Zygote **8** (February), pp 87–96. © 2000 Cambridge University Press Printed in the United Kingdom

Zygotic and embryonic gene expression in cow: a review of timing and mechanisms of early gene expression as compared with other species

Erdogan Memili and Neal L. First
Endocrinology and Reproductive Physiology Program, Department of Animal Sciences, University of Wisconsin-Madison, Madison, Wisconsin, USA

Date submitted: 22.8.99. Date accepted: 6.11.99

Summary

Early embryonic development is largely dependent on maternal RNAs and proteins synthesised during oogenesis. Zygotic transcription is an essential event that occurs at a species-specific time after fertilisation. In the absence of zygotic transcription the embryo dies since it can no longer support requirements for successful embryo development. Molecular genetics of gene expression during early embryogenesis, especially in the bovine species, remain one of the unsolved questions in modern biology. Earlier studies suggested that embryonic transcription in cattle begins at the late 4-cell or 8-cell stage. However, more recent studies suggest that bovine zygotes and 2-cell embryos are both transcriptionally and translationally active. Moreover, changes in chromatin structure due to acetylation of core histones and DNA replication play important roles in the regulation of zygotic/embryonic gene expression. This review will summarise results of recent studies about the timing and mechanisms of zygotic/embryonic gene expression in cattle. In addition, terminology in the literature regarding gene expression during early embryogenesis will be clarified. These terminologies include: 'zygotic/embryonic gene expression', 'maternal to embryonic transition in control of development (MET)' and 'zygotic/embryonic genome activation (ZEGA)'.

Key words: Bovine, Embryo, Gene expression, mRNA, Transcription

Introduction

Early embryonic development in many species is supported by maternal RNAs and proteins synthesised during oogenesis. Depending on the species, zygotic/embryonic transcription starts at a specific time after fertilisation. Activation of zygotic/embryonic genes is accompanied by a gradual degradation of maternal mRNAs and proteins and reprogramming of gene expression. Zygotic/embryonic gene expression is important not only in its own right but also for a dramatic reprogramming of gene expression that sets the stage for later developmental events. Regulation of the zygotic/embryonic gene expression has been studied mainly in *Xenopus*, *Drosophilia* and mouse. Studies of gene expression during bovine embryogenesis have been elusive. Since studies in only one species would be inadequate to provide a model for the control of gene expression during early development in mammals, zygotic/embryonic gene activation in cattle needs to be elucidated. Detailed analyses of gene expression during bovine embryogenesis will be valuable for understanding basic cellular and molecular mechanisms of control of gene expression, development of better embryo culture systems and better strategies for transgenic and cloning studies. Topics reviewed in this paper include the timing of zygotic/embryonic gene expression in mammalian species and proposed mechanisms underlying zygotic/embryonic expression in *Xenopus*, *Drosophila* and mouse embryos with emphasis on specific elements involved in mRNA transcription. Timing of RNA polymerase I dependent transcription (ribosomal RNA synthesis) is reviewed as well.

In this paper we review the timing and mechanism(s) of gene expression as transcription of mRNA during zygotic/embryonic stages of development. Throughout the literature there have been specific

All correspondence to E. Memili. Current address: Harvard Medical School, Harvard Institute of Human Genetics, Room 441, 4 Blackfan Circle, Boston, MA 02115, USA.

terms used to define the developmental transition. The terms used are transition from maternal to embryonic control of development (MET) and zygotic/embryonic genome activation (Z/EGA). Midblastula transition (MBT) has been used to explain the same event in *Drosophila*. These terms have been used to explain zygotic or embryonic transcription as one or two steps of major onset of gene activation. However, evidence reviewed in this paper shows that embryos can survive in the absence of zygotic or embryonic transcription (when transcription is inhibited with α-amanitin) until a certain cell stage. In other words, maternal messages and proteins are able to support cell cleavage without zygotic or embryonic transcription until a certain embryonic cell stage. Even though mRNA synthesis starts at the late 1-cell stage, in the absence of zygotic or embryonic transcription, embryos can cleave until the 2-cell stage in the mouse (Aoki *et al*., 1997) and 9- to 16-cell stages in cattle (Memili & First, 1998, 1999). In summary, initiation of zygotic or embryonic transcription does not coincide with the dependence of embryo development on zygotic or embryonic transcription. Therefore, it would be more appropriate to use the terms MET or Z/EGA to define either initiation of zygotic/embryonic transcription or the time when embryo development depends on zygotic/embryonic transcription. The other alternative is not to use any specific terms such as the MET or Z/EGA at all. Evidence for this alternative comes from the fact that activation of zygotic/embryonic transcription and degradation of maternal messages occur gradually (Aoki *et al*., 1997; Memili *et al*., 1998).

Characteristics of zygotic/embryonic gene expression and the onset of RNA polymerase II mediated transcription in mammals

It has long been known that mRNAs and proteins used during early embryogenesis are produced in oocytes. As embryonic development proceeds and maternally inherited RNAs and proteins degrade, embryonic development becomes dependent on expression of embryonic genes. The maternal contribution to development was first discovered in amphibian studies wherein enucleated oocytes underwent cleavage (Briggs *et al*., 1951). Qualitative changes in polypeptide profiles and poly(A+) mRNA occurring during oocyte maturation were evidence for similar events taking place in mammalian oocytes (Cullen *et al*., 1980; Bachvarova & De Leon, 1980). Stored maternal products include diverse proteins ranging from histone variants in *Xenopus* to tubulins in the mouse (Woodland & Adamson, 1977; Schultz *et al*., 1979). Major maternal proteins and their functions in early development are reviewed by Dworkin & Dworkin-Rastl (1990).

RNA polymerase II dependent transcription is inhibited by α-amanitin. RNA polymerase III dependent transcription is inhibited at higher concentrations of α-amanitin while RNA polymerase I dependent transcription is not inhibited by α-amanitin at any tested concentrations (Lindel *et al*., 1970; Barnes & First, 1991). Therefore, α-amanitin can be used to differentiate transcriptional activity of different RNA polymerases. Culturing mouse embryos in the presence of absence of α-amanitin followed by polyacrylamide gel electrophoresis resulted in inhibition of proteins resulting from the zygotic/embryonic transcription that have been used as some of the markers of embryonic transcription in the mouse. These proteins are about 70 kDa and called the transcription-requiring complex (TRC); their identities are not known (De Souse *et al*., 1998). However, TRC is expressed at a relatively high level and can easily be detected on a one-dimensional polyacrylamide gel.

