

Ring chromosomes: vicious circles at the end and beginning of life

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Chromosomes are often regarded merely as static containers for genetic information. However, it is now becoming increasingly clear that chromosomes are highly dynamic structures with a tightly regulated organisation. Chromatin conformation is known to play an important role for the regulation of gene expression and untranscribed chromosomal elements have been shown to have crucial functions in the cellular life cycle. For instance, the centromeric repeat sequences provide anchor-points for the kinetochore proteins and the reduction of telomeric repeat length during proliferation sets limits to the life span of human cells. The topology of chromosomes also plays a vital role. Normal DNA replication results in two linear sister chromatids organised in a parallel configuration so that symmetrical separation can occur at the metaphase-anaphase transition. However, a change in topology from linear to circular may totally disrupt this sequence of events.

The following text is an attempt to delineate the consequences of such ring-shaped chromosomes in human cells. On rare occasions, ring chromosomes are found as constitutional aberrations in foetuses or new-borns with developmental abnormalities. More commonly, they may arise as acquired genetic abnormalities in cells from tumours or leukaemias (Table 1).

Mechanisms of ring formation

Ring chromosomes may be formed in two ways (Fig. 1):

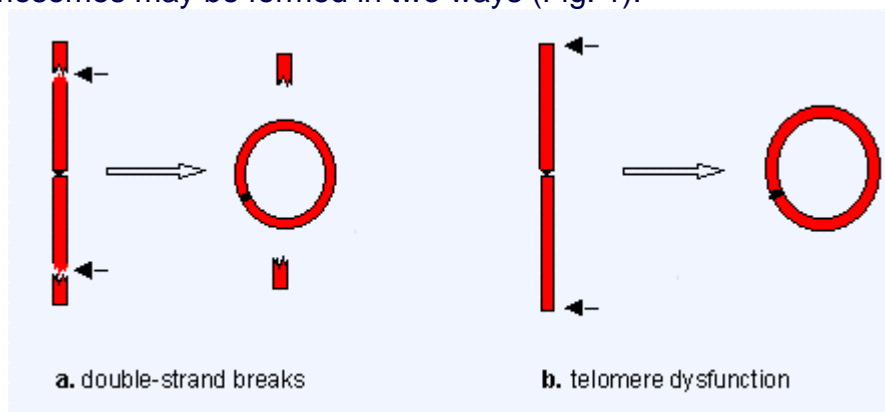


Figure 1 : Ring chromosome formation may occur through breaks in the chromosome arms and fusion of the proximal broken ends, leading to loss of distal material (a). Rings may also be formed by telomere dysfunction triggering fusion of reactive chromosome ends without major loss of genetic material (b).

1. By two DNA breaks, one in each arm of the same chromosome, followed by fusion of the proximal broken ends. The causes of these DNA breaks are usually unknown and so is the mechanism behind ligation of the ends. It is possible that the non-homologous end-joining machinery plays a role in this process (Smith et al. 2001). A ring can also be formed by fusion at two breakpoints in the same chromosome arm. However, only few examples of such rings have been described. Most probably, this is because they are acentric and will lack attachment point for the cell division machinery. Unless there is a different anchorage sequence for the kinetochore complex they will be lost in subsequent mitoses. Such *Neocentromere* sequences have, however, been described in rare cases of constitutional (Slater et al. 1999) and acquired (Gisselsson et al. 1999) ring chromosomes.
2. By fusion of dysfunctional telomeres from the same chromosome. Several in vitro and animal models have shown that shortening of telomeric DNA repeats leads to the detachment of protective proteins from the chromosome ends (Counter et al. 1992). This renders the chromosome ends prone to recombination with DNA either from other chromosomes leading to formation of a dicentric or with the other arm of the same chromosome leading to formation of a ring.

Ring chromosomes at cell division

In contrast to linear chromosomes, rings may undergo cell division in three different ways (McClintock 1938; Lejeune 1968). Which of these pathways a ring chromosome will follow depends on the number of sister chromatid exchanges (SCE) that has occurred in the ring before cell division:

1. No SCE or an even number of SCEs in the same direction will enable normal, symmetrical segregation of the chromatids.
2. An even number of SCEs in different directions will lead to the formation of interlocked rings.
3. An odd number of SCEs will lead to transformation from two parallel chromatids into one continuous ring, similar to a Möbius band with the double size of the original rings (Fig. 2).

