

Expression and Potential Role of GATA Factors in Trophoblast Development

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Abstract. Despite exhaustive studies, molecular mechanisms governing blastocyst formation, implantation to the uterine endometrium and placentation have not been definitively characterized. GATA family proteins are a group of zinc finger transcription factors, for which gene ablations eventually result in embryonic death later in pregnancy. These findings suggested that GATA factors are not essential for early embryonic development. However, recent studies from our laboratory and others have revealed that GATA proteins are involved in the regulation of key genes expressed by the trophoctoderm that underpin the transition from the morula to trophoblast, and trophoctoderm maintenance. Consequently, it is important to consider the current understanding how GATA factors govern early trophoctoderm development.

Key words: Development, GATA factors, Mammals, Transcriptional regulation, Trophoctoderm (TE)

(J. Reprod. Dev. 59: 1–6, 2013)

In most mammals, conceptus implantation to the uterine endometrium consists of blastocyst hatching, migration, apposition/attachment, invasion, and subsequent placental formation. It is known that close to 50% of fertilized preimplantation embryos in mammals, including humans, fail to implant [1]. Although numerous transcription factors and their downstream genes involved in trophoblast development have been identified [2, 3], the regulation of trophoctoderm (TE)-specific gene expression has not been definitively characterized. A lack of our knowledge on implantation mechanisms and TE-specific gene regulation may have limited the improvements in pregnancy success.

GATA transcription factors are so named for their highly conserved zinc finger domains that bind to the consensus DNA sequence W(A/T)GATAR(A/G) (GATA motif), resulting in transcriptional regulation of downstream genes [4, 5]. They have been found throughout the eukaryote spectrum, including fungi and plants as well as invertebrates and vertebrates [6]. In vertebrates, including mammals, six GATA factors (GATA1 through GATA6) have been identified, and based on sequence homology and tissue distribution, these GATA factors have been divided into two subfamilies. In mice, the mRNA and proteins of all six GATA factors were detected during the embryonic development process (Table 1). GATA1, GATA2 and GATA3 regulate development and differentiation of hematopoietic lineages [7–9], while GATA4, GATA5 and GATA6 are involved in cardiac development and endodermal derivatives [10–12]. In *Gata* gene ablation studies, with the exception of *Gata5*, lack of each *Gata* gene resulted in mid-gestation lethality [7–12] (Table 2). For these reasons, GATA factors had been considered not important for early embryonic and/or trophoblast development.

Over the past three years, GATA3 was found to assist TE differentiation in mice [13, 14], while our laboratory was discovering that GATA2 and GATA3 regulate interferon tau (IFNT) and other TE-specific gene transcriptions in ruminants [15, 16]. Because of these findings, we believe that GATA factors play roles, yet unidentified, in peri-implantation development, including both species-specific functions and those universal across vertebrate species. This review details the currently ascribed functions of the GATA factors during the peri-implantation period with emphasis on the hematopoietic group of GATA1, GATA2, and GATA3 in TE development and TE-specific gene expressions.

Trophoblast Lineage Development

The trophoblast lineage, derived from the extraembryonic trophoctoderm, is the first differentiated cells arising from the pre-implantation embryos in mammals [17]. Trophoblast development and differentiation in the mouse have been well studied. The blastocyst hatches from the zona pellucida at 3.5 day post coitum (dpc) in mice [18], and trophoctodermal cells that line the blastocoel cavity (mural trophoctoderm) differentiate into trophoblast giant cells (TGCs) coincident with the implantation process around 5–6 dpc. Among numerous factors well studied in mice, a caudal-type homeodomain transcription factor *Cdx2*, expressed from 4-cell stage embryos predominantly in the outer blastomeres, has been characterized as the factor involved in the decision of TE cell lineage [13, 14, 19–21]. Recently, GATA3 was found to be capable of inducing trophoblast fate in embryonic stem cells and driving trophoblast differentiation in trophoblast stem cells (TS) [14]. In addition to these observations, we also confirmed that all six *GATA* mRNAs exist in bovine and/or ovine conceptuses during the peri-implantation periods [15, 22 and unpublished observations]. The presence of six *GATA* mRNAs in the bovine and/or ovine conceptuses suggests that these factors

