
The Human Artificial Chromosome

Talia Mota

Jesse Gelsinger died September 17, 1999 after he received a trial gene therapy for ornithine transcarbamylase (OTC). The vector that transferred the OTC cDNA into Gelsinger's liver mimicked the human adenovirus type 5 (1). Even with critical adenoviral disease-causing regions deleted (E1 and E5), the virus still proved troublesome. Gelsinger experienced a systemic inflammatory response and multiple organ failures that left him comatose two days before he died (2). Three years later, two boys treated with therapy for X-linked severe combined immune deficiency (SCID), the "bubble boy" disease, developed leukemia (3). The delivered DNA integrated into the host LMO2 gene, which naturally plays a role with cell division. Leukemia developed because over-stimulated mitotic events caused extra-proliferated white blood cell growth. Cases such as these in which gene therapy has turned fatal show that technology has not proven to be safe enough for human trials—and therefore, better techniques in gene therapy must be developed and mastered.

The FDA has approved approximately 300 gene therapy experiments since 1990 (3). Current research on the human artificial chromosome (HAC) has shown that HAC therapy avoids the problems associated with older gene therapy techniques. As its own chromosome, "cousin 47" does not present naked DNA that could randomly integrate into the host genome. Instead, it promotes its own replication and transcription using the host's cellular mechanics (4). Ikeno et al showed that human cultured cells transfected with HACs remain mitotically stable after many cell divisions. Ikeno's experiments indicate that 95 percent of HACs survived after 30 days without selection. Chromosome loss per day was only 0.2 percent (5). Even after 60 days, 90 percent of cells still had their HACs, showing good kinetochore establishment (6). HACs do not interrupt normal cellular functioning, and, along with their mitotic stability, they have the ability to help overcome genetic predispositions.

Although artificial chromosomes are capable of using much more DNA than just cDNA (DNA reverse transcribed from mRNA exons)—current gene therapy is forced to use cDNA because of its condensed size. Larin and Mejia used the entire genomic sequence from the hypoxanthine phosphoribosyltransferase I (HPRT1) gene. While engineering the whole HPRT1 gene into a HAC, Larin and Mejia also included necessary regulatory elements: DNA insulators, promoter and enhancer regions, and locus control regions (7). All these mechanisms help control transgene expression. Grimes et al engineered human cultured HT1080 cells, deleting the HPRT1 gene, which in humans can mutate and cause Lesch-

Nyham syndrome (a self-mutilating disease). After creating their HAC with HPRT1 DNA, they transfected HT1080 cells. A Northern blot showed the fully expressive HPRT1 message. The HAC containing the HPRT1 gene complemented the deficiency previously engineered in the HT1080 cells (6). Grimes and Larin have both shown that, for the HPRT1 gene, HACs can replace or introduce gene function.

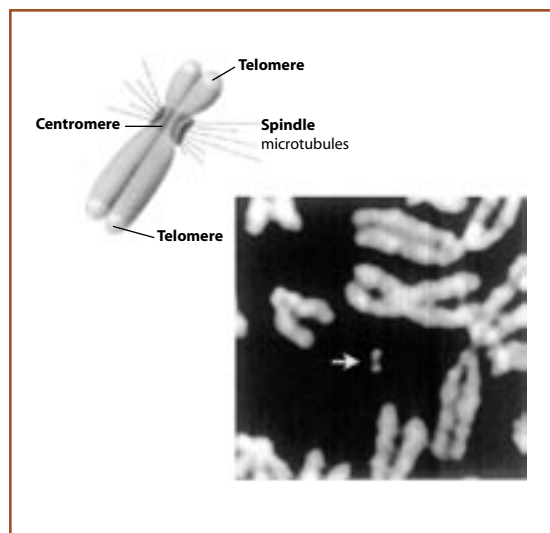


Figure 1. Functional human artificial chromosome (arrow). Normal chromosomes range from ~50 to ~230 Mb. Artificial chromosomes range from 2 to 6 Mb⁽⁸⁾.

is heterogeneous within the human population (10). Telomeres cap chromosome ends with TTAGGG tandem repeats that range from 5 to 20 kb. With replication they stabilize the ends and protect against chromosomal fusion and DNA loss (11).

