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Nanog: A New Recruit to the Embryonic Stem Cell Orchestra

In this issue of *Cell*, Chambers et al. (2003) and Mitsui et al. (2003) add a new transcriptional operating system to the known Oct4 and Stat3 systems required for early embryonal stem cell potency and self-renewal. Nanog, a homeobox transcription factor, plays a crucial role in the second embryonic cell fate specification following formation of the blastocyst.

Development of a healthy organism requires that cell fate be specified at the correct place and time in the embryo. In mammals, the first differentiation event occurs when the outer morula cells compact to form an extraembryonic epithelial layer—the trophectoderm—required for implantation into the mother's uterus. The inner part of the morula develops into the inner cell mass (ICM) of the blastocyst and contains the founder cells of the embryo proper. Prior to implantation, the ICM splits into two lineages: the epiblast (primitive/embryonic ectoderm) which will eventually form the three somatic germ layers and primordial germ cells, and the hypoblast (primitive/embryonic endoderm) which will generate two extraembryonic lineages, the visceral and parietal endoderm. Embryonic stem (ES) cell lines derived from the ICM are considered to be the immortal ex vivo counterpart of early embryo stem cells (Smith, 2001), and represent a powerful tool with which to address scientific and medical issues (Boiani and Schöler, 2002).

Current knowledge of what defines the potency of mouse embryonic stem cells revolves around a quartet of critical players—the transcription factors Oct4, Sox2, FoxD3, and Stat3—but the concert cannot begin with these factors alone, because it is apparent that some players are yet to come. Oct4 is required for regulation of cell fate in the early embryo; it is expressed in the ICM and downregulated upon differentiation into trophoblast cells (Nichols et al., 1998). Sox2 and FoxD3 are involved somewhat later in the maintenance of the epiblast after implantation (Avilion et al., 2003; Hanna et al., 2002). In cultured ES cells, Stat3 activation by the cytokine LIF is required to sustain self-renewal (Williams et al., 1988; Niwa et al., 1998; Matsuda et al., 1999), although LIF is not required for normal mouse development (Stewart et al., 1992).

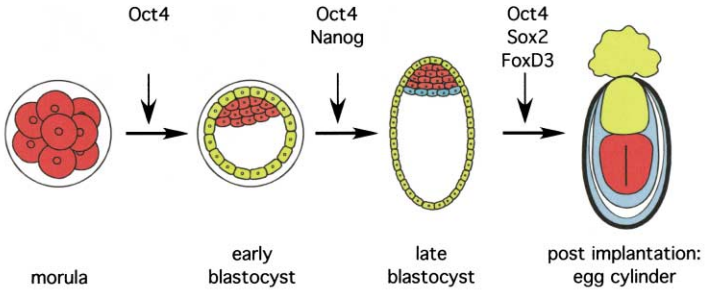
Thus, to date, two transcription factors are known to participate in the self-renewal of embryonic stem cells—Oct4 and Stat3. However, there is room for new players in the orchestra, especially if they receive as warm a reception as the one introduced in two papers published in this issue of *Cell*. Chambers et al. (2003) and Mitsui et al. (2003) describe a divergent homeobox transcription factor expressed in the inner cells of a compacted morula and blastocyst, and early germ cells, as well as in the ES and embryonic germ (EG) cell lines derived from these stages. They named the factor Nanog, after the mythological Celtic land of the ever-young, "Tir nan Og."

The studies not only describe Nanog, but venture as far as showing how embryo cell fate specification and ES cell self-renewal may be related. Mitsui et al. (2003) show that Nanog ablation in vivo causes a failure in the specification of early embryo pluripotent cells, which adopt a differentiated visceral/parietal endodermal fate. Thus, Nanog plays a crucial role in the second embryonic cell fate specification event (Figure 1). Oct4 plays a role in this step and also the preceding one. The phenotypes of both Oct4- and Nanog-deficient embryos confirms that embryonic stem cell identity depends on keeping the stem cell regulatory system active and the differentiation system silent.

