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Overreplication and recombination of DNA in higher eukaryotes: Potential consequences and biological implications

(chromosome rearrangements/gene amplification/evolution/aging/cancer)

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ABSTRACT **We propose that a fundamental problem in the faithful replication of complex chromosomes of higher eukaryotes is the proper control of both the number and timing of the multiple initiations of replication on single chromosomes. When replication patterns are disrupted by any of a variety of agents, overreplication of DNA can occur. We propose a model that accounts for the generation of a wide variety of chromosomal aberrations–rearrangements, resulting from the various ways in which the overreplicated strands can undergo recombination. We also discuss certain implications of the generation of chromosomal alterations in higher eukaryotes as they may relate to cancer chemotherapy, cancer progression, aging, and rapid speciation-evolution.**

Previous studies in our laboratory have examined the process of gene amplification in cultured mammalian cells (for reviews, see refs. 1 and 2). The frequency of a spontaneous doubling of the dihydrofolate reductase (DHFR) gene is 1×10^{-3} per cell generation (3), and this frequency can be increased 10-fold or more by pretreatment of cells with agents such as hydroxyurea (4), UV light, and carcinogens (5). Mariani and Schimke (6) provided evidence that when DNA synthesis was inhibited during hour 2 of S phase in synchronized Chinese hamster ovary (CHO) cells, DNA synthesized before the hydroxyurea block was rereplicated once the hydroxyurea was removed. This overreplication resulted in an increase in the number of DHFR genes and led to enhanced resistance to methotrexate (MTX). This overreplication involves not only the DHFR gene, which is replicated early in S phase, but also a large proportion of the DNA replicated prior to the inhibition of DNA synthesis.

More recently Hill and Schimke (7) have extended these studies to show that treatment of cells with hydroxyurea results in the generation of a wide variety of chromosomal aberrations–rearrangements observed in the first M phase following inhibition of DNA synthesis. The aberrations include normal chromosomes with extrachromosomal DNA, increased frequencies of sister chromatid exchange, polyploidization, breakage–bridge fusion chromosomes, and gapped–fragmented chromosomes. Most significant in their results is the observation that the cells in which the chromosomal alterations are observed are derived from that subset of the treated cells that contains more than the G_2 content of DNA as studied with DNA fluorochromes and flow cytometric techniques.

We observed additionally (S.W.S., A.B.H., and R.T.S., unpublished data) that the increased DNA content of cells previously treated with hydroxyurea (or aphidicolin) does not result from fusion of cells or uptake of DNA from killed cells. The finding that the chromosomal alterations occur only within the subset of cells with additional DNA leads us to propose that the chromosomal alterations are the consequence of recombination events involving the strands of overreplicated DNA, and not that the additional DNA occurs after the generation of the chromosomal aberrations. Thus, we propose that gene amplification is only one consequence of the overreplication process and that the same initial processes of overreplication and recombination can result in a number of chromosomal alterations, which have major consequences for surviving somatic and germ cells.

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Perturbation of Cell Replication Patterns and Overreplication of DNA

The overreplication of DNA is a phenomenon intimately tied to the regulation of the number and timing of origins of replication in "complex" chromosomes. The effects of perturbing these processes are particularly important. Pritchard and Lark (8) and Billen (9) have shown that transient inhibition of DNA replication in *Escherichia coli* results in replication from the arrested replication forks as well as additional initiations. A graphic illustration of multiple initiations are the studies of Evenson and Prescott (10), which show visual evidence in the protozoa *Euplotes* of multiple initiations of replication following recovery from brief heat treatment. Treatment of cells with inhibitors of DNA synthesis likewise result in overreplication of DNA (6). Rice *et al.* have shown that transient (12–48 hr) exposure of cells to hypoxia results in overreplication of DNA in hamster cells (11). Additionally, Lavi (12) has shown that a number of agents facilitate amplification of simian virus 40 (SV40) sequences integrated into CHO cells. Common to these diverse physical and pharmacologic agents is the production of a transient inhibition of DNA synthesis. Agents that introduce adducts into DNA (e.g., UV light and carcinogens) transiently inhibit DNA synthesis and facilitate gene amplification (5) and may induce overreplication of DNA (9). That inhibition of DNA synthesis results in altered replication control has been documented by Laughlin and Taylor (13), who showed by DNA fiber autoradiographic techniques that, after treatment of cultured cells with 5-fluorouracil, an increased number of initiations of DNA synthesis occurs.

