

# CHROMATIN DYNAMICS '97

## Centromere DNA Dynamics: Latent Centromeres and Neocentromere Formation

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The centromere is a vital chromosomal structure that provides all living cells with the ability to faithfully partition their genetic material during mitotic and meiotic cell divisions. It functions by holding newly replicated sister chromatids together, allowing the attachment of spindle microtubules, and orchestrating the ordered movement of chromosomes to the daughter cells. The centromere has also been recognized as a "marshalling station" for a host of "passenger proteins" that appear transiently on the centromere during specific stages of the cell cycle (Earnshaw and Mackay 1994). Through the study of these and several of the known constitutive centromere proteins, diverse roles for these proteins have been defined, such as formation of centromere-specific chromatin, cohesion and release of sister chromatids, control of cell-cycle checkpoint, motor movement of chromosomes, modulation of spindle dynamics, organization of nuclear structure and intercellular bridge, and cytokinesis (reviewed by Earnshaw and Mackay 1994; Pluta et al. 1995; Choo 1997). This review will focus on the unusual properties of the DNA that underlies centromere function and will discuss the implications of recent studies on neocentromeres, in light of our new understanding of the dynamic nature of the centromere DNA.

### The CEN-DNA Paradox

All eukaryotic centromeres, except those of the budding yeast *Saccharomyces cerevisiae*, are known to contain a great abundance (as much as 5%–50% of each chromosome) of repeat DNA sequences. In humans, a typical centromere carries as many as 2,000–4,000 kb of a 171-bp repeat, known as " $\alpha$ -satellite" (e.g., see Ty-

ler-Smith and Brown 1987; Wevrick and Willard 1991; Jackson et al. 1993; Trowell et al. 1993). Several lines of evidence suggest that this DNA has a role in centromere function: (i) transfection of this DNA into cultured mammalian cells has shown that it confers centromere activity at the sites of DNA integration (Heartlein et al. 1988; Haaf et al. 1992; Larin et al. 1994); (ii) analysis of rearranged (Tyler-Smith et al. 1993) or in vitro-truncated (Brown et al. 1994) Y chromosomes has demonstrated that retention of 150–200 kb of  $\alpha$ -satellite is essential for centromere activity; and (iii) cotransfection of  $\alpha$ -satellite with telomeric DNA and total human genomic DNA into human cell culture has resulted in the formation of a stable and independently segregating minichromosome (Harrington et al. 1997). However, conflicting evidence has come from observations in human dicentric chromosomes, in which this DNA is present on both the active centromere and the inactive centromere, suggesting that  $\alpha$ -satellite per se does not confer centromere function (Earnshaw et al. 1989; Page et al. 1995; Sullivan and Schwartz 1995). Moreover, an increasing number of stable human marker chromosomes (discussed below) have now been identified that lack detectable  $\alpha$ -satellite, indicating that this DNA is not mandatory for centromere function.

In other species, studies using a minichromosome in *Drosophila* have demonstrated that ~200 kb of a simple, A/T-rich satellite DNA is important for centromere function and stable inheritance of the minichromosome (Murphy and Karpen 1995). Similarly, investigation of the centromere DNA of the fission yeast *Schizosaccharomyces pombe* has indicated that a "K-type" repeat is essential for centromere function (Hahnenberger et al. 1991). In addition to these organisms, centromeric repeats from a wide range of organisms have now been isolated and characterized (reviewed by Choo 1997). The analyses of these sequences (including those of the relatively simple, repeat DNA-free but A/T-rich, 125-bp centromeres of *S. cerevisiae*; Clarke and Carbon 1985) have revealed a rather perplexing outcome, in that no discernible homology is seen between the centromeric DNA of the different species. This apparent lack of phylogenetic conservation of the DNA sequence of a struc-

