HHS Public Access

Author manuscript

Endocrinol Metab Clin North Am. Author manuscript; available in PMC 2019 December 01.

Published in final edited form as:

Endocrinol Metab Clin North Am. 2018 December ; 47(4): 733–742. doi:10.1016/j.ecl.2018.07.002.

Embryology of the parathyroid glands

Kristen Peissig, M.S. [Graduate student],
Dept of Genetics, University of Georgia, Athens, GA USA

Brian G Condie, PhD [Associate Professor], and
Dept of Genetics, University of Georgia, Athens, GA USA

Nancy R Manley, PhD [Professor]
Dept of Genetics, University of Georgia, Athens, GA USA

Keywords

Parathyroid; organogenesis; GCM2; PTH; SHH

Introduction

Maintaining a stable level of serum calcium is important for proper muscle function, neurotransmission, and enzyme and hormone secretion, among many other functions [1]. Calcium homeostasis in terrestrial vertebrates is regulated by the parathyroid glands, endocrine organs located in the neck near the thyroid gland. Parathyroid glands express Calcium-Sensing Receptors (CaSR) that monitor serum calcium levels [2]. When these receptors detect low levels of calcium, the parathyroids secrete parathyroid hormone (PTH), which interacts with G-protein-coupled receptors on bone cells to release calcium from long bones into the bloodstream. In the kidney, PTH increases resorption of calcium in the ascending limb of the loop of Henle and in the distal tube as well as increasing excretion of inorganic phosphate into the urine [3]. When the parathyroids do not function properly, calcium/phosphorus homeostasis is disrupted. This results in one of two parathyroid disorders: hyper- or hypoparathyroidism. Hyperparathyroidism is usually caused by parathyroid adenomas, which are benign parathyroid tumors that cause an excess of PTH to be produced and secreted. For hyperparathyroidism, the current treatment option is to surgically remove the affected parathyroid, and the patient is cured following this procedure. Hypoparathyroidism is observed much less frequently in the clinic and has a number of

CORRESPONDING AUTHOR: 500 DW Brooks Drive, Coverdell Bldg Ste. 270, University of Georgia, Athens, GA, 30602; nmanley@uga.edu.
AUTHOR CONTACT INFORMATION
1. 500 DW Brooks Drive, Coverdell Bldg Ste. 270, University of Georgia, Athens, GA, 30602; kpeissig@uga.edu
2. 500 DW Brooks Drive, Coverdell Bldg Ste. 270, University of Georgia, Athens, GA, 30602; bcondie@uga.edu
3. 500 DW Brooks Drive, Coverdell Bldg Ste. 270, University of Georgia, Athens, GA, 30602; nmanley@uga.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

DISCLOSURE STATEMENT
The authors have nothing to disclose.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

causes, including mutations in genes required for proper parathyroid development [4]. There is currently no permanent cure for hypoparathyroidism, and patients are managed by daily use of activated forms of vitamin D, calcium supplements, and/or recombinant parathyroid hormone in order to help regulate their calcium levels (please see Muriel Babey, Maria-Luisa Brandi and Dolores Shoback's article "Conventional Treatment of Hypoparathyroidism" and Gaia Tabacco and John P. Bilezikian's article "New Directions in Treatment of Hypoparathyroidism," in this issue.).

Understanding the normal process of parathyroid development can provide important information about how and why parathyroid disorders manifest. Much of the research done in this area has been done in mice. Embryonic development of the parathyroid is a topic that has been studied since the 1930s, and the findings up to the present will be summarized in this review.

Major Events during Parathyroid Organogenesis and Morphogenesis in Mice

Beginning around embryonic day 9 (E9.0) in mouse development, bilateral endodermal outpocketings called pharyngeal pouches form on the lateral aspects of the pharynx. These pouches are epithelial structures surrounded by neural-crest-derived mesenchymal cells (NCC). Both pharyngeal endoderm and NCC provide signals that play an important role in initial pouch patterning. In mice, the parathyroid glands develop in the 3rd pharyngeal pouches, yielding one set of bilateral parathyroid glands [5]. Humans have two pairs of parathyroid glands, developing from both the 3rd and 4th pouches [6–8]. In mice, the anterior-dorsal region of each 3rd pharyngeal pouch forms a parathyroid gland, while the posterior-ventral region develops into one lobe of the thymus, the organ responsible for T cell development[9]. Early parathyroid organogenesis initiates by specifying parathyroid and thymus cell fates, and by E11.5 essentially all cells in the developing primordium have assumed either GCM2+ parathyroid or FOXN1+ thymus fates (Figure 1). This stage is followed by separation of the combined parathyroid-thymus primordia from the pharynx, which occurs by coordinated apoptosis [10] and is regulated in part by FGF [11] and BMP4 [12] signaling, and possibly other signals from the neighboring mesenchyme.