Recent studies in our laboratory suggest a possible mechanism whereby inhibition of DNA synthesis can result in overreplication of DNA. An important parameter in "inducing" overreplication of DNA by hydroxyurea and aphidicolin is the length of time replication is inhibited (R.N.J., J. N. Feder, and R.T.S.; unpublished data). During the period of inhibition, there is a 10-fold increase in dihydrofolate reductase enzyme levels that results from comparable in-

Abbreviations: DHFR, dihydrofolate reductase; CHO cells, Chinese hamster ovary cells; MTX, methotrexate; SV40, simian virus 40.

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creases in DHFR mRNA levels. DHFR mRNA transcription occurs predominately in a short window of time at the G_1–S boundary of the cell cycle (14), and we suggest that the inhibition of DNA synthesis "freezes" cells in the cell cycle where the DHFR gene is transcriptionally active. Alternatively, mRNA may continue to accumulate because the perturbation of the cell cycle results in an inhibition of DHFR mRNA degradation. Gel electrophoresis analysis of soluble proteins extracted from [^{35}S]methionine pulse-labeled cells indicate that at least five proteins accumulate during a replication block in addition to DHFR. We suggest that these proteins are involved in regulating DNA replication and that elevated amounts of these proteins allow for overreplication of DNA once the drug (hydroxyurea or aphidicolin) is removed from the medium. It follows from this proposal that perturbations of cells that predominately affect DNA synthesis relative to RNA or protein synthesis will be particularly effective in stimulating overreplication (i.e., when an unbalanced inhibition is induced). In fact, when inhibitors of protein synthesis are used in addition to inhibitors of DNA synthesis, the overreplication process can be markedly inhibited in cultured animal cells (S.W.S., A.B.H., and R.T.S., unpublished data).

The extent to which specific DNA sequences (genes) are potentially vulnerable to overreplication may well vary, depending on the time of their replication in the cell cycle (15) and the kinetic properties (i.e., "strength") of individual replication origins. Lavi (12) has shown that the addition of a wide variety of agents to the medium of CHO cells containing integrated SV40 sequences results in extensive amplification of the SV40 and adjacent hamster DNA sequences over a 24- to 48-hr period. This overreplication requires a functional SV40 replication origin. It remains to be determined whether DNA sequences in mammalian cells possess differential affinities for rate-limiting components for initiation of DNA replication. That limited overreplication of specific sequences results from reinitiation at a single chromosome site is shown dramatically in the case of *Drosophila* chorion genes (16). Interestingly, Thireos *et al.* (17) showed that, prior to amplification of the chorion genes, there is a transient expression of chorion mRNA from the nonamplified gene. This suggests that gene expression and replication may be coupled in such a way that an actively expressed gene may have a higher probability of overreplication when cells are perturbed.

The Model of Overreplication of DNA and Generation of Chromosomal Rearrangements–Aberrations

This model is an extension of the model presented previously (1) that was used to show how amplification of genes might occur. This model proposes that multiple strands of overreplicated DNA are ligated together and subsequently recombined into the chromatid to generate expanded chromosomal regions containing amplified genes. Alternatively, the overreplicated strands can circularize to form extrachromosomal elements. For present purposes, the model deals only with consequences of recombination. It does not imply specific mechanisms for overreplication of DNA or for recombination.

Fig. 1*A* depicts the situation in which overreplication of a DNA sequence has already occurred. Each line represents double-stranded DNA. To simplify the model, the sister chromatids have been completely replicated and ligated; additionally, only a single rereplicated chromatid strand is depicted (the dark line). The model is presented in the form in which rereplicated DNA occurs in "loop" structures, a model consistent with the studies of Vogelstein *et al.* (18), which indicate that the DNA undergoing replication is moving relative to the replication complexes. The consequence of such a "loop" structure is the close approximation of free-ended DNA strands, the presence of which is known to be highly recombinogenic in mammalian cells (19). Additionally, the model proposes that the recombinations occur within the replication loop structure (i.e., during S phase). The recombination structures proposed are analogous to Holliday structures (20) except that they are generated from overreplicated DNA strands. Although the model shows only a single overreplication complex, the length of the recombined DNA can vary and potentially can involve overreplicated DNA constituting multiple replicon lengths.

