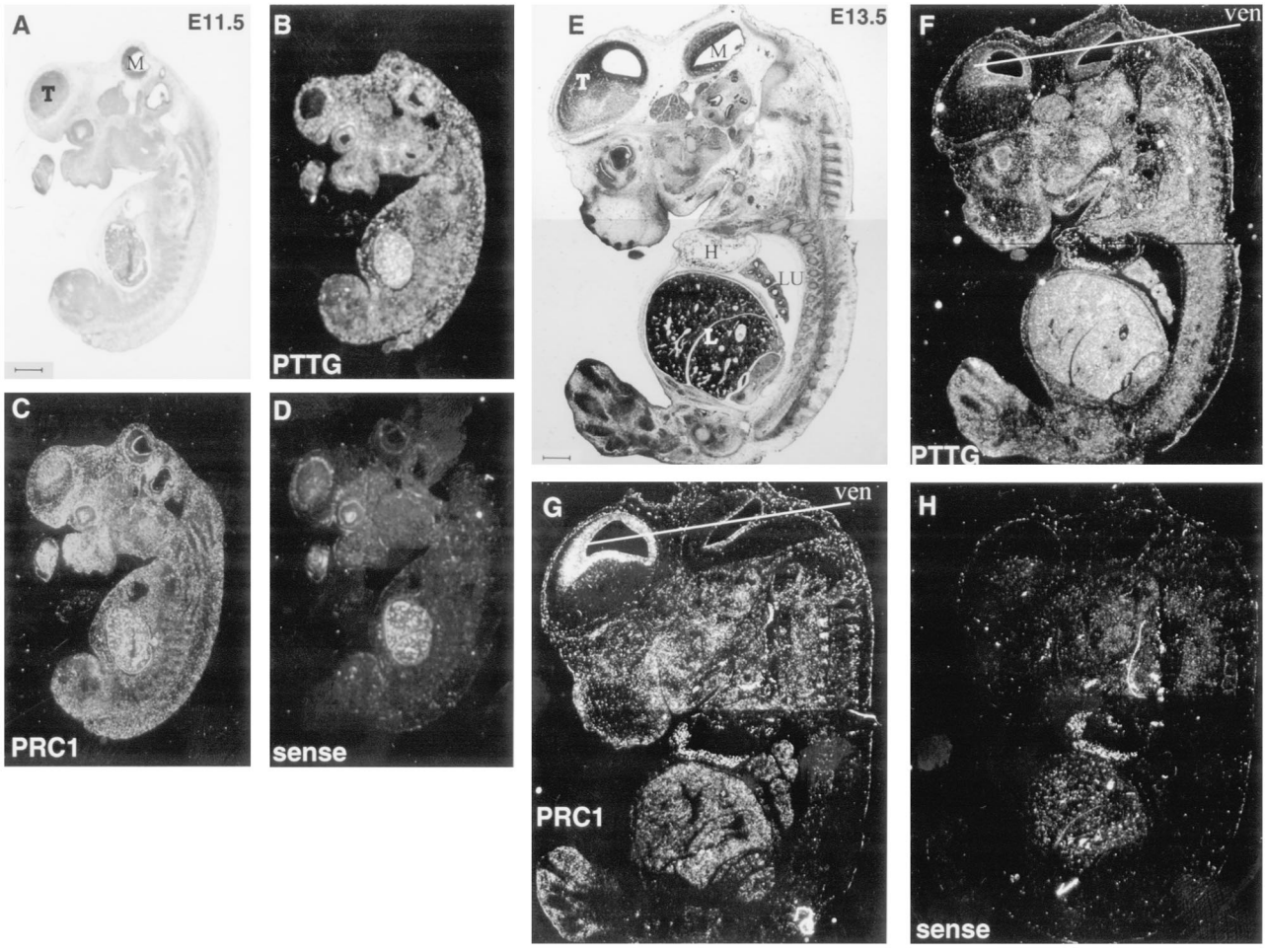

Fig. 1. Localization of PTTG and *prc1* mRNAs in E11.5 (B,C) and E13.5 (F,G) embryos by in situ hybridization (A,E, bright field, D,H, PTTG sense probe control; PRC sense probe gave the same results). T, telencephalon; M, midbrain; H, heart; LU, lung; L, liver; ven, ventricular zone of telencephalon.

