Chapter 3

Transgenesis and Genome Editing in Poultry

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76961

Abstract

The transgenic approach and precise editing of specific loci in the genome have diverse practical uses in animal biotechnology. Recent advances in genome-editing technology, including clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) have helped to generate highly valuable and quality-improved poultry. The production of transgenic and genome-edited birds mainly depends on primordial germ cells (PGCs), which are the progenitor cells of gametes, due to the unique system that is quite different from the mammalian system. This chapter introduces the basic physiology of avian PGCs and the latest PGC-mediated methodologies in transgenesis and genome editing of birds. Based on these techniques, future applications of precisely genome-modulated poultry are discussed to provide opportunities and benefits for humans.

Keywords: avian primordial germ cells, CRISPR/Cas9, genome editing, poultry, transgenesis

1. Introduction

The ability to genetically modify and precisely edit the genomes of animals has revolutionized various fields in which the genotypes, phenotypes, and traits of animals can be easily modified. Traditional animal breeding has been dependent on selective or artificial breeding for improvements in productivity, food quality, and other economical traits of the offspring [1]. However, transgenic and precise genome-editing tools facilitate improvements in genetic traits of animals when combined with conventional breeding systems. Recent technological progress of programmable nucleases, particularly the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system, has enabled much higher frequencies of homologous recombination events and targeted mutagenesis through

a highly efficient generation of double-stranded DNA breaks (DSBs) in specific regions and genetic modifications at targeted loci in the genome [2, 3]. This system has been adopted for programmable genome editing in various organisms, including humans [4]. More recently, programmable one-base pair conversion into another without DNA cleavage has been reported [5, 6]. This novel base editing system overcomes the low efficiency of correcting a point mutation using Cas9-mediated genome editing by delivering a homology-directed repair (HDR) donor template.

Germ-line modification is essential for the application of transgenic and genome-editing technologies in animals and to transmit modified and improved genetic traits from generation to generation. Germ-line modification methods differ between mammals and birds. The first transgenic mouse was generated by microinjecting the target DNA into the pro-nucleus of a fertilized embryo [7]. Livestock, including rabbits, sheep, and pigs, have been genetically modified using this technique [8]. This classical strategy is still widely used in animal transgenesis despite several disadvantages, such as the low efficiency of producing founder animals and the random integration of foreign DNA. Another popular method is the use of embryonic stem cells (ESCs) to modify the germ line, especially in mice (**Figure 1a**). Following genetic modification of ESCs in vitro, the cells are injected into the recipient blastocyst. Then, germ-line chimeras composed of germ cells originating from both endogenous and exogenous sources are produced to generate transgenic offspring derived from genetically modified ESCs [9, 10].

Unlike mammals, a unique system is used for transgenesis and genetic modification in avian species (**Figure 1b**) due to their oviparity and the physiological properties of the ovum [11]. As the avian zygote is surrounded by a large amount of yolk and a small germinal disc, introducing foreign DNA or microinjecting avian ESCs into the zygote is quite difficult [12–14]. Alternatively, the first transgenic chicken was produced via retroviral injection into the subgerminal cavity of