There are six general consequences of overreplication–recombination of DNA as depicted in Fig. 1.

(i) Extrachromosomal elements are generated. If the free strands of DNA recombine, they can form extrachromosomal circular structures (Fig. 1*B*) that, if capable of autonomous replication and of sufficient size, would constitute minute chromosomes. Beverley *et al.* (21) have provided evidence in MTX-resistant *Leishmania tropica* for the presence of supercoiled structures consistent with this model. In addition, Hamkalo *et al.* (22) have found that the ultrastructural features of minute chromosomes in a MTX-resistant mouse cell line are consistent with their being circular structures. Noncircularized DNA segments, DNA circles without origins of replication, or minute chromosomes not subject to selection pressure will be lost rapidly in dividing cells. In postreplicative cells, they may simply be retained in the cells.

(ii) Single-ended recombination into the chromatid results in breakage of the chromatid. Fig. 1*C* depicts the recombination of one end of the overreplicated DNA into the parental chromatid (for convenience denoted "site 0"). If the second end of this strand does not recombine, the backbone of the chromatid is broken. If this same event occurs at multiple sites on the same or both chromatids, the result will be varying degrees of fragmentation of sister chromatids. Note that maintenance of chromosome integrity would be better served if there were no recombination of overreplicated DNA into the chromatids.

(iii) Double-ended recombination at sites 0 and 1 can be silent or result in an inversion. Fig. 1*D* depicts recombination of the overreplicated DNA strand at sites 0 and 1 (i.e., the same chromatid). In one orientation of ligation, the recombined strand will be present in the same orientation as the parental (excised) strand. This constitutes a "silent" event. If ligation occurs in the alternative orientation, the consequence is an inversion. Inasmuch as the size of the overreplicated DNA strand can be highly variable in length (23), an inversion may be observed cytologically. In addition, if the overreplicated strand were displaced relative to the similar sequence on the chromatid and underwent a double-ended recombination event within a gene (e.g., homologous recombination within a coding sequence or nonhomologous recombination within an intervening sequence), the consequence would be a nonreciprocal "gene conversion" event (20, 24). Additional consequences of this (or any other recombination) would include deletions and insertions as well as recombinational point mutations. Interestingly, Turner *et al.* (25) have reported that at the hypoxanthine/guanine phosphoribosyl transferase locus in humans, 57% of naturally occurring mutations involve major deletions–rearrangements, some of which are also associated with amplification events.

(iv) Double-ended recombination at sites 0 and 2 results in a sister chromatid exchange. Fig. 1*E* shows the recombination between sites 0 and 2, resulting in a sister chromatid exchange between chromatid a and b′. Note that such a recombination results in a break in the sister chromatid. Thus, if the reciprocal chromatid recombination (b to a′) does not occur, a broken chromatid is generated. As in the case of a site 0 and 1 recombination, the recombined strand may

