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Satellite DNA and Heterochromatin Variants: The Case for Unequal Mitotic Crossing Over

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Summary. Variations of constitutive heterochromatin (heteromorphisms) appear to be a general feature of eucaryotes. A variety of molecular and cytogenetic evidence supports the hypothesis that heteromorphisms result from unequal double-strand exchanges during mitotic DNA replication. Constitutive heterochromatin consists of highly repeated DNA sequences that are not transcribed. Thus, heteromorphisms are tolerated without overt phenotypic effect. Several of the highly repeated DNAs that comprise constitutive heterochromatin have been shown to contain site-specific endonuclease recognition sequences interspersed at regular intervals dependent upon nucleosome structure. These interspersed short repeated sequences could mediate unequal crossovers, resulting in quantitative variability of constitutive heterochromatin and satellite DNA. De novo variations of constitutive heterochromatin may be useful as markers of exposure to mutagens and/or carcinogens.

Introduction

Following the inception of chromosome banding techniques, careful examination showed quantitative variations of constitutive heterochromatin, designated 'heteromorphisms' (Craig-Holmes and Shaw, 1971; Paris Conference, 1975). Although heteromorphisms were originally detected in clinical material from retarded and/or dysmorphic subjects, subsequent work has shown that heteromorphisms occur with equal frequency among phenotypically normal controls (Brogger et al., 1977; Jacobs, 1977; Lubs et al., 1977; Tharapel and Summitt, 1978). The extent of this variability is underscored by the finding that the heteromorphism pattern of each individual human karyotype is virtually unique (Muller et al., 1975). The existence of such widespread variability poses fundamental questions about the organization of eucaryotic genomes in general and constitutive heterochromatin in particular. This article summarizes recent molecular and cytogenetic studies on the properties and evolution of constitutive

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heterochromatin. These studies result in a model for variation of constitutive heterochromatin based on unequal mitotic crossovers determined by short repeated sequences in the DNA sequences that comprise constitutive heterochromatin.

Constitutive Heterochromatin and Satellite DNA

Constitutive heterochromatin, defined by the pycnotic staining qualities of both (maternal and paternal) homologous chromosomes (Brown, 1966), has been of interest to cytologists for 50 years (Heitz, 1928). Cytologists of that period accumulated a rich literature, especially in plants and insects, detailing the marked variability of constitutive heterochromatin that occurs with little or no phenotypic effect. From these studies, constitutive heterochromatin has long been thought to be transcriptionally inert (Brown, 1966). In the same year that Brown summarized nearly a half-century of cytologic endeavor, Waring and Britten (1966) reported the existence of repeated sequences in eucaryotic DNA. Subsequent investigations by these and other authors showed that highly repeated DNAs, termed satellite or simple-sequence DNAs, were widespread in eucaryotes (for review, see Skinner, 1977).

In the past decade, several techniques have been introduced to probe the intranuclear organization of DNA sequences and nucleoproteins; these techniques include in situ hybridization (Gall and Pardue, 1969; Jones, 1970), the C-band stain for constitutive heterochromatin (Arrighi and Hsu, 1971) and subnuclear fractionation of chromatin (Frenster et al., 1963; Yunis and Yasmineh, 1971). Application of these techniques has resulted in the ascertainment of a general one-to-one relationship between highly repeated satellite DNAs and darkly staining constitutive heterochromatin. Occasional exceptions to this rule exist: Chinese hamster constitutive heterochromatin is apparently not comprised of repeated sequences (Arrighi et al., 1974), and several mammalian satellite DNAs are not strictly localized to constitutive heterochromatin (Kurnit et al., 1973; Kurnit and Maio, 1974; Gosden et al., 1977). However, this strong correlation between satellite DNA and constitutive heterochromatin remains a good rule of thumb for most eucaryotes (Yunis and Yasmineh, 1971; Pardue, 1975; Miklos and Nankivell, 1976; Nagl, 1977). This correlation is in agreement with molecular evidence that highly repeated DNAs are not transcribed (Flamm et al., 1969; Ryffel and McCarthy, 1975). *Conclusion 1:* Constitutive heterochromatin and highly repeated satellite DNA are most often coincident. These entities are apparently not transcribed.

