The transcription factor GCMa is a member of a new small family of transcription factors with a conserved zinc-containing DNA-binding domain. All members of this transcription factor family play crucial roles as master regulators during development. GCMa is restricted to placenta during development and to kidney and thymus at postnatal stages. It is essential for the formation of the placental labyrinth and as a consequence for survival of the embryo from mid-embryogenesis onwards. Here, we identify Pitx transcription factors as GCMa-interacting proteins. We show that Pitx proteins interact via their conserved homeodomain with the DNA-binding domain of GCMa. As a consequence, Pitx proteins and GCMa exhibit cooperative DNA binding. Furthermore, Pitx proteins influence GCMa-dependent promoter activation in a cell-specific manner. One of the three Pitx paralogues in mice, Pitx2, is the predominant Pitx member present in the placenta and colocalizes on the cellular level with GCMa in the kidney. This is the first description of a regulatory cross-talk between a transcription factor of the GCM family and a homeodomain protein.

GCM1 (gial cells missing) proteins are a small group of transcription factors containing a highly conserved DNA-binding domain with novel mode of conformation and DNA interaction (1–6). Contrary to their structural conservation, GCM proteins are involved in very different biological events as key regulators (7–19). Whereas the prototype member GCM/glide was identified in the nervous system of Drosophila and shown to play a role in gliogenesis, vertebrate homologs turned out to be located and to function in tissues such as placenta, kidney, thymus, and parathyroid gland (7–19). Knockout mice proved to lethality around E9.5 (11, 14). Postnatally, GCMa is expressed in neonatal mice and is restricted to the S3 segment of proximal tubules (18). GCMb knockout mice lack the parathyroid gland and suffer from partial neonatal lethality caused by defects in calcium and phosphate homeostasis (12). Although the octameric binding motif (ATGGGGGT) of GCM proteins has been identified and its main biochemical properties have been thoroughly studied, up to now only two potential target gene promoters have been described for GCMa, namely aromatase and syncytin (1, 20, 21). No target gene is known for GCMb.

Transcriptional regulators of the Pitx group also exert crucial roles during mammalian development (22–32). Their common structural characteristic is a bicooid-related homeodomain. There are three Pitx paralogues in mice, which are highly conserved within their amino-terminally located homeodomain (>97% identity) and still well conserved over the whole remaining carboxyl-terminal length (55–70% identity). A short region near the carboxyl-terminal end has been termed OAR (Otx, Aristaless, Rex) and might modulate transactivation capacities or be involved in protein-protein interactions (28, 33). Pitx1 is expressed in many tissues, including the stomodeum and its derivatives such as the pituitary, where a large set of Pitx1 target genes has been identified. Pitx3 is expressed in the midbrain dopaminergic system and in the eyes (34, 35). Pitx2 was originally identified by positional cloning in patients with Axenfeld-Rieger syndrome, a rare autosomal dominant hereditary condition characterized by ocular anomalies as well as dental hypoplasia and defects in umbilical development (27, 28, 30, 36, 37). The Pitx2 gene encodes three isoforms in mice (Pitx2a–Pitx2c), and a fourth isoform (Pitx2d) has been identified in humans (27, 38–42). The different isoforms arise from alternative splicing and use of different promoters. All isoforms can combine as homodimers, and heterodimers are formed with Pitx2b (43).

We searched for proteins interacting with GCMa and identified Pitx proteins. At least one Pitx protein is localized in the same tissues where GCMa is expressed. Furthermore, the interaction of GCMa and Pitx modulates GCMa-dependent promoter activation in reporter gene assays. We mapped the GCMa DNA-binding domain and the homeodomain of Pitx proteins as the regions that physically associate with each other. Our findings that GCMa interacts and cooperates with a member of the Pitx transcription factor family might open a new avenue for the identification of GCMa target genes and will yield insight into the mechanistic aspects of GCMa function.

**Experimental Procedures**

Plasmid Constructs and Yeast Two-hybrid Experiments—Bait plasmids were generated by subcloning full-length GCMa and parts thereof into pGBKT7 (Clontech) using restriction sites EcoRI and SalI. DNA fragments were amplified from the GCMa cDNA (Fig. 1) by use of the following 5′-primer: 5′-agt aag taa tgg aac tgg acg act ttg a-3′ and 3′-primers: 5′-gca ggt gct cga ctt ctt gag cat gaa gag cag agg tt-3′ (full-length) or 5′-gca ggt gta ctt gtt cgg cag agg-3′ (GCMa220, Fig. 1).