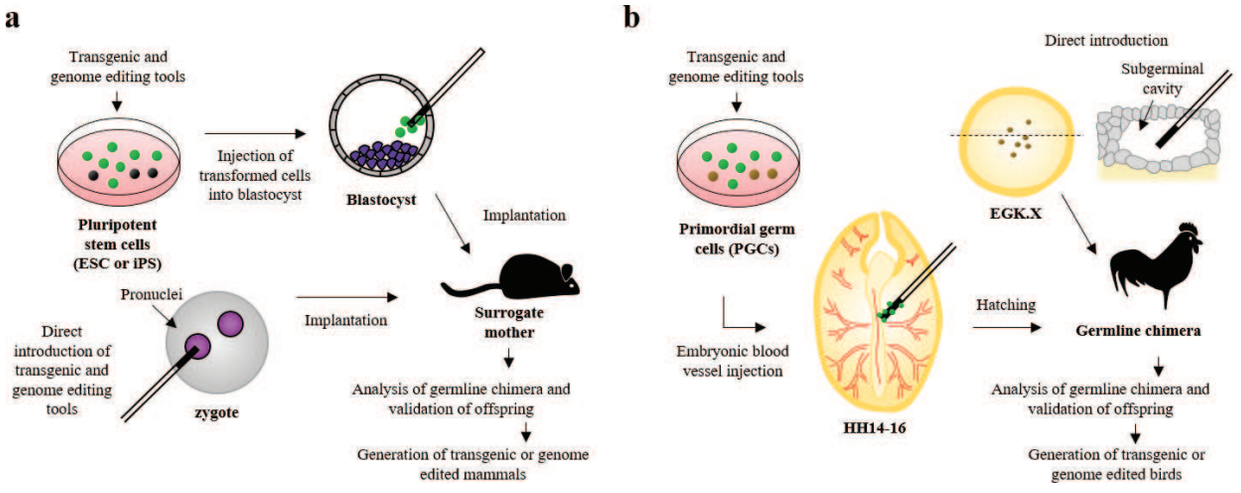

Figure 1. The different transgenic and genome-editing system between mammalian and avian species. (a) In mammals, transgenic and genome-edited systems are based on direct introduction of genome-editing tool into the zygote or microinjection of genome-edited ESCs into the recipient blastocyst. (b) In aves, those systems can be applied via injection of genome-edited primordial germ cells (PGCs) into the blood vessel of recipient. This figure is adopted from [135].

Eyal-Giladi and Kochav (EGK) [15] stage X embryos [16]. Then, various strategies, including viral infection of stage X embryos [17–19], microinjection of transgenes into fertilized eggs [14, 19], and ESCs [20], have been applied to produce genetically modified transgenic birds. However, because of the low efficiency of germ-line transmission, these strategies have not been appropriate for the production of genome-modified birds until recently. As an alternative cell source comparable to ESCs in mammals, primordial germ cells (PGCs) in avian species have overcome this limitation [21]. Here we present an overview of PGC physiology, recent advances in transgenesis and genome editing, and potential strategies for programmable genetic modulation in poultry.

2. Physiological overview of avian PGCs

2.1. Early development of avian PGCs

Since the first examination of the origin of PGCs in chicken germinal epithelium [22], chickens have been used as a valuable germ cell model (**Figure 2**). In initial studies on the origin of avian PGCs, only the central region of the blastoderm was considered to give rise to PGCs [23, 24], until the discovery and tracing of the chicken VASA homolog (CVH) in 2000 [25]. CVH is used as a PGC marker during the early developmental stages in chickens. CVH mRNA and protein expression can be consistently detected during early embryogenesis, from functional oocyte to fertilized embryo. The CVH protein was observed in granulofibrillar structures surrounding the mitochondrial cloud and the spectrin protein-enriched structure of oocytes, suggesting a CVH-containing structure in the germplasm of chickens. During early cleavage, CVH was found in cleavage furrows and restricted to about 6–8 cells at the 300-cell stage. According to

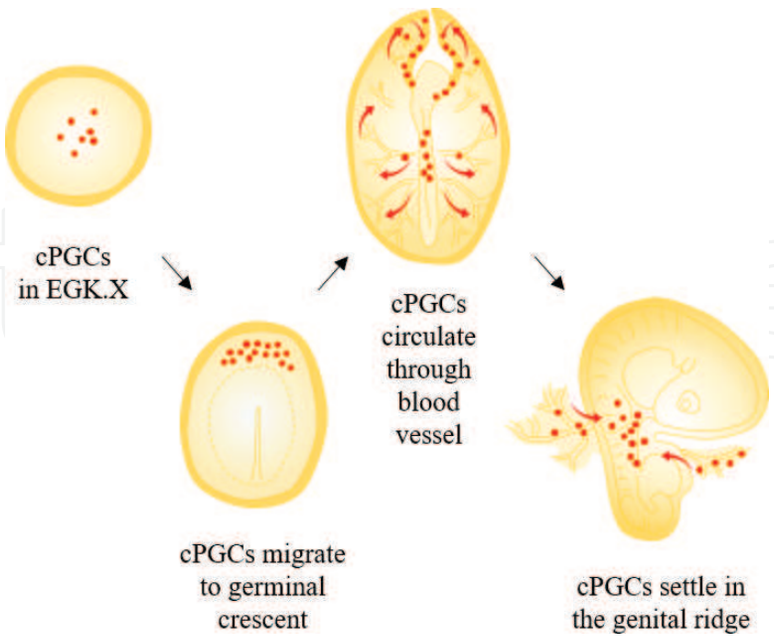

Figure 2. Schematic representation of the development and migration of PGCs in chicken. Chicken PGCs (cPGCs) are dispersed at stage X and move to the germinal crescent at HH stage 4. They then undergo circulation via extra-embryonic blood vessels until settlement in embryonic gonads at HH stage 17. This figure is adopted from [135].