Variability of Constitutive Heterochromatin

Variability of constitutive heterochromatin appears to be a general characteristic of eucaryotes. Such variations, termed heteromorphisms (Paris Conference, 1975), have been demonstrated in plants (Natarajan and Natarajan, 1972; Filion, 1974; Marks and Schweizer, 1974; Vosa, 1974; Linde-Laursen, 1975; Weimarck, 1975; Sachan and Tanaka, 1977), invertebrates (Klasterska et al., 1974; Beer-

mann, 1977), and higher animals (Craig-Holmes and Shaw, 1971; Lubs et al., 1977). Twin and family studies, and also diploid human tissue-culture cell analyses, demonstrate a Mendelian inheritance of heteromorphisms, with only rare exceptions (Muller et al., 1975; Verma and Lubs, 1976; Van Dyke et al., 1977; Hoehn et al., 1977; Mikelsaar et al., 1977; Phillips, 1977; Robinson et al., 1978). In agreement with the evidence that the highly repeated DNA sequences that comprise constitutive heterochromatin are not transcribed, no clinical phenotype has been ascribed to quantitative variation of constitutive heterochromatin (Brogger et al., 1977; Jacobs, 1977; Lubs et al., 1977; Tharapel and Summitt, 1978). In contrast, variations of euchromatin are accompanied by altered transcription of the affected DNA sequences (Kurnit, manuscript in preparation) and are associated with specific abnormal phenotypes (Schinzel, 1976; de Grouchy and Turleau, 1977). *Conclusion 2:* Nontranscribed constitutive heterochromatin shows widespread variation, usually without phenotypic effect, among members of a given species.

Chromosome Pairing and Meiosis

The function, if any, of highly repeated DNAs in constitutive heterochromatin is unknown. Earlier speculations concerning effects on chromosome pairing and centromere strength (Walker, 1971) are not supported by recent data showing that chromosome segregation of the X chromosome in *Drosophila melanogaster* is not appreciably altered by deletion of centromeric heterochromatin (Yamamoto and Miklos, 1977). This hypothesis was proposed when early studies on animals showed that most constitutive heterochromatin was pericentromeric. Subsequent studies have shown that most plants, birds, and invertebrates also have prominent interstitial and telomeric constitutive heterochromatin, as do several mammalian chromosomes, including the human Y (Arrighi and Hsu, 1971; Natarajan and Natarajan, 1972; Arnason, 1974; Filion, 1974; Klasterska et al., 1974; Marks and Schweizer, 1974; Stack et al., 1974; Vosa, 1974; Linde-Laursen, 1975; Weimarck, 1975; Beermann, 1977; Fussell, 1977; Lau et al., 1977; Mascarello and Mazrimas, 1977; Sachan and Tanaka, 1977; Raman et al., 1978; Yen and Filion, 1977).

Although constitutive heterochromatin is not transcribed and therefore does not result in gross phenotypic changes, this does not mean that it is without genetic effect. Several workers have documented statistically significant effects of heterochromatin on chiasma frequency and location in invertebrates and plants (John, 1973; Klasterska et al., 1974; Miklos and Nankivell, 1976; Brandham and Bhattarai, 1977; Roupakias and Kaltsikes, 1977; Yamamoto and Miklos, 1978). Neither the significance of nor the mechanisms by which diverse effects of heterochromatin on meiotic recombination occur are currently known. However, both cytological and molecular evidence show that these effects are not mediated by meiotic recombination within heterochromatin. In plants, insects, and mammals, careful cytology demonstrates early repulsion and lack of chiasmata in heterochromatic chromosomal segments (Zenzes and Wolf, 1971; Natarajan and Gropp, 1971; Schindler et al., 1973; Klasterska et al., 1974; John, 1976; Jones, 1978). Stern and co-workers have demonstrated pachytene repair-type DNA synthesis in

mouse main band DNA, but have not detected such synthesis in mouse satellite DNA (Hotta et al., 1977; Y. Hotta and H. Stern, personal communication). *Conclusion 3:* Meiotic crossing-over in constitutive heterochromatin is rare or absent.

Somatic (Mitotic) Origin of Constitutive Heterochromatin Variability

As noted above, crossing-over and chiasma formation in heterochromatin during meiosis is rare or nonexistent. This makes meiosis an unlikely source for unequal crossovers and resultant rearrangements of constitutive heterochromatin. In contrast, evidence for a mitotic origin of constitutive heterochromatin variation includes:

