Oct-3 and the Beginning of Mammalian Development

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MAMMALIAN DEVELOPMENTAL BIOLOGISTS CONTINUE TO search for regulatory proteins that are responsible for the transformation of a fertilized egg into an embryo. A common approach has been to characterize the mammalian counterparts of *Drosophila* developmental control genes, many of which encode transcription factors (*1*). The mammalian genes that have been so identified are expressed in the developing embryo after gastrulation, that is, after pluripotent embryonic stem cells have given rise to the three germ layers of the embryo. In an effort to isolate regulatory genes active at earlier stages in mammalian development, recent investigations have focused on transcription factors that are expressed in undifferentiated, but not differentiated, embryonal carcinoma (EC) and embryonic stem (ES) cell lines (*2–12*). This review focuses on the first regulatory gene to be identified in this way, *Oct-3* (*2–9, 13*), which is expressed in totipotent and pluripotent stem cells before gastrulation and in the germ cell lineage. Recent experiments have demonstrated that maternally derived Oct-3 is required for mouse development to proceed beyond the one-cell stage (*9*).

Oct-3 was first detected as a DNA binding protein in undifferentiated EC and ES cells; Oct-3 specifically binds the octamer DNA motif, ATTTGCAT (*2, 3, 5*). Because several members of the POU domain family of transcription factors (*14*), including Oct-1 (*15*), Oct-2 (*16*), SCIP (also known as Tst-1 or Oct-6) (*17*), and Pit-1 (*18*), can bind to the octamer motif, it was suspected that Oct-3 would belong to this family. The *Oct-3* gene was cloned by probing EC cell complementary DNA libraries with DNA fragments derived from the POU domain of Oct-2 (*5–7*). The *Oct-3* gene encodes two structural motifs characteristic of the POU domain transcription factors, a 75–amino acid POU-specific domain and a 60–amino acid POU homeodomain, both of which are required for high-affinity binding to the octamer motif (*3, 5, 7*).

Oct-3 can function as an octamer-specific transcription factor in transient transfection experiments (*5, 7, 8*). In addition, reporter constructs dependent on the presence of the octamer motif are transcribed efficiently in the Oct-3–expressing cells of the blastocyst (*4*). The transcriptional control elements of some genes that are active in undifferentiated EC cells contain an octamer motif or a related sequence and may be regulated by Oct-3 (*5*).

That *Oct-3* might be involved in early mammalian development was suggested by its expression in undifferentiated EC and ES cell lines (*2, 3, 5–7*). ES cell lines are derived directly from the inner cell mass (ICM) of the mouse blastocyst and are true totipotent stem cells; if they are re-introduced into a murine blastocyst, they can give rise to all somatic and germ cell lineages (*19*). EC and ES cell lines can be induced to differentiate in vitro with retinoic acid; during this process, *Oct-3* is down-regulated (*2, 3, 5–7*). In contrast, other homeobox genes are expressed at low or undetectable levels in undifferentiated EC and ES cells and are activated during differentiation (*1*).

Studies of EC and ES cell lines suggested a relationship between Oct-3 expression and a highly undifferentiated phenotype. This correlation was extended by analyzing Oct-3 messenger RNA (mRNA) expression during mouse development (*7, 8*) (Fig. 1). From the one-cell stage to the morula stage the embryonic cells are believed to be totipotent, and all cells appear to express Oct-3. At the early blastocyst stage, the totipotent cells that give rise to the embryo proper are located in the ICM and express high amounts of Oct-3. The ICM subsequently differentiates into two cell types: primitive ectoderm, which is pluripotent, and primitive endoderm, which is committed to differentiate into extraembryonic endoderm. Oct-3 expression remains high in the primitive ectoderm but decreases in the primitive endoderm. The trophectoderm of the early blastocyst initially expresses low levels of Oct-3 that become undetectable when these cells differentiate into extraembryonic tissues. After implantation during gastrulation, mesoderm is formed by differentiation from the pluripotent primitive ectoderm. Oct-3 is abundant in the primitive ectoderm, but its expression is down-regulated in the mesoderm. In 8.5-day-old embryos, low amounts of Oct-3 are detectable in ectodermal cells, but after this time Oct-3 is undetectable in any somatic cells. The decrease of Oct-3 expression during differentiation and loss of pluripotency in the embryo suggest that Oct-3 may be required to maintain a highly undifferentiated state.

An important clue to the function of Oct-3 comes from its expression in the germ-cell lineage. Primordial germ cells express Oct-3 throughout their migration from the allantois to the genital ridges (*7, 8*). In the adult, Oct-3 is found in both the ovary and the testis (*7, 8*). In the ovary, Oct-3 is confined to oocytes, and maturing oocytes express higher amounts of Oct-3 than resting oocytes do (*7*). The identity of the cells expressing Oct-3 in the testis has not yet been defined, although neither Sertoli cells nor mature spermatozoa have detectable Oct-3 (*3, 20*).