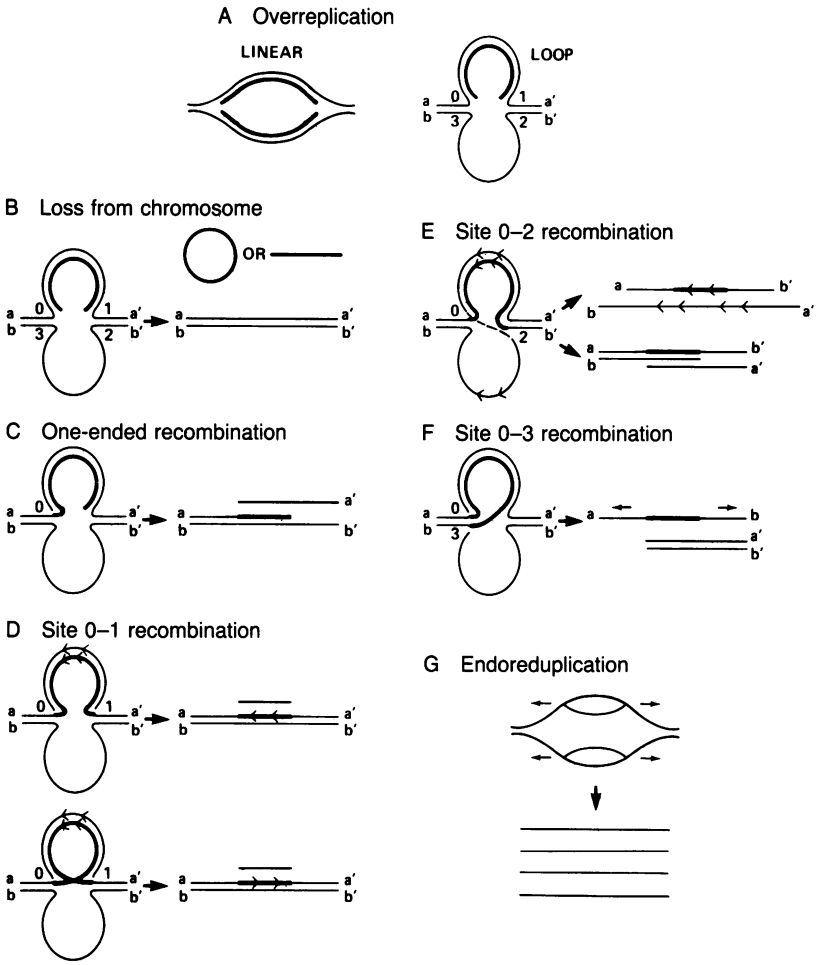

FIG. 1. Model of overreplication–recombination leading to chromosomal aberrations. See the text for a description of this model. Each thin line represents completed chromatids (double-stranded DNA), and the darker lines represent the overreplicated (double-stranded) strands.

result in an inversion of the overreplicated–recombined DNA sequence (not depicted). Note also that a site 0–2 recombination can result in a duplication (Fig. 1*E*).

(v) Double-ended recombination at sites 0 and 3 results in a breakage–bridge fusion chromosome. Fig. 1*F* shows the double-ended recombination at sites 0 and 3. The consequence is the generation of a dicentric chromosome and loss of the chromatids distal to the recombination.

(vi) Overreplication of DNA can result in partial or complete endoreduplication of a chromosome or multiple chromosomes. Pritchard and Lark (8) showed that, when DNA synthesis is inhibited in an *E. coli* thymine auxotroph by removal of thymine, upon restoration of DNA synthesis the blocked replication forks continue from the point of inhibition, and there is overreplication of DNA from reinitiation processes. We propose a similar mechanism for the generation of complete or partially endoreduplicated chromosomes. The length of the overreplicated DNA can vary dramatically, resulting in some instances in endoreduplication of the entire chromosome complement or in complete or partial duplication of individual chromosomes. The consequence of such an event is the generation of various forms of polyploidy in cells, including so-called B chromosomes. In addition, if major regions of a chromosome are endoreduplicated and undergo chromatid pairing, a number of aberrant structures can be generated (so-called mitotic recombination events). Many such aberrant structures will be resolved during mitosis of viable cells, including loss of partially reduplicated chromosomes lacking centromeric regions. One can readily envisage that translocations could result from recombination of chromatid fragments in the occasional cell in which chromosome (and gene) integrity is maintained to allow replicative viability of cells (see ref. 26 for further discussion).

Hill and Schimke (7) have analyzed metaphase spreads of cells subjected to hydroxyurea and provide (*i*) numerous examples of virtually all of the above predicted chromosomal alterations often combined in the same metaphase spreads and (*ii*) an extended discussion of relevant literature. The same spectrum of abnormalities are present after treatment of cells with aphidicolin (27). It is interesting to note that there are a variety of chromosomal aberrations in MTX-resistant cell lines with chromosomally localized, amplified DHFR genes in so-called homogeneously staining regions. Some chromosomes contain large inversions within such expanded chromosomes (28). Other chromosomes with amplified genes occur as dicentric chromosomes in which the fusion has occurred in the expanded region containing amplified genes (i.e., breakage–bridge fusion chromosomes) (29, 30). In addition, karyological changes (e.g., translocations, expanded regions, ring chromosomes) that are not associated with DHFR gene amplification are often seen in MTX-resistant cell lines (30–32).