1. The excision of large blocks of heterochromatin during mitotic cleavage divisions in a number of *Diptera, Copepoda,* and *Nematoda,* a process termed 'chromatin diminution' (for review, see Beermann, 1977). In the case of the nematodes *Ascaris* and *Parascaris*, this has been shown to involve deletion of highly repeated satellite DNA sequences (Moritz and Roth, 1976).
2. The creation of large heterochromatin 'megachromosomes' in interspecific tobacco hybrids. These megachromosomes are found in somatic tissue and are not transmitted through the germ line (Gerstel and Burns, 1967).
3. Cells in tissue culture can show alterations of constitutive heterochromatin, including deletion of the heterochromatic long arm of the *Microtus agrestis* X chromosome (Cooper, 1977), intercalary location of C bands in cells derived from animals with only pericentromeric C bands in the natural state (Bianchi and Ayres, 1971; Popescu and DiPaolo, 1972; White et al., 1975; Levan et al., 1977), and translocation of a known human heteromorphism (Nielsen et al., 1974).
4. Small supernumerary B chromosomes that stain darkly after C banding have appeared in tumor cells in vivo (Atkin and Baker, 1978) and in vitro (Levan et al., 1977).
5. Sister chromatid exchanges (SCEs) have been observed in *Drosophila melanogaster* ring Y chromosomes, which consist almost entirely of constitutive heterochromatin comprised of satellite DNA. Since these chromosomes do not undergo meiotic recombination, these exchanges must be mitotic in origin (Yamamoto and Miklos, 1978).
6. Cultured human fibroblasts show a variety of intra- and interchromosomal rearrangements involving constitutive heterochromatin upon recovery from mitomycin C (MMC) treatment (Hoehn and Martin, 1972, 1973). This phenomenon will be discussed in detail below. *Conclusion 4a:* De novo rearrangements of constitutive heterochromatin, which occur rarely, if ever, in meiotic cells, are well-documented in mitotically dividing cells.

Mitomycin C Increases Variability of Constitutive Heterochromatin

Although both intra- and interspecies variations of constitutive heterochromatin are commonly found, the actual events yielding constitutive heterochromatin variations must be rare in nature, as evidenced by overall intraspecies conservation of karyotype and satellite DNA content and the general stability and

Mendelian inheritance of heteromorphisms. Yamamoto and Miklos (1978) estimated the frequency of spontaneous SCE in the heterochromatic *Drosophila* Y chromosome they examined to be 0.3% per cell division. Treatment of cells with MMC speeds up evolution, as it were, and has enabled researchers to examine the variability of constitutive heterochromatin in a laboratory setting. Initial studies on MMC-treated human cells used unbanded karyotypes and were focused on chromosome breaks and interchanges, which occurred primarily in the large constitutive heterochromatin blocks of chromosomes 1, 9, and 16 (Shaw and Cohen, 1965; Morad et al., 1973). Finer structural analysis of the karyotypes by means of C banding demonstrated that in fact, intrachromosomal rearrangement was more frequent than interchromosomal rearrangement following exposure to MMC (Hoehn and Martin, 1972, 1973). A wide variety of constitutive heterochromatin heteromorphisms were observed, including interstitial C bands and sister chromatid asymmetry of C bands early after MMC treatment (Fig. 1 C of Hoehn and Martin, 1972). This early sister-chromatid asymmetry is cytologic evidence for either unequal sister-chromatid interchange or unequal replication of constitutive-heterochromatin DNA sequences as the basis of constitutive heterochromatin heteromorphism; a subsequent round of DNA replication would result in the formation of two chromosomes, which would be heteromorphic with respect to each other though each would have isomorphic sister chromatids. Presumably, sister chromatids with asymmetry of constitutive heterochromatin are not observed in natural populations, because this primary event is rare without artificial induction by MMC. Once again, it should be emphasized that these heritable alterations of constitutive heterochromatin stimulated by MMC reflect somatic (mitotic) variability. In this regard, it is of note that mice treated chronically with MMC do not show significant meiotic chromosome abnormalities, although MMC inhibited mitotic division and caused germ cell mortality (Savkovic et al., 1977). *Conclusion 4b:* Mitomycin C, a bifunctional alkylator, is a potent stimulator of somatic (mitotic) constitutive heterochromatin variability.

Mechanism of Mitomycin-Stimulated Rearrangements

A clue to the underlying mechanism of these interchanges came from the studies of Latt (1974), who demonstrated that MMC treatment results in a dramatic increase of SCEs. Further, both Latt (1974) and Huttner and Ruddle (1976) showed that intrachromosome exchanges (which occurred over the entire length of the chromosome) greatly exceeded interchromosome exchanges in quantity, in agreement with the findings of Hoehn and Martin (1972, 1973) for regions of constitutive heterochromatin. Although the mechanism by which bifunctional alkylating agents such as MMC induce SCE has not yet been fully elucidated, several points are clear: monofunctional alkylating agents, which do not cross-link DNA, are far less potent stimulators of SCEs than their bifunctional counterparts (Perry and Evans, 1975). The repair process following treatment with bifunctional alkylators appears to involve excision of one end of the cross link, followed by a double-strand exchange associated with DNA replication (Cole,

1973; Wolff et al., 1974; Kato, 1974; Cole et al., 1976). The role of repair synthesis in this process is still contested (Rommelaere and Miller-Faures, 1975; Moore and Holliday, 1976; Shafer, 1977). In summary, although the precise molecular details are still unknown, repair following MMC treatment is associated with replication-mediated exchanges of DNA duplexes. These exchanges occur most often, but not always, as intrachromosomal events between sister chromatids. Such exchanges may be unequal, as demonstrated by the cytologic findings of Hoehn and Martin (1972, 1973). *Conclusion 5:* Somatic (mitotic) rearrangements stimulated by MMC include SCEs and appear to represent double-strand exchanges occurring during the DNA replication process at the site of cross-linked DNA.