Received: June 14, 2012

Accepted: August 22, 2012

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Table 1. GATA Transcription factor mRNA and protein expression in mice

GATA factors	RNA expression detected	Methods utilized	References	Protein expression detected	Methods utilized	References
GATA1	E1.5 (2-cell)	R	[53]	E6.75	Im	[56]
GATA2	E1.5 (2-cell)	R	[54]	E10.5	Im	[57]
GATA3	E2.0 (4-cell)	R	[13]	E3.5 (blastocyst)	Im	[13]
GATA4	E1.5 (2-cell)	R	[53]	E4.0	Im	[58]
GATA5	E7.0	ISH	[55]	E9.5	Im	[59]
GATA6	E1.5 (2-cell)	R	[54]	E4.0	Im	[58]

Methods: Im, immunohistochemical staining; ISH, *in situ* hybridization; R, RT-PCR.

Table 2. Embryonic lethality of *Gata* gene knockout mice

GATA factors	Lethality of knockout mouse	Cause	References
<i>Gata1</i>	E10.5 – E11.5	Embryonic erythropoiesis arrest	[7]
	E12.5 (Hematopoietic promoter-specific disruption)	95% reduction of <i>Gata1</i> mRNA maturation arrest in proerythropoiesis	[60]
<i>Gata2</i>	E10.5	Failure to develop all hematopoietic lineages (severe anemia)	[8]
<i>Gata3</i>	E11.0 – 12.0	Massive internal bleeding, marked growth retardation, severe deformities of the brain and spinal cord, and gross aberrations in fetal liver hematopoiesis	[9]
	E12.0	Failure to give rise to thymocytes or mature peripheral T cells	[61]
<i>Gata4</i>	E7.0 – E9.5	Lack primitive heart tube and foregut, developed partially outside the yolk sac	[10]
	E8.5 – E10.5	Defect in rostral-to-caudal and lateral-to-ventral folding	[62]
<i>Gata5</i>	No embryonic lethality	Abnormalities of genitourinary tract (female)	[11]
<i>Gata6</i>	E6.5 – E7.5	Defects in visceral endoderm function and extraembryonic development	[12]
<i>Gata4^{+/-} Gata6^{+/-}</i>	E13.5	Abnormal vascular development	[63]
<i>Gata4^{+/-} Gata5^{-/-}</i>	E14.5	Cardiovascular defects	[64]

may play roles other than those already known for erythropoiesis and heart formation.

GATA-regulated Cellular Events

The GATA factor is associated with differentiation processes in various cells and tissues. GATA1 is critical for terminal maturation of erythroid and megakaryotic cells [23, 24], the early stage of eosinophil differentiation [25], and the late stage of mast cell differentiation [26]. GATA2 is expressed in undifferentiated hematopoietic cells and is involved in the maintenance of these cells at the undifferentiated state, while GATA3 is involved in the differentiation of Th2 cells from immature T cells [27]. The demise of transgenic mice seems to be unrelated to apparent defects in early TE development (Fig. 1, Table 2). However, since GATA factors have both distinct and overlapping expression and biological functions [7, 8, 28–31], it is possible that redundant expression and functions of other GATA factors might compensate for those inactivated in knockout mice.

GATA-regulated Genes

It is thought that GATA factors contribute to regulation of gene expression while balancing with an expression pattern and the expression level, and the expression level is important in GATAs' functions [32]. A number of genes regulated by GATA2 and/or GATA3 in trophoblast cells and placental tissues are shown in Table 3. GATA2 and GATA3 are expressed in TGC of the mouse placenta,