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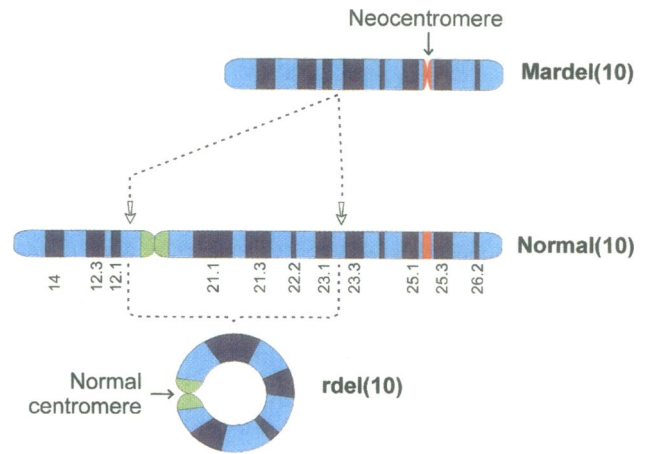
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ture that performs such a universal function as the centromere therefore seriously challenges conventional evolution dogma and highlights a “CEN-DNA paradox.” At present, the basis for this paradox is not fully understood, although some light is beginning to emerge from various recent studies described below.

### Analphoid Neocentromere Cases

In recent years, an increasing number of supernumerary human marker chromosomes with centromeres that contain no detectable  $\alpha$ -satellite have been reported. These “analphoid” marker chromosomes carry newly derived centromeres (or “neocentromeres”) that are apparently formed within interstitial chromosomal sites that have not previously been known to express centromere function. A well-characterized analphoid neocentromeric marker chromosome is the mardel(10) chromosome described by Voullaire et al. (1993) (fig. 1). This neocentromere forms a distinct primary constriction and is negative for  $\alpha$ -satellite DNA, satellite 3 DNA (a simple repeat often found in the pericentric regions of human centromeres), CENPB (a functionally unknown centromere protein that binds a subset of  $\alpha$ -satellite DNA), and C-banding (which detects repeat DNA—dense chromosomal regions, including all normal human centromeres). The marker chromosome is 100% stable in both short-term lymphocyte cultures and long-term cultures of fibroblast and lymphoblast cells, indicating that its neocentromere is fully functional in mitosis. The activity of this neocentromere is further revealed by the detection (du Sart et al. 1997; E. Earle and K. H. A. Choo, unpublished data) of three functionally important centromere proteins: CENPA, CENPC, and CENPE. In other studies, CENPA has been shown to be a centromere-specific core histone that serves to differentiate the centromere from the rest of the chromosome, at the chromatin level (Sullivan et al. 1994); CENPC has been shown to be an essential centromeric protein for mitosis, as is evident from the inhibition of mitotic progression in cells microinjected with anti-CENPC antibodies (Bernat et al. 1990; Tomkiel et al. 1994); and CENPE has been shown to be a motor molecule that is important for chromosome movement (Lombillo et al. 1995; Thrower et al. 1995). In addition, CENPC and CENPE are known to associate with the active—but not with the inactive—centromeres of dicentric chromosomes (Earnshaw et al. 1989; Page et al. 1995; Sullivan and Schwartz 1995).

Maraschio et al. (1996) recently described a different case of a stable, chromosome 3–derived, analphoid marker chromosome that was formed through a rearrangement similar to that described for mardel(10). In addition, 16 other analphoid marker chromosomes, originating from 9 different chromosomes, have now been reported (fig. 2 and table 1). These marker chro-

