体細胞核の初期化に関与する卵内因子に関して

誌名	Journal of mammalian ova research = 日本哺乳動物卵子学会誌
ISSN	13417738
著者名	宮本,圭
発行元	日本哺乳動物卵子学会
巻/号	30巻3号
掲載ページ	p. 68-78
発行年月	2013年10月

J. Mamm. Ova Res. Vol. 30 (3), 68–78, 2013

—Mini Review—

Maternal Factors Involved in Nuclear Reprogramming by Eggs and Oocytes

Kei Miyamoto*

Wellcome Trust/Cancer Research UK Gurdon Institute and Department of Zoology, University of Cambridge, Cambridge CB2 1QN, UK

Abstract: When a somatic cell nucleus is transplanted into an egg or an oocyte, the transplanted nucleus can be reprogrammed to support early embryonic development so that the reconstructed embryo gives rise to a cloned animal. Nuclear reprogramming of somatic nuclei is induced by maternal components stored in eggs and oocytes. These endogenous reprogramming factors and mechanisms have been explored for decades in mammals and amphibia. There are several ways of investigating reprogramming mechanisms, including nuclear transfer to eggs/oocytes and incubation in egg/oocyte extracts. In this review I describe the type of reprogramming events induced in each system and what factors in eggs and oocytes are responsible for these. Based on our current knowledge, I propose a model for the early phase of nuclear reprogramming in eggs and oocytes.

Key words: Cell-free extract, Egg and oocyte, Nuclear reprogramming, Nuclear transfer, Chromatin remodelling

Introduction

Scientists have long been intrigued by the fact that an egg has the remarkable ability to confer totipotency not only to sperm, but also to a transplanted somatic nucleus. This capacity of an egg was first demonstrated by John Gurdon using *Xenopus laevis* more than 50 years ago [1, 2]. Gurdon performed ingenious nuclear transfer (NT) experiments in which an unfertilised egg nucleus was inactivated by UV irradiation and a somatic cell nucleus was injected into the enucleated egg. These NT embryos developed into normal cloned frogs. His experiments provided the proof that cell differentiation is not necessarily accompanied by the loss of the genetic content. Furthermore, his study demonstrated that adult cells can be reversed to an embryonic state to acquire totipotency. Nuclear reprogramming of somatic cells towards totipotency has been successfully shown in many mammalian species [3, 4], which brings the idea of applying reprogramming technologies to medical applications, such as autologous cell replacement therapy. A major step forward towards medical applications has been made by the discovery of induced pluripotent stem (iPS) cells [5, 6].

Although reprogramming research has been rapidly advancing since the discovery of iPS cells, we still have not answered a fundamental question: how are somatic nuclei reprogrammed in eggs and oocytes? One may argue that this question is no longer of interest since we can obtain reprogrammed cells for future medical applications by the iPS route without using eggs and oocytes. However, there are several reasons why this question is still very important. Firstly, the factors and mechanisms that eggs and oocytes use to reprogram somatic nuclei relate to those used for reprogramming sperm at fertilisation. Therefore, studying such factors and mechanisms would also provide important insights into understanding normal development. Secondly, nuclear reprogramming by the iPS cell technology currently works at a low efficiency, and iPS cells sometimes carry somatic cell features [7] indicative of incomplete reprogramming. Moreover, one study reported that somatic nuclei are more efficiently reprogrammed by nuclear tranfer than by the iPS route [8]. It is, therefore, of great interest to investigate egg/oocyte factors and mechanisms that support efficient reprogramming, since it might be possible to apply these maternal factors to the improvement of iPS-mediated reprogramming. Thirdly, eggs and oocytes utilise germ cell-specific or germ cell-enriched factors, which often induce biologically unique phenomena, such as rapid chromatin decondensation and DNA demethylation. Studying reprogramming in eggs and oocytes thus provides a great opportunity to reveal such interesting cellular events. Fourthly, nuclear transfer remains the only established way to reprogram somatic nuclei to to-

Received: May 28, 2013
Accepted: July 16, 2013
*To whom correspondence should be addressed.
e-mail: k.miyamoto@gurdon.cam.ac.uk

tipotency. Understanding how totipotency is restored to somatic nuclei can lead to the improvement of the current cloning technology. If animal cloning technology is improved, we might be able to use this for propagating domestic animals with useful traits as well as endangered species. Lastly, the recent breakthrough study by Tachibana et al. [9] demonstrates that human embryonic stem (ES) cells are obtained by nuclear transfer to human oocytes. Importantly, their improved NT method allows the efficient derivation of human ES cells. Therefore, it is still possible that human NT could be used for future medical applications.

How can we study reprogramming mechanisms in eggs and oocytes? There have been many studies of the changes occurring in somatic nuclei upon nuclear transfer. Analyses of transplanted nuclei in NT embryos have revealed many characteristic features of reprogramming. It is however difficult to identify a specific egg/oocyte component in this way, since biochemical approaches cannot be easily applied to NT embryos due to a lack of materials. In addition, reprogramming of gene expression is associated with cell divisions, which makes it difficult to evaluate whether a given factor has a direct effect on gene expression. To overcome these obstacles, two other systems are being used. One is to inject hundreds of mammalian nuclei into the nucleus of the *Xenopus* oocyte [10]. *Xenopus* oocyte nuclear transfer allows direct induction of reprogrammed transcripts from mammalian embryonic genes without the need of cell divisions and new protein synthesis. The other is to incubate permeabilised nuclei in egg and oocyte extracts [11, 12]. The reprogramming events associated with nuclear transfer to eggs/oocytes are at least partially recapitulated on a million somatic cells in these extracts and hence many biochemical assays can be used to analyse reprogramming. In this review, I summarise the reprogramming factors and mechanisms identified in each reprogramming system. This classification of experimental systems would allow us to properly understand the roles of each maternal factor in reprogramming somatic nuclei. I also propose a model of nuclear reprogramming in eggs and oocytes.

Nuclear Transfer to Eggs/Oocytes at the Metaphase II Stage

Most of the successfully cloned animals have been obtained by injecting a somatic nucleus into an enucleated egg/oocyte at the metaphase II stage (MII stage; the term "MII egg" is used for frogs, while "MII oocyte" is used for mammals). Here, I briefly summarise the changes that happen to transplanted nuclei during reprogramming in NT embryos, and then discuss the factors and mechanisms involved in these nuclear changes.

Reprogramming of somatic nuclei after nuclear transfer

When mammalian somatic nuclei are injected into MII oocytes, the injected nuclei undergo nuclear envelope breakdown and premature chromosome condensation (PCC) due to strong maturation promoting factor (MPF) activity [13, 14]. PCC is not necessary for complete reprogramming at least in some mammalian species [15], but the studies using frog egg extracts indicate that it is important for subsequent DNA replication [16, 17] (which is discussed in a later section). Moreover, the recent human cloning study supports the necessity of PCC for the development of NT embryos [9]. After activation of NT oocytes, injected nuclei form pseudo-pronuclei and somatic chromatin is decondensed. In the pseudo-pronuclei, somatic nuclear architecture is remodelled to resemble the embryonic one [18]. Epigenetic changes, such as DNA demethylation and histone tail modifications, are induced in pseudo-pronuclei. After NT embryos divide, embryonic gene expression is induced. Then, cloned embryos can develop to blastocyst embryos from which embryonic stem cells are derived in mammals [9, 19]. More details of each process are explained in our recent article [20]. In this review, I focus on the known maternal factors that induce nuclear reprogramming events.

