The Review of Epigenetic and Genetic Limits to Indefinite Mammalian Cloning

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***Abstract*—Cloning via Somatic Cell Nuclear Transfer (SCNT) represents a landmark biotechnology with transformative implications for regenerative medicine and developmental biology. Beginning with the principle of cellular totipotency established by Gurdon's nuclear transplantation experiments and culminating in the generation of Dolly the sheep. However, despite its promise, mammalian cloning remains constrained by fundamental biological barriers. Serial cloning studies in mice and pigs reveal progressive epigenetic degradation across successive generations—including aberrant DNA remethylation, histone modification defects, and imprinting disruptions that compromise embryonic genome activation and developmental competence—and these observations align closely with Muller's ratchet, which predicts the irreversible accumulation of deleterious mutations in asexual lineages, ultimately driving mutational meltdown and extinction. Notably, a single round of sexual reproduction effectively resets these epigenetic lesions, underscoring the evolutionary indispensability of genetic recombination. This paper reviews the theoretical foundations, historical milestones, and persistent limitations of mammalian cloning, tracing its evolution from a proof of concept to a tool with therapeutic applications, and further examines the mechanistic basis of SCNT inefficiency, particularly the retention of hypermethylated states that suppress key pluripotency genes. Collectively, these findings elucidate the intrinsic limits of clonal propagation and emphasize the need for refined epigenetic modulation strategies to improve cloning efficiency and support responsible clinical and conservation applications.**

***Keywords*—totipotency, clone, somatic cell nuclear transfer, serial cloning, muller's ratchet**

I. Introduction

Since the elucidation of DNA as the universal genetic substrate across both prokaryotic and eukaryotic organisms, biologists have relentlessly pursued molecular interventions to ameliorate physiological decline, combat hereditary pathologies, and enhance organismal fitness. Among nucleic acid-based technologies, cloning has emerged as a particularly transformative modality, distinguished by its capacity to reprogram somatic nuclei and generate genetically identical organisms. The maturation of Somatic Cell Nuclear Transfer (SCNT) has created unprecedented opportunities to address longstanding biomedical challenges—ranging from producing patient-specific pluripotent stem cells for regenerative therapy and generating transgenic livestock for biopharmaceutical synthesis to the theoretical prospect of combating senescence and improving health. Yet, despite its promise, cloning remains a technology with intrinsic constraints. Fundamental obstacles persist, including incomplete epigenetic reprogramming, aberrant genomic imprinting, and the progressive accumulation of replication-induced mutations across successive clonal generations. These biological ceilings not only compromise developmental efficiency but also raise profound questions regarding the long-term viability of clonal lineages. Consequently, a critical inquiry emerges: can cloning technology transcend its current limitations to achieve indefinite application, or does it remain inherently bounded by the evolutionary imperatives of sexual reproduction? This paper systematically examines the theoretical framework and practical constraints of mammalian cloning, with particular emphasis on the epigenetic and genetic mechanisms that define its operational frontiers.

II. Biological Basis and Development of Cloning Technology

A. Cellular Totipotency: The Theoretical Basis of Cloning

The history of cloning is relatively brief: after Oswald Avery and his team first proved that DNA, not protein, is the true genetic material, scientists have been committed to changing the biological characteristics of organisms at the molecular level. Meanwhile, some people put forward an interesting idea: since a complete set of chromosomes includes all the genes necessary for an organism, why can't a single cell divide to form a complete individual? This ability is called totipotency. It is widely recognized that all plant cells can grow into complete individuals. In contrast, only a small number of animal cells, such as embryonic stem cells, possess this ability. Kenneth D. Birnbaum and Alejandro Sánchez Alvarado have clarified why plant and animal cells differ in their capacity to realize cell totipotency. They concluded that "The lack of cell migration in plants, which is precluded by rigid cell walls, would prevent movement of dispersed stem cells toward injured sites" [1]. They explained the reason from

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the perspective of regeneration, which clarifies the underlying logic of why these two biological forms share the same theoretical basis but differ in degree.

