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Application of DNA array technology to mammalian embryos

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Abstract

Early embryogenesis depends on a tightly choreographed succession of gene expression patterns which define normal development. Fertilization and the first zygotic cleavage involve major changes to paternal and maternal chromatin and translation of maternal RNAs which have been sequestered in the oocyte during oogenesis. At a critical species-specific point known as the major onset of embryonic expression, there is a dramatic increase in expression from the new diploid genome. The advent of array technology has, for the first time, made possible to determine the transcriptional profile of all ~20,000 mammalian genes during embryogenesis, although the small amount of mRNA in a single embryo necessitates either pooling large numbers of embryos or a global amplification procedure to give sufficient labeled RNA for analysis. Following array hybridization, various bioinformatic tools must be employed to determine the expression level for each gene, often based on multiple oligonucleotide probes and complex background estimation protocols. The grouped analysis of clusters of genes which represent specific biological pathways provides the key to understanding embryonic development, embryonic stem cell proliferation and the reprogramming of gene expression after somatic cloning. Arrays are being developed to address specific biological questions related to embryonic development including DNA methylation and microRNA expression. Array technology in its various facets is an important diagnostic tool for the early detection of developmental aberrations; for improving the safety of assisted reproduction technologies for man; and for improving the efficiency of producing cloned and/or transgenic farm animals. This review discusses current approaches and limitations of DNA microarray technology with emphasis on bovine embryos.

Keywords: Preimplantation embryo; Transcriptome; RNA amplification; DNA microarray; Bioinformatics; Plasticity of gene expression; Maternal and embryonic gene expression

1. Introduction

Sequencing and annotation of the human genome resulted in a downward estimation of the total number of protein-coding genes to approximately 20,000 [1]. This is much lower than previous estimates which ranged to as many as 150,000 genes and is similar to estimates for other vertebrates' species including mouse, chicken and pufferfish [2–6], or bovine and dog (http://www.hgse.bcm.tmc.edu/projects/bovine [7,8]). It is clear that complex organisms exploit a variety of regulatory mechanisms to extend the functionality of their genomes [9], including differential promoter activation, alternative RNA splicing, RNA modification, RNA editing, localization, translation and stability of RNA, expression of non-coding RNA, antisense RNA and microRNAs [10–16]. It is important to note that most of these mechanisms work together at the RNA level to produce the functional transcriptome of an organism. Tissue-specific transcriptomic profiles indicate important features of the regulatory process. During the past decade, efficient high-throughput methods have been developed for whole genome sequencing, transcriptome

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and proteome analysis [17–19]. These technologies are crucial to improve the efficiency and safety of assisted reproduction techniques, to facilitate the development of novel therapies based on regenerative medicine and to insure the quality of biotechnological products at the molecular level.

The application of array technology to the analysis of preimplantation mammalian embryos poses-specific challenges associated with the picogram levels of mRNA in a single embryo, the plasticity of the embryonic transcriptome and the difficulties in obtaining in vivo developing embryos in some species. Gene expression patterns in mammalian embryos are more complex than those in most somatic cells due to the dramatic restructuring of the paternal chromatin, the major onset of embryonic transcription, events related to the cellular differentiation at the early morula stage, blastocyst formation, expansion and hatching and finally implantation. Embryo development is first dependent on maternally stored transcripts which are gradually depleted until the embryo produces its own transcripts after a switch to the embryonic expression program. The gene expression patterns and RNA stability in oocytes and early embryos prior to the activation of embryonic expression are dramatically different from what is seen after major onset of embryonic transcription. The onset of embryonic gene transcription occurs at a species-specific time point; in mice, the major activation starts in late 1-cell embryos, in pigs it occurs at 4-cell stage, in men at the 4- to 8-cell stage and in bovine embryos it is delayed until the 8- to 16-cell stage [20] (Fig. 1). Due to the late onset of this maternal-to-embryonic transition in bovine embryos, they provide an excellent model in which to study early reprogramming events in detail. Major epigenetic reorganization also occurs during preimplantation development. This includes DNA demethylation and methylation as well as targeted modifications of histone methylation and acetylation all of which play a role in controlling chromatin remodeling and X chromosome inactivation [21].

Another challenge for applying DNA arrays to preimplantation embryos is the high degree of expression plasticity seen in early stage embryos. Preimplantation stages of several species can be cultured in vitro. However, this is frequently associated with changes in chromatin configuration and gene expression [22–24]. In vitro production of bovine embryos is usually

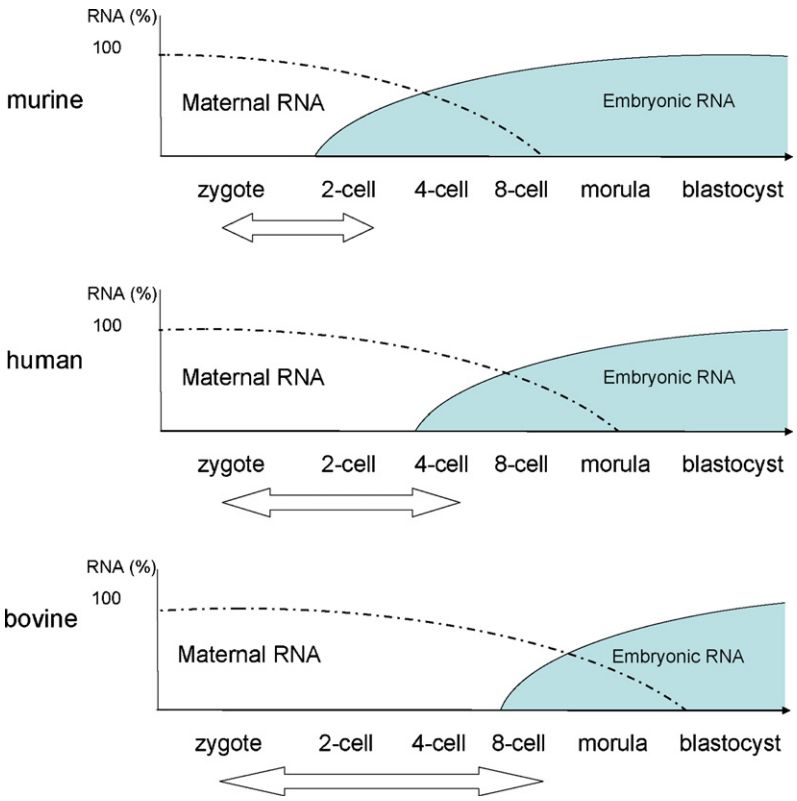

Fig. 1. Early reprogramming steps of maternal and embryonic transcriptomes are extended in bovine embryos (bottom) compared to mouse (upper) and human embryos (middle panel).