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Faithful artificial chromosome propagation using spermatogonial stem cells

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Abstract

Artificial chromosomes are useful in making functional vectors for very large genes, studying chromosome physiology, and modeling chromosomal disorders. Shinohara et al. have succeeded for the first time in creating transchromosomal mice by manipulating spermatogonial stem cells, which exhibited superior chromosomal stability compared to embryonic stem cells.

Keywords

spermatogonia; artificial chromosomes; karyotype; spermatogenesis; stem cells

Chromosome structure evolved to optimize gene maintenance and faithful transmission into daughter cells. Three key components of the chromosome, the centromere, telomeres, and its replication origins, collaborate to ensure precise replication and segregation during the cell cycle. Based on knowledge obtained from engineered chromosome constructs in yeasts, the first mammalian artificial chromosomes (MACs) were engineered in 1997 and shown to propagate genes in a transgenerational manner [1, 2]. Since then, the techniques were refined for production both of MACs and the engineered cells and animals that harbor MACs. Megabase-sized MACs can be generated either by modifying endogenous chromosomes or assembling large DNA sequences de novo. MACs are partitioned into microcells (membrane enclosed structures produced from donor cells [e.g., Chinese hamster ovary cells]), and the microcells fuse to target cells, such as embryonic stem cells (ESCs), by virtue of a fusogen (e.g., murine leukemia virus envelope protein) [3]. Based on their ability to self-renew in vitro and tolerate genetic manipulation, ESCs, which originate from the inner cell mass of the early embryo, can be injected back into the blastocyst and employed for production of transgenic mouse lines carrying MACs.

Transchromosomal animals and cells are invaluable in studying chromosome homeostasis, in evaluating functional vectors for large genes and regulatory elements, and in simulating chromosomal disorders, such as Down syndrome and other diseases. In theory, stable artificial chromosomes could be used for therapeutic purposes to avoid the pitfalls of other

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gene delivery strategies, such as inadvertent disruption of endogenous genes. However, either chromosome loss or gain is detrimental to cell survival. While a normal karyotype is critical for ESC function, aneuploidy occurs frequently during culture of ESC lines (for a review, [4]). Not surprisingly, chromosomal abnormalities of ESCs preclude efficient germ line transmission. Since the advent of induced pluripotent stem (iPS) cells, rapid progress has been made in developing regenerative strategies, in which iPS cells differentiate into specific cell types in vitro and can subsequently be delivered in vivo to repair tissues. For such translational applications, chromosomal stability is a major concern. Notably, iPS cells seem to harbor chromosomal and subchromosomal abnormalities, similarly to ESCs [4].

In a new article by Shinohara et al. [5], a MAC was successfully introduced into cultured mouse spermatogonial stem cells (SSCs), representing the first such milestone in the field (Figure 1). SSCs reside along the basement membrane of the seminiferous tubules of the testes, continuously self-renew, and supply sperm. Long-term mouse SSC lines can be derived from either neonatal or adult testes and are capable of differentiating into mature sperm to produce offspring upon transplantation into recipient testes [6]. By transplanting donor SSCs into recipient testes, the authors of the new study subsequently produced offspring, in which the artificial chromosomes were stably inherited. In contrast to ESCs/iPS cells, the integrity of chromosomes in SSCs in culture appears to be maintained. Yet, few studies have been done to address the mechanisms underlying the genomic stability of SSCs. One set of experiments showed that mouse SSCs in vitro, unlike ESCs, retained intact chromosome numbers (i.e., euploid karyotype) over two years, equivalent to 139 passages [7]. Shinohara et al. [5] compared karyotypic changes in transchromosomal ESCs and SSCs over time and observed that SSCs indeed retained their host karyotype far better than ESCs, and the retention of transchromosomes by SSCs was also improved over ESCs. These data suggest that, among other germline-competent cell types, SSCs are unusually good at preserving their chromosomes.

Despite the unusual chromosomal stability of SSCs, recent genome sequencing studies have revealed that paternal germlines steadily acquire mutations. A substantial burden of mutations, particularly single nucleotide variants, originate in the paternal lineage, likely in SSCs, and the mutations increase with paternal age [8]. Even though the rate of overall mutation may not be high (a few mutations per generation per additional year of paternal age), there is solid evidence that individual mutations can accumulate exponentially, due to stem cell competition [9]. Also, several studies observed that offspring of older fathers have longer telomeres in leukocytes, suggesting that telomere length may change in the SSCs [10].

Based on their promise for the creation of novel disease models, for studying germline biology, and as a potential alternative to ESCs, it is critical to understand how the genomes of SSCs change temporally. Yet, the factors that influence genomic stability in SSCs are poorly understood. To ensure the quality and scope of SSCs as a technical resource, many questions should be addressed. Shinohara et al. [5] studied phenotype of the transchromosomal SSC lines and found that they had more proliferative capacity and also more apoptosis than control SSCs. Since these features were not attributable to obvious genomic alterations, epigenetic modifications would be important to elucidate in this

context. Additional quality controls are necessary. Karyotype analysis does not detect small deletions, insertions, and mutations acquired after long-term cell line maintenance. High-resolution analyses of whole genomes of SSCs, such as SNP arrays and DNA sequencing, serially over the course of culture could be applied to detect subtle variations, the functional consequences of which could then be evaluated.

The capacity to generate transchromosomal offspring using SSCs circumvents a number of challenges associated with prior approaches. SSC lines can be derived from a number of different species and enable production of F1 transgenic offspring without first creating F0 chimeras, as required by the use of ESCs. Going forward, the new strategy for propagating transchromosomes should be applicable to other species and may ultimately allow delivery of xenogeneic chromosome constructs into experimental animal models.

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Figure 1. Generation of transchromosomal mice using spermatogonial stem cells (SSCs)

After constructing producer cell lines carrying artificial chromosomes, chromosome are separated into microcells. SSCs are fused with the microcells carrying artificial chromosomes in the presence of fusogens. SSCs harboring the artificial chromosomes are selected and transplanted into sterile testes. Transplanted SSCs differentiate into sperm and can produce offspring by natural mating or in vitro fertilization. F1 transchromosomal animals (gray) can be analyzed in subsequent studies.