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Review

Cell cycle control in the early embryonic development of aquatic animal species☆,☆☆

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ABSTRACT

The cell cycle is integrated with many aspects of embryonic development. Not only is proper control over the pace of cell proliferation important, but also the timing of cell cycle progression is coordinated with transcription, cell migration, and cell differentiation. Due to the ease with which the embryos of aquatic organisms can be observed and manipulated, they have been a popular choice for embryologists throughout history. In the cell cycle field, aquatic organisms have been extremely important because they have played a major role in the discovery and analysis of key regulators of the cell cycle. In particular, the frog *Xenopus laevis* has been instrumental for understanding how the basic embryonic cell cycle is regulated. More recently, the zebrafish has been used to understand how the cell cycle is remodeled during vertebrate development and how it is regulated during morphogenesis. This review describes how some of the unique strengths of aquatic species have been leveraged for cell cycle research and suggests how species such as *Xenopus* and zebrafish will continue to reveal the roles of the cell cycle in human biology and disease.

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1. Introduction

Any biologist who has had the opportunity to watch and experiment with a developing fish or frog embryo would appreciate why embryologists have studied the development of aquatic animals for centuries. The embryos of many aquatic species are extraordinarily accessible for observation and manipulation. Aristotle (384 BC–322 BC) studied embryos from aquatic animals, comparing their development with other

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animals including humans and chickens. Even without the use of the microscope, Aristotle observed how species begin embryonic development by one of two major cell division patterns: holoblastic (in which the entire egg is divided into smaller cells, as in frogs) and meroblastic (in which the egg is separated into a yolk cell and the cells of the embryo proper, as in fish). The early cell divisions that Aristotle could observe in aquatic embryos are still the focus of many biologists today. In fact, studies of the easily observable cell divisions in the early embryos of sea urchins, frogs, and starfish, along with landmark studies in yeast and *Drosophila melanogaster*, have contributed greatly to our understanding of how the human cell cycle is controlled at the molecular level. We have much to learn about how the cell cycle is regulated during embryonic development and tissue homeostasis, and it is likely that aquatic model systems will continue to serve us well. The purpose of this review is to highlight how aquatic species have contributed to our understanding of the molecular mechanisms of cell cycle control and how aquatic models can still be used to address unanswered questions about how the cell cycle is coordinated with morphogenesis and cellular differentiation.

2. Fundamentals revealed through studying the "simple" embryonic cell cycle

A major advantage of aquatic model organisms is that their oocytes and embryos develop externally and are relatively large, so they are particularly amenable to experimental manipulations such as microinjection. It is also extremely helpful that large numbers of oocytes, eggs or embryos can be collected at the same stage. This feature has enabled the biochemical analysis of the cell divisions occurring during oocyte maturation and early embryonic development. Such analyses have been important for the discovery of the fundamental mechanisms of the cell cycle.

Many aquatic organisms begin development with a period of exponential cell expansion via a series of synchronous and rapid embryonic cell cycles. These early embryonic cell cycles of frogs and other aquatic organisms are simplified versions of the cell cycles of somatic cells (Newport and Kirschner, 1982a; Evans et al., 1983; Kane and Kimmel, 1993). These cycles differ from the canonical four-phase cell cycle in four important ways. First, an autonomous biochemical oscillator drives them, which is unaffected by developmental signals or checkpoints (Newport and Kirschner, 1982a; Newport and Kirschner, 1984; Kimelman et al., 1987; Kane and Kimmel, 1993; Ikegami et al., 1997). Second, they are extremely rapid and lack the gap phases (G1 and G2), which separate S-phase from mitosis (Fig. 1). Third, they are characterized by a lack of growth in cytoplasmic volume, so the large egg cytoplasm is progressively cleaved into a large number of smaller nucleated cells. Finally, they occur without zygotic transcription, so they are entirely controlled by maternally provided mRNA and protein. For example, the frog *Xenopus laevis* undergoes twelve cleavage cycles to produce thousands of cells; and the zebrafish (*Danio rerio*) undergoes ten cleavage cycles (Newport and Kirschner, 1982a; Kane and Kimmel, 1993) (Fig. 1). Thus, in these aquatic species, the egg cytoplasm contains all the protein and mRNA necessary for multiple complete embryonic cell cycles. The "loaded" egg cytoplasm of aquatic species, especially amphibians, has enabled cell biologists and biochemists to develop cell-free systems to study the embryonic cell cycle. These widely used systems were developed during the discovery of key regulators of the cell cycle, the Cyclins.

