Section 1 Historical perspective

Chapter 1

Insights into the amphibian egg to understand the mammalian oocyte

Kei Miyamoto and John B. Gurdon

Abstract

Amphibian eggs and oocytes have been widely used as a model system for understanding animal development. They have led to numerous major discoveries in cellular and developmental biology. These findings have greatly helped us to understand the physiology of mammalian oocytes. Amphibian eggs have also played an important role not only in revealing genomic conservation and plasticity historically, but also in gaining a mechanistic insight into nuclear reprogramming. This chapter summarizes major findings using amphibian eggs and oocytes, focusing on reprogramming aspects. We also discuss how *Xenopus* eggs can be used to study mammalian oocytes.

Introduction

For over 100 years, amphibian embryos have been the favored choice of material for research into mechanisms of early vertebrate animal development. This is because amphibian embryos are unusually large, being about 1 mm in diameter. The whole amphibian egg divides into an embryo whereas, in birds, for example, only a very small amount of material in the huge egg actually forms an embryo. All mammalian eggs are relatively inaccessible and are very small, usually 70–120 μm in diameter. European amphibia include the Urodeles (salamanders, newts, *Triturus*, etc.) as well as Anura (frogs, toads, *Rana*, *Bufo*). Members of these groups usually lay abundant eggs in natural pond water in the northern-hemisphere spring. The eggs are easy to culture. Their large size and consistency make them exceptionally favorable for microdissection and other manipulative experiments. This was the material used by Spemann, Hamburger, and Holtfreter and others.

The only disadvantage of most anuran species is that they produce eggs naturally only in the European spring, amounting to one or two months during the year. Soon after World War II, *Xenopus* became the favored choice for amphibian research. The interesting history of how this happened was largely coincidental [1]. The huge advantage of *Xenopus* is that it can be induced to lay eggs at any time of year, following an injection of mammalian pituitary hormone. The species is permanently aquatic, making its laboratory maintenance a great deal easier than for land-living amphibia. Since it naturally lives in highly infected pond water (in Africa) *Xenopus laevis* is exceptionally disease-free and easy to culture. Over the last 50 years, nearly all amphibian research has come to be conducted on *Xenopus* species.

The majority of experimental interventions now carried out on a range of vertebrate species, and especially in mammals, have their origin in work that started with amphibia. Moreover, many scientific discoveries and knowledge in amphibia have been extended to mammals. In this review, we trace back the origin of many experimental procedures and scientific findings that are now in widespread use in mammals, and find that these were first pioneered in amphibia.

Meiotic prophase germ line in *Xenopus laevis* and the mouse

In *Xenopus laevis*, the female germ cell, the so-called oocyte, is arrested in prophase of meiosis I (MI) in the ovary of the adult frog (Figure 1.1; stage I to VI). During this period, oocytes accumulate a stockpile of macromolecules and organelles that are required to support early embryonic development. Stage VI oocytes are fully grown and capable of reacting to progesterone from the surrounding follicle cells. They complete MI and are subsequently arrested in metaphase of meiosis II (MII). These matured

Biology and Pathology of the Oocyte, 2nd edn., ed. Alan Trounson, Roger Gosden, and Ursula Eichenlaub-Ritter.
Published by Cambridge University Press.

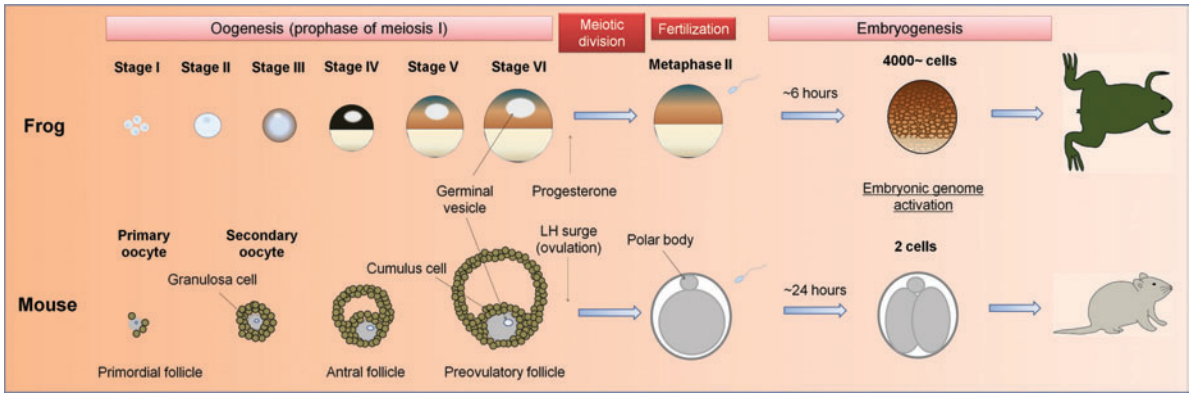

Figure 1.1 Oogenesis and embryogenesis in frog and mouse. Oocytes contain a giant nucleus referred to as the germinal vesicle. Upon resumption of meiosis, germinal vesicles are broken down and oocytes are matured to the metaphase II stage, followed by fertilization. Fertilized embryos undergo early cleavages directed by maternally stored factors without conspicuous transcription. Embryonic genome activation happens at the indicated cell stages, allowing embryos to develop further. LH, luteinizing hormone.

oocytes are then ovulated as unfertilized eggs (MII eggs). Upon fertilization, the egg is released from meiotic arrest and enters interphase. Early embryonic development is characterized by rapid progression through the cell cycle, consisting of repeated S- and M-phases. The stockpile of components present within the eggs supports this early development until the mid-blastula transition (MBT). Major embryonic gene activation starts at this MBT (Stage 8–8.5 embryos; 4000–8000 cells) and embryonic gene products then direct further embryonic development.