On the Possible Consequences of Overreplication–Recombination

In the preceding section, we have suggested that a chain of causal relationships extends from perturbation of DNA replication to overreplication of DNA to the generation of various forms of genomic rearrangements and aberrations. Because numerous agents can alter replication patterns in cells and because genomic rearrangements are significant in a number of contexts, we wish to comment and speculate on

the consequences of this process in cancer, aging, and evolution.

Cancer and Cancer Chemotherapy. The concept that overreplication–amplification is a mechanism for the generation of malignant phenotypes was first proposed formally in terms of "replicon misfiring" by Varshavsky (33). DHFR gene amplification has been shown to be a mechanism of clinical resistance to MTX. In addition, evidence is accumulating that various cellular oncogenes are amplified in a variety of human tumors (see refs. 1 and 2). The two types of phenomena are analogous inasmuch as in both cases the overproduction of protein products by virtue of gene amplification can be envisaged as a means to overcoming growth control. Schimke (34) has discussed some of the implications for rapid emergence of drug resistance based on the fact that many agents used in cancer chemotherapy regimens are inhibitors of DNA synthesis. Since carcinogens (5, 12) can facilitate overreplication–amplification events, we raise the question as to whether DNA adduct formation might also function in part through the consequences of such adducts acting to inhibit replication fork movement, resulting in overreplication due either to increased "normal" replication or induction of a repair–replication function. Among other possible consequences of such a process might be recombinational mutagenesis of oncogenes and recombinational activation of and/or inactivation of growth-controlling genes.

Additional consequences of perturbed replication include the generation of aneuploidy in tumor cells and of cellular heterogeneity within tumor populations (35). Treatments known to induce chromosome fragile sites indirectly influence DNA replication (36), and evidence has been presented suggesting a relation between fragile sites and generation of specific chromosomal rearrangements correlated with human cancer (37). Such sites may constitute chromosomal regions subject to a higher probability of overreplication.

An important question to raise is whether normal cells are capable of the overreplication phenomenon. It is possible that an important component in the alteration of cells to generate an overt cancer and/or in its subsequent malignant progression is the relaxation or loss of "replication control" (i.e., control of the number and timing of initiations of DNA replication). If so, then some examples of cellular oncogene amplification in tumors may be a consequence of overreplication of particularly amplification-prone genes. Thus, while oncogene amplification may be causally related to tumor progression, it could also reflect a loss of replication control.

We would argue that overreplication–recombination does occur in nontumorigenic cells as an occasional consequence of improper replication control. There are various reports documenting the existence of small, circular DNA molecules in a variety of mammalian cells, both in intact organisms and in cells in culture (37–40). We suggest that such structures are, indeed, a "successful" consequence of the overreplication phenomenon inasmuch as strand recombination can lead to chromosome fragmentation (Fig. 1*C*), a detrimental consequence for dividing cells. Bloom *et al.* (41) reported year-to-year variation in chromosomal breakage in leukocytes in South American Indians. It remains to be determined to what extent such circular DNAs and aberrant chromosomes are a consequence of "normal" replication and to what extent they are the result of drug ingestion, exposure to pollutants, or physical perturbations (such as intercurrent infections and febrile episodes).