Characteristic events that occur during transcriptional activation of zygotic/embryonic genes include loss or decay of maternally inherited mRNA molecules and qualitative changes in protein profiles at different cell stages. The mouse has been the most studied species since it is relatively inexpensive and various inbred lines with unique genetic characteristics are available. Although some strain differences have been reported, mouse embryos cultured with α-amanitin do not develop beyond the 2-cell stage (Rambhatla & Latham, 1995). Zygotic/embryonic transcription in mouse occurs as a minor activation in the late 1-cell stage and as a major activation during G_2 of the second cell cycle. However, translation of zygotic transcripts does not begin until the 2-cell stage (Davis & Schultz, 1997; Aoki *et al*., 1997).

Even though studies using [^{3}H]uridine labelling have shown that embryonic transcription in cattle starts at the 8-cell to 16-cell stage (Kopecny *et al*., 1989), recent studies have shown that embryonic transcription is evident at or earlier than the 4-cell stage, with detection of eight embryonic proteins at this stage followed by 23 embryonic proteins at the 8-cell stage (Barnes & First, 1991). The most recent studies with [^{3}H]uridine suggest that embryonic transcription may be active as early as the 2-cell stage (Viuff *et al*., 1996; Hyttel *et al*., 1996). These results are consistent with the results of our studies. We labelled bovine immature, mature and early embryos with [^{35}S]UTP and showed that bovine immature oocytes and early embryos are transcriptionally active (Memili *et al*., 1998). Also in this study, transcriptional activity per cell did not increase from the 2-cell to 8-cell stage. However, if the data were plotted per oocyte or embryo, then transcriptional activity increased significantly from the 2-

https://doi.org/10.1017/S0967199400000861 Published online by Cambridge University Press

cell to 8-cell stage embryos. An attempt was also made to determine whether this early transcriptional activity was essential for embryo development by treating the embryos with α-amanitin for specific times during the first four cell cycles and then rescue. The results indicated that this early transcription was indeed essential for embryo development beyond the 9- to 16-cell stages (Memili & First, 1998). Results of this study also suggested that maternal RNAs and proteins were able to support cell cleavage until the 9- to 16-cell stages. Detection of transcriptional activity at the 2-cell stage of bovine embryos led us to question whether there was any transcription at 1-cell stage zygotes. To answer this question, bovine 1-cell zygotes and other early embryos including blastocysts (as control) were labelled with [^{3}H]uridine. Detection of [^{3}H]uridine incorporation into RNA showed that 1-cell zygotes were transcriptionally active (Memili & First, 1999). A further attempt was made to determine the timing of translation of these early transcripts by labelling 1-cell zygotes and 2-cell embryos with [^{35}S]methionine followed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and autoradiography. Results of this study showed that the zygotic/embryonic transcripts were translated during the 1- and 2-cell stages as evidenced by the presence at these cell stages of polypeptides sensitive to α-amanitin.

Sheep embryos cultured with α-amanitin arrest at the 8- to 16-cell stages while human embryos treated with α-amanitin did not develop beyond the 4-cell stage (Crosby *et al*., 1988). Protein synthesis during the first three cell cycles has been shown to be relatively constant while a distinct pattern of protein synthesis was observed at the 16-cell and later cell stages. The beginning of embryonic transcription has been documented by [^{3}H]uridine incorporation and autoradiography at the 4-cell stage in pig (Kopecny *et al*., 1989). Major qualitative changes in the pattern of radiolabelled polypeptides at the 4- to 8-cell stage of human embryos have been reported and some of these changes were shown to be inhibited by α-amanitin, indicating that they were due to translation of embryonic messages (Braude *et al*., 1988; reviewed by Pergament & Fiddler, 1998). Consistent results have been obtained from studies with [^{3}H]uridine incorporation and autoradiography (Tesarik *et al*., 1986). Manes (1971) showed that there was a progressive increase in the amount of total RNA in rabbit embryos. This feature of rabbit embryos is in contrast to a decrease in total RNA in early cleavage stage embryos followed by progressive increase in late stage embryos in the other species described above. RNA transcriptional units have been directly examined using electron microscopic analysis of spreads. This approach showed a α-amanitin sensitive transcription (transcriptional units were seen as 'Christmas-tree like' structures) at the 2-cell stage of embryonic development in the rabbit (Cotton *et al*., 1980).

There is a direct correlation between the transcriptional activation of embryonic genes and an increase in cell cycle length. Cell cycle and embryonic gene expression in bovine species have been documented in detail by Barnes & Eyestone (1990). briefly, bovine embryos have a total length for the first cell cycle of 28 h; pronuclei form at 10 h after fertilisation followed by the S phase for 8–10 h and a G_2 period of 4–6 h. The second cell cycle has no G_1 or G_2, and lasts for 12 h with an S phase of 8–10 h and an M phase of 2–4 h. The third cycle lasts 14 h without a G_1 period while the S phase lasts for 8–10 h followed by a G_2 phase of 4 h. The total length of the fourth cell cycle is 24–28 h; asynchronous cell divisions beginning at the fourth cell cycle make it difficult to determine exact cell cycle phases. Therefore mouse embryos have a short G_2 period lasting only 1–2 h for the first cell cycle and a long G_2 period lasting 12 h for the second cell cycle. DNA synthesis lasts 7 h for each cell stage (Smith & Johnson, 1986).

Exact mechanisms of a 'developmental block' that coincide with the time of a major activation of the embryonic genome and arrest with α-amanitin culture *in vitro* cultured embryos are not known. The developmental block occurs at the 8-cell stage in bovine embryos (Camous *et al*., 1984), the 8- to 16-cell stage in ovine embryos (Gandolphi & Moor, 1987), the 4- to 8-cell stage in human embryos (Braude *et al*., 1988), the 4-cell stage in porcine embryos (Davis, 1985), the 8- to 16-cell stage in goat embryos, and the 2-cell stage in the mouse (Telford *et al*., 1990). The timing of the 'developmental block' suggests that embryonic transcription is particularly sensitive to culture conditions. It has been shown that the 'developmental block' can be relieved by co-culture systems, addition of fetal calf serum to the culture medium and a reduction of glucose in the culture medium (Gandolphi & Moor, 1987).