Mouse oocytes, as well as *Xenopus* oocytes, are arrested at prophase of MI in the ovary. During oogenesis, mouse oocytes increase their size from ~10 μm to 80 μm while actively transcribing the maternal genome for subsequent embryonic development (Figure 1.1). When the luteinizing hormone (LH) surge stimulates the resumption of meiosis, oocytes surrounded by cumulus cells are released from fully grown follicles. Oocytes are re-arrested at the MII stage until fertilization takes place. Major embryonic genome activation is first observed at the 2-cell stage.

As summarized above, maternal factors required for early embryonic development are accumulated in both *Xenopus* and mouse oocytes. During this oogenesis period, oocytes at the first meiotic prophase contain a giant nucleus referred to as the germinal vesicle (GV). The *Xenopus* GV reaches a diameter of 400 μm, which is more than 100 times larger than that of a mature mouse oocyte itself. It also stores huge amounts of macromolecules and nuclear organelles for intensive transcription; these include extrachromosomal nucleoli (~1500), Cajal bodies (50–100), and RNA polymerase II whose activity is sufficient for 100 000 somatic nuclei [2]. Notably, *Xenopus* GV oocyte genomes form so-called lampbrush chromosomes with actively transcribing chromatin loops and are found throughout chromosomes. Chromatin loops are maximally extended during early oogenesis and retracted towards the fully grown stage (stage VI). In the mouse, although lampbrush-like chromosomes have not been identified, oocyte genomes are also actively transcribed and produce a large stockpile of maternal RNA and protein. The chromatin structure of a mouse oocyte has been extensively studied. Follicular activation, at the beginning of oogenesis, is characterized by the loading of an oocyte-specific linker histone H1foo (closely related to the *Xenopus* histone B4) into the oocyte nucleus. Chromatin in growing mouse oocytes is initially decondensed and supports active transcription. As oogenesis proceeds, chromatin becomes progressively condensed and transcriptionally silenced, forming a heterochromatin rim around the oocyte nucleolus. Recent research suggests that histone-modifying enzymes play roles in the mouse oocyte chromatin remodeling associated with changes in transcriptional abilities [3]. Revealing mechanisms of oocyte transcription and its associated chromatin structure helps our understanding not only of germ cell development but also of the maternal contribution to early embryonic development.

Signaling in early embryogenesis

The first pivotal experiment which demonstrated signaling in development was that of Spemann and Mangold [4]. By transplanting tissue from one embryo

into another (distinguished by pigment markers), it was proved that one set of cells can alter the fate of other cells placed near them. The Spemann signaling center exists in early amphibian embryos at the early gastrula stage. Subsequently, Nieuwkoop [5] demonstrated that signaling also occurs much earlier in development from the vegetal cells to the overlying animal cells. This Nieuwkoop center is the first known source of signaling in animal development and is responsible for the formation of the mesoderm layer.

In more recent years the mechanism of the Spemann signaling process has become greatly clarified. This is particularly due to the work of De Robertis [6] who has identified a number of signaling and other molecules that regulate the signaling process and in particular the distance in an embryo over which a signal factor acts. A network of such signaling centers and of the counteracting molecules that restrict the strength or distance of signaling has been identified [7].

Many such signaling processes work as morphogen gradients. This means that the concentration of signaling factor decreases with distance from its source. Most importantly, cells are able to sense the strength of the signal, at the position in which they lie, and differentiate in directions related to the strength of the signal that they receive. The mechanisms of morphogen gradient interpretation continue to attract wide interest [8]. The phenomenon is of great importance because the single source of signal can generate several different cell types according to the strength and duration of signal that a cell receives. The regulation of morphogen gradient interpretation is complex because it depends on the rate of movement of the morphogen, its stability, and particularly on the abundance of counteracting factors which can inactivate the morphogen [9].

Signaling in embryos is now well established in mammalian development. Gene ablation technologies and the availability of cultured pluripotent stem cells, such as embryonic and epiblast stem cells, in mice accelerated our understanding of how signaling pathways function in mammals [10, 11]. Signaling pathways that play a key role in early post-implantation development, such as Wnt and transforming growth factor beta (TGFβ), have been extensively studied [12].

Cell-free system

Components of amphibian eggs can be efficiently extracted by crushing them in an appropriate buffer. These cell-free extracts retain many egg proteins intact and as a result numerous cellular events, such as transcription, translation, cell-cycle progression, chromatin remodeling, and even reprogramming, are reproduced in the extracts to some extent. *Xenopus laevis* eggs have been widely used as a source of extracts due to their large size and abundance. Egg extracts are valuable for identifying molecules and molecular mechanisms involved in cellular events since many biochemical approaches can be applied to extracts. For example, specific proteins can be depleted from extracts by immunodepletion using antibodies in order to assess the roles of these proteins. To carry out such knockout experiments is very challenging in living embryos. In addition, the complexity of a live cell or egg can be somewhat simplified in extracts. We summarize below major discoveries and recent applications of egg extracts to understand reprogramming.