Isolation of Nanog also produced a surprising insight into ES cell culture. Until now, it was known that the maintenance of self-renewing mouse ES cells requires a combination of activated STAT3 (by LIF) and Oct4 expression. However, both Mitsui et al. (2003) and Chambers et al. (2003) demonstrate that Nanog overexpression relieves ES self-renewal from dependence on LIF/STAT3 stimulation. Furthermore, Chambers et al. (2003) show that the differentiation potential of ES cells overexpressing Nanog is both reduced and retarded, but removal of Nanog overexpression reverses the cells' status to that of the parental stem cell. Nanog deletion triggers differentiation of ES cells into parietal/visceral endoderm, confirming its role in the second embryonic differentiation event. This result, together with the observation that LIF and Nanog have an additive effect on ES cell self-renewal, suggests that the two factors control two different but partially overlapping pools of target genes.

Chambers' report elucidates the role of Nanog in the transcriptional hierarchy that controls ES cell identity and maintenance of embryonic stem cell potency. Nanog overexpression does not affect self-renewal by activating Stat3, and vice versa, indicating that Nanog and Stat3 act independently. Furthermore, Nanog and Oct4 work in concert to support stem cell potency and self-renewal. This conclusion is based on the two observations: first, Nanog is expressed in Oct4-deficient embryos, and second, Nanog overexpression cannot revert the differentiation program induced in ES cells by Oct4 downregulation.

Thus, Nanog, Oct4, and STAT3 appear to define three different transcriptional pathways operating in ES cells (Figure 1). The studies by Chambers et al. (2003) and Mitsui et al. (2003) add a new contributor to the orchestra regulating stem cell potency and self-renewal. However, not surprisingly, important questions still remain. Why is an embryonic stem cell capable of differentiating in so many directions, whereas a differentiated cell is not? Is there a link between any of these transcription factors and chromatin to keep it open to be conducted in a new direction? Insight into the regulation of FoxD3-, Sox2-, Oct4-, Nanog-, Stat3-directed transcriptional pathways and the network of crosstalk between them might con-



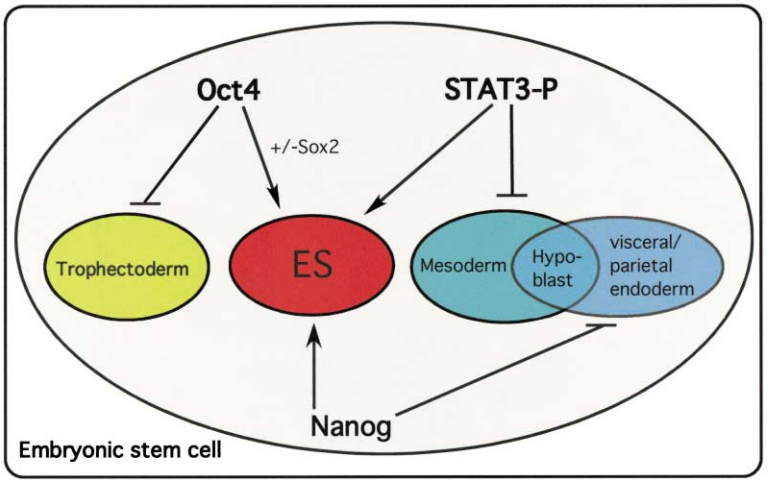


Figure 1. Model Of Transcription Factor Operating System in Early Mouse Development and ES Cells

Oct4 is crucial for the first embryonic lineage specification, and Nanog is crucial for the second. Maintenance of the pluripotent epiblast of postimplantation embryos requires Oct4, Sox2, and FoxD3. In the ES cell Oct4, Sox2, Stat3, and Nanog are essential for self-renewal: the pools of target genes controlled by each transcription factor or combination of factors are shown in color.

tribute to a better understanding of how stem cell renewal and differentiation are related to downstream target genes. At this stage, any new composition beyond the transcriptional tune we currently hum is bound to be instrumental.

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Fingering the Ends: How to Make New Telomeres

Telomerase-mediated healing of broken chromosomes gives rise to terminal deletions and is repressed in most organisms. In ciliated protozoa, however, chromosome fragmentation and de novo telomere addition are part of the developmental program. Work by Karamysheva et al. (2003) in this issue of *Cell* indicates that in *Euplotes crassus*, this is mediated through switching between different telomerase reverse transcriptase isoforms.

Double-stranded DNA breaks can be repaired either by homologous recombination or by non-homologous end-joining. Alternatively, telomere sequences can be added