Onset of RNA polymerase I dependent transcription during embryogenesis

RNA polymerase I dependent transcription, i.e. ribosomal RNA (rRNA) synthesis, is characterised by formation of the nucleolus precursor body (NPB), which is a dense network of filaments arranged in a spherical form that eventually becomes the nucleolus. A functional nucleolus is formed upon differentiation of the NPB into a structure that has a fibrillar centre surrounded by a granular component. Nucleolar transcription can be detected by [^{3}H]uridine incorporation during the early phase of NPB formation. Soon after nucleolar transcription, mature nucleoli are formed which are characterised by penetration of DNA into

https://doi.org/10.1017/S0967199400000861 Published online by Cambridge University Press

the NPB. species-specific differences exist along mammalian NPBs: size, morphology, pattern of embryonic nucleogenesis and molecular composition of different NPB compartments. Two different NPB types have been described: the mouse-type NPB and the cow-type NPB. The mouse-type NPB is characterised by the presence of detectable original compact material of the NPB in the early functional nucleolus in which the core contains neither DNA nor argentophilic-nucleolar organising region proteins. Formation of the nucleolus at the periphery of the NPB coincides with initiation of nucleolar transcription. The cow-type NPB contains a homogeneous distribution of nucleolar proteins in its body from the pronuclear to early 8-cell stages. At the onset of nucleolar transcription in the cow-type NPB, perinucleolar DNA penetrates into the NPB and nucleolar transcription is localised deep inside the functional nucleolus (Flechon & Kopecny, 1998). Nucleolar transcription is first detected at the late 8-cell stage in the cattle (Kopecny *et al*., 1989), 2-cell stage in the mouse (Geuskens & Alexandre, 1984) and 4-cell stage in the pig (Tomanek *et al*., 1989). RNA polymerase II mediated transcription precedes polymerase I mediated transcription.

Mechanisms of RNA polymerase II dependent transcription during embryonic development

In search of an understanding of the mechanism(s) of zygotic/embryonic gene expression, which is an essential event for successful development, there have been numerous investigations leading to theories to explain this transition in early development. Several points regarding these theories will be discussed in this review. These will include: (1) the nuclear:cytoplasmic ratio that affects transcription, (2) a zygotic clock that measures time passed after fertilisation and in turn affects transcription, (3) the effect of cell cycle regulation on transcriptional control, and finally (4) post-translational modifications of transcriptional machinery and effects of changes in chromatin structure on transcription.

Nuclear : cytoplasmic ratio, cytoplasmic/zygotic clock, and cell-cycle-mediated mechanisms of the onset of zygotic/embryonic mRNA transcription

Effects of changing nuclear:cytoplasmic ratio in *Xenopus* embryos have been the subjects of intense research. In *Xenopus*, fertilisation initiates 11 cycles of rapid cell division that occur without zygotic gene transcription. Embryonic transcription, which is termed the mid-blastula transition (MBT:4000-cell stage), starts during cell cycle 12. There are neither G_1 nor G_2 periods before the MBT; however, during the MBT, G_1 and G_2 periods are acquired (Newport & Kirschner, 1982*a*). Therefore the MBT appears to be coupled to the nuclear:cytoplasmic ratio or number of rounds of DNA replication. This interpretation has come under question since it has been shown that injection of exogenous DNA or arrest of DNA cleavage does not influence the temporal control of snRNA, tRNA or rRNA synthesis (Shiokawa *et al*., 1989). Treatment of embryos with α-amanitin did not inhibit the cell cleavage and cell motility which were observed in the control embryos (Newport & Kirschner, 1982a). Experiments with injection of a plasmid that had a yeast leucine tRNA encoding gene into *Xenopus* embryos showed that transcription from the plasmid was not detected until the twelfth-cell cycle. However, if DNA equal to the amount that is present after 12 cleavages was co-injected with the plasmid, transcription was prematurely initiated (Newport & Kirschner, 1982*b*). This led to the conclusion that there is a transcriptionally repressive maternal factor that must be titrated by the increasing amounts of DNA, which doubles with each cleavage division. The simplest and most current model is that rapid DNA synthesis during *Xenopus* embryogenesis titrates out large amounts of histones stored in the embryo (Thompson *et al*., 1998). However, expression of some specific genes prior to the MBT led researchers to conclude that specific genes may be activated early depending on their transcriptional activators or levels of transcriptional repressors (Wolffe, 1996). Embryonic transcription in *Drosophila* is initiated at low levels at the tenth cell cycle followed by high levels of transcription at the fourteenth cell cycle which coincides with the appearance of G_1 and G_2 periods (Newport & Kirschner, 1982*b*). However, premature induction of transcription by aphidicolin treatment provided evidence against the nuclear:cytoplasmic ratio hypothesis in *Drosophila* embryonic gene activation (Newport & Kirschner, 1982*b*).

Another hypothesis for the zygotic/embryonic transition in gene expression is the presence of a 'zygotic clock' that measures elapsed time after fertilisation and delays zygotic/embryonic transcription by a time-dependent mechanism. However, the zygotic clock may not be the model of activation of zygotic/embryonic genes for *Xenopus* and *Drosophila* because of the fact that inhibition of DNA synthesis caused by aphidicolin treatment prematurely induced embryonic transcription in both these species. Similar results were obtained with bovine embryos, in which ephidicolin treatment induced transcription of an 8-cell specific mRNA in 2-cell bovine embryos (Jones & First, 1995). Timing of zygotic gene activation (ZGA) as indicated

https://doi.org/10.1017/S0967199400000861 Published online by Cambridge University Press

by expression of the TRC in mouse seems to be related to the zygotic clock (Schultz, 1993). The TRC is expressed at the same time in control embryos and embryos treated either with cytochalasin D (inhibitor of cytokinesis) or aphidicolin (inhibitor of DNA synthesis). The zygotic clock is not simply the time that is required to convert sperm and egg chromatin into a transcribable form but a mechanism that may involve *trans*-acting factors that are required for either transcription or suppression of transcription. The zygotic clock may regulate activity of general transcription factors such as TATA binding protein (TBP) or it may delay the ZGA until chromatin is remodelled for transcription (Nothias *et al*., 1995). Newport & Kirschner (1982*b*) proposed that the MBT in *Xenopus* does not depend on a zygotic clock or rounds of DNA synthesis after fertilisation. In this study early embryos were partially constricted such that only one-half of the embryo had a nucleus. A nucleus was forced across the constriction after four to eight cleavage divisions, and this resulted in induction of cleavage in the half that initially lacked a nucleus. The half with the original nucleus reached the MBT two-cell divisions before the other half that originally lacked a nucleus. The authors then proposed that the MBT is not caused by a zygotic clock since the MBT occurred at different times in this experiment. They also concluded that the MBT is not caused by the number of rounds of DNA synthesis since the two halves had undergone different numbers of cleavage divisions when the MBT occurred.