Studies of frog oocyte maturation laid the foundation for the discovery of Cyclins. Oocytes are arrested in prophase of meiosis I. The hormone progesterone triggers oocyte prophase exit and cell division (Masui, 1967). Using the frog *Rana pipiens*, Yoshio Masui showed that effects of progesterone could be mimicked by injecting cytoplasm from a maturing oocyte into a resting one, demonstrating that a soluble cytoplasmic factor, which was named maturation promoting factor (MPF), could induce meiotic progression (Masui and Markert, 1971). This result was repeated in the frog *X. laevis*, and through microinjection experiments in these two species, it was demonstrated that MPF activity is also present in the cytoplasm of mitotic cells, and it oscillates during the cell cycle, peaking in metaphase and disappearing with the completion of mitosis (Sunkara et al., 1979; Gerhart et al., 1984). MPF was shown to be an enzymatic activity that causes an increase in overall protein phosphorylation (Maller et al., 1977). We now know that MPF is a Cyclin-dependent kinase (CDK), which is composed of the Cdc2 catalytic subunit and Cyclin B regulatory subunit. Like the MPF, the discovery of Cyclin could be credited to the clever exploitation of highly accessible and manipulable oocytes and embryos of aquatic animals.

Tim Hunt and colleagues discovered Cyclin proteins when they noticed that a few proteins were preferentially translated upon sea urchin and clam egg fertilization, and those proteins were destroyed during the first mitosis and then resynthesized in the next interphase (Evans et al., 1983). The periodic expression of those proteins mirrored the periodic MPF activity that had been described earlier. Indeed, Cyclins activate MPF, but proving that fact involved the development of cell-free systems that recapitulate the embryonic cell cycle in frogs.

To study the MPF, Manfred Lohka and Yoshio Masui took advantage of the transformative activity of frog egg cytoplasm to develop a system in which a single cell cycle takes place in vitro (Lohka and Masui, 1983). Improvement of that system by Lohka and Maller enabled the first biochemical purification of MPF, which was comprised of two proteins (Lohka et al., 1988). One of the two proteins was demonstrated to be the *Xenopus* homolog of the *Schizosaccharomyces pombe* cell cycle kinase p34cdc2; the other protein was shown to be Cyclin B (Dunphy et al., 1988; Gautier et al., 1988; Lohka et al., 1988; Gautier et al., 1990). Together Cdc2 and Cyclin B form the prototypical Cyclin-dependent kinase (CDK) complex that drives mitosis. Cyclin B–Cdc2 is just one of several CDK complexes that promote cell cycle progression; other CDKs are critical for G1- and S-phase progression.

The discovery and molecular definition of MPF and Cyclin demonstrate the power of aquatic model systems for both embryology and biochemistry. Derivatives of the frog egg cytoplasmic extracts have continued to be extraordinarily productive in the cell cycle field. Blow and Laskey showed that the *Xenopus* derivative of the Lohka and Masui system could be used to study the initiation and completion of DNA replication in a cell-free system (Blow and Laskey, 1986). Murray and Kirschner developed the egg extract system even further, and showed that their improved system could drive multiple successive cell cycles (Murray and Kirschner, 1989). Variations in the *Xenopus* egg extract protocols have been optimized to study different aspects of cell biology including DNA replication licensing and initiation; sister chromatin cohesion; mitosis; the DNA damage and cell cycle checkpoints; and DNA repair (Sheehan et al., 1988; Dasso and Newport, 1990; Hyrien et al., 1995; Kumagai et al., 1998; Walter et al., 1998; Hekmat-Nejad et al., 2000; Raschle et al., 2008; Lafont et al., 2010). Just as aquatic model organisms have been used to study the fundamental mechanisms of cell cycle control, they have also been used to understand how the cell cycle changes during development. Organisms such as *Xenopus* and zebrafish have been useful for studying how the basic embryonic cell cycle is remodeled into the more complex somatic cell cycle.

3. Using aquatic model organisms to understand cell cycle remodeling

Early development is associated with dramatic changes in cell cycle dynamics. The cell cycle is remodeled throughout the cleavage, blastula, gastrula, and segmentation stages, during which time critical changes in transcription, cell motility and cellular differentiation also occur. The early embryonic cell cycles of cleavage and early blastula embryos, which have been useful for elucidating the mechanisms of the "core" cell cycle engine, lack additional layers of regulation that are found in somatic proliferating cells. Ultimately the rapid cleavage embryo cell cycles with alternating S and M phases are transformed into mature cell