Variability of Satellite DNAs

Excluding the phenomenon of the underreplication of satellite DNAs during polytenization (Gall et al., 1971), variability of satellite DNA within a given species has not been as well-documented as variability of constitutive heterochromatin. Perhaps this is because satellite DNAs have been studied mainly in higher animals, which show less variation in constitutive heterochromatin than insects and plants. The variations of satellite DNA expected from most higher animal heteromorphisms are too small to be detected accurately given the preparation variability involved in the isolation and characterization of satellite DNAs. Nevertheless, examples of intraspecies variability of satellite DNA have been documented:

1. As noted before, chromatin diminution in *Ascaris* and *Parascaris* is associated with a specific loss of highly repeated satellite DNA (Moritz and Roth, 1976).
2. The presence of grasshopper heterochromatic-supernumerary B chromosomes has been correlated with the presence of a highly repeated satellite DNA (Gibson and Hewitt, 1972), although this result has been disputed (Dover and Henderson, 1976).
3. A heavy component of African green monkey kidney DNA, consisting of two satellite DNAs, was observed to comprise a small but variable portion of the monkey genome. This variability existed both within and among different monkey cell lines. In situ hybridization showed this difference among cell lines to be quantitative rather than qualitative (i.e., all cell lines contained these sequences) (Kurnit and Maio, 1974). There is one report of variation of mouse satellite DNA in a tissue culture line adapted from an experimentally induced tumor that produced C-type particles (Manuelidis and Manuelidis, 1976).
4. Variability of human Y chromosome-specific reiterated DNA correlates with variability of the constitutively heterochromatic long arm of the Y (Kunkel et al., 1977; McKay et al., 1978).
5. Loss of satellite DNA sequences has been observed following a human 13:14 Robertsonian fusion (Gosden et al., 1978).
6. Restriction enzyme analysis has demonstrated heterogeneity of the satellite-like spacer regions interspersed among 5S and ribosomal DNAs (Carroll and Brown, 1976; Arnheim and Southern, 1977; Botchan et al., 1977; Stambrook, 1978). In yeast, such heterogeneity in ribosomal DNA appears to result from mitotic crossovers and is inherited in a stable Mendelian fashion (Petes and Bot-

stein, 1977). In *Drosophila,* ribosomal DNA magnification results from unequal mitotic sister chromatid exchanges (Tartof, 1973). Examples 1, 3, and 6 provide evidence of highly repeated DNA variability in mitotically dividing cells.

In addition to the intraspecies data, a large number of observations have been made demonstrating quantitative differences of identical or closely related satellite DNAs among closely related species. Crustaceans, rodents, bovids, and primates all show the same phenomenon: different members of these groups share several satellite DNAs, which are present in variable amounts among different species within a group (Sutton and McCallum, 1972; Graham and Skinner, 1973; Prosser et al., 1973; Gosden et al., 1977; Mazrimas and Hatch, 1977; Kurnit et al., 1978).

Fine sequence analyses show that many of the satellite DNAs within a species are closely related even though they appear distinct when examined by density gradient or molecular hybridization techniques (Gall and Atherton, 1974; Fry and Salser, 1977). Thus, multiple satellites in a given species may result from amplification of a common predecessor sequence. These data are consistent with the hypothesis that rapid fluctuations of satellite DNAs among closely related species may reflect quantum jumps in amounts of preexisting satellite DNA sequences rather than de novo formation of new sequences (Mazrimas and Hatch, 1977; Fry and Salser, 1977). This hypothesis predicts discrete homogeneous families of satellite DNAs within a species, which has been confirmed by careful analysis of hybridization data in several plants (Bendich and Anderson, 1977). Further confirmation comes from the observation that in the *Bovoidea* quantitative change (loss) of satellite DNA sequences (sheep satellite I, which shares extensive homology with goat satellite I) apparently accompanied the centric fusions that led to formation of the large sheep metacentrics (Kurnit et al., 1978). Thus quantum jumps in satellite DNA sequences may be reflected in discrete karyotypic changes by Robertsonian fusion (Gosden et al., 1978; Kurnit et al., 1978) or by the creation of new chromosome arms as in *Dipodomys* and *Peromyscus* (Prescott et al., 1973; Hatch et al., 1976; Hazen et al., 1977). Such karyotypic changes may be important in speciation (Bush et al., 1977). *Conclusion 6:* Intra- and interspecies data both indicate the high quantitative variability of satellite DNA sequences. Mitotic variability of satellite DNA occurs.

