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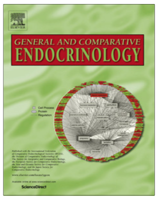

Comparative genomics of hormonal signaling in the chorioallantoic membrane of oviparous and viviparous amniotes

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ABSTRACT

In oviparous amniotes (reptiles, birds, and mammals) the chorioallantoic membrane (CAM) lines the inside of the egg and acts as the living point of contact between the embryo and the outside world. In livebearing (viviparous) amniotes, communication during embryonic development occurs across placental tissues, which form between the uterine tissue of the mother and the CAM of the embryo. In both oviparous and viviparous taxa, the CAM is at the interface of the embryo and the external environment and can transfer signals from there to the embryo proper. To understand the evolution of placental hormone production in amniotes, we examined the expression of genes involved in hormone synthesis, metabolism, and hormone receptivity in the CAM of species across the amniote phylogeny. We collected transcriptome data for the chorioallantoic membranes of the chicken (oviparous), the lizards *Lerista bougainvillii* (both oviparous and viviparous populations) and *Pseudemoia entrecasteauxii* (viviparous), and the horse *Equus caballus* (viviparous). The viviparous taxa differ in their mechanisms of nutrient provisioning: *L. bougainvillii* is lecithotrophic (embryonic nourishment is provided via the yolk only), but *P. entrecasteauxii* and the horse are placentotrophic (embryos are nourished via placental transport). Of the 423 hormone-related genes that we examined, 91 genes are expressed in all studied species, suggesting that the chorioallantoic membrane ancestrally had an endocrine function. Therefore, the chorioallantoic membrane appears to be a highly hormonally active organ in all amniotes. No genes are expressed only in viviparous species, suggesting that the evolution of viviparity has not required the recruitment of any specific hormone-related genes. Our data suggest that the endocrine function of the CAM as a placental tissue evolved in part through co-option of ancestral gene expression patterns.

1. Introduction

Reproduction is fundamental for organisms because it is the only mechanism that allows genes to be transmitted through generations. The ancestral reproductive mode for amniote vertebrates (reptiles, birds, and mammals) is egg-laying (oviparity), but live birth (viviparity) has evolved independently multiple times in reptiles, and once in mammals (Blackburn, 2014; Griffith et al., 2015). Embryonic development requires a suitable supply of respiratory gases and water. In viviparous species where the embryo is not exposed to the external environment, adequate structures are required to facilitate the exchange of materials between mother and offspring. In oviparous species, the chorioallantoic membrane (CAM, Fig. 1) is an embryonic tissue that lines the majority of the internal surface of the eggshell late in incubation, where it is the primary gas exchange organ between the embryo and the external environment (Piiper et al., 1980).

In contrast, in viviparous amniotes transfer of materials between mother and offspring occurs in a placenta composed of both maternal (uterine) and embryonic (chorioallantoic or yolk sac membranes) tissues (Blackburn, 2006; Van Dyke et al., 2014a; Wake, 1992). In eutherian mammals, the definitive placenta is produced by the uterus and the embryonic CAM. Chorioallantoic placentae are also found in all viviparous squamate reptiles, with the placental region in some species having morphological and physiological specializations for gas exchange and nutrient

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transport (Adams et al., 2005; Biazik et al., 2010; Blackburn, 1993; Griffith et al., 2013a; Van Dyke et al., 2015). Viviparous amniote placentae are extremely diverse in structure and function, with some placentae transporting respiratory gases, water, and small amounts of other molecules, and others transporting all classes of nutrients required for embryonic development (placentotrophy) (Ferner and Mess, 2011; Thompson and Speake, 2002; Thompson et al., 2002). Placentotrophy has evolved relatively few times in amniotes, and exists along a continuum, with complete reliance on egg yolk resources (lecithotrophy, in most squamates) at one end and complete reliance on placental nutrient transfer on the other end (as in eutherian mammals and some lizards). There are also species with intermediate reliance on both placental transfer and ovulated yolk resources, such as the lizard *Pseudemoia entrecasteauxii* (Ferner and Mess, 2011; Thompson et al., 2000; Van Dyke et al., 2014b).

To understand the evolution of viviparity, it is essential to understand the processes that regulate key pregnancy functions and how these processes have been modified during evolutionary transitions (Thompson and Speake, 2006). Hormones play an essential role in almost every step of amniote reproduction. Hormonal signals are responsible for egg production, vitellogenesis, ovulation, maintenance of the egg in utero, and oviposition/parturition (Callard et al., 1992; Custodia-Lora and Callard, 2002; Jones, in press; Licht, 1979). Furthermore, hormones are required to induce tissue-level changes that facilitate pregnancy, including, for example, cellular changes in the uterus for nutrient transport to offspring. Finally, hormones facilitate communication between maternal and embryonic tissue throughout gestation, which is important for coordination of developmental features such as apposition of vascular beds within the placenta (Murphy et al., 2011).

