Biochimica et Biophysica Acta 1859 (2016) 780–791

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Sox2/Oct4: A delicately balanced partnership in pluripotent stem cells and embryogenesis☆

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ARTICLE INFO

Article history:
Received 12 January 2016
Received in revised form 10 March 2016
Accepted 11 March 2016
Available online 23 March 2016

Keywords:
Sox2
Oct4
Embryonic stem cells
Pluripotent stem cells
Embryogenesis
Negative feedback loop

ABSTRACT

Considerable progress has been made in understanding the roles of Sox2 and Oct4 in embryonic stem cells and mammalian embryogenesis. Specifically, significant progress has been made in answering three questions about the functions of Sox2 and Oct4, which are the focus of this review. 1) Are the first or second cell lineage decisions during embryogenesis controlled by Oct4 and/or Sox2? 2) Do the levels of Oct4 and Sox2 need to be maintained within narrow limits to promote normal development and to sustain the self-renewal of pluripotent stem cells? 3) Do Oct4 and Sox2 work closely together or is the primary role of Sox2 in pluripotent cells to ensure the expression of Oct4? Although significant progress has been made in answering these questions, additional studies are needed to resolve several important remaining issues. Nonetheless, the preponderance of the evidence suggests there is considerable crosstalk between Sox2 and Oct4, and further suggests Sox2 and Oct4 function as molecular rheostats and utilize negative feedback loops to carefully balance their expression and other critical genes during embryogenesis. This article is part of a Special Issue entitled: The Oct transcription factor family, edited by Dr. Dean Tantin.

1. Introduction

One of the most intensely studied periods of mammalian development is the stage between fertilization and the implantation of the early embryo. Besides the intrinsic interest in understanding the very first events that shape mammalian embryogenesis, a deeper understanding of this period has the potential to accelerate advances in regenerative medicine. Over the past 10 years, considerable progress has been made in understanding the molecular and cellular changes that control the first two embryonic cell lineage decisions. During mouse development, these decisions occur between the third and fourth days of development. As development proceeds from the 8-cell stage to the morula, an increasing number of cells become localized within the interior of the developing embryo (Fig. 1). By the early blastocyst stage, this inner cluster of cells has formed the inner cell mass (ICM), which gives rise to each of the three embryonic germ-layers and some extraembryonic tissues, while the outer layer of cells, the trophectoderm, will eventually contribute to the fetal portion of the placenta. The specification of the trophectoderm and the ICM represents the first critical cell lineage decision made during embryogenesis. Shortly thereafter, the second major cell lineage decision is made. Just prior to implantation of the blastocyst, the ICM gives rise to two distinct cell populations, the pluripotent (naïve) epiblast cells, which form the embryo proper, and a layer of primitive (extraembryonic) endoderm (PrE) that faces the blastocoel cavity (Fig. 1). The partitioning of the ICM into the naïve epiblast and PrE represents the second cell lineage decision. By this point in development, pluripotency (the ability to form all cell types in the body) has already been established.

Our understanding of the molecular and biochemical changes leading up to the first two cell lineage decisions has grown rapidly, due in large measure to several major advances, including major advances in the methods needed to maintain pluripotent stem cells in culture, in particular those that represent different stages of mammalian embryogenesis. Although pluripotent mouse embryonal carcinoma cells (ECC) and pluripotent mouse embryonic stem cells (ESC) were first established in culture over 35 years ago [1–5], progress has accelerated with the development of human ESC, mouse epiblast stem cells (EpiSC) and induced pluripotent stem cells (iPSC). Germane to the focus of this review, mouse ESC are believed to be similar to the ICM of the early epiblast at the pre/peri-implantation blastocyst stage [6]; whereas, mouse EpiSC, and presumably human ESC, are believed to closely resemble the ectoderm at the late gastrula stage [7]. Due to prominent differences in their developmental potential [8], mouse ESC are also referred to as naïve ESC and mouse EpiSC are referred to as primed pluripotent stem cells. Additionally, there are significant differences in the signaling pathways needed to sustain their self-renewal in culture. Mouse ESC depend on LIF and BMP signaling; whereas mouse EpiSC and human ESC depend on FGF and

Abbreviations: ICM, inner cell mass; PrE, primitive extraembryonic endoderm; ECC, embryonal carcinoma cells; ESC, embryonic stem cells; EpiSC, epiblast stem cells; iPSC, induced pluripotent stem cells.