Once separated from the pharynx, the combined parathyroid-thymus primordial begin to migrate ventrally and posteriorly, mediated by Ephrin signaling between NCC and thymic epithelium [13]. During this migration at about E12.5, the parathyroid and thymus primordia begin to separate from each other. Unlike the separation from the pharynx, this separation event does not involve apoptosis, but rather occurs via a combination of differential cell adhesion mediated by higher levels of E-caherin expression on thymus cells compared to parathyroid cells [12]. BMP4 signaling in particular modulates formation of a 'wedge' of mesenchyme expressing F-actin filaments [14], implying that physical forces are also involved in this separation event. Separation is complete between E12 and E13, during the migration period. After separation is complete, the thymic lobes continue in their migration to the anterior mediastinum above the heart, while the parathyroids do not continue to migrate.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Variability in the timing of this separation leads to a somewhat variable location of the parathyroids in the neck region, usually lateral to the thyroid lateral lobes[15].

The physical separation of the thymus and parathyroids, coupled with the low expression of homotypic adhesion molecules on parathyroid cells, leads to the generation of a ‘trail’ of small clusters of parathyroid cells between the two organs along the migratory path of the thymus [8]. These small clusters of cells can either maintain their parathyroid differentiation state or, at a low frequency, can down-regulate the parathyroid gene expression program and undergo spontaneous transdifferentiation to a thymic epithelial cell fate, resulting in parathyroid-derived cervical thymi[16]. The data from mice suggest that fetal parathyroid cell fate is unstable, but is stabilized postnatally [17], although the mechanism behind this instability and subsequent fate switch is unclear.

The common embryonic origin and process of thymus-parathyroid separation has also led to the idea that the thymus itself makes physiologically relevant levels of PTH[18]. PTH was first detected in the thymus in the original analysis of *Gcm2* null mutants, which lack parathyroid glands. However, subsequent investigation revealed that thymus PTH originated from two sources: (1) parathyroid cells that were ‘trapped’ underneath the thymic capsule during the separation of the parathyroid and thymus during embryonic development, and (2) medullary thymic epithelial cells (mTECs) that express PTH as a self-antigen and do not have any endocrine function [8]. Therefore, the sole source of endocrine-active PTH is the parathyroid.

Gcm2: The Key Regulator of Parathyroid Development

Glial cells missing (GCM) was first discovered in *Drosophila* as a transcription factor that acts as a binary switch for neural cells to become either neurons or glial cells [19,20]. Characterization of the mammalian homologs of *Drosophila* GCM called *Gcm1* and *Gcm2* revealed high expression of Gcm2 in parathyroid glands in developing mice, suggesting a non-neuronal function for this transcription factor [21]. *Gcm2* is expressed at very low levels in the dorsal 2nd and 3rd pharyngeal pouches at E9.5, and by E10.5 its expression is up-regulated in and restricted to approximately the dorsal-anterior third of the 3rd pouch endoderm [9]. *Gcm2* null mice fail to develop parathyroid glands leading to primary hypoparathyroidism [18]. In humans *GCM2* mutations are associated with hypo- and hyperparathyroidism (OMIM 603716). *Gcm2* is required for both parathyroid cell differentiation and survival. In *Gcm2* null mice, and in *Hoxa3* mutants in which *Gcm2* fails to be up-regulated, the parathyroid domain is specified, but these cells undergo rapid and coordinated programmed cell death [22]. PTH is a direct target of GCM2, and its expression is never activated in parathyroid-fated cells in *Gcm2* null mice.

Patterning in the 3rd Pharyngeal Pouch and Initiation of Gcm2 Expression

Initial patterning of the 3rd pharyngeal pouch at E9.5 determines the cell fate of the endodermal cells within it, with cells being fated to either parathyroid or thymus lineages. Parathyroid development occurs at the dorsal end marked by Gcm2 and thymus development at the ventral end marked by Foxn1. These domains are specified and subsequent differentiation determined by a network of signaling pathways and transcription factors

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(Figure 2). One of the earliest genes that affects 3rd pharyngeal pouch patterning is the signaling molecule Sonic Hedgehog (SHH), which is expressed throughout the pharyngeal endoderm but is excluded from the 3rd pharyngeal pouch [23,24] In *Shh* null mutants, the parathyroid domain is not specified and Gcm2 is never activated [23,25]. *Shh* signaling occurs in both the dorsal endoderm and the adjacent NCC mesenchyme, and recent evidence using tissue-specific deletion or activation of SHH signaling showed that signaling from either location is sufficient to specify parathyroid fate and activate *Gcm2* expression [26]. In addition to its role in initiating parathyroid differentiation, SHH has been shown to prevent parathyroid-specific genes such as *Gcm2* from being activated in other pharyngeal pouches [27], suggesting that its role in *Gcm2* expression could be dosage-sensitive.

SHH signaling may act in part through activation of the T-box transcription factor TBX1 in the parathyroid domain. *Tbx1* is expressed throughout the pharyngeal pouches at their earliest stages of development, and is required for pouch outgrowth [28,29]. However, in the 3rd pharyngeal pouch, *Tbx1* is down regulated in the ventral pouch during outgrowth, resulting in its restriction to the parathyroid domain [30]. This restriction may be necessary for specification of thymus fate, as ectopic *Tbx1* is sufficient to block thymic epithelial cell differentiation [31]. However, neither ectopic SHH signaling nor ectopic TBX1 is sufficient to expand *Gcm2* expression into the ventral region [26,31], indicating that either additional positive regulators are present in the dorsal domain, or that negative regulators are present in the ventral domain that block parathyroid fate and/or *Gcm2* expression.