HACs are created in two different ways. The less popular “bottom-up” approach includes de novo chromosome formation, where alphoid and telomeric DNA are put together (Fig. 2). Harrington et al. combined cloned alphoid and telomeric DNA arrays and co-transfected HT1080 cells with the mixture. The resulting chromosomes showed mitotic and cytogenetic stability, with CENP proteins interacting with the de novo centromeres. These artificial chromosomes lasted six months in culture (12). Uncontrolled chromosome formation within the cell, however, can cause problems if the transgene is not stable inside the artificial chromosome and integrates itself into the host genome (4).

“Top-down” construction uses a different strategy (Fig. 2). An already existing chromosome is truncated around the centromere in situ. Telomeric sequences introduced at truncation sites can shorten the chromosome (4). For example Heller et al used telomeric DNA and fragmented the human Y chromosome’s long arm. Homologous recombination helped the telomeric DNA truncate the arm and attach itself after the Y alphoid DNA interval (7). Minichromosomes were produced in this way, via a procedure developed in 1991 called telomere-associated chromosome fragmentation (TACF). Farr et al and Heller et al created human X and Y minichromosomes as small as 0.5 Mb. For stable chromosomal function, the HAC needs a minimum of 100 kb of alphoid DNA. At first, TACF minichromosomes were created using human cells or hybrid lines from hamster-human somatic cells (7). For more efficient “chromosome shuttling,” Dieken et al used cells from a chicken pre-B-cell line, DT 40, which provided better homologous recombination (13). When creating their HAC, Larin et al used a 162 kb fragment from Xq

Ikeno et al created a HAC expressing the guanosine triphosphate cyclohydrolase I (GCH1) gene. Such mutations cause hereditary dopa-responsive dystonia, or Segawa’s syndrome, and Parkinsonism (5). Again, the HAC balanced the deficiency within cultured cells. Dr. Larin is developing a HAC with the type VII collagen gene (COL7A1). This inherited mutation characterizes epidermolysis bullosa, causing extremely fragile skin where fingers fuse together. With more research, more genes are becoming acceptable for HAC technologies.

Human chromosomes have components important for mitotic stability: the centromere and the telomere (Fig. 1) (9). Centromeres are necessary for spindle attachment at pole migration during anaphase. Without a centromere, the chromosome would degrade when the cell divides. The major component of human centromeres is alpha-satellite DNA, also known as alphoid DNA (7). Alphoid DNA constitutes 2- to 5-percent of the genome. It forms 171-bp arrays, which organize into monomeric, dimeric, or pentameric repeat families, and more, higher order repeats. Alphoid DNA

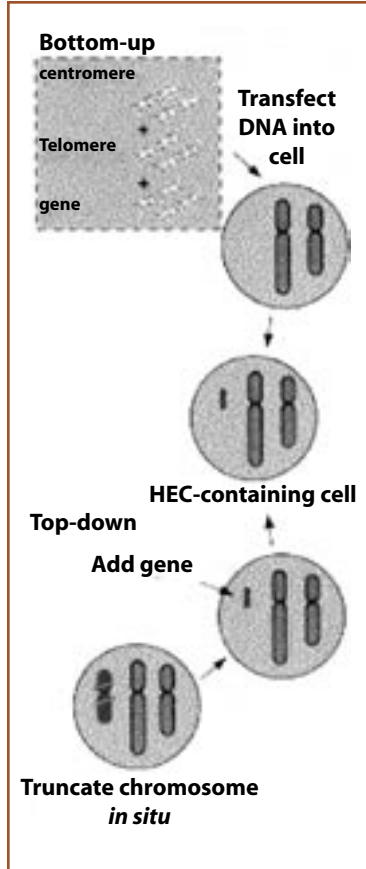


Figure 2. “Bottom up” (creating *de novo* chromosomes) and “Top Down” (truncating existing chromosomes into minichromosomes) approaches for creating HACs ⁽⁴⁾.

not harm the individual at all, and blocks HIV from entering the cell (15). Chemokine receptor CCR5 is a G protein-coupled receptor involved mainly with trafficking for inflammatory processes. CCR5’s seven trans-membrane domain makes three extra cellular loops. Individuals with the deletion only display one loop, and HIV cannot bind (16). This system, however, affects macrophages. Once HIV establishes itself, it can switch tropism and kill T-cells, causing AIDS (15). Although patients with the CCR5 deletion have not progressed to AIDS, they do not show 100 percent resistance (15). If a natural mutation arose also for CXCR4—the coreceptor HIV uses for T-cell infection—then a promising advantage would be offered by efficiently inducing mutations in both receptors. For example, an engineered HAC could turn off CCR5 and CXCR4 promoters and the new mutations would inhibit HIV entry. Thus, a HAC engineered into embryos could make the individual immune to AIDS. Parents would naturally want this therapy for their children, especially if HIV were one day to mutate into a bacterial vector, perhaps from bioterrorism.

