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Sequence of centromere separation another mechanism for the origin of nondisjunction

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Summary. The most commonly accepted view about the origin of aneuploidy is that it is due to errors in meiotic division. However, its rare occurrence makes it difficult to explain recurrent births of trisomic children to some parents. This problem causes more serious concern when one accepts that an abnormal (n+1 or n−1) sperm would enter fertilization by overriding thousands, or even millions, of normal haploid sperms. Also, the failure of aneuploidy to be induced in the offspring of mammals treated with mutagens raises questions about the effectiveness of the accepted mode of origin of errors. Current concepts also do not explain why one observes more errors of meiotic I, than of meiotic II, origin. It is known that most chromosomes separating at meta-anaphase junction in mitosis follow a nonrandom, genetically controlled sequence of separation. The present proposal makes use of out-of-phase separation of a rare chromosome, like premature separation in mitosis of the X in elderly humans or of an 18 in parents of trisomy 18 children. The suggestion is made that such out-of-phase separation results in aneuploid cell lines by total failure of the centromere to separate or by it separating too early, before the spindle is formed. The prematurely separating centromeres, it appears, do not attach to spindle fibers and hence cause nondisjunction. Such nondisjunction in embryonic stages will produce apparently normal individuals with mosaicism in somatic and/or gametic tissue. An individual carrying mosaicism in gonadal tissue will produce a large number of abnormal gametes, one of which may have a reasonable chance of entering fertilization. This mode of origin of aneuploidy takes care of all questions raised above and finds support in the data available in the literature. Several of the suggestions made in the hypothesis are easily testable.

Introduction

An understanding of the mechanism(s) of origin of nondisjunction has defied the efforts of cytogeneticists in many ways. Whereas it is now possible to trace the origin of an extra chromosome to the contributing parent in some cases, it is not yet possible to trace the time of origin with accuracy. Nor is it possible to differentiate between nondisjunction originating in premeiotic mitotic *vs* meiotic cells. Whereas concepts like the presence of a recessive homozygous combination resulting in meiotic nondisjunction have been proposed (Alfi et al. 1980), it is not at all clear why only a few select chromosomes are the target of malfunction of the centromere or the spindle as seen in man (Chandley 1982) and why some human chromosomes are involved extremely rarely (e.g., no. 3 or 19), or not at all (e.g., no. 1) in nondisjunction. The old belief that all chromosomes participate in nondisjunction with equal frequency does not find support even in analyses of abortuses. One may, of course, argue for the currently untestable hypothesis that preimplantation wastage carries all or a majority of the chromosomes unaccounted for by analyzable materials. However, some recent data, howsoever limited, using analysis of sperm chromosomes, do not reflect an equal involvement of all chromosomes.

Errors of chromosome distribution in meiosis I or meiosis II have been implicated repeatedly as *the* cause of nondisjunction. Perhaps it reflects our ability to analyze these stages with ease and to correlate the origin of marker chromosomes in trisomic/monosomic individuals to one of the parents. Nontheless, this "fashionable" approach has overlooked the silent message in some communications (e.g., Hecht 1981) that there exists a correlation between errors of presumptive meiotic origin with those in mitotic tissues. The concept put forth is that nondisjunction in mitotic tissues (e.g., lymphocytes) is related to aneuploidy in gametic tissues. An example can be found in an early study by Prodescue et al. (1969) for the X chromosome.

The present paper attempts to bridge the gap between mitotic errors and presumptive meiotic nondisjunction. A hypothesis is proposed which relates centromere separation with the origin of aneusomic cell lines resulting eventually in the formation of mosaics. This hypothesis is an extensive elaboration of an idea presented elsewhere (Vig 1983).

Background

It is becoming increasingly clear that chromosomes in a given genome separate in a nonrandom, sequential, genetically controlled manner. Examples can be cited in man (Vig 1981a; Vig and Woinicki 1974; Méhes 1975), Chinese hamster (Vig and Miltenburger 1976; Singh and Miltenburger 1977), *Potorus tridactylus* (Vig 1981b), *Rana radibunda* (Belcheva et al. 1980), *Haplopappus gracilis* and *Crepis capillaris* (Farook and Vig 1980) and *Vicia faba* (Murata and Vig 1980).