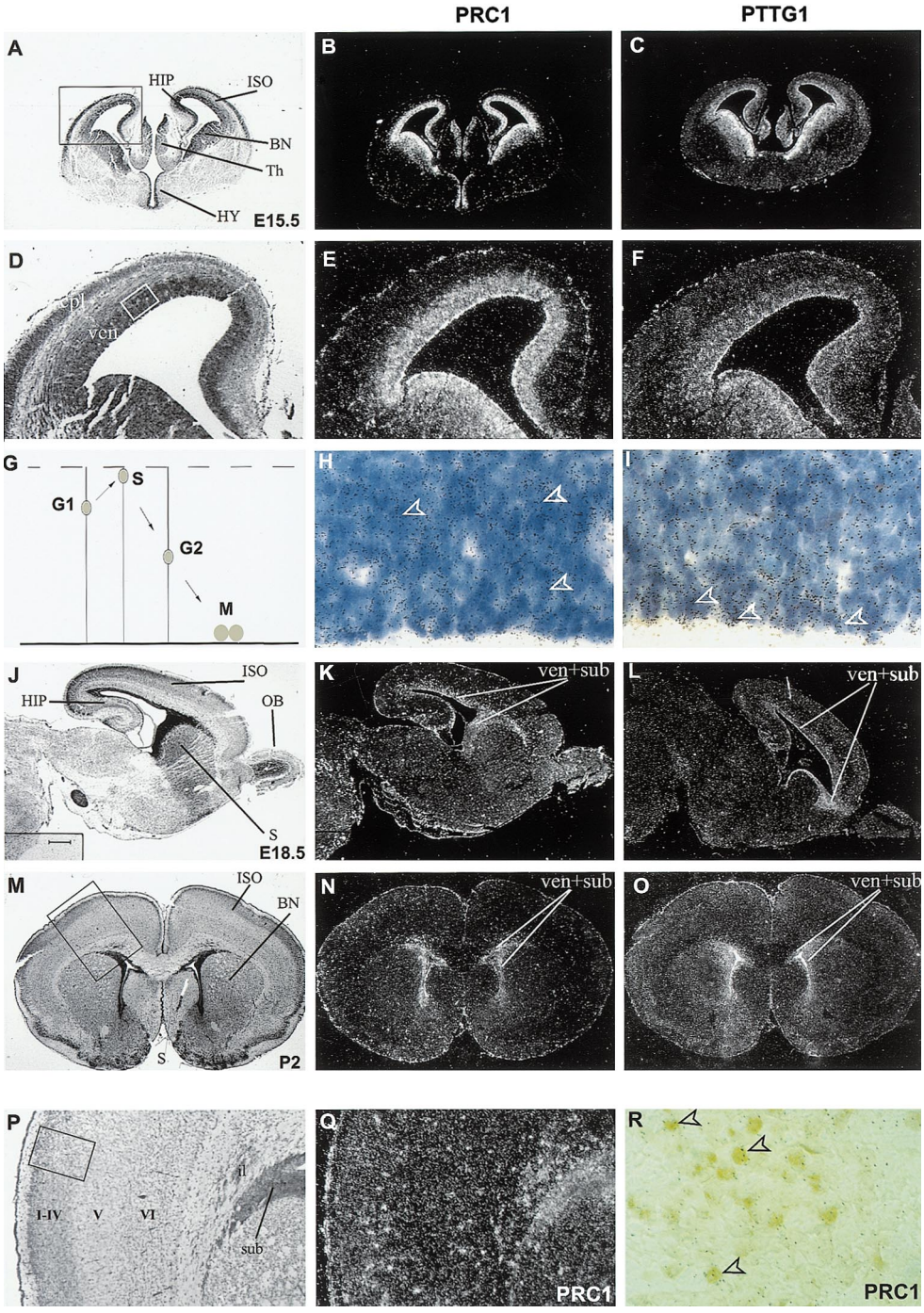

Fig. 2. Expression of *prc1* and PTTG in fetal and early postnatal brains demonstrated by in situ hybridization. (A–C) Transverse sections through a mouse E15.5 telencephalon (A, bright field; B,C, dark field). (D–F) High magnification view of region depicted on (A). (H,I) High magnification view of region depicted on (D). (G) Scheme illustrating positions of cells in the ventricular zone during different phases of mitotic cycle. Note the differences between PTTG and *prc* expression. Examples of cells expressing PTTG and *prc1* are indicated with arrowheads. (J–L) Saggital sections through the E18.5 brain (J, bright field; K,L, dark field). (M–O) Transverse sections through a mouse P2 telencephalon (M, bright field; N,O, dark field). (P,Q) High magnification view of region depicted on (M). Note the cells positive for *prc1* mRNA in superfisial layers and within the intermediate layer. (R) Immunohistochemistry with anti-BrdU antibody combined with radioactive in situ hybridization with *prc1* probe. Cells positive for both labels indicated with arrowheads. The cortical region is the same as indicated by the box in P. I–VI, isocortical layers 1–6; BN, basal nucleus; ISO, isocortex; cpl, cortical plate; Th, thalamus; HIP, hippocampus; il, intermediate layer; HY, hypothalamus; OB, olfactory bulb; S, septum; sub, subventricular layer; ven, ventricular layer (neuroepithelium).

mitosis are located (Fig. 2G; Sidman et al., 1959). In the cells of upper two thirds of the ventricular zone the PTTG gene is down-regulated (2–4 silver granules per a cell (Fig. 2I). This data indicates that the level of PTTG mRNA is regulated differentially during the phases of the mitotic cycle.

During the stages E18.5–P2 (Fig. 2J,Q), transcripts of both

genes still persist in the ventricular/subventricular zone, though their abundance is considerably diminished compared to the stage E15 (Fig. 2A,F). Few cells positive for *prc1* but not for PTTG could also be found inside the cortical plate and intermediate zone (Fig. 2K) of the E18.5 cortex. By P2, the amount of non-subventricular zone cells positive for *prc1* was increased. These cells were especially abundant in superficial cortical layers (Fig. 2K,M,N) and in the part of intermediate zone adjacent to the subventricular