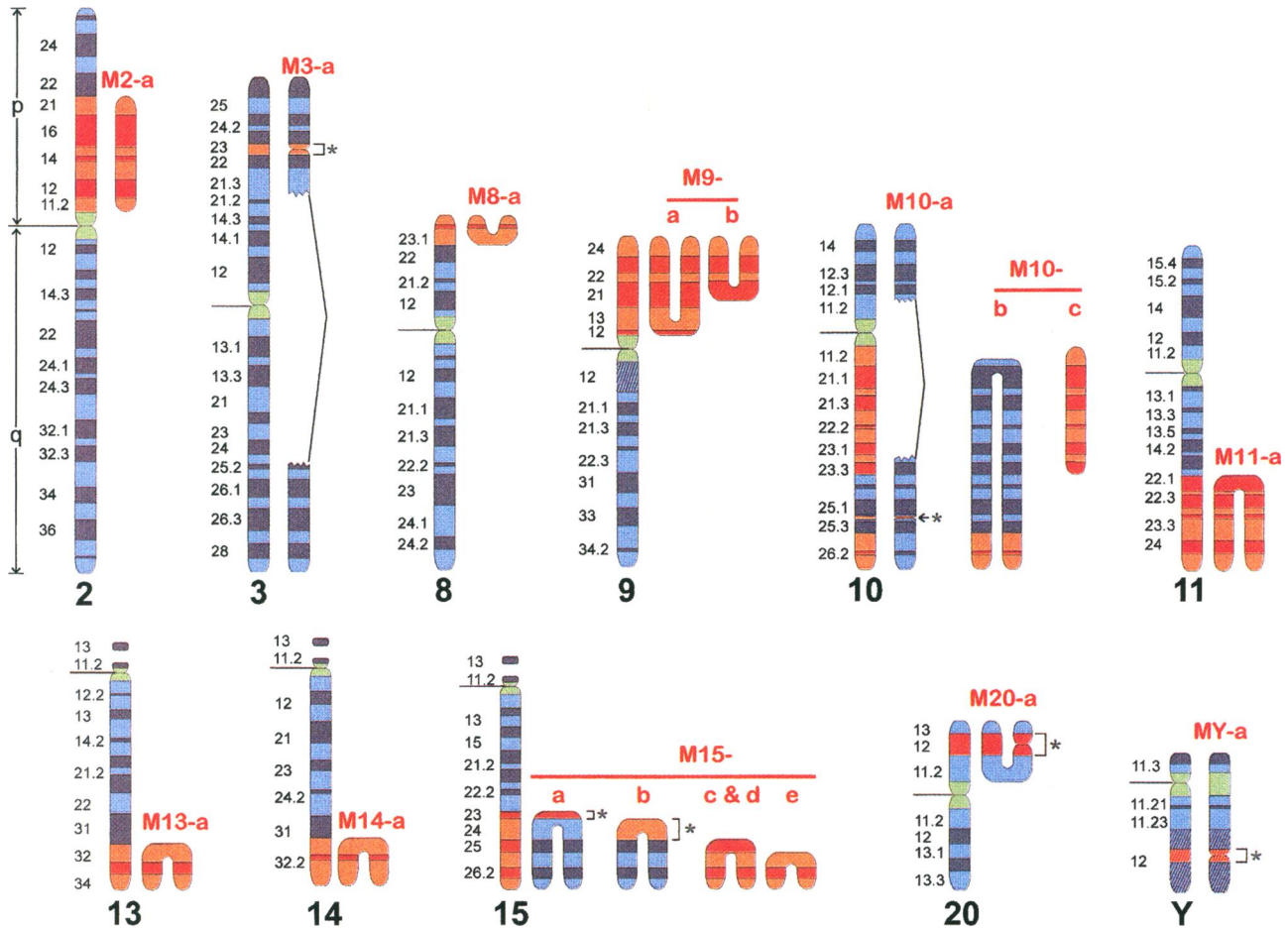


**Figure 1** Formation of the chromosome 10–derived, analphoid, neocentromeric marker mardel(10) (Voullaire et al. 1993). Two breaks (downward-pointing, unblackened arrows), one on each side of the normal centromere, release a centric fragment that forms a ring chromosome, rdel(10), while the two distal fragments rejoin to form an analphoid marker chromosome that carries a functional neocentromere at band 10q25.2.

