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# Early Gene Expression and Morphogenesis of the Murine Chorioallantoic Placenta *In vivo* and *In vitro*

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ABSTRACT

*Background:* In mice the exchange of oxygen and nutrients between mother and fetus occurs in the chorioallantoic placenta where fetal capillaries come in close proximity with maternal blood perfusing trophoblast-lined sinusoids. Despite its critical importance, quantitative *in vivo* gene expression over the initial stages of chorioallantoic placental development has not been described, nor are there *in vitro* systems recapitulating the critical syncytiotrophoblast differentiation step in its formation. Here we describe molecular events that occur during the onset of chorioallantoic morphogenesis in mice *in vivo*, and in placental explant and whole conceptus cultures *in vitro*.

*Results:* Chorioallantoic morphogenesis began immediately following allantoic fusion with the chorion *in vivo*, and was associated with significant upregulation of syncytiotrophoblast associated mRNA (*Gcm1* and *Syncytin A*). However mouse placentas with chorioallantoic point attachment cultured with the allantois or as whole conceptuses did not upregulate *Gcm1* and/or *Syncytin A*, suggesting that syncytiotrophoblast differentiation did not occur *in vitro*. Failure of morphogenesis appeared to be due to failure to sustain *in vitro* the chorionic trophoblast cells from which the syncytiotrophoblast cells are derived. *In vitro* culture conditions did support the upregulation of ectoplacental cone marker *Tpbpα*, maintenance of giant cell marker *Pl1*, and maintenance of *Fgfr2* expression; all of which mimicked *in vivo* events observed over this developmental interval.

*Conclusions:* We conclude that chorionic trophoblast maintenance and the early events that occur *in vivo* between chorioallantoic point attachment and primary villous formation are dependent on undefined intrauterine factors that were not present in the *in vitro* culture system. Nevertheless, *in vitro* culture conditions were appropriate to reproduce *in vivo* expression levels of *Fgfr2*, *Pl1*, and *Tpbpα* in placental explants.



## 1. Introduction

An essential function of the mammalian chorioallantoic placenta is to facilitate the exchange of oxygen, nutrients, and wastes between the mother and her developing fetus [1]. In the human placenta this occurs at the chorionic villi, a highly branched villous structure perfused by the fetal placental vasculature. The trophoblast-lined intervillous spaces surrounding the chorionic villi are perfused by maternal blood. In humans, maternal blood is separated from fetal blood by three cell layers: a continuous layer of syncytiotrophoblast, an underlying cytotrophoblast cell layer, and an endothelial cell layer of the fetal capillary [1]. Distal hypoplasia of the chorionic villi, and aging of the syncytiotrophoblast are often present in placental-mediated diseases such as severe early-onset intrauterine growth restriction, and are associated with fetal hypoxia, preterm delivery, and/or fetal death [1,2]. The etiology of such diseases is multifactorial and our understanding of their pathophysiology is rudimentary. However it has been suggested that dysregulation of the gene Glial cells missing 1 (*GCM1*), a transcription factor of particular importance in syncytialization, may account for the deficit of normal syncytiotrophoblast differentiation in such cases [3].

Mouse models are proving valuable model systems for advancing our understanding of the molecular mechanisms regulating normal, and abnormal, chorioallantoic placental development [4,5]. Although the placentas of no two species are identical [6], the mouse placenta shares considerable structural and functional similarities with that of the human [7,8]. The labyrinth is the

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site of nutrient and gas exchange in the mouse placenta and is comprised of 4 cell layers separating the maternal and fetal blood spaces: a discontinuous layer of sinusoidal trophoblast giant cells (TGC), two continuous layers of syncytiotrophoblast, and the endothelium of the fetal capillary [8,9]. The fetal villous component of the labyrinth originates from allantoic mesoderm extending from the embryo which attaches to, transforms, and interdigitates with the chorionic ectoderm underlying the ectoplacental cone (Fig. 1). Chorioallantoic morphogenesis is initiated following the fusion of the allantois to the chorion [5], and is followed by the formation of primary villi across the chorionic plate.