The fundamental difference between oviparous and viviparous species is that in viviparous species eggs are retained in utero until development is complete. As embryonic development in ectotherms is temperature rather than time-dependent, viviparous mothers cannot precisely determine the developmental state of offspring without relying on embryonic cues. Hormone production by embryos therefore represents one possible way for eggs to be retained in utero and deposited at the correct developmental stage which would ultimately give rise to viviparity. For example, progesterone is a key hormonal regulator of pregnancy/gravidity in both mammals and reptiles (Custodia-Lora and Callard, 2002). Genes involved in progesterone biosynthesis have been identified in the chorioallantoic tissue of the chicken, suggesting that this tissue may ancestrally produce progesterone (Albergotti et al., 2009). Production of progesterone by the chorioallantoic placenta could play a significant role in the evolution of viviparity, because egg retention could be achieved in part by simply increasing the transfer of progesterone from embryo to mother by, for example, reducing eggshell thickness.

As well as facilitating maternal-offspring communication, embryonic production of hormones allows embryos to manipulate maternal physiology, which can facilitate parent-offspring conflict. Parent-offspring conflict is a major driver of the evolution of viviparity and placental functions in vertebrates (Blackburn, 2015; Crespi and Semeniuk, 2004; Zeh and Zeh, 2008). Conflict arises when offspring demand more resources than is optimal for the lifetime reproductive success of the parent (Trivers, 1974). For maternal-offspring conflict to occur during pregnancy, embryos must be able to manipulate maternal reproductive physiology, such as nutrient transport across the placenta. This manipulation can be achieved either by gaining direct access to maternal resources through invasive placentation, or through embryonic secretions that change the physiology of maternal tissue. In species with non-invasive epitheliochorial placentation, such as those examined in this study, nutrients must be actively transported across maternal tissue to the embryo, restricting embryonic control. However, embryonic control of transport could still occur in these species (Fowden et al., 2006): In the horse, which has non-invasive epitheliochorial placentation, embryos actively manipulate placental nutrient supply, likely by the production of hormones such as insulin like growth factor 2 (Allen et al., 2002).

In species with non-invasive placentation, if offspring are able to manipulate the activity of maternal tissues by the production and transport of hormones to maternal tissues, conflict may occur. Extensive embryonic production of growth factors occurs in the embryonic trophoblast of mammals (Fowden and Forhead, 2009; Fowden et al., 2006). Understanding how the production of growth factors correlates with different reproductive modes and the evolution of placentotrophy will allow us to understand if embryonic manipulation, through growth factor production, is a novel function of the chorioallantois in placentotrophic lineages. Alternatively, the production of growth factors may be the result of modifications to processes that occurred ancestrally in chorioallantoic tissue, with growth factors expressed in the chorioallantois of oviparous species.

We measured gene expression in the embryonic chorioallantoic tissue of species across the amniote phylogeny. To characterize the hormonal activity of the chorioallantoic membrane in amniotes we quantified the expression of genes which encode peptide hormones, proteins responsible for hormone synthesis, and hormone receptors. From these data we addressed two specific questions: 1) Is hormone production by the chorioallantoic placenta widespread in viviparous amniotes and 2) Has placental hormonal signaling evolved following the recruitment of hormone related genes in viviparous lineages, or is expression of hormone-related genes a result of coopting ancestral gene expression patterns.

2. Materials and methods

2.1. Species selection

Recent advances in next-generation sequencing technology make it possible to simultaneously measure the expression of most genes in a given tissue (transcriptome). We evaluated the expression of hormone-related genes in the CAM of multiple species using newly-generated and published transcriptome data for the chorioallantoic membrane (Fig. 2). We sampled two oviparous taxa including the chicken (*Gallus gallus*) and an oviparous population of

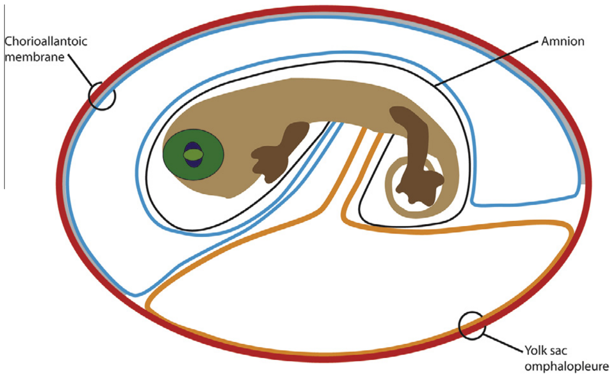

Fig. 1. Simplified diagram showing the generic layout of embryonic membranes in amniotes, modified from Ferner and Mess (2011). In oviparous taxa, these structures sit inside the eggshell membrane; in viviparous species the egg is maintained in the uterus. The shape, size, and layout of each membrane differs between amniotes (see Ferner and Mess (2011) for comparisons between major amniote groups). Red – embryonic ectoderm; grey –embryonic endoderm; blue – allantoic membrane; orange – yolk sac membrane.

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