However, the fact that realizing totipotency in animal cells is difficult does not mean it is impossible. John B. Gurdon, a British developmental biologist, proved that the animal cell nucleus is totipotent. He transplanted the intestinal epithelial cell nucleus of an African clawed frog tadpole into an enucleated oocyte, and successfully cultured complete tadpoles and frogs. This discovery subverts the traditional view that "cell differentiation is irreversible" and lays the theoretical foundation for cloning technology and cell reprogramming. Hence, cloning technology is no longer limited to plants only, but also extends to animals.

B. Development of Mammalian Cloning Technology

After demonstrating that the nucleus of an animal cell is totipotent, many biologists became dedicated to cloning a mammal, aiming to prove that scientists could create an identical life form. After years of research and exploration, Dolly the sheep was born on July 5th, 1996, at the Roslin Institute in Edinburgh. Dolly the sheep was the first artificially cloned mammal in the world, and it carries profound significance for biology: it not only confirmed Gurdon's conclusion that "animal cell differentiation is reversible", but also opened the era of Somatic Cell Nuclear Transfer (SCNT), starting a new chapter for biological research.

III. Negative Effects of Continuous Cloning in Mammals

A. Telomere Dynamics in Cloned Animals

Before examining the adverse effects of serial cloning, it is necessary to clarify a widely suspected but experimentally disproved limitation-telomere shortening. Several issues emerged after Dolly the sheep's birth, and its short lifespan has been one of the most widely discussed concerns. Telomere shortening is considered a potential underlying mechanism for this phenomenon. In accordance with telomere shortening theory, Paul G. Shiels, Alexander J. Kind, Keith H. S. Campbell, and their team found that the mean Terminal Restriction Fragment (TRF) length of all three nuclear-transfer animals was the shortest [2]. Surprisingly, this phenomenon did not strongly correlate with the average lifespan of cloned individuals in the results. They concluded that telomere shortening may not be a core factor contributing to reduced lifespan in nuclear-transfer individuals. This conclusion is not isolated: another study conducted by Wakayama, which involved nuclear-transferred mice, has yielded consistent findings [3]. The results validate the original hypothesis: even animals derived from nuclear transfer are capable of producing normal offspring and do not experience adverse effects associated with the nuclear transfer procedure.

Existing studies have clearly confirmed that telomere shortening is not an essential factor affecting the lifespan of cloned individuals, and the life quality of cloned individuals has been proved to be no different from that of normal individuals. A team from the University of Nottingham claimed that the emerging consensus, supported by the current data, is that cloned animals are healthy and appear to age normally [4]. They monitored various data in aged cloned sheep and contemporary Embryo Transfer (ET) controls, including body fat, glucose tolerance, insulin sensitivity, ambulatory and stimulated blood pressure. "Importantly, despite their advanced age (7–9 years), none of the clones showed any clinical signs of disease, being euglycaemic, insulin sensitive and normotensive," they concluded. The above experimental results indicate no significant difference in quality of life between cloned individuals and embryo transfer control individuals. To further verify this conclusion, two clones were selected for the study—one with osteoarthritis and one without. Mediolateral radiographic views of their elbow joints were collected for the experiment, which was designed with reference to a similar study published by Gordon *et al*. [5] in 2003. "It is not surprising that no sheep showed marked lameness, as radiographic changes indicative of OA do not necessarily correlate with the extent of clinical disease" [5]. These findings indicate no inherent difference between cloned and normally developed individuals, and currently, no empirical evidence supports the assertion that cloned individuals have shorter lifespans and lower quality of life.

B. Adverse Effects of Serial Cloning

Although cloning technology has made profound progress in recent years, there are still a series of issues that need to be solved, especially in the area of serial cloning. Among these obstacles, the most fundamental issue is how to address the inevitable errors that occur during DNA replication. Accordingly, researchers at the University of Yamanashi conducted a series of studies tracking comprehensive data on cloned mice starting in January 2005 and achieved a key breakthrough by identifying the root cause of the limitations of serial cloning in mammals. While the success rate of serial cloning increased at first, it suddenly decreased in the last few generations, indicating an insurmountable barrier to serial cloning [6]. In contrast, the lifespan results of the re-cloned mice were unexpected: the lifespans fluctuated across generations and did not necessarily decline with successive cloning. Soon after, researchers collected additional data to identify the underlying cause of these phenomena.