Since cell cycle lengths increase during the period of zygotic/embryonic transcription in many species, it was proposed that this activation is mediated in part by a mechanism that involves the cell cycle in that the rapid cell divisions are suppressive for transcription. As mentioned previously, *Drosophila* embryos undergo rapid cell divisions that have no G_1 or G_2 until the eleventh cell cycle when a low level of transcription is detected. Then the cell cycle length increases gradually until the fourteenth cell cycle, which is accompanied by a large burst of transcription (McNight & Miller, 1976). Aphidicolin or cycloheximide treatment was shown to induce premature transcription in *Xenopus*, and nocodazole (an inhibitor of microtubule polymerisation and cell cleavage, but not an inhibitor of DNA synthesis) treatment did not affect the MBT (Kimmelman *et al*., 1987). It was then suggested that aphidicolin and cycloheximide caused an artificial G_1 period in the cell cycle that led to a longer cell cycle which in turn induced transcription. It was shown that CDC25, a cell cycle regulator, was maternal in origin until the fourteenth cell cycle in *Drosophila* and was degraded during this cell cycle. CDC25 was then required for further development (Edgar & O'Farrel, 1990). Maternal CDC25 was shown to last until the late 8-cell stage in bovine embryos and embryonic CDC25 was first synthesised at the 8-cell stage (Jones & First, 1995). Furthermore, overexpression of CDC25 in the late 4- or 8-cell stages modified these cell cycles to be more like the second cell cycle and transcription was suppressed. Taken together all of the observations above suggest that the delay in the cell cycle or increase in cell cycle lengths is due to diminishing amount of maternal CDC25 which also coincides with expression of CDC25 and activation of the embryonic genome (Jones, 1994).

Post-translational modifications of transcriptional machinery and the effect of changes in chromatin structure on transcription

Chromosomal architecture has been known to be one of the most important factors for developmental control of gene expression. Chromosomes and chromatin undergo major transitions during development. Changes in histones play key roles in determining the pattern of gene activity and directing repression of specific eukaryotic genes. Studies in *Xenopus* and mouse have shown that biochemical composition of nucleosomes is a very important part of the mechanisms that regulate gene transcription during early embryogenesis (Wolffe, 1996). Differences in the type and modification of chromosomal and chromatin structural proteins provide the dominant means for controlling transcriptional activity in a promoter-specific manner. There are three factors involved in differential transcription of a gene: (1) availability of specific *trans*-acting factors used by a specific gene to recruit transcriptional activators or repressors, (2) the transcriptional machinery itself including the transcriptional factors such as TFIID, TFIIB and TFIIH, and (3) the chromosomal environment into which a gene is introduced.

Some integral components of the transcription initiation complex, such as the SWI/SNF protein complex (subunit of RNA polymerase II holoenzyme), have been shown to cause changes in chromatin structure when they bind to transcription factors. Such changes provide the transcription initiation complex the opportunity to start transcription and displace nucleosomes during transcriptional elongation (Brownell & Allis, 1996; Stein *et al*., 1997). Transcriptional competency (presence of competent transcriptional machinery) during early bovine embryogenesis was tested by assaying the presence of RNA polymerase IIA by immunoblotting. The results of this study showed that the IAI form was expressed at all early embryonic stages from 2- to 8-cell stages and it is at the highest level in 2-cell embryos (Memili & First, 1998).

https://doi.org/10.1017/S0967199400000861 Published online by Cambridge University Press

Formation of core histones and linker histones is a fundamental part of chromosomal architecture. The core histones, histone H2A and H2B, form a dimer while histones H3 and H4 form a tetramer. These dimers and tetramers wrap 160 base pairs of DNA, and together with a single linker histone they organise 180–190 base pairs of DNA into a nucleosome. The N-terminal domains of the histones lie outside the nucleus and this positively charged tail influences the path of DNA and interacts with specific *trans*-acting factors (Hecht *et al*., 1995). The N-terminal domains of the core histones are also subject to post-translational modifications such as acetylation/deacetylation and phosphorylation. It appears that the global changes in gene activity observed during early embryogenesis are dependent on developmentally regulated changes in the type or modification of histone or other basic chromatin proteins. In mammalian somatic cells histone H4 is acetylated in the order lysine 16, followed by lysine 8 or 12 and then by lysine 5 (Turner & Fellows, 1989). Acetylation of histones causes a loose chromatin structure and this provides an opportunity for transcription initiation factors to be able to bind DNA. Deacetylation of histones leads to transcriptional silencing indicating a direct link between histone acetylation and the transcriptional process (Wolffe & Prus, 1996). Histone deacetylase is inhibited by trapoxin, tricostatin A and butyrate (Yoshida *et al*., 1990; Kijima *et al*., 1993). Mouse embryos treated with butyrate had increased levels of reporter gene expression from reporter genes not bearing an enhancer, the increase being to a level similar to that of untreated embryos injected with an enhancer-bearing reporter gene (Weikowski *et al*., 1993). Consistent with this, trapoxin prevented a decrease in expression of endogenous genes in which expression transiently increases during the 2-cell stage (Davis *et al*., 1996). How chromatin is remodelled at the onset of mammalian development is important not only for a better understanding of how the embryo progresses through the preimplantation period, but also for long-lasting epigenetic effects appearing only in adults (Roemer *et al*., 1997; Thompson *et al*., 1998).