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§ The abbreviations used are: GCM, glial cells missing; CMV, cytomegalovirus; RT, reverse transcription; PBS, phosphate-buffered saline; GST, glutathione S-transferase.
For expression and immunoprecipitation, GCMa constructs were myc-tagged and transferred to pCMV5 by PCR amplification using the 5’-primer: 5’-tat aga tct gac acc atg gag gag cac aa-3’ annealing 5’ to the myc-epitope of pG5K7 and the above-mentioned 3’-primer, shown with one of the different bait plasmids as template. Additionally a myc-tagged GCMa167, containing only the DNA-binding domain of GCMa was created by using the same 5’-primer and 5’-cag tac cag atc acctg tgg act ttc ttc atg-3’ as 5’-primer.

The insert of the prey plasmid containing human Pitx1 (Acc. No. XM_047746.1, pos. 560–1273) was transferred to pCMV5 and at the same time fused at the 5’-end with a myc-tagged att-3 primer (gatc aac cag cag gcc gag ctg; 2, gat cgg atc cct gtc ctc tca gag tat gtt t; XM_047746.1, pos. 560–1273) was transferred to pCMV5 and at the 5’-end with a gat cgt cga ctt agc gtt ccc gct ttc tcc-3’ primer fused to the Gal4-DNA-binding domain as bait. Potential candidates that positive clones do interact with the bait and not with the Gal4-DNA-binding domain. Selected positive clones were used to subclone parts of Pitx2 into pGEX-KG to create GST fusions.

Yeast two-hybrid analysis was performed mainly according to the manufacturer’s instructions (Clontech). In brief, after yeast transformation, transformants were selected on agar synthetic complete medium lacking leucine, tryptophan or both. Screening of a human HeLa cDNA Matchmaker library (Clontech) was performed with GCMa220 bait-3 primer.

For GST pull-downs, bacteria were grown in 200-ml cultures until the reaction mixture. Samples were loaded onto a 10% polyacrylamide gels and electrophoresed in 0.5× TBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.3) at 120 V for 1.5 h. Gels were dried and exposed for autoradiography.

For immunoprecipitation, transfected COS7 cells grown on 100-mm plates were lysed in the presence of 2 μg/ml leupeptin and aprotinin each in ice-cold 10 mM Hepes (pH 7.9), 0.2 mM EDTA, 2 mM MgCl2, 1 mM dithiothreitol, and 1% Nonidet P-40. Immediately after 4°C overnight. One-fifth of the extract was used for immunoprecipitation after adding 1 μl of a monoclonal antibody against the 5’- (Novagen) or myc-epitope (Cell Signaling) in 500 μl of HNTG buffer (50 mM Hepes, pH 7.5, 150 mM NaCl, 1 mM EDTA, 10% glycerol, 0.1% Triton X-100) (Invitrogen) and 1% Nonidet P-40. Complete protease inhibitor mixture (Roche Applied Science). Homogenization was carried out at 4°C using a Dounce homogenizer with a typical tissue to buffer ratio of 8 ml of buffer per gram of tissue. After 20 strokes of homogenization, homogenates were centrifuged at 10,000 × g for 5 min, and the supernatants were collected. To bind antibodies to protein A-Sepharose, 40 μl of protein A beads were first incubated overnight with an equal mixture of goat-anti-Pitx2-C16 (Santa Cruz Biotechnology) and goat-anti-Pitx2-N17 (Santa Cruz Biotechnology) at 4°C in PBS. After removal of unbound supernatant, the antibody-loaded protein A-Sepharose was washed three times with PBS. The following steps were the same as for immunoprecipitation of cell extracts (see above). For detection of endogenous GCMa protein in Western blots a rabbit anti-GCMa antibody (3) was used.