Aging. If there is any generalization that describes the overall phenomenon of aging at the cellular level, it would be that there is a progressive decrement in both the number of cells and in the functions of the existing cells. A number of investigators have shown that "older" cells have a higher incidence of chromosomal aberrations, including, for example, sister chromatid exchange (42). Harnden *et al.* (43) have shown that clonal chromosomal aberrations were not detected in primary cultures of human fetal fibroblasts, whereas 25% of primary cultures from adult fibroblasts showed clonal chromosomal aberrations. Martin *et al.* (44) have recently studied chromosomal aberrations in first mitotic divisions of primary cultures of mouse kidney cells in progressively aging mice and found that ≈5% of metaphase spreads contained aberrations in young mice, whereas in 40-month-old mice 27% of metaphase spreads were aberrant. Such aberrations include sister chromatid exchanges, gapping–fragmentation of chromosomes, as well as normal chromosomes with extrachromosomal DNA—aberrations whose relative frequencies are similar to those described by Hill and Schimke (7) as a consequence of treatment of cells with hydroxyurea (see above).

We suggest that the decrement in number and function of cells during aging may reflect genomic changes caused by overreplication–recombination. The most frequently observed form of aberration seen in hydroxyurea-treated cells is chromosome gapping–fragmentation (7), as proposed from one-ended recombination (Fig. 1*C*). Mitosis in cells with such gaps will result in unequal assortment of chromosome segments, partial haploidy, and the death of cells in subsequent mitoses. In cell types where stem-cell proliferation occurs (for instance the immune and hematologic systems and with certain neuroendocrine functions), the consequence of extensive chromosome gapping–fragmentation would be a diminution in the number of differentiated cells. A "partial haploidy" as generated by limited chromosomal fragmentation may have important detrimental effects in a variety of postreplicative cells whose function is critical to the homeostatic regulation of organismal functioning. Inasmuch as a number of studies have indicated the existence of an age-related increase in the number of chromosomal aberrations, the question must again be posed, as is the case with cancer, whether loss of replication control is primary in the accumulation of cells with chromosomal damage. Such a loss of replication control could be programmed or stochastic and could be the consequence of a variety of fundamental "damaging" mechanisms.

Evolution. A great deal of genetic–genomic change of evolutionary significance is not accounted for by the accumulation of point mutations within coding sequences of genes and/or regulatory regions. Likewise, the inactivation of genes by transposon mutagenesis cannot account for the variety of these genomic changes. Thus, the generation of multigene families, changes in genome size and complexity, rapid change in sequence copy number, maintenance of homogeneity in gene clusters, ploidy changes, and multiple chromosome rearrangements are significant aspects of genome evolution, for which a unifying mechanistic basis is lacking. However, such gross genomic change can be explained by loss of replicative control in germ cells, leading to overreplication–recombination and the generation of heritable genomic rearrangements. Among treatments of cells/organisms that result in overreplication of DNA are heat (10) and alterations in the partial oxygen tension (11), and we suggest that other environmental perturbations may do likewise (45). In organisms in which development occurs in the external environment, the germ cells are potentially subject to direct environmental perturbation of DNA replication. Plant germ cells may be particularly prone to such disturbance with the potential for rapid genomic change. Major genomic change often is an aspect of population divergence in plants (46), and, for example, Lewis (47) has argued that speciation in the genus *Clarkia* resulted from the "simultaneous" generation and fixation of multiple chromosome rearrangements. The rapid mitotic expansion of the germ-cell lineage in homeotherms is presumably buffered against direct environmental influences. However, it has

been shown that metabolic perturbation of proliferating primordial germ cells can induce an extremely high level of chromosomal abnormality in the gametes of adult female mice (48).

An important consequence of the hypothesis we are presenting is that if a chromosomal rearrangement occurred early in the expansion of the germ-cell lineage, a significant number of the resulting gametes present in a single individual could carry the same and/or other chromosomal alteration(s). If such gametes were viable in generating reproductively competent offspring, chromosome changes could be rapidly introduced into a population and quickly fixed in the homozygous state by brother–sister mating.

It is, perhaps, difficult to accept the notion that over-replication–recombination occurs at a high frequency in mitotically dividing germ-cell lineages, inasmuch as there is maintenance of overall genome structure and developmental programs within each species. However, in mammals it is well documented that the vast majority of primordial germ cells degenerate during primordial germ-cell mitosis and in meiosis. This so-called germ-cell "atresia" accounts for the loss of up to 90–98% of the primordial germ cells (49). It is dividing rather than interphase cells that degenerate, and degenerating mitotic and meiotic prophase cells show chromosomal abnormalities (49–51). Germ cell atresia has also been observed in birds (52) and in cyclostomes (53).