The role of DNA replication in the transcriptional activation of zygotic/embryonic genes has been studied in detail in mouse. It was shown that the first round of DNA replication was critical for reprogramming of the pattern of gene expression in mouse zygotes by disrupting nucleosomes, thereby providing opportunity for maternally inherited transcription factors to bind their cognate *cis*-binding sequences (Wolffe, 1994; Davis & Schultz, 1997). Expression of some endogenous genes has been proposed to be coupled to the first round of DNA replication while others are not coupled in the mouse (Aoki *et al*., 1997). DNA replication thus changes chromatin structure in such a way as to generate a transcriptionally permissive chromatin structure. The challenging question was to determine how embryonic gene expression is initiated in bovine embryos, i.e. what the mechanism(s) is that controls gene expression at the onset of embryonic development. To test this, 1-cell zygotes and 2-cell embryos were cultured in the presence or absence of α-amanitin, aphidicolin and Tricostatin A (TSA), and labelled with [^{3}H]uridine and [^{35}S]methionine to determine the effect(s) of inhibition of the first and second rounds of DNA replication and histone deacetylases on the expression of zygotic/embryonic transcription and translation respectively. Results of these experiments indicated that inhibition of DNA replication decreased transcriptional activity in the zygotic stage while inhibition of histone deacetylases increased transcriptional activity in the same cell stage. In the second cell cycle, inhibition of DNA replication decreased the transcriptional activity and inhibition of histone deacetylases decreased the transcriptional activity, but not as much as that of inhibition of DNA replication. We also observed translation of some of these zygotic transcripts during 1- and 2-cell stages (Memili & First, 1999). Previously, Barnes & First (1991) showed translation of embryonic messages as eight new polypeptides in the late 4-cell stage and 23 new polypeptides in 8-cell stage bovine embryos. However, better resolution of 2D-PAGE enabled us to detect translation of embryonic messages earlier than the 4-cell stage. Results of our experiments were confirmed by others in an experiment in which reverse transcription polymerase chain reaction (RT-PCR) and sequential southern blot analysis indicated that transcripts of luciferase cDNA were detected in DNA microinjected bovine embryos at 24, 36, 48, 60 and 72 h post-insemination (Saeiki *et al*., 1999). In our study both aphidicolin and TSA decreased expression of the number of zygotic/embryonic proteins. Even though inhibition of DNA replication was shown to induce expression of specific genes (Jones & First, 1995; Davis & Schultz, 1997), our study suggests that inhibition of the first and second rounds of DNA replication decreases general transcriptional activity as well as translation of the majority of zygotic/embryonic genes. DNA replication facilitates embryonic gene activation, as our control embryos without aphidicolin treatment had higher transcriptional and translational activities. It has been proposed that DNA replication provides an opportunity for transcription factors that are maternal in origin to gain access to their cognate *cis*-binding DNA sequences (Davis & Schultz, 1997); however, inhibition of DNA replication may selectively activate some genes. This may be the reason for induction of some specific genes observed by Jones & First (1995). A number of investigators have suggested that simple titration of maternally derived core histones regulates transcription and accounts for transcriptional silencing

in *Xenopus* (Prioleau *et al*., 1994). Almouzni & Wolffe (1995) titrated or re-added the core histones and showed that these proteins indeed have a necessary, but far from sufficient role in generating a transcriptionally inactive state prior to the MBT. The same study showed that artificial increase in DNA content within embryos prior to the MBT led to detection of significant transcription from certain genes (Prioleau *et al*., 1994). The same authors microinjected c-*myc* promoter prior to the MBT and detected significant transcriptional activity in the presence of mass excess of histones (> 24-fold) over DNA. Thus it was proposed that titration of another, more limiting transcriptional repressor or some other independent process determines basal transcriptional activity. Therefore, the activation of c-*myc* before the MBT was shown to be a promoter-specific phenomenon and the presence or level of a particular transcriptional activator was suggested to play an important role that can be dominant over inhibitory chromatin structures and can recruit the basal transcriptional machinery to activate transcription. Furthermore, selective use of particular transcription factors during early embryogenesis may have important consequences for the regulation of gene expression in vertebrates (Landsberger & Wolffe, 1995). Many lines of evidence now indicate that acetylation of the N-terminal tails of core histones facilitates access of transcription factors and RNA polymerase II to DNA in the nucleosome. It was shown that when DNA replication was inhibited, histone H4 was acetylated to the same level as in control mouse embryos (Thompson *et al*., 1998). This may explain why transcription of some genes is activated in aphidicolin-treated embryos. Histone deacetylases (HDACs) deacetylate all core histones and in turn repress transcription. DNA-binding proteins such as MeCP2 interact with proteins such as sin3A, which in turn recruits HDACs. Levels of HDACs and histone acetyltransferases exist at an equilibrium (Wolffe, 1996) and presence of all HDACs in bovine oocytes and early embryos suggests that a transcriptionally repressive environment is active in early embryogenesis (E. Memili & N.L. First, unpublished data).

Conclusions and future directions

In *Xenopus* and *Drosophila* embryos, zygotic transcription occurs after a series of rapid cell divisions in which DNA replication occupies most of the cell cycle. It has been proposed that activation of the zygotic genes at the transcriptional level does not begin until a threshold nuclear:cytoplasmic ratio has been obtained in which transcriptionally repressive factors (such as histones) are titrated out and interphase (G_1 and G_2) becomes long enough to allow synthesis of transcripts. In mammalian embryos, however, a model for threshold nuclear:cytoplasmic ratio does not seem to apply. As early as the beginning of the 1-cell stage, there are regulated cell cycle with the expression of zygotic transcripts during the cleavage period. Changes in transcriptional machinery and chromatin structure have essential roles during transition from acquisition of transcriptional competency to minor and eventually major embryonic genome activation. Translation of zygotic transcripts occurs in late 1-cell stage in cattle (Memili & First, 1999) and 2-cell stage in the mouse (Davis & Schultz, 1997). Even though the timing is different, the zygotic/embryonic gene expression in cattle is similar to the mouse model (Aoki *et al*., 1997; Thompson *et al*., 1998). Data obtained in our laboratory (Barnes & First, 1991; Memili *et al*., 1998; Memili & First, 1998, 1999) and elsewhere (Kopecny *et al*., 1989; Plante *et al*., 1994; Hyttel *et al*., 1996; Viuff *et al*., 1996) suggest that there is a low level of transcriptional activity (mRNA synthesis, i.e. RNA polymerase II dependent transcription) that can be called 'minor gene activation' between the 1- and late 4-cell stages, and a high level of transcriptional activity that can be called 'major gene activation' at the 8-cell stage in bovine embryos (Fig. 1).