For GST pull-downs, bacteria were grown in 200-ml cultures until A600 reached 0.4 and induced by 1 mM isoproply 1-thio-β-n-galactopyranoside for 4 h to express the GST fusion proteins. Bacteria were collected by centrifugation, incubated in sonication buffer (50 mM NaH2PO4, 300 mM NaCl, 25 units/ml benzonase, 10 μg/ml leupeptin, 10 μg/ml aproitin, 1 μl/ml Triton X-100, 10 μg/ml DNase I, 15 units/ml RNase A, 0.5% Triton X-100) for 30 min on ice. The supernatants containing the GST fusion proteins were supplemented by 30 μl of equilibrated glutathione beads and incubated under constant rotation at 4°C. After washing three times with washing buffer (4.3 mM NaH2PO4, 1.47 mM KH2PO4, 1.37 mM NaCl, 2.7 mM KCN) an aliquot of the beads, now carrying the GST fusion protein, was incubated together with 25 μl of extract from COS7 cells or mouse kidney tissue expressing the desired protein after transient transfection.
GCMa and Pitx Proteins Interact in Vivo through Their DNA-Binding Domains—To confirm the interaction between GCMa and Pitx proteins, we transiently transfected COS7 cells with part of the open reading frame of human Pitx1 (prey, Fig. 1b) coding for residues 77–315 and corresponding to the homeodomain plus the following regions until the regular stop codon (Fig. 1, b and d).

Only One Pitx Member, Pitx2, Is Present in the Developing Placenta and in the Kidney—To analyze whether Pitx genes are transcribed in the same tissues as GCMa, we performed RT-PCR studies (Fig. 2a). First we used a mismatch primer pair capable of amplifying the homeodomains of all three Pitx members. Using these primers, we detected signals in tissues of adult mice, namely in brain, kidney, and spinal cord. We failed to detect any mRNA for Pitx members in adult liver and thymus (Fig. 2). Additionally, we observed Pitx transcripts in placenta of GCMa<sup>+/−</sup> and GCMa null animals (GCMa<sup>lacZ/lacZ</sup>), see Fig. 2) at E9.5. Cloning and sequencing of the PCR product from kidney and placenta yielded fragments corresponding to Pitx2. No other member of the Pitx family could be detected in the cloned PCR products. By repeating RT-PCRs with specific primers for the three Pitx family members, we detected Pitx2 in all of the tissues previously identified as expressing a Pitx member, but neither Pitx1 nor Pitx3, indicating that Pitx2 is the predominantly expressed Pitx protein in these tissues (Fig. 2b). Due to technical limitations, the presence of very low levels of Pitx1 and Pitx3 in kidney and placenta cannot be excluded. Because several Pitx2 isoforms have been reported, we also used isoform-specific primers for detection of Pitx2a, Pitx2b, or Pitx2c and mainly detected Pitx2c in kidney and placenta (data not shown). For the following experiments we cloned full-length Pitx2 from mouse kidney.

**RESULTS**

Pitx Homeodomain Proteins Interact with GCMa—To isolate cofactors interacting with GCMa, we set up a yeast two-hybrid screen. Because fusions of the Gal4 DNA-binding domain to carboxyl-terminal GCMa regions containing the protein’s transactivation domains (44) induced Gal4-dependent reporters in transformed yeast, even in the absence of a Gal4-activating partner, we fused different amino-terminal portions of GCMa to the Gal4 DNA-binding domain. The amino-terminal part of GCMa up to the first transactivation domain exhibited tolerable autonomous transactivation and could be used as bait for the Gal4-based yeast two-hybrid system (GCMa220, Fig. 1, a and d). Expression of the bait (GCMa<sub>220</sub>) was verified by Western blot. Furthermore, growth kinetics of transformed yeast cells on agar lacking histidine and adenine. Note that growth occurs only in yeast that express GCMa and Pitx<sub>1</sub> (Fig. 1, b and d).

**FIG. 1.** GCMa and Pitx as interaction partners. a–c, schematic representation of constructs. a, full-length GCMa is shown at the top. Carboxyl-terminal truncations of GCMa are depicted below the full-length construct. GCMa<sub>220</sub> fused to Gal4-DNA-binding domain has been used as bait in the yeast-two-hybrid assay. b, full-length Pitx1 and the part identified by the yeast-two-hybrid screen are shown. c, Pitx2 constructs are depicted that were used for mapping the epitope that interacts with GCMa. DBD, GCMa DNA-binding domain; TA1, TA2, GCMa transactivation domains; HD, homeodomain; OAR, conserved modulatory domain of Pitx proteins. d, interaction of GCMa and Pitx1 in yeast leading to growth of transformed yeast cells on agar lacking histidine and adenine. Note that growth occurs only in yeast that express GCMa and Pitx1 (A) or p53 and SV40 T as a positive control for interaction (E).
GCMa alone or with GCMa in combination with Pitx1 or Pitx2. We immunoprecipitated proteins from these transfected cell extracts with an antibody reactive against GCMa and observed coprecipitation of Pitx1 as well as Pitx2 proteins (Fig. 3a). In complementary immunoprecipitations we used an antibody against the T7 tag fused to the amino termini of the Pitx proteins. Now, we transiently transfected COS7 cells with Pitx members alone or with Pitx members in combination with GCMa. Full-length GCMa coprecipitated with both Pitx1 and Pitx2 (Fig. 3b).