mosomes have been recognized through their lack of detectable  $\alpha$ -satellite DNA and their C-band–negative nature. They are characterized by the formation of functional neocentromeres in chromosome regions that are not discernibly rearranged at the cytogenetic level. These neocentromeres have conferred to the marker chromosomes a significant, although sometimes variable, level of mitotic stability (see below). Where tested (markers M9-a, M10-c, M11-a, M13-a, M15-c, and M20-a), the neocentromeres, like that of mardel(10), demonstrated the presence of centromere protein(s) CENPC and/or CENPE, but not CENPB, although, in two cases (markers M8-a and M14-a), only the presence of some unspecified centromere proteins was reported, when anti-centromere antibodies derived from the sera of patients with the “CREST” form of autoimmune disease were used (Moroi et al. 1980).

By far the most common mechanism for the formation of analphoid marker chromosomes is the de novo inverted duplication of some distal segments of chromosomes. This mechanism, which results in mirror-image chromosomes, accounts for 13 of the currently described markers (fig. 2). In 11 of these mirror-image chromosomes, the neocentromeres are found on one of the duplicated arms. In the remaining two cases (M15-a and M15-b), which were presented as metacentric chromosomes, the neocentromeres are at or near the breakpoints of the inverted duplications at 15q23 and 15q24, respectively.

The inferred chromosomal structures of markers M2-a, M10-c, and MY-a are different from those depicted by the two mechanisms described above and are



**Figure 2** Putative latent-centromeric sites within the human genome. These sites were deduced from their involvement in the formation of neocentromeres in a number of independent aneuploid marker chromosomes derived from chromosomes 2, 3, 8–11, 13–15, 20, and Y. Thirteen of these markers (indicated by the U-shaped configurations) were formed as a result of inverted duplications of distal chromosome fragments. In the remaining markers, M3-a and M10-a were formed through the mechanism shown in fig. 1. M2-a and M10-c were formed from an internal deletion of 2p11→p21 and 10q11→q23, respectively; interestingly, both these markers lacked detectable telomeric DNA, suggesting that they may be ring chromosomes. MY-a was formed through an unknown mechanism that reconstituted a morphologically normal Y chromosome carrying an inactivated normal centromere. In some of these cases, the banding patterns of the marker chromosomes drawn are based on limited published information and are therefore approximate. For the karyotypes, references, and other properties of these marker cases, refer to table 1. Areas shown in green designate normal centromeres, whereas those shown in orange designate chromosomal regions carrying one or more potential latent-centromeric sites. Asterisks (\*) denote regions where more-refined localization of the neocentromeres on the marker chromosomes has been described.

formed through different types of rearrangements (see the legend to fig. 2). In addition to these marker chromosomes, other, less well-characterized but stable aneuploid markers, of undefined chromosomal origin and/or morphology, have been reported (Callen et al. 1992; Crolla et al. 1992; Rauch et al. 1992).

Only limited information on the time of formation of aneuploid neocentromeric marker chromosomes is available. The mirror-image chromosome M10-b was reported as an acquired event found in the leukemic cells of a patient with acute myeloid leukemia (Abeliovich et al. 1996). This suggests that the chromosome has a mitotic origin and that the steps leading to neocentromere

formation can occur in cancerous cells. In addition to this marker, four other neocentromeric chromosomes have been studied by DNA polymorphism analyses (Depinet et al. 1997). Three of these markers (M13-a, M15-c, and M15-e) have been shown to have a probable mitotic origin, whereas one (M15-d) appeared to be formed during meiosis. Further studies on the mitotic/meiotic origins of these and other neocentromeric marker chromosomes will be important for the understanding of the mechanisms underlying their formation.

