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The Problem of Partial Endoreduplication

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Summary. Partial endoreduplication (PE) as defined by Lejeune et al. (1966) has only been found in a few instances. Similar configurations, also called PEs, seem to originate from a different process. A series of 12 PEs is presented in this paper, discovered in metaphases from healthy individuals, and in patients with or without chromosome-breakage syndrome and after treatment with chromosome-breaking agents. Interpretations of the microscopic appearance of each configuration led to the conclusion that there are three different modes of origin for such rare events, one being true partial endoreduplication, the second a partial pseudoendoreduplication, and the third a homologous triradial chromatid translocation.

Introduction

Partial or selective endoreduplications have been defined by Lejeune et al. (1966): in a regular mitosis one chromosome or segment of a chromosome divides into four instead of two chromatids. The reduplication information is interrupted at a certain site in the chromosome. This leads to false reduplication. Ferguson-Smith (1973) suggested that this type of chromatid aberration arises from chromatid breakage followed by mitotic nondisjunction of the distal fragment rather than by partial endoreduplication. Reports of so-called partial endoreduplication (PE) are rare: discussions on its origin and consequences have been published in seven instances; other examples have been shown in illustrations without comment. Most authors follow the first definition of Lejeune and co-workers, but discuss nondisjunction of fragments.

Although discussing a selective endoreduplication in their case, Magensis et al. (1970) describe a chromosome abnormality, which seems not to belong into either group of PE's: The fragile site of a chromosome 16 is reported in several members of a family and no convincing picture of a PE is demonstrated, except for

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widely spread minute double fragments in one metaphase, none of them showing the expected four-chromatid configuration.

Reeves et al. (1970) also found a fragile site on a chromosome 2 and demonstrated breakage within the secondary constriction. Loss of the acentric fragment (2q) produced a cell type containing only the centric fragment with the short arms of chromosome 2. Moreover, another cell line with one centric and two acentric fragments in end-to-end association with a possible structural continuity was observed.

Golob et al. (1970) also reported one case with repeated chromatid aberrations in chromosome 2, again at a fragile site near the centromere on q. The authors described all possible consequences after breakage at the fragile site and nondisjunction of the acentric fragment, except for cells that lost the centric fragment and kept one acentric fragment. Apart from possible PEs, as in the figures of Lejeune's PEs, associated double fragments at the fragile site were also shown. These observations in this case favor Ferguson-Smith's explanation of breakage with subsequent nondisjunction and close association of the fragments.

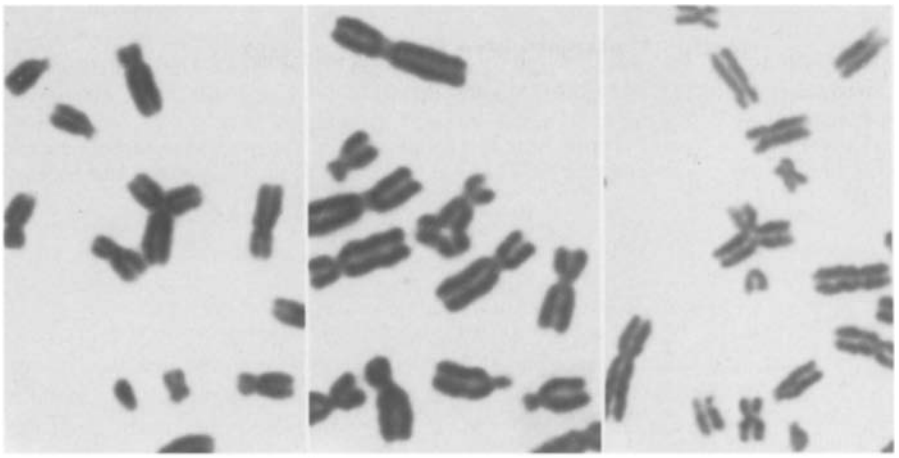
A special case is described by Drets et al. (1970), who observed a terminal amplification of chromosome 16q in three generations of a family. Four cells contained the amplified chromosome 16 with partially endoreduplicated fragments on both chromatids, while in another cell the total aberrant chromosome was endoreduplicated. These observations collaborate very well with the definition given by Lejeune et al.: in each instance the amplified fragments consist of four chromatids, two of each connected with the original chromatids of chromosome 16q. Other double fragments were found close to chromosome 16, without a visible filament. In this case, however, nondisjunction of the fragments was not found.