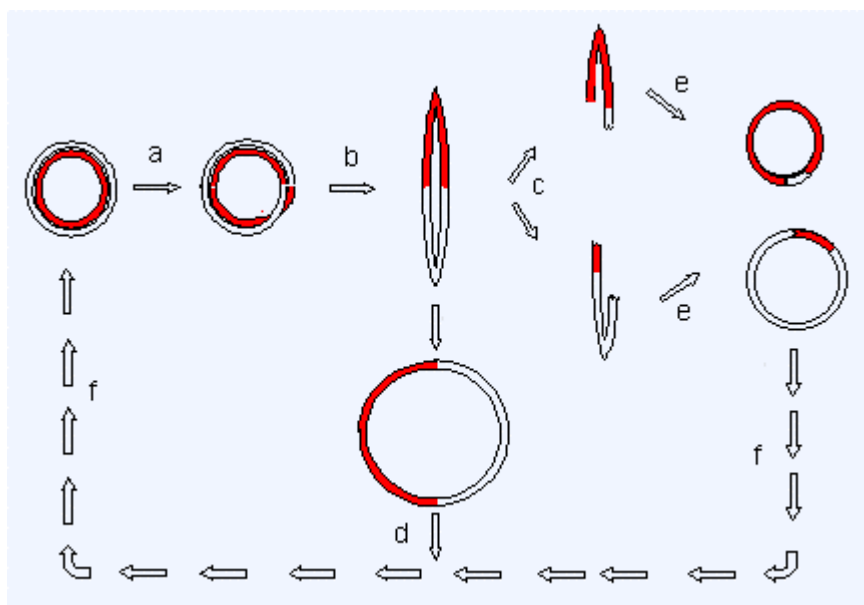


Figure 2 : Breakage-fusion-bridge cycle triggered by a sister chromatid exchange (a) leading to bridge formation (b) and breakage (c), or nondisjunction (d) at anaphase. Broken ends fuse in the daughter cells (e) and form novel ring structures, which can again undergo the same series of events (f).

The ring in (1) will undergo a normal mitotic division with equal distribution of chromosome material to the daughter cells at anaphase. In the other situations, the sister chromatids will not be capable of normal separation. Instead the interlocked (2) or continuous (3) ring will be suspended as a chromosome bridge between the two poles of the mitotic spindle at anaphase. Depending on the balance between the pulling forces of the cell division machinery and the chemical bonds within the anaphase bridge, one of the following scenarios will occur (Fig. 3; McClintock 1938):

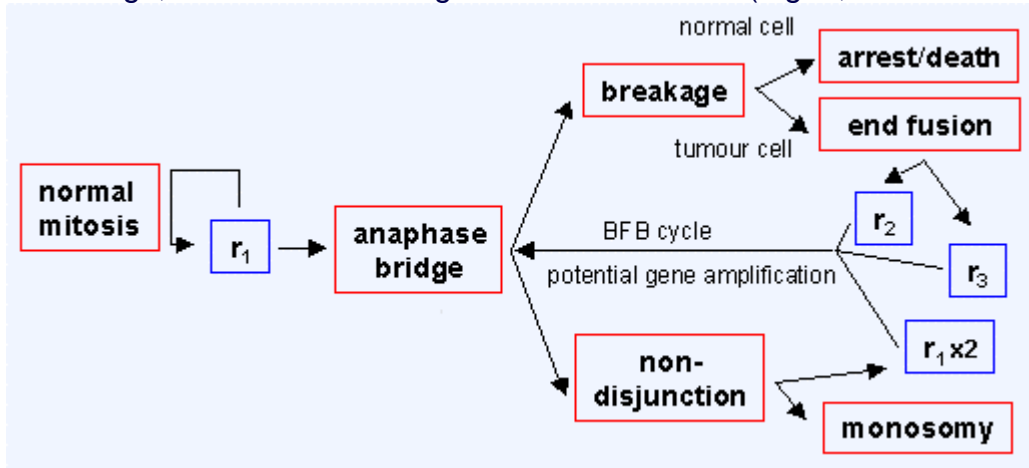


Figure 3 : Flow chart of potential ring chromosome dynamics. The ring (r_1) can either undergo normal mitosis or form a bridge at cell division. If the bridge breaks, the cell may die, arrest, or survive and allow fusion of broken ends into novel rings (r_2 and r_3). The bridge can also undergo nondisjunction so that a double-sized ring ($r_1 \times 2$) is formed. All these rings may continue through breakage-fusion-bridge (BFB) cycles, potentially leading to amplification of genes.