In man, for example, the chromosomes separating the earliest at their centromeres in mitotic meta-anaphase are nos. 18 and 2 (Vig 1981; Méhes 1975). The last separating chromosomes in this species are the acrocentrics, the members of the D group being the latest. Chromosome 1 separates quite late but is not among the last ones. The picture that emerges is interesting in that, with the exception of chromosome 16, the earliest separating and the last separating chromosomes constitute the bulk of human aneusomies (see Chandley 1982). Such a correlation is not simply a fortuitous one.

The separation of centromeres appears to be governed by the quantitiy as well as quality of the paracentromeric constitutive heterochromatin (CH) (Vig 1982). In species with qualitatively uniform CH, a good correlation exists between the increasing quantity of CH and delayed separation of the centromere of the bearer chromosome. The Y chromosome in many species of mouse, for instance, carries no detectable CH

and, in all species so far studied, it is the first chromosome in the genome to separate. Similarly, sex chromosomes of cattle (which lack any CH) always separate before any autosome (all of which carry some quantity of CH). A more clear-cut situation is present in the wood lemming, *Myopus schisticolor*. In this species, the 30 chromosomes in the somatic cell can be divided into three groups, viz., those with light, medium, and heavy C bands. The earliest separating chromosomes are the ones which belong to the first group, followed by those of the second group. Those with a large quantity of C-band material separate the last. In this species the X carries the largest quantity of C-band material, but it is divided into two blocks: medium quantity in the paracentromeric region and a large block at the distal end, separated by euchromatin from the CH. This chromosome behaves as if it has only a moderate quantity of C band, and there appears to be no influence of the distal heterochromatin on the sequence of separation. It is suggested that the CH in *Myopus schisticolor* also belongs to only one type of satellite DNA (Vig 1982).

An analogous situation exists in *Potorus*. Here also, the bearer of the largest quantity of CH (viz., Y_2) separates the last, and those with little or no CH (viz., nos. 4 and 5) separate the earliest (Vig 1981b).

The situation in man, however, is not so simple. Chromosomes 16 and 9, with large C bands, separate somewhere in the middle of the sequence, though chromosome 1, another carrier of a large C band, separates late. In individuals heterozygous for the quantity of C-band material on chromosome 1, the chromosome with the lesser amount separates earlier than its homologue (Vig 1981a). Perhaps this lack of simple correlation between separation and quantity of CH in man reflects qualitative differences. After all, our species has at least four satellite DNA fractions (Miklos and John 1979), not considering five cytologically detectable regions even within the CH segment of chromosome 1 (Wegner and Pawlowitzki 1981).

It has been shown that colcemid has no detectable influence on the sequence of centromere separation (Belcheva et al. 1980; Figueroa and Vig, unpublished data; also see Fitzgerald et al. 1975). It must also be recognized that sister chromatid separation and anaphase movements are two different, uncoupleable phenomena. Simple experiments, like the addition of colcemid before fixation of cells, clearly show that whereas centromeres can separate in the presence of colcemid, the separated centromeres do not move as far away as is seen if a spindle is allowed to form.