these observations, the specification of germ cells in chickens seems to follow a pre-determined model from maternally inherited material. More recently, another germ cell marker detected in various species, deleted in azoospermia-like (DAZL), was identified in pre-PGCs of chicken embryos during intrauterine development prior to oviposition [26]. Using DAZL as an early germplasm marker, the germ granule was determined to be asymmetrically localized in oocytes, with a shift to a diffused form during early cleavage when the zygotic genome is activated. Moreover, knockdown of DAZL expression in chicken PGCs affects germ-cell integrity, such as proliferation, gene expression, and apoptosis. These findings further demonstrate that the origin of PGCs in birds is mediated by maternally inherited determinants, which is required to examine specific functions of germplasm components and to clarify the mechanisms.

In vertebrates, germ cells arise in a specific region of the embryo and then migrate to the genital ridges during early development [27]. Avian PGCs are clustered and derived from the epiblast layer [28, 29]. Then, the PGCs migrate toward the germinal crescent region at Hamburger and Hamilton (HH) stage 4 [30–32]. The PGCs are located in this extraembryonic region from HH stages 4–10 during formation of the primitive streak [15, 31, 33]. Using chicken fibroblast cell-line DF-1 and PGC transplantation into the embryos, PGCs are shown to passively reach the anterior region but, later, are actively incorporated into the germinal crescent compared to DF-1 [32]. However, the detailed mechanism of active migration, which may be guided by attractive and repulsive cues, remains poorly understood. Several studies have shown that migrating and in vitro-cultured PGCs generate pseudopodia, suggesting germ-cell migration in birds occurs via amoeboid movement [34–36]. Subsequently, PGCs enter the embryonic blood vessels through the anterior vitelline vein during HH stages 10–12 [37, 38], contrary to mammalian PGC migration from the hindgut endoderm to the mesentery [27, 39]. The PGCs enter blood vessels and are most abundant at HH stage 12 [35, 40]. PGCs circulating in the embryonic bloodstream start to settle in the genital ridge and invade the thickened coelomic epithelium during HH stages 15–18 [41, 42]. Research suggests that coelomic epithelium releases a chemical cue to attract PGCs to the gonads [43]. Later, the main factors guiding chicken PGCs to the genital ridges are chemokine stromal cell-derived factor 1 (SDF1) and its receptor, C-X-C motif chemokine receptor 4 (CXCR4) expressed by migrating PGCs [44]. One study revealed that the anterior vitelline vein plays a key role directly accumulating migrating PGCs, which reach the genital ridges during circulation [37]. Thus, compared with mammalian PGCs, the unique migratory pathway of avian PGCs through the bloodstream enables the generation of germ-line chimeras or genetically modified birds via an exogenous injection of PGCs into the blood vessel of a recipient embryo.

2.2. Germ-line chimera production via PGCs

Due to difficulties in the application of the mammalian system for highly efficient production of transgenic birds, many researchers have focused on improving the efficiency of germ-line transmission. In 1976, the colonization of germinal crescent-derived donor turkey PGCs was examined in recipient chicken gonads following intravascular injection, and a germ-line chimera chicken was produced from functional gametes derived from turkey PGCs [45]. In addition, PGCs from the germinal crescent have been successfully transplanted into recipient embryos to produce germ-line chimeras in quail [46]. Germ-line chimeras and donor-derived progeny

in birds have been generated by transplanted blood PGCs from the HH stage 14–16 embryos [47, 48] and gonadal PGCs (gPGCs) of HH stage 26–28 embryos [49, 50] in chickens and quail. Germ-line chimeras using cryopreserved PGCs or interspecific germ-line chimera enables the preservation of avian genetic resources and restoration of endangered bird species [51, 52]. Furthermore, endogenous PGCs are depleted in recipient embryos to improve the efficiency of germ-line chimera production. Various approaches, such as exposure to gamma rays [53], administration of busulfan to embryos [54], and removal of blood from recipient embryos at HH stages 14–15 [55], have been used to eliminate endogenous germ cells in birds. One report showed that the germ-line chimera efficiency of a busulfan-treated founder was approximately 99% [56]. These efforts have promoted the development of transgenic and genome-edited birds.