A. The bridge will break in two or several pieces. Breakage may occur at any point between the chromosome-spindle attachments. Broken fragments that are still attached to the spindle will each be pulled into one of the daughter cells (Fig. 2c). B. Detachment occurs between one kinetochore and the mitotic spindle. The double-sized ring/interlocked rings will then be pulled into one of the daughter cells, whereas the other cell becomes monosomic for the corresponding chromosome (fig. 2d). These dramatic distortions of chromosomal morphology may, however, only be the beginning of a chain of further recombination events. The broken ends in each daughter cell in (A) may heal by fusion to each other (McClintock 1938, 1940) forming a novel ring chromosome that can again give rise to bridges at the next cell division (Fig. 2e, f). The ring chromosome can thus trigger a series of breakage-fusion-bridge (BFB) events, causing continuous DNA breakage and recombination of the chromosomal material. The ring in (B) was not broken but may again form a bridge at any forthcoming cell division.

Constitutional ring chromosomes

Constitutional ring chromosomes occur in 1/50,000 human fetuses (Jacobs et al. 1975). In most instances, these rings are formed by breakpoints in both arms, followed by fusion of the proximal ends into a ring with loss of the distal material. Such rings may thus result in clinical features mimicking terminal deletion syndromes. Alternatively, congenital ring chromosomes are supernumerary, i.e. they occur together with two normal homologues of the corresponding chromosome (Anderlid et al. 2001), and the consequences will be similar to partial trisomies or duplications. The ring syndromes are thus a very heterogeneous group, with different

characteristics depending, not only, on which chromosome is involved, but also on the position of breakpoints within the chromosome.

However, ring syndrome patients do not only display diverse symptoms resulting from deletions or duplications. Most of them have one feature in common. In a meta-study including more than two-hundred patients with congenital ring chromosomes it has been demonstrated that the majority of children with rings show a failure to thrive beyond the extent expected from their chromosomal imbalances (Kosztolanyi 1987). It has been suggested that this is due to the mitotic instability of rings, preventing somatic cells to proliferate normally. The hypothesis is supported by the fact that growth failure is more common among patients with large ring chromosomes, than among those with small ones (Kosztolanyi 1987). This is in accordance with the BFB model of ring chromosome dynamics. Statistically, large rings will undergo more SCEs per cell cycle than small rings and would thus have a higher propensity for breaking at anaphase. In a normal cell, this provokes a physiological DNA damage response leading to either cell cycle arrest or apoptosis (Cohen-Jonathan et al. 1999).

From the reasoning above, it follows that a cell population carrying a ring chromosome would proliferate slower than a population without rings; the population with rings would be less fit and be at a selective disadvantage. Interestingly, ring chromosome loss or size reduction is not uncommon in cases with congenital rings. In particular, cases with small rings often exhibit a subclone without the ring chromosome and these patients are thus ring/monosomy mosaics (Gisselsson et al. 1999). In cases with large rings and prominent growth failure, heterogeneity of ring size is a more common feature. Children with ring chromosomes are thus illustrative examples of how natural selection at the cellular level may play a role for the symptoms and signs of human disease.

Acquired ring chromosomes

Table 1. Ring chromosome prevalence (%) in human tumours*

Hematological neoplasms	
Acute lymphoblastic leukaemia	0.7
Chronic lymphocytic leukaemia	1.1
Acute myelogenous leukaemia	2.2
Chronic myelogenous leukaemia	1.0
Carcinomas	
Breast	5.7
Colon & rectum	4.6
Gallbladder	21.1
Kidney	13.0
Larynx	5.2
Liver	13.0
Lung	8.8
Mouth	1.9
Ovary	3.9
Pancreas	11.5

Prostate	0.0
Skin	3.7
Stomach	1.7
Thyroid	1.1
Uterine cervix	0.0
Urinary bladder	4.5
Uterus	2.2
Sarcomas	
Chondrosarcoma	5.6
Dermatofibrosarcoma protuberans	70.3
Ewing sarcoma	0.6
Leiomyosarcoma	11.2
Liposarcoma	21.1
Malignant fibrous histiocytoma	11.5
Mesothelioma	14.2
Osteosarcoma	12.2
Central nervous system tumours	
Astrocytoma	0.0
Glioma	0.0
Oligendroglioma	0.0
Meningioma	4.0

* Data from the Mitelman Database of Chromosome Aberrations in Cancer, October 4, 2001.