Another transcription factor that is required for *Gcm2* activation is the transcription factor GATA3. In *Gata3* null mice, *Gcm2* never is never activated, and *Gata3* heterozygotes have fewer *Gcm2*-expressing cells during embryonic development and have hypoparathyroidism [32]. This regulation is via GATA3 binding sites in the *Gcm2* promoter. Furthermore, human mutations in *Gata3* cause hypoparathyroidism, indicating that this aspect of *Gcm2* regulation is conserved in humans and mice [33]. As SHH signaling induces *Gata3* expression in the brain [34], it is possible that *Gata3* is also acting downstream of SHH signaling during early parathyroid development, although this possibility has not been experimentally tested.

From E10.5 to E11.5, the signaling molecule BMP4 is expressed in both NCC and endoderm at the ventral end of the 3rd pharyngeal pouch where the thymus will develop [23,35], and multiple lines of evidence suggest that BMP signaling is a positive regulator of thymus differentiation[12,25,36–38]. As opposing gradients of SHH and BMP4 signaling are antagonistic during neural tube patterning, a similar model has been proposed to operate during 3rd pharyngeal pouch patterning [12,23,26]. This model was supported by expansion of *Bmp4* expression into the pharynx in *Shh* null mutants [23], and by the expression of the BMP inhibitor NOGGIN in the dorsal domain during pouch patterning and early organogenesis [35]. However, little functional evidence has supported the possibility that BMP4 signaling suppresses parathyroid fate or *Gcm2* expression.

Gcm2 is Up-Regulated by HOXA3 and Interacting Transcription Factors

Gcm2 is initially expressed at a low level at the dorsal end of the E9.5 3rd pharyngeal pouch, but by E10.5 is up regulated dramatically. Failure to up-regulate *Gcm2* leads to coordinated

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

apoptosis in the parathyroid-fated cells, emphasizing the importance of *Gcm2* up regulation in parathyroid cells. *Hoxa3* was the first *Hox* gene to be knocked out in mice via homologous recombination, and has been shown to play a role in the development of multiple pharyngeal organs, and is required for both parathyroid and thymus organogenesis [15,39,40]. Although there is overlapping function between the group 3 HOX paralogs, only *Hoxa3* has a specific role in the development of the parathyroid and thymus [15]. The formation of the 3rd pharyngeal pouch is normal in *Hoxa3* null mice. However, by E12, the parathyroids are absent, and the thymus rudiments are severely hypoplastic. By E12.5 in Hoxa3 mutants all of the 3rd pharyngeal pouch derivatives have disappeared due to coordinated apoptosis [40–42].

Detailed analysis of global and tissue-specific null alleles for *Hoxa3* showed that *Hoxa3* is not required to specify the parathyroid domain or for initial *Gcm2* expression. In HoxA3 null mice, *Gcm2* expression is initiated, but fails to be up-regulated at E10.5, and by E11.5 *Gcm2* expression is undetectable [42,43]. Analysis of inefficient *Hoxa3* deletion in endoderm via tamoxifen-inducible Cre recombinase showed that HOXA3 up-regulation of *Gcm2* expression at E10.5 is cell-autonomous [42,44] although it remains to be shown that *Gcm2* is a direct HOXA3 target.

HOXA3 has been shown to work in concert with a network of PAX, EYA, and SIX family transcriptional regulators to regulate many of its downstream functions, including thymus and parathyroid organogenesis [45]. *Pax1* null mutants show decreased *Gcm2* expression at E11.5 and parathyroid hypoplasia [46]. *Pax1* expression is reduced in the 3rd pharyngeal pouch in *Hoxa3* null mutants [40], and *Gcm2* expression is further reduced or absent by E11.5 in *HoxA3*$^{+/-}$*Pax1*$^{-/-}$ compound mutants [46], suggesting that *Pax1* may be downstream of *Hoxa3. Pax1* expression is also dependent on the expression of *Eya1* and *Six1* in the 3rd pharyngeal pouch. In *Six1* null mutants, *Gcm2* is initiated but cannot be maintained [47], and the cells subsequently undergo apoptosis consistent with loss of *Gcm2* expression. These results suggest that a HOX-PAX-EYA-SIX network interacts to up-regulate *Gcm2* expression.