DNA replication

Xenopus egg extracts that are widely used at present were first reported by Lohka and Masui [13]. Unfertilized frog eggs are collected in a test tube and crushed by centrifugation. After centrifugation, the cytoplasmic fraction is used as an extract (Figure 1.2A). When demembranated sperm nuclei are incubated in this extract, these nuclei are immediately decondensed (Figure 1.2B) and start to form nuclear envelopes and pronuclei, accompanied by DNA replication. This is followed by breakdown of nuclear envelopes and chromosome condensation. This egg extract was further developed and could also replicate purified DNA [14]. Egg factors required for the DNA replication, such as Orc (origin recognition complex, subunit 2) [15], cdc6 (cell division cycle 6) [16], and MCMs (minichromosome maintenance proteins) [17], have been found and characterized in this system.

Cell-cycle analysis

Many important findings using *Xenopus* egg extracts have been achieved in the field of cell-cycle analysis. After the first egg extract, in which a single cell cycle is reproduced [13], Hutchison *et al.* [18] and Murray and Kirschner [19] developed and established the cycling extracts in which multiple cell cycles are reproduced. This led to the identification of cyclin-dependent protein kinase, CDK1, and cyclin B as necessary regulators for mitotic entry. CDK1 and cyclin B are also known as maturation-promoting factor (MPF). Molecules that modulate MPF activity have been extensively studied. Extracts have been also prepared from eggs arrested in

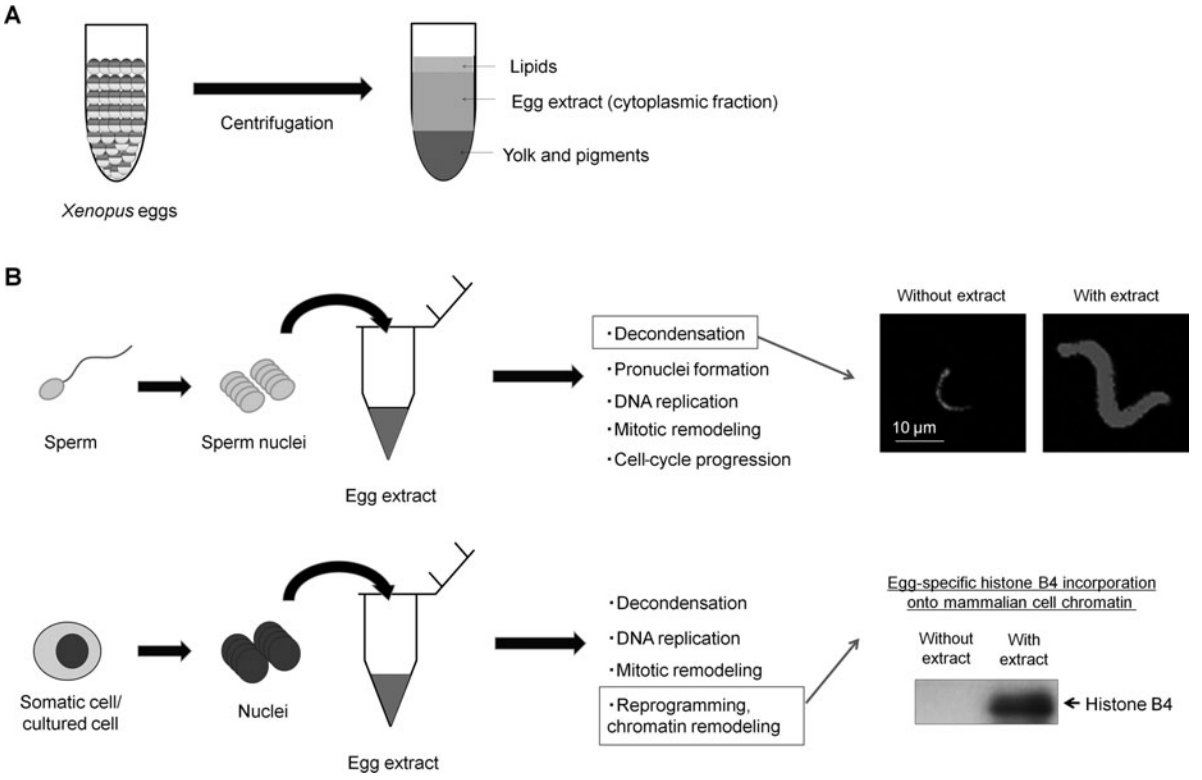

Figure 1.2 *Xenopus* egg extracts and their utility. (A) *Xenopus* eggs collected in a test tube are crushed by centrifugation and separated into three fractions. The middle fraction containing cytoplasm and membranes is used as an egg extract. (B) Various kinds of cellular and molecular events are reproduced in *Xenopus* egg extracts. When sperm nuclei are incubated in egg extracts, rapid decondensation of sperm nuclei is observed as shown. Egg extracts also induce several changes in somatic nuclei. Notably, a part of reprogramming, which includes oocyte linker histone B4 incorporation onto chromatin as revealed by the western blotting, is induced in somatic nuclei.

metaphase of the second meiotic division by an activity called cytostatic factor (CSF) [20]. CSF in connection with MPF activity plays an essential role in MII arrest to prevent parthenogenesis. Although some differences in molecular behavior between *Xenopus* and the mouse have been reported [21], the *Xenopus* egg cell-free system is a powerful tool to analyze biochemical interactions and signaling pathways involved in this meiotic arrest, egg activation, and early embryonic cell cycles.