☆ This article is part of a Special Issue entitled: The Oct transcription factor family, edited by Dr. Dean Tantin.

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http://dx.doi.org/10.1016/j.bbagrm.2016.03.006
1874-9399/

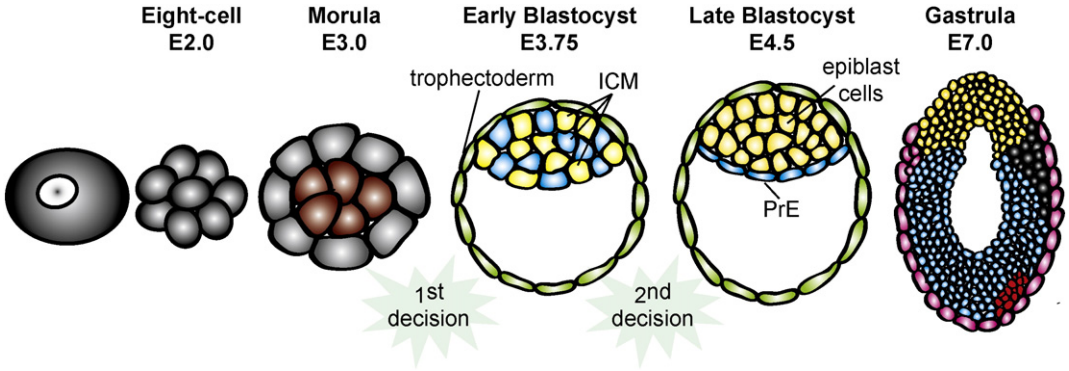

Fig. 1. Early stages of mammalian embryogenesis and pluripotent stem cells. Stages of mouse development from the one-cell fertilized egg to the early gastrula. Approximate developmental stages are shown from E2.0 to E7.0. The inner cluster of cells at the morula stage is shown in reddish-brown. The first cell lineage decision occurs during the early blastocyst stage with the specification of the ICM and the trophectoderm. The second cell lineage decision occurs when the cells of the ICM develop into distinct cell populations, the early epiblast and PrE. The trophoblast giant cells and parietal extraembryonic endoderm are not shown at the gastrula stage. The three embryonic germ layers, ectoderm (blue), mesoderm (black) and endoderm (red) are shown in the gastrula stage embryo. Also shown are the ectoplacental cone and extraembryonic ectoderm (yellow), and extraembryonic endoderm (pink). ESC most closely resemble cells of the early epiblast, and EpiSC most closely resemble the ectoderm at the gastrula stage.

TGFβ signaling. Although naïve and primed pluripotent stem cells represent different stages of mammalian development, each can be converted into the other in culture [8].

Progress in defining the molecular machinery that regulates self-renewal and pluripotency of ESC, has also led to a much deeper understanding of the genes that control embryogenesis. Although it is now clear that many transcription factors function as master regulators during embryogenesis and in the biology of pluripotent stem cells, two transcription factors, Sox2 and Oct4, have taken center stage. Oct4 and Sox2 have generated considerable interest because blocking their expression in pluripotent stem cells and during the initial stages of embryogenesis has dire developmental consequences. Furthermore, numerous studies conducted in ESC support the widely held view that Oct4 and Sox2 work together closely to regulate a large set of genes that are essential for the self-renewal and pluripotency of ESC and EpiSC. As a result, it is widely believed that they function in a similar manner during embryogenesis.

