Stable dicentric X chromosomes with two functional centromeres

Naturally occurring dicentric chromosomes allow examination of complex cis-acting effects that regulate kinetochore function and chromosome segregation. Dicentric chromosomes with well-separated centromeres are functionally monocentric, stabilized by the poorly understood phenomenon of centromere inactivation. We show here that dicentric X chromosomes with closely apposed centromeres remain functionally dicentric.

Centromeres in multicellular eukaryotes, including humans, are structurally complex and are associated with large blocks of repeated DNA (ref. 1). Although the precise nature of human centromeres remains unknown, α-satellite DNA may have a role in centromere function2,3, in concert with centromere proteins (CENPs) involved in assembling functional centromeres and kinetochores4. CENP-A, CENP-C and CENP-E localize to kinetochore plates and are exclusively associated with functional centromeres5,6.

In many organisms, dicentric chromosomes are unstable and have two functional centromeres. Such functionally dicentric can manifest as anaphase bridging and chromosome breakage7,9. In mammals, however, dicentrics can be stable and may reflect inactivation of one of the two centromeres. For example, nonhomologous dicentric chromosomes (such as Robertsonian translocations) are generally mitotically stable, and CENP-A, C and/or E are detected only at one centromere7,8,9. In contrast, dicentric X chromosomes (dic(X)s), detected in approximately 15% of patients with Turner syndrome, typically contain two identical copies of the X centromere separated by a variable amount of material from the proximal short arm (Xp) and may be partially unstable, as they are frequently found in mosaic karyotypes10-13. We used antibodies to human CENP-C and CENP-E to examine Turner syndrome-derived dic(X)s with only 2-12 Mb of Xp material separating the two centromeres13 (Fig. 1a). CENP antibody staining typically appears as a pair of fluorescent dots at active centromeres5,10 (Fig. 1b). Here, CENP-C and -E were detected at both centromeres in most cells of the four dic(X) examined (Table 1; Fig. 1a,b), suggesting that both centromeres can assemble kinetochores. These dic(X)s were different from those with well-separated centromeres (for example, DIC34) in which two sets of CENP signals were never detected (Fig. 1a, and data not shown), and which are, therefore, considered functionally monocentric.

Several of the dic(X) lines are karyotypically mosaic, with both a dic(X)-containing line and a 45,X line (Table 1). Such mosaicism may reflect chromosome loss at the time of dicentric formation or ongoing instability due to the presence of two functional centromeres11,12. To address the proposed instability of these dic(X)s, we monitored movement of functionally monocentric and dicentric Xs using a novel procedure to enrich for anaphase and telophase cells. With this assay, segregation of chromosomes to daughter cells could be monitored directly, and control chromosomes (either the normal X or representative autosomes) segregated normally (that is, one copy to each daughter cell) in more than 98-99% of mitoses. Functionally monocentric dic(X)s segregated as accurately as control chromosomes (Fig. 1c, Table 1, and data not shown). Two of three functionally dicentric dic(X)s showed elevated levels of dic(X) lag (Table 1). In approximately 25% of anaphase/telophase cells, DIC4 (Fig. 1d,e) and DIC10 were observed either at the spindle midzone in anaphase or between two newly formed daughter cells in telophase, clearly separated from the other chromosomes.

Behaviour of dic(X) in anaphase/telophase did not correlate with the
presence of a mosaic karyotype (Table 1). Therefore, although some functionally dicentric dic(X)s show retarded segregation during mitosis, they are not inherently unstable and must, in most instances, overcome the evident anaphase lag. We propose that the mosaicism associated with most cases of dic(X) reflects chromosome malsegregation and loss at the time of dicentric formation, rather than an ongoing instability.

Our findings indicate several different mechanisms involved in ensuring the stability of dicentric chromosomes. Further, as the distance between centromeres in these dic(X)s has been established by physical mapping, these data provide evidence for an influence of inter-centromere distance on centromere function. Larger dic(X)s appeared to be stabilized by centromere inactivation, characterized by the absence of kinetochore proteins at one centromere (data not shown). As the distance between centromeres decreased, however, centromere inactivation was not consistently observed, and both centromeres appeared capable of kinetochore assembly, as judged by the presence of CENP-C and CENP-E at two α-satellite regions (Fig. 1a,b). That such functionally dicentric dic(X)s are mitotically stable implies a high degree of coordination between the two sets of active kinetochores over molecular distances of up to 12 Mb, ensuring ultimate orientation of the two kinetochores on each chromatid to the same spindle pole. Although all dic(X)s were stable, some experienced anaphase lag, perhaps indicating additional heterogeneity in the stabilization process. Similar heterogeneity in CENP staining has been noted for some dicentric human Robertsonian translocations, although centromeric distances were not reported in that study.

Our results indicate that the presence of two functional kinetochores on a single chromosome does not invariably lead to chromosome instability and loss. As such, there may be degrees of dicentric instability determined by inter-centromere distance, the nature of the two centromeres and/or potential cell cycle checkpoint mechanisms. Severe instability may result in anaphase bridge formation, chromosome breakage and chromosome loss, and less extreme instability in chromosome lag without chromosome loss.

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Table 1 • Segregation of normal and dicentric X chromosomes in anaphase

<table>
<thead>
<tr>
<th>Line</th>
<th>Karyotype</th>
<th>Correct segregation (2:2 or 1:1) (%)</th>
<th>Normal X lag (%)</th>
<th>Dic(X) lag (%)</th>
<th>Control autosome lag (%)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>46,XY</td>
<td>92</td>
<td>1</td>
<td>n/a</td>
<td>1</td>
</tr>
<tr>
<td>DIC34</td>
<td>46,X, dic(X)</td>
<td>93</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>DIC4</td>
<td>46,X, dic(X)</td>
<td>76</td>
<td>1</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>DIC8</td>
<td>45,X/46,X, dic(X)</td>
<td>94</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>DIC10</td>
<td>45,X/46,X, dic(X)</td>
<td>68</td>
<td>2</td>
<td>28</td>
<td>0</td>
</tr>
</tbody>
</table>

Chromosome segregation was monitored in anaphase/telephase preparations using FISH to identify the normal X, dic(X), or control autosome X (chromosome 7 or 8). At least 100 anaphase cells containing the dic(X) were scored in each experiment. *Chromosome 7 or 8, as detected with specific probes (Fig. 1, legend). Frequency of lag of other autosomes (scored only as DAPI-stained chromosomes) was less than 1% per chromosome. ‡Similar data were obtained for Dic4 without drug treatment or when treated with nocodazole for only 16 h.