The expression of *Oct-3* in oocytes suggested that maternally derived Oct-3 might regulate early zygotic development. In many

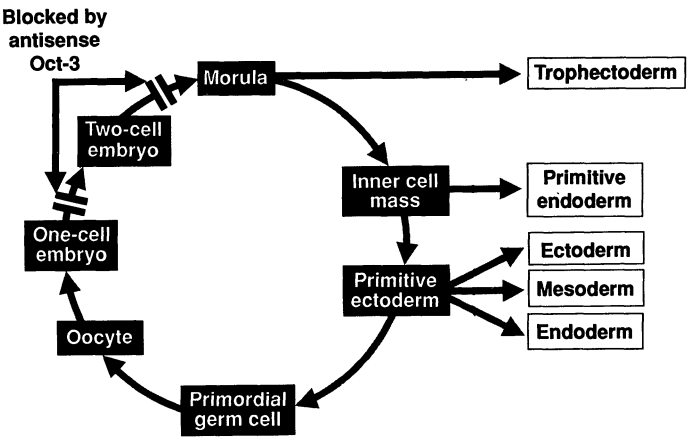

Fig. 1. Expression pattern of Oct-3 mRNA during mouse development. The black boxes at left indicate those stages that express Oct-3. The white boxes at right indicate those cell types that have little or no expression of Oct-3.

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organisms, maternally transcribed mRNA present in the oocyte is translated by the embryo before the onset of transcription from the embryonic genome (*21*). In *Drosophila*, such maternal effect genes are critical for the establishment of the anterior-posterior and dorsal-ventral axes (*22*). In the mouse, maternally derived mRNA is believed to be required for development to proceed to the two-cell stage, at which time the zygotic genome becomes transcriptionally active (*23*) .

The effect of maternally derived Oct-3 mRNA on mouse development was addressed by injecting antisense Oct-3 oligonucleotides into one-cell mouse embryos (*9*). The antisense oligonucleotides induce specific degradation of Oct-3 mRNA. The loss of Oct-3 mRNA resulted in an arrest in development at the one-cell stage, which could be reversed by co-injecting in vitro–synthesized Oct-3 mRNA. Oct-3 mRNA containing a frame-shift mutation was unable to rescue the antisense-induced arrest. Thus, Oct-3 protein is required to go through the first embryonic cell cycle. Injection of antisense Oct-3 oligonucleotide into one-cell embryos just before mitosis did not block the first cell division but did arrest development at the two-cell stage. This result suggests an additional requirement for Oct-3 during the second embryonic cell cycle.

The requirement for Oct-3 in one-cell embryos is surprising in view of studies suggesting that transcription of the mouse genome begins at the two-cell stage (*23*). Little, if any, transcription of the embryonic genome can be detected in one-cell embryos. Furthermore, treatment of one-cell mouse embryos with high concentrations of α-amanitin, which inhibits RNA polymerases II and III, does not block the first cell division but does arrest development at the two-cell stage. Thus, if the critical function of Oct-3 in one-cell embryos is as a transcription factor, then the assumption that embryonic transcription begins at the two-cell stage would have to be re-evaluated. Clearly, any Oct-3–mediated transcription in one-cell embryos would have to be at a low level and would apparently be mediated by a form of RNA polymerase that is insensitive to α-amanitin.

A provocative alternative hypothesis is that Oct-3 regulates DNA replication in one-cell embryos. This possibility was suggested by studies that showed regulation of adenovirus DNA replication by the octamer motif (*24–26*). In support of this hypothesis, injection of antisense Oct-3 oligonucleotide into one-cell embryos led to an 87% inhibition of DNA replication (*9*). The injection of DNA fragments containing the octamer motif into one-cell embryos also blocked DNA replication, raising the possibility that Oct-3 may control DNA replication by binding to octamer motifs in chromosomal DNA. In many cells, mitosis is coupled to the completion of DNA replication (*27*). Therefore, one scenario is that loss of Oct-3 leads to a primary inhibition of DNA replication that causes a secondary block in cell division.

Transcription factors have been shown to regulate DNA replication in several viral systems (*28*). In particular, both Oct-1 and Oct-2 can stimulate the replication of adenovirus DNA in vitro (*25, 26*) and the octamer motif in the simian virus 40 (SV40) enhancer has been implicated in SV40 viral DNA replication in vivo (*29*). The ability of transcription factors to stimulate viral replication is independent of transcription (*26, 28, 30*). Studies of viral replication have led to proposed mechanisms by which transcription factors can facilitate the interaction of the replication machinery with an origin of replication. Transcription factors can perturb nucleosomal assembly, thus making an origin more accessible (*31*). Alternatively, transcription factors may act directly by contacting components of the replication machinery and promoting their binding to the origin of replication (*32*).

Mammalian origins of replication are now being defined (*33*), but little is known about regulatory proteins involved in initiation of replication at these origins. The one-cell mouse embryo may provide a unique opportunity to study chromosomal DNA replication in the absence of transcription. Analysis of *Oct-3* mutants and their ability to stimulate replication in one-cell embryos may provide insight into the control of mammalian chromosomal DNA replication.

Many POU domain genes encode octamer-binding proteins, and these factors might regulate DNA replication as Oct-3 does. Because each octamer-binding protein has a unique pattern of expression and responsiveness to external stimuli, these factors might contribute to cell type–specific differences in the regulation of DNA replication. The octamer-binding protein encoded by the *SCIP* gene is expressed in proliferating Schwann cells but not in terminally differentiated, resting Schwann cells, thus raising the possibility that SCIP may regulate replication in this cell type (*34*). The dual roles of octamer-binding proteins as regulators of replication and transcription would provide a method to integrate changes in cellular proliferation with changes in gene expression.

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