STELLA-positive subregions of the primitive streak contribute to posterior tissues of the mouse gastrula

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ABSTRACT

The developmental relationship between the posterior embryonic and extraembryonic regions of the mammalian gastrula is poorly understood. Although many different cell types are deployed within this region, only the primordial germ cells (PGCs) have been closely studied. Recent evidence has suggested that the allantois, within which the PGCs temporarily take up residence, contains a pool of cells, called the Allantoic Core Domain (ACD), critical for allantoic elongation to the chorion. Here, we have asked whether the STELLA-positive cells found within this region, thought to be specified PGCs, are actually part of the ACD and to what extent they, and other ACD cells, contribute to the allantois and fetal tissues. To address these hypotheses, STELLA was immunolocalized to the mouse gastrula between Early Streak (ES) and 12-somite pair (-s) stages (~6.75–9.0 days post coitum, dpc) in histological sections. STELLA was found in both the nucleus and cytoplasm in a variety of cell types, both within and outside of the putative PGC trajectory. Fate-mapping the headfold-stage (~7.75–8.0 dpc) posterior region, by which time PGCs are thought to be segregated into a distinct lineage, revealed that the STELLA-positive proximal ACD and intraembryonic posterior primitive streak (IPS) contributed to a wide range of somatic tissues that encompassed derivatives of the three primary germ layers. This contribution included STELLA-positive cells localizing to tissues both within and outside of the putative PGC trajectory. Thus, while STELLA may identify a subpopulation of cells destined for the PGC lineage, our findings reveal that it may be part of a broader niche that encompasses the ACD and through which the STELLA population may contribute cells to a wide variety of posterior tissues of the mouse gastrula.

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Introduction

A range of embryonic and extraembryonic tissues, including the amnion, allantois, blood vessels, hindgut, surface ectoderm, tailbud, and primordial germ cells (PGCs), are deployed and develop together in the posterior region of the mammalian conceptus. However, with the exception of the PGCs, the origin of these posterior cell types is still largely obscure.

PGCs are thought to originate from the proximal epiblast of the mouse conceptus at ~6.25–6.5 dpc (Lawson and Hage, 1994; Ohinata et al., 2005). Although the timing of PGC specification is controversial (Lawson and Hage, 1994; Ohinata et al., 2005), epiblast cells assumed to be destined for the gonads migrate through the primitive streak, and then reside temporarily within the base of the allantois/posterior embryonic region (Chiquoine, 1954; Ginsburg et al., 1990; Lawson and Hage, 1994; Ozdzenski, 1967; Saitou et al., 2002), where additional signals may induce PGC specification (Fujiwara et al., 2001). From the allantois/posterior embryonic region, the putative PGCs translocate into the hindgut endoderm (~8.5 dpc); between ~9.5 and 10.0 dpc, the PGCs exit the hindgut and migrate towards the developing genital ridges, which they colonize between ~10.0 and 11.5 dpc (reviewed in Molyneaux and Wylie, 2004). Within the gonads, PGCs complete their development as germ cells and form sperm and eggs.

The PGC trajectory was established using tissue non-specific alkaline phosphatase (TNAP) activity (Macgregor et al., 1995) as a "marker" for PGCs (Chiquoine, 1954; Ozdzenski, 1967; Tam and Snow, 1981) in conjunction with *Dominant white spotting* (*W*) and *Steel* (*Sl*) mutants (McCoshen and McCallion, 1975; Mintz, 1957; Mintz and Russell, 1957). In these sterile mutants, TNAP-positive cells formed in the posterior region of the conceptus in numbers similar to wildtype. During hindgut migration, the number of TNAP-positive cells became greatly reduced in the mutants, and then significantly reduced or absent in the early gonad. Yet, although TNAP is thought to mark PGCs, it is not required for successful PGC formation, migration, and colonization of the gonads (Macgregor et al., 1995).

Recently, other proteins have been reported to be associated with the emergence of the germ line, including BLIMP1 (PRDM1) and STELLA (DPPA3, PGC7). BLIMP1, a transcriptional repressor of the somatic program within putative PGCs (Kurimoto et al., 2008; Ohinata et al., 2005), is thought to be required for the formation of the nascent putative PGC population, as *Blimp1* null mutants exhibit fewer TNAP-positive cells in the posterior region as early as ~7.5–7.75 dpc (Ohinata et al., 2005; Vincent et al., 2005). STELLA, a maternally inherited factor required for preimplantation development that protects the early embryo against DNA demethylation (Bortvin et al., 2004; Nakamura et al., 2007; Payer et al., 2003), is also found in presumptive PGCs (Saitou et al., 2002; Sato et al., 2002). However, STELLA is not required for germline development, as *stella* null mutants are viable and fertile (Payer et al., 2003). During putative PGC localization to the posterior region and prior to migration to the hindgut, only a subpopulation of TNAP-positive cells expressed *stella* (Saitou et al., 2002) while the majority (i.e., 80–100%) of *Blimp1*-expressing cells exhibited STELLA protein (Ohinata et al., 2005; Seki et al., 2007). At the same time, all STELLA-positive cells were reported to be TNAP- and *Blimp1*-positive (Ohinata et al., 2005; Saitou et al., 2002; Seki et al., 2007). Whether these relationships persist at later stages of PGC development, for example, as they migrate through the hindgut, is obscure.