To analyze whether the interaction between GCMa and Pitx2 also occurred in a physiological context, we used tissue extract from kidney, where GCMa and Pitx2 are coexpressed (Fig. 2). First, we performed immunoprecipitations. When precipitating endogenous Pitx2 from kidney extract, we were able to coprecipitate endogenous GCMa (Fig. 3c). Concurrent electrophoresis of an extract of transiently transfected COS7 cells by GCMa served as a size-control (Fig. 3c). These data demonstrate that endogenous amounts of GCMa and Pitx2 are sufficient to interact with each other. We also successfully used a GST-Pitx2 fusion protein to pull down endogenous GCMa from kidney extracts (Fig. 3d).

To map the interacting epitopes we used truncated versions of GCMa and Pitx2 proteins (Fig. 1, a and c). The original GCMa bait is composed of the DNA-binding domain and the region between the DNA-binding domain and the first transactivation domain (GCMa220). Similar to full-length GCMa, GCMa220 can be coprecipitated with full-length Pitx2 (Fig. 4a). Coprecipitation of GCMa220 was also observed with the homeodomain of Pitx2 (Fig. 4a). In contrast, carboxy-terminal parts of Pitx2 corresponding to amino acid residues 211–324 or 268–324 were not able to coprecipitate GCMa220 (Pitx2- Δ211 and Pitx2-Δ324 in Fig. 4a). Having identified the homeodomain of Pitx2 as the region interacting with GCMa220, we asked which area of GCMa220 might be responsible for this contact. We created a shorter version containing only the DNA-binding domain of GCMa (GCM167, Fig. 1a). Even GCMa167 was sufficient for interaction with Pitx2 proteins (Fig. 4b). Because the DNA-binding domain is a conserved structural element of GCM family members, we investigated if the DNA-binding domain of GCMb is also able to interact with Pitx2. Indeed we observed that the DNA-binding domain of GCMb interacted with Pitx2 (Fig. 4b).

In addition to immunoprecipitations, we also performed GST-pull-down experiments using fusions between GST and full-length Pitx2 and parts thereof. As already observed in immunoprecipitations, GCMa220 bound to full-length Pitx2 and the homeodomain of Pitx2, but not to carboxy-terminal parts of Pitx2 corresponding to amino acid residues 153–324, 211–324, or 268–324 (Fig. 5a). We could also confirm in GST-pull-downs that the DNA-binding domain of GCMa is sufficient for the interaction between GCMa and Pitx2 as GCMa167 binds to both full-length Pitx2 and the homeodomain (Fig. 5b). We also inspected the binding ability of GCMb180 by GST-pull-down and confirmed that GCMb180 is able to interact with...
full-length Pitx2 and with the homeodomain of Pitx2 (Fig. 5c). Our GST-pull-downs and immunoprecipitations consistently reveal that the DNA-binding domains of the two respective transcription factors mediate their interaction.

Pitx2 and GCMa Bind Cooperatively to DNA—To assess whether the observed interaction between GCMa and Pitx2 might influence DNA binding characteristics of the two proteins, we performed electrophoretic mobility shift assays. Using an oligonucleotide containing GCMa and Pitx binding motifs separated by 6 bp (Fig. 6a), we first showed that both GCMa and Pitx2 are able to bind to this oligonucleotide. However, GCMa appears to bind to this oligonucleotide with higher affinity (Fig. 6b). When incubating a low amount of Pitx with the labeled probe, the amount of the dimeric complex was below the detection limit. But when increasing amounts of GCMa were added in the presence of this low amount of Pitx2, we detected a ternary complex consisting of GCMa, Pitx2, and the probe (Fig. 6b). The amount of this ternary complex strongly exceeded the amount of Pitx2-DNA complex formed with the same amount of Pitx2 in the absence of GCMa indicating that increasing amounts of GCMa allowed more Pitx2 protein to bind to the probe (Fig. 6b). To verify the composition of the ternary complex we performed supershift studies. An antibody that detects the myc tag fused to GCMa, recognized GCMa bound to DNA, and caused a significantly slower migration of this binary complex. When an antibody was used that recognizes the T7 tag fused to Pitx2, it also supershifted the ternary complex. Additionally, most of the GCMa was released from the ternary complex and re-appeared in a binary complex with the labeled probe (Fig. 6c).