Mechanism of Satellite DNA Variability

Satellite DNAs were originally shown to be highly repeated by DNA-DNA reassociation techniques (Waring and Britten, 1966). Subsequent studies have demonstrated both short-range and long-range periodicities. The short-range periodicities revealed by direct nucleotide-sequencing techniques demonstrate that a significant portion of a number of satellite DNAs from diverse organisms consist of very short (2—12 nucleotides) segments repeated in tandem (Swartz et al., 1962; Gall and Atherton, 1974; Skinner et al., 1974; Biro et al., 1975; Fry and Salser, 1977); such satellites are often termed 'simple-sequence' DNAs. The origin and significance of this peculiar form of organization remain a matter of speculation; most popular current theories propose amplification of tandem repeats by unequal sister chromatid exchanges in germ line cells (Southern, 1975; Smith,

1976; Perelson and Bell, 1977). When Smith (1976) proposed his model, he chose not to specify whether the unequal crossovers occurred during meiosis or germ line mitosis. As noted above, subsequent data favor a mitotic origin.

The long-range periodicities of satellite DNAs have been elucidated with the use of restriction enzymes. Maio et al. (1977) have analyzed restriction-endonuclease digestion patterns of monkey component α, mouse satellite, calf satellite I, human and rat highly repetitive fractions, and *Apodemus* satellite DNAs (Botchan, 1974; Southern, 1975; Gruss and Sauer, 1975; Cooke, 1975; Horz and Zachau, 1977; Igo-Kemenes et al., 1977; Fittler, 1977; Maio et al., 1977). On the basis of these analyses, Maio et al. (1977) proposed a long-range periodicity for many satellite DNAs based upon even-numbered integral multiples of nucleosome-length DNA sequences, or in the case of a human highly repetitive fraction, on a geometric progression of nucleosome lengths. This organization based on an even number or geometric progression of nucleosome segments led these authors to suggest that an unequal interchange event involved in amplification of satellite DNA is related to the DNA replication process. The recombinational events yielding the proposed unequal interchange could be mediated by short site-specific endonuclease recognition sites, often palindromes, which are interspersed at regular intervals in satellite DNAs, as demonstrated by the above restriction endonuclease data. Recently, these authors isolated site-specific nucleases from monkey and mouse testes, giving further credence to this hypothesis (Brown et al., 1978). Sobell (1972) and Wagner and Radman (1975) have both proposed models detailing how palindromic sequences could mediate recombination. *Conclusion 7:* The evolution of both short- and long-range periodicities of satellite DNAs is best explained by unequal crossovers. Analysis of long-range periodicity evolution suggests a model in which the recombinational event is linked to the replication process. This process may involve short repeats interspersed at regular intervals corresponding to nucleosome lengths or even multiples of such lengths.

Model for Variability of Constitutive Heterochromatin and Satellite DNAs

Comparison of conclusions 5 and 7 (vide supra) shows that current hypotheses for the mechanisms of amplification (and de-amplification) of constitutive heterochromatin and satellite DNAs are remarkably similar: both invoke double-strand exchanges occurring during the DNA replication process. The evidence relating to constitutive heterochromatin variability implies a mitotic process; the evidence from satellite DNA organization suggests the involvement of short site-specific endonuclease recognition sequences interspersed at regular intervals (Table 1).

Figure 1 shows a highly schematized version of how recombination involving interspersed short repeated sequences could explain the kinds of variability of constitutive heterochromatin and satellite DNAs. In this version, the simplest model for recombination (identity of short single-stranded whiskers following the staggered cuts generated by some restriction enzymes) is used. This schematization is not meant to specify the actual mechanism by which sequences such as palindromes might stimulate chromatid exchanges (Sobell, 1972; Wagner and

Table 1. Summary of conclusions from text[a]

Property of variability	Constitutive heterochromatin	Satellite DNA
Degree	2. High	6. High
Phenotypic effect	2. No major effects	6. None documented
Origin	3—5. Mitotic	6. Mitotic
Mechanism	5. Double-strand exchanges during DNA replication	7. Double-strand exchanges during DNA replication

[a] Arabic numerals refer to conclusion numbers in text

Radman, 1975), but is intended to facilitate visualization of the underlying concepts and polarity of the exchange event. Figure 1A demonstrates an unequal SCE. A 180° rotation (in the plane of the paper) of the interstitial fragment before insertion into the sister chromatid (or re-integration into the original chromatid from which it had been excised) would yield an inversion with transposition of DNA chains. If the original chains differed significantly in thymine content, this event would be detected as compound lateral asymmetry (Angell and Jacobs, 1975; Galloway and Evans, 1975). Figure 1B demonstrates Robertsonian fusion with the generation of 1 dicentric metacentric chromosome plus an acentric fragment from 2 acrocentrics (Ferguson-Smith, 1974). This schema is not meant to exclude alternative locations for recombination in the pericentromeric area which would yield monocentric chromosomes (Lau and Hsu, 1977). Figure 1C demonstrates supernumerary B chromosome formation. Figure 1D demonstrates chromatin diminution (Beermann, 1977). Figure 1E demonstrates formation of a simple inversion without transposition of DNA chains. This requires a specialized palindrome which is itself an inverted repeat. Thus, the specialized palindrome 5′-ATTA-3′ was used in Figure 1E.

