Transcriptional, post-transcriptional and epigenetic control of porcine oocyte maturation and embryogenesis

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Embryogenesis is a complex process that is controlled at various levels. As new discoveries are made about molecular mechanisms that control development in other species, it is apparent that these same mechanisms regulate pig embryogenesis as well. Methylation of DNA and modification of histones regulate transcription, and mechanisms such as ubiquitinization, autophagy and microRNAs regulate development post-transcriptionally. Each of these systems of regulation is highly dynamic in the early embryo. A better understanding of each of these levels of regulation can provide tools to potentially improve the reproductive process in pigs, to improve methods of creating pig embryos and cloned embryos *in vitro*, and to provide markers for predicting developmental competence of the embryo.

Introduction

Development of the embryo and the relative likelihood that it will give rise to a viable offspring is dependent upon factors that include, but are not limited to, the general health status of the sire and dam during gametogenesis and their genetic contributions. These factors set the stage for the oocyte to mature with the proper stores of information to support complete development. Factors that regulate development will be presented with a focus on transcriptional, post-transcriptional and epigenetic regulation of development in the pig. Finally, examples of how this information can be used to our advantage will be presented.

Overview of embryogenesis

An oocyte competent to develop to term is the result of adequate growth of the oocyte, and subsequent nuclear and cytoplasmic maturation. During this time the information necessary for early development is transcribed and stored in the form of RNA, or translated and stored in the form of protein. Upon germinal vesicle breakdown (GVBD) transcription ceases and nuclear maturation progresses until arrest at metaphase II of meiosis. Fusion of the sperm and oocyte plasma membrane results in the deposition of the sperm chromatin in the ooplasm, and resumption and completion of meiosis of the maternal chromatin. The maternally- and paternally-derived chromatin each forms a separate pronucleus that migrates to the center of the oocyte. At first mitosis the pronuclear envelopes breakdown and for the first time the maternally- and paternally-derived chromatin mix as the chromosomes align on the metaphase plate and subsequently segregate to the two poles prior to cytokinesis. In the pig, after the first mitosis the embryo immediately begins DNA synthesis without a G1 phase. After DNA

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synthesis is completed (about 12 hours (Prather *et al.* 1996)) the embryo enters directly into mitosis without an intervening G2 phase. Entry into the 4-cell stage is the first time that a G1 phase is inserted and corresponds with the onset of transcription (Schoenbeck *et al.* 1992). The length of the 4-cell stage is over twice that of the 2-cell stage; the difference being the result of a short G1 phase and a long G2 phase (Anderson *et al.* 2001). When the chromatin is not encumbered with DNA polymerases, i.e. G1 and G2, the transcriptional machinery can gain access to the chromatin and RNA can be synthesized (Prather 1993).

Since there is little or no transcription between GVBD and the 4-cell stage, it is imperative that the germinal vesicle stage oocyte (GV) be poised to control development for the first few divisions. Thus, the information stored in the oocyte must be sufficient to direct the first few cellular divisions and establish the correct chromatin configuration so that the symphony of gene expression can begin. If the chromatin configuration is not correct, the embryo may develop to varying degrees, but it may not have normal transcription (Tian *et al.* 2009) or it may not thrive and reproduce - as observed with some cloned animals (Carter *et al.* 2002). Thus it is imperative that the correct developmental pattern of gene expression be established during the first few cleavage divisions.

Upon successful activation of the embryonic genome (ZGA) the pig embryo continues to divide to the 8-, 16- and subsequently the 32-cell stage. Compaction of the blastomeres occurs during this time and the embryo continues the differentiation process by forming a blastocyst with two distinct cell types, each with its own transcriptional repertoire. Dramatic changes in mRNA abundance occur between the oocyte and 4-cell stage and blastocyst stage and these changes are driven by both transcriptional and post-transcriptional regulation (Whitworth *et al.* 2005).

Transcriptional regulation

Mammalian oogenesis, oocyte maturation and early embryo development are distinct processes, each carefully coordinated, and bound by a common thread: the need to supply an appropriately programmed genome that permits proper gene expression in time and space. In addition to transcription factor binding to promoters, regulation of transcription early in development is achieved via epigenetic mechanisms. The term epigenetics, as used and accepted today, is defined as "heritable changes in gene function that cannot be explained by changes in DNA sequence" (Russo *et al.* 1996). Epigenetic mechanisms in the oocyte and early embryo include DNA methylation, histone modification, chromatin remodeling, and non-coding RNAs (ncRNA) (Li 2002; Morgan *et al.* 2005). Space limitations prevent a thorough treatment of each of these topics. Rather a brief summary of the status of our collective understanding of how these mechanisms are/could be involved in controlling transcription in the mammalian oocyte and early embryo will be provided. Most of the information will focus on porcine embryo development.