Chromatin remodeling (sperm decondensation, mitotic remodeling and chromatin assembly)

As previously mentioned, sperm decondensation and male pronucleus formation were induced in frog egg extracts. By utilizing this property, nucleoplasmin in egg extracts was identified as a factor to decondense sperm nuclei and remove protamines from sperm nuclei [22, 23]. Oocyte linker histone B4 is also involved in sperm chromatin remodeling [24].

In addition to the above-mentioned replicating cell-cycle extracts, CSF extracts maintain the metaphase state so that they can induce nuclear envelope breakdown, chromosome condensation, and spindle assembly [20]. The roles of chromosomal proteins, such as topoisomerase IIα and histone H1, in mitotic chromosome assembly have been examined [25, 26]. Condensin necessary for mitotic chromosome condensation was identified using mitotic extracts [27].

Chromatin is formed when double-stranded or single-stranded plasmid DNA molecules are incubated in egg extracts [28, 29], providing unique opportunities to study chromatin assembly. Histones stored in eggs carry distinct patterns of histone modification [30]. Changes in histone modification are related to those of transcriptional activities in oocytes.

Therefore, it would be interesting to study chromatin structures in eggs/oocytes using this system.

Recapitulation of reprogramming in egg/oocyte extracts

Since *Xenopus* egg extracts mimic sperm nuclear remodeling after fertilization, it is reasonable to speculate that egg extracts, at least to some extent, can recapitulate somatic nuclear reprogramming that is induced after nuclear transplantation to eggs. Kikyo *et al.* [31] first reported that somatic nuclei incubated in *Xenopus* egg extracts are remodeled towards an embryonic state in which somatic proteins are lost, while egg proteins are incorporated into somatic chromatin. They have shown that the ATP-dependent chromatin remodeling factor ISWI plays a key role in this process. This system has also led to the identification of FRGY2a/b as a critical factor for nucleolar disassembly [32]. These are the first reports to identify actual egg factors involved in somatic cell reprogramming in vitro, proving that the egg cell-free system is a good route to manifest reprogramming mechanisms.

Subsequently, several reports have shown that reprogramming activities of egg extracts are conserved in mammalian somatic nuclei. The incorporation of *Xenopus* egg factors into mammalian chromatin was observed, including oocyte type lamin LIII [33] and histone B4 (Figure 1.2B) [34]. Moreover, the ability of egg extracts to trigger induction of mammalian embryonic gene expression has been shown [34, 35]. Egg and oocyte extracts from another amphibian species, the axolotl, also exhibit strong epigenetic reprogramming activities in mammalian nuclei [36]. These findings emphasize the utility of amphibian egg extracts as a tool to study reprogramming of mammalian nuclei, especially for the purpose of identifying egg factors with reprogramming activities.

Application of cell-free systems for understanding mammalian oocytes

The *Xenopus* egg cell-free system has greatly advanced molecular understanding of many cellular events, as mentioned above. Factors and mechanisms originally found in this system have been extensively tested and validated in mammalian in vivo systems. Therefore, the *Xenopus* cell-free system has served as a foundation for revealing molecular mechanisms. If a similar kind of cell-free system can be developed in mammals, our molecular understanding of mammalian oocytes may advance rapidly. This idea has been hampered by the fact that we cannot collect enough mammalian oocytes for making functional extracts. Nevertheless, some attempts to produce these have been made [37, 38], although further sophistication is needed. It might be a good idea to start first with one specialized oocyte extract that can reproduce only one aspect of cellular events.

Special manipulations

Nuclear transfer in eggs and oocytes

Spemann did an ingenious delayed nucleation experiment in which the nucleus of one of the first eight cells of an amphibian embryo was shown to lead to the formation of a normal embryo [39]. This demonstrated the totipotency of one of the first eight cells of an embryo, but did not test later stages. The first major success in nuclear transplantation was that of Briggs and King [40] when they were able to transplant the nuclei of *Rana pipiens* blastula cells into enucleated eggs of that species and obtain normal embryos. When they tried the same experiment using nuclei from slightly later stages, they were no longer able to obtain normal development [41]. They reached the entirely reasonable conclusion that, as development proceeds, the nuclei of somatic cells lose their totipotency. In 1958 nuclear transplantation had succeeded in *Xenopus*. A series of experiments culminated in the finding that totally normal, sexually mature adult animals could be obtained by transplanting the nuclei of embryo endoderm cells into enucleated eggs (Figure 1.3A) [42]. Subsequently it was found that the nuclei of differentiated intestinal epithelium cells could also yield normal, sexually mature animals. This was the proof that cell differentiation does not necessarily involve any loss of genetic totipotency. It is now generally accepted that, with very special exceptions like antibody-producing cells, all cells of the body have the same complete genome. In recent time, notably following the work of Takahashi and Yamanaka [43], the principle of totipotency of somatic cell nuclei has led to extensive work aiming to derive embryonic stem cells from adult tissue cells, with a view to drug testing and possibly cell replacement therapy.