and involved in placental development. Placentation sites lacking GATA2 have significantly less neovascularization compared with the wild-type placenta [33]. GATA2 was shown to contribute to both positive and negative regulation of mouse trophoblast cell-specific gene expressions [34]. GATA2 and GATA3 regulate trophoblast specific *PL-1* (*Pr13d1*) and *proliferin* (*Pr12c2*) gene expression *in vivo* and *in vitro* in the mouse [33, 35], the rat [36] and the ovine [37]. We also found that GATA2 and/or GATA3 regulate TGC related factors such as *PL-1*, and *HAND1* in bovine trophoblast CT-1 cells [16]. Furthermore, we examined whether or not GATA2 and GATA3 directly regulated TE-specific genes such as *IFNT*, *CDX2*, and *PL-1* in bovine trophoblast CT-1 cells. Over-expression of GATA2 and/or GATA3 effectively upregulated these TE-specific gene-reporter constructs, transfected into bovine non-trophoblast ear fibroblast (EF) cells [15, 16]. These results are similar to previous studies in which GATA2 and GATA3 induced *PL-1* transcription in transfected mouse non-trophoblast (fibroblast) cells [35]. These studies indicate that forced expression of GATA2 and/or GATA3 in non-trophoblast EF cells conditions the non-trophoblast cells to support TE-specific gene transcription. This does not preclude the possibility of other functions; GATA factors may control many other genes. In fact, DNA microarray and/or chromatin immunoprecipitation (ChIP) assays revealed that GATA proteins are involved in transcriptional regulation of many genes in erythroid cells [38, 39]. For these reasons, GATA proteins should deserve deeper research into their ability to control TE differentiation and TE-specific gene transcription.

Table 3. Genes regulated by GATA factors in the trophoblast

GATA factors	Target gene (Symbol)	Species	Methods	References
GATA2	Proliferin (<i>Prl2c2</i>)	m	Im, ISH, NB	[33]
GATA2	Placental lactogen-I (<i>LOC44319</i>)	s	D, E, L	[37]
GATA2	Prolactin-like protein-A (<i>Prl4a1</i>)	m	ISH	[34]
GATA2	P450 side chain cleavage (<i>Cyp11a1</i>)	m, r	C, E, L	[65]
GATA2	Placental lactogen-I (<i>Prl3b1</i>)	r	L, E	[36]
GATA3	17 β -Hydroxysteroid dehydrogenase Type1 (<i>HSD17B1</i>)	h	C, E	[66]
GATA3	Caudal type homeobox 2 (<i>Cdx2</i>)	m	Ch, Im, L, R, RI	[13]
GATA2, GATA3	Placental lactogen-I (<i>Prl3d1</i>)	m	C, D, E, ISH, NB	[35]
GATA2, GATA3	GnRH receptor (<i>GNRHR</i>)	h	D, E, L	[67]
GATA2, GATA3	Syncytin (<i>ERVW</i>)	h	D, E, L	[68]
GATA2, GATA3	GATA binding protein (<i>Gata2</i>)	m	Ch, D, L, NB, R	[47]
GATA2, GATA3	Gonadotropin alpha subunit gene (<i>CGA</i>)	h	C, D, E, NB	[69]

Species: h, humans; m, mice; r, rats; s, sheep. Methods: C, CAT assay; Ch, ChIP assay; D, DNase footprinting; E, EMSA; Im, immunohistochemical staining; ISH, *in situ* hybridization; L, Luciferase assay; NB, Northern blotting; R, RT-PCR; RI, RNA interference.

Self-regulation of GATAs in Hematopoiesis

The GATA proteins share conserved zinc finger DNA-binding domains that recognize the same GATA motif, by which they can regulate multiple developmental processes by binding to GATA motif regulatory element, and thereby, these proteins can regulate multiple developmental processes [4, 5, 40]. GATA proteins have both distinct and overlapping biological activities, and changes in occupancy in GATA protein at its binding site often affect the degree of target gene transcription [7, 8, 28–31]. During erythroid differentiation in mice, GATA1 and GATA2 directly regulate *Gata2* transcription. GATA1 represses *Gata2* transcription in association with four conserved GATA binding sites on the upstream region (–77, –3.9, –2.8, and –1.8 kb) along with an intron (+9.5 kb) region [41–44]. GATA2 is associated with these sites when *Gata2* is in a transcriptionally active state [41–44]. The “GATA switch” is well stated by Brennick *et al.* [45, 46].