Now that general transcriptional activity is detected as early as the 1-cell stage, it would be interesting to determine the identities of specific genes that are activated at the onset of embryonic development. Expression of these genes could be determined *in vivo* and in several kinds of *in vitro* culture systems. These genes would serve as markers for normal embryo development. It would also be interesting to explore the regulation of these specific genes by culturing the cells with aphidicolin and TSA. Another set of future studies would be to search transcriptional activity in male and female pronuclei of the 1-cell zygote. This can be accomplished by labelling the sperm with Mito Tracker (to differentiate the male pronucleus from the female pronucleus since male and female pronuclei are about the same size in bovine zygotes), culturing zygotes with BromoUTP for a short time followed by immunocytochemistry and confocal microscopy. This would answer the intriguing question of whether the male and female pronuclei have the same levels of transcriptional activity. In the mouse the male pronucleus is transcriptionally more active than the female pronucleus due to replacement of protamines with maternally derived histones and hyperacetylation of these histones (Aoki *et al*., 1997). Live offspring were born as a result of nuclear transfer studies in which a somatic cell was able to support full term development after transplantation into an enucleated oocyte (Wilmut *et al*., 1997; Cibelli *et al*., 1998; Wakayama *et al*., 1998. However, the success rates in these studies is very low (~1%). This may be improved by better

https://doi.org/10.1017/S0967199400000861 Published online by Cambridge University Press

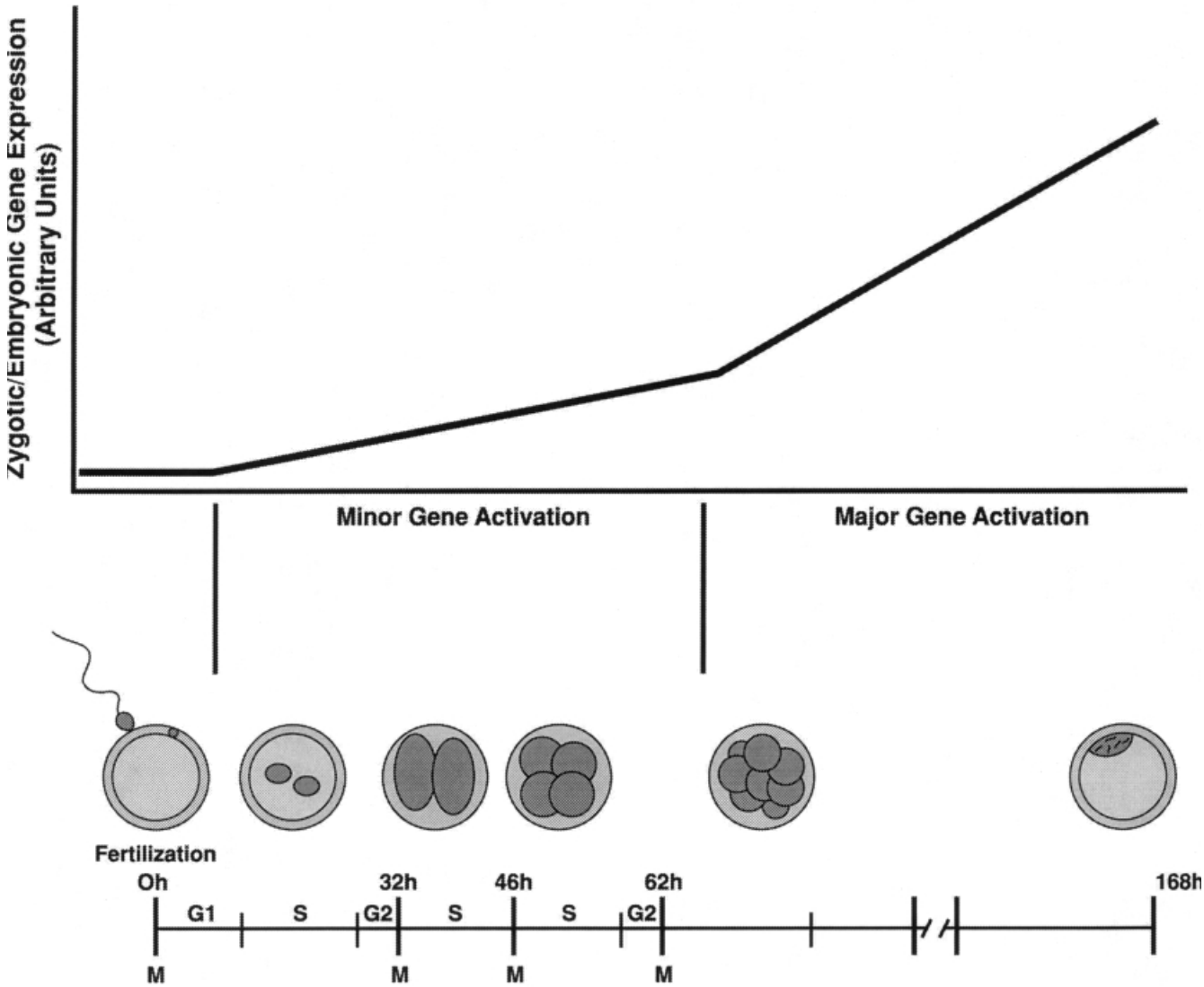

Figure 1 Bovine embryonic cell cycles and zygotic/embryonic gene expression (mRNA synthesis) in cattle. The cell cycle was adapted from Barnes & Eyestone (1990). Data from the literature suggest that there is a 'minor gene activation' between the 1- and 4-cell stages. Changes in the transcriptional machinery and chromatin structure play an important role in the control of early gene expression. Several references reviewed here suggest that there is a 'major gene activation' starting at the 8-cell stage.

understanding of early embryo development and regulation of zygotic/embryonic gene expression.

References

Almouzni, G. & Wolffe, A.P. (1995). Constraints on transcriptional activator function contribute to transcriptional quiescence during early *Xenopus* embryogenesis. *EMBO J.* **14**, 1752–65.