In total, ≥ 11 different human chromosomes are now known to display neocentromeric activity (fig. 2 and table 1). The number of individual neocentromeric sites

**Table 1**  
**Properties of Anaphoid Marker Chromosomes Shown in Figure 2**

MARKER	KARYOTYPE <sup>a</sup>	STABILITY OF MARKER IN <sup>b</sup>		CENTROMERE PROTEIN <sup>c</sup>		REFERENCE	
		Lymphocyte Fibroblast		Lymphoblast	GENPB		Other <sup>d</sup>
		Fibroblast (%)	Lymphoblast (%)				
M2-a	47,XY,del(2)(p11-p21),+der(2)(p11-[neocent]-p12)	NA	100	NA	NA	NA	Petit and Fryns (in press)
M3-a	47,XX,-3,+r(3)(p21.3-q25),+rea(3)(pter-p23[neocent]p23-p21.3::q25-qter)	100	100	NA	NA	NA	Maraschio et al. (1996)
M8-a	47,XX,+der(8)(pter-p23.1::p23.1-[neocent]-pter)	100	NA	NA	NA	+	Ohashi et al. (1994)
M9-a	47,XY,del(9)(p12),+der(9)(pter-p12::p12-[neocent]-pter)	100	NA	100	-	+	Vance et al. (1997)
M9-b	47,XY,+der(9)(pter-p21.2::p21.2-[neocent]-pter)	100	NA	NA	NA	NA	Depinet et al. (1997)
M10-a	48,XY,-10,+rdel(10)(p11.2-q23.2),+mardel(10)(pter-p11.2::q23.2-q25.2[neocent]q25.2-qter),+bisatellited marker	100	100	100	-	+	Voullaire et al. (1993)
M10-b	dup(10)(qter-q11.2::q11.2-q26[neocent]q26-qter)	90 <sup>e</sup>	NA	NA	NA	NA	Abeliovich et al. (1996)
M10-c	47,XX,del(10)(q11-q23),+der(10)(q11-[neocent]-q23)	62	80	NA	-	+	Depinet et al. (1997)
M11-a	47,XY,del(11)(q22),+der(11)(qter-q22::q22-[neocent]-qter)	100	100	100	-	+	Depinet et al. (1997)
M13-a	47,XY,+der(13)(qter-q32::q32-[neocent]-qter)	98	8	32	-	+	Depinet et al. (1997)
M14-a	47,XX,del(14)(q32.1-qter),+der(14)(qter-q32.1::q32.1-[neocent]-qter)	100	NA	100	NA	+	Sacchi et al. (1996)
M15-a	47,XY,+der(15)(qter-q23::q23[neocent]q23-qter)	70	11	0	NA	NA	Blennow et al. (1994)
M15-b	47,XX,+der(15)(qter-q24::q24[neocent]q24-qter)	80	NA	0	NA	NA	Blennow et al. (1994)
M15-c	47,XX,+der(15)(qter-q25.3::q25.3-[neocent]-qter)	82	NA	33	-	+	Depinet et al. (1997)
M15-d	47,XY,+der(15)(qter-q25.3::q25.3-[neocent]-qter)	74	NA	3	NA	NA	Depinet et al. (1997)
M15-e	47,XY,+der(15)(qter-q26.1::q26.1-[neocent]-qter)	86	NA	0	NA	NA	Depinet et al. (1997)
M20-a	47,XX,del(20)(p11.2),+der(20)(pter-p11.2::p11.2-p12[neocent]p12-pter)	100	100	100	-	+	L.E. Voullaire and K. H. A. Choo (unpublished data)
MY-a	45,X/46,XY/47,XY+mar(Y)(pter-psucent-q12[neocent]q12-qter)	5	NA	NA	NA	NA	Bukvic et al. (1996)

<sup>a</sup> Inferred positions of putative neocentromeres are given.

<sup>b</sup> NA = not available.

<sup>c</sup> A plus sign (+) denotes presence of the protein; and a minus sign (-) denotes absence of the protein. NA = not available.

<sup>d</sup> GENPC, GENPE, or GREST (see text).

<sup>e</sup> Detected in leukemic cells of a patient with acute myeloid leukemia.

within the human genome is expected to be higher, since some of these chromosomes (e.g., chromosomes 10 and 15) have already been shown to contain multiple potential sites for such activity, and there seems no reason not to believe that this number will increase as new anaphoid neocentromeric markers are reported.