They monitored multiple characteristics of re-cloned mice, including the effect of Trichostatin A (TSA) on G50 re-cloned mice, and the production of sexually reproduced offspring from re-cloned mice. Some data showed no difference between re-cloned mice and controls in the effect of TSA. In contrast, other data revealed a strong association regarding offspring production: the first finding indicated that even after more than 50 nuclear transfer cycles, the somatic cell nuclei of G50 retained sensitivity to TSA; the second finding showed that when the next generation (grandchildren) was produced from offspring of G55, litter size increased. This means that

outcrossing with natural individuals can help eliminate the side effects of serial nuclear transfer cloning. Interestingly, researchers found that when re-cloned individuals underwent sexual reproduction, their offspring developed normally. This suggests that a single round of sexual reproduction can effectively eliminate the adverse effects accumulated through serial cloning.

This is not unique to mice; it has counterparts in other mammals, such as pigs. A scientific group from the Yunnan Key Laboratory of Porcine Gene Editing and Xenotransplantation used pig somatic cells as the sample to perform serial cloning. "Evidence indicates that incomplete epigenetic reprogramming, including abnormal histone modifications, disrupted DNA methylation, and imprinting defects, is a major limiting factor," they summarized [7]. In normal fertilized embryos, parental genome methylation undergoes a global erasure through active and passive mechanisms followed by re-establishment during implantation.

However, this dynamic reprogramming process is disrupted in the re-cloned embryos. This is caused by SCNT; the hypermethylated state may still be retained, suppressing promoter regions of key pluripotency genes (OCT4, NANOG) required for Embryonic Genome Activation (EGA) and preventing their proper transcription. Thus, during the early developmental stage of SCNT embryos, an abnormal wave of DNA "remethylation" occurs. This premature and erroneous remethylation may entrench an epigenetic state unfavorable for development and contribute to subsequent developmental failure [8]. These SCNT-stage defects therefore hinder serial cloning and restrict its translation to clinical applications, where reliable animal models are needed to validate new therapies.

C. *Mechanism of Adverse Effects: Muller's ratchet theory*

When these experiments were carried out, researchers from the University of Yamanashi noted: "It has long been unclear whether mammals, unlike plants and some lower animals, could sustain their species through clonal reproduction alone." This finding reveals the inherent limitations of asexual reproduction and the critical evolutionary significance of sexual reproduction. Then, what are the underlying mechanisms driving the inability of species to maintain asexual reproduction over the long term? Muller's ratchet theory offers an explanatory framework. As a well-established classical theory, it elucidates the genetic damage induced by DNA replication errors. Muller's ratchet is a fascinating concept in evolutionary biology, named after the geneticist Hermann Joseph Muller, who proposed it in 1964. It describes a process in which the genome of an asexual population accumulates harmful mutations irreversibly. Recent theoretical work further formalizes this trade-off, showing that the continual accumulation of deleterious mutations ultimately bounds the rate of adaptive evolution [9]. This has a profound influence on explaining the damage of asexual reproduction to sexually reproducing animals.

Four main steps illustrate how Muller's ratchet works. First, in an asexual population, offspring are essentially clones of their parents, so that no genetic recombination can generate new, potentially mutation-free haplotype combinations—this forms the theory's foundation. Second, by chance, the individuals carrying the fewest mutations in the population may fail to reproduce or die. Third, once this least-mutated class is lost, the population's minimum mutation count increases by one, meaning the ratchet has clicked one turn forward. Fourth, after this click, because sexual recombination cannot reconstruct genomes and regenerate a mutation-free individual, the population can never revert to a state with fewer mutations. Hence, the ratchet only turns forward, accumulating deleterious mutations across successive generations. This relentless buildup of harmful mutations drives a severe decline in population fitness, and eventually leads to the extinction of the asexual lineage.