Aoki, F., Worrad, D.M. & Schultz, R.M. (1997). Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. *Dev. Biol.* **307.**

Bachvarova, R. & De Leon, V. (1980). Polyadenylated RNA of mouse ova and loss of maternal RNA in early development. *Dev. Biol.* **74**, 1–8.

Barnes, F.L. & Eyestone, W.H. (1990). Early cleavage and the maternal to zygotic transition in bovine embryos. *Theriogenology* **33**, 141–52.

Barnes, F.L. & First, N.L. (1991). Embryonic transcription in *in vitro* cultured bovine embryos. *Mol. Reprod. Dev.* **29**, 117–23.

Braude, P., Bolton, V. & Moore, S. (1988). Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature* **332**, 459–61.

Briggs, R., Green, E.U. & King, T.J. (1951). An investigation of the capacity for cleavage and differentiation in *Rana pipiens* eggs lacking 'functional' chromosomes. *J. Exp. Zool.* **116**, 455.

Brownell, J.E. & Allis, C.D. (1996). Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. *Curr. Opin. Genet. Dev.* **6**, 176–84.

https://doi.org/10.1017/S0967199400000861 Published online by Cambridge University Press

Cibelli, J.B., Stice, S.L., Golueke, P.J., Kane, J.J., Jerry, J., Blackwell, C., de Leon, A.P. & Robl, J.M. (1998). Cloned transgenic calves produced from nonquiescent fetal fibroblasts. *Science* **22**, 1256–8.

Cotton, R.W., Manes, C. & Hamkalo, B.A. (1980). Electron microscopic analysis of RNA transcription in preimplantation rabbit embryos. *Chromosoma (Berl.)* **79**, 169–78.

Crosby, I.M., Gandolfi, F. & Moor, R.M. (1998). Control of protein synthesis during early cleavage of sheep embryos. *J. Reprod. Fertil.* **82**, 769–75.

Cullen, B., Emigholz, K. & Monahan, J. (1980). The transient appearance of specific proteins in one-cell mouse embryos. *Dev. Biol.* **76**, 215–21.

Davis, W. Jr & Schultz, R.M. (1997). Role of first round of DNA replication in reprogramming gene expression in the preimplantation mouse embryo. *Mol. Reprod. Dev.* **47**, 430–4.

Davis, W. Jr, De Sousa, P.D. & Schultz, R.M. (1996). Transient expression of translation initiation factor eIF-4C during the 2-cell stage of the preimplantation mouse embryo: identification by mRNA differential display and the role of DNA replication. *Dev. Biol.* **174**, 190–201.

De Sousa, P.A., Caveney, A., Wsethusin, M.E. & Watson, A.J. (1998). Temporal patterns of embryonic gene expression and their dependence on oogenetic factors. *Theriogenology* **49**, 115–28.

Dworkin, M.B. & Dworkin-Rastl, E. (1990). Functions of maternal mRNA in early development. *Mol. Reprod. Dev.* **26**, 261–97.

Edgar, B.A. & O'Farrel, P.H. (1990). The three postblastoderm cell cycles of *Drosophila* embryogenesis are regulated in G_2 by *string*. *Cell* **62**, 469–80.

Flechon, J.-E. & Kopecny, V. (1998). The nature of the 'nucleolus precursor body' in early preimplantation embryos: a review of fine-structure cytochemical, immunochemical and autoradiographic data related to nucleolar function. *Zygote* **6**, 183–91.

Gandolphi, F. & Moor, R.M. (1987). Stimulation of early development in sheep by co-culture with oviduct epithelial cells. *J. Reprod. Fertil.* **81**, 23–8.

Geuskens, M. & Alexandre, H. (1984). Ultrastuructural and autoradiographic studies of nucleolar development and rRNA transcription in preimplantation mouse embryos. *Cell Differ.* **14**, 125–34.

Hecht, A., Laroche, T., Strahl-Bolsinger, S., Gasser, S.M. & Grustein, M. (1995). Histone H3 and H4 N-terminal interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin yeast. *Cell* **80**, 583–92.

Hyttel, P., Viuff, D., Avery, B., Laurincik, J. & Greve, T. (1996). Transcription and cell cycle-dependent development of intranuclear bodies and granules in two-cell bovine embryos. *J. Reprod. Fertil.* **108**, 263–70.

Jones, J.M. (1994). Cell cycle repression of transcription in cleavage stage bovine embryos. PhD thesis, University of Wisconsin-Madison.

Jones, J.M. & First, N.L. (1995). Expression of cell cycle control protein CDC25 in cleavage stage bovine embryo. *Zygote* **3**, 133–9.

Kato, Y., Tani, T., Sotomaru, Y., Kurokawa, K., Kato, J., Doguchi, H., Yasu, H. & Tsunoda, Y. (1998). Eight calves cloned from somatic cells of a single adult. *Science* **282**, 2095–7.

Kijima, M., Yoshida, M., Sugita, K., Horinoichi, S. & Beppu, T. (1993). Trapoxin, an antitumour cyclic tetrapeptide, is an irreversible inhibitor of mammalian histone deacetylase. *J. Biol. Chem.* **268**, 22429–35.

Kimmelman, D., Kirschner, M. & Scherson, T. (1987). The events of the midblastula transition in *Xenopus* are regulated by changes in the cell cycle. *Cell* **48**, 399–407.

Kopecny, V., Flechon, J.E., Camous, S. & Fulka, J. (1989). High-resolution autoradiographic studies of comparative nucleogenesis and genome reactivation during embryogenesis in pig, man and cattle. *Reprod. Nutr. Dev.* **29**, 589–600.

Landsberger, N. & Wolffe, A.P. (1995). Chromatin and transcriptional activity in early *Xenopus* development. *Semin. Cell Biol.* **6**, 191–9.

Lindel, T.J., Weinberg, F., Morris, P.W., Roeder, R.G. & Rutter, W.J. (1970). Specific inhibition of nuclear RNA polymerase II by alpha-amanitin. *Science* **170**, 447–9.

Manes, C. (1971). Nucleic acid synthesis in preimplantation rabbit embryos. II. Delayed synthesis of ribosomal RNA. *J. Exp. Zool.* **176**, 87–96.

McNight, S.L. & Miller, O.L. (1976). Ultrastructural patterns of RNA synthesis during early embryogenesis of *Drosophila melanogaster*. *Cell* **8**, 305–19.