Methylation of the cytosine of CpG dinucleotides plays a central role in transcriptional regulation in mammals. In most situations, methylation of DNA in and near genes is associated with repression of transcriptional activity of those genes (Iager *et al.* 2008). DNA methyltransferases are responsible for establishing (DNMT3a and DNMT3b) and maintaining (DNMT1) methylation patterns within DNA (Li 2002). Interestingly, mRNA levels for DMNT1 are 20-fold higher in the pig GV oocyte to 4-cell stage as compared to the blastocyst stage, whereas DMNT3b levels are not different (Whitworth *et al.* 2005). Four general mechanisms have been proposed for the methylation-mediated silencing of gene expression: 1) direct inhibition of transcription factor binding of methylated gene promoter regions; 2) recruitment of co-repressors to methylated regions by DNA methyl-binding proteins; 3) DNA methyltransferase-mediated chromatin

remodeling; and 4) dampened efficiency of transcriptional elongation in methylation-rich gene bodies. A detailed summary of these mechanisms has been reviewed (Klose & Bird. 2006).

It has become apparent in recent years that, in addition to DNA methylation, histone modification plays an important role in controlling gene expression in both gametes (DeJong 2006) and early embryos (Nowak-Imialek *et al.* 2008; Ooga *et al.* 2008; Thomas *et al.* 2008). Nuclear DNA is packaged in the cell as chromatin – octamers of predictably-organized histone proteins wrapped by 146 bp of DNA. The N-termini of the histone proteins are highly susceptible to post-translational modifications, including (but not limited to) lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, sumoylation, ubiquitination and ADP-ribosylation (Kouzarides 2007). The effects of histone modifications on gene expression may not be predictable enough to consider these patterns of modifications as a "code" in the truest sense, as once proposed (Strahl & Allis 2000). Rather it seems that genomic and physiologic context play a significant role in determining the effect that individual modifications might have on chromatin function. Some generalizations can be made: histone acetylation is almost universally associated with increased transcription; methylation of lysine residues can either activate (H3K4me, H3K36me, H3K79me) or repress (H3K9, H3K27, H4K20) transcription; while histone ubiquitination and sumoylation are generally considered to be repressive to transcription. Control over histone modifications is exquisite, with an impressive array of enzymes dedicated to establishing, maintaining and/or removing histone marks (Kouzarides 2007).

In addition to these primary marks, there are a number of facilitator processes and molecules that are involved in the establishment, maintenance, and transmission of epigenetically silenced (or activated) genes. These include polycomb group (PcG) proteins, trithorax group (TrxG) proteins, chromatin remodeling complexes, and ncRNAs. Polycomb group and TrxG proteins function essentially antagonistically to repress or maintain activity of genes that are important during development. Some members of the PcG and TrxG protein families have histone methyltransferase activities, with PcG proteins methylating K27 of H3 – a generally repressive mark – and TrxG proteins catalyzing H3K4 methylation, which is permissive to transcription (Kiefer 2007). Chromatin remodeling complexes (de la Serna *et al.* 2006) such as the ISWI and various SWI/SNF complexes interact with and modulate the activity of histone arginine methyltransferases (Pal & Sif. 2007) and acetyltransferases (Brockmann *et al.* 2001) which are generally associated with increased transcription. These complexes are necessary for transcriptional regulation that occurs after fertilization (de la Serna *et al.* 2006). SMARCA4, an SNF2 chromatin remodeling ATPase, is an important determinant of early porcine embryogenesis in both *in vitro*-fertilized and somatic cell nuclear transfer (SCNT) embryos (Magnani *et al.* 2008). Finally ncRNAs result in gene silencing in a variety of scenarios important to development, such as X-chromosome inactivation (Boumil & Lee. 2001) and gene imprinting (Nagano *et al.* 2008; Pandey *et al.* 2008), in addition to their role in RNA-induced gene silencing (RNAi).