For technical reasons, it was nearly 40 years after the first successful nuclear transplantation in amphibia

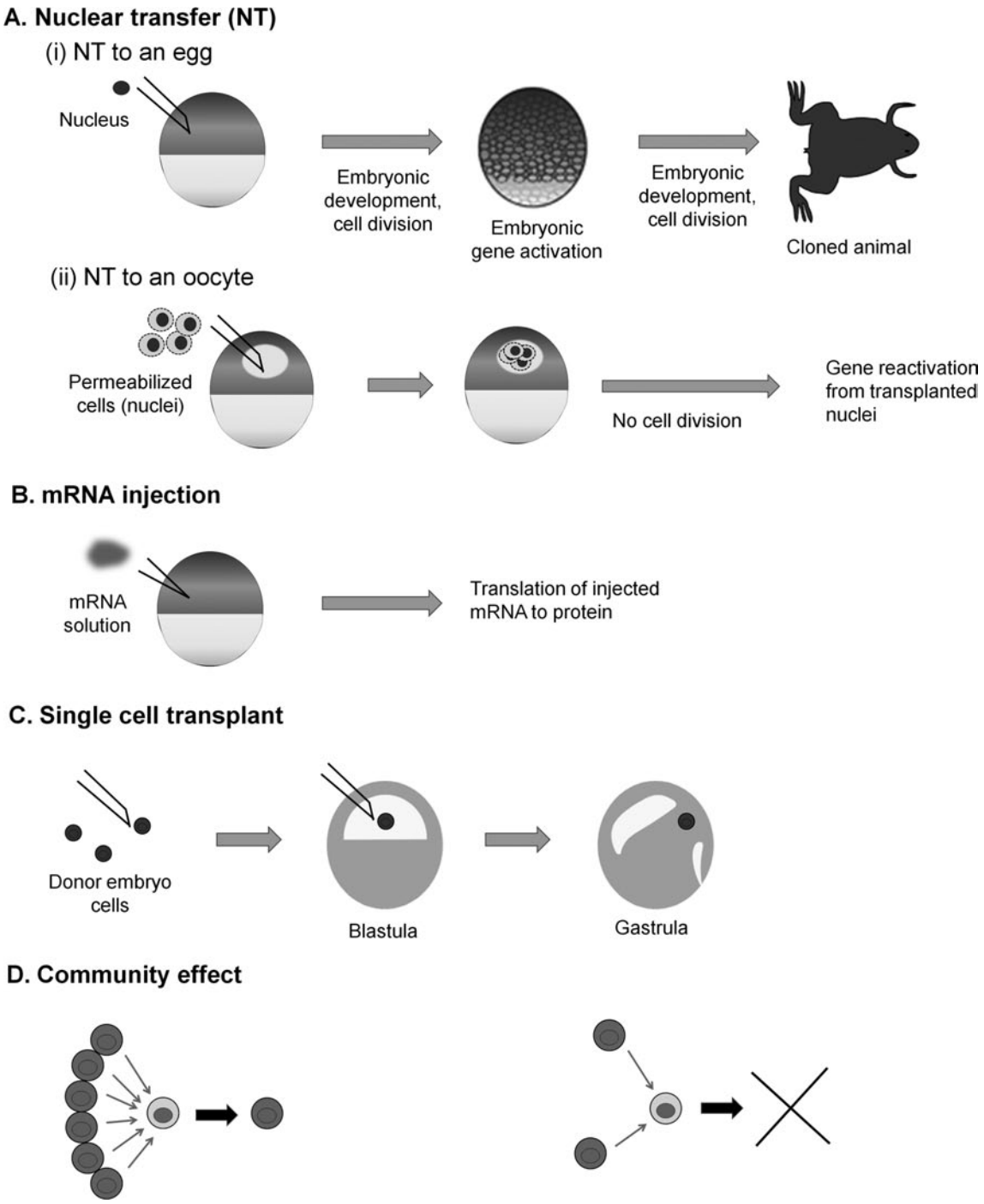

Figure 1.3 Special manipulations using *Xenopus* eggs and oocytes. (A) Two types of nuclear transfer (NT) are available in *Xenopus*. NT to MII eggs generates NT embryos, which finally give rise to cloned animals. Hundreds of nuclei can be injected into a nucleus of the *Xenopus* oocyte. Injected nuclei do not change to another cell type, but, instead, previously silenced genes are reactivated. Direct transcriptional reprogramming of somatic nuclei without the need for cell division is induced in this oocyte NT unlike NT to an egg, in which cell divisions are required before initiation of embryonic gene transcription. (B) In vitro synthesized mRNAs are injected into the cytoplasm of eggs/oocytes and are readily translated. (C) Embryo cells are separated from each other. A single cell is injected into the cavity of a blastula embryo and the fate of the injected cell can be traced. (D) Cell transplantation experiments led to the finding of the community effect, the phenomenon in which cells in close proximity to each other contribute some signal factor and receive more signals from their neighbors, thereby allowing efficient differentiation.

that Dolly the sheep was produced [44]. This demonstrated that a differentiated adult tissue, in this case mammary gland, could yield normal, fertile animals by nuclear transfer. Since then, nuclear transfer in a wide range of mammals has been successful. Byrne *et al.* has reported the successful derivation of embryonic stem cells from monkey nuclear transfer embryos [45]. Recently, Tachibana *et al.* have succeeded in establishing human embryonic stem cells from nuclear transfer embryos with high efficiency [46].