Nuclear remodelling by maternal factors

One of the earliest changes occurring in transplanted somatic nuclei is the exchange of somatic type linker histone H1 for oocyte type linker histone H1foo (Table 1) [21, 22]. Association of H1foo with somatic chromatin starts immediately after nuclear transfer (within 5 min). H1foo is more mobile than somatic H1 in NT embryos, which may contribute to making somatic chromatin accessible for other maternal reprogramming factors. Oocyte-specific linker histone is also found in other species such as bovine [23] and *Xenopus laevis* [24]. In *Xenopus*, removal of somatic type histone H1 is mediated by the histone chaperone nucleoplasmin (Table 1) [25]. Amphibian nucleoplasmin is also necessary for decondensing sperm and embryonic cell chromatin [26]. Although the extent of sperm chromatin decondensation by nucleoplasmin is less prominent in mammals, three nucleoplasmin family proteins cooperatively work to decondense sperm chromatin [27, 28]. One report has suggested that injection of *Xenopus* nucleoplasmin into bovine oocytes enhances reprogramming in NT embryos [29]. It would be interesting to test whether nucleoplasmin also plays a role in somatic chromatin decondensation in NT embryos.

Table 1. Maternal factors that are involved in somatic cell nuclear reprogramming*

Name of factors	Species	Systems	Requirement for development of NT embryos	Functions
H1foo/B4	Murine, Bovine, Xenopus	NT to MII oocytes, NT to Xenopus GV oocytes, Xenopus egg extract	Not determined	Chromatin mobility change
H3.3	Murine, Xenopus	NT to MII oocytes, NT to Xenopus GV oocytes	Somatic derived H3.3 prevents normal development	Gene activation, organisation of chromatin
Nucleolus	Murine, Porcine	NT to MII oocytes	Required (arrest at the early cleavage stage)	Not determined
Tpt1	Bovine, Xenopus	NT to MII oocytes, NT to Xenopus GV oocytes	Required for efficient development in cow	Activation of embryonic genes in Xenopus oocytes
DJ1	Porcine	NT to MII oocytes	Required (arrest at the early cleavage stage)	Inhibit P53 activation in early embryos
Tet3	Murine	NT to MII oocytes	Not determined	Hydroxymethylation for DNA demethylation on developmentally important genes
HIRA	Xenopus	NT to Xenopus GV oocytes	Not determined	Deposition of H3.3 for subsequent gene activation
Nuclear actin	Xenopus	NT to Xenopus GV oocytes	Not determined	Activation of embryonic genes in Xenopus oocytes
Nucleoplasmin	Xenopus	Xenopus egg extract	Not determined	Chromatin decondensation, Histone chaperone
Brg1	Xenopus	Xenopus egg extract	Not determined	Activation of embryonic genes
Frgy2a/2b	Xenopus	Xenopus egg extract	Not determined	Disassembly of somatic nucleoli
ISWI	Xenopus	Xenopus egg extract	Not determined	Release TBP from somatic chromatin
Topoisomerase II	Xenopus	Xenopus egg extract	Not determined	Resetting somatic replication origins

*Maternal factors listed in this Table have been functionally validated in egg/oocyte reprogramming systems. Factors validated in more than one species or one system are shown at the top of the list.

Maternal factors that induce epigenetic reprogramming

Somatic nuclei undergo global and active DNA demethylation after NT in some mammalian species although the extent of DNA demethylation is less in NT embryos than in fertilised embryos [30, 31]. In normal fertilisation, several maternal factors have been found to be important for active DNA demethylation of sperm nuclei [32–37]. Among them, Tet3 DNA dioxygenase has been proven to be responsible for DNA demethylation in mouse NT embryos (Table 1) [32, 35]. Tet3 can convert methylated cytosine (5-methylcytosine) to 5-hydroxymethylcytosine for subsequent DNA demethylation. Tet3 is enriched in pseudo-pronuclei where 5-hydroxymethylcytosine is accumulated in NT embryos. Deficiency of Tet3 impairs DNA demethylation of the *Oct4* promoter. In addition to DNA demethylation, histone tail modifications, such as histone acetylation, phosphorylation and methylation, are dynamically changed during reprogramming in NT embryos. Although maternally stored enzymes that directly induce these changes in somatic chromatin have not been clearly identified, a proteomic study of mouse oocytes revealed that oocytes possess a variety of histone modifying enzymes such as Kdm6a, Hdac1 and Mll3 [38]. Considering the crucial roles of histone modifications and DNA methylation in gene regulation, maternal factors that control these important epigenetic modifications in the context of reprogramming may greatly affect the development of NT embryos. In fact, treatment of NT embryos with histone deacetylase inhibitors significantly improves cloning efficiency [39].

Maternal factors required for the development of NT embryos

One clear example of a maternal component neces-

sary for the development of NT embryos is the nucleolus (Table 1) [40]. When the nucleolus is removed from oocytes, these enucleolated oocytes cannot support the development of NT embryos. This also means that oocyte nucleoli components cannot be substituted by those of somatic nuclei. It would be interesting to ask how and why maternal nucleoli play such an essential role in embryonic development. Proteomic analyses of early embryos led to the identification of phosphorylated TPT1 as a candidate reprogramming factor in bovine embryos (Table 1). Overexpression of TPT1 in donor cells improves the development of bovine NT embryos to term [41]. A proteomic approach in combination with the porcine oocyte cell-free system (discussed in a later section) enabled us to identify maternal protein DJ-1 as a protein associating with somatic nuclei during reprogramming (Table 1) [42]. Inhibition of DJ-1 by antibody injection or dominant negative overexpression impairs the development of NT embryos, but not that of fertilised embryos. DJ1-deficient embryos upregulate P53, and P53 inhibition rescues developmental arrest of DJ1-deficient embryos. Most likely, many more maternal factors are required for the successful development of NT embryos. It is therefore important to search for other maternal factors necessary for the development of NT embryos. The list of candidate reprogramming factors produced by proteomic analyses [38, 42], transcriptome analyses [43] and a systems genetics approach [44] will help to identify such factors.

Nuclear Transfer to Embryos

Cloned embryos can also develop after nuclear transfer to the cytoplasm of fertilised zygotes as long as pronuclear factors are not removed during enucleation [45, 46]. These experiments indicate that the factors required for reprogramming somatic nuclei are present in the pronuclei. The existence of reprogramming factors in the pronuclei has been verified by ingenious serial manipulation experiments [47]. Brg1 has been suggested as one of such pronuclear factors [47]. Furthermore, the blastomeres of 2-cell stage mouse embryos can also support reprogramming when the chromosomes has been removed from mitotic embryos and replaced by mitotic donor nuclei [48]. Interestingly, using this NT system, the *Oct4* transgene in T cells is activated within 20 h after NT. Rapid activation of embryonic genes in NT embryos has also been demonstrated using a different NT method [49]. These studies suggest that maternal reprogramming factors might be identified or at least narrowed down by screening pronuclear factors, which are distributed to the cytoplasm during mitosis.