### Latent-Centromere Activation and Epigenetic Control

du Sart et al. (1997) investigated the DNA of the mardel(10) neocentromere. These workers employed centromere-specific anti-CENPA and anti-CENPC antibodies to tag the neocentromere. By chromosome walking using cloned single-copy normal DNA, an 80-kb region containing the core antibody-binding domain of the neocentromere was identified. Extensive restriction mapping (du Sart et al. 1997) and high-density PCR analyses (M. R. Cancilla and K. H. A. Choo, unpublished data) of this core region indicated an identical genomic structure for the neocentromere and the corresponding normal chromosomal DNA at 10q25.2. These results, together with those of FISH studies reported, have indicated that the neocentromere DNA is specific to 10q25.2 and does not cross-hybridize with the DNA of the normal centromeres. The du Sart et al. study therefore provided direct molecular evidence that a previously noncentromeric region of the human genome is nonetheless capable of forming a neocentromere.

On the basis of the existing data (Voullaire et al. 1993; du Sart et al. 1997), the most plausible explanation for the formation of a neocentromere at a hitherto noncentromeric chromosomal site in mardel(10) is that a "cryptic" or "latent" centromere becomes activated in situ within band 10q25.2 (fig. 1). Direct molecular evidence for the other neocentromeric marker chromosomes is eagerly awaited, but their cytogenetic and biochemical characteristics suggest that they too are formed through a similar mechanism involving latent-centromere activation. Furthermore, since all the known neocentromeres have originated from chromosomes' euchromatic regions (except for marker MY-a, which was formed within Yq12 heterochromatin) that are generally known to be chromosome specific, it can be inferred that the DNA sequences for these putative latent centromeres are different from one another.

At present, the mechanism responsible for the activation of latent centromeres is not clear. Given that such a mechanism has the property of conferring heritable changes to otherwise identical DNA sequences, it must, by definition, be "epigenetic" in nature (Steiner and Clarke 1994). This epigenetic mechanism would submit the centromere DNA—or the preferred active configuration of the centromeric DNA–protein complex—to a type of modification that is self-replicating. The nature of this modification is equally unknown, although mech-

anisms involving either methylation of centromeric DNA (Mitchell et al. 1996) or acetylation of centromeric chromatin (Allshire 1997) have been proposed.

This epigenetic modification may, besides its possible role in the formation of neocentromeres, also help control centromere activity in human dicentric chromosomes containing normal centromeres. It is generally assumed that true dicentrics with two active centromeres are rare, since they are unstable and prone to anaphase bridge formation and breakage due to forces generated by two active centromeres pulling the chromosomes toward opposite spindle poles. As such, most dicentric chromosomes are pseudodicentrics, in which one of the centromeres has become inactivated (e.g., see Earnshaw et al. 1989; Therman and Susman 1993; Page et al. 1995). Furthermore, cases have been described in which different cells from a given individual have inactivated different centromeres on a pseudodicentric chromosome (e.g., see Dewald et al. 1979; Ing and Smith 1983; Rivera et al. 1989). It is unclear whether such mosaicism results from successive inactivation-reversion processes or from a functionally dicentric chromosome that has persisted long enough to be acted on by random centromere inactivation to produce the distinct cell lines. These observations indicate that the functional potentials of both the centromeres of a dicentric chromosome remain intact and that centromere inactivation does not appear to involve overt modification of centromere DNA content. This situation is somewhat analogous to the cases in which (a) an inverted duplication creates a second, presumably identical copy of a latent centromere but (b) only one is activated. Thus, as with the formation of neocentromeres, epigenetic mechanisms offer the simplest explanation for centromere inactivation in the dicentric chromosomes. Interestingly, inactivation of the normal centromere can also occur in neocentromeric marker chromosomes. This is exemplified by marker MY-a (fig. 2), where the normal centromere of the Y chromosome has been retained and has become inactivated. The investigation of the molecular nature of centromere-targeted epigenetic mechanisms, as well as the determination of whether a common mechanism is responsible for the activation/inactivation of normal and latent human centromeres, should constitute an exciting new area of research.

