Identification and characterization of the t(w73) candidate gene Ortc3
Verhaagh, S.F.M.J.

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The extraneuronal monoamine transporter Slc22a3/Orc3 co-localizes with the Maoa metabolizing enzyme in mouse placenta

S. Verhaagh, D.P. Barlow and R. Zwart

The extraneuronal monoamine transporter *Slc22a3/Orct3* co-localizes with the *Maoa* metabolizing enzyme in mouse placenta

S. Verhaagh, D.P. Barlow and R. Zwart

Department of Molecular Genetics, The Netherlands Cancer Institute, Amsterdam, The Netherlands

1 Present address: Department of Developmental Genetics, ÖAW Institute of Molecular Biology, Salzburg, Austria

Monoamine clearance is a combined function of uptake mechanisms in the plasma membrane with intracellular metabolizing enzymes. Two different uptake mechanisms have been described. Uptake_1 is located in presynaptic neurons, whereas uptake_2 is extraneuronal. Recently, the *Slc22a3/Orct3* gene was identified as the extraneuronal monoamine transporter. In mouse embryonic development *Orct3* expression is restricted to the placenta, which is also a site of expression of neuronal transporters. We have used RNA blots and *in situ* hybridization to examine the expression of *Orct3* and other members of the monoamine uptake and metabolizing pathways in mouse placenta. The results show that *Orct3* expression overlaps that of the monoamine metabolizing enzyme *Maoa* in the labyrinth layer of the placenta with an expression pattern distinct from that of the neuronal transporters *Slc6a2/Net* and *Slc6a4/Sert*.

Keywords: placenta, catecholamine clearance, *Orct3*, *Slc22a3*, EMT, mouse, organic cation transporter, extraneuronal monoamine transporter, uptake_2, *Net*, *Sert*, *Maoa*
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Results

Recently, the Slc22a5 (Oct3/EMT) gene has been identified in mouse, rat, and man (1-3). Both rat and human Oct3 have the capacity to transport monoamines in vitro (3,4). Based on kinetic parameters, inhibitor profile, and expression pattern, it was proposed that Oct3 is the extraneuronal monoamine transport system known as uptake2, that is involved in inactivating monoamines (3,5). Northern blot analysis has shown that Oct3 is expressed abundantly and uniquely in placenta during mouse embryonic development (1,2).

RNA in situ hybridization shows expression of Oct3 in a subset of cells in placenta at day 12.5 of development (Fig. 1A,B). Hybridization of adjacent sections shows that the Oct3 expression pattern resembled that of the labyrinth trophoblast marker Tfeb (Fig. 1D and (6)) but not that of Mash2 (Fig. 1C), which is expressed in the spongiotrophoblast (7). Thus, Oct3 expression is restricted to the labyrinth layer, where trophoblast cells are in contact with both the maternal and the fetal circulation to allow an exchange of nutrients, gases, and waste products.

Monoamine clearance by the placenta from both the maternal and the fetal circulation has been described (reviewed in (8,9)). Northern blot analysis of human placenta has also shown the expression of the neuronal transporters NET (noradrenaline transporter) and SERT (serotonin transporter)(10-12). In addition, biochemical and immunohistochemical studies have shown that the intracellular enzymes, catechol-O-methyltransferase (COMT) and

**Figure 1** Oct3 is expressed in the labyrinth layer.
Orct3 co-localizes with Maoa in mouse placenta

Figure 2 Placental expression of monoamine transporters and metabolizing enzymes.
Northern blot analysis of 12.5, 15.5, 17.5, and 18.5 dpc placenta RNA with probes for the monoamine transporters Orct3, Net, Sert, and the monoamine metabolizing enzymes Maoa and Comt. Plasminogen activator inhibitor type I (Pai1) served as a loading control. Sizes of detected mRNAs are indicated.

Figure 3 Differential expression of the extraneuronal and neuronal monoamine transporters, but co-localization of Orct3 and Maoa.
RNA in situ hybridizations of (A,E) Net, (B,F) Sert, (C,G) Orct3, and (D,H) Maoa on 12.5 (A-D) and 17.5 (E-H) dpc placentas. Scale bar, 0.1 mm.
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monoamine oxidase A (MAOA), that inactivate monoamines after transport across the cell membrane, are also present in the human placenta (5,13-15). However, with the exception of MAOA, the cell types in the human placenta that express these transporters and metabolizing enzymes have not been identified. To gain more insight into the process of monoamine clearance in the mouse placenta, the expression of monoamine transporters and metabolizing enzymes was studied by Northern blot and in situ hybridization.