Nuclear Transfer to Oocytes at the Germinal Vesicle Stage

Hundreds of mammalian somatic nuclei can be injected into an oocyte nucleus, called the germinal vesicle (GV), of a *Xenopus* oocyte arrested in the prophase of meiosis I. Injected somatic nuclei initiate new transcription from previously silenced mouse genes including embryonic genes like *Oct4* [50]. An appealing point of using this NT system is that reprogrammed transcripts are detected without the need for cell division and new protein synthesis. Therefore, this type of nuclear transfer does not yield any new cell types, but instead allows us to analyse reprogramming of transcription in a direct manner. The major reprogramming events and factors working in this reprogramming system are explained in their chronological order.

Oocyte-specific linker histone B4 is incorporated soon after NT [51]. This incorporation of histone B4 is associated with an increase in linker histone mobility and is required for transcriptional reprogramming of embryonic genes. Subsequently, Histone H3.3, but not H3.2, is incorporated into transplanted nuclei (Table 1) [52]. Histone variant H3.3 is enriched in oocytes. H3.3 loading onto somatic chromatin is mediated by the histone chaperone, HIRA, and this HIRA-mediated H3.3 deposition is required for transcriptional reprogramming (Table 1). Incorporation of these oocyte-enriched histone variants probably makes somatic chromatin more accessible to other oocyte factors. Epigenetic modifications of chromatin, such as DNA demethylation [53] and histone H3 lysine 4 methylation [54], similarly accelerate transcriptional reprogramming. Transcriptional reprogramming is also enhanced by other oocyte factors. DNA oligonucleotides corresponding to the *Oct4* regulatory sequence were used to search for oocyte proteins that bind to this sequence [55]. Interestingly, tpt1, which plays a role in the development of bovine NT embryos [41], was identified by this screening method (Table 1). Tpt1 in *Xenopus* oocytes is important for activating *Oct4* and *Nanog* from transplanted human nuclei. Recently, we found that polymerised nuclear actin is formed in transplanted nuclei (Table 1) [56]. *Xenopus* oocytes contain an unusually large amount of nuclear actin [57]. If the polymerisation of nuclear actin is impaired, activation of *Oct4* is also inhibited. At the moment it is still not clear how nuclear actin polymerisation affects gene activation, but it seems that changing polymerisation affects the activity of actin-containing chromatin remodelling factors. Additionally, we have recently found that an actin-binding protein that can change polymerisation states of nuclear actin has a

significant impact on transcriptional reprogramming [58].

Incubation in Egg/Oocyte Extracts

Active maternal components can be efficiently extracted without losing their activities by crushing eggs or oocytes using ultra-high speed centrifugation. *Xenopus laevis* eggs/oocytes are used for making extracts due to their large size and abundance. *Xenopus* egg extracts can reproduce early embryonic cell cycle and sperm chromatin remodelling after fertilisation. Kikyo et al. first showed that *Xenopus* egg extracts can also induce reprogramming events in *Xenopus* somatic cells [11]. The reprogramming activities of *Xenopus* egg extracts are active even towards mammalian cells [59–61]. Oocyte extracts from axolotl also have the ability to reprogram mammalian cells [62, 63]. To make functional extracts from mammalian oocytes is challenging because of their size and availability. However, several reports have suggested that partial reprogramming events can be reproduced in mammalian oocyte extracts [12, 64, 65]. The advantages of using extracts for analysing reprogramming are as follows: 1) reprogramming is induced in a large number of cells and hence using extract is adequate for investigating reprogramming by biochemical means; 2) factors can be easily depleted from extracts (especially *Xenopus* extracts) by immunodepletion; and 3) the complexity of a live cell or egg can be somewhat simplified in extracts. In the following sections I summarise reprogramming factors and the mechanisms elucidated in extracts.

Reprogramming in Xenopus egg extracts

When *Xenopus* somatic nuclei are incubated in *Xenopus* egg extracts, chromatin proteins that support active transcription in somatic nuclei, such as TATA-binding protein (TBP) and the general transcription factor TFIIB, are released [11]. This removal of the transcription machinery is in good agreement with the lack of apparent transcriptional activity in early embryos, and is also observed in NT embryos [66]. While the transcription machinery is being released, egg-specific or egg-enriched proteins, such as histone B4, nucleoplasmin and nucleosomal ATPase ISWI, are incorporated into somatic nuclei. Interestingly, egg extract-derived ISWI plays a key role in TBP removal from somatic chromatin (Table 1). Apart from somatic chromatin remodelling, nucleoli in somatic nuclei are disassembled during incubation in egg extracts and nucleolus disassembly is known to be induced in NT embryos [67]. Fractionation of extracts has led to the identification of FRGY2a/b as a critical factor for nucleolar disassembly (Table 1) [68]. FRGY2a/b cooperates with nucleolar protein B23 for nucleolar disassembly [69]. The studies cited above were the first to identify the actual egg factors involved in somatic cell reprogramming, proving that using an egg cell-free system is a valid method for identifying reprogramming mechanisms. Egg extracts have also been used to study reprogramming of DNA replication [17]. In embryos, DNA is replicated more rapidly than in somatic nuclei, suggesting that after NT somatic nuclei need to change their pattern of replication to that of embryos. If erythrocyte nuclei are incubated in S phase egg extracts, they replicate less efficiently than sperm nuclei do. Exposure of erythrocyte nuclei to metaphase egg extracts prior to incubation in S phase extracts increases replication origins and DNA replication in erythrocyte nuclei occurs as rapidly and efficiently as in sperm nuclei. Topoisomerase II in metaphase extracts is involved in resetting somatic DNA replication origins and contributes to subsequent, efficient DNA replication (Table 1).

The ability of *Xenopus* egg extracts to induce reprogramming is not limited to intraspecies somatic nuclei. Mammalian nuclei incubated in *Xenopus* egg extracts are also reprogrammed although the extent of reprogramming is reduced compared to intraspecies nuclei [68]. After incubation of mammalian nuclei in egg extracts, the incorporation of *Xenopus* egg factors to mammalian chromatin is observed, including nucleoplasmin [26], egg type lamin LIII [60] and histone B4 (Table 1) [61]. Nucleoplasmin is important for decondensing heterochromatin [26]. Simultaneously, the removal of somatic nuclear proteins, such as lamin A/C [60], HP1β [26] and TBP [70], is observed. Histone modifications, such as acetylation [61, 62], phosphorylation [54] and methylation [62], also change during incubation in amphibian egg and oocyte extracts. Aurora B kinase activity is important for histone H3 serine 10 phosphorylation during oocyte extract treatment [54]. In addition, the cell membrane of mammalian cells can be mildly permeabilised with a low concentration of Streptolysin O [71, 72] or digitonin [59, 70] allowing permeabilised cells to be returned to culture after extract treatment. Although reversible permeabilisation is technically difficult and requires careful optimisation, this technique makes it possible to assess the functional significance of extract treatment on changes in gene expression. After several days of culture, cells treated with egg extracts start to express embryonic genes including *Oct4* and *Nanog* [16, 59, 61, 70]. Egg-derived chromatin-remodelling factor Brg1 has been shown to be important for *Oct4* upregulation in extract-treated cells in culture [59]. Interestingly, the extract-treated cells can support

better development of NT embryos [73–76], suggesting that the nuclear states of somatic cells after extract treatment are more amenable to reprogramming in mammalian oocytes. In summary, *Xenopus* egg extract-mediated reprogramming includes chromatin remodelling and embryonic gene activation, but the extract treatment itself is not enough to make somatic cells pluripotent. *Xenopus* egg extracts are suited to the analysis and identification of the maternal factors that are responsible for somatic chromatin remodelling. In contrast, the cell proliferation that accompanies cell culture makes it difficult to use this system for elucidating the molecular mechanisms of mammalian embryonic gene activation induced by egg factors. Axolotol oocyte extracts have been reported to induce embryonic gene activation in mammalian nuclei within 6 h without the need for subsequent cell culture [62, 63]. This direct induction of embryonic gene transcription makes extracts ideal for investigating transcriptional reprogramming.