Epigenetic mechanisms have also been implicated in the functional regulation of alternative centromere states in lower organisms, where such regulation appears not to be accompanied by alteration in the sequence content, structural organization, or chemical state of the chromosomal DNA. In *S. pombe*, activation of a nonfunctional centromere on a minichromosome to a functional one has been observed (Steiner and Clarke 1994; Ngan and Clarke 1997), whereas, in *Drosophila*, acentric chromosome fragments that exhibit residual centromere

activity have been generated (Murphy and Karpen 1995). The centromeres of the holocentric chromosomes (i.e., chromosomes with centromeres that are present diffusely along their entire length rather than regionally at a single differentiated point) of the horse parasitic nematode *Parascaris univalens* have also been shown to undergo drastic activation/inactivation cycles in different cell types (Goday and Pimpinelli 1989; Goday et al. 1992).

From the aforementioned studies, it is clear that eukaryotic cells have the ability to selectively regulate their centromere activity, through an operation that not only searches chromosomes for suitable DNA sequences for adoption into functional centromeres but also restricts any “unnecessary,” or “surplus,” centromeric sites that are present on the same chromosomes from expressing centromere activity. In future studies, it will be of interest to determine—for example, by use of neocentromere formation as a model—both the prevalence of this “*search-adopt-restrict*” mechanism and whether neocentromeric hotspots exist within the genomes. Another pertinent question is the stage of the cell cycle or window of embryonic development at which such a mechanism will operate. Since, to effect the imprinting of functional centromeric status, this mechanism requires direct access to chromosomal DNA sequences during both the “search” step and the assembly of specific chromatins, it is conceivable that the mechanism will be cell-cycle dependent or embryonic-stage dependent.

### Biological Significance of the Latent-Centromere System

The biological significance of having an intrinsic system whereby latent centromeres exist alongside a normal centromere is not entirely clear. At the population level, it has been proposed that activation of latent centromeres, by providing newly arisen acentric chromosome fragments with the ability to segregate autonomously, may constitute an important mechanism for karyotype evolution (Dutrillaux 1979). For example, this mechanism offers a reasonable explanation for the observed progressive increase in chromosome numbers during the evolution of a group of Old World monkeys, *Cercopithecidae* (Dutrillaux 1979), and for the appearance, in humans but not in mice, of a centromere within two syntenically conserved regions (Searle et al. 1989). However, since the centromeres of the present-day chromosomes in these species contain centromeric repeat sequences, the possibility that the original acentric chromosomes have acquired some normal centromeric repeat DNA through translocation cannot be excluded. Alternatively, the formation of neocentromeres might have been accompanied by additional unknown mech-

anisms that facilitated the gain of centromeric repeats over time.

At the individual level, it may be speculated that the ability to activate latent centromeres in otherwise acentric chromosomes that erroneously arise from time to time may provide an effective way to salvage these chromosomes to protect against genomic imbalance. Insight into this possibility can be gained from the observation that the formation of neocentromeres in a number of human supernumerary marker chromosomes has indeed served a vital role in that, in some cases (e.g., markers M3-a and M10-a), a cytogenetically balanced karyotype is restored and, in others (markers M9-a, M11-a, M14-a, and M20-a), a trisomic state of the duplicated region is achieved. In all these cases, loss of the marker chromosome would result in a monosomic condition for a substantial portion of the genome. Consistent with an essential role of the neocentromeres, these markers have been shown to be 100% stable in both short-term and long-term cultures. However, in a number of other cases, the retention of the neocentromeric marker chromosomes appears to have a negative effect. In these cases, the presence of the marker chromosomes seriously upset karyotype balance, whereas their loss would in fact have restored the balance. Examples are the inverted duplications that give rise to tetrasomies of the duplicated regions (fig. 2, markers M8-a, M9-b, M13-a, and M15-a–M15-e). These markers show varying degrees of stability (i.e., 70%–100%) in short-term lymphocyte cultures and, when tested in fibroblast or long-term lymphoblast cultures (markers M13-a and M15-a–M15-e), are quite unstable and often totally lost (table 1). These markers therefore reinforce the idea that, if activation of latent centromeres serves the purpose of counteracting genomic imbalance, then its mode of regulation is imperfect, because of its seeming inability to discriminate between essential and nonessential acentric chromosome fragments.