Nuclear reprogramming by the induced pluripotent stem (iPS) cell technology currently works at a low efficiency. There is a strong desire to understand the mechanism of this route towards deriving pluripotent stem cells from adult cells, in the hope that the efficiency of the procedure might be improved. By comparison, the transplantation of somatic nuclei to enucleated eggs, whether amphibian or mammalian, works at a much higher efficiency and therefore gives a special opportunity to understand the mechanism of this route towards nuclear reprogramming. Amphibian eggs and oocytes are very favorable for an analysis of nuclear reprogramming mechanisms. This is because a *Xenopus* egg is approximately 4000 times larger than a mouse or other mammalian egg, and one frog can provide as many as 25 000 eggs or oocytes. An attempt to make use of these conspicuous advantages has led to the development of nuclear transfer to oocytes (first meiotic prophase egg progenitors). Multiple somatic nuclei (including those of mammals) can be transplanted to the GV of an oocyte (Figure 1.3A). There is no DNA replication or cell division, characteristics which cause much of the abnormality in nuclear transfer to egg (second meiotic metaphase) experiments with amphibia. Somatic nuclei transplanted to oocytes undergo transcriptional reprogramming by inducing the expression of pluripotency genes characteristic of embryonic stem cells. A large number of new transcripts (~1000 *Sox2* transcripts per gene per day) are produced after transfer to the oocyte GV, and therefore provide a special opportunity to analyze mechanisms of transcriptional reprogramming. Work with oocytes has revealed some of the mechanisms by which the specialized state of a somatic nucleus can be reversed to allow reactivation of embryo-specific genes. This is the initial step of nuclear reprogramming. Current progress in this direction has recently been reviewed by Pasque *et al.* [47].

The oocyte route of nuclear reprogramming has not yet been extended to work with mammals, but some of the reprogramming factors identified in this oocyte system have been shown to be involved in reprogramming in mammalian oocytes. In conclusion, the success of Dolly the sheep, and subsequently of work with other mammals, was clearly initiated by work with amphibia some 40 years earlier. This is therefore an area in which early work with amphibia has helped to lead success with mammals.

Messenger RNA (mRNA) injection

The first success of injecting mRNA into living eggs was in 1971 (Figure 1.3B) [48]. This experiment was undertaken primarily because, in previous years, success had been achieved by injecting purified DNA into eggs as an attempt to analyze the mechanism of DNA replication that immediately follows nuclear transfer to eggs. Surprisingly, the mRNA injection experiment worked remarkably well. It was even possible to derive swimming *Xenopus* tadpoles in which as much as half of their soluble protein was derived from the injection of rabbit globin mRNA at the egg stage. The "transplanted" mRNA turned out to be very stable and translated with very high efficiency after injection into living eggs or oocytes. Evidently the microinjection of eggs and oocytes does not release any of the ribonuclease activity which is carefully sequestered in living eggs and embryos.

The use of mRNA injection into eggs and oocytes was much enhanced by the finding of Krieg and Melton [49] which showed that mRNA could be synthesized in vitro from any cloned gene and then overexpression of that gene achieved with high efficiency after mRNA injection into amphibian eggs or oocytes. This procedure has now become extensively used for the overexpression of any gene that might be important in development. In just the same way, the overexpression of dominant negative constructs is an effective way of knocking down the expression of genes that might be important in development.

In due course, this procedure for message injection was extended to mammals where it is also widely used for gene overexpression and cell tracing experiments [50].

Cell separation, reaggregation, and rearrangement

Much of what we now know about mechanisms of development has depended on experiments in which

cells are moved from their normal in vivo environment to be cultured in vitro or transplanted to the vicinity of other kinds of cells. In general, groups of cells or tissues are explanted or transplanted. While informative, these experiments are not ideal. This is because a cell within a group will always be surrounded by other cells of the same source. Therefore, it is unclear whether an individual cell is truly surrounded by cells of another germ layer or by cells of its own origin. This uncertainty is compounded by the fact that most tissues consist of cells which are not identical. Therefore, interactions between cells of different kinds may continue even if the group of cells is now transplanted to an ectopic site or cultured in vitro. The most informative experiments are those in which a single cell is transplanted to a new environment.

Nowadays, it is not uncommon to carry out single cell transplants. It is therefore interesting to consider the origin of single cell transplant experiments. Looking back to the time of Spemann, Holtfreter, etc., much of the work published between 1900 and 1940 involved tissue, that is, multicell, transplants or explants [51]. The earliest experiments involving single cell isolation in vertebrates go back to a landmark paper by Townes and Holtfreter [52] that involved isolating embryo cells from different germ layers, mixing them together, and then reaggregating them to determine their fate. For an appreciation of this paper, see Steinberg and Gilbert [53].

The difference between rearranging whole multicellular tissues from embryos and separating single cells so that they can be transplanted or recombined is not trivial. For example, in 1953, Grobstein and Zwilling showed the so-called "mass effect" [54]. This meant that the differentiation of cells is much less successful when they are cultured as progressively smaller groups ranging from large pieces of tissue containing several 1000 cells to small groups of only 100 cells or less. Therefore, single cells do not like being cultured on their own. Furthermore, groups of cells are often heterogeneous and unknown interactions may take place between them. To accurately determine the state of determination or specification of cells requires that single cells are used.

An important paper was published by Heasman *et al.* [55], in which they implanted single amphibian cells of known germ layer origin into the *Xenopus* blastocoel cavity at the blastula stage (Figure 1.3C). The embryos were cultured and the fate of the transplanted single cell and its immediate descendants was determined. The results showed that endoderm cells become irreversibly committed (determined) remarkably early in development. However, this experiment was not perfect. This is because, when dropping a cell into a blastocoel cavity, it is not certain to which host cells it will become attached. For example, in zebrafish experiments, a single transplanted cell tends to migrate back into its own tissue of origin, even if it has been transplanted to an ectopic position in an embryo. In amphibia, it was possible to transplant single embryonic cells into sandwiches of unlike cells so that they could not migrate back to their own preferred environment. This kind of experiment led to the description of the community effect (see below).