Almost by definition, cellular differentiation is an epigenetic phenomenon: phenotypic and functional differences between cell types arise without reordering the sequence of the DNA. Thus for each distinct cell population, there is an associated cadre of epigenetic modifications that determine which genes are turned on/off thus giving each cell population its phenotype. As somatic cells differentiate into germ cells, somatic-specific epigenetic marks are erased and at the end of gametogenesis there is a paternal-specific pattern of epigenetic modifications established in each sperm, and a maternal-specific pattern established in each oocyte. Upon fertilization, the genome of the embryo is demethylated and otherwise reprogrammed to replace the extensive germ cell-specific epigenetic marks with those conducive to pluripotency. De novo reestablishment of epigenetic marks in appropriate cell lineage-specific patterns occurs

in the inner cell mass (ICM) and to a lesser extent in the trophectoderm of the blastocyst (Dean *et al.* 2001; Santos et *al.* 2002). A global survey of DNA methylation during early development in the pig showed that 18% of the randomly selected genomic regions changed from the gametes to the blastocyst stage (Bonk *et al.* 2007a). Some DNA methylation in early embryos is sensitive to the culture conditions, i.e. cultured embryos can have a different pattern of DNA methylation compared to *in vivo*-collected embryos (Mann *et al.* 2003; Mann et *al.* 2004).

Expression of imprinted genes– genes that are expressed in a parent-of-origin, monoallelic pattern - show how epigenetics can impact development. Many imprinted genes regulate fetal and placental growth, development, and function. Establishment of imprinting appears to be fairly conserved across species, and one such imprinted region is the well-characterized IGF2/H19 locus (Sasaki *et al.* 2000; Engel *et al.* 2004), which in most mammalian species – including pig (Han *et al.* 2008) – is heavily methylated on the paternal allele, and relatively unmethylated on the maternal allele. In the mouse the unmethylated maternal allele can bind to an insulator (CTCF) which prevents the IGF2 promoter from interacting with an upstream enhancer, thus preventing maternal allele expression of IGF2. An inactive IGF2 allele is conducive to H19 expression and is therefore expressed highly from the maternal chromosomes. Heavy methylation on the paternal allele is repressive to H19 expression, while conducive to IGF2 expression because the CTCF insulator does not bind to methylated DNA. Another imprinted locus is IGF2R/KCNQ1, which is silenced at the paternal allele by the interaction of ncRNAs with histone methyltransferases and members of the PcG complex (Nagano *et al.* 2008; Pandey *et al.* 2008). While the sperm and oocyte chromatin are hypermethylated relative to the early embryo, the demethylation that occurs after fertilization does not result in a loss of the imprint (Nakamura *et al.* 2006; Santos *et al.* 2002). The mechanisms by which these epigenetic 'memories' are maintained are poorly understood, but additional examples of how epigenetic mechanisms impact development are abundant (Hansen *et al.* 2008). For example, increased methylation of arginine residues on histone H3 predisposes blastomeres of the early embryo to contribute to the pluripotent cells of the ICM, whereas lower levels of H3 arginine methylation direct cells to a mural trophectoderm state (Torres-Padilla *et al.* 2007). Histone methyltransferase EHMT2 is a 'master regulator' of early embryonic genes: 126 genes (including POU5F1) expressed in undifferentiated mouse embryonic stem cells were converted to heterochromatin and silenced by EHMT2 upon differentiation (Epsztejn-Litman *et al.* 2008). A common mark on these gene promoters was trimethylation on lysine 9 of histone H3. Interestingly, the promoters of these 126 silenced genes exhibited de novo DNA methylation in addition to the H3K9 trimethylation. This case also serves to exemplify the functional interplay that exists between these supposed 'separate' mechanisms of epigenetic gene control, in that EHMT2 – a *histone* methyltransferase – also appears to be able to direct de novo DNA methylation by autonomously recruiting DNA methyltransferases to the silenced loci.

Much of what we know about epigenetic control of gene activity in germ cells and early embryo development comes from studies investigating the nuclear reprogramming associated with SCNT. The inefficiency of SCNT has been correlated with aberrant patterns of DNA methylation, histone modification and, consequentially, gene expression (Santos et *al.* 2003; Wrenzycki *et al.* 2006; Bonk *et al.* 2007b). Upon transfer of a somatic nucleus into oocyte cytoplasm an epigenetic reprogramming event must take place to revert the somatic chromatin back to a pluripotent-like state. This is an inefficient process, with the transferred nucleus often retaining a proportion of the somatic epigenetic marks, resulting in mis-regulated gene expression, which in turn can cause abnormalities in the embryo or fetus (Niemann *et al.* 2002). The most severe of these abnormalities result in failed development very early. However, less severe epigenetic aberrations can allow development to proceed, but often the resulting offspring have obvious