We shall now discuss an additional hypothesis for the origin of PEs, which could explain the microscopic appearance in chromosome preparations from lymphocyte cultures of different individuals, including the cases reported in the literature. A scheme is offered that demonstrates the production of the different chromosome configurations after the three modes of origin:

1. PEs according to Lejeune's hypothesis (true partial endoreduplication);
2. So-called PEs according to Ferguson-Smith's hypothesis (partial pseudoendoreduplication);
3. A configuration arising from another possible mechanism of origin, resulting in a very similar appearance to what has been referred to in the literature as a PE without further differentiation: the homologous triradial interchange. It will be considered that the last two types do not represent a partial endoreduplication that fits the definition given by Lejeune et al. (1966).

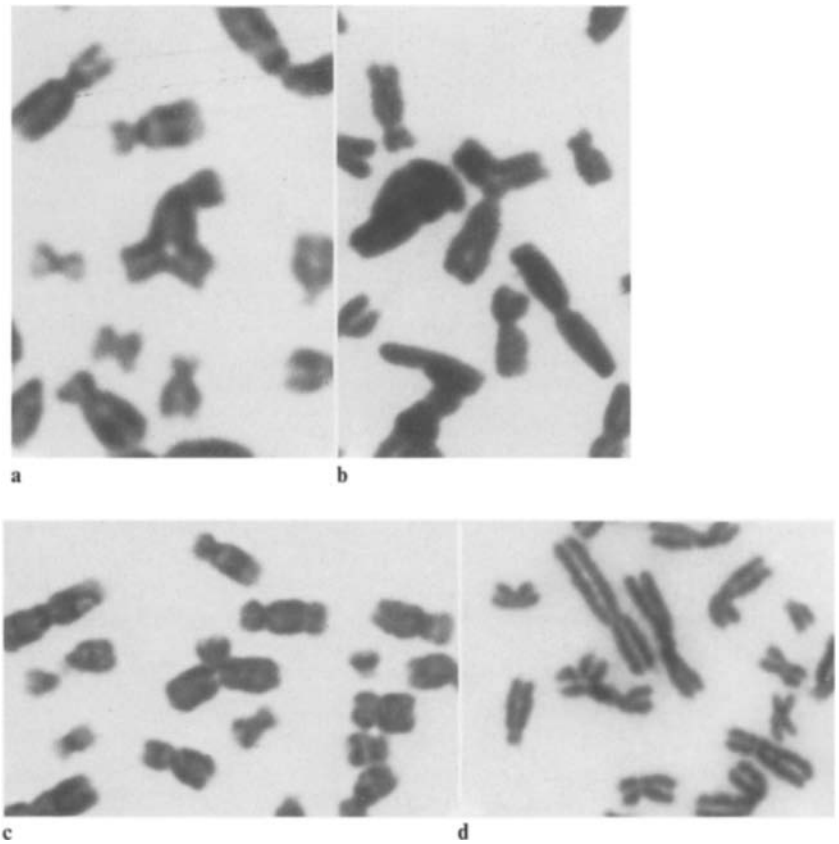
Materials and Methods

PEs were collected over five years from individuals karyotyped for various reasons in our cytogenetic laboratory. We found a remarkably high number of these configurations in chromosome preparations of patients with Fanconi's anemia and Bloom's syndrome. One PE was found in a series of lymphocyte cultures treated for 24 h with mutagens (mitomycin C 1.8×10^{-6} M, trenimon $1—2 \times 10^{-7}$ M). Lymphocytes were cultured in Chromosome Medium IA (GIBCO), harvested and prepared according to the method of Moorehead et al. (1960), and



a b c

Fig. 1a—c. Partially endoreduplicated chromosomes. **a** chromosome 2, healthy individual; **b** a C-group chromosome, healthy individual; **c** a C-group chromosome after treatment with mitomycin C



a b

c d

Fig. 2a—d. Partially endoreduplicated chromosomes from metaphases of patients with Bloom's syndrome. **a** a B-group chromosome; **b** chromosome 2; **c** a chromosome 4; **d** a C-group chromosome

stained in a 2% Giemsa solution. A small fraction of lymphocytes from healthy individuals was grown in Ham F 10, TC 199, and MEM + 15% fetal calf serum and stimulated with phytohemagglutinin.

Three PEs were found in chromosome preparations from patients with Fanconi's anemia and Bloom's syndrome stained with a GTG-banding method described by Wang and Federoff (1972).