While the artificial chromosome will benefit the individual, future progeny must also be considered. “Cousin 47” causes a problem with fertilization. If the individual with the HAC is a man, his wife may or may not contain an extra chromosome, especially in technology’s early stages. When their gametes

that contained HPRT1 and 220 kb-alphoid DNA from chromosome 17. Stability lasted for months and loss rates per day were as low as 0.0049, showing 98 percent accurate HAC mitotic activity (7).

Even when these promising HAC technologies become fully capable and safely make up for inherited animal deficiencies, targeting the correct cells proves a major obstacle. Gelsinger received a gene therapy dosage containing 38 trillion virus particles, yet only one percent reached the targeted cells. The other 17 participants also did not show significant gene expression (1). Current techniques include liposome-mediated transfer, the targeting of specific cell receptors, and adenoviral-mediated delivery (7). The latter caused Gelsinger’s death. The first two show success, but HAC construct transfection efficiencies lurk between 0.0000001 and 0.00001, showing just how much need there is for improvement (7).

Germline gene therapy would guarantee that a transgene reaches specified target cells. Introducing a HAC into the six- to eight-celled blastocyst in vitro means the gene would penetrate all future adult cells. Therapy can potentially step further than just treatment for disease. Using in vitro fertilization (IVF), available testing can conclude whether an embryo carries a predisposition to a particular disease (14). Embryo selection can therefore allow a couple to choose to implant only the healthy one(s). A HAC can change the embryo’s genetics, but because genetic disease can be prevented, HAC technologies introduced to the germline would most likely be used beyond their medical applications; a HAC might have advantages that humans can not now foresee. While this futuristic scenario seems far away, “potential human germline interventions might become feasible in a time frame that is meaningful to us and our children” (14).

Future HAC use could also protect us against pathogens. With AIDS, a natural mutation confers resistance to HIV in one percent of northern-European Caucasians. HIV uses CD4 and coreceptor CCR5 to penetrate white blood cells. A 32 bp deletion in the CCR5 gene does

form, the unequal chromosome number could suppress fertilization. Mario R. Capecchi recognized that deleting transgenic information from the gametes would make such technologies reversible. The Cre/loxP system allows recombination events. A gene of interest, for example in a mouse, can be flanked by loxP sites in vitro, while Cre recombinase is also engineered into the mouse. When triggered by specific chemical signals, the Cre cuts out everything between the loxP sites (leaving one loxP in place), cutting out the gene of interest. Using the Cre/loxP recombinase system, Capecchi successfully cut out changes he made to a transgenic mouse, only in gametic cells (17). A mouse gene, tACE, makes a murine angiotensin-converting enzyme, and only becomes transcriptionally active during spermatogenesis. After placing the Cre gene with a neomycin resistance selectable marker after the tACE, Bunting et al flanked the region with loxP sites. Initiating the tACE effectively excised the region, leaving only one loxP site among the progeny DNA (17). With HACs, such loxP sites could flank the centromere. As already mentioned, without the centromere the chromosome is lost during mitosis and meiosis. Capecchi showed that this system can be time and tissue specific (14). Cre/loxP provides a deletion mechanism for the HAC when and where the extra chromosome is no longer needed. In humans, the inability to reverse a genetically engineered extra has been a major ethical issue, but this system holds the promise of being able to delete any added information.

Jesse Gelsinger was under the impression that he would have only flu-like side effects, and, because Dr. James Wilson told him his participation could help 12 million people worldwide, he was eager to join the OTC gene therapy study. However, Gelsinger fell into a coma because his ammonia level was 393 micromoles per deciliter, 10 times the normal reading. His lungs failed and even with 100 percent oxygen they were not able to penetrate his blood cells. By the time his father, Paul, was allowed to see him, he described Jesse as bloated beyond recognition. Dr. Wilson failed to mention that he knew Gelsinger's dosage of adenoviral particles could put him at great risk for liver failure (18).