<http://cgap.nci.nih.gov/Chromosomes/Mitelman>

Tumours with ring frequency >10% are in red.

Congenital rings appears to be a burden for normal proliferating cells. The high prevalence of rings in neoplastic cells (Table1) thus appears paradoxical at first glance. Rings are rare in benign tumours, whereas they are common in certain invasive tumours. Rings are even so common in certain subgroups of sarcomas that they may be used as diagnostic indicators for these lesions. Well-differentiated (also referred to as atypical lipomas) are borderline malignant tumours occurring primarily in the thigh and retroperitoneum (Enzinger and Weiss 1994). More than 90% of these tumours contain large supernumerary ring chromosomes, typically as the sole abnormalities or together with giant marker chromosomes (Fig. 4; Heim et al. 1987). They rarely metastasise but show a locally aggressive growth behaviour and frequently recur after surgery. A virtually identical cytogenetic scenario is found in two other low-grade malignancies: parosteal and well-differentiated (...ndal et al. 1992; Sinovic et al. 1992).

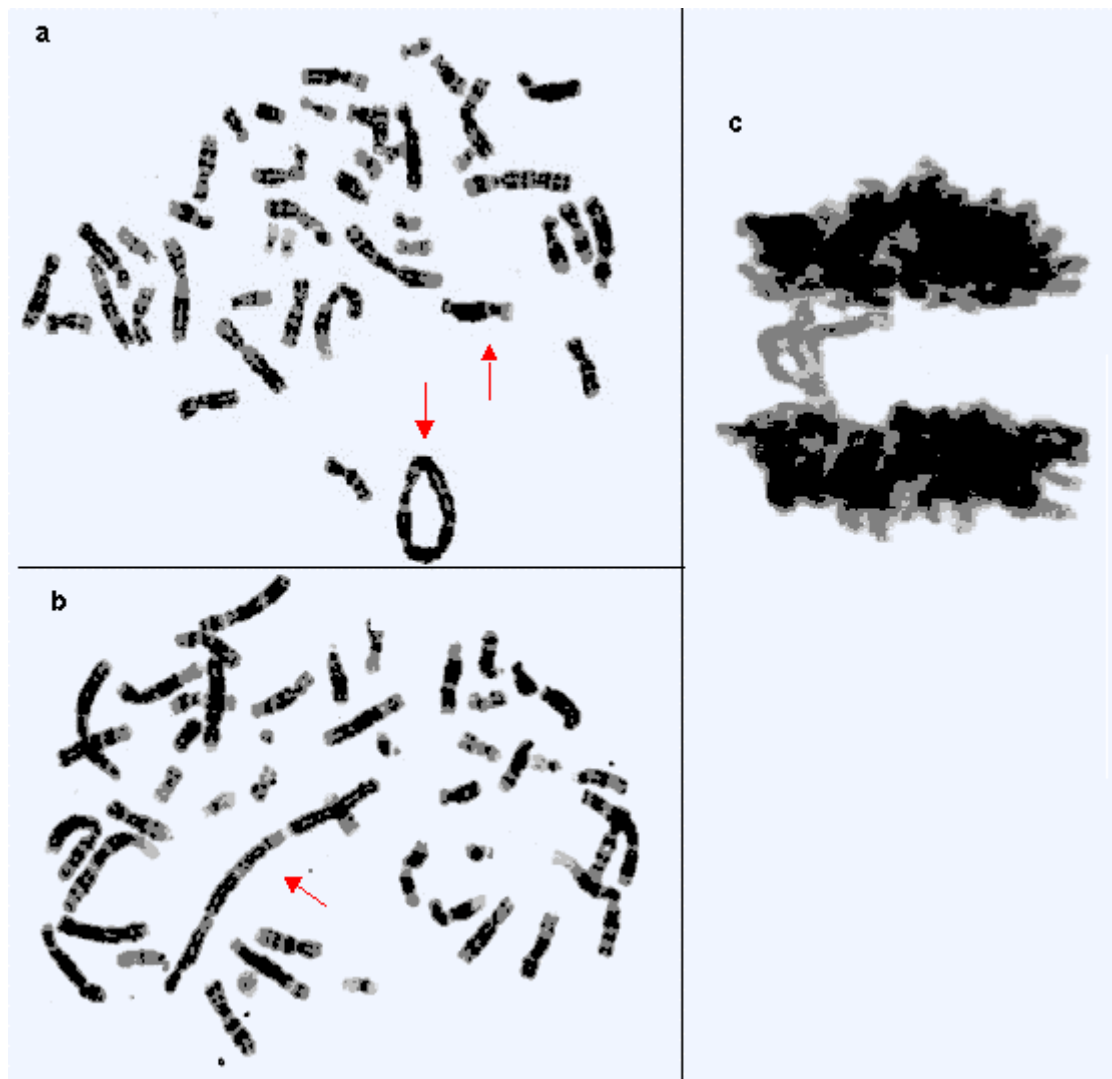


Figure 4 : Ring chromosome (a), giant marker chromosome (b), and anaphase bridge (c) in a well-differentiated liposarcoma. The former metaphase cell also contains telomeric associations between chromosomes. Courtesy of Prof. N. Mandahl.

