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Regionalised signalling within the extraembryonic ectoderm regulates anterior visceral endoderm positioning in the mouse embryo

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Abstract

The development of the anterior–posterior (AP) axis in the mammalian embryo is controlled by interactions between embryonic and extraembryonic tissues. It is well established that one of these extraembryonic tissues, the anterior visceral endoderm (AVE), can repress posterior cell fate and that signalling from the other, the extraembryonic ectoderm (ExE), is required for posterior patterning. Here, we show that signals from the prospective posterior ExE repress AVE gene expression and affect the distribution of the AVE cells. Surgical ablation of the prospective posterior, but not the anterior, extraembryonic region at 5.5 days of development (E5.5) perturbs the characteristic distal-to-anterior distribution of AVE cells and leads to a dramatic expansion of the AVE domain. Time-lapse imaging studies show that this increase is due to the ectopic expression of an AVE marker, which results in a symmetrical positioning of the AVE. Surgical ablation of this same ExE region after the distal-to-anterior migration has already commenced, at E5.75, does not affect the localisation of the AVE, indicating that this effect takes place within a short time window. Conversely, transplanting the prospective posterior, but not the anterior, extraembryonic region onto isolated E5.5 embryonic explants drastically reduces the AVE domain. Further, transplantation experiments demonstrate that the signalling regulating AVE gene expression originates from the posterior ExE, rather than its surrounding VE. Together, our results show that signals emanating from the future posterior ExE within a temporal window both restrict the AVE domain and promote its specific positioning. This indicates for the first time that the ExE is already regionalised a day before the onset of gastrulation in order to correctly set the orientation of the AP axis of the mouse embryo. We propose a reciprocal function of the posterior ExE and the AVE in establishing a balance between the antagonistic activities of these two tissues, essential for AP patterning.

Keywords: Mouse embryo; AVE; ExE; Cer-l; AP patterning

1. Introduction

Shortly after implantation, the mouse embryo develops an asymmetric pattern of gene expression that is essential for the development of the AP axis. Thus far, two signalling domains have been identified that are critical for the establishment of the anterior and posterior regions of the embryo, both located within extraembryonic tissues. One is the AVE and another is within the distal ExE (Ang and Constam, 2004; Beddington and Robertson, 1999). A number of genes that are specifically expressed within the AVE have been identified, including *Cerberus-like* (*Cer-l*), *Lefty-1*, *Dickkopf homolog 1 (Dkk1)* and *Hex* (Lu et al., 2001). Of these, the first three participate in the inhibition of Nodal and Wnt signalling in the adjacent epiblast and promote anterior patterning through the restriction of the expression of posterior markers (Glinka et al., 1998; Perea-Gomez et al., 2002; Yamamoto et al., 2004). Expression of genes in the ExE, such as *Bmp4*, is essential for the induction of posterior patterning (Winnier et al., 1995). Furthermore, proteases such as PACE4 and Furin that are expressed within the ExE, are important for the establishment of the AP axis through their influence on Nodal signalling (Beck et al., 2002).

AP asymmetry is first evident along the proximal–distal axis in the E5.5 embryo. Genes such as *Cer-l* and *Hex* are first expressed within a distal subset of VE cells, which begin to move proximally after E5.5 to define the anterior pole of the embryo (Beddington and Robertson, 1999; Thomas et al., 1998). This unilateral movement of cells expressing these distal markers establishes AP axis orientation prior to gastrulation (Rivera-Perez et al., 2003; Srinivas et al., 2004).

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Thus, the future AVE cells are initially located at the distal tip of the egg cylinder. In mutants in which this asymmetric cell movement is inhibited, correct AP patterning is prevented (Ding et al., 1998; Huelsken et al., 2000; Kimura-Yoshida et al., 2005; Perea-Gomez et al., 2001). It has been well documented that Nodal signalling is essential for AVE specification (Beddington and Robertson, 1999; Brennan et al., 2001; Yamamoto et al., 2004). However, when the AVE becomes positioned asymmetrically, Nodal is expressed throughout the epiblast and surrounding VE (Varlet et al., 1997). This suggests that, further asymmetries are required for ensuring the specific localisation of the AVE domain.

Recent studies that have followed the development of AP axis orientation from its initial proximal–distal positioning have shown that the signals required for the distal-to-anterior movement of the AVE relate to the polarity of the embryo, rather than its specific orientation within the uterus (Mesnard et al., 2004). This indicates that signals regulating AP axis formation are intrinsic to the embryo itself. Another recent study indicates that a potential source of such signals may come from the ExE, as removal of the entire ExE results in the upregulation of *Hex* expression in isolated embryonic explants (Rodriguez et al., 2005). Since AVE positioning is asymmetric, we questioned whether the source of the signals within the ExE is also asymmetric.