Extracts from mammalian oocytes

Considering that interspecies NT works much less efficiently than intraspecies NT [77], it is reasonable to postulate that mammalian somatic nuclei are better reprogrammed in mammalian oocyte extracts than in *Xenopus* egg extracts. However, it is practically challenging to collect enough mammalian oocytes for extract preparation since a mammalian oocyte is approximately 4,000 times smaller than a *Xenopus* egg/oocyte. Nevertheless, some progress has been made in mammalian oocyte extract-mediated reprogramming. An initial attempt to use mammalian oocytes for analysing reprogramming was done using porcine oocytes, which can be obtained in abundance from local slaughterhouses [78]. Oocytes were disrupted with repeated freeze-thaw cycles and somatic nuclei were incubated with oocyte extracts. Many oocyte proteins that were associated with somatic nuclei during incubation were identified. Although the ability of porcine oocyte extracts to reprogram somatic nuclei was not tested, this study suggests the potential use of mammalian oocyte extracts for analysing reprogramming. That study was followed by the use of lysates of porcine oocytes [79] and mouse oocytes [64] for reprogramming permeabilised somatic cells. Interestingly, lysates from the cytoplasm of mouse GV oocytes induced demethylation of histone H3 lysines 9, and somatic cells incubated in these lysates support higher development of NT embryos when used as donor cells [64]. The first functional extracts used to analyse reprogramming were made from both porcine MII oocytes and GV oocytes by following the protocol used for *Xenopus* egg extract preparation with modifications [12]. Extracts from MII oocytes induce epigenetic reprogramming such as histone deacetylation and TBP removal, while extracts from GV oocytes trigger activation of embryonic genes from extract-treated cells after culture. These results suggest that GV and MII oocytes possess differential reprogramming abilities towards somatic nuclei. Porcine oocyte extracts were used to identify oocyte proteins that were incorporated into somatic nuclei during extract-mediated reprogramming [42]. This approach successfully identified oocyte proteins that are associated with reprogramming. Protein DJ-1 was identified as an important maternal protein for the development of NT embryos (Table 1, explained in the first section). Interestingly, many proteins related to DNA replication, such as PCNA and GINS complex, are identified after treatment with MII oocyte extracts, suggesting that reprogramming of DNA replication may start at the metaphase stage in mammals, as is the case in *Xenopus* [17]. This procedure of preparing mammalian oocyte extracts has also been used in bovine. For example, bovine oocyte extracts retain some reprogramming activities [80, 81]. Recently, porcine oocyte extracts from the cytoplasm of GV oocytes have been used to induce dedifferentiation in porcine somatic cells, and porcine pluripotent cells have been successfully obtained [65]. This interesting study suggests that the sophistication of the mammalian oocyte cell-free system may enable us to reproduce a wider range of reprogramming events in oocyte extracts, and may be used as a tool to dedifferentiate somatic cells.

Conclusions and Perspectives

Reprogramming of somatic cells to an embryonic state by eggs and oocytes entails multiple and complex processes. Transplanted somatic nuclei have to erase their differentiated cell identity, including epigenetic memories of a somatic transcription state, and build an embryonic one to successfully activate embryonic genes that lead to the establishment of pluripotency during early embryonic development. In order to understand such complicated processes, systems that can recapitulate each phase of reprogramming, such as the egg/oocyte cell-free system and the *Xenopus* oocyte nuclear transfer, have been used. Our current knowledge of maternal factors involved in reprogramming is summarised in Table 1. Based on our current understanding, below I propose a model for the early phase of nuclear reprogramming in NT embryos (Fig. 1).

Reprogramming initiates soon after transplanting a somatic nucleus to an enucleated egg/oocyte. The earli-

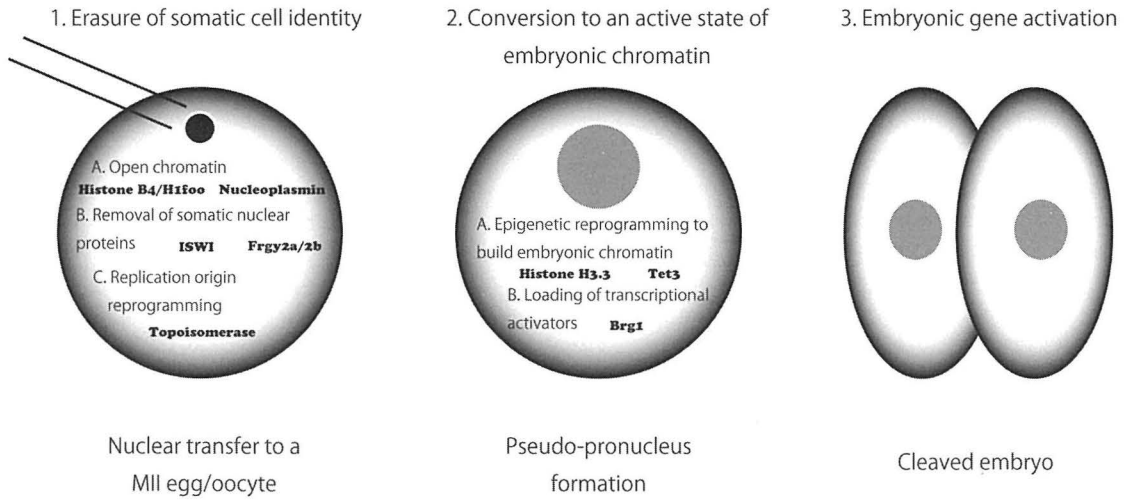

Fig. 1. A simplified model of the early phase of nuclear reprogramming in nuclear transfer embryos. Nuclear events related to reprogramming of somatic cells are shown. There are three major processes for the early phase of reprogramming in NT embryos: 1) erasure of somatic cell identity, 2) building embryonic chromatin for subsequent gene activation, and 3) activation of embryonic genes. Each process consists of multiple nuclear events, which are induced by distinct maternal reprogramming factors. Reprogramming factors are shown in bold letters. A detailed explanation of each factor or process is given in the corresponding part of the main text.