deformities and/or are compromised physiologically after birth. The term Large Offspring Syndrome (LOS) was coined as a result of the large birth weights of cloned calves, although the characterization of this syndrome as dealing exclusively with "Large Offspring" is inappropriate since cloned offspring in some species (e.g. pigs) tend to have lower birth weights relative to their naturally-conceived counterparts (Estrada *et al.* 2007); additional manifestations of LOS include hydroallantois, hydrops fetalis, hyperplasia in various organ systems, compromised immune function, respiratory distress and other skeletal and soft tissue deformations (Carter *et al.* 2002; Wrenzycki & Niemann 2003; Carroll *et al.* 2005; Wrenzycki *et al.* 2006). Of interest is the association of similar developmental defects with offspring arising from other less invasive assisted reproductive technologies such as *in vitro*-oocyte maturation, -fertilization, -culture, and intracytoplasmic sperm injection (Farin *et al.* 2006; Fernandez-Gonzalez *et al.* 2007; Lawrence & Moley. 2008). While the etiology of these disturbances is not known it is clear that epigenetics play a role. In one recent study, bisulfite sequencing at distinct genomic loci revealed significant differences in levels of DNA methylation in comparisons made between *in vivo*-produced and parthenogenetic blastocysts (5/12 loci were differentially methylated), and between *in vitro* fertilized blastocysts (6/8 loci) and SCNT blastocysts (4/12 loci) (Bonk *et al.* 2007a). Success at increasing 'normal' developmental patterns of cloned embryos is achievable by treating donor cells and/or reconstructed embryos with inhibitors of DNA methylation and histone acetylation (Ding *et al.* 2008; Iager *et al.* 2008). It has yet to be demonstrated whether such treatments can restore 'normal' transcription profiles in cloned embryos, or reduce the occurrence or severity of LOS.

The study of the epigenetic control of embryonic and germ cell transcription is a nascent field of research. What has emerged is a picture of enormous complexity as there is significant functional overlap and cooperation between marking mechanisms. The practical application of this knowledge is now beginning to be developed. This will be an area of intense interest into the foreseeable future, with continued emphasis being placed on the epigenetic control of development, and especially pluripotency.

Post-transcriptional regulation

In addition to proper chromatin configuration, many maternally-derived RNAs and proteins must be degraded by microRNAs or the binding of regulatory proteins to the 3' untranslated region of the message (Schier 2007; Stitzel & Seydoux 2007). Degradation of the maternal proteins begins immediately after fertilization and is mediated partially via ubiquitin-proteasome mediated processes (Huo *et al.* 2004) and macroautophagy. There are at least two periods of degradation; the first is at the time of fertilization, and the second is at the time of ZGA. In drosophila the main degradation of transcripts and proteins occurs at ZGA and a third of the genes whose maternal transcripts are degraded also begin transcription at the same time (De Renzis *et al.* 2007).

Macroautophagy results from the sequestration of proteins into an autophagosome that fuses with a lysosome where the proteins are degraded (Mizushima *et al.* 2008). This system, mediated by autophagy-related 5 protein (ATG5), is up-regulated after fertilization or parthenogenetic activation in the mouse, and a deficiency in this system results in embryos that die by the 4- to 8-cell stage (Tsukamoto *et al.* 2008). In the pig, ATG5 (aka APG5L) decreases ($p = 0.080$) from a ratio of 10.2 in the GV to 3.5 at the 4-cell stage, and then decreases further to 1.3 by the blastocyst stage (Whitworth *et al.* 2005). High levels of mRNA for ATG5 show that a macroautophagy system may also function in the pig.

MicroRNA regulation of oocyte maturation and embryonic development

Specificity in cellular phenotype, as affected by differentiation and cellular lineage, is the result of a particular combination mRNA transcript abundance and controlled translation of those mRNAs to produce proteins capable of eliciting biological function. Transcription and translation are both critical for specificity in cellular phenotype and miRNAs have been shown to be potent regulators of transcript abundance and protein translation, particularly during cell lineage progression and differentiation (Neilson *et al.* 2007).

What are miRNAs?