In an effort to provide a better understanding of the roles of Oct4 and Sox2 during embryogenesis, as well as their roles in pluripotent stem cells, this review focuses on three questions. 1) Are the first or second cell lineage decisions during embryogenesis controlled by Oct4 and/or Sox2? 2) Do the levels of Oct4 and Sox2 need to be maintained within narrow limits to promote normal development and to sustain the self-renewal of pluripotent stem cells? 3) Do Oct4 and Sox2 work closely together or is the primary role of Sox2 in pluripotent cells to ensure the expression of Oct4? As discussed in this review, significant progress has been made in addressing each of these questions, but there is far more to learn before we will fully know the answers. Clearly, answers to these questions require an understanding of other master regulators, in particular Nanog. Therefore, where possible, other important players are incorporated into the discussion. Finally, Section 7 provides an updated model for the roles of Sox2 and Oct4 in pluripotent stem cells and embryogenesis. Specifically, it is proposed that one of the essential functions of Sox2 and Oct4 is to delicately balance their expression and the expression of other essential genes that enable pluripotent cells of the early embryo to proliferate yet undergo differentiation in response to appropriate developmental signals.

2. Developmental expression of Sox2 and Oct4

Sox2 and Oct4 each belong to a large family of transcription factors that play key roles in most, if not all, stages of mammalian development. Oct4 (also known as Oct3 or Oct3/4) is encoded by the *Pou5f1 gene*. Oct4 belongs to the POU transcription factor family, which includes the prototypic members Pit-1, Oct1, and Unc-86. Each of the 14 POU family members contains a well-conserved bipartite POU domain, which consists of 150 to 160 amino acids, that is responsible for binding to DNA. The POU domain of Oct4 consists of a POU-specific subdomain (60 amino acids) and a POU homeodomain (75 amino acids) that are separated by a flexible linker (17 amino acids). These two subdomains fine tune Oct4 binding to DNA, as well as its activation of transcription in conjunction with other transcription factors that bind to adjacent DNA sequences.

Unlike Oct1, which is ubiquitously expressed, Oct4 expression is far more restricted. Oct4 protein is present at fertilization from maternal sources, but maternally derived Oct4 begins to decay by the late 2-cell stage and it is lost by the 8-cell stage [9,10]. Embryonic expression of Oct4 appears to be initiated at the 8-cell stage and it is expressed in all cells at the 16- and 32-cell stage [10,11]. By the blastocyst stage, Oct4 is expressed in the ICM and it is excluded from the trophectoderm [9], where its expression is repressed by Cdx2 [12]. However, recent studies report that Oct4 is present at the protein level in the trophectoderm, at least during the early blastocyst stage [13]. Just prior to implantation, Oct4 is expressed in the naïve epiblast and in the PrE. As development proceeds, Oct4 continues to be expressed through the early somite stages. Thereafter, it is believed to be expressed solely in the germ cell lineage. Oct4 has not been studied extensively after birth, but it has been shown to be expressed in at least some multipotent stem cells in the adult [14–16]. Given the large number of reports of OCT4 expression in many types of human cancer, further study of Oct4 in the adult is warranted.

Similar to Oct4, Sox2 expression is initiated early during embryogenesis. Sox2 is a member of the SRY-related gene family, each member of which contains a well-conserved high mobility group domain (HMG box, 79 amino acids), which mediates its binding to DNA. This family comprises ~20 members and Sox2 belongs to the B1 subgroup composed of Sox1, Sox2, and Sox3. Sox2 expression during development was extensively characterized by Avilion et al. [17]. Sox2 protein is present at fertilization from maternal sources. Zygotic *Sox2* expression (mRNA and protein) begins at the morula stage where it is preferentially localized to the inner cells of the morula, which will give rise to the ICM [11,13,17]. By the blastocyst stage, Sox2 is restricted to a subset of cells in the ICM. As the ICM develops into the epiblast and PrE, Sox2 is expressed only in the epiblast. Interestingly, *Sox2* expression is initiated after that of *Oct4*, but Sox2 becomes restricted to the cells that will ultimately give rise to the ICM before Oct4 [11].

Beyond the egg cylinder stage, Sox2 continues to be expressed in both embryonic and extraembryonic (extraembryonic ectoderm) tissues. After gastrulation, Sox2 expression in the embryo is observed in the nervous system, sensory placodes, primitive foregut endoderm, and branchial arches [17,18]. Like Oct4, Sox2 is expressed in the germ cell lineage [17]. After birth, Sox2 expression has been reported in