HOXA3 may also interact with PBX1, a TALE-class transcription factor. *Pbx1* null mutants have parathyroid and thymic hypoplasia, and reduced *Gcm2* expression [30]. As HOX and PBX proteins are known to act together to regulate multiple developmental processes, these data suggest that PBX1 is also a member of the transcriptional network regulating early *Gcm2* expression [45,48]

Differentiation of Parathyroid Cells

After up-regulation of Gcm2, downstream parathyroid genes are activated including those encoding PTH and CaSR. In addition, the chemokine CCL21 is expressed in the early parathyroid domain at E11.5, and plays a role in initial attraction of lymphoid progenitors to the thymus domain, although this expression is transient and does not have a known role in parathyroid biology [49]. The initiation of *Casr* and *Ccl21* expression in the parathyroid domain is *Gcm2*-independent, but maintenance of their expression is *Gcm2*-dependent [22]. In

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

contrast, *Pth* expression is initiated later, at E12, and is dependent on Gcm2 for both initiation and maintenance [22]

The transcriptional activator MAFB was also found to be important for the activation of PTH [50]. *MafB* is expressed in the parathyroid after E11.5 and its expression is *Gcm2*-dependent. In *MafB* null mutants, PTH expression and secretion are greatly reduced. MAFB was shown to physically associate with GCM2 in order to interact with the PTH promoter via a Maf-recognition element and a GCM2 binding site to turn on its expression [51]. GATA3 also physically interacts with MAFB and GCM2 together with the ubiquitous transcription factor, SP1, in order to activate PTH expression [52].

Parathyroid Development in Humans

Current evidence suggests that parathyroid development in humans occurs in a similar manner as in mice. In humans, the parathyroid glands develop in tandem with the thymus in the 3rd pharyngeal pouch and with the ultimobranchial bodies in the 4th pharyngeal pouch, yielding two sets of bilateral parathyroid glands [53–56]. This was further strengthened when expression of the parathyroid marker, Gcm2, was shown in both the 3rd and 4th pharyngeal pouches in human embryos [8]. This study also showed the presence of 'trailing' *Gcm2* positive parathyroid cells, similar to those seen during mouse parathyroid organogenesis; these small cluster of parathyroid cells were proposed to be the source of many parathyroid adenomas in humans [8]. Several genes known to be important in mouse parathyroid development have also been shown to be associated with human parathyroid disease. Loss of function mutations in *GCMB, GATA3,* and *TBX1* have all been shown to cause hypoparathyroidism in humans [4], consistent with data from mouse studies.

Summary/Discussion

The field of parathyroid organogenesis is small, and there is still much to be discovered. The framework of the regulatory network controlling the establishment of parathyroid cell fate, and the central role of GCM2/GCMB in parathyroid survival and differentiation are now established. For the genes that have already been identified as playing a role in parathyroid development, the precise pathways and mechanisms of action need to be elucidated. For example, TBX1 has been implicated in the regulation of *Gcm2* expression, but further evidence is required to confirm this hypothesis. The interaction between HOXA3, PAX1, and PBX1 also needs to be clarified in terms of their role in *Gcm2* up regulation.

It will be important to identify all of the transcription factors and signaling pathways that play a role in parathyroid cell differentiation during embryonic development. With –omics techniques, researchers will be able identify all of the genes that are being expressed, which genes are in areas of open chromatin, and thus the structure of the network controlling parathyroid organogenesis. Elucidation of the transcriptional network controlling parathyroid differentiation could enable the generation of parathyroid cells from pluripotent cells in culture. The lack of an *in vitro* system for studying parathyroid cells remains a challenge. The only cell lines available are from human adenomas, likely because of the tight proliferation control in parathyroid cells. The question of parathyroid cell fate stability

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

still needs to be addressed as parathyroid cell fate has been shown to be unstable with some parathyroid cells switching to a thymus cell fate[16]. The mechanism of this fate switch will need to be elucidated in order for cell therapy to become a viable treatment option. Solving these problems in basic parathyroid biology could lead to the development of new *in vitro* systems, which would have both research and therapeutic benefits.