Compared to constitutional rings, the ring chromosomes occurring in these tumours are highly unstable. The rings are rarely lost. Instead, they are frequently present in more than one copy and there is wide variability in ring size and structure within each case (Gisselsson et al. 1998) and chromosome bridges occur frequently at mitosis (Gisselsson et al. 1999). There is also evidence that the rings may break up into large linear marker chromosomes by capturing telomeric sequences from other chromosomes. Thus, BFB events do not seem to have the same negative consequences on cell proliferation as in non-neoplastic cells. Although these tumours all have an indolent growth pattern, they are still able to invade surrounding tissues and frequently reach a considerable size before clinical presentation.

Function and origin of ring chromosomes in tumours

The mechanism behind the great ring chromosome variability in some neoplastic cells is not completely understood. Several studies have shown that the normal DNA damage response is disrupted in many malignant tumours. BFB events may thus occur at a high frequency without substantial negative consequences for cell proliferation (Artandi and DePinho 2000; Gisselsson et al. 2001). There is evidence that one common mechanism behind the deficient response to DNA damage is inactivation of the protein by point mutations (Stark 1993; Gisselsson et al. 2000). In

this context, it is of interest to note that the large unstable rings in the previously mentioned bone and soft tissue tumours contain amplified sequences from the region in 12q14-15 in more than 90% of cases (Fig. 5; Berner et al. 1996). MDM2 binds to the TP53 protein and either directly inhibits its transcription factor activity or targets it for destruction (Oliner et al. 1993; Haupt et al. 1997; Kubbutat et al. 1997).