Many attempts have been made to develop an alternative system for producing germ-line chimera using other germ-line competent cells, such as blastodermal cells [57], embryonic germ cells [58], germ-line stem cells, and spermatogonial stem cells [59]; however, the efficiency of using these cells is lower than that of the PGC-mediated method. In addition to efficient germ-line chimera production, a PGC culture system, which has been optimized and proven to maintain germ-line competency after expansion in vitro [36, 60–62] despite differences in efficiency, provides many advantages of the use of PGCs in terms of transgenesis and precise genome modulation in birds. Although there are challenges to overcome, including the relationship between the in vitro culture of PGCs and germ-line competency and the absence of germ-line competency-associated markers, the PGC-mediated germ-line transmission system is the most efficient method to establish transgenic and genome-edited birds, until now.

3. Transgenesis and programmable genome editing in poultry

3.1. Primordial germ-cell isolation and in vitro culture in birds

Avian PGCs are generally obtained from three different stages, such as the germinal crescent in HH stage 4–8 embryos, embryonic blood in HH stage 14–16 embryos, and gonads in HH 26–28 embryos. Before the PGC cell-surface antigens were identified, PGCs were isolated using a density gradient-dependent centrifugation method [63, 64]. However, this method was limited due to low yield rates, purity, and viability of isolated PGCs. After the discovery of PGC-specific surface markers such as chicken stage-specific embryonic antigen-1 (SSEA-1) and the quail germ-cell-specific marker, QCR1, magnetic-activated cell sorting or fluorescence-activated cell sorting systems were used to isolate highly pure avian PGCs [65–67]. Nevertheless, isolating PGCs from wild or endangered birds, in which PGC-specific markers have not yet been fully determined, is difficult using such cell-sorting systems. To overcome this problem, a Transwell-mediated size-dependent isolation method was recently developed in various avian PGCs from HH stage 14–16 embryonic blood based on the larger size of PGCs compared to whole blood cells at that stage [68].

Since in vitro culture of PGCs without loss of germ-line competency was successfully established in 2006 [60], many studies have focused on optimizing PGC culture systems and revealing the detailed signaling mechanisms related to the proliferation and maintenance of

germ-line competency in vitro. For example, basic fibroblast growth factor is essential for in vitro proliferation and survival through the MEK/ERK signaling pathway in chicken PGCs [36, 61]. Furthermore, in vitro self-renewal of chicken PGCs requires complex pathways composed of MEK1, AKT, and SMAD3 signaling to maintain germ-line competency [69], and Wnt/β-catenin signaling is also required for the proliferation of PGCs in vitro [70]. This system could be developed for various avian species and would be useful to apply to PGC-mediated avian transgenesis and genome editing.

3.2. PGC-mediated transgenesis in birds

Before the establishment of in vitro PGC culture systems, the major method for transgenesis in birds relied on injecting viruses into EGK stage X embryos. The first transgenic chicken was generated by microinjecting recombinant avian leukosis virus into the subgerminal cavity of EGK stage X embryos [16]. In addition, transgenic quail were produced using direct injection of a replication-defective retrovirus into the embryonic blastoderm [71]. Due to frequent silencing of the transgene, which is randomly integrated in the genome of the transgenic animal [17, 71–73], the lentivirus-mediated method has been recognized as the most efficient viral transduction system for avian transgenesis. This system successfully produces diverse transgenic chickens without silencing gene expression [74–77]. In the case of zebra finch, microinjecting lentivirus into blastodermal stage embryos generated the first transgenic finch expressing green fluorescent protein (GFP) [78]. The transgenic birds, using PGCs from the germinal crescent of HH stage 5 chicken embryos, was firstly produced [79]. Furthermore, gPGC-mediated transgenesis in quail was successfully established via the lentiviral system [80]. In that study, although the efficiency of the gPGC-mediated method was comparable to the blastoderm-mediated method in quail, the production of transgenic birds through viral infection into non-cultivated PGCs after purification has been demonstrated.