MicroRNAs are ncRNA that are processed into a functional size of 18-24 nucleotides (Bartel 2004). The mature sequence confers significant biological impact on the cells in which they are synthesized and processed through perfect or imperfect pairing to the 3'UTR of a target mRNA. The binding of a miRNA and its target mRNA 3'UTR results in posttranscriptional gene silencing (PTGS) through the action of several mechanisms, including translation inhibition, target transcript degradation and in some cases, chromatin silencing via methylation (Jackson & Standart 2007). Since the discovery of the first miRNA (Lee *et al.* 1993; Wightman *et al.* 1993) significant advancements have been made into the understanding of miRNA prevalence, biogenesis and function in the Plantae and Animalia kingdoms, largely due to the overlapping pathways and mechanisms involved with RNA interference (RNAi). Current estimates predict that 2-3% of human genes represent miRNAs which are collectively capable of conferring PTGS on an estimated 30% of the human genome (Rajewsky 2006).

MicroRNA biogenesis

Similar to mRNA transcription, microRNA expression is RNA polymerase II dependent (Bartel 2004). The capped, polyadenylated RNA transcripts containing the primary miRNA, have been coined 'pri-miRNA' (Lee *et al.* 2002). All pri-miRNAs have the spatial sequence complementation necessary to form secondary hairpin structures that are recognized by nuclear RNASEN (aka Drosha RNase III endonuclease) (Lee *et al.* 2003). RNASEN cleavage, which requires the functional cooperation of DGCR8 (Han *et al.* 2004), results in the nuclear release of ~60-70 nt stem loops, referred to as pre-miRNA (Lee *et al.* 2002; Zeng & Cullen 2003). Following RNASEN/DGCR8-mediated cleavage, pre-miRNAs are exported from the nucleus (Lund *et al.* 2004) into the cellular cytoplasm, DICER1, also an RNase III endonuclease, typically cleaves both strands resulting in 18-24 nt dsRNA molecules possessing 3' overhangs. These molecules are referred to as mature microRNAs (Grishok *et al.* 2001; Hutvagner *et al.* 2001; Ketting *et al.* 2001). Argonaute proteins (EIF2C1) then interact with mature miRNAs as the foundational component to the formation of a RNA-induced silencing complex (RISC) capable of conferring PTGS (Liu *et al.* 2008).

MicroRNA mechanism of action

The mechanism of action of microRNAs was demonstrated when the abundance of lin-4, having sequence complementary to *lin*-14 and *lin*-28, was shown to be involved with C. elegans larval stage transition from L1 to late L2/early L3 (Lee *et al.* 1993; Wightman *et al.* 1993). During this developmental transition both LIN-14 and LIN-28 abundance was reduced 90 percent while mRNA abundance of lin-14 was unchanged compared to only a 50% reduction of *lin-28*

mRNA (Seggerson *et al.* 2002). Both transcriptional degradation and translational repression are widely involved in miRNA function. In addition, targeted deadenylation, which accelerates mRNA decay, also occurs (Zhang *et al.* 2007). MicroRNA target recognition occurs primarily through the binding of residues on the 5′ end of the miRNA to complementary sequence in the 3′UTR of mRNA target for PTGS (Brennecke *et al.* 2005).

Biological importance of miRNAs

The biological importance of miRNA function is the suggested role of conferring robustness to a given cell's mRNA and protein profile (Stark *et al.* 2005) and is accomplished by providing alternative molecular networks that can contribute to the mRNA abundance and protein production. With respect to embryogenesis, miRNA functions are strategically involved with cell fate and are required for cell lineage destinations. This is evidenced by the tissue specificity of many miRNAs and is further supported by the fact that genes associated with tissue specific Gene Ontology (GO) categories have 3′UTRs enriched for miRNA binding sites whereas genes associated with non-tissue specific GO categories lack significant miRNA complementation sites in the 3′UTR (Beuvink *et al.* 2007; Kawahara *et al.* 2007; Wang *et al.* 2007; Zhao *et al.* 2007).

Importance of miRNA function for oocyte and early embryonic development

Successful embryonic development in mammals requires broad translational arrest and mRNA clearance to deplete maternally derived mRNAs and proteins in coordination with ZGA and protein production; as shown in the pig (Schoenbeck *et al.* 1992). Maternal depletion of mRNA is in part controlled via the 3′UTR of the expressed transcripts (Brevini *et al.* 2007). So it is not surprising that differential expression of microRNAs is temporally associated with oocyte maturation and early embryonic development in a variety of species (Biemar *et al.* 2005; Watanabe *et al.* 2005; Giraldez *et al.* 2006; Tang *et al.* 2007).