Pitx2 Changes GCMa-dependent Promoter Activation in a Cell-specific Manner—GCM proteins bind to the octamer AT-GCGGGT motif on DNA and can thus activate luciferase reporter genes whose expression is under the control of a promoter containing six tandemly arranged GCM-binding sites (6xgbs-luc) (1). This reporter was used in transfections of two different cell lines to examine the influences of cotransfected Pitx proteins (Fig. 7). Whereas Pitx1 and Pitx2 do not influence
transcription from the 6xgbs reporter in HEK293 cells, a robust 151-fold increase of luciferase activity was detected with GCMα alone (Fig. 7a). In the presence of either Pitx1 or Pitx2, the GCMα-dependent activation rates declined to 2.7- or 43.9-fold, respectively, arguing that Pitx proteins inhibit GCMα-mediated transactivation of the reporter (Fig. 7a).

Transfection of GCMα, Pitx1, or Pitx2 together with the same 6xgbs-luciferase reporter into JEG-3 yielded a different picture. GCMα increased the promoter activity by 33-fold, whereas Pitx1 and Pitx2 even repressed promoter activity by 10- and 5-fold, respectively (Fig. 7b). Surprisingly, GCMα together with either Pitx1 or Pitx2 increases the promoter activity up to three times more than GCMα alone (Fig. 7b). This Pitx-mediated increase of GCMα-dependent promoter activation is all the more noteworthy, because Pitx1 and Pitx2 alone have a repressive effect (Fig. 7b). The results demonstrate that Pitx proteins increase the capacity of GCMα to turn on promoters in JEG-3 cells, whereas in HEK293 cells Pitx proteins inhibit GCMα-dependent promoter activation.

Additional transactivation assays were performed using a reporter containing in its promoter five tandemly repeated composite elements each consisting of a GCM and a Pitx-binding site (5x(gbs/H11001pbs)-luc, see Fig. 7, c and d). This reporter was designed in analogy to Lamolet et al. (45), who described the requirement of a T-box-binding site for Pitx1 activation of POMC transcription. The spacing of 10 bp between the centers of GCM- and Pitx-binding sites ensures close apposition of GCMα and Pitx2 on DNA, because the motifs will be presented one turn apart on the same site of the DNA double helix. Whereas GCMα itself was unable to change promoter activity of 5x(gbs+pbs)-luc in JEG-3 cells, Pitx1 and Pitx2 elicited 35- or 17-fold inductions of the reporter, respectively (Fig. 7d). Cotransfection of GCMα with either Pitx1 or Pitx2 in JEG-3 cells exhibited robust 162- or 165-fold activations (Fig. 7d). Again, synergistic activation was only observed in JEG-3 but not in HEK293 cells. In HEK293 cells GCMα, Pitx1, and Pitx2 activate the 5x(gbs+pbs)-luc reporter 5.1-, 5.5-, and 1.7-fold, respectively (Fig. 7c). In the presence of both Pitx and GCMα proteins, induction corresponds to the additive activation rates (Fig. 7c).

GCMα and Pitx2 Colocalize in the kidney—Our RT-PCR data show the presence of Pitx2 in the kidney. The presence of GCMα in the kidney was shown previously (18). Therefore we examined if both transcription factors colocalize to the same...
FIG. 8. Colocalization of GCMa and Pitx2 in mouse kidney. a, PCR with first strand cDNA generated from RNA of dissected kidney regions. Abbreviations describe different sections of the kidney: CO, cortex; OM, outer medulla; IM, inner medulla. For amplification of GCMa- and Pitx2-specific products, 40 cycles of PCR were performed. b, quantitative evaluation of the amount of GCMa and Pitx2 transcripts were performed by real-time PCR (LightCycler). Amounts were determined relative to the inner medulla which arbitrarily was set to 1. c, immunohistochemistry on cryosections of adult kidney. 12-μm cryosections of the adult mouse kidney were incubated with antibodies that specifically recognize β-galactosidase (green) or Pitx2 (red) as indicated. β-Galactosidase is expressed from the GCMa locus and mimics GCMa expression. An overlay of the two images is shown on the right. Magnification, ×200.