est possible event is the incorporation of maternal linker histone H1foo/B4 into somatic chromatin. This maternal linker histone binding seems to happen at a genome-wide level and globally destabilises somatic chromatin, making it accessible to other maternal factors. At the same time, histone chaperones like nucleoplasmin decondense somatic heterochromatin. These maternal factors thus make somatic chromatin more accessible and allow subsequent chromatin remodelling. For example, ISWI, which mediates TBP removal from somatic chromatin, is active on B4-containing chromatin, but not on somatic H1-containing chromatin [82]. Such chromatin remodelling actively contributes to the erasure of transcriptional memories of somatic chromatin. At the same time, NT embryos have to prepare for the rapid DNA replication. Topoisomerase II can induce changes from somatic DNA replication origins to embryonic ones, resulting in the erasure of somatic replication memory. When NT embryos are activated, pseudo-pronuclei are assembled and an embryonic type of nuclear organisation is formed [18]. For the formation of this unique nuclear structure, somatic nucleoli are disassembled with the help of maternal factors like FRGY2a/b, and maternally derived nucleoli are utilised instead. In pseudo-pronuclei, epigenetic reprogramming continues for subsequent embryonic genome activation. HIRA-mediated deposition of H3.3 onto somatic chromatin further enables the accessible states of chromatin. Tet3-meditaed active DNA demethylation is important for activation of developmentally important genes. Transcriptional activators including BRG1 are gradually incorporated into pseudo-pronuclei [66], allowing embryonic genome activation. Nuclear actin may work at this stage by enhancing chromatin remodelling via actin-containing remodelling complexes and the transcription of embryonic genes [83]. Successful embryonic gene activation allows further development of NT embryos towards blastocyst embryos from which ES cells are derived. It is most likely that reprogramming of somatic nuclei continues throughout early embryonic development, since the number of abnormally expressed genes often decreases towards the blastocyst stage [84, 85]. It is also noteworthy that NT embryos seem to be more susceptible to cellular stresses [86–88]. Therefore, other maternal factors, such as DJ-1 and Tpt1, are needed to cope with stress-induced embryonic arrest. This notion also opens up an interesting idea, that the requirement of maternal factors for development and reprogramming might be slightly different between fertilised and NT embryos.

Although the nuclear transfer technique has long been used to produce cloned animals, we only have a partial understanding of which molecules can induce reprogramming in eggs and oocytes (Table 1). Presumably, many other maternal factors that are involved in reprogramming have not been identified or characterised yet. In fact, a large-scale screening of the transcription

factors that enhance iPS-mediated reprogramming has identified maternal transcription factor Glis1 [89]. This not only represents a new way of finding maternal reprogramming factors without using eggs and oocytes, but also exemplifies that maternal factors can be used for improving reprogramming of somatic cells towards pluripotency. In addition, the recent success of deriving human embryonic stem cells using human somatic cell nuclear transfer with a very high efficiency [9], strongly supports the idea that revealing maternal reprogramming factors will be invaluable for achieving efficient reprogramming of somatic cells in future regenerative medicine.

Acknowledgements

I am grateful to Dr JB Gurdon for useful discussions and Dr J Jullien for his critical reading of the manuscript. K.M. is a Research Fellow at Wolfson College in the University of Cambridge and is funded by the Herchel Smith Postdoctoral Fellowship and Great Britain Sasakawa Foundation.

References

1) Gurdon, J.B., Elsdale, T.R. and Fischberg, M. (1958): Sexually mature individuals of Xenopus laevis from the transplantation of single somatic nuclei. Nature, 182, 64–65.
2) Gurdon, J.B. (1962): The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. J. Embryol. Exp. Morphol., 10, 622–640.
3) Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J. and Campbell, K.H. (1997): Viable offspring derived from fetal and adult mammalian cells. Nature, 385, 810–813.
4) Wakayama, T., Perry, A.C., Zuccotti, M., Johnson, K.R. and Yanagimachi, R. (1998): Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature, 394, 369–374.
5) Takahashi, K. and Yamanaka, S. (2006): Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 126, 663–676.
6) Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. and Yamanaka, S. (2007): Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell, 131, 861–872.
7) Polo, J.M., Liu, S., Figueroa, M.E., Kulalert, W., Eminli, S., Tan, K.Y., Apostolou, E., Stadtfeld, M., Li, Y., Shioda, T., Natesan, S., Wagers, A.J., Melnick, A., Evans, T. and Hochedlinger, K. (2010): Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nat. Biotechnol., 28, 848–855.
8) Kim, K., Doi, A., Wen, B., Ng, K., Zhao, R., Cahan, P., Kim, J., Aryee, M.J., Ji, H., Ehrlich, L.I., Yabuuchi, A., Takeuchi, A., Cunniff, K.C., Hongguang, H., McKinney-Freeman, S., Naveiras, O., Yoon, T.J., Irizarry, R.A., Jung, N., Seita, J., Hanna, J., Murakami, P., Jaenisch, R., Weissleder, R., Orkin, S.H., Weissman, I.L., Feinberg, A.P. and Daley, G.Q. (2010): Epigenetic memory in induced pluripotent stem cells. Nature, 467, 285–290.
9) Tachibana, M., Amato, P., Sparman, M., Gutierrez, N.M., Tippner-Hedges, R., Ma, H., Kang, E., Fulati, A., Lee, H.S., Sritanaudomchai, H., Masterson, K., Larson, J., Eaton, D., Sadler-Fredd, K., Battaglia, D., Lee, D., Wu, D., Jensen, J., Patton, P., Gokhale, S., Stouffer, R.L., Wolf, D. and Mitalipov, S. (2013): Human embryonic stem cells derived by somatic cell nuclear transfer. Cell, 153, 1228–1238.
10) Jullien, J., Pasque, V., Halley-Stott, R.P., Miyamoto, K. and Gurdon, J.B. (2011): Mechanisms of nuclear reprogramming by eggs and oocytes: a deterministic process? Nat. Rev. Mol. Cell Biol., 12, 453–459.
11) Kikyo, N., Wade, P.A., Guschin, D., Ge, H. and Wolffe, A.P. (2000): Active remodeling of somatic nuclei in egg cytoplasm by the nucleosomal ATPase ISWI. Science, 289, 2360–2362.
12) Miyamoto, K., Tsukiyama, T., Yang, Y., Li, N., Minami, N., Yamada, M. and Imai, H. (2009): Cell-free extracts from mammalian oocytes partially induce nuclear reprogramming in somatic cells. Biol. Reprod., 80, 935–943.
13) Campbell, K.H., Loi, P., Otaegui, P.J. and Wilmut, I. (1996): Cell cycle co-ordination in embryo cloning by nuclear transfer. Rev. Reprod., 1, 40–46.
14) Wakayama, T. (2007): Production of cloned mice and ES cells from adult somatic cells by nuclear transfer: how to improve cloning efficiency? J. Reprod. Dev., 53, 13–26.
15) Sung, L.Y., Shen, P.C., Jeong, B.S., Xu, J., Chang, C.C., Cheng, W.T., Wu, J.S., Lee, S.N., Broek, D., Faber, D., Tian, X.C., Yang, X. and Du, F. (2007): Premature chromosome condensation is not essential for nuclear reprogramming in bovine somatic cell nuclear transfer. Biol. Reprod., 76, 232–240.
16) Ganier, O., Bocquet, S., Peiffer, I., Brochard, V., Arnaud, P., Puy, A., Jouneau, A., Feil, R., Renard, J.P. and Mechali, M. (2011): Synergic reprogramming of mammalian cells by combined exposure to mitotic Xenopus egg extracts and transcription factors. Proc. Natl. Acad. Sci. U.S.A., 108, 17331–17336.
17) Lemaitre, J.M., Danis, E., Pasero, P., Vassetzky, Y. and Mechali, M. (2005): Mitotic remodeling of the replicon and chromosome structure. Cell, 123, 787–801.
18) Martin, C., Brochard, V., Migne, C., Zink, D., Debey, P. and Beaujean, N. (2006): Architectural reorganization of the nuclei upon transfer into oocytes accompanies genome reprogramming. Mol. Reprod. Dev., 73, 1102–1111.
19) Wakayama, T., Tabar, V., Rodriguez, I., Perry, A.C., Studer, L. and Mombaerts, P. (2001): Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer. Science, 292, 740–743.
20) Teperek, M. and Miyamoto, K. (2013): Nuclear reprogramming of sperm and somatic nuclei in eggs and oocytes. Reprod. Med. Biol., in press.
21) Gao, S., Chung, Y.G., Parseghian, M.H., King, G.J., Adashi, E.Y. and Latham, K.E. (2004): Rapid H1 linker histone transitions following fertilization or somatic cell nuclear trans-