Results

The pictures of the 12 PEs are given in Figures 1—4. One chromosome 1, two B chromosomes, five C chromosomes, and two D chromosomes are involved in our material. Six of the 12 PEs show doubling of the total chromatid arms, with spreading at or near the centromere. One chromosome 14 shows doubling of the

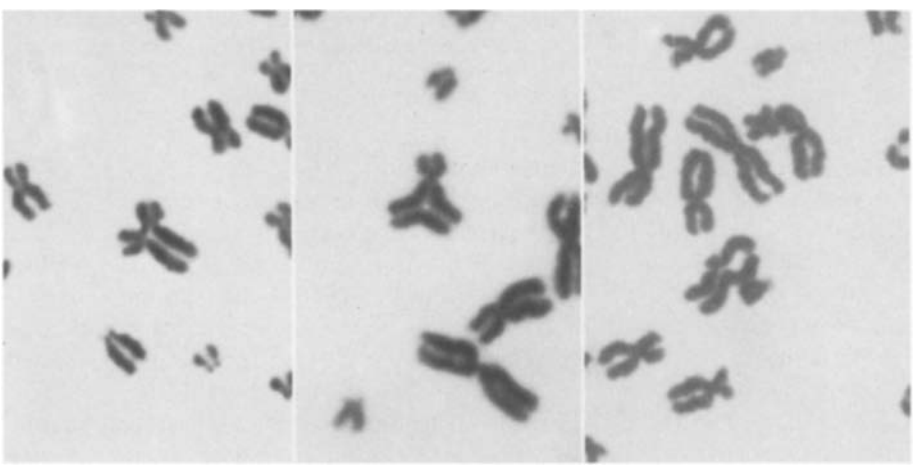


Fig. 3a—c. Partially endoreduplicated chromosomes from metaphases of patients with Fanconi's anemia. **a** a B-group chromosome; **b** a C-group chromosome; **c** a D-group chromosome

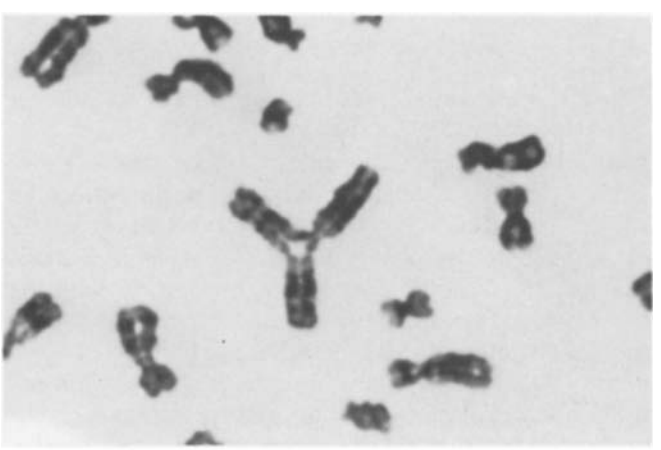
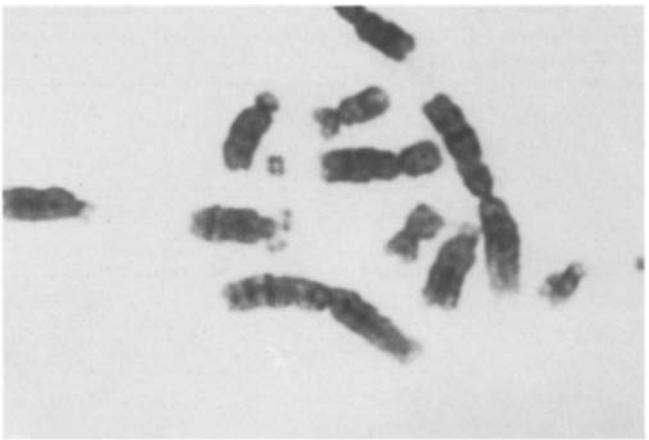


Fig. 4 a



b

Fig. 4a and b. Two Giemsa-banded chromosomes with partially endoreduplicated segments. **a** a chromosome 1, Fanconi's anemia; **b** a chromosome 14, Fanconi's anemia

satellites. Four of the chromosome configurations begin the duplication of the chromatids in the intermediate segment of the long arm. Almost all of them demonstrate spreading of the duplicated chromatid arms to about 120°, and three duplications near the centromere reach almost 180°. When cells of healthy individuals contained a PE, they invariably showed 46 chromosomes.