Much effort has also been made to develop non-viral transgenic systems without PGCs, such as sperm-mediated gene transfection [81, 82] and direct microinjection of transgenes into fertilized eggs [14]. However, these approaches appear to have low germ-line chimerism and transmission efficiency compared with that of the PGC-mediated method. Furthermore, because of long-term *in vitro* PGC culture systems, it is possible to develop more optimal approaches to produce genetically modified birds compared with other germ-line cells. Combined with a culture system, the highly efficient non-viral transposable systems, such as *piggyBac* and Tol2, have been developed for stable transgene integration into the genome of chicken PGCs [83, 84]. The transgenic efficiency in cultured PGCs using lipofection or electroporation is remarkably higher than that of the virus-mediated methods to produce transgenic chickens. Moreover, using site-specific gene cassette exchange in transgenic chicken genomes via PGCs with the flippase recombinase system was introduced [85]. Alternatively, transgenic birds have been produced by direct transfection into circulating PGCs at HH stages 14–16 [86–88], although transgenic efficiency is usually lower than that of the cultured PGC-mediated method. This approach could be applied to establish a transgenesis system in avian species, as PGCs are difficult to manipulate in vitro in birds.

3.3. Precise genome-editing tools

Programmable DNA nucleases, such as ZFN and TALEN, have made genome editing in the target region possible over the last decade. Briefly, ZFN is the first programmable genome-editing tool. ZFN is a fusion protein with zinc finger proteins that bind to specific DNA and nuclease domains, such as the FokI endonuclease [89]. The second generation of programmable genome-editing tools is TALEN, in which the TAL effector of a DNA-binding domain derived from *Xanthomonas* is fused with a DNA cleavage nuclease domain [90]. Generally, ZFN and TALEN specifically recognize target sequences, resulting in the generation of DSBs to enable efficient gene targeting in specific genomic loci compared with natural homologous recombination, although the DNA binding mechanism is different between the systems. Compared to ZFN, TALEN is a more flexible tool for editing genome sequences in the target site because the TAL effector contains one repeat domain that binds to one nucleotide each [91]. Furthermore, customized TALEN can be easily synthesized using an assembly kit for precise genetic modifications [92–94].

The CRISPR/Cas9 system is considered the most revolutionary tool and has been developed to carry out highly efficient and specific genome editing as a simple RNA-guided platform. This system is derived from prokaryotic DNA, which is involved in acquired resistance against exogenous plasmid DNA and phages. These bacteria possess clustered repeats called CRISPRs that bind to the viral RNA to disrupt it with the Cas9 protein to defend [95]. CRISPR/Cas9 also leads to DNA cleavage at a specifically recognized target site, resulting in the generation of DSBs, similar to ZFN and TALEN. However, unlike ZFN and TALEN, which require paired units to induce DSBs at the target region, the CRISPR/Cas9 system, a type-II CRISPR system, includes the Cas protein, CRISPR RNA (crRNA), trans-activating CRISPR RNA (tracrRNA), and a protospacer adjacent motif (PAM) sequence for targeted genome editing [2] (**Figure 3a**). The PAM sequence is in the upstream region of the crRNA-binding site and guides the Cas9 protein to the target site. The target DNA sequence is specifically recognized by the CRISPR/Cas9 complex through base pairing with a guide RNA and subsequently induces DSBs at the targeted genomic loci. Then, these DSBs activate the cell's DNA repair system, which includes random indels at the site of DNA cleavage via non-homologous end joining or replacement of a homologous DNA template in the DNA surrounding the cleavage site via HDR. Compared to ZFN and TALEN, which rely on DNA-binding specificity and were developed through expensive and time-consuming processes, the CRISPR/Cas9 system is convenient for targeted genome editing because it is extremely easy to synthesize crRNA and tracrRNA and it is easy to construct thousands of customized CRISPR/Cas9 systems depending on the targeted genes. Furthermore, the CRISPR/Cas9 system is simple and practically easy to use with robust cutting activity, leading to a fast and cost-effective system for modifying the genomes of various organisms [96, 97]. Among the ZFN, TALEN, and CRISPR/Cas9 systems, CRISPR/Cas9 is now the most powerful method to precisely edit in a targeted manner and has been applied in diverse organisms, including animals, plants, and humans [4, 98, 99].

More recently, a programmable base editing system leading to precise and efficient nucleotide conversion was developed and applied to various species to minimize DNA damage