fer: evidence for a uniform developmental program in mice. Dev. Biol., 266, 62–75.
22) Teranishi, T., Tanaka, M., Kimoto, S., Ono, Y., Miyakoshi, K., Kono, T. and Yoshimura, Y. (2004): Rapid replacement of somatic linker histones with the oocyte-specific linker histone H1foo in nuclear transfer. Dev. Biol., 266, 76–86.
23) McGraw, S., Vigneault, C., Tremblay, K. and Sirard, M.A. (2006): Characterization of linker histone H1FOO during bovine in vitro embryo development. Mol. Reprod. Dev., 73, 692–699.
24) Smith, R.C., Dworkin-Rastl, E. and Dworkin, M.B. (1988): Expression of a histone H1-like protein is restricted to early Xenopus development. Genes. Dev., 2, 1284--1295.
25) Dimitrov, S. and Wolffe, A.P. (1996): Remodeling somatic nuclei in Xenopus laevis egg extracts: molecular mechanisms for the selective release of histones H1 and H1(0) from chromatin and the acquisition of transcriptional competence. EMBO J., 15, 5897–5906.
26) Tamada, H., Van Thuan, N., Reed, P., Nelson, D., Katoku-Kikyo, N., Wudel, J., Wakayama, T. and Kikyo, N. (2006): Chromatin decondensation and nuclear reprogramming by nucleoplasmin. Mol. Cell Biol., 26, 1259–1271.
27) Inoue, A., Ogushi, S., Saitou, M., Suzuki, M.G. and Aoki, F. (2011): Involvement of mouse nucleoplasmin 2 in the decondensation of sperm chromatin after fertilization. Biol. Reprod., 85, 70–77.
28) Okuwaki, M., Sumi, A., Hisaoka, M., Saotome-Nakamura, A., Akashi, S., Nishimura, Y. and Nagata, K. (2012): Function of homo- and hetero-oligomers of human nucleoplasmin/nucleophosmin family proteins NPM1, NPM2 and NPM3 during sperm chromatin remodeling. Nucleic. Acids. Res., 40, 4861–4878.
29) Betthauser, J.M., Pfister-Genskow, M., Xu, H., Golueke, P.J., Lacson, J.C., Koppang, R.W., Myers, C., Liu, B., Hoeschele, I., Eilertsen, K.J. and Leno, G.H. (2006): Nucleoplasmin facilitates reprogramming and in vivo development of bovine nuclear transfer embryos. Mol. Reprod. Dev., 73, 977–986.
30) Dean, W., Santos, F., Stojkovic, M., Zakhartchenko, V., Walter, J., Wolf, E. and Reik, W. (2001): Conservation of methylation reprogramming in mammalian development: aberrant reprogramming in cloned embryos. Proc. Natl. Acad. Sci. U.S.A., 98, 13734–13738.
31) Chan, M.M., Smith, Z.D., Egli, D., Regev, A. and Meissner, A. (2012): Mouse ooplasm confers context-specific reprogramming capacity. Nat. Genet., 44, 978–980.
32) Gu, T.P., Guo, F., Yang, H., Wu, H.P., Xu, G.F., Liu, W., Xie, Z.G., Shi, L., He, X., Jin, S.G., Iqbal, K., Shi, Y.G., Deng, Z., Szabo, P.E., Pfeifer, G.P., Li, J. and Xu, G.L. (2011): The role of Tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. Nature, 477, 606–610.
33) Hajkova, P., Jeffries, S.J., Lee, C., Miller, N., Jackson, S.P. and Surani, M.A. (2010): Genome-wide reprogramming in the mouse germ line entails the base excision repair pathway. Science, 329, 78–82.
34) Wossidlo, M., Arand, J., Sebastiano, V., Lepikhov, K., Boiani, M., Reinhardt, R., Scholer, H. and Walter, J. (2010): Dynamic link of DNA demethylation, DNA strand breaks and repair in mouse zygotes. EMBO J., 29, 1877–1888.
35) Wossidlo, M., Nakamura, T., Lepikhov, K., Marques, C.J., Zakhartchenko, V., Boiani, M., Arand, J., Nakano, T., Reik, W. and Walter, J. (2011): 5-Hydroxymethylcytosine in the mammalian zygote is linked with epigenetic reprogramming. Nat. Commun., 2, 241.
36) Hatanaka, Y., Shimizu, N., Nishikawa, S., Tokoro, M., Shin, S.W., Nishihara, T., Amano, T., Anzai, M., Kato, H., Mitani, T., Hosoi, Y., Kishigami, S. and Matsumoto, K. (2013): GSE is a maternal factor involved in active DNA demethylation in zygotes. PLoS One, 8, e60205.
37) Okada, Y., Yamagata, K., Hong, K., Wakayama, T. and Zhang, Y. (2010): A role for the elongator complex in zygotic paternal genome demethylation. Nature, 463, 554–558.
38) Pfeiffer, M.J., Siatkowski, M., Paudel, Y., Balbach, S.T., Baeumer, N., Crosetto, N., Drexler, H.C., Fuellen, G. and Boiani, M. (2011): Proteomic analysis of mouse oocytes reveals 28 candidate factors of the "reprogrammome". J. Proteome. Res., 10, 2140–2153.
39) Kishigami, S., Mizutani, E., Ohta, H., Hikichi, T., Thuan, N.V., Wakayama, S., Bui, H.T. and Wakayama, T. (2006): Significant improvement of mouse cloning technique by treatment with trichostatin A after somatic nuclear transfer. Biochem. Biophys. Res. Commun., 340, 183–189.
40) Ogushi, S., Palmieri, C., Fulka, H., Saitou, M., Miyano, T. and Fulka, J., Jr. (2008): The maternal nucleolus is essential for early embryonic development in mammals. Science, 319, 613–616.
41) Tani, T., Shimada, H., Kato, Y. and Tsunoda, Y. (2007): Bovine oocytes with the potential to reprogram somatic cell nuclei have a unique 23-kDa protein, phosphorylated transcriptionally controlled tumor protein (TCTP). Cloning Stem Cells, 9, 267–280.
42) Miyamoto, K., Nagai, K., Kitamura, N., Nishikawa, T., Ikegami, H., Binh, N.T., Tsukamoto, S., Matsumoto, M., Tsukiyama, T., Minami, N., Yamada, M., Ariga, H., Miyake, M., Kawarasaki, T., Matsumoto, K. and Imai, H. (2011): Identification and characterization of an oocyte factor required for development of porcine nuclear transfer embryos. Proc. Natl. Acad. Sci. U.S.A., 108, 7040–7045.
43) Awe, J.P. and Byrne, J.A. (2013): Identifying candidate oocyte reprogramming factors using cross-species global transcriptional analysis. Cell Reprogram, 15, 126–133.
44) Cheng, Y., Gaughan, J., Midic, U., Han, Z., Liang, C.G., Patel, B.G. and Latham, K.E. (2013): Systems genetics implicates cytoskeletal genes in oocyte control of cloned embryo quality. Genetics, 193, 877–896.
45) Greda, P., Karasiewicz, J. and Modlinski, J.A. (2006): Mouse zygotes as recipients in embryo cloning. Reproduction, 132, 741–748.
46) Egli, D., Rosains, J., Birkhoff, G. and Eggan, K. (2007): Developmental reprogramming after chromosome transfer into mitotic mouse zygotes. Nature, 447, 679–685.
47) Egli, D. and Eggan, K. (2010): Recipient cell nuclear factors are required for reprogramming by nuclear transfer. Development, 137, 1953–1963.
48) Egli, D., Sandler, V.M., Shinohara, M.L., Cantor, H. and Eggan, K. (2009): Reprogramming after chromosome trans-