zone. To investigate whether these cells were mitoticaly active ones, we combined in vivo BrdU pulse labeling of P2 mice with radioactive in situ hybridization (see Methods). One hour after the pulse, all BrdU positive cells were also positive for *prc*.1. However, there were some cells that did not incorporate BrdU, but were positive for *prc1* (Fig. 2R). Most of the cells expressing *prc1* were mitoticaly active. We detected neither *prc1* nor PTTG expressing cells in the adult telencephalon.

2. Methods

Ferret ventricular zone tissue was dissected under sterile conditions as previously described (Katz, 1987). RNA was isolated as described by Chomczynski et al. (1987). Differential display was performed as described (Liang and Pardee, 1992). Ds cDNA was synthesized with SuperScript Reverse Transcriptase and reagents for the second strand Gubler & Hoffman synthesis, which were supplied in the Marathon cDNA Amplification Kit (CLONTECH) following the supplier's protocol. RT-PCR was performed as described (Lukyanov et al., 1997). Oligonucleotides were designed on a base of murine EST sequences. The final products of 700 bp (*prc1*) and 400 bp PTTG were cloned into pAtlas plasmid and thereafter sequence confirmation was used as in vitro transcription templates for in situ hybridization. In situ hybridization experiments on whole-mounts or tissue sections were performed as previously described (Simmons et al., 1989; Nieto et al., 1996). Identification and naming of brain structures were done after Alvarez-Bolado and Swanson (1996). For the BrdU labeling combined with in situ hybridization P2 mice were injected with 100 μg/g bodyweight bromodeoxyuridine (BrdU). One hour later the animals were sacrificed and brains were processed for in situ hybridization on tissue sections (see above). Before dipping the sections were processed for BrdU immunohistochemistry with anti-BrdU antibody (Bio-Science Products), color reactions were performed using ABC kit (Vector Laboratories). Sections were processed for autoradiography as described (Simmons et al., 1989; Nieto et al., 1996).

Acknowledgements

We thank Dr. N. Usman, Dr. G. Alvarez-Bolado and Dr. T. Thomas for fruitful discussion. V.T. was supported by an EMBO postdoctoral fellowship.

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