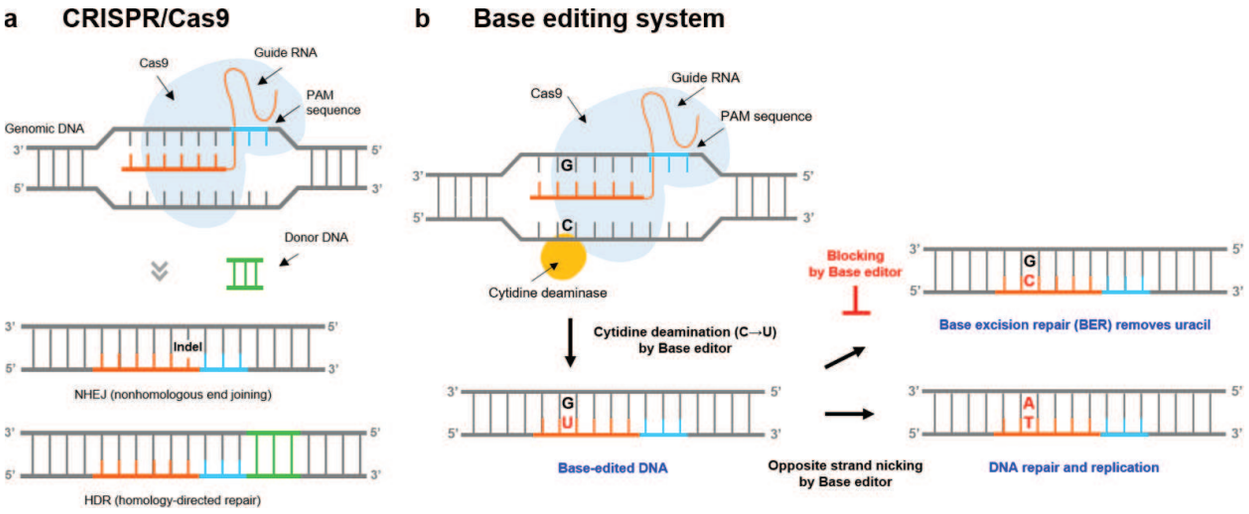

Figure 3. Principles of clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9) and development of the base editing system. (a) CRISPR/Cas9 system enhances the DNA mutation through the creation of a double-stranded DNA breaks (DSBs) at a specific locus in the genome and generates highly efficient genetic modification in a targeted manner. (b) A single base editing system is generated by fusion of cytidine deaminase to the catalytically inactivated Cas9 (dead Cas9) and provides a valuable tool for precise genome editing with regard to highly targeted single-base changes. This figure is modified from [136].

and acquire point-mutation corrections without HDR donors during genome editing. The strategies for modifying a single base pair by CRISPR/Cas9 are difficult to apply for various purposes, although efficiency is 60% in cultured cells [100]. However, the base editing system is composed of a group of cytidine deaminases, including the apolipoprotein B mRNA editing enzyme (APOBEC) 1–4 or activation-induced deaminase, resulting in deamination of cytidine to uridine [101] (**Figure 3b**). The deaminases fuse with CRISPR/Cas9 substitute C in a target site with T (or G to A) without breaking the DNA [5, 102, 103]. A base editing system is advantageous because indel formation rates are <0.1% [5]. Until now, the improved base editing systems have been advanced up to four generations. The first-generation base editor (BE1) involves the rat APOBEC1 fused with the N-terminus of catalytically dead Cas9 by a 16-residue peptide of the XTEN linker [5]. BE1 converts C to U with an activity window of approximately five nucleotides. However, base excision repair removing U from DNA decreases intracellular efficiency. To increase the low-editing efficiency of BE1, BE2, which fuses uracil glycosylase inhibitor (UGI) to the C-terminus of BE1, was created. In human cells, BE2 increases the editing efficiency threefold compared with BE1. Moreover, BE3, which involves Cas9 nickase (A840H)-UGI-generated nicks in an unmodified DNA strand, results in 37% efficiency, which is from three to sixfold to that of BE2. Subsequently, BE4 was developed to increase efficiency to 50% compared with BE3 and decrease undesired products [104]. Moreover, the Mu protein Gam, which binds DSBs and protects their ends from degradation during base editing, was fused to the N-terminus of BE4 resulting in a reduction of indel frequency [104]. In addition, A·T to G·C conversion was recently developed to broaden the application of the base editing system [105]. This simple system for base conversion has been applied to precisely modify the human and mice genomes [106]. In addition, base editing has been successfully applied in various

plants [5, 6, 99, 107–113]. In the near future, the ability to modify single-base changes in the base editing system will be widely used for precise genome editing and specialized purposes by substituting amino acids.