Muller's ratchet is one of the leading evolutionary explanations for the existence of sexual reproduction: sex enables genetic recombination, which can assemble beneficial mutations and eliminate harmful ones, effectively resetting the ratchet. Without this mechanism, asexual species would theoretically be doomed to mutational meltdown, which explains why long-term asexual lineages are quite rare in nature. "In a finite population, Muller's ratchet eventually results in the extinction of the population if compensatory or beneficial mutations do not oppose it" [10]. Matuszewski *et al*. [10] identified the key mechanism by which Muller's ratchet affects populations. They showed that the ratchet can trigger mutational meltdown, causing a population to decline until it gradually goes extinct. Moreover, this theory explains the core reason for the failure of serial cloning, since this biotechnology is still essentially a form of asexual reproduction. Accordingly, given the current biotechnology, serial SCNT cloning remains an immature and unpredictable procedure, limiting progress in many relevant research fields. Despite these inherent biological limits, cloning technology retains substantial value in controlled biomedical contexts, where its benefits can be realized without aspiring to indefinite propagation.

IV. Applications of Cloning Technology

Since the advent of cloning technology, scientists have focused on its applications, and clinical treatment is one of its core domains. Relevant experiments were conducted decades ago. In 1999, three years after the birth of Dolly, Robert Lanza, Jose Cibelli, and Michael West devoted themselves to exploring the proper application of cloning technology to benefit medical treatment, and made profound progress in cross-species transfer, stem cell differentiation, and tissue engineering [11]. They successfully developed a variety of treatment methods, including the development of patient-specific therapies with reduced risks of immune rejection. For instance, stem cells can play an essential role in treating diseases such as diabetes: by guiding cells along the endoderm lineage and exposing endoderm cells derived from embryonic stem cells to pancreatic bud tissue, researchers

can induce these cells to differentiate into pancreatic cell precursors [12]. This therapy did not emerge independently; only after cloning technology matured could it become an approach to address new challenges in biomedicine. Therefore, cloning truly plays a significant role in specific fields, helping solve problems from an entirely new perspective.

A recent study demonstrated that epigenetic modulation can substantially improve the efficiency of mammalian cloning. Li *et al*. [13] combined Trichostatin A (TSA) treatment with the overexpression of the histone demethylases Kdm4d and Kdm5b to correct abnormal histone modifications in mouse SCNT embryos. The researchers further used tetraploid complementation to address imprinting defects during post-implantation development. This combined strategy achieved an approximately 30% full-term development rate among reconstructed embryos, representing a substantial improvement over conventional SCNT [13]. The findings suggest that some barriers to cloning efficiency can be mitigated through targeted epigenetic intervention. However, this result should not be interpreted as evidence that indefinite cloning or clinical reproductive cloning has become feasible. Instead, it supports the more cautious view that cloning technology may have practical value in controlled biomedical research, regenerative medicine, and animal biotechnology.

V. Conclusion

Cloning via somatic cell nuclear transfer has undeniably transformed biomedical research, validating cellular reprogramming and opening avenues for regenerative medicine and therapeutic development. However, the collective evidence examined in this review demonstrates that mammalian cloning remains fundamentally constrained by intrinsic biological barriers that limit its indefinite application. Serial cloning experiments in mice and pigs reveal progressive epigenetic deterioration across successive generations—manifested as aberrant DNA remethylation, histone modification defects, and imprinting disruptions—that compromise embryonic genome activation and developmental competence. These empirical findings align closely with Muller's ratchet, which predicts the irreversible accumulation of deleterious mutations in asexual lineages, ultimately driving mutational meltdown and extinction. Critically, the observation that a single round of sexual reproduction effectively resets these epigenetic lesions underscores the evolutionary indispensability of genetic recombination and explains why long-term clonal propagation remains biologically unattainable in mammals. While SCNT efficiency may be improved through refined epigenetic modulation strategies, the fundamental ceiling imposed by replication-associated mutagenesis and incomplete reprogramming cannot be fully circumvented. Consequently, cloning technology, despite its remarkable achievements, operates within inherent boundaries defined by evolutionary imperatives. Its responsible application must therefore be directed toward contexts where these limitations are acknowledged and managed, rather than toward aspirations of indefinite clonal propagation. Future progress will therefore depend on integrating epigenetic optimization with careful experimental design to maximize therapeutic benefits while respecting biological constraints.

Conflict of Interest

The author declares no conflict of interest.

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