No constant abnormality at the site of the PE was found in other metaphases. Cells from patients with Fanconi's anemia or Bloom's syndrome and cells treated with mitomycin C contained some other chromatid aberrations.

The Giemsa-banded PE in Figure 4a demonstrates for the first time that the duplicated long arms of chromosome 1 are identical. The banded B chromosome in Figure 2c may be a chromosome 4, and the D chromosome in Figure 4b is probably a chromosome 14.

Discussions

In our material, the described PEs are rare events. The frequencies of PEs in the various cell types can only be estimated (Table 1).

Lejeune's (1966) estimate of the frequency of PEs from accumulated data of normal individuals was 1×10^{-4}, which is about three times our result, but still in the same order of magnitude. In Fanconi's anemia and Bloom's syndrome, PEs are about ten times as frequent. Cells treated with chromosome-breaking agents show also an increased number of PEs. Perhaps the frequency varies with the kind of damage and the concentration of the chemical. Hence, the overall incidence in our material (12 in 80,000 metaphases $= 1.5 \times 10^{-4}$) is of little importance. Cells of patients with Fanconi's anemia and Bloom's syndrome suffer from increased chromosomal instability. In both cell types, fragments of various lengths are frequently found; chromatid aberrations such as gaps, breaks, and chromatid interchanges are typical findings.

Table 1. Frequency of PE in the samples studied

	Lymphocyte metaphases from				
	Control individuals[a]		Patients with		Total
	Untreated	Treated with clastogens[b]	Fanconi's anemia	Bloom's syndrome	
Cells	53,000	5,000	12,000	10,000	80,000
PEs	2	1	5	4	12
Frequency	0.38×10^{-4}	2.0×10^{-4}	4.17×10^{-4}	4.0×10^{-4}	1.50×10^{-4}

[a] Control individuals are healthy persons and patients without a chromosome-breakage syndrome

[b] Clastogens: mitomycin C and trenimon

According to the hypothesis of Ferguson-Smith (1973), the PEs seen in Fanconi's anemia and Bloom's syndrome cells probably derive from nondisjunction of a distal chromatid fragment, which stays in close association with the sister chromatid.

Reduplication during the next S phase leads to the microscopic picture of all but one of the PEs demonstrated in this paper. On the other hand, there is not even one that would fit the hypothesis of Lejeune et al. (1966), which requests that one chromosome segment divides into four instead of two chromatids. This does not however, exclude, the possibility that this hypothesis might hold true in rare instances not observed in our material.

One PE (Fig. 3a) convinced us that there must be another mechanism in addition to the mentioned above (Lejeune et al., 1966; Ferguson-Smith, 1973). This PE was found in a B-group chromosome, in which the centromere and the short arms are doubled. At first glance, the figure resembles a PE because of the similar spread of the short arms. A closer view discriminates this aberrant chromosome from the two types of PEs: attempts to reconstruct this figure according to Lejeune's hypothesis or according to Ferguson-Smith's supposed mechanism of origin fail. There is no duplication of each chromatid—including the centromere—and no independent associated double fragment can be seen. To demonstrate the microscopic appearance of PEs originating from the two mechanisms under discussion, in contrast to the actual picture of this chromosome aberration, Figure 5 shows a scheme of the different ways in which PEs can be reconstructed: the development in column a leads to PEs, which originate from a duplication of each chromatid arm at a specific site (Lejeune hypothesis, 1966) certainly observed by Drets et al. (1970), and possibly by Lejeune et al. (1968). Column c demonstrates the origin of such figures when chromatid fragments stay with the unbroken chromosome and reduplicate close to the homologue chromosome segments (nondisjunction of fragments according to Ferguson-Smith's suggestion, 1970), which have been reported by Lejeune et al. (1966), Golob et al. (1970), Fraccaro et al. (1971), and Ferguson-Smith (1973).