Northern blot analysis shows that besides Orct3, the specific transporters for norepinephrine (Net) and serotonin (Sert) are expressed in mouse placenta (Fig. 2). A decline in the expression of Orct3 was seen towards the end of gestation (Fig. 2 and (1)). The expression of Net also showed a slight decrease in later stages in contrast to the expression of Sert that shows a large increase at day 18.5. Finally, no expression of the dopamine transporter (Slc6a3/Dat, data not shown) could be detected. Of the metabolizing enzymes, Maoa expression showed a decline with developmental age that was very similar to that of Orct3, whereas Comt levels were not developmentally regulated. These results show that both uptake and uptake components as well as the metabolizing enzymes are present and developmentally regulated in the mouse placenta.

To determine the cellular expression pattern of these monoamine clearance components in placenta, RNA in situ hybridization was performed at two developmental stages. At day 12.5 of gestation, the neuronal transporter Net was expressed primarily in the endothelial cells surrounding the maternal blood sinuses in the decidua basalis. Lower levels were detected in trophoblast giant cells and in large rounded cells in the labyrinth layer (Fig 3A). At day 17.5, Net expression is no longer detected in the giant cells and in the labyrinth layer, but expression in the decidual endothelial cells remains high (Fig 3E). In contrast to Net, Sert expression was seen in a small number of solitary cells in the labyrinth at day 12.5 of development (Fig. 3B). The increase of Sert expression during late gestation is the result of an induction in the spongiotrophoblast layer (Fig. 3F). Comparison of 12.5 and 17.5 dpc placental sections shows that Orct3 is expressed in a reduced number of cells in the labyrinth layer at later gestation (Fig. 3C,G). These results show that the monoamine transporters Net, Sert, and Orct3 are located in different compartments in the mouse placenta. Furthermore, the in situ data provide support for the functional activities described for NET and SERT at the maternal side of the human placenta based on brush border vesicles (11,16,17). For the metabolizing enzyme Comt, sensitivity fell below detection levels in placenta (data not shown). Maoa expression was detected in the labyrinth layer at day 12.5 in a pattern that overlapped that of Orct3 (Fig. 3C,D), but was reduced below detection levels at 17.5 dpc (Fig. 3H).

These results show that monoamine uptake and metabolizing pathways are present in the mouse placenta. The uptake1 transporter Net is expressed primarily in endothelial cells surrounding the maternal blood sinuses in the decidua basalis. The expression of the uptake2 extraneuronal monoamine transporter Orct3 co-localizes with the monoamine metabolizing enzyme Maoa in the labyrinth layer, where trophoblast cells mediate transport from both the maternal and fetal circulation. The co-localization of Orct3 and Maoa and their synchronous developmental regulation indicates the presence of a monoamine clearance pathway in mouse placenta. Mice, in which Orct3 is inactivated by homologous recombination, will provide an in vivo model to test the role of Orct3 in placental monoamine transport.

Materials and Methods
Orct3 co-localizes with Maoa in mouse placenta

RNA in situ hybridization

Dioxigenin-labeled probes were generated from the following cDNA fragments: mOrct3 from 2766 to 3499 (Accession No. AF078750); mMash2 complete cDNA from 1 to 1594 (Accession No. AF139595); mTfeb from 868 to 1532 (Accession No AF079095); mNet from 1272 to 1725 (Accession No. U76306); mSert from 1874 to 2465 (Accession No. AF013604); mDat from 2753 to 3287 (Accession No. AF109072); mouse Maoa from nucleotide 1502 to 2093 on the rat sequence (Accession No. D00688); mComt from 723 to 1197 and from 178 to 802 (Accession No. AF076156). RNA in situ hybridization was performed on 20 μm cryosections of day 12.5 and 17.5 pc mouse placentas as previously described (18).

Northern blotting

RNA was isolated by lithium chloride extraction (19), and analyzed on formaldehyde-agarose gels. Hybridizations were performed as described (20). Fragments described above were used as probes. The mouse Pail gene (Accession No. M33960) was used as a loading control.

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References