In this study, we present data demonstrating for the first time binding of Pitx proteins to transcription factors belonging to the family of GCM proteins. In particular, Pitx2, which is expressed together with GCMa in kidney and in placenta interacts with GCMa. No interactors have been described so far for GCMa and only a limited number for Pitx proteins. One of them is the pituitary-specific POU-homeodomain protein Pit-1 (28). The other two described Pitx-binding partners are either the basic helix-loop-helix proteins NeuroD1/Pan1 or Tpit, which belongs to the T-box transcription factor family (45, 46).

GST-pull-down data showed binding between Pitx2 and Pit-1, but the interaction was not narrowed down to any particular region (28, 46). In the case of NeuroD1/Pan1, evidence for a direct physical interaction between the basic helix-loop-helix domain of Pan1 and the homeodomain of Pitx1 was presented (28, 46). This latter interaction seems to be specific for the two protein classes rather than for the two proteins, because both proteins are exchangeable by other basic helix-loop-helix and Pitx proteins (46). In analogy, we observed binding between all analyzed GCM and Pitx members, obviously because the conserved DNA-binding domains of the two proteins mediate the interaction between them. Regardless of the ability of GCMa to bind all Pitx members, Pitx2 probably is the only interactor in vivo, because other Pitx paralogues are not expressed at sites of GCMa expression. On the other hand, the fact that all GCM and Pitx members are able to interact with each other makes an interaction between GCMb and Pitx protein members in the parathyroid gland possible. It remains to be seen which of the Pitx proteins, if any, are expressed in the parathyroid gland. If there really is an interaction between GCMb and a Pitx protein, it might also be of relevance for the function of GCMb in a number of human tumor cell lines and in parathyroid adenoma (47, 48), where GCMb has been found to be expressed.

In the case of Pit1 it has been previously observed that, as a consequence of protein-protein interaction, binding of Pitx2 to its cognate DNA-binding site is facilitated (49). We also observed that GCMa helps recruiting Pitx2 to its binding site. This might argue that Pitx proteins generally need coactivators to bind to DNA. But it could also mean that in cases where binding of Pitx proteins is necessary to modulate gene expression, Pitx proteins only occupy their binding sites if their partner transcription factor is already present. Furthermore, the interaction between Pitx and its binding partners has been reported to result in a transcriptional synergism (30, 45, 46). This synergism was detected on either the prolactin- or pro-opiomelanocortin-promoter containing reporters. In line with these previous observations, the interaction between GCMa and Pitx2 synergistically induced transcription of promoters containing solely GCM DNA-binding sites or GCM and Pitx DNA-binding sites. However, in contrast to published cases the transcriptional synergism observed between GCMa and Pitx proteins is cell-specific and was only observed in JEG-3 cells. In HEK293 cells we even measured repressive effects of Pitx proteins on GCMa-dependent promoters of certain configurations. Such repressive effects have not been observed so far for Pitx proteins but might be more common than previously thought.

Pitx2-deficient mice have primarily been studied for their defects in laterality and organogenesis (50). Thus it is difficult to compare placental phenotypes of Pitx2- and GCMa-deficient animals. The observed early mortality of a fraction of Pitx2 embryos around E10 is compatible with the existence of additional placental defects. These have, however, not been investigated so far. Similarly, functions of both proteins in the kidney cannot be compared in the currently available mouse mutants, because GCMa knockout mice do not survive long enough.

Recently we speculated that GCMa is involved in mediating physiological rather than developmental functions of the kidney, because GCMa is not expressed in the embryonic kidney, but begins to be expressed perinatally (18). Now that we identified GCM and Pitx family members coexisting in the same cells in the kidney, potential target genes for both transcription factors might be postulated to contain adjacent GCM and Pitx-binding sites in their regulatory elements. Therefore, computational searches for adjacent GCM and Pitx-binding sites
might enable the identification of such target genes in the kidney.

The presence of Pitx2 transcripts in placenta is compatible with the assumption that GCMa and Pitx2 might also colocalize in the placenta and jointly regulate common target genes. Due to limitations in the quality of antibodies we could not directly investigate this. However, if true, comparison of GCMa- and Pitx2-dependent target genes in kidney and placenta might help to understand the biological significance of the interaction between these two transcription factors.

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