Jesse Gelsinger was sacrificed for gene therapy. Similar concerns will surround human artificial chromosomes even after they prove safe and efficient for human trial. This new type of gene therapy, however, will avoid the problems associated with the current techniques. The HAC will not cause a viral inflammatory response like the adenovirus did in Gelsinger. The DNA, recognized by the immune system as human, will avoid killer T-cell attack, allowing more transgene expression (14). HACs use host cellular transcription mechanisms; thus, their transgenes will not incorporate into the host genome and will not interrupt sequences important for normal cellular function. While HAC might be put to use beyond simply therapeutic treatments, its use may make it possible not only to prevent future disease, but also to provide benefits based on individuals' choices. Ethicists will no doubt have a field day over the situation, but if HACs gain acceptance for germline use, humans begin to seize control of our own evolution. Raising an important question, Gregory Stock, Director of Medicine, Technology and Society at UCLA's School of Medicine asks, "To what extent will we transform ourselves?" (14)

References:

1. Raper SE, Chirmule N, Lee FS, Wivel NA, Bagg A, Gao GP, Wilson JM, Batshaw ML. *Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer*. Mol Genet Metab. 2003 Sep-Oct;80(1-2):148-158.
2. Eliot Marshall. *Gene Therapy Death Prompts Review of Adenovirus Vector*. Science. 1999 Dec 17;286(5448):2244-45.
3. <http://www.mindfully.org/GE/GE4/Gene-Therapy-Leukemia-Cancer11oct02.htm>

4. Andy Choo. *Engineering human chromosomes for gene therapy studies*. Trends in Mol Med. 2001 June;7(6):235-237.
5. Masashi Ikeno, Hidehito Inagaki, Keiko Nagata, Miwa Morita, Hiroshi Ichinose, Tuneko Okazaki. *Generation of human artificial chromosomes expressing naturally controlled guanosine triphosphate cyclohydrolase*. Genes to Cells. 2002;7:1021-32.
6. Brenda R. Grimes, Eirk Schindelhauer, Nio I. McGill, Andrew Ross, Thomas A. Ebersole, Howard J. Cooks. *Stable gene expression from a mammalian artificial chromosome*. EMBO. 2001 Oct;2(10):910-914.
7. Zoia Larin, Jose Mejia. *Advances in human artificial chromosome technology*. Trends in Gen. 2002 Jun;18(6):313-319.
8. Huntington F. Willard. *Genomics and Gene Therapy: Artificial Chromosomes Coming to Life*. Science. 2000 Nov 17;290(5495):1308-9.
9. Karla A. Henning, Elizabeth A. Novotny, Sheila T. Compton, Xin-Yuan Guan, Pu P. Liu, Melissa A. Ashlock. *Human artificial chromosomes generated by modification of a yeast artificial chromosome containing both human alpha satellite and single-copy DNA sequences*. Genetics. 1999;96(2): 592-597.
10. Dirk Schindelhauer, Tobias Schwarz. *Evidence for a Fast, Intrachromosomal Conversion Mechanism From Mapping of Nucleotide Variants Within a Homogeneous alpha-Satellite DNA Array*. Genome Res. 2002 Dec;12(12):1815-26.
11. MJ Greenwood, PM Lansdorp. *Telomeres, telomerase, and hematopoietic stem cell biology*. Arch Med Res. 2003 Nov-Dec;34(6):489-495.
12. Harrington JJ, Van Bokkelen G, Mays RW, Gustashaw K, Willard HF. *Formation of de novo centromeres and construction of first-generation human artificial microchromosomes*. Nat Genet. 1997 Apr;15(4):345-355.
13. Dieken ES, Epner EM, Fiering S, Fournier RE, Groudine M. *Efficient modification of human chromosomal alleles using recombination-proficient chicken/human microcell hybrids*. Nat Genet. 1996 Feb;12(2):174-82.
14. *Engineering the Human Germline*. (2000). Edited by Gregory Stock and John Campbell. Oxford: Oxford University Press.
15. *Evolutionary Analysis 3rd ed.* (2004). By Scott Freeman and Jon C. Herron. Upper Saddle River: Pearson Prentice Hall.
16. Monsef Benkirane, Dong-Yan Jin, Rene F. Chun, Richard A. Koup, and Kuan-Teh Jeang. *Mechanism of Transdominant Inhibition of CCR5-mediated HIV-1 Infection by ccr5Δ32*. J Biol Chem. 1997 Dec 5;272(49):30603-6.
17. Michaeline Bunting, Kenneth E. Bernstein, Joy M. Greer, Mario R. Capecchi, and Kirk R. Thomas. *Targeting genes for self-excision in the germ line*. Genes Dev. 1999 Jun 15;13(12):1524-28.
18. <http://www.primr.org/jessesintent.html>