3.4. The recent progress of genome-edited poultry

In birds, combining an in vitro culture system for PGCs and an efficient genome-editing system can produce programmable genome-edited poultry, especially chickens. Although total germ-line transmission efficiency from targeted PGCs is approximately 0.1% due to natural homologous recombination that occurs with very low frequency, the immunoglobulin gene knockout chicken was first produced via the PGC-mediated method in 2013 [114]. However, applying TALEN technology to in vitro-cultured PGCs improved germline transmission efficiency of mutant progeny to 8% of the donor-derived knockout chicks in the ovalbumin locus in 2014 [115]. This case is the first programmed DNA nuclease-mediated knockout chicken, and the TALEN-mediated gene knockout appeared to be much more efficient than the conventional homologous recombination-mediated system. Later, the CRISPR/Cas9 system was used to efficiently generate ovomucoid gene-targeted chickens by transferring transiently drug-selected PGCs into recipient embryos using gamma-ray irradiation to deplete endogenous PGCs [116]. Here, the G0 founders had 93% mutant sperm and produced 53% ovomucoid gene mutant offspring, indicating a highly efficient CRISPR/Cas9 system in birds. Furthermore, through HDR insertion of an additional loxP site into the loxP variable region segment previously inserted into the joining gene segment of the chicken immunoglobulin heavy chain (IgH) locus and Cre recombination, a 28-kb genomic DNA sequence at the IgH locus was deleted in CRISPR/Cas9-mediated genome-modified chickens [114, 117]. More recently, CVH gene-targeted chickens via the TALEN-mediated HDR system were produced using 2-week-recovered PGCs with GFP transgene knockin at the CVH locus with 8.1% efficiency [118]. They generated 6% CVH-targeted progeny from one G0 male founder showing 10% of genomic equivalents in its semen. Germ-line transmission efficiency varies among genome-edited PGC lines compared with TALEN- and CRISPR-mediated genome modification. Because of possible loss of germline competency during long-term in vitro culture and genetic modification, it is crucial to optimize the conditions for establishing stable PGC lines during genome editing. On the other hand, a recent method, called sperm transfection-assisted gene-editing based on direct delivery of the CRISPR/Cas9 complex, is a potential alternative for avian transgenesis and genome editing without culturing PGCs, despite the low efficiency of genome editing and germ-line transmission [119].

3.5. Further applications of genome editing in poultry

After completion of the chicken genome sequencing project in 2004 and the subsequently available genomic sequences of the zebra finch and turkey, infinite possibilities and multiple opportunities are available to access invaluable genetic information from birds [120]. The bird 10 K genome sequencing project was initiated in 2015 based on recent next-generation sequencing technology. The progress of efficient genome-editing technologies in birds synergizes the

value of avian genetic information by avian genome manipulation for the development of beneficial poultry breeds (**Figure 4**).

We expect to establish an efficient bioreactor system to produce valuable proteins through genome-editing technology in chickens, which has the well-known advantage that egg white protein is easy to purify and eggs are produced daily by chickens [11, 121]. Bioreactors producing target proteins under the strong ovalbumin promoter have interested researchers for a long time [77, 122]. Thus, HDR-mediated target gene insertion into the ovalbumin locus could be an ideal bioreactor system to cost-effectively produce more than 1 g of target protein from an egg. In addition, genome editing has been used to remove or enhance targeted nutrients in meat and eggs of chickens. Knocking out allergen-related genes, such as ovalbumin and ovomucoid, has been achieved and could be used to generate allergen-free chicken meat and eggs [115, 116]. Additionally, muscle-related genes, such as myostatin, could be used to generate double-muscled and muscle hypertrophied chickens via genome editing, as in other livestock [123–125]. The conventional genetically modified organism (GMO) containing a foreign gene has been a concern due to a safety issue from unknown allergic reactions or the use of antibiotic resistance genes. Genome-edited poultry can be produced with a controllable genome-editing system, which is similar to natural mutations rather than foreign gene insertion as in conventional GMO. Moreover, an advanced base editing system may be more suitable for slight modifications of nucleotides without HDR in some cases. After scientists convince the public that genome-edited animals are similar to naturally selected animals, genome-edited poultry will be profitable for consumers.