To address this question, we conducted a series of microsurgical ablations and transplantation experiments that were carried out in a specific time and place. This led us to find that signals emanating from the prospective posterior, but not anterior, ExE repress anterior gene expression within the VE in the posterior region of the embryo. This provides the first evidence that the ExE signals asymmetrically as early as E5.5. Moreover, this regionalisation of the ExE is critical for the specific positioning of the AVE domain.

2. Results

2.1. Ablation of the prospective posterior, but not anterior, extraembryonic region affects Cer-l/GFP expression

We first wished to examine whether there is any asymmetry within the extraembryonic region of the egg cylinder that could influence AVE positioning. To this end, we ablated either the prospective posterior or anterior extraembryonic tissue just above the embryonic/extraembryonic boundary. To monitor the AVE behaviour directly, we performed our experiments using a transgenic line of mice in which GFP expression is driven by the promoter of *Cer-l*, an AVE marker, which has been shown to faithfully indicate endogenous *Cer-l* mRNA (Mesnard et al., 2004). In agreement with this, a localised domain of GFP-positive cells corresponding to the AVE was observed at E5.5 (Fig. 1A,B). We found that by E5.5, this domain was already located slightly, but clearly asymmetrically, towards one side of the distal tip of the embryo (Fig. 1A—black arrow). This early asymmetry relates to the orientation of the anterior–posterior axis (Mesnard et al., 2004; Rivera-Perez et al., 2003) and thus provided us with the marker enabling identification of the prospective anterior and posterior poles of the embryo at E5.5.

In the first set of experiments, we ablated the prospective posterior extraembryonic region (ExE and surrounding VE) by mechanically destroying the region of extraembryonic tissue opposite the characteristic thickening of the distal AVE. After 6 h of culture, the asymmetric shift of *Cer-l*/GFP-expression was evident in 100% of the control non-manipulated embryos ($n=18$) (Fig. 1C,E). In contrast, when we ablated the posterior extraembryonic region, only 11% of the embryos had the same distal-to-anterior pattern of *Cer-l*/GFP expression 6 h following ablation (Fig. 1C,E). In 89% of these embryos, ($P<0.0001$), *Cer-l*/GFP expression extended along the circumference of the egg cylinder from its original distal position symmetrically towards both prospective 'anterior' and 'posterior' poles of the embryo (Fig. 1C,E).

After 18 h of culture, cells expressing *Cer-l*/GFP had reached the embryonic/extraembryonic boundary in 96% of the control embryos (Fig. 1C,E). At this time, the lateral movement of VE cells at the embryonic/extraembryonic boundary was evident (Thomas et al., 1998; Weber et al., 1999), due to 'residual' GFP protein in these cells. In contrast, in 83% of ablated embryos ($n=23$), *Cer-l*/GFP expression was observed circumferentially towards both 'anterior' and 'posterior' regions of the VE overlying the epiblast ($P<0.0001$) (Fig. 1C,E). Only 13% of these embryos had distal-to-anterior positioning of *Cer-l*/GFP positive cells and in 4% of the embryos, the GFP domain remained distal. In addition, in 26% of the embryos subjected to ablation of the posterior extraembryonic region, the domain of *Cer-l*/GFP expression expanded into VE in the extraembryonic region of the egg cylinder (data not shown). This is in contrast to the control embryos, where *Cer-l*/GFP expressing cells were never found in the extraembryonic VE. This indicates that the prospective posterior extraembryonic region has the ability to modulate the distribution of anterior gene expression within the VE.

To address whether the prospective anterior extraembryonic region could also influence the AVE, we then conducted a similar series of experiments in which we ablated anterior extraembryonic tissue on the same plane as the distal AVE. After 6 h, all of the control embryos developed with the expected unilateral shift in *Cer-l*/GFP expression ($n=5$) (Fig. 1D,E). Moreover, all of the experimental embryos subjected to the ablation of anterior extraembryonic region showed the characteristic distal-to-anterior shift in *Cer-l*/GFP expression as the control embryos after 6 h of culture ($n=11$) (Fig. 1D,E). When examined 18 h after ablation of the anterior extraembryonic region, all of the experimental embryos ($n=17$) showed a similar asymmetric domain of *Cer-l*/GFP positive cells to that of the control embryos ($n=10$) (Fig. 1D,E).

These data demonstrate that embryos subjected to the ablation of the prospective posterior—but not anterior—extraembryonic region displayed a greater domain of *Cer-l*/GFP expressing cells. Thus, our results indicate firstly that the prospective posterior extraembryonic region functions to restrict AVE character to the distal region of the embryo and to influence the distribution of AVE cells; and secondly,