fer into mouse blastomeres. Curr. Biol., 19, 1403–1409.
49) Egli, D., Chen, A.E., Saphier, G., Ichida, J., Fitzgerald, C., Go, K.J., Acevedo, N., Patel, J., Baetscher, M., Kearns, W.G., Goland, R., Leibel, R.L., Melton, D.A. and Eggan, K. (2011): Reprogramming within hours following nuclear transfer into mouse but not human zygotes. Nat. Commun., 2, 488.
50) Byrne, J.A., Simonsson, S., Western, P.S. and Gurdon, J.B. (2003): Nuclei of adult mammalian somatic cells are directly reprogrammed to oct-4 stem cell gene expression by amphibian oocytes. Curr. Biol., 13, 1206–1213.
51) Jullien, J., Astrand, C., Halley-Stott, R.P., Garrett, N. and Gurdon, J.B. (2010): Characterization of somatic cell nuclear reprogramming by oocytes in which a linker histone is required for pluripotency gene reactivation. Proc. Natl. Acad. Sci. U.S.A., 107, 5483–5488.
52) Jullien, J., Astrand, C., Szenker, E., Garrett, N., Almouzni, G. and Gurdon, J.B. (2012): HIRA dependent H3.3 deposition is required for transcriptional reprogramming following nuclear transfer to Xenopus oocytes. Epigenetics Chromatin, 5, 17.
53) Simonsson, S. and Gurdon, J. (2004): DNA demethylation is necessary for the epigenetic reprogramming of somatic cell nuclei. Nat. Cell Biol., 6, 984–990.
54) Murata, K., Kouzarides, T., Bannister, A.J. and Gurdon, J.B. (2010): Histone H3 lysine 4 methylation is associated with the transcriptional reprogramming efficiency of somatic nuclei by oocytes. Epigenetics Chromatin, 3, 4.
55) Koziol, M.J., Garrett, N. and Gurdon, J.B. (2007): Tpt1 activates transcription of oct4 and nanog in transplanted somatic nuclei. Curr. Biol., 17, 801–807.
56) Miyamoto, K., Pasque, V., Jullien, J. and Gurdon, J.B. (2011): Nuclear actin polymerization is required for transcriptional reprogramming of Oct4 by oocytes. Genes Dev., 25, 946–958.
57) Bohnsack, M.T., Stuven, T., Kuhn, C., Cordes, V.C. and Gorlich, D. (2006): A selective block of nuclear actin export stabilizes the giant nuclei of Xenopus oocytes. Nat. Cell Biol., 8, 257–263.
58) Miyamoto, K., Teperek, M., Yusa, K., Allen, G.E., Bradshaw, C.R. and Gurdon, J.B. (2013): Nuclear Wavel is required for reprogramming transcription in oocytes and for normal development. Science, 341, 1002–1005.
59) Hansis, C., Barreto, G., Maltry, N. and Niehrs, C. (2004): Nuclear reprogramming of human somatic cells by xenopus egg extract requires BRG1. Curr. Biol., 14, 1475–1480.
60) Alberio, R., Johnson, A.D., Stick, R. and Campbell, K.H. (2005): Differential nuclear remodeling of mammalian somatic cells by Xenopus laevis oocyte and egg cytoplasm. Exp. Cell Res., 307, 131–141.
61) Miyamoto, K., Furusawa, T., Ohnuki, M., Goel, S., Tokunaga, T., Minami, N., Yamada, M., Ohsumi, K. and Imai, H. (2007): Reprogramming events of mammalian somatic cells induced by Xenopus laevis egg extracts. Mol. Reprod. Dev., 74, 1268–1277.
62) Bian, Y., Alberio, R., Allegrucci, C., Campbell, K.H. and Johnson, A.D. (2009): Epigenetic marks in somatic chromatin are remodelled to resemble pluripotent nuclei by amphibian oocyte extracts. Epigenetics, 4, 194–202.
63) Allegrucci, C., Rushton, M.D., Dixon, J.E., Sottile, V., Shah, M., Kumari, R., Watson, S., Alberio, R. and Johnson, A.D. (2011): Epigenetic reprogramming of breast cancer cells with oocyte extracts. Mol. Cancer, 10, 7.
64) Bui, H.T., Wakayama, S., Kishigami, S., Kim, J.H., Van Thuan, N. and Wakayama, T. (2008): The cytoplasm of mouse germinal vesicle stage oocytes can enhance somatic cell nuclear reprogramming. Development, 135, 3935–3945.
65) Bui, H.T., Kwon, D.N., Kang, M.H., Oh, M.H., Park, M.R., Park, W.J., Paik, S.S., Van Thuan, N. and Kim, J.H. (2012): Epigenetic reprogramming in somatic cells induced by extract from germinal vesicle stage pig oocytes. Development, 139, 4330–4340.
66) Gao, T., Zheng, J., Xing, F., Fang, H., Sun, F., Yan, A., Gong, X., Ding, H., Tang, F. and Sheng, H.Z. (2007): Nuclear reprogramming: the strategy used in normal development is also used in somatic cell nuclear transfer and parthenogenesis. Cell Res., 17, 135–150.
67) Gurdon, J.B. and Brown, D.D. (1965): Cytoplasmic regulation of Rna synthesis and nucleolus formation in developing embryos of Xenopus laevis. J. Mol. Biol., 12, 27–35.
68) Gonda, K., Fowler, J., Katoku-Kikyo, N., Haroldson, J., Wudel, J. and Kikyo, N. (2003): Reversible disassembly of somatic nucleoli by the germ cell proteins FRGY2a and FRGY2b. Nat. Cell Biol., 5, 205–210.
69) Gonda, K., Wudel, J., Nelson, D., Katoku-Kikyo, N., Reed, P., Tamada, H. and Kikyo, N. (2006): Requirement of the protein B23 for nucleolar disassembly induced by the FRGY2a family proteins. J. Biol. Chem., 281, 8153–8160.
70) Miyamoto, K., Yamashita, T., Tsukiyama, T., Kitamura, N., Minami, N., Yamada, M. and Imai, H. (2008): Reversible membrane permeabilization of mammalian cells treated with digitonin and its use for inducing nuclear reprogramming by Xenopus egg extracts. Cloning Stem Cells, 10, 535–542.
71) Walev, I., Bhakdi, S.C., Hofmann, F., Djonder, N., Valeva, A., Aktories, K. and Bhakdi, S. (2001): Delivery of proteins into living cells by reversible membrane permeabilization with streptolysin-O. Proc. Natl. Acad. Sci. U.S.A., 98, 3185–3190.
72) Taranger, C.K., Noer, A., Sorensen, A.L., Hakelien, A.M., Boquest, A.C. and Collas, P. (2005): Induction of dedifferentiation, genomewide transcriptional programming, and epigenetic reprogramming by extracts of carcinoma and embryonic stem cells. Mol. Biol. Cell, 16, 5719–5735.
73) Liu, Y., Ostrup, O., Li, J., Vajta, G., Kragh, P.M., Purup, S. and Callesen, H. (2011): Cell colony formation induced by Xenopus egg extract as a marker for improvement of cloned blastocyst formation in the pig. Cell Reprogram, 13, 521–526.
74) Liu, Y., Ostrup, O., Li, J., Vajta, G., Lin, L., Kragh, P.M., Purup, S., Hyttel, P. and Callesen, H. (2012): Increased blastocyst formation of cloned porcine embryos produced with donor cells pre-treated with Xenopus egg extract and/or digitonin. Zygote, 20, 61–66.
75) Yang, X., Mao, J., Walters, E.M., Zhao, M.T., Teson, J., Lee, K. and Prather, R.S. (2012): Xenopus egg extract treatment