References

1. Ramasamy I Recent advances in physiological calcium homeostasis. Clin Chem Lab Med 2006;44(3):237–273. [PubMed: 16519596]
2. Chen RA, Goodman WG. Role of the calcium-sensing receptor in parathyroid gland physiology. Am J Physiol Renal Physiol 2004;286(6):F1005–1011. [PubMed: 15130894]
3. Houillier P, Nicolet-Barousse L, Maruani G, Paillard M. What keeps serum calcium levels stable? Joint Bone Spine. 2003;70(6):407–413. [PubMed: 14667548]
4. Grigorieva IV, Thakker RV. Transcription factors in parathyroid development: lessons from hypoparathyroid disorders. Ann N Y Acad Sci 2011;1237:24–38. [PubMed: 22082362]
5. Cordier A, Haumont S. Development of thymus, parathyroids, and ultimo-branchial bodies in NMRI and nude mice. The American Journal of Anatomy. 1980;157:227–263. [PubMed: 7405870]
6. Gilmour J The embryology of the parathyroid glands, the thymus, and certain associated rudiments. journal of Pathology. 1937;45:507–522.
7. Weller G Development of the thyroid, parathyroid and thymus glands in man. Contrib Embryol 1933;24:93–142.
8. Liu Z, Farley A, Chen L, et al. Thymus-associated parathyroid hormone has two cellular origins with distinct endocrine and immunological functions. PLoS Genet 2010;6(12):e1001251. [PubMed: 21203493]
9. Gordon J, Bennett AR, Blackburn CC, Manley NR. Gcm2 and Foxn1 mark early parathyroid- and thymus-specific domains in the developing third pharyngeal pouch. Mech Dev 2001;103(1-2):141–143. [PubMed: 11335122]
10. Gordon J, Wilson VA, Blair NF, et al. Functional evidence for a single endodermal origin for the thymic epithelium. Nat Immunol 2004;5(5):546–553. [PubMed: 15098031]
11. Gardiner JR, Jackson AL, Gordon J, Lickert H, Manley NR, Basson MA. Localised inhibition of FGF signalling in the third pharyngeal pouch is required for normal thymus and parathyroid organogenesis. Development. 2012;139(18):3456–3466. [PubMed: 22912418]
12. Gordon J, Patel SR, Mishina Y, Manley NR. Evidence for an early role for BMP4 signaling in thymus and parathyroid morphogenesis. Dev Biol 2010;339(1):141–154. [PubMed: 20043899]
13. Foster KE, Gordon J, Cardenas K, et al. EphB-ephrin-B2 interactions are required for thymus migration during organogenesis. Proc Natl Acad Sci U S A. 2010;107(30): 13414–13419. [PubMed: 20616004]
14. Gordon J, Manley NR. Mechanisms of thymus organogenesis and morphogenesis. Development. 2011;138(18):3865–3878. [PubMed: 21862553]
15. Manley NR, Capecchi MR. Hox group 3 paralogs regulate the development and migration of the thymus, thyroid, and parathyroid glands. Dev Biol 1998;195(1):1–15. [PubMed: 9520319]
16. Li J, Liu Z, Xiao S, Manley NR. Transdifferentiation of parathyroid cells into cervical thymi promotes atypical T-cell development. Nat Commun 2013;4:2959. [PubMed: 24343363]
17. Li J, Liu Z, Xiao S, Manley NR. Transdifferentiation of parathyroid cells into cervical thymi promotes atypical T-cell development. Nat Commun 2013;4:2959. [PubMed: 24343363]
18. Gunther T, Chen ZF, Kim J, et al. Genetic ablation of parathyroid glands reveals another source of parathyroid hormone. Nature. 2000;406(6792):199–203. [PubMed: 10910362]
19. Hosoya T, Takizawa K, Nitta K, Hotta Y. Glial cells missing: a binary switch between neuronal and glial determination in Drosophila. Cell. 1995;82(6):1025–1036. [PubMed: 7553844]
20. Jones BW, Fetter RD, Tear G, Goodman CS. Glial cells missing: a genetic switch that controls glial versus neuronal fate. Cell. 1995;82(6):1013–1023. [PubMed: 7553843]

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

21. Kim J, Jones BW, Zock C, et al. Isolation and characterization of mammalian homologs of the Drosophila gene glial cells missing. Proc Natl Acad Sci U S A. 1998;95(21): 12364–12369. [PubMed: 9770492]
22. Liu Z, Yu S, Manley NR. Gcm2 is required for the differentiation and survival of parathyroid precursor cells in the parathyroid/thymus primordia. Dev Biol 2007;305(1):333–346. [PubMed: 17382312]
23. Moore-Scott BA, Manley NR. Differential expression of Sonic hedgehog along the anterior-posterior axis regulates patterning of pharyngeal pouch endoderm and pharyngeal endoderm-derived organs. Dev Biol 2005;278(2):323–335. [PubMed: 15680353]
24. Grevellec A, Graham A, Tucker AS. Shh signalling restricts the expression of Gcm2 and controls the position of the developing parathyroids. Developmental biology. 2011;353(2):194–205. [PubMed: 21349263]
25. Figueiredo M, Silva JC, Santos AS, et al. Notch and Hedgehog in the thymus/parathyroid common primordium: Crosstalk in organ formation. Dev Biol 2016;418(2):268–282. [PubMed: 27544844]
26. Bain VE, Gordon J, O'Neil JD, Ramos I, Richie ER, Manley NR. Tissue-specific roles for sonic hedgehog signaling in establishing thymus and parathyroid organ fate. Development. 2016;143(21):4027–4037. [PubMed: 27633995]
27. Grevellec A, Graham A, Tucker AS. Shh signalling restricts the expression of Gcm2 and controls the position of the developing parathyroids. Dev Biol 2011 ;353(2): 194–205. [PubMed: 21349263]
28. Jerome LA, Papaioannou VE. DiGeorge syndrome phenotype in mice mutant for the T-box gene, Tbx1. Nat Genet 2001;27(3):286–291. [PubMed: 11242110]
29. Xu H, Cerrato F, Baldini A. Timed mutation and cell-fate mapping reveal reiterated roles of Tbx1 during embryogenesis, and a crucial function during segmentation of the pharyngeal system via regulation of endoderm expansion. Development. 2005;132(19):4387–4395. [PubMed: 16141220]
30. Manley NR, Selleri L, Brendolan A, Gordon J, Cleary ML. Abnormalities of caudal pharyngeal pouch development in Pbx1 knockout mice mimic loss of Hox3 paralogs. Dev Biol 2004;276(2): 301–312. [PubMed: 15581866]
31. Reeh KA, Cardenas KT, Bain VE, et al. Ectopic TBX1 suppresses thymic epithelial cell differentiation and proliferation during thymus organogenesis. Development. 2014;141(15):2950–2958. [PubMed: 25053428]
32. Grigorieva IV, Mirczuk S, Gaynor KU, et al. Gata3-deficient mice develop parathyroid abnormalities due to dysregulation of the parathyroid-specific transcription factor Gcm2. J Clin Invest 2010;120(6):2144–2155. [PubMed: 20484821]
33. Van Esch H, Groenen P, Nesbit MA, et al. GATA3 haplo-insufficiency causes human HDR syndrome. Nature. 2000;406(6794):419–422. [PubMed: 10935639]
34. Liu J, Wang X, Li J, Wang H, Wei G, Yan J. Reconstruction of the gene regulatory network involved in the sonic hedgehog pathway with a potential role in early development of the mouse brain. PLoS computational biology. 2014;10(10):e1003884. [PubMed: 25299227]
35. Patel SR, Gordon J, Mahbub F, Blackburn CC, Manley NR. Bmp4 and Noggin expression during early thymus and parathyroid organogenesis. Gene Expr Patterns. 2006;6(8):794–799. [PubMed: 16517216]
36. Bleul CC, Boehm T. BMP signaling is required for normal thymus development. J Immunol 2005;175(8):5213–5221. [PubMed: 16210626]
37. Tsai PT, Lee RA, Wu H. BMP4 acts upstream of FGF in modulating thymic stroma and regulating thymopoiesis. Blood. 2003;102(12):3947–3953. [PubMed: 12920023]
38. Neves H, Dupin E, Parreira L, Le Douarin NM. Modulation of Bmp4 signalling in the epithelial-mesenchymal interactions that take place in early thymus and parathyroid development in avian embryos. Developmental biology. 2012;361(2):208–219. [PubMed: 22057081]
39. Chisaka O, Capecchi MR. Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene hox-1.5. Nature. 1991;350(6318):473–479. [PubMed: 1673020]
40. Manley NR, Capecchi MR. The role of Hoxa-3 in mouse thymus and thyroid development. Development. 1995;121(7):1989–2003. [PubMed: 7635047]