Memili, E. & First, N.L. (1998). Developmental changes in RNA polymerase II in bovine oocytes, early embryos and effect of alpha-amanitin on embryo development. *Mol. Reprod. Dev.* **51**, 381–9.

Memli, E. & First, N.L. (1999). Control of gene expression at the onset of bovine embryonic development. *Biol. Reprod.*, in press.

Memili, E., Dominko, T. & First, N.L. (1998). Onset of transcription in bovine oocytes, 2-, 4- and 8-cell embryos. *Mol. Reprod. Dev.* **51**, 36–41.

Newport, J.W. & Kirschner, M. (1982*a*). A major developmental transition in early *Xenopus* embryos. I. Characterization and timing of cellular changes at the midblastula stage. *Cell* **30**, 675–86.

Newport, J.W. & Kirschner, M. (1982*b*). A major developmental transition in early *Xenopus* embryos. II. Control of the onset of transcription. *Cell* **30**, 687–96.

Nothias, J.Y., Majumder, S., Koneko, K. & DePamphilis, M.L. (1995). Regulation of gene expression at the beginning of mammalian development. *J. Biol. Chem.* **270**, 22077–80.

Pergament, E. & Fiddler, M. (1998). The expression of genes in human preimplantation embryos. *Prenat. Diagn.* **18**, 1366–73.

Plante, L., Plante, C., Shepherd, D.L. & King, W.A. (1994). Cleavage and ^{3}H-urudine incorporation in bovine embryos of high *in vitro* developmental potential. *Mol. Reprod. Dev.* **39**, 375–83.

Rambhatla, L. & Latham, K.E. (1995). Strain-specific progression of alpha-amanitin-treated mouse embryos beyond the two-cell stage. *Mol. Reprod. Dev.* **41**, 16–19.

Roemer, I., Reik, W., Dean, W. & Wklose, J. (1997). Epigenetic inheritance in the mouse. *Curr. Biol.* **7**, 277–80.

Saeki, K., Matsumoto, K., Kaneko, T., Hosoi, Y., Kato, H. & Iritani, A. (1999). Onset of RNA synthesis in early bovine embryos detected by reverse transcription-polymerase chain transcription following introduction of exogenous gene into their pronuclei. *Theriogenology* **51**, 192.

Schultz, R.M. (1993). Regulation of zygotic gene expression in the mouse. *BioEssays* **8**, 531–8.

Schultz, R.M., Letourneau, G.E. & Wassarman, P.M. (1979). Program of early development in the mammal: changes in the patterns and absolute rates of tubulin and total protein synthesis during oogenesis and early embryogenesis in the mouse. *Dev. Biol.* **68**, 341–59.

Shiokawa, K., Fu, Y., Nakakura, N., Tashiro, K., Sameshima, M. & Hosokawa, K. (1989). Effects of the injection of exogenous DNAs on gene expression in early embryos and coenocytic egg cells of *Xenopus laevis*. *Arch. Dev. Biol.* **198**, 78–84.

Smith, R.K.W. & Johnson, M.H. (1986). Analysis of the third and fourth cell cycle of mouse early development. *J. Reprod. Fertil.* **76**, 393–9.

Stein, P., Worrad, D.M., Belyaev, N.D., Turner, B.M. & Schultz, R.M. (1997). Stage-dependent redistributions of acetylated histones in nuclei of the early preimplantation mouse embryo. *Mol. Reprod. Dev.* **47**, 421–9.

Telford, N.A., Watson, A.J. & Schultz, G.A. (1990). Transition from maternal to embryonic control of development: a comparison of several species. *Mol. Reprod. Dev.* **26**, 90–100.

Tesarik, J., Kopecny, V., Plachot, M., Mandelbaum, J., Dalage, C. & Flechon, J-E. (1986). Nucleogenesis in the human embryo developing *in vitro*: ultrastructural and autoradiographic analysis. *Dev. Biol.* **1152**, 193–203.

Thompson, E.M., Legouy, E. & Renard, J.P. (1998). Mouse embryos do not wait for the MBT: chromatin and RNA polymerase remodeling in genome activation at the onset of development. *Dev. Genet.* **22**, 31–42.

Tomanek, M., Kopecny, V. & Kanka, J. (1989). Genome reactivation in developing early pig embryos: an ultrastructural and autoradiographic analysis. *Anat. Embryol.* **180**, 309–16.

Turner, B.M. & Fellows, G. (1989). Specific antibodies reveal ordered and cell-cycle-related use of histone-H4 acetylation sites in mammalian cells. *Eur. J. Biochem.* **79**, 131–9.

Viuff, D., Avery, B., Greve, T., King, W.A. and Hyttel, P. (1996). Transcriptional activity *in vitro* produced bovine two- and four-cell embryos. *Mol. Reprod. Dev.* **43**, 171–9.

Wakayama, T., Perry, A.C.F., Zuccotti, M., Johnson, K.R. & Yanagimachi, R. (1998). Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* **394**, 369–73.

Wiekowski, M., Miranda, M. & DePamphilis, M.L. (1993). Requirements for promoter activity in mouse oocytes and embryos distinguish paternal pronuclei from maternal and zygotic nuclei. *Dev. Biol.* **159**, 366–78.

Wilmut, I., Schnieke, A.E., McWjir, J., Kind, A.J. & Campbell, K.H.S. (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature* **385**, 810–3.

Wolffe, A.P. (1994). The transcription of chromatin templates. *Curr. Opin. Genet. Dev.* **4**, 245–54.

Wolffe, A.P. (1996). Chromatin and gene regulation at the onset of embryonic development. *Reprod. Nutr. Dev.* **36**, 581–606.

Wolffe, A.P. & Prus, D. (1996). Targeting chromatin disruption: transcription regulators that acetylate histones. *Cell* **84**, 817–19.

Woodland, H.R. & Adamson, E.D. (1977). The synthesis and storage of histones during the oogenesis of *Xenopus laevis*. *Dev. Biol.* **57**, 118–35.

Yoshida, M., Kijima, M., Akita, M. & Beppu, T. (1990). Potent and specific inhibition of mammalian histone deacetylase both *in vivo* and *in vitro* by tricostatin A. *J. Biol. Chem.* **265**, 17174–9.