The concept of isolating, rearranging, and transplanting single cells was soon taken up by early mammalian embryologists. Notably Tarkowski [56, 57] was able to separate early mammalian cells and rearrange them into different embryo aggregates. By combining this kind of experiment with appropriate genetic markers, it was possible to analyze the fate and differentiation potential of single cells in mammalian embryos. Indeed, such a design of experiment was central to the pioneering work of Gardner [58] in which he transplanted single embryo cells into the blastocyst of an early mouse embryo. These experiments now form a central part of the whole field of mouse embryonic stem cells. Experiments of this kind are now routinely used in mammalian embryo and stem cell research.

Community effect

This phenomenon describes a situation in which like cells in proximity to each other each contribute a small amount of some signal factor. So long as many cells close to each other each contribute a low concentration of factor, the concentration of that factor in the cell group will increase to a higher level than could be achieved by the factor production from one single cell on its own (Figure 1.3D).

The community effect should not be confused with the so-called "mass effect." The community effect first came to light in experiments in which single cells were transplanted to different parts of an embryo (see above); even though not exposed to any artificial environment, single transplanted cells failed to differentiate in the way that members of a group did. A range of experiments was subsequently carried out and led

to the conclusion that secreted signal factors account most readily for the community effect. In one case [59] there was evidence that a particular variety of basic fibroblast growth factor was the secreted factor involved. There are many instances in normal development when a community effect may apply. In any case where a group of cells needs to differentiate in a coordinated way, the building up of a sufficient concentration of factor by a group of cells, but not by single cells, would account for the effect. This applies to many tissues in embryos, such as muscle tissue.

Conclusions and perspectives

Xenopus research has been developed by taking advantage of large and abundant eggs and easy manipulation procedures. With this useful experimental material, many breakthrough discoveries have been achieved, such as signaling pathways that govern the cell fate decision, the egg cell-free system, tissue or cell grafts, mRNA injection, and nuclear transfer. Since the whole genome sequence of *Xenopus tropicalis* has been decoded, *Xenopus* research is now compatible with the genome-wide sequencing technology, making it possible to draw global views of gene expression and chromatin signature in early embryos. We will be able to envisage more detailed molecular networks underlying development. In addition, genetic approaches such as mutation screening for finding developmentally important genes can now be applied [60]. These useful features together with the state-of-the-art technologies can make it possible to answer questions that are currently difficult to answer using mammalian oocytes, such as the genome-wide ChIP-seq analysis of chromatin factor binding in early embryonic development. For using *Xenopus* eggs/oocytes as a model to understand mammalian oocytes, it is important to study conserved mechanisms between these species. In this sense, reprogramming is appropriate since many reprogramming factors that play a role in *Xenopus* have been also shown to work in mammalian systems. We therefore believe that *Xenopus* eggs work well together with mammalian oocytes to understand animal development and reprogramming.

Acknowledgments

We thank Marta Teperek for her careful reading of the manuscript. K.M. is supported by the Japan Society for the Promotion of Science (International Research Fellowship Program). Gurdon laboratory is supported by a grant from the Wellcome Trust (Grant RG54943) to J.B.G.

References

1. Gurdon JB, Hopwood N. The introduction of *Xenopus laevis* into developmental biology: of empire, pregnancy testing and ribosomal genes. *Int J Dev Biol* 2000; **44**: 43–50.
2. Sommerville J. Using oocyte nuclei for studies on chromatin structure and gene expression. *Methods* 2010; **51**: 157–64.
3. De La Fuente R. Chromatin modifications in the germinal vesicle (GV) of mammalian oocytes. *Dev Biol* 2006; **292**: 1–12.
4. Spemann H, Mangold H. Über Induktion von Embryonanlagen durch Implantation artfremder Organisatoren. *Wilhelm Roux's Arch Dev Biol* 1924; **100**: 599–638.
5. Nieuwkoop PD. Inductive interactions in early amphibian development and their general nature. *J Embryol Exp Morphol* 1985; **89** Suppl: 333–47.
6. De Robertis EM. Spemann's organizer and self-regulation in amphibian embryos. *Nat Rev Mol Cell Biol* 2006; **7**: 296–302.
7. De Robertis EM. Spemann's organizer and the self-regulation of embryonic fields. *Mech Dev* 2009; **126**: 925–41.
8. Rogers KW, Schier AF. Morphogen gradients: from generation to interpretation. *Annu Rev Cell Dev Biol* 2011; **27**: 377–407.
9. Muller P, Schier AF. Extracellular movement of signaling molecules. *Dev Cell* 2011; **21**: 145–58.
10. Goumans MJ, Mummery C. Functional analysis of the TGFbeta receptor/Smad pathway through gene ablation in mice. *Int J Dev Biol* 2000; **44**: 253–65.
11. Pera MF, Tam PP. Extrinsic regulation of pluripotent stem cells. *Nature* 2010; **465**: 713–20.
12. Tam PP, Loebel DA, Tanaka SS. Building the mouse gastrula: signals, asymmetry and lineages. *Curr Opin Genet Dev* 2006; **16**: 419–25.
13. Lohka MJ, Masui Y. Formation in vitro of sperm pronuclei and mitotic chromosomes induced by amphibian ooplasmic components. *Science* 1983; **220**: 719–21.
14. Blow JJ, Laskey RA. Initiation of DNA replication in nuclei and purified DNA by a cell-free extract of *Xenopus eggs. Cell* 1986; **47**: 577–87.