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

41. Kameda Y, Arai Y, Nishimaki T, Chisaka O. The role of Hoxa3 gene in parathyroid gland organogenesis of the mouse. J Histochem Cytochem 2004;52(5):641–651. [PubMed: 15100241]
42. Chojnowski JL, Masuda K, Trau HA, Thomas K, Capecchi M, Manley NR. Multiple roles for HOXA3 in regulating thymus and parathyroid differentiation and morphogenesis in mouse. Development. 2014;141(19):3697–3708. [PubMed: 25249461]
43. Chen L, Zhao P, Wells L, Amemiya CT, Condie BG, Manley NR. Mouse and zebrafish Hoxa3 orthologues have nonequivalent in vivo protein function. Proc Natl Acad Sci U S A. 2010;107(23): 10555–10560. [PubMed: 20498049]
44. Chojnowski JL, Trau HA, Masuda K, Manley NR. Temporal and spatial requirements for Hoxa3 in mouse embryonic development. Dev Biol 2016;415(1):33–45. [PubMed: 27178667]
45. Manley NR, Condie BG. Transcriptional regulation of thymus organogenesis and thymic epithelial cell differentiation. Prog Mol Biol Transl Sci 2010;92:103–120. [PubMed: 20800818]
46. Su D, Ellis S, Napier A, Lee K, Manley NR. Hoxa3 and pax1 regulate epithelial cell death and proliferation during thymus and parathyroid organogenesis. Dev Biol 2001 ;236(2):316–329. [PubMed: 11476574]
47. Zou D, Silvius D, Davenport J, Grifone R, Maire P, Xu PX. Patterning of the third pharyngeal pouch into thymus/parathyroid by Six and Eya1. Dev Biol 2006;293 (2):499–512. [PubMed: 16530750]
48. Moens CB, Selleri L. Hox cofactors in vertebrate development. Developmental biology. 2006;291(2): 193–206. [PubMed: 16515781]
49. Liu C, Saito F, Liu Z, et al. Coordination between CCR7- and CCR9-mediated chemokine signals in prevascular fetal thymus colonization. Blood. 2006;108(8):2531–2539. [PubMed: 16809609]
50. Kamitani-Kawamoto A, Hamada M, Moriguchi T, et al. MafB interacts with Gcm2 and regulates parathyroid hormone expression and parathyroid development. J Bone Miner Res 2011;26(10): 2463–2472. [PubMed: 21713993]
51. Kamitani-Kawamoto A, Hamada M, Moriguchi T, et al. MafB interacts with Gcm2 and regulates parathyroid hormone expression and parathyroid development. J Bone Miner Res 2011;26(10): 2463–2472. [PubMed: 21713993]
52. Han SI, Tsunekage Y, Kataoka K. Gata3 cooperates with Gcm2 and MafB to activate parathyroid hormone gene expression by interacting with SP1. Molecular and cellular endocrinology. 2015;411:113–120. [PubMed: 25917456]
53. Phitayakorn R, McHenry CR. Incidence and location of ectopic abnormal parathyroid glands. Am J Surg 2006;191(3):418–423. [PubMed: 16490559]
54. Weller G Development of the thyroid, parathyroid and thymus glands in man. Contributions to Embryology. 1933;24:93–142.
55. Gilmour J The embryology of the parathyroid glands, the thymus, and certain associated rudiments. Journal of Pathology. 1937;45:507–522.
56. Norris E The morphogenesis and histogenesis of the thymus gland in man: in which the origin of the Hassall's corpuscles of the human thymus is discovered. Contributions to Embryology. 1938;27:193.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

