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Identification and Characterization of Zygotic Genome Activation Gene in Preimplantation Stage Mouse Embryo

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SUMMARY

Preimplantation development encompasses the period from fertilization to implantation andis marked by a number of critical events, both from the gene expression and from the morphological point of view. Mouse preimplantation embryogenesis is marked by the waves of gene expression: the massive degradation of maternal RNAs, the zygotic genome activation (ZGA) at the 2 cell stage, and the mid-preimplantation gene activation (MGA) (Hamatani *et al.*, 2004). Global expression profiles of mouse preimplantation embryos by microarrays have been reported recently (Hamatani *et al.*, 2004; Wang *et al.*, 2004; Wang *et al.*, 2005; Zeng *et al.*, 2004), providing a great opportunity to search for genes that show specific expression patterns during preimplantation development. However, global gene expression profiling by DNA microarrays has recently revealed that nearly all genes identified for their increase of expression at the 1-cell stage were insensitive to inhibition by alpha-amanitin, which blocks RNA polymerase II (Hamatani *et al.*, 2004a; Zeng and Schultz, 2005).

Therefore, de novo transcription of zygotic genome seems to begin during the 2-cell stage of mouse development (Hamatani *et al.*, 2004; Zeng and Schultz, 2005). Furthermore, the major burst of ZGA occurs only in the late 2-cell stage (Hamatani *et al.*, 2004). Utilizing DNA microarray data, we looked for genes that are expressed only during ZGA and found Zscan4, whose expression is restricted to late 2-cell stage embryos. Sequence analysis of genomic DNA and cDNA clones revealed nine paralogous genes tightly clustered in 0.85 Mb on mouse Chromosome 7. Three genes are not transcribed and are thus considered pseudogenes. Among the six expressed genes named Zscan4a-Zscan4f, three -- Zscan4c, Zscan4d, and Zscan4f -- encode full-length ORFs with 506 amino acids (Falco *et al.*, 2007). Zscan4d is a predominant transcript at the late 2-cell stage, whereas Zscan4c is a predominant transcript in embryonic stem (ES) cells. No transcripts of any Zscan4 genes are detected in any other cell types.

Reduction of Zscan4 transcript levels by siRNAs delays the progression from the 2-cell to the 4-cell stage and produces blastocysts that fail to implant or proliferate in blastocyst outgrowth culture.

And we sought MGA genes, which should be expressed uniquely in the later stages of preimplantation development. Here we studiedthe identification and characterization of Trim43, whose expression is restricted to preimplantation stages and peaks at the 8-cell to morula stage. Sequence analysis of genomic DNA and cDNA clones revealed the presence of three gene copies, named Trim43a, Trim43b, and Trim43c, on mouse Chromosome 9. We also identified a 5 kb DNA fragment that covers upstream region of Trim43 as a putative promoter, which can drive the expression of mStrawberry fluorescent protein in a manner similar to endogenous Trim43 gene. In conclusion, Zscan4 thus seems to be essential for preimplantation development, and Trim43 will be a useful stage-specific marker for preimplantation embryos.

REFERENCES

Hamatani T, Carter MG, Sharov AA *et al.* 2004 Dev Cell 6, 117-31.

Wang QT, Piotrowska K, Ciemerych MA *et al.* 2004 Dev Cell 6, 133-44.

Wang S, Cowan CA, Chipperfield H *et al.* 2005 Reprod Biomed Online 10, 607-16.

Zeng F, Baldwin DA, Schultz RM 2004 Dev Biol 272, 483-96.

Zeng F, Schultz RM 2005 Dev Biol 283, 40-57.

Falco G, Lee SL, Stanghellini I, Bassey UC, Hamatani T and Ko MS. 2007 Dev Biol 307, 539-50.