reduced global DNA methylation of donor cells and enhanced somatic cell nuclear transfer embryo development in pigs. Biores. Open Access, 1, 79–87.

76) Rathbone, A.J., Fisher, P.A., Lee, J.H., Craigon, J. and Campbell, K.H. (2010): Reprogramming of ovine somatic cells with Xenopus laevis oocyte extract prior to SCNT improves live birth rate. Cell Reprogram, 12, 609–616.

77) Narbonne, P., Miyamoto, K. and Gurdon, J.B. (2012): Reprogramming and development in nuclear transfer embryos and in interspecific systems. Curr. Opin. Genet Dev., 22, 450–458.

78) Novak, S., Paradis, F., Savard, C., Tremblay, K. and Sirard, M.A. (2004): Identification of porcine oocyte proteins that are associated with somatic cell nuclei after co-incubation. Biol. Reprod., 71, 1279–1289.

79) Tong, G.Q., Heng, B.C. and Ng, S.C. (2006): Exposure of mouse cumulus cell nuclei to porcine ooplasmic extract eliminates TATA box protein binding to chromatin, but has no effect on DNA methylation. J. Assist Reprod. Genet, 23, 413–419.

80) Xiong, X.R., Li, J., Fu, M., Gao, C., Wang, Y. and Zhong, J.C. (2013): Oocyte extract improves epigenetic reprogramming of yak fibroblast cells and cloned embryo development. Theriogenology, 79, 462–469.

81) Tang, S., Wang, Y., Zhang, D., Gao, Y., Ma, Y., Yin, B., Sun, J., Liu, J. and Zhang, Y. (2009): Reprogramming donor cells with oocyte extracts improves in vitro development of nuclear transfer embryos. Anim. Reprod. Sci., 115, 1–9.

82) Saeki, H., Ohsumi, K., Aihara, H., Ito, T., Hirose, S., Ura, K. and Kaneda, Y. (2005): Linker histone variants control chromatin dynamics during early embryogenesis. Proc. Natl. Acad. Sci. U.S.A., 102, 5697–5702.

83) Miyamoto, K. and Gurdon, J.B. (2013): Transcriptional regulation and nuclear reprogramming: roles of nuclear actin and actin-binding proteins. Cell Mol. Life Sci., 70, 3289–3302.

84) Cao, F., Fukuda, A., Watanabe, H. and Kono, T. (2013): The transcriptomic architecture of mouse Sertoli cell clone embryos reveals temporal-spatial-specific reprogramming. Reproduction, 145, 277–288.

85) Smith, S.L., Everts, R.E., Tian, X.C., Du, F., Sung, L.Y., Rodriguez-Zas, S.L., Jeong, B.S., Renard, J.P., Lewin, H.A. and Yang, X. (2005): Global gene expression profiles reveal significant nuclear reprogramming by the blastocyst stage after cloning. Proc. Natl. Acad. Sci. U.S.A., 102, 17582–17587.

86) Hao, Y., Lai, L., Mao, J., Im, G.S., Bonk, A. and Prather, R.S. (2003): Apoptosis and in vitro development of preimplantation porcine embryos derived in vitro or by nuclear transfer. Biol. Reprod., 69, 501–507.

87) Boiani, M., Gentile, L., Gambles, V.V., Cavaleri, F., Redi, C.A. and Scholer, H.R. (2005): Variable reprogramming of the pluripotent stem cell marker Oct4 in mouse clones: distinct developmental potentials in different culture environments. Stem Cells, 23, 1089–1104.

88) Rodriguez-Osorio, N., Wang, Z., Kasinathan, P., Page, G.P., Robl, J.M. and Memili, E. (2009): Transcriptional reprogramming of gene expression in bovine somatic cell chromatin transfer embryos. BMC Genomics, 10, 190.

89) Maekawa, M., Yamaguchi, K., Nakamura, T., Shibukawa, R., Kodanaka, I., Ichisaka, T., Kawamura, Y., Mochizuki, H., Goshima, N. and Yamanaka, S. (2011): Direct reprogramming of somatic cells is promoted by maternal transcription factor Glis1. Nature, 474, 225–229.

体細胞核の初期化に関与する卵内因子に関して……68–78

宮本　圭　　ケンブリッジ大学ウェルカムトラスト／英国癌研究基金ガードン研究所，ケンブリッジ CB2 1QN

体細胞核を卵子内に移植することによって，移植された核に初期化が誘導され，再構築胚（核移植胚）の胚発生が開始する．初期化された核によって核移植胚は発生を進め，最終的にはクローン動物へと発生する．この体細胞核の初期化は卵子内に蓄えられた因子によって誘導されることが分かっている．卵子に内在する初期化因子と初期化のメカニズムは，何十年もの間哺乳類や両生類を使用して研究されてきた．その方法としては卵子への核移植や卵子抽出液中での培養といった実験系があげられる．本稿ではそれぞれの実験系においてどのような初期化現象が誘導されるのかについて説明し，卵内の初期化因子に関して考察する．最終的には，卵内での体細胞核初期化のモデルを提唱する．

キーワード：無細胞抽出液，卵子と卵母細胞，核の初期化，核移植，クロマチンリモデリング