The proposal

It is proposed that whereas every chromosome separates at meta-anaphase of mitosis at its destined position, a chromosome may make an error of separation in that it may separate earlier or later than its normally assigned position in the genome. This out-of-phase separation, I propose, is ultimately responsible for "misdivision" of the centromere in question. Firstly, a delay in separation, e.g., for chromosome 21, 22, 13, 14, or 15 in man, Y in *Potorus,* etc., would result in unequational separation through typical mitotic nondisjunction, viz., failure of the centromere to split in time to coincide with the activity of the spindle. Secondly, for chromosomes which separate earlier than their destined position, the centromere is not "mature" enough to receive the spindle fibers for attachment, or premature separation excludes the two daughter centromeres from attaching to the spindle fibers. The second alternative has some experimental support and can be verified by further experiments (see below). This proposal also requires that (1) only a centromere not already separated into two at the time of availability of spindle fibers will be capable of normal functioning; (2) the separated (half) centromeres do not (or can not) attach to the spindle; (3) the numerical aberrations so arising will result from random inclusion of both (or no) separated centromeres into one daughter cell; (4) the majority of numerical errors arising from this phenomenon will affect the chromosomes which prematurely separate, and thus the earliest separating chromosomes will be most often involved, since the time required between their regular, normal separation and premature separation can be only minimal; and (5) premature separation of chromosomes dividing in the middle of the genome will not be much affected because spindle-centromere attachment has already taken place. The last point considers that spindle fibers become available to all centromeres almost simultaneously (see Reider 1982). Possibly, chromosome 16 in man either separates out-of-phase more frequently or is affected by an alternate mechanism. No data on the relative frequency of out-of-phase separation of various chromosomes are available yet.

The most critical point in this suggestion is the lack of attachment of spindle fibers to prematurely separated centromeres, which, I suggest, do not mature to the point of "synthesizing" or assembling material for the synthesis of kinetochores. Some evidence to support this notion, though at this time only indirect, is available from two studies, i.e., premature separation of the X chromosome in man and premature separation of "accessory", "recessive", or "nonfunctional" centromeres in multicentric chromosomes of a mouse cell line. These are briefly discussed below.

Fitzgerald and colleagues in Australia discovered that some lymphocytes from elderly women showed a long isochromosome-like fragment along with 45 somatic chromosomes (Fitzgerald and McEwan 1977; Fitzgerald et al. 1975). Studies using G bands, C bands, karyotyping, and DNA replication pattern showed this fragment to be an X chromosome which had separated at its centromere prematurely. It was either the active or inactive X (Fitzgerald et al. 1975; Galloway and Buckton 1978). Several cells in these individuals showed multiple Xs as well as 45/XO constitution. The two complementary types can be obtained through random segregation of both "fragments" during mitosis. A similar phenomenon, though observed to a more modest degree, was also found for the Y chromosomes in elderly men. The control, comprising younger individuals, did not exhibit such aberrations to any appreciable degree. It is logical to assume that these prematurely separated chromosomes were included randomly in daughter cells because these lack binding to, and any directive movement by, the spindle fibers.

The various sublines of mouse L cells have a long isochromosome which shows as many as five C and Cd bands (Lau and Hsu 1977), in another case as many as four kinetochores stained by $AgNO_3$ (Zheng and Burkholder 1982), and in a third case, in our laboratory, as many as eight C bands, $AgNO_3$-staining regions, and primary constrictions in premetaphase cells (Vig and Zinkowski, unpublished). This chromosome is present in 100% of cells, testifying to its capability for equational division. These cells show no bridges or any consequence of multicentricity of this chromosome. In our cell line we also have several bi-armed chromosomes which upon extension of centromeric heterochromatic region by BrdU or Hoechst 33258 clearly show two C bands and two centromeric regions adjacent to each other. It is possible that

the two closely associated centromeres in dicentric chromosomes separate (and function) as one unit. The multicentric chromosome with eight centromeres (present as individual units along the length of the entire chromosome) is unlikely to make use of this "neighborhood cooperation" phenomenon. What we have observed to happen in this instance is interesting. At the promethaphase stage, all the centromeres function as normal and hold the two chromatids together at respective positions i.e., three along the length of one arm, three along the other, and two in the middle as in the case of dicentrics. Before the onset of metaphase-anaphase movements, and apparently before complete spindle formation and fiber attachment takes place at the centromeric regions or kinetochores, the six centromeres in the arms separate prematurely, leaving the two in the middle intact (Vig, unpublished). This separation is suggestive of some sort of control exerted in every cell by the "dominant" centromeres, located in the middle, over the function of the others, or "recessive" centromeres. The separation of these six centromeres is more or less sequential even at this early stage of mitosis. However, when spindle activity ensues, there appears to be only one point of attachment left at a position in the middle of the chromosome. Since spindle attachment to any more centromeres (separated or not) along the length of the chromosome is likely to cause bridges, etc. (and we have not seen one in thousands of cells analyzed), the only logical conclusion is that the spindle fibers do not attach to these prematurely separated centromeres.