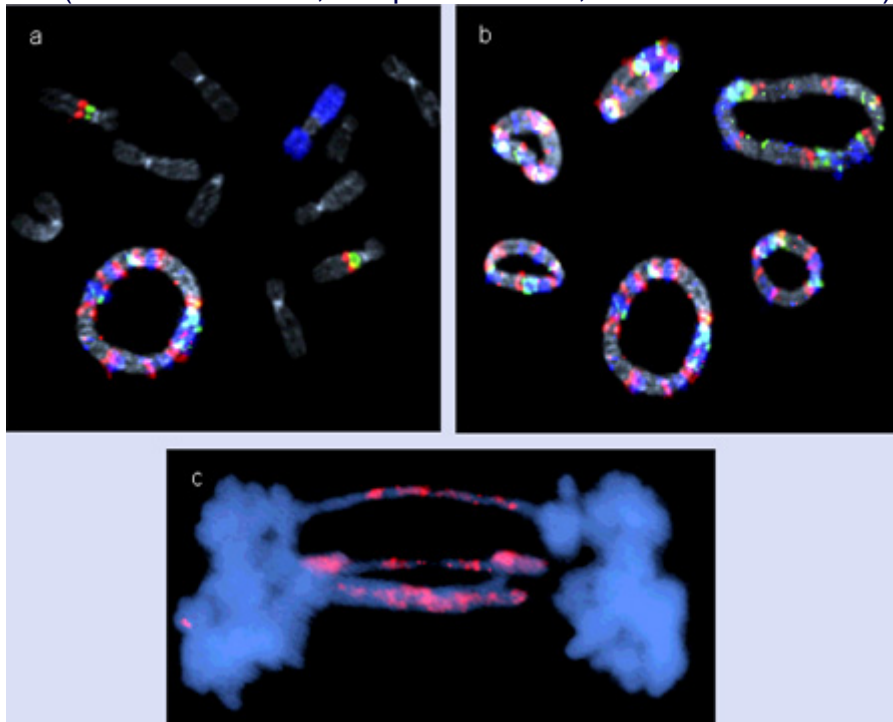


Figure 5 : Amplification of MDM2 (red), CDK4 (green), and chromosome 9 sequences (blue) in a low-grade malignant fibrous histiocytoma (a). Rings from six different cells from the same tumour showing extensive variability in size and structure (b). Anaphase bridges containing amplified MDM2 sequences (red) in a well-differentiated liposarcoma.

It is tempting to suggest that the amplified sequences carried in the rings are also the very prerequisite for ring variability and size expansion. It has been shown that the elevated copy-number of MDM2 genes in the rings correlates with an increased production of the MDM2 protein. Cells with large rings will over-express MDM2 and may thereby, at least partly, disable the DNA damage response. These rings may break at cell division with little, if any, impact on cellular survival and novel rings may form in the daughter cells after fusion of the broken ends (Fig. 3). A wide variety of rings may then occur after only a few cell divisions. If MDM2 and/or other genes in the 12q14-15 region are favourable for the growth of the tumours, cells with rings containing several copies of these genes will have a selective advantage. In this way, repeated BFB events could actually contribute to the high-level of gene amplification in low-grade bone and soft tissue tumours. Similar mechanisms have been demonstrated in several in vitro systems with amplification of drug-resistance genes (Smith et al. 1995; Coquelle et al. 1997). However, this reasoning does not resolve the issue of how the rings are originally generated.

Recent studies have demonstrated that the initial formation of ring and dicentric chromosomes in malignant tumours can result from telomere shortening and subsequent fusion of chromosome ends (Gisselsson et al. 2001). The same bone and soft tissue tumours that exhibit ring chromosomes also show a high frequency of telomeric fusions. This results in the formation of dicentric chromosomes and it has been suggested that gene amplification is initiated by the involvement of these chromosomes in BFB events, in a way similar to that of the rings (Pedeutour and

Turc-Carel 1997). After a number of BFB cycles, when the amplified arrays have reached a large size, the two ends of some fragments may fuse after anaphase breakage to form ring chromosomes. However, two arguments may be raised against this sequence of events: (1) The rings typically occur together with an apparently normal chromosome complement; at least one of the chromosome 12 homologues should be rearranged if the above hypothesis is correct. (2) It still does not explain why the tumour cell tolerates chromosome breakage at this early stage of neoplastic development; to claim so would be circular reasoning.

