MOLECULAR BIOLOGY

Engineering better artificial chromosomes

Constructing human artificial chromosomes in yeast avoids unintended multimerization

By **R. Kelly Dawe**[1,2]

Artificial chromosomes can carry large numbers of engineered genes and have been proposed as an alternative technology for adding or recoding genetic information in human cell lines (*1*, *2*). Early studies showed that when sequences from human centromeres were introduced along with a marker gene into cell lines, they formed stable artificial chromosomes (*1*). However, human artificial chromosomes (HACs) formed this way were often large concatenated (linked together) mixtures of the introduced DNA (*1*–*3*), reducing their usefulness for precision genome engineering. On page 1344 of this issue, Gambogi *et al.* (*4*) describe a substantially improved method that results in HACs that are small and structurally well defined. The work is likely to reinvigorate efforts to engineer artificial chromosomes in both animals and plants.

A typical eukaryotic chromosome contains many genes, two telomeres that stabilize the ends, and a centromere that interacts with the spindle during mitosis. In budding yeast (*Saccharomyces cerevisiae*), artificial chromosomes were created by placing centromere sequences onto circular or linear DNA molecules (with telomeres) that then segregated as normal chromosomes (*5*). Scaling this technology to the human genome, which is two orders of magnitude larger than the yeast genome, proved to be more difficult (*1*, *2*). Early efforts to make HACs involved extracting ~200–kilo–base pair (kbp) segments of the native centromeres and transforming the molecules back into human cultured cells along with a selectable marker. The artificial chromosomes that emerged from selection often contained up to 40 or more copies of the original molecule in complex multimerized forms (*3*). These results suggested that the 200-kbp units used might have been too small or that the method of transformation and selection promoted multimerization.

Gambogi *et al.* used a different approach to creating HACs (see the figure). To form centromeres, they used a technique called protein tethering, in which a small region of the artificial chromosome contains an array of 256 DNA binding sites (LacO sites) that bind to the bacterial protein lactose repressor (LacI) (*6*). The host cell expresses a specialized fusion protein (LacI-HJURP), which binds to the DNA array and recruits the key endogenous centromere protein CENP-A (centromere protein A), which subsequently spreads to nearby sequences and forms a functional centromere. This approach to HAC formation is effective because a variety of sequences inserted into a HAC will support functional centromere formation when CENP-A is present (*7*).

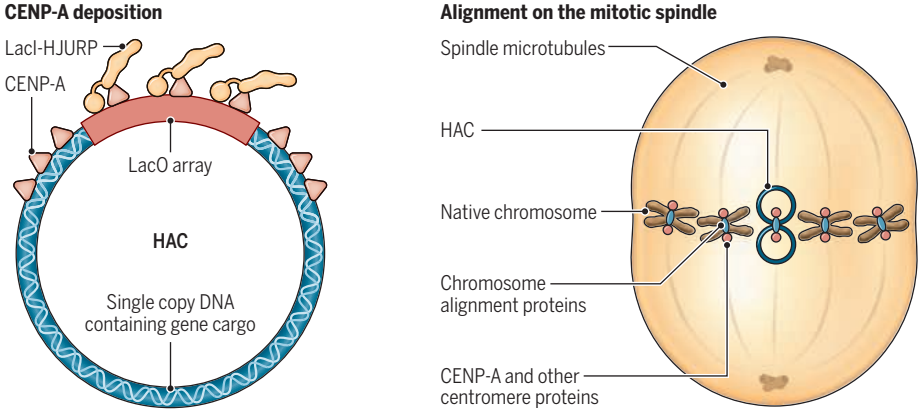
The HAC produced by Gambogi *et al.* was initially assembled as a circular artificial chromosome in budding yeast. It contained 760 kbp of DNA, which is about 1/200th the size of a human chromosome. To transfer the HAC into human cell lines, they passed the chromosome directly from yeast into cultured cells using cell fusion. Fusion was achieved by removing the yeast cell wall and applying chemical treatments that promote the merging of cell membranes (*8*). The newly formed HACs rarely multimerized after being transferred to human cells; instead, the majority were maintained as single-copy circular molecules. The single-copy HACs recruited endogenous regulatory proteins that facilitate chromosome alignment on the mitotic spindle and segregated at mitosis in a manner similar to that of endogenous chromosomes.

The major advantages of single-copy HACs are their size and defined structure. Traditional genome engineering efforts are limited by the size of the DNA pieces that can be moved from one source to another, which is generally defined by bacterial cloning vectors. The most advanced bacterial cloning vectors have a maximum size of ~200 kbp. By contrast, using the method reported by Gambogi *et al.*, it is theoretically possible to manipulate molecules up to ~1500 kbp or larger (the size of the largest yeast chromosome). Traditional genetic modification strategies are further complicated by engineered genes usually being inserted into the native genome, often at random locations, which can affect their expression in different ways. In an artificial chromosome, genes can be assembled in precise and predetermined arrangements so that their expression is predictable. Once an artificial chromosome is demonstrated to be useful in one cell line or individual, it could be reused in another and be expected to have the same properties.

More work is needed to better understand the strengths and limitations of the new approach reported by Gambogi *et al.* One area of concern is the stability of the HACs during mitotic segregation. The authors showed

Single-copy human artificial chromosome

The human artificial chromosome (HAC) is a large circular molecule. An array of 256 LacO sites binds lactose repressor (LacI)–HJURP fusion proteins, which deposit native centromere protein A (CENP-A) that can spread over nearby single-copy DNA. CENP-A defines the location of the new centromere. During mitosis, the replicated HAC aligns on the mitotic spindle and interacts with inner centromere proteins that assure accurate chromosome alignment. Thus, the HAC can be transferred to daughter cells.



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that these HACs, similar to the earlier multimerized versions, are lost during mitosis more frequently than normal chromosomes, perhaps because of their structure or relatively small size. Some of this instability can be attributed to the behavior of circular chromosomes, which spontaneously form double-ring structures and undergo cycles of breakage (*9*). Linearizing the circular chromosome and adding telomeres may add more stability (*10*). In general, the lower limits on chromosome size are not understood, nor are the limits that may apply to the relative sizes of the centromere, pericentromeric regions (adjacent to centromeres), and gene cargo areas. It is also not yet known how the compact size of artificial chromosomes might affect the regulation of gene expression, particularly when genes are placed near the centromeres or pericentromeric regions. It should be possible to further increase the size of the artificial chromosomes, alter the relative sizes of their components, and add genes and telomeres to better understand their performance.

Future applications of HACs will likely focus on introducing long genes or multigene clusters into cell lines or individuals. Such artificial chromosomes may enable the creation of versatile cell lines that better model human disease or can be used to produce pharmaceuticals and vaccines (*11*). Moreover, the implications of the work by Gambogi *et al.* are not limited to what can be done in animals. The principles of centromere design, use of yeast for engineering, and cell fusion to transfer large molecules should be applicable across kingdoms. In higher plants, a CENP-A tethering method for engineering centromeres has already been demonstrated (*12*), and cell-cell fusion is achievable after removal of the cell walls (*13*). It may soon be possible to include artificial chromosomes as a part of an expanding toolkit to address global challenges related to health care, livestock, and the production of food and fiber. ■

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ECOLOGY

Collateral impacts of organic farming

Clustering organic cropland can reduce pesticide use on nearby conventional farms

By **Erik Lichtenberg**

Organic and conventional farms are frequently located close to one another because of suitable climate and soil conditions, proximity to marketing channels, ownership, and other local factors. Yet proximity does not necessarily make for compatibility. Drift from pesticide sprays or pollen from genetically modified crops can threaten organic certification status (*1*). Conversely, insects, fungal spores, and weed seeds from flower strips maintained for natural pesticide control in organic fields can be sources of pest infestation in conventional fields (*2*), as can mobile insect pests that are inadequately controlled in organic fields. On page 1308 of this issue, Larsen *et al.* (*3*) used field observations from Kern County, California, to show that being surrounded by organic fields can help reduce the use of pesticides by organic crop producers but increases their use on conventional fields. Clustering organic cropland has the potential to mitigate the collateral impact on pesticide use for conventional cropland.

"When pests are mobile, collective action may be the only efficient or effective way to achieve control."

When pests are mobile, collective action may be the only efficient or effective way to achieve control. Collective strategies can differ qualitatively from control strategies that make sense on an individual farm basis. For example, in California, efficient control of alfalfa weevil (*Hypera postica*) relies on the suppression of mobile adults before reproduction rather than the juveniles that actually cause crop damage (*4*). By contrast, eradication of boll weevil (*Anthonomus grandis*) requires the use of intensive sprays of early-season trap crops to kill weevils emerging from dormancy on a county-wide basis, followed by no attempts to control any survivors to avoid selecting for resistance (*5*, *6*). Unfortunately, individual incentives often fail to align sufficiently to enable voluntary collective action (*7*). Furthermore, differences in practices allowed in conventional and organic farming can make collective pest control infeasible, even when individual farmers would like to coordinate actions.

The potential for pest spillovers between organic and conventional fields has been established in principle, with simulations suggesting that pests in organic fields can migrate to nearby conventional ones (*8*), but the extent to which they occur is not well documented in practice. To address this knowledge gap, Larsen *et al.* used field observations from neighboring organic and conventional fields in Kern County, California, between 2013 and 2019. Located at the southern end of the San Joaquin Valley, Kern County produces a wide variety of high-value crops, including grapes, citrus, almonds, pistachios, carrots, tomatoes, potatoes, stone fruits, pome fruits, watermelons, and alfalfa and other forage. Many have a short growing season and are highly susceptible to damage from insects and diseases. Some number among the most pesticide-intensive crops grown in the United States. Conventional and organic fields are intermingled throughout the county, with some growers operating both conventional and organic fields.

Larsen *et al.* analyzed data on roughly 14,000 individual fields that were derived from public records, including Kern County's digitized maps of agricultural fields and the crops grown on them, records of pesticide applications (required under California law), and records of fields with organic certifications provided by the California Department of Food and Agriculture. They then conducted careful statistical analyses to investigate how pesticide use in both conventional and organic fields varies with the prevalence of organic fields nearby.

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