We suggest that germ cells are subject to the basic problem of control of number and timing of replication origins, which, if altered, can lead to death and chromosomal rearrangements, most of which may be of little or no consequence to the resulting offspring. As has been suggested by Bernstein (54), meiosis may have an important "repair" function by means of which pairing of chromosomes has an "editing" function to either rearrange chromosomal aberrations or to insure that such potential gametes do not progress to the state of reproductive capability. We can only assume that the meiotic editing function is not foolproof, inasmuch as 60% of spontaneous abortions in humans have gross karyotypic abnormalities (55).

This paper is an attempt to provide new views on the generation of rapid genome changes in higher eukaryotes. As complex organisms have evolved, with an increase in genome size, the requirement for the retention of genetic material in a form that can be replicated faithfully and segregated properly into daughter cells has required the evolution of chromosomes with multiple initiation sites for DNA replication within each chromosome. We suggest that the problems attendant to the replication of such complex chromosomes and the potential for generation of chromosomal aberrations, in particular under circumstances where DNA replication patterns are perturbed, are critical to maintaining genomic constancy from generation to generation in both somatic cells and germ cells. Deviation from this constancy is, indeed, an aberration with potential adverse effects for both somatic and germ cells. Space constraints do not allow for an extensive development of the concepts mentioned here, and we must apologize for the inability to provide all relevant references or give suitable acknowledgments for many investigations highly relevant to these speculations.