Several lines of evidence suggest the above idea workable. Thus in humans, very many instances of more than one centromere have been reported. In many of these cases the metaphase chromosomes show only one centromere and even one Cd band [e.g., dicentric X, reported by Sinha et al. (1976); t(5;15), reported by Dewald et al. 1979)]. In other cases isodicentric chromosomes have been reported to have only one functional centromere [e.g., X chromosome, reported by Therman et al. (1974)]. These studies have not been extended to prophase chromosomes, where one has good chance of observing two primary constrictions in these chromosomes. Also, there are reports of a dicentric Y which separates equationally at mitotic anaphase (Ying and Ives 1971) as well as that of a ring chromosome 12 which shows up to four centromeres with evidence of bridges or other anaphase anomalies associated with the separation of this chromosome (Zuffardi et al. 1980). Possibly, in all these instances premature separation of secondary centromeres precludes them from being active during anaphase movements.

The concept that prematurely separated centromeres do not attach to spindle fibers is easily testable by study of electron microscopic preparations. The prematurely separating centromere in human X or multicentric chromosome in mouse is not expected to show any fibers attached to this region. It is likely, also, that premature separation excludes these centromeres from forming a functional kinetochore structure and, hence, the failure of functionality of these centromeres. Contrary to popular belief, our data (Vig, unpublished) with the L cells clearly show that the centromeres are neither lost nor lose their function if allowed to reach metaphase as one unit. But in cases involving multicentricity, the main centromere, somehow, instructs the accessory or secondary units so that their function is nullified. The elucidation of the nature of such a control would be exciting.

The second part of my proposal is that centromeres do make errors of separation, primarily during embryogenesis, when rapid cell division is in progress. (Errors can occur later in the life of the organisms as well as during meiosis II. The former type of errors may have only somatic effects, and the latter would be too infrequent to be a statistically significant factor contributing to nondisjunction in the egg or sperm). The errors during embryogenesis may lead to nondisjunction so that the individual becomes a mosaic of disomic, trisomic, and monosomic cell lines. The final outcome and stability of aneusomic lines would depend upon several factors, e.g., gene content of the affected chromosome, the stage of development, etc. Trisomic lines will have advantage over the nullisomic lines. This pattern of cellular development would thus result in an individual who may be a mosaic in the somatic tissue, or gametic tissue, or both, depending upon the time of the initial out-of-phase separation event and the formation of two aneuploid cells.

A gametic mosaic would thus produce a large number of aneuploid gametes along with normal, haploid gametes. Again the ratio between the two types (aneuploid : haploid) of gametes would depend upon the relative numbers of primary meiocytes of the aneusomic and disomic types in the germ line. Thus, a female may produce a "normal" egg during one menstrual cycle and an abnormal one during the other. A male will produce a mixture of sperms of two types, and fertilization will now depend upon the relative frequency of the two.

Even though it is not easy to determine the exact stage of original aneuploidy and, hence, readily find proof of out-of-phase separation during embryogenesis, it is possible to test for aneusomic cells by carrying out meiotic analysis of spermatocytes and oocytes. This proposal also explains several observed dilemmas. Some of these are discussed below:

1) It is known that about one-third of all Down syndrome children receive the extra chromosome from their father. Considering that the changes for error of reduction division or equational division in meiosis I or meiosis II, respectively, are in the neighborhood of 10^{-5} for all chromosomes in the genome, the number of abnormal sperms for a given chromosome will be about 1 in 5000 at most. It is too much to assume that a sperm that is genetically handicapped to start with will be able to overcome the competition from such an overwhelming majority of normal sperms and succed in fertilization, some times twice in a row. This mere numbers game speaks against the meiosis I or II origin of errors in the majority of cases, if not in all. Assuming that a person is a mosaic for disomic and trisomic germ cell populations, it is easy to conceive how he would produce a respectable proportion of aneusomic sperms, one of which will have an even chance of entering the fertilization process.