Self-regulation of GATAs in Trophoblast Stem (TS) Cells and Trophoblast Cells

Besides this hematopoietic GATA switch, evidence of a GATA2/GATA3 switch has been gathered through studies of mouse TS cells. It was reported that changes in GATA2 or GATA3 occupancy occur at the –3.9 kb and +9.5 kb regions of the *Gata2* gene during the differentiation process from TS cells to TGCs [47]. Binding of GATA3 directly represses the *Gata2* gene in undifferentiated TS cells, and a switch in chromatin occupancy between GATA3 and GATA2 (GATA3/GATA2 switch) induces *Gata2* transcription during TS cell differentiation. Recently, we also demonstrated that as bovine and/or ovine conceptus attachment begins, *GATA2* and *GATA3* mRNAs decrease when *GATA1* mRNA increases concurrent with erythroid development (Fig. 2). Because high *GATA1* mRNA appeared to coincide with reduced *GATA2* and *GATA3* mRNA expression at this time period, the effect of GATA1 was examined through over-expression of *GATA1* in bovine trophoblast F3 cells, resulting in the down-regulation of endogenous *GATA2* transcripts [22]. Although roles of GATA1 during conceptus attachment processes have not

been characterized, these observations suggest that GATA1 is likely integral to conceptus development through the down-regulation of *GATA2* transcription and possibly other developmentally important genes. Moreover, *in situ* hybridization studies revealed that both sense and antisense *GATA1* [22] and *GATA2* (unpublished observations) transcripts were present in trophoblast cells. It is now recognized that the natural antisense transcripts are important in governing cellular and organismal processes through transcriptional regulation [48] (see review, and references therein). These natural anti-sense transcripts may be involved in the regulation of *GATA* gene transcriptions.

Because trophoblast cells are unique to mammalian species, we examined the existence of the –3.9 kb and +9.5 kb regions of the *Gata2* gene in several species. Interestingly, although the +9.5 kb GATA binding site of the *Gata2* gene is preserved in several mammalian species, birds (*Gallus gallus*), and Zebra fish (*Danio rerio*), the –3.9 kb regulatory element is found in humans and mice, but not in birds or Zebra fish (Table 4), suggesting that the –3.9 kb GATA site functions in a trophoblast cell-specific manner.

Ruminants as an Animal Model

Rodents have been used as the primary animal models to study implantation processes. In mice, implantation occurs soon after blastocyst hatching from the zona pellucida. Within a span of a few embryonic days that extends from implantation to placentation, several dramatic and concurrent events occur in rodents. Therefore, it is difficult in rodents to delineate the underpinning molecular and accompanying cellular changes during this time period. However, in ruminants, the peri-implantation period is prolonged compared to rodents (Fig. 2), and thus, identification of key gene expression changes and developmental progression can be determined in these species. Although the duration of peri-attachment periods and types of implantation (invasive vs. non-invasive) differ, processes leading to conceptus implantation into the maternal endometrium are similar in most mammalian species [49]. In addition, the integrity of the bovine conceptus can be monitored through measurement of IFNT, the major protein implicated in the process of maternal recognition of pregnancy in ruminants [50–52]. For these reasons, ruminants