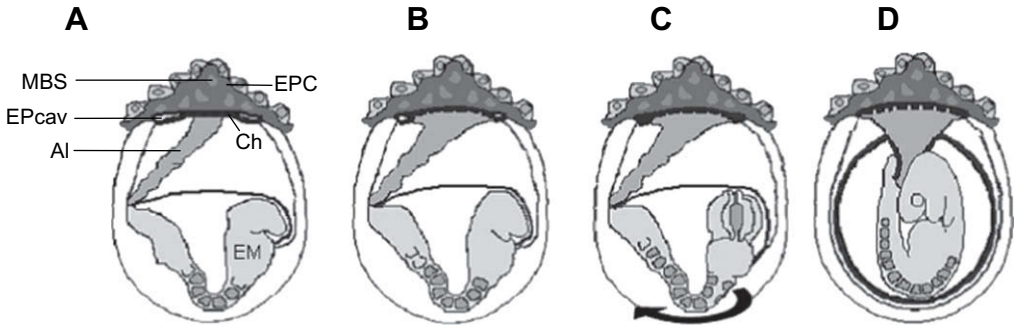
The period of development between chorioallantoic point attachment and primary villous formation (~E8.0–E9.0) includes critical morphogenic events in the development of the labyrinth. Chorioallantoic fusion is followed by closure of the ectoplacental cleft, degeneration of the chorionic mesothelium, and the formation of syncytiotrophoblast-lined invaginations along the chorion at the sites of expression of the murine homologue *Gcm1* (Fig. 1) [5]. *Gcm1* is later confined to syncytiotrophoblast layer-II of the labyrinth, adjacent to the fetal capillary. *Syncytin A* and *B* are fusogenic membrane proteins which have a similar expression pattern to *Gcm1* and are later expressed in syncytiotrophoblast layer-I and -II of the labyrinth, respectively [10]. However, the quantification and time-course of gene expression changes *in vivo* over this critical period of chorioallantoic development are unknown.

The first aim of this study was to describe the molecular events that occur at the onset of chorioallantoic morphogenesis *in vivo*. Our second aim was to characterize differentiation events in placentas in *in vitro* culture systems. Murine placental explants are commonly used to investigate TGC differentiation because cultured ectoplacental cones/chorions tend to spontaneously differentiate into secondary TGC [11–13]. *Gcm1* protein translation has been observed in placental explants cultured with the allantois [14], and syncytialization occurs in mouse labyrinthine cell lines *in vitro* [15]. Thus, we hypothesized that trophoblast cells in mouse placental explants and/or whole conceptus cultures in which the allantois was intact would upregulate *Gcm1*, differentiate into syncytiotrophoblast, and initiate chorioallantoic morphogenesis *in vitro*. The long term goal was to establish an *in vitro* model of early chorioallantoic morphogenesis in the mouse to facilitate studies of the molecular regulation of early events in placental branching morphogenesis as has previously been achieved for the kidney, lung, and mammary gland [16–18]. Such a model could be used to elucidate the molecular mechanisms that may underlie placental abnormalities in the human intrauterine growth restricted pregnancies.

## 2. Methods

### 2.1. Animals

CD-1 (ICR) mice were purchased from Charles River Canada, housed conventionally, and handled according to the guidelines established by the Canadian Council on Animal Care. CD-1 is an outbred strain that breeds well and is commonly used in reproductive research. Pregnant mice were sacrificed by cervical dislocation on the 8th day following overnight mating (i.e. 4 days after implantation). The animals' decidual swellings were immersed in dissection medium consisting of DMEM (Invitrogen, Burlighton, ON, Canada), 0.04 mM MEM non-essential amino acids (Invitrogen), 1 mM NaPyruvate (Invitrogen), 100 U/ml penicillin and 100 mg/mL streptomycin (Wisent Inc., ST-BRUNO, QC, Canada), 4 mM L-glutamine (Invitrogen), 10 mM HEPES (Wisent Inc.), and 7.5% FBS (Wisent Inc.). Conceptuses were separated from their deciduas and Reichert's membranes using #55 forceps. Embryos at different stages of development from allantoic point attachment to primary villous formation were usually observed in any given mother on this day. The stage of development of each conceptus was classified based on the headfold




| | Class 1 | Class 2 | Class 3 | Class 4 |
|---|---|---|---|---|
| **Degree of C-A Fusion** | C-A point attachment | C-A fusion and lateral spreading of the allantois | Medial primary C-A villous formation | Primary C-A villous formation extends laterally |
| **Somite Pairs** | 5 to 7<br>*6 ± 0.08 SEM*<br>*N = 23* | 7 to 9<br>*8 ± 0.09 SEM*<br>*N = 27* | 8 to 11<br>*9 ± 0.3 SEM*<br>*N = 10* | 11 to 15<br>*12 ± 0.3 SEM*<br>*N = 20* |
| **Embryonic Features** | Head folds expose a region of flat neural tissue | Head folds become more prominent and approach the midline | Embryo in mid-turn | Embryo has completed turning |
| **Theiler Stage** | 12 | 12-13 | 13 | 13-14 |

**Fig. 1.** Classification of stages of chorioallantoic placental morphogenesis. (A) Class 1: The allantois makes "point" attachment to the chorion (5–7 somite pairs). (B) Class 2: Fusion of the allantois across the chorion (7–9 somite pairs). (C) Class 3: Primary villi form in the centre of the chorion as the embryo begins to turn (arrow) (8–11 somite pairs). (D) Class 4: Primary villous formations have spread laterally across the chorion as embryos complete turning (12–15 somite pairs). Al, allantois; Ch, chorion; EM, embryo; EPC, ectoplacental cone; EPcav, ectoplacental cavity; MBS, maternal blood space. Representative ranges and mean ± SEM somite pairs in each Class are presented.