15. Carpenter PB, Mueller PR, Dunphy WG. Role for a *Xenopus* Orc2-related protein in controlling DNA replication. *Nature* 1996; **379**: 357–60.
16. Coleman TR, Carpenter PB, Dunphy WG. The *Xenopus* Cdc6 protein is essential for the initiation of a single round of DNA replication in cell-free extracts. *Cell* 1996; **87**: 53–63.
17. Chong JP, Mahbubani HM, Khoo CY, *et al.* Purification of an MCM-containing complex as a component of the DNA replication licensing system. *Nature* 1995; **375**: 418–21.
18. Hutchison CJ, Cox R, Drepaul RS, *et al.* Periodic DNA synthesis in cell-free extracts of *Xenopus* eggs. *EMBO J* 1987; **6**: 2003–10.
19. Murray AW, Kirschner MW. Cyclin synthesis drives the early embryonic cell cycle. *Nature* 1989; **339**: 275–80.
20. Lohka MJ, Maller JL. Induction of nuclear envelope breakdown, chromosome condensation, and spindle formation in cell-free extracts. *J Cell Biol* 1985; **101**: 518–23.
21. Perry AC, Verlhac MH. Second meiotic arrest and exit in frogs and mice. *EMBO Rep* 2008; **9**: 246–51.
22. Philpott A, Leno GH, Laskey RA. Sperm decondensation in *Xenopus* egg cytoplasm is mediated by nucleoplasmin. *Cell* 1991; **65**: 569–78.
23. Ohsumi K, Katagiri C. Characterization of the ooplasmic factor inducing decondensation of and protamine removal from toad sperm nuclei: involvement of nucleoplasmin. *Dev Biol* 1991; **148**: 295–305.
24. Dimitrov S, Dasso MC, Wolffe AP. Remodeling sperm chromatin in *Xenopus laevis* egg extracts: the role of core histone phosphorylation and linker histone B4 in chromatin assembly. *J Cell Biol* 1994; **126**: 591–601.
25. Adachi Y, Luke M, Laemmli UK. Chromosome assembly in vitro: topoisomerase II is required for condensation. *Cell* 1991; **64**: 137–48.
26. Ohsumi K, Katagiri C, Kishimoto T. Chromosome condensation in *Xenopus* mitotic extracts without histone H1. *Science* 1993; **262**: 2033–5.
27. Hirano T, Kobayashi R, Hirano M. Condensins, chromosome condensation protein complexes containing XCAP-C, XCAP-E and a *Xenopus* homolog of the *Drosophila* Barren protein. *Cell* 1997; **89**: 511–21.
28. Laskey RA, Mills AD, Morris NR. Assembly of SV40 chromatin in a cell-free system from *Xenopus* eggs. *Cell* 1977; **10**: 237–43.
29. Almouzni G, Mechali M. Assembly of spaced chromatin promoted by DNA synthesis in extracts from *Xenopus* eggs. *EMBO J* 1988; **7**: 665–72.
30. Nicklay JJ, Shechter D, Chitta RK, *et al.* Analysis of histones in *Xenopus laevis*. II. Mass spectrometry reveals an index of cell type-specific modifications on H3 and H4. *J Biol Chem* 2009; **284**: 1075–85.
31. Kikyo N, Wade PA, Guschin D, *et al.* Active remodeling of somatic nuclei in egg cytoplasm by the nucleosomal ATPase ISWI. *Science* 2000; **289**: 2360–2.
32. Gonda K, Fowler J, Katoku-Kikyo N, *et al.* Reversible disassembly of somatic nucleoli by the germ cell proteins FRGY2a and FRGY2b. *Nat Cell Biol* 2003; **5**: 205–10.
33. Alberio R, Johnson AD, Stick R, *et al.* Differential nuclear remodeling of mammalian somatic cells by *Xenopus laevis* oocyte and egg cytoplasm. *Exp Cell Res* 2005; **307**: 131–41.
34. Miyamoto K, Furusawa T, Ohnuki M, *et al.* Reprogramming events of mammalian somatic cells induced by *Xenopus laevis* egg extracts. *Mol Reprod Dev* 2007; **74**: 1268–77.
35. Hansis C, Barreto G, Maltry N, *et al.* Nuclear reprogramming of human somatic cells by *Xenopus* egg extract requires BRG1. *Curr Biol* 2004; **14**: 1475–80.
36. Bian Y, Alberio R, Allegrucci C, *et al.* Epigenetic marks in somatic chromatin are remodelled to resemble pluripotent nuclei by amphibian oocyte extracts. *Epigenetics* 2009; **4**: 194–202.
37. Miyamoto K, Tsukiyama T, Yang Y, *et al.* Cell-free extracts from mammalian oocytes partially induce nuclear reprogramming in somatic cells. *Biol Reprod* 2009; **80**: 935–43.
38. Bui HT, Wakayama S, Kishigami S, *et al.* The cytoplasm of mouse germinal vesicle stage oocytes can enhance somatic cell nuclear reprogramming. *Development* 2008; **135**: 3935–45.
39. Spemann H. *Embryonic Development and Induction*. New Haven: Yale University, 1938.
40. Briggs R, King TJ. Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. *Proc Natl Acad Sci USA* 1952; **38**: 455–63.
41. Briggs R, King TJ. Changes in the nuclei of differentiating endoderm cells as revealed by nuclear transplantation. *J Embryol Exp Morphol* 1957; **100**: 269–312.
42. Gurdon JB, Elsdale TR, Fischberg M. Sexually mature individuals of *Xenopus laevis* from the transplantation of single somatic nuclei. *Nature* 1958; **182**: 64–5.
43. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663–76.