Mouse

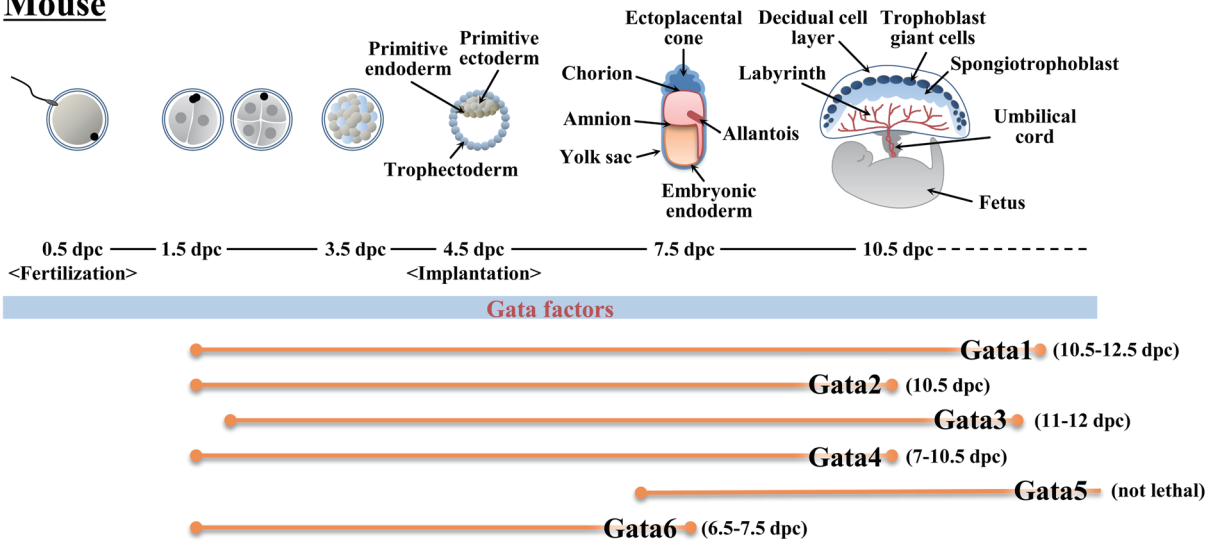


Fig. 1. Embryonic and extraembryonic development, and GATA transcription factor expression in mice. Early development of the mouse embryo from 0.5 dpc to 10.5 dpc is shown. Upper: Mouse conceptus developments. Following the first lineage decision to trophoblast (TE) and inner cell mass (ICM), the ICM differentiates into the primitive ectoderm, which gives rise to the embryo proper and the yolk sac. After implantation, the trophoblast differentiates into subtypes consisting of trophoblast giant cells, chorionic ectoderm, and ectoplacental cone. The yolk sac membranes consist of the parietal yolk sac (trophoblast giant cells and parietal endoderm) and the visceral yolk sac (visceral endoderm and extraembryonic mesoderm). The allantoic mesoderm forms the endothelial cell lining of fetal blood vessels in the labyrinth zone. Distinct regions of the placenta include the labyrinth, the spongiotrophoblast and a discontinuous layer of trophoblast giant cells. Lower: GATA transcription factor expression in mice. Expression of GATA transcription factors in mice is shown. Days on the right indicate days post coitus (dpc) when embryonic death occurs in mutant mice for various *Gata* genes.