### CEN-DNA Dynamics, Neocentromere Formation, and CEN-DNA Paradox

The study of neocentromeres in humans and lower organisms, described in this communication, has unraveled a previously unsuspected phenomenon depicting the dynamic nature of the centromere DNA. At the core of this phenomenon, there appears to be no specific DNA sequence requirement for centromere function; rather, a new class of DNA, containing a variety of sequences that can respond to the appropriate epigenetic influence, appears to provide this function. As with the epigenetic influence, the characteristics of this proposed class of DNA are unclear. One possibility is that this class of DNA may be enriched for A/T nucleotides that are or-

ganized in a special and presently esoteric spatial manner, since sequence analyses of the normal centromeric DNA from many different organisms have indicated that A/T richness is a widespread feature. Another possibility is that the DNA may possess special bending properties that would allow the DNA to fold into the appropriate centromere-active conformation. Whatever the nature of this DNA is, this phenomenon of "CEN-DNA dynamics" offers a reasonable explanation for the formation of neocentromeres at widely different alphoid chromosomal locations in the human genome. In addition, it provides a plausible explanation for the observed lack of sequence conservation (i.e., the CEN-DNA paradox) among the centromeres of different species. The investigation of this phenomenon will be aided by the cloning of neocentromeric DNA from different chromosomal locations, to allow detailed comparison of nucleotide sequences and functional domains. It will also be important to identify neocentromere-binding proteins and to study the biochemistry of protein-DNA interactions at the different centromeric sites. In recent years, a number of centromere-targeted proteins have been reported to have homologues in widely separated species (reviewed in Choo 1997), although none of these proteins has as yet been shown to procure the proposed epigenetic functions.

### Practical Implications for Artificial-Chromosome Construction

An artificial chromosome is a defined structure that carries all the necessary functional elements for its long-term survival, replication, and division in a cell. There has been an increasing interest in the construction of such artificial chromosomes in mammalian/human cells, for a variety of important reasons, including structural and functional analysis of chromosomes, study of gene expression, genetic manipulation of animals, and stable transmission of therapeutic genes in human gene therapy. Despite the fact that artificial chromosomes have been created in budding and fission yeasts since the early 1980s (Murray and Szostak 1983; Hahnenberger et al. 1989), the construction of mammalian artificial chromosomes has so far met with less success. A major reason is the ill-defined nature of the mammalian centromere DNA, since strategies based on the use of normal centromeric repeat DNA have to contend with uncertainty of centromere function and the instability of such DNA in conventional cloning systems. The discovery of latent-centromeric DNA and the realization of the dynamic property of the centromere DNA promise to offer an expanded repertoire of sequences that can be used to make artificial chromosomes. A caveat to this, however, is that we do not yet understand the mechanisms

whereby centromere latency and activation are controlled. Although current evidence suggests that once a latent centromere becomes activated the change is heritable through mitosis, it remains to be determined whether such a change can be maintained through cloning in microorganisms or whether the cloned DNA, when returned to a mammalian nucleus, will be subject once again to the activation of one latent-centromeric site and the silencing of any other such sites. The pursuit of answers to these and many new questions that will arise from the recognition of the existence of latent centromeres and of the dynamic nature of the centromere DNA is likely to have a profound impact not only on artificial-chromosome construction but also on our understanding of genome evolution and the control of centromere functions.

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