KEY POINTS

- Parathyroid cell fate specification in the endodermal pharyngeal pouches is dependent on signaling from the Sonic hedgehog (SHH) pathway and modulated by Fibroblast Growth Factor (FGF) signaling, leading to initiation of *Gcm2* gene expression.
- Gcm2 up regulation is dependent on the HOXA3 transcription factor, working with a suite of other transcriptional regulators. In the absence of *Gcm2* up regulation, parathyroid cells undergo early and coordinated apoptosis.
- GCM2 also regulates parathyroid cell differentiation, regulating the expression of key functional genes including Parathyroid Hormone (PTH). GCM2, with another transcription factor MAFB, directly regulate *Pth* gene expression.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

SYNOPSIS

The parathyroid glands are essential for regulating calcium homeostasis in the body. During mouse development, the parathyroid develops from the 3rd pharyngeal pouch along with the thymus. Parathyroid organogenesis involves several key morphogenetic stages including differentiation from the 3rd pouch, separation from the thymus primordium and the pharynx, and migration to their final location in the neck near the thyroid gland. to develop, separate from thymus and pharynx, and migrate to their final location in the neck near the thyroid gland. The genetic programs that control parathyroid fate specification, morphogenesis, differentiation, and survival are only beginning to be delineated, but are all centered around a key transcription factor, GCM2. Mutations in the *Gcm2* gene as well as in several other genes involved in parathyroid organogenesis have been found to cause parathyroid disorders in humans. Therefore, understanding the normal development of the parathyroid will provide insight into the origins of parathyroid disorders.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