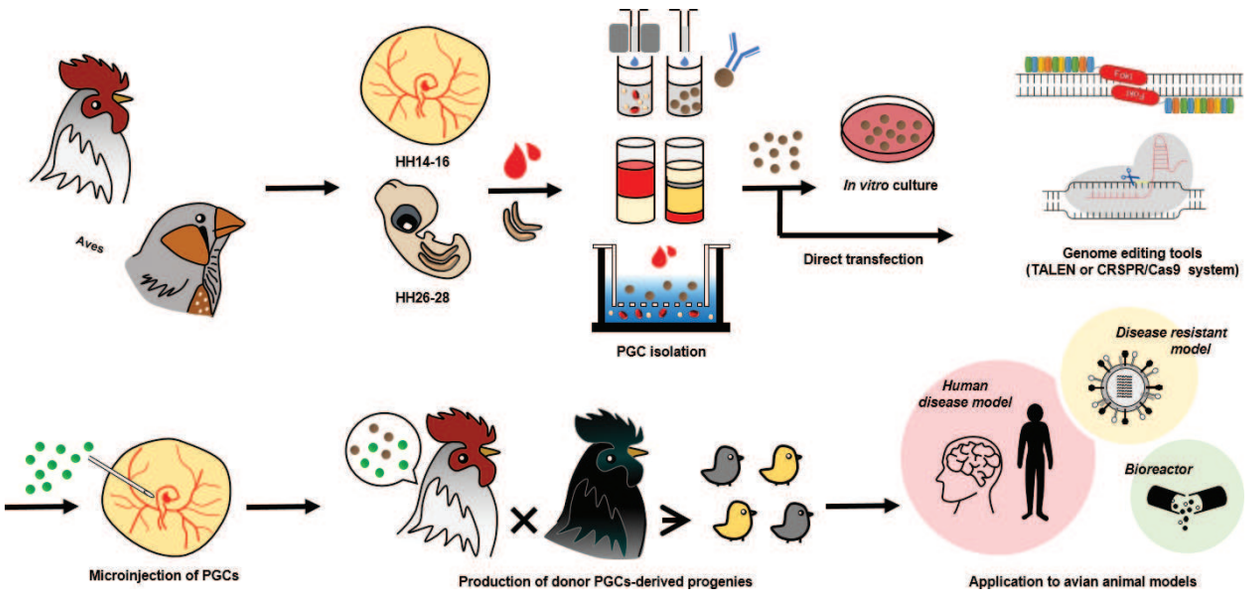

Figure 4. Strategies for the production of genome-edited poultries. PGCs in poultry can be obtained from embryonic blood and embryonic gonads. After the delivery of genome editing tools, genome-edited poultry can be established by microinjection of directly isolated or *in vitro* cultured PGCs into the blood vessels of recipient embryos. Avian genome editing systems can be applied to produce various avian models and poultry. This figure is adopted from [135].

In addition, it will be possible to control avian-specific diseases, such as avian influenza and Marek's disease, which cause serious problems in the poultry industry. Although understanding the mechanism of avian virus pathogenesis is essential for the application [126, 127] and limited in vitro results have been achieved [98, 128], avian genome-editing technology is expected to be used to develop avian disease-resistant birds by eliminating host factors or receptors of avian viruses. Lastly, because birds lay a large number of eggs and have a short ovulation cycle, they are considered the best model organism for studying human ovarian cancer [129]. With precise genome modulation of ovarian cancer-related genes in an avian model, especially chickens, it is possible to reveal the genetic mechanisms of ovarian cancer. In addition, avian genome-editing tools will gradually be applied to other birds, such as zebra finch, which is an exclusive non-human model organism for investigating the biological basis of speech learning and neurobehavioral research and disease [130–134]. Until now, direct injection of virus-mediated transgenesis into embryos has been used in zebra finch [132]. Genome-editing technology delivered by both in vivo and in vitro strategies will be widely applied to reveal the function and mechanism of neuron-related genes in zebra finch.

4. Conclusions

Poultry is important not only as a food resource but also as a valuable model animal for diverse disciplines, such as human disease, neurological research, and developmental biology. Until a few years ago, the difficulties in transgenesis and genome editing of birds limited their use as model animals. State-of-the-art technologies, such as CRISPR/Cas9 and the base editing system, have provided new insights into avian models when combined with PGC culture and other reliable germ-line systems. The novel genome-edited birds, including specific-gene knockout, human disease models, allergen-free, and disease-resistant poultry and egg-based bioreactors, are expected to be developed. Although the challenges in improving germ-line transmission strategies remain for many poultry species, programmable genome-editing tools will be useful in the development of genetically modulated poultry, together with efficient delivery and germ-line modification. Therefore, applying genome editing technology to birds will contribute to the poultry industry and ultimately provide benefits to humans.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF), grant funded by the Korean government (MSIP) (No. 2015R1A3A2033826).

Conflict of interest

The authors declare that they have no competing interests.

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