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1. Schimke, R. T. (1984) *Cell* **37**, 705–713.
2. Stark, G. R. & Wahl, G. (1985) *Annu. Rev. Biochem.* **53**, 447–492.
3. Johnston, R. N., Beverley, S. M. & Schimke, R. T. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3711–3715.
4. Brown, P. C., Tlsty, T. D. & Schimke, R. T. (1983) *Mol. Cell. Biol.* **3**, 1097–1107.
5. Tlsty, T. D., Brown, P. C. & Schimke, R. T. (1984) *Mol. Cell. Biol.* **4**, 1050–1056.
6. Mariani, B. D. & Schimke, R. T. (1984) *J. Biol. Chem.* **259**, 1901–1910.
7. Hill, A. B. & Schimke, R. T. (1985) *Cancer Res.* **45**, 5050–5057.
8. Pritchard, R. H. & Lark, K. G. (1964) *J. Mol. Biol.* **9**, 288–307.
9. Billen, D. J. (1969) *Bacteriology* **97**, 1169–1175.
10. Evenson, D. P. & Prescott, D. M. (1970) *Exp. Cell Res.* **63**, 245–252.
11. Rice, G. C., Spiro, I. J. & Ling, C. C. (1985) *Int. J. Radiat. Oncol.* **11**, 1817–1822.
12. Lavi, S. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6144–6148.
13. Laughlin, T. J. & Taylor, J. H. (1979) *Chromosoma* **75**, 19–35.
14. Farnham, P. J. & Schimke, R. T. (1985) *J. Biol. Chem.* **260**, 7675–7680.
15. Goldman, M. A., Holmquist, G. P., Gray, M. C., Caston, L. A. & Nag, A. (1984) *Science* **224**, 686–890.
16. Osheim, Y. N. & Miller, O. J. (1983) *Cell* **33**, 543–553.
17. Thireos, G., Griffin-Shea, R. & Kafatos, F. C. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5789–5793.
18. Vogelstein, B., Pardoll, D. M. & Coffey, D. S. (1980) *Cell* **22**, 79–85.
19. Folger, K. R., Thomas, K. & Capecchi, M. (1985) *Mol. Cell. Biol.* **5**, 59–69.
20. Holliday, R. (1964) *Genet. Res.* **5**, 282–304.
21. Beverley, S. M., Coderre, J. A., Santi, D. V. & Schimke, R. T. (1984) *Cell* **38**, 431–439.
22. Hamkalo, B., Farnham, P., Johnston, R. & Schimke, R. T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1126–1130.
23. Federspiel, N. A., Beverley, S. M., Schilling, J. W. & Schimke, R. T. (1984) *J. Biol. Chem.* **259**, 9127–9140.
24. Jackson, J. A. & Fink, G. R. (1981) *Nature (London)* **292**, 306–311.
25. Turner, D. R., Morley, A. A., Heliandros, M., Kutlaca, R. & Sanderson, B. J. (1985) *Nature (London)* **315**, 343–345.
26. Schimke, R. T. (1982) in *Gene Amplification*, ed. Schimke, R. T. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 313–333.
27. Rainaldi, G., Sessa, M. R. & Mariani, T. (1984) *Chromosoma* **90**, 46–55.
28. Biedler, J. L., Melera, P. W. & Spengler, B. A. (1980) *Cancer Genet. Cytogenet.* **2**, 47–60.
29. Kaufman, R. J., Latt, S. A. & Sharp, P. A. (1982) in *Gene Amplification*, ed. Schimke, R. T. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 245–250.
30. Fougere-Deschatrette, C., Schimke, R. T., Weil, D. & Weiss, M. C. J. (1984) *Cell Biol.* **99**, 497–502.
31. Nunberg, J. N., Kaufman, R. J., Schimke, R. T., Urlaub, G. & Chasin, L. (1979) *Proc. Natl. Acad. Sci. USA* **75**, 5553–5556.
32. Tyler-Smith, C. & Bostock, C. J. (1981) *J. Mol. Biol.* **153**, 219–230.
33. Varshavsky, A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3673–3677.
34. Schimke, R. T. (1984) *Cancer Res.* **44**, 1735–1741.
35. Nowell, P. C. (1978) *Virchows Arch. B* **29**, 145–150.
36. Sutherland, G. R. (1983) *Int. Rev. Cytol.* **81**, 107–138.
37. Yunis, J. H. & Sooreng, A. L. (1985) *Science* **226**, 1199–1202.
38. Bertelsen, A. H., Humayun, M. Z., Karfopoulos, S. G. & Rush, M. G. (1982) *Biochemistry* **21**, 2076–2082.
39. Riabowal, K. T., Schmookler Reis, R. J. & Goldstein, S. (1985) *Nucleic Acids Res.* **13**, 5563–5584.
40. Toda, M. & Yamagishi, H. (1984) *Dev. Growth Differ.* **26**, 195–202.
41. Bloom, A. D., Neel, J. V., Tshuchimoto, T. & Meilinger, K. (1973) *Cytogenet. Cell Genet.* **12**, 175–183.
42. Schneider, E. L., ed. (1978) *The Genetics of Aging* (Plenum, New York), pp. 27–49.
43. Harnden, D. G., Benn, P. A., Oxford, J. M., Taylor, A. M. R. & Webb, T. P. (1976) *Somatic Cell Genet.* **2**, 55–61.
44. Martin, G. M., Smith, A. C., Ketterer, D. J., Ogburn, C. E. & Disteche, C. M. (1985) *Israel J. Med. Sci.* **21**, 296–303.
45. McClintock, B. (1984) *Science* **226**, 792–801.
46. Stebbins, G. H. (1971) *Chromosomal Evolution in Higher Plants* (Addison-Wesley, New York).
47. Lewis, H. (1966) *Science* **152**, 162–172.
48. Rohrnborn, G. & Hansmann, I. (1971) *Humanagenetik* **13**, 184–198.
49. Beaumont, H. M. & Mandl, A. M. (1962) *Proc. R. Soc. London Ser. B* **155**, 557–579.
50. Baker, T. G. (1963) *Proc. R. Soc. London Ser. B* **158**, 417–433.
51. Baker, T. G. & Franchi, L. L. (1967) *J. Cell Sci.* **2**, 213–224.
52. Hughes, G. C. (1963) *J. Embryol. Exp. Morphol.* **11**, 513–536.
53. Hardisty, M. W. (1971) in *The Biology of the Lampreys*, ed. Hardisty, M. W. & Potter, J. C. (Academic, New York), Vol. 1, pp. 295–359.
54. Bernstein, H. (1977) *J. Therm. Biol.* **69**, 371–380.
55. Boue, A. & Boue, J. (1981) *Chromosomes Today* **7**, 281–290.