Bovine

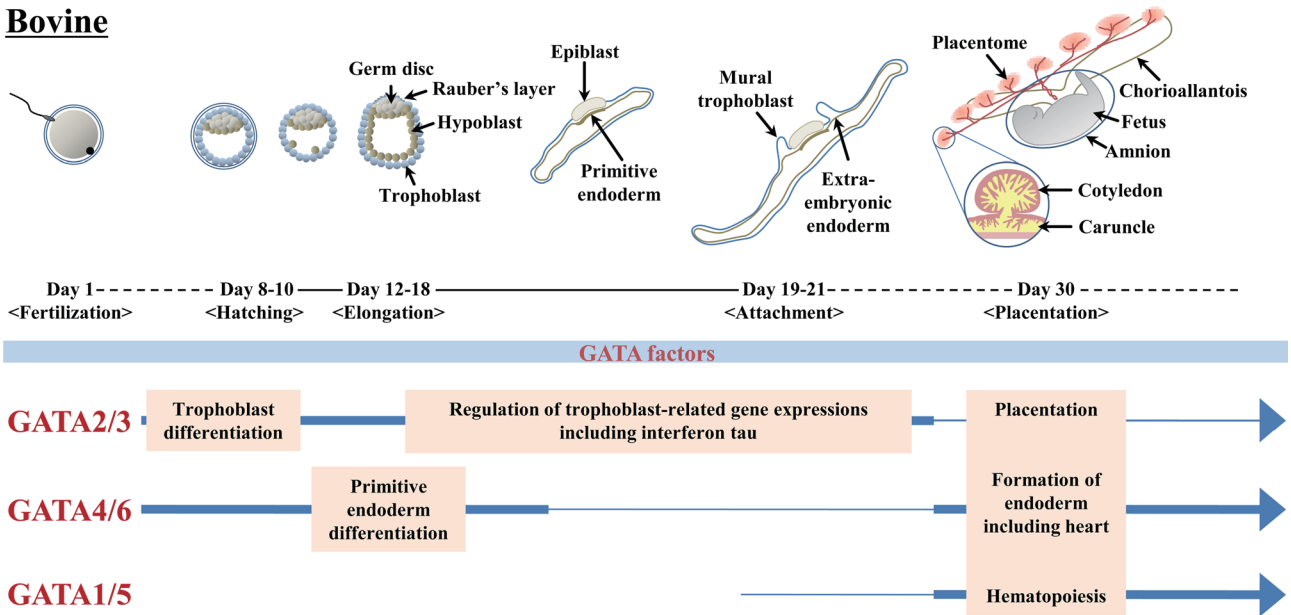


Fig. 2. Embryonic and extraembryonic development, and GATA transcription factor expression in the cow. In ruminant species (bovine, ovine, and caprine), the blastocyst is formed several days after fertilization, but placentation starts on day 21, approximately two weeks later than in mice. Upper: Bovine conceptus developments. One of the unique features seen in ruminant conceptus development is trophoblast elongation. The trophoblast elongates exponentially and reaches a length of more than 150–300 mm before the initiation of its attachment to the uterine epithelium. Lower: Developmental events associated with bovine conceptuses, and GATA transcription factor expression. Arrows indicate the presence or increase (bold) in GATA expression during bovine conceptus development.

Table 4. Homology of candidate GATA switching sites

Species	-3.9 kb region	+9.5 kb region
Humans	GAGATAGG --- GCTATCTG	CCTATCCG---TAGATAAG---TGTATCTG
Mice	GAGATAGG --- GCTATCTG	CCTATCCG---TAGATAAG---TGTATCTG
Rats	GAGATAGG --- GCTATCTG	CCTATCCG---TAGATAAG---TGTATCTG
Canis	GAGATAGG --- GCTATCTG	CCTATCCG---TAGATAAG---CGTATCTG
Equine	GAGATAGG --- ACTATCTG	CCTATCCG---TAGATAAG---TGTATCTG
Bovine	No data available	CCTATCCG---TAGATAAG---CGTATCTG
Gallus	Not found	CCTATCCG---TAGATAAG---CGTATCTG
Danio	Not found	CCTATCCG---CAGATAAG---CTCCCGCC

may provide major advantages in characterizing processes associated with peri-implantation periods, possibly allowing the identification of a phenomenon and/or its gene expression overlooked in rodents.

Conclusions

Significant improvements in reproductive success are unlikely without first characterizing the complex interactions leading to successful implantation and eventual placentation. Because of mid-gestation embryonic loss in mouse gene ablation studies, GATA proteins have been considered not essential in mediating these processes. Recently, however, GATA proteins have emerged from scientific obscurity to be at the forefront of conceptus development studies. Although the various additional roles each GATA may undertake remain to be definitively established, the tantalizing insights into roles played by various GATAs provide strong impetus to clarify their effects on the peri-implantation process.

There are many factors involved in the maintenance, proliferation and differentiation of the trophoblast cells. We would like to emphasize the point that GATA factors regulate the expression of trophoblast-specific factors in many species including humans, mice and ruminants during several stages of their development. More importantly, insights gained from ruminants can be applied to elucidation of molecular mechanisms associated with conceptus implantation in other mammalian species. Further research into GATA factors may allow us to more accurately identify pathways separating pregnancy success or failure, and thereby, potentially improve fertility rates in humans and in agriculturally important animals.

Acknowledgements

The authors would like to thank Mr R Moriarty for his critical reading of the manuscript. Our appreciation is extended to Drs A Ideta and Y Aoyagi (Zen-Noh ET Center, Hokkaido, Japan) for critical discussions throughout the course of these studies. We also thank the members of the Laboratory of Animal Breeding at The University of Tokyo for their support throughout the course of GATA studies.

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