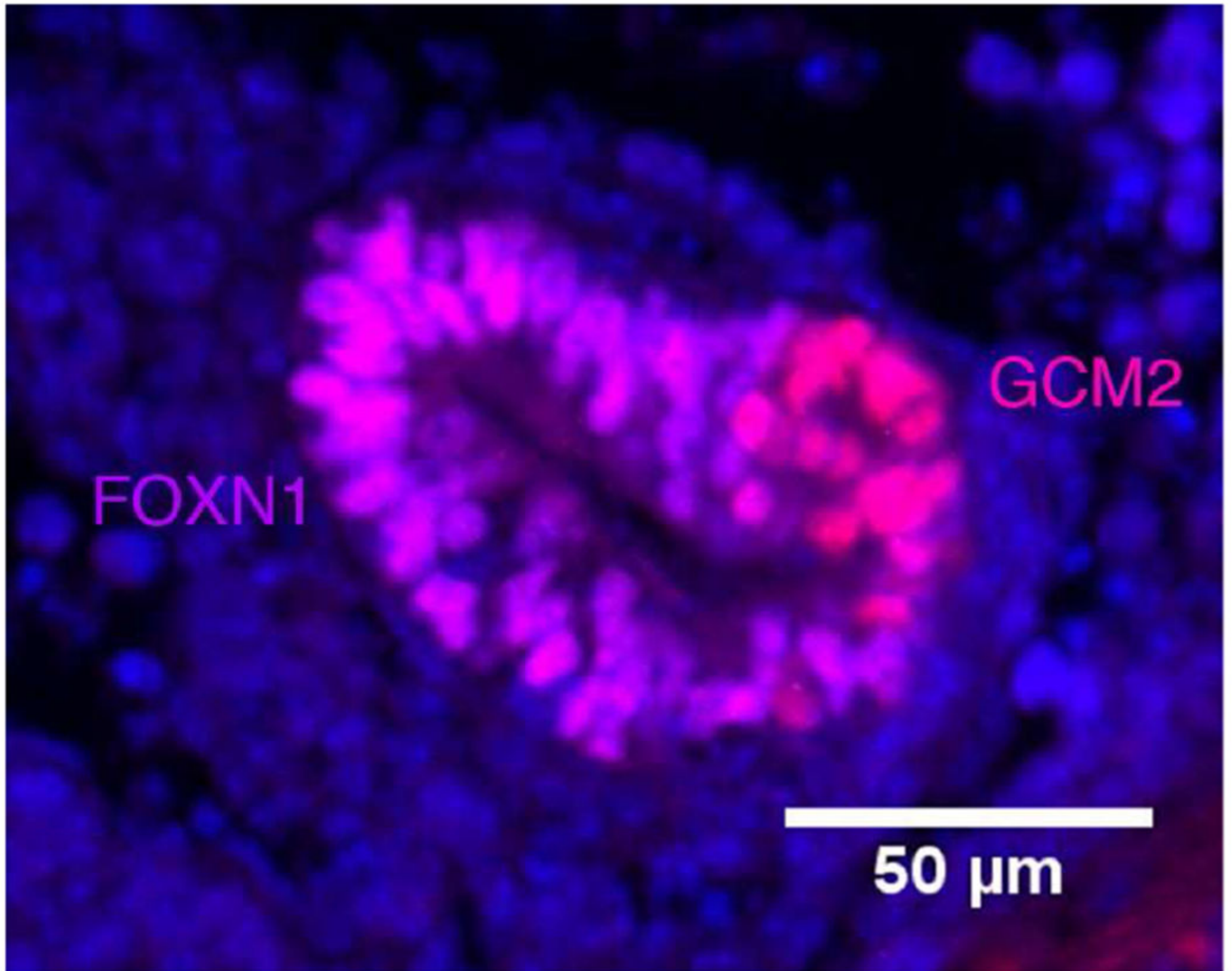

Figure 1.
3rd pharyngeal pouch-derived primordium from an E11.5 mouse embryo. Dorsal parathyroid-fated cells marked with anti-GCM2 antibody shown in magenta; ventral thymus-fated cells marked with anti-FOXN1 antibody shown in purple.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

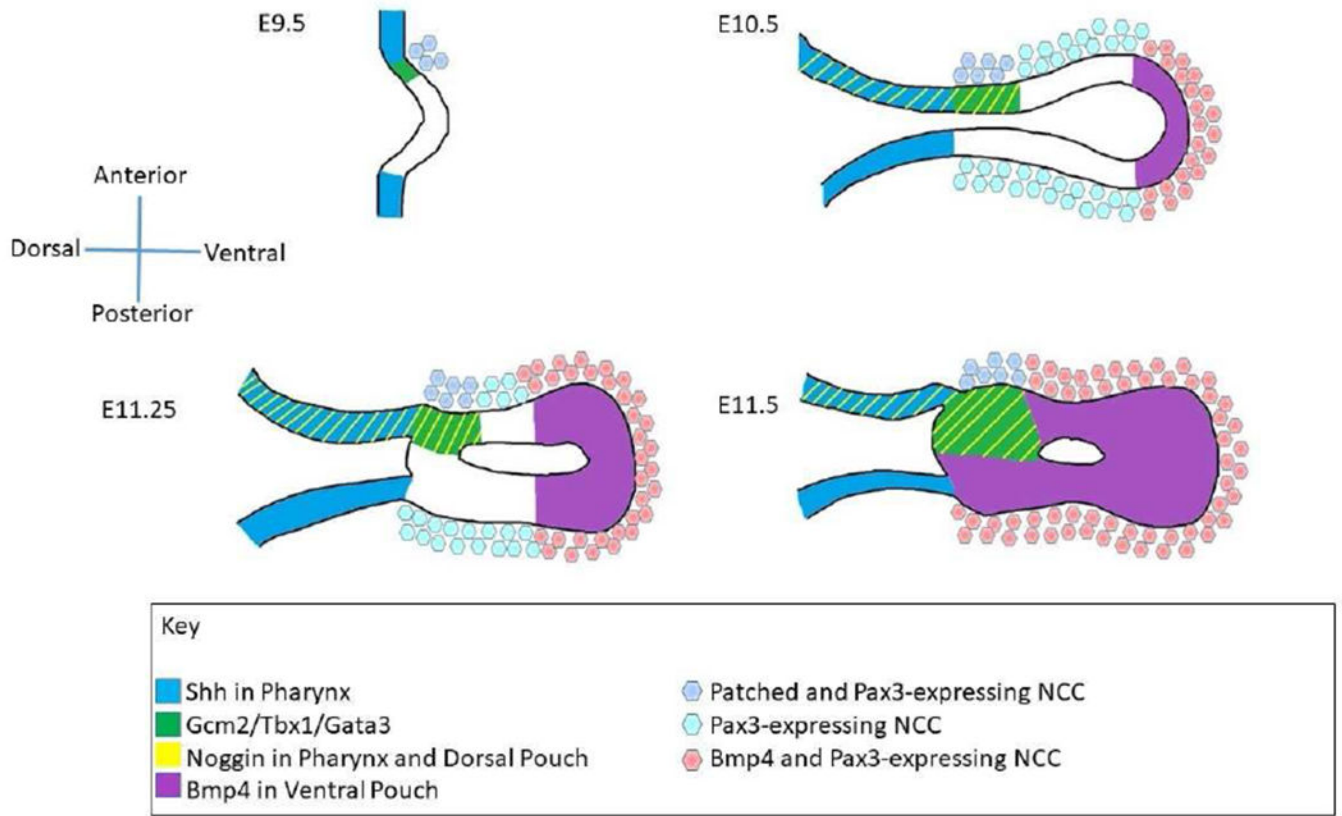

Figure 2.
Current model for early patterning and organogenesis of the 3rd pharyngeal pouch in mice.