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High-throughput screen for genes predominantly expressed in the ICM of mouse blastocysts by whole mount in situ hybridization

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Abstract

Mammalian preimplantation embryos provide an excellent opportunity to study temporal and spatial gene expression in whole mount in situ hybridization (WISH). However, large-scale studies are made difficult by the size of the embryos (~60 μm diameter) and their fragility. We have developed a chamber system that allows parallel processing of embryos without the aid of a microscope. We first selected 91 candidate genes that were transcription factors highly expressed in blastocysts, and more highly expressed in embryonic (ES) than in trophoblast (TS) stem cells. We then used the WISH to identify 48 genes expressed predominantly in the inner cell mass (ICM) and to follow several of these genes in all seven preimplantation stages. The ICM-predominant expressions of these genes suggest their involvement in the pluripotency of embryonic cells. This system provides a useful tool to a systematic genome-scale analysis of preimplantation embryos.
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Keywords: Preimplantation embryo; Whole mount in situ hybridization; High-throughput screen; Hybridization chamber; ICM; TE

1. Introduction

Preimplantation development encompasses the period from fertilization to implantation, and is marked by a number of critical events, including the degradation of maternally stored RNAs, zygotic genome activation (ZGA), compaction, and blastocyst formation (reviewed in Edwards, 2003). From the viewpoints of developmental potency (potential), fertilized eggs are the ultimate totipotent cells, giving rise to all cell types. The loss of totipotency occurs during preimplantation development, marked by the segregation of two distinct cell lineages in the blastocyst: the inner cell mass (ICM), which gives rise to the embryo proper and is thus pluripotent, and the trophectoderm (TE), which contributes to the trophoblast portion of the placenta and is thus lineage-restricted (Fig. 1B). Genes that are important for cellular pluripotency, such as *Pou5f1/Oct4* (Pesce and Scholer, 2000) and *Nanog* (Chambers et al., 2003; Mitsui et al., 2003), are predominantly expressed in the ICM, and thus, the identification of genes expressed in the ICM will be an important first step towards understanding the cellular potency. Whether the emergence of such asymmetry between the ICM and TE originates from an earlier event, such as fertilization, is still controversial (Gardner, 2001; Hiiragi and Solter, 2004; Piotrowska et al., 2001).

Large-scale systematic analysis holds great promise for understanding preimplantation embryos as a whole (Ko, 2001). A large number of cDNA clones have been identified from mouse preimplantation embryos and mapped to the mouse genome (Ko et al., 2000; Sharov et al., 2003; Solter et al., 2002). Microarray analysis of the preimplantation embryos has provided global picture of expression changes during preimplantation mouse development (Hamatani et al., 2004; Tanaka and Ko, 2004; Wang et al., 2004; Zeng et al., 2004). The knowledge of genes expressed in preimplantation mouse embryos has increased dramatically. However, because RNA samples are taken from homogenized tissues, spatial information is lost, and thus, questions of their asymmetric expression cannot be directly addressed.

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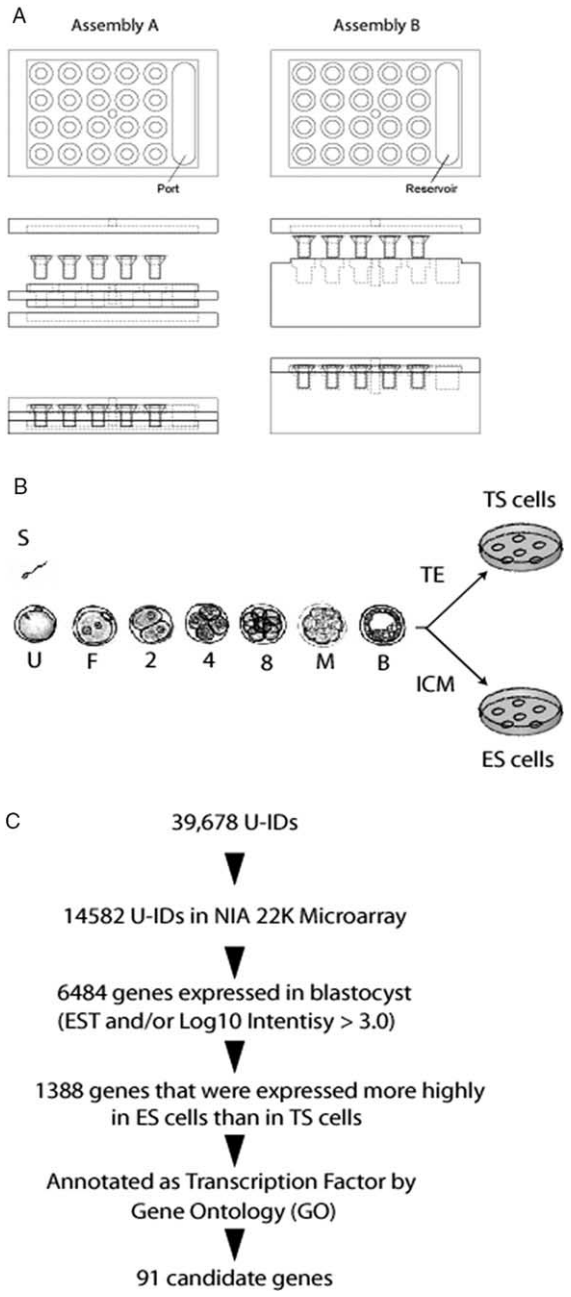