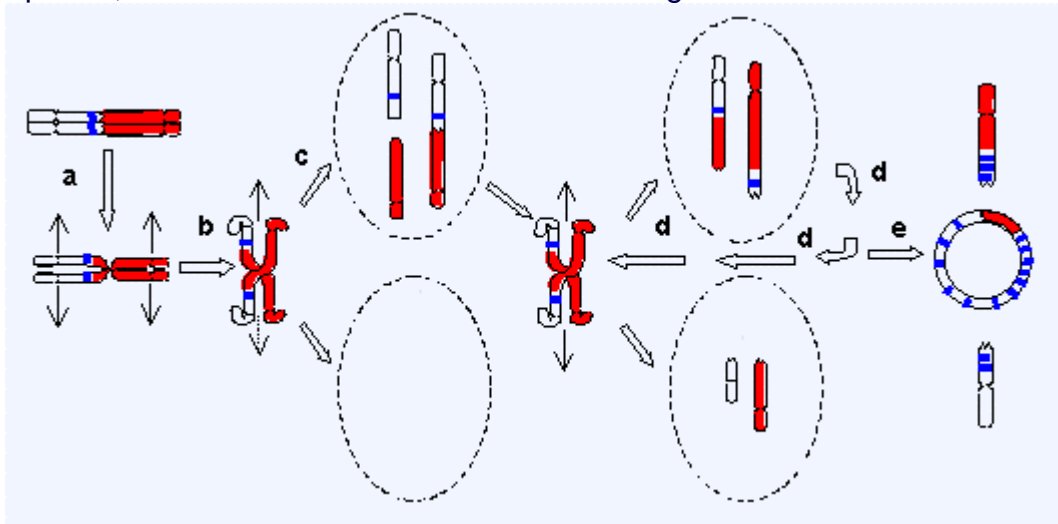


Figure 6 : Hypothetical mechanism of gene amplification: Telomeric association between two chromosomes produces a functional dicentric (a), which may form a bridge at mitosis (b) and undergo nondisjunction (c), leading to an extra copy of the MDM2 gene (blue). This renders the cell permissive for structural rearrangements through breakage-fusion-bridge events and potential amplification of the gene (d). The dicentric structures may finally convert into a ring and marker chromosomes with a high MDM2 copy-number (e). The ring will, in turn, initiate another series of breakage-fusion-bridge events (Fig. 2).

A theoretically plausible scenario for the amplification would most probably include an early event leading to increased copy-number of MDM2 sequences without chromosomal breakage. As a first step, one might image that the dicentric chromosome loses the attachment to one of the poles of the mitotic spindle and undergoes nondisjunction, instead of anaphase breakage, which would lead to a trisomy for both chromosomes involved in the dicentric (Fig. 6). For a dicentric including chromosome 12, this would imply gain of one MDM2 allele in one daughter cell and loss of one in the other. If we assume that one of the dicentric chromatids remains intact, it may again form a bridge at the next cell division. If the increased copy-number of MDM2 now provides sufficient inhibition of the DNA damage checkpoint, anaphase breakage could occur without apoptosis or cell cycle arrest. Once this threshold of tolerance has been surpassed, additional BFB events could generate large amplified arrays that may break into fragments, which could then close into rings. As such rings will have evolved through a large number of random breakage events their structure would be expected to show a high level of complexity. Indeed, when the structure of rings in well-differentiated liposarcomas and parosteal osteosarcomas was studied in detail, the normal order of loci along the 12q arm was extensively disrupted even below the megabase level (Gisselsson et al. 1998). It has also been demonstrated that genes amplified in the rings may actually be structurally rearranged, having recombined with loci from other parts of chromosome 12 or even other chromosomes (Berner et al. 1997).

Summary

Ring chromosomes come to clinical attention either in association with developmental anomalies at the beginning of life or with telomere shortening in ageing and neoplastic cells. It is likely that a transition from a DNA-damage sensitive to a DNA-damage tolerant state explains the high instability of rings in some tumour cells compared to those in non-neoplastic cells. However, many tumours show a pattern similar to that of normal cells. One might tentatively distinguish two main modes for chromosomal reorganisation in tumours:

Type 1: Where simple chromosome rearrangements lead to either the formation of a chimaeric gene or a dysregulated oncogene expression with potent transforming capability. The *BCR/ABL* fusion in and the *RET/PTC* fusions in are examples of this mode. In general, such tumours show few additional chromosomal abnormalities and ring and dicentric chromosomes are rare.

Type 2: Where vast chromosomal instability lead to formation of complex karyotypes and multiple gene changes including activation of oncogenes and loss of tumour suppresser genes. This mechanism of constant chromosome evolution most probably acts against a background of disrupted DNA damage and/or mitotic checkpoints (Gisselsson et al. 1998). It is common in many aggressive solid tumours, e.g. *glioblastoma*, *colorectal cancer*, and a number of sarcomas.

In type 1 lesions and non-neoplastic cells, ring chromosome structure is relatively conserved: rings may be duplicated or lost, but structural rearrangements are rarely maintained in the cell population (Gisselsson et al. 2000). In type 2 lesions, rings show extensive structural variability and provide a means for gene amplification. Ring chromosomes are thus illustrative proofs that chromosomal behaviour is not only a function of straightforward molecular interactions; chromosomal topology and the physiological context in which a certain chromosome aberration occurs must also be taken into consideration.

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