2) A couple with a trisomic child is usually counseled that the chances of recurrence of the problem are about three times as high as those for the general population under the similar situation. This requires the errors of meiosis I or meiosis II to be speeded up to three times in the contributing parent. The concept of recessive genes causing such problems can not satisfactorily explain this increase because of the diversity of pregnancy outcomes seen in these couples. This is true even after careful allowance is made for genetic background, for which we have no basis except in a few studies (e.g. Alfi et al. 1980). It is more reasonable to assume the existence of differential degrees of mosaicism in these individuals as the basis for increased chances of repeated occurrences.

3) It is possible to trace the origin of extra chromosomes in trisomies (e.g., in case of chromosome 21) to errors in meiosis I or meiosis II in one of the parents. It is interesting that meiosis I errors outnumber meiosis II errors by a factor of 2 : 1 (see

Chandley 1982). Actually, however, if the frequency of cells making errors is equal in the two divisions, the ratio should be the reverse. The existence of mosaicism and consequent production of aneusomic gametes can explain this dilemma. The various final combinations produced by trisomic meiocytes would show twice the frequency of errors, as if happening in meiosis I-type nondisjunction, as those for meiosis II-type. [Thus if C1 and C2 are two homologous chromosomes, then C1C2 (diploid cell) → C1C2C2 (trisomic cell population)]. This in turn will produce gametes of the constitution C1C2 + C2, C2C2 + C1; C2C1 + C2. These results are equivalent to those generated by twice as many errors in meiosis I as in meiosis II. It might be added that most XO mice result from loss of a paternal chromosome after sperm entry into the egg (Russell and Montgomery 1974) and, hence, have their origin in somatic errors.

4) The efforts of cytogeneticists in inducing aneuploidy by mutagenesis in mammals have so far largely failed (see, for example, Russell and Montgomery 1974; Sankaranarayanan 1979), in spite of a few random reports of minor successes. Also, there is no evidence that exposure of humans or mice to mutagens during their life time (after birth) has significantly increased the incidence of nondisjunction. A case in point is the population exposed to radiation from atomic bombs in Japan in 1945. No increase in the frequency of nondisjunction among the offspring of the survivors has been observed (for details see Denniston 1982). These data can be easily accomodated if one accepts the idea of mosaicism occurring before birth. The incidence of such mosaicism, clearly, can not be increased by exposure to mutagens. The minor increases seen in mammalian experiments can be accounted for by errors actually taking place in meiosis I or meiosis II.

Unquestionably, all trisomics do not result from mosaicism as suggested above. Errors of meiosis in older females certainly are well established as a source of trisomy. Also, the ideas presented above are entirely compatible with low frequencies of certain trisomies observed in man (e.g., of trisomy 13) as also with high frequencies (e.g., trisomy 16). And there is ample literature now showing that parents of many trisomic children are actually mosaics. Additionally, in several trisomics whose lymphocytes do not show any chromosome abnormalities, the skin fibroblasts do.

Not only the observations on somatic errors of premature separation of the X chromosome in elderly individuals support the origin of trisomy through this mechanism, but evidence is accumulating that out-of-phase separation is actually observed in parents of trisomic children. Thus, Méhes (1978) has reported an exessive out-of-phase separation of chromosome 18 in parents of three trisomy 18 children. Similarly, he observed premature, early separation of chromosome 21 in parents of Down syndrome babies. These data, howsoever limited, do shed some light on the consequences of out-of-phase separation in humans. Bajnoczky et al. (1980) also reported artificial induction of out-of-phase separation in some subjects exposed to prednisolone. However, no data pertaining to nondisjunction in the progeny are available since the subjects were only around 12 years old. Of course, the current hypothesis excludes any induction of transmissible aneusomy from treatment with mutagens.

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