Fig. 1. (A) Assembly A: Washing chamber. Assembly B: Hybridization chamber. (B) Schematic drawings of mouse preimplantation development. At blastocyst stage, there are two different cell types: Trophectoderm (TE) and inner cell mass (ICM). ES cells are derived from the ICM, whereas TS cells are derived from the TE cells. (C) Bioinformatic selection of candidate genes.

WISH allows localization of gene transcripts in the individual cell, enabling the study of the heterogeneity of cells and/or their polarity at very early stages of the embryo, in which no morphological differences are seen among cells.

Large-scale in situ hybridizations have been performed on mouse intestine (Komiya et al., 1997), E9.5 embryos (Gitton et al., 2002; Neidhardt et al., 2000), and E9.5 and E10.5 embryos (Reymond et al., 2002), and mouse brain as well as on other species, such as Drosophila (Tomancak et al., 2002), Zebrafish (Kudoh et al., 2001), Xenopus (Gawantka et al., 1998), Medaka Fish (Quiring et al., 2004), Chick retina (Shintani et al., 2004), Ascidian (Mochizuki et al., 2003), Chicken embryos (Bell et al., 2004). A robotic workstation is available, but due to its larger filter pore size (35 μm) it cannot be used for small embryos, such as mammalian preimplantation embryos. Due to the technical difficulty of handling small embryos, WISH data for mouse preimplantation embryos is scarce even with small-scale methods based on individual genes. During the pipetting procedure, embryos are often lost. This has been addressed by using a microcentrifuge tube, which was cut at the bottom and attached to a 20 μm pore membrane (Newman-Smith and Werb, 1995). The method has successfully circumvented laborious micropipetting work, but the microtubes were made by hand each time and were not suited for parallel processing. While a pore size of 20 μm is necessary for achieving efficient drainage without special instruments, much smaller pores are preferable to maintain the best morphology of small samples. As a result, transwell with pore size 12 μm which are originally designed for cell culture were introduced into WISH (Hanna et al., 2002) to retain embryos. Although, solution changes were achieved by manually transferring the transwell from one well to another, it is difficult to have good buffer exchange through smaller pores without the assistance of a special device. Here we report the development of a chamber system that utilizes both the transwell inserts for parallel processing and capillary action for gentle buffer exchanges. Using this method, we have identified 48 genes that are expressed predominantly in the ICM.

2. Results

2.1. Design and fabrication of WISH chamber system

To perform a high-throughput WISH for preimplantation embryos (up to 100 μm diameter), we developed a chamber system that can run multiple probes in parallel without microscope-assistance (Fig. 1A). Embryos can be placed in plastic Transwell-inserts with 8 μm pore-size membrane on the bottom. Up to 20 inserts can be placed in one aluminum chamber, which allows analysis of up to 20 different probes in parallel. The small pore size helps maintain good embryo morphology while minimizing the chance of embryo loss during the WISH procedure. However, the small pore size makes it difficult to drain the solution through the bottom membrane. Initial design used negative air pressure by vacuum pump, resulting in poor morphology of embryos. We then devised a chamber system so that the distance between the bottom of the