The main difference between a and c is the sister chromatid reunion of the additional fragments (c) in contrast to the doubling of the chromatids without

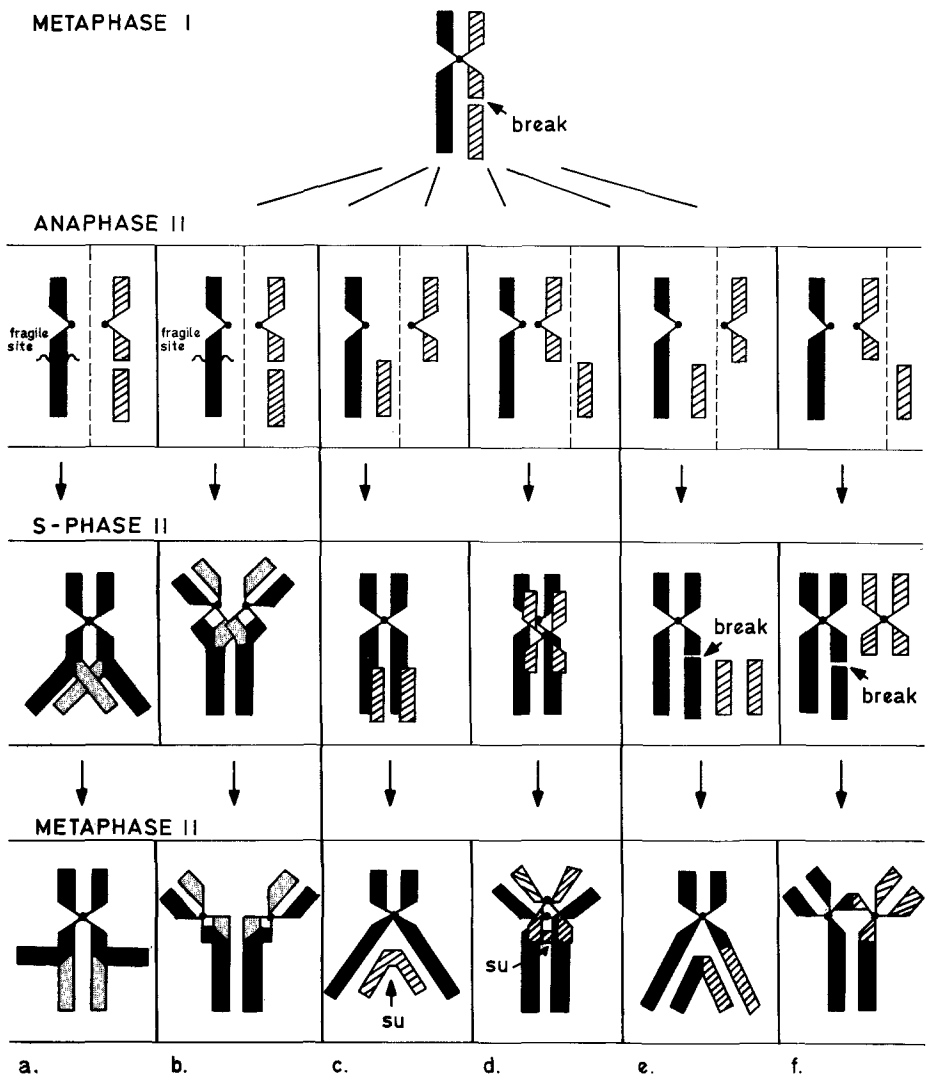


Fig. 5a–f. Scheme for the origin of chromosomal configurations, representing **a** true partial endoreduplication (TPE) after Lejeune's hypothesis, with splitting of chromatid arms at fragile site of chromosome; **c** partial pseudoendoreduplication (PPE), adherent homologous double fragment lying between spread chromatid arms after hypothesis of Ferguson-Smith. Fragments often show sister reunion; **e** triradial homologous chromatid interchanges, composed of one whole chromosome and a homologous double fragment. Columns **b**, **d**, and **e** demonstrate configurations of an endoreduplicated chromosome according to the three possibilities of origin; if, in column **b**, doubling point starts proximal to centromere and centromere and short arms are endoreduplicated as true endoreduplication; in **d** if a centric fragment, containing short arms and centromere, functions like adherent double fragments, comparable to a partial pseudo-endoreduplication; and in **e** a triradial configuration with reunion of breakpoints of whole chromosome and the centric fragments. Only this configuration is comparable to configuration actually seen in Figure 3a (see text)

any connection between the branched chromatid arms (a). In Drets' cases, the reduplication of the amplified fragments started at a filament, similar to a secondary constriction of an elongated chromosome 16. In almost all other observations in familial cases, it is reported that the starting points are the sites of a secondary constriction, an especially fragile region, or a depressed second centromere. The difference between true partial endoreduplication (TPE) in Figure 5a and (as we refer to it from now on) a partial pseudoendoreduplication (PPE), originating from nondisjunction of associated homologous fragments, is easily distinguishable.

