cation of the introduced DNA and its segregation into daughter cells. At least two approaches are being explored towards this goal. The first uses plasmid vectors carrying parts of the Epstein–Barr-virus genome that allow the retention and replication of DNA under the control of the host cell-cycle[3]. Another is to re-create the natural cellular vehicle for DNA replication and segregation — the human chromosome.

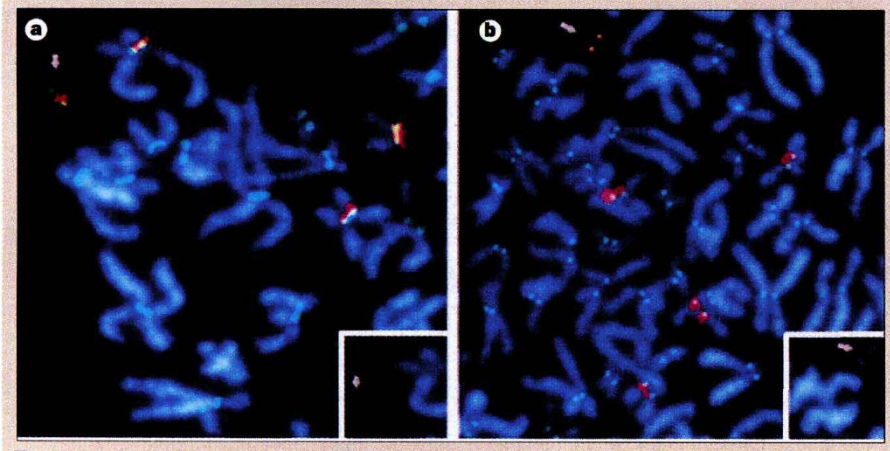
The minimal requirements for an artificial chromosome include sequences to allow for the maintenance of its ends (telomeres), replication of its DNA, and mitotic segregation to daughter cells (centromeres) (Fig. 1). Yeast artificial chromosomes (YACs) have been available for many years, and they function when introduced into cells as naked DNA. Mammalian geneticists, on the other hand, suffer from species envy, largely having had to be content with functional reintroduction of telomeres[4]. Moreover, whereas origins of replication are defined sequence elements in yeast, the existence of analogous mammalian origins remains controversial. One suggestion is that potential sequence-specific origins are frequent in the genome. Another is that replication simply requires a threshold amount of mammalian DNA, with the specific patterns of replication initiation that are observed being created by overall chromosome structure[5]. In either case, the replication of MACs may not require careful provision for origins.

The main stumbling block for the construction of MACs, which Harrington *et al.*[1] have now overcome, has been the provision of a centromere and the kinetochore that assembles there. The centromere is not simply a passive point at which the chromosome attaches to the microtubules of the mitotic spindle — it actively participates in the segregation of chromosomes to daughter cells. The centromeric region contains at least one motor, which is involved in chromosome movement during cell division. The centromere also mediates a critical checkpoint that prevents completion of cell division until all chromosomes are properly attached to the mitotic spindle[6]. The centromere (*CEN*) in *Saccharomyces cerevisiae* is a 125-base-pair DNA sequence; human centromeres, on the other hand, contain up to several million base-pairs of alpha satellite DNA, a large, tandemly repeating 171-bp DNA family[7]. Previous reintroduction studies had indicated that alpha satellite DNA could provide some aspects of centromere function[8,9], but the microchromosomes constructed by Harrington *et al.* (see panel) are the first to have all the features necessary to designate them MACs.

The successful creation of microchromosomes with centromere function from introduced naked DNA is a considerable step towards a MAC-based vector system for gene therapy. Some problems remain, such as the incompletely defined DNA that is used, and the variable and unpredictable structure, and relatively low yield, of the microchromosomes. This is further compounded by undesirable genomic rearrangements, which are caused by the introduced telomeric sequences. Ultimately, a practical MAC system for gene therapy must use defined DNA and yield microchromosomes in a predictable and efficient fashion. Even then, it still faces the problems of immune reactions against the therapeutic gene product, cell-specific targeting and so forth. Manipulation and delivery of such large DNA molecules is a technological challenge — these MACs are the whoppers of gene-therapy vectors — and it will be interesting to determine just how small a centromere-containing human chromosome can be. MACs do not have the stringent size limits of many viral-delivery systems, so they may allow for natural patterns of gene expression by introducing large genomic fragments carrying the therapeutic gene in its natural genomic environment.

Microchromosomes should also aid our understanding of chromosome maintenance and transmission in both mitosis and meiosis. For example, by custom-building arrays of mutated centromeric sequences from human and other species, the sequence

Ordering up a MAC



To make an artificial chromosome, Harrington *et al.*[1] obtained megabase-sized alpha satellite arrays, approaching the size of those at natural centromeres. Starting with a single characterized alpha satellite repeat unit, they used a directional-cloning method in a low-copy bacterial artificial chromosome vector, followed by *in vitro* ligation, to make tandem arrays up to 1 Mbp in size. This DNA was then mixed with human $(TTAGGG)_n$ telomeric sequences and high molecular-weight genomic DNA, and introduced into cultured human cells using cationic liposomes. Although most clones represent integration or chromosome-fragmentation events, at least two cases of *de novo* centromere formation seemed to occur on the introduced alpha satellite DNA. One such microchromosome, created from transfected DNA including alpha satellite from human chromosome 17, is shown. In most cells only a single microchromosome is seen, indicating faithful maintenance of copy-number control. These microchromosomes are stably inherited for many generations in the absence of selection, with a loss rate of less than 0.5 per cent per cell division. The formation of centromeres was further shown by the ability of the microchromosomes to bind two known centromere-associated proteins, CENP-C and CENP-E. **a**, The microchromosome (arrow) was detected using antibodies against CENP-C (green), and the chromosome-17 alpha satellite was detected by *in situ* hybridization (red). **b**, A similar experiment using antibodies against CENP-E (green). Co-localization of green and red signals leads to a yellow signal. In both cases, the chromosomes were stained with the DNA dye DAPI (blue). These microchromosomes seem to be linear and they are 6–10 Mbp in size, but they are composed of a complex arrangement of sequences, characteristic of non-homologous recombination of the introduced DNA cocktail. (Figure reproduced courtesy of Harrington *et al.*[1].) **P.E.W. & D.K.**