To the best of our knowledge, there is no evidence demonstrating that the TNAP-, BLIMP1-, and STELLA-positive putative PGC populations actually translocate from the allantois/posterior primitive streak to the hindgut and gonads to contribute to the germ line. Although results from grafting experiments demonstrated that the posterior primitive streak/allantois of neural plate-stage conceptuses (~7.5 dpc) contributes TNAP-positive cells to the hindgut, whether these were derived from the posterior region's TNAP-positive, and not TNAP-negative, cell population is not known (Copp et al., 1986). Furthermore, if these cell populations represent the germ line, it remains unclear whether they are a homogeneous population that gives rise exclusively to germ cells, or whether they contribute to other cell types.

Nevertheless, despite the uncertainty of the precise nature of these markers vis-à-vis the PGCs, two studies have employed them to explore the timing of PGC lineage restriction. In the first, clonal analysis was used to fate-map a single cell per embryo (Lawson and Hage, 1994). Dextran labeling in conjunction with endogenous TNAP activity in the base of the allantois led to the conclusion that PGCs are specified as a distinct cell lineage at the neural plate stage, ~7.2 dpc, in the base of the allantoic bud. In the second study, genetic lineage tracing of the *Blimp1*-expressing population and identification of "specified PGCs" via STELLA localization led to a significantly different conclusion, i.e., that lineage restriction occurs prior to gastrulation, as early as ~6.25 dpc, in the small *Blimp1*-expressing population of the proximal epiblast (Ohinata et al., 2005). Differences in "markers" used may explain this major discrepancy, as *stella* was expressed only in a subpopulation of the posterior region's TNAP-positive cells (Saitou et al., 2002). In addition, clonal analysis might have missed the small specified PGC population claimed to exist at ~6.25 dpc.

Regardless of the discrepancies in these conclusions, the morphological endpoint in both studies was the allantois, prior to PGC translocation to the hindgut. As it is not known whether the putative PGCs

scored in these studies would have ever translocated to the hindgut and ultimately colonized the gonads, it is possible that the scored TNAP- or STELLA-positive cells are not PGCs but actually part of a larger cell pool used to build the posterior region. Moreover, in the case of STELLA, previous expression and localization studies relied on whole mount analysis (Saitou et al., 2002) and limited sectional analysis (Sato et al., 2002), respectively. Therefore, STELLA's spatiotemporal pattern within the posterior region has not been examined in sufficient detail to conclude that STELLA exclusively localizes to the PGC trajectory or whether it may be found in other tissues of the posterior conceptus.

Recent evidence has suggested that the posterior primitive streak extends into the base of the allantois, where it establishes a precursor pool of cells, called the Allantoic Core Domain (ACD), used to build the allantois (Downs et al., 2009). The presence of the ACD in the allantois coincides with the localization of the putative PGCs within the allantois/posterior embryonic region (Anderson et al., 2000; Chiquoine, 1954; Ginsburg et al., 1990; Saitou et al., 2002). Like the PGCs, the ACD is positive for OCT-3/4 (Downs, 2008; Downs et al., 2009), which is found in relatively undifferentiated cells (Scholer et al., 1990). In addition, the ACD exhibits dynamic localization of extracellular matrix molecules COLLAGEN TYPE IV, E-CADHERIN, and PERLECAN (Daane et al., 2011; Mikedis and Downs, 2009), which are known to regulate many stem cell properties, such as proliferation and differentiation (reviewed in Kruegel and Miosge, 2010; Marthiens et al., 2010). Uniquely, only the region containing the ACD rescues allantoic elongation after grafting to microsurgically foreshortened allantoises in normal embryos and to genetically foreshortened allantoises in *Brachyury* (*T*) mutants (Downs et al., 2009). When the lipophilic tracer dye, DiI, was introduced into the ACD, labeled cells both persisted there and put forth a file of descendant cells through the allantoic midline that branched throughout the distal allantoic region (Downs et al., 2009). Finally, when ectopically grafted, some cells of the proximal allantois, and no other region, contributed to descendants of all three primary germ layers, suggesting the presence of a pluripotent population there (Downs and Harmann, 1997). Whether pluripotency was an intrinsic property of this tissue or due specifically to the presence of putative PGCs was not investigated.

Given the uncertainties concerning the true identity of the cells thought to be PGCs within the allantois, we decided to test the hypothesis that STELLA-positive cells may be found outside of the PGC trajectory, and/or are part of the ACD, the latter of which may be a larger, more general population used to build the posterior region of the mouse conceptus. Toward this end, we designed three experimental strategies. First, we localized STELLA to the mouse conceptus (Early Streak (ES)–12-s stages; ~6.75–9.0 dpc), focusing on the posterior region of the embryo and allantois. Second, to discover the fate of the ACD and surrounding regions, we fate-mapped the posterior region of the headfold-stage conceptus (~7.75–8.0 dpc), subdividing it into the distal allantois, distal ACD, proximal ACD, and intraembryonic posterior primitive streak (IPS). Third, to discover whether STELLA-positive cells in these regions contributed only to the hindgut, or whether they contributed to other cell types, we immunostained a subset of grafted chimeras to localize STELLA at the end of the culture period. Our results revealed that the STELLA-positive population is found in a variety of sites outside of the PGC trajectory, and that STELLA protein localized both within and outside of the cell nucleus. In addition, the proximal ACD and IPS, which together contained the majority of STELLA-positive cells, contributed to a range of tissues, both STELLA-positive and -negative, encompassing derivatives of the three primary germ layers within the posterior conceptus.

Materials and methods

Animal husbandry, embryo dissections, and staging

All animals were treated in accordance with Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (Public