Ten of our 12 PEs can be classified as PPE type. None of them fits into Lejeune's category of TPEs; however, the doubling of the satellites on chromosome 14 in Figure 4b could be interpreted as a TPE, since the stalks seem to start in pairs from each short arm of the chromosome.

The PE shown in Figure 3a, a B-group chromosome with two centromeres and doubling of the short arms, as described before, does not follow the formation of either a or c of the scheme. If this aberrant chromosome is a true PE, then there should be no connection between the sister chromatids (Fig. 5b). If this aberrant chromosome is a PPE, the centric fragment should be merely in association with the whole homologous chromosome, without any connecting chromatin bridges. A sister chromatid reunion could show up as in other PPEs drawn in the scheme (Fig. 5c and d).

However, this is not the case. In contrast, this configuration can be constructed only under the assumption of an accidental chromatid interchange between two homologous chromosomes, one intact, the other being a centric fragment.

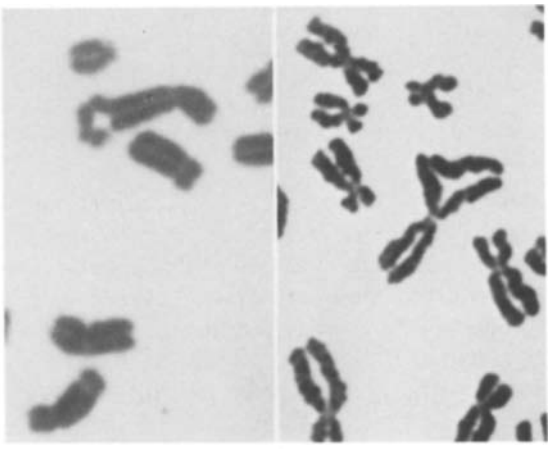
This type of formation, which looks like a PE, was discovered only because it seemed to fall within the definitions of PEs. The main argument that an interchange involving two homologous chromosomes is the only possible explanation for this triradial configuration is the presence of chromatid bridges between the short arms of the centric fragment in the identical site of the structurally normal homologue. Looking at the construction of a true PE involving the same chromosome and chromosome fragment, it becomes quite evident that the figure observed here does not represent a TPE: it would have to show a split at the starting point of the endoreduplicated part and should not include an interchromosomal bridge (Fig. 5b).

Drets et al. (1970) found a filament connecting the inner endoreduplicated parts in one of four PEs. However, the starting site in this case was a terminal secondary constriction of chromosome 16q. Lejeune et al. (1968) also described a secondary constriction near the centromere of chromosome 2q. In these cases, the endoreduplicated fragments or the filaments may break off more easily from the original chromosome. On the other hand, it is hard to decide from the microscopic picture whether there is actually a filament bridge. In all other observations cited from the literature, including our own cases, a chromatidlike continuity can undoubtedly be seen. A chromatidlike continuity between the fragments represents, in our opinion, the main difference between TPEs and other formations of chromosomes and fragments resembling a PE only at first glance. A TPE—according to Lejeune's definition—is obviously the great ex-

ception compared with other configurations found in chromosome preparations of normal individuals and patients with increased chromosomal instability. In the majority of cases, breaks and reunion are necessary to form such a triradial figure.

Golob et al. (1970) discussed this possibility; Ferguson-Smith (1973) arrived at the definition of PEs originating from mitotic nondisjunction of fragments. If mitotic nondisjunction of double fragments leads very often to association of the fragments with the homologous parts of intact chromosomes, then one would expect to find double fragments close to the homologous chromosomes, especially in patients with increased chromosome instability. However, we have never seen double fragments associated with the homologous part of a chromosome like that in Figure 2d in the scheme. We have never seen double fragments very close to a possible homologous chromosome side by side, as shown in Figure 5e, S phase II, which must happen accidentally if the homologous association is the major mechanism and the whole chromosome has no gap, break, or secondary constriction. Ferguson-Smith repeatedly observed the phenomenon of triradial configurations in members of a family during three generations. The chromosome findings very much resemble those observed by Lejeune et al. (1968).

As seen in the scheme, there is no difference in the microscopic picture—either with conventional staining or with banding methods—between the two mechanisms of origin shown in Figure 5c and e, under the assumption that the fragments are reduplicated homologous chromosome segments. Figure 5c represents the PPR after nondisjunction of a chromatid fragment according to the hypothesis of Ferguson-Smith. Figure 5e is a triradial chromatid interchange composed of a chromosome with a chromatid break and a double acentric fