the entropic point of view, but the Y's are energetically more favorable than the ends. The Y's and ends may thus phase separate as the temperature is lowered, resulting in a dense connected network (the Y's) in equilibrium with a dilute gas of polymers (the ends). This scenario is not unique: Identical symmetry and global topological arguments describe a very different system, namely microemulsions, which are (at least) ternary systems of oil, water, and surfactant (*8*).

The Tlusty-Safran transition may be viewed as a specific example of a phase transition in a hierarchical system, in which the basic elements involved in the phase transition are composite objects. Such hierarchical assemblies are characteristic of biological structures (*9*), and it is likely that analogous transitions will be observed in biogels. The Tlusty-Safran transition can also be viewed as a topological transition, similar to the Blue liquid crystalline phases (*10*), which are ordered arrays of disclinations (the typical defects of orientationally aligned liquid crystals). As the soft condensed matter community becomes familiar with topological transitions, the development of general models providing a unified description of these diverse and seemingly distinct phenomena may be expected.

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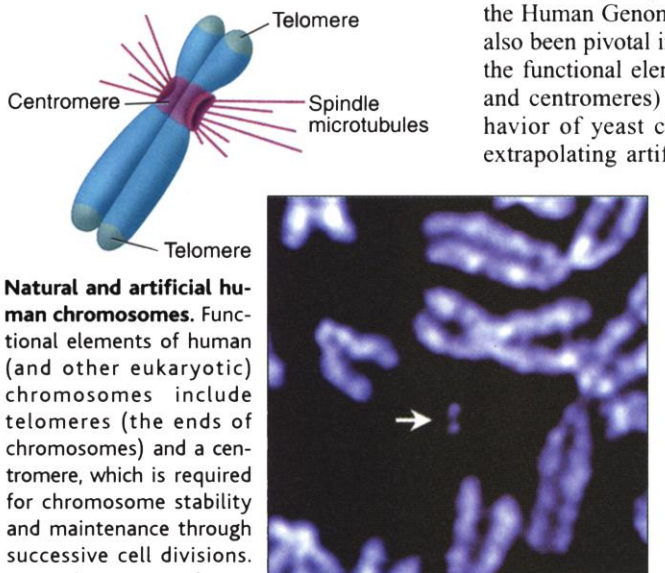
PERSPECTIVES: GENOMICS AND GENE THERAPY

Artificial Chromosomes Coming to Life

Huntington F. Willard

Most advertisements that arrive on my desk rapidly find their way into the trash with barely a glance; however, occasionally a piece will catch my eye. "Make Biology Come Alive," cried the headline of an advertisement for educational software that promised to teach the reader about Genetic Engineering, including Gene Therapy. Following on the footsteps of the first reported death of a gene therapy patient, Jesse Gelsinger, at a time when nearly everyone is urging caution and calling for more balanced reporting of results of gene therapy research (*1*), I was curious to see how "Gene Therapy" would be portrayed.

"Gene therapy is the use of genes for correcting genetic disorders." So far, so good, although the lead statement ignores the fact that, with two exceptions, no such correction (at least not to the point of proven clinical benefit) has been convincingly demonstrated by any of the hundreds of gene therapy protocols currently being pursued. Indeed, the first two clinical gene therapy successes were reported only earlier this year (*2*). The software then invites the reader/viewer to click on different "techniques for transferring DNA to humans," including "DNA coated in lipids," "viruses," and "artificial human chromosomes." What? Artificial human chromosomes being transferred to human beings?



Natural and artificial human chromosomes. Functional elements of human (and other eukaryotic) chromosomes include telomeres (the ends of chromosomes) and a centromere, which is required for chromosome stability and maintenance through successive cell divisions. Inset shows several normal human chromosomes and a single artificial human chromosome (arrow). Natural human chromosomes range in size from ~50 to ~250 megabases. The artificial human chromosomes constructed so far range from 2 to 6 megabases in size.

Have I been missing something?

In the early 1980s, artificial chromosomes came to life with the construction of a fully functional yeast chromosome from its component parts (*3*). Yeast artificial chromosomes (YACs) can be assembled from cloned telomeres (the specialized DNA sequences found at the ends of chromosomes), a centromere (to ensure proper segregation through each cell division), and genomic fragments carrying origins of DNA replication (so that DNA synthesis is correctly initiated at sites along the chromosomal DNA). YACs are fully stable in both types of cell division (mitosis and meiosis) and have proven to be of inestimable value as a workhorse for assembling overlapping clones of DNA as part of the Human Genome Project (*4*). They have also been pivotal in defining and dissecting the functional elements (such as telomeres and centromeres) required for normal behavior of yeast chromosomes. However, extrapolating artificial chromosome technology to mammalian cells and chromosomes has proven to be a difficult task.

Mammalian chromosomes are two to three orders of magnitude larger than yeast chromosomes. The molecular components of their telomeres—well conserved among a wide range of eukaryotic organisms (*5*)—have been identified and are increasingly well understood. In contrast, the composition of mammalian and other non-yeast centromeres has been much more difficult to determine. Mammalian centromeres are likely to be structurally complex because they have to attach large and complex chromosomes to the spindle apparatus during cell division.

Without a functional centromere, artificial chromosomes are unstable, fail to attach to the spindle, and are quickly lost. Thus, many researchers are seeking to identify the sequence elements in centromeres that enable them to work properly during cell division. Attempts to define the mammalian centromere have taken one of two

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converging experimental paths. Analysis of either naturally occurring (*6*) or engineered (*7*) rearrangements of human chromosomes has delimited the centromere locus to the vicinity of the megabase-sized arrays of alpha satellite DNA (the dominant class of tandemly repeated DNA found at or near the primary constriction of each human chromosome). Such studies, although eliminating much or all of the DNA in the chromosome arms from consideration, cannot distinguish whether alpha satellite sequences themselves constitute the centromere or whether a different functional sequence might be embedded within the alpha satellite array. Indeed, detailed mapping of centromeric satellite DNA arrays (both in humans and in other organisms) has indicated the presence of at least some nonsatellite sequences, most often members of retrotransposon (mobile DNA) families (*8*).

An alternative approach has been to test alpha satellite sequences directly by incorporating them into artificial chromosomes. At least three groups have now demonstrated that large blocks of alpha satellite DNA contribute to the formation of mitotically stable, megabase-size artificial human chromosomes that have a fully functional centromere (and, in some cases, fully functional telomeres) (*9*). However, despite the conceptual simplicity of this approach, the results of these studies do not amount to definitive proof that alpha satellite DNA "is" the functional centromere. In all cases, the efficiency of artificial chromosome formation is lower than one would desire and the artificial human chromosomes are larger than the sum of their DNA components. This indicates that at least some DNA rearrangement or amplification has taken place. Given the size and complexity of these mammalian artificial chromosomes, it has not been possible to establish definitively that their only functional sequences are those that were present in the original DNA components. This would require controlled manipulation of both the amount and type of input sequences, as well as a more complete understanding of the determinants of and steps involved in the formation of a competent centromere (*10*). The establishment of centromeric chromatin seems to be critical for the formation of a functional centromere (perhaps even more critical than the actual DNA sequences themselves) because nonsatellite sequences can, at an undetermined frequency, form competent centromeric DNA in some abnormal human chromosomes (*11*). In the case of both satellite and nonsatellite centromeres, incorporation of a specific centromere histone protein, CENP-A, appears to be a necessary requirement (*10*).

One of the substantial promises of artificial chromosome research (already realized in yeast) is to provide an experimental approach for defining the organization of complex genomes. Continued development of these systems in mammalian cells, using genomic manipulations to interrogate aspects of chromosome biology, may enable an integrated "chromonomics" approach to a number of complex biological and medical problems. These include imprinting (an inherited pattern defining whether the maternal or paternal allele of a gene is expressed), X chromosome inactivation, position effects (by which expression of a gene is affected by other distant sequences), disorders of chromatin assembly or function, and other examples of long-range control of gene expression. Analysis of artificial chromosomes containing different fragments of (for example) human genomic DNA should permit the process of chromatin assembly (about which we are generally quite ignorant) to be followed. In addition, this may provide a way to systematically evaluate the genomic determinants of active or inactive chromatin and their effects on gene expression in a variety of developmental, ontological, or pathological systems.

From a more applied standpoint, why would one want to make artificial human chromosomes? This brings us back (albeit gingerly) to "Gene Therapy." Recent versions of human artificial chromosomes introduced into cultured cells express the human *HPRT* gene (*12*). This gene—cloned into the artificial chromosome as a ~90-kilobase genomic fragment—is mutated in the inherited neurological disorder Lesch-Nyhan disease. These studies provide proof-of-principle that it will be possible to design and assemble a variety of different artificial human chromosomes carrying and expressing genes of biological or therapeutic interest. Given this development, how realistic is it to consider artificial human chromosomes as vectors (gene carriers) for gene therapy (*13*)? Notwithstanding the pronouncement of the educational software that trumpets artificial chromosomes as an off-the-shelf technique soon to be ready for humans, the promise of such constructed chromosomes for gene therapy remains unproven. They have many potential advantages over other vectors (particularly those based on viruses), including capacity for carrying much bigger genes, maintenance of gene copy number, absence of side effects, and long-term regulated gene expression. But a number of barriers block their introduction as gene therapy vectors, not the least of which is the necessity for new delivery systems capable of introducing these giant DNA molecules intact into recipient cells. Furthermore, centromeres of artificial chromosomes appear to exhibit at least some species-specific features that will need to be fully understood before testing artificial chromosome vectors in non-human animal models (*14, 15*). Fragments of natural human chromosomes have recently been introduced into mice and transmitted to progeny (*16*). In addition, a well-defined, engineered minichromosome containing both human and mouse centromeric elements has been transmitted through the mouse germ line (*15*). These data are promising and suggest that evaluation of the stability and expression of human genes in artificial chromosomes, both in vitro and in vivo, should soon be possible (*5, 12*).

Notwithstanding recent successes (*2*), setbacks for gene therapy associated with the death of Jesse Gelsinger and with the limitations of gene therapy protocols underscore the need for renewed and comprehensive research into the most fundamental mechanisms of gene delivery and expression (*1*). It is in this context that artificial chromosome technology—itself less than 3 years old and still very much in the realm of basic gene therapy research—may come to life.

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17. I disclose a financial interest in Athersys Inc., a Cleveland-based biopharmaceutical and gene therapy company.