Figure 2, also highly schematized, is adapted from models of Keyl (1965), Thomas (1970), and Maio et al. (1977). Again, the orientation of palindromic sequences is given to determine polarity rather than to specify the precise mechanism of the event resulting in interchange of DNA duplexes. Figure 2 illustrates how interactions of different DNA duplexes at the replication fork would result in: unequal SCE (strand ends 2 and 3); chromatin diminution or B chromosome formation (looping out of strand ends 2 and 4); or simple inversion (3 joins 4, and 2 then breaks and reinserts below, at the unreplicated sequence where the breaks at 3 and 4 had occurred) to give an inverted unequal chromatid exchange. This last exchange, while requiring more breakage and reunion events than in Figure 1E, would not require an inverted position or unusual sequence of the recombining palindrome to generate a simple inversion. With the exception of simple inversion, Figures 1 and 2 are complementary, with Figure 1 detailing the polarity of the exchange event and Figure 2 detailing the topology of the interacting DNA duplexes at the replication loop.

One aspect of the model is that following one of the exchanges in Figure 2, the resulting structure could be recognized by the cell as an unreplicated region of DNA; re-replication of this region would result in symmetrical duplication of the

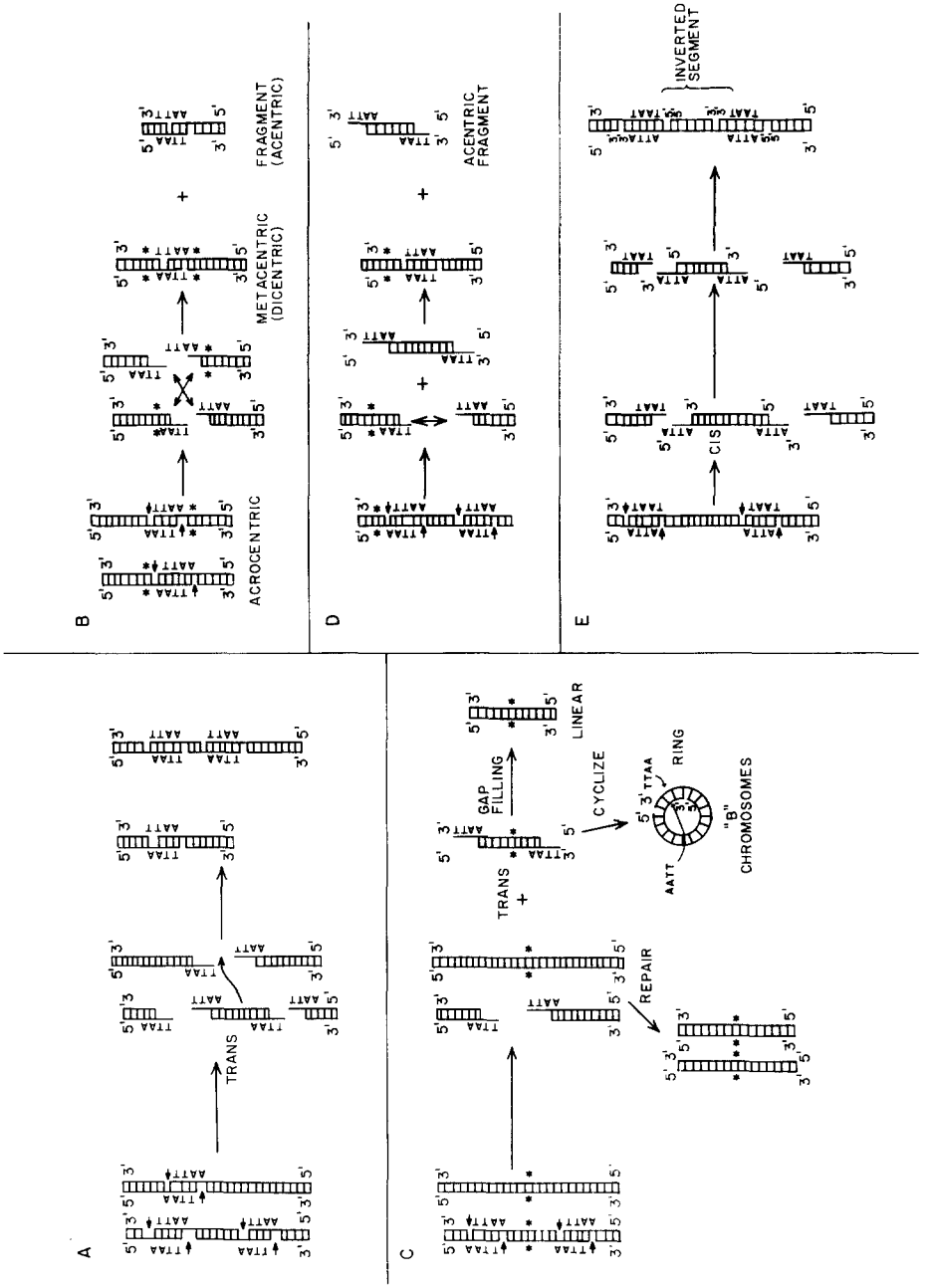

Fig. 1A—E. Involvement of palindromes in chromosome rearrangements. A highly schematized view of how palindromic sequences might stimulate chromosome rearrangements is presented. In this diagram, small arrows mark the site of staggered nicks yielding single-stranded whiskers with 'sticky ends.' This representation is useful to identify the polarity of the event, but should not be construed as the actual mechanism of the event. The palindromic sequence 5'-AATT-3' has been used for purposes of illustration. For fragments with whiskers at both ends, *trans* indicates that