The 3′UTR repertoire of the mRNAs present in porcine oocytes suggests a potential role during oocyte maturation and embryonic development. In addition the presence of DICER1 mRNA in GV oocytes at 2.3 times the abundance after ZGA suggests biological importance (Whitworth *et al.* 2005). Biological importance of DICER1 is demonstrated by the ability of long double-stranded RNAs injected into porcine zygotes to induce knockdown of corresponding target mRNA (Cabot & Prather. 2003). The required role of DICER1 function for successful development (Tang *et al.* 2007) suggests that specific mature miRNAs are responsible for PTGS necessary for embryo survival. While the average miRNA is estimated to have recognition sites for approximately 100 target mRNAs, specific individual miRNAs have the predicted ability to confer PTGS on a group of genes varying from only a few to more than 800 (Rajewsky 2006). Thus the alteration of only a few miRNA during embryonic development may contribute to large changes in transcript abundance during developmental progression or between embryo production methods (Whitworth *et al.* 2005). The loss of developmental capacity in mouse oocytes lacking functional DICER1 during oogenesis is associated with disorganized spindles, lack of chromosome alignment, as well as transcriptome and proteome alterations (Tang *et al.* 2007) . In addition to DICER1 function being required for oocyte maturation and fertility, the maternal expression of EIF2C1, an RNA binding protein essential to the formation of RNA-induced silencing complexes, is required for early embryonic development in the mouse (Lykke-Andersen *et al.* 2008). Loss of function of EIF2C1 results in the stabilization of specific maternal mRNAs and arrested development during ZGA. EIF2C1 mRNA levels in the pig GV oocyte are 10- to 13-fold higher than in a reference sample (Whitworth *et al.* 2005)

The utility of understanding transcript population changes during embryo development

At least three major benefits are derived from understanding alterations in transcript abundance in the early embryo. The first is the possibility that such information can be used to improve reproduction by explaining the ~30% loss of potential conceptuses that occurs during the first month of development. Toward that end, we have been engaged in EST projects to identify genes in reproductive tissues and early embryos of pigs (Jiang *et al.* 2001; Jiang *et al.* 2004; Whitworth *et al.* 2004). Through these efforts many tissue-specific and novel (previously uncharacterized) genes were identified. Follow-up transcriptional profiling experiments with cDNA microarrays confirmed the involvement of many of these genes in embryo development, as well as providing a better understanding of the genetic pathways that are involved in embryo development (Whitworth *et al.* 2005). Secondly, such information can provide clues to improve assisted reproductive technologies, such as altering culture conditions to improve fertilization parameters (Hao *et al.* 2006). A third benefit is the opportunity to identify gene products that are predicted to be secreted into the culture medium. If such products happen to be correlated with developmental competence, they provide a potential means to non-invasively screen *in vitro* produced embryos to better define their quality.

Identification of transcribed genes that encode secreted molecules that are higher in *in vivo* produced embryos compared to *in vitro* produced embryos (Whitworth *et al.* 2005) may permit identification of molecule(s) that could be correlated with developmental completence. Several abundant and/or differentially transcribed genes were identified that are candidate markers because many:

- possess enzymatic activity that can be detected (e.g. PAGs, cathepsin D and ADAMs),
- possess enzyme *inhibitory* activity (e.g. Bikunin) that can be quantified,
- possess a defined biological activity that can be measured (e.g. IFN-gamma),
- exhibit relatively specific binding characteristics that might be exploited (e.g. Galectin 1 and IGFBP7), and
- are known to be bound by antibodies (e.g. CD9 antigen, Relaxin, Laminin receptor) that could be used to measure marker release.

Our group is currently working to determine if the data generated from transcriptional profiling can be leveraged to identify markers, such as those above, that can serve as way to identify embryos with low or high developmental potential (e.g. Telugu *et al.* 2009).

Conclusion

Control of development through the cycle of embryonic cells to somatic and germ cells is incredibly complex. Early thought was focused on transcriptional control of development. Now we have learned that transcriptional control is just one component. The factors that regulate transcription are very complex (histone modifications, DNA methylation) and post-transcriptional controls (miRNAs) appear to be just as important as transcriptional regulation alone. Nevertheless, a more complete understanding of the global control of differentiation will provide tools to improve the efficiency of *in vivo* processes. These efforts will also lead to improvements in systems of embryo production and will likely lead to the identification of factors that can be used to predict the developmental competence of embryos and gametes.

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