the whiskers are on opposite single strands; *cis* indicates that the whiskers are on the same strand. Kinetochores are designated by asterisks. **A** Unequal sister chromatid exchange. **B** Robertsonian fusion. **C** Supernumerary ('B') chromosome formation. **D** Chromatin diminution. **E** Simple inversion. Note specialized palindromic sequence required in **1E**

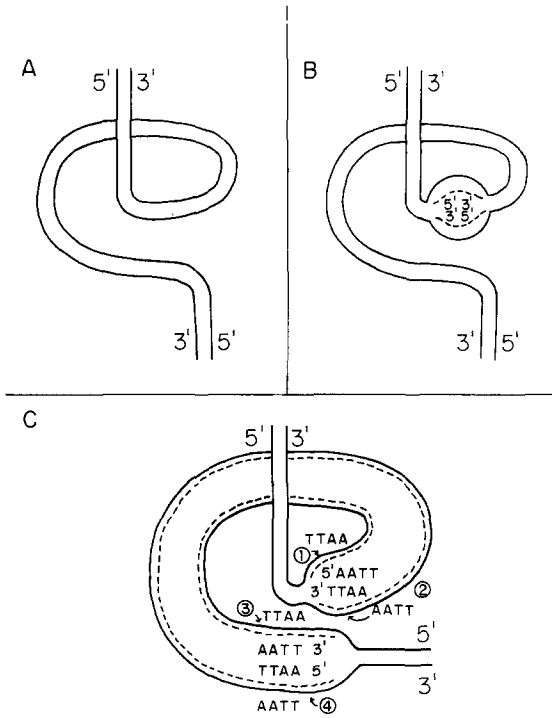

Fig. 2A–C. Involvement of DNA replication in chromosome rearrangements. **A** A chromosome is depicted in a conformation that brings palindromic sequences into apposition. **B** DNA replication commences: newly synthesized strands are indicated by broken lines, parental strands by solid lines. **C** The replication bubble has progressed so that both sets of palindromic sequences are now duplicated. Breakage at strand ends 2 and 3, followed by union of these two strand ends, would give rise to an unequal sister chromatid exchange. Breakage at strand ends 2 and 4, followed by union of these two strands, would give rise to a separate circular molecule, which could result in chromatin diminution or supernumerary chromosome formation. Breakage at strand ends 3 and 4, followed by union of these two strands, followed by a break at strand end 2 which would then re-insert at the lower terminus of the replication bubble from which strands 3 and 4 have detached, would result in an inverted unequal sister chromatid exchange

sequences following an unequal exchange or prevent chromosomal loss of constitutive heterochromatin following formation of B chromosomes. Another aspect of the model is that it implies amplification of a block of sequences on one chromosome at a time. Such segmental amplification of satellite DNA sequences is consistent with the nonrandom clustered organization of restriction sites that has been found in subsegments of a given satellite DNA (Fittler, 1977; Horz and Zachau, 1977; Carlson and Brutlag, 1977) or ribosomal spacer DNA (Junakovic et al., 1978).

Implications and Clinical Relevance

Since the model emphasizes the DNA replication process rather than interphase pairing as the usual locus for chromatid interchange (Comings, 1976), the model

predicts that intrachromosome sister-chromatid interchange will be more frequent than interchromosome exchange. In fact, chromosome heteromorphisms are clearly more frequent than translocations in natural populations, and MMC-treated cells show many more SCEs and heteromorphisms than interchromosome exchanges (Hoehn and Martin, 1973; Latt, 1974; Huttner and Ruddle, 1976). Although rarer, interchromosomal exchanges do occur (Shaw and Cohen, 1965; Morad et al., 1973), which could explain the spread of a given satellite DNA to different chromosomes of a karyotype.

The data generated from investigation of MMC treatment imply that SCE and constitutive heterochromatin heteromorphism are closely correlated. In fact, mutagenic agents or diseases (e.g., Bloom's syndrome: Chaganti et al., 1974) that result in increased SCEs might be expected to increase the frequency of de novo heteromorphisms. Given the rarity of de novo heteromorphisms both in vivo and in vitro (Van Dyke et al., 1977; Hoehn et al., 1977; Robinson et al., 1978), studies of Bloom's syndrome patients to detect de novo heteromorphisms and satellite DNA variability could provide supporting evidence for this model. Since heteromorphisms localized strictly to variations of constitutive heterochromatin do not appear to be associated with abnormal phenotypes (Jacobs, 1977; Lubs et al., 1977; Brogger et al., 1977; Tharapel and Summitt, 1978), nonrandom association of fresh heteromorphisms with dysmorphism or malignancy would imply an underlying common mutagenic event rather than cause and effect. Thus, de novo heteromorphisms might be useful clinically as indicators of an earlier mutagenic event. The observations of Atkin and Baker (1977, 1978) on increased frequencies of heteromorphisms among individuals with cancer are in keeping with this hypothesis. This increased frequency could result from an inherent predisposition for chromosome rearrangement, primary exposure to carcinogens, or chemotherapy.

Conclusion

Cytogenetic studies on the variability of constitutive heterochromatin and molecular studies on the variability of satellite DNA have yielded virtually identical models. This synthesis of 'molecular cytogenetic' knowledge is logically satisfying in view of the proposed identity of constitutive heterochromatin and satellite DNAs. The widespread and common variability of constitutive heterochromatin and satellite DNA is best accounted for by a model that postulates interchange of double-stranded DNA duplexes during the mitotic DNA replication process. Such events may be particularly common in satellite DNA sequences, due to the interspersion of site-specific endonuclease recognition sequences at regular intervals related to nucleosome structure.

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Notes Added in Proof

1. The claim that the sex chromosomes of Chinese hamster contain constitutive heterochromatin deficient in repetitive DNA sequences (cited on page 170 of proofs) requires re-investigation given the recent discovery of repetitive restriction fragments in the Chinese hamster genome [Wolgemuth et al.: J. Cell Biol. **79**, 138a (1978)].

2. The difficulties involved in understanding the actual mechanism by which the exchanges discussed in Conclusion 5 occur are underscored by a recent study of Loveday and Latt [Nucleic Acids Res. **5**, 4087—4104 (1978)]. These authors demonstrate that a fraction of DNA which Moore and Holliday (1976) isolated as putative recombinant DNA structures is apparently not in main the result of SCE's as formerly thought. Nevertheless, the overall concept that these exchanges involve double strands of DNA and require DNA replication remains valid.

3. Stable Mendelian inheritance of compound lateral asymmetry has been found in 2 recent studies [Lin and Alfi: Cytogenet. Cell Genet. **21**, 243—250 (1978); Angell and Jacobs: Am. J. Hum. Genet. **30**, 144—152 (1978)]. However, de novo variations of compound lateral asymmetry were observed in mouse embryos following administration of mitomycin C to their mothers on day 10 of gestation [Tucci and Kaye: J. Cell Biol. **79**, 120a (1978)]. This is in accord with the hypothesis that de novo heteromorphisms may be useful clinically as indicators of previous mutagenic exposure.

4. Quantitative variation of euchromatin has recently been demonstrated in mitotically-dividing cells. Schimke and co-workers [Science **202**, 1051—1055 (1978)] have shown that in vitro drug resistance to methotrexate can occur by amplification of DNA fragments containing the structural gene for dihydrofolate reductase. This amplification could be explained by the same model proposed for quantitative variation of constitutive heterochromatin: unequal mitotic exchanges mediated by interspersed repeated DNA sequences. Such interspersed repeated DNA sequences would have to flank the dihydrofolate reductase gene as the DNA sequence which is actually amplified is considerably larger than the reductase gene itself [Nunberg et al.: Proc. Natl. Acad. Sci. USA **75**, 5553—5556 (1978)]. A similar tandem amplification of ribosomal genes also involves fragments of DNA larger than the rDNA gene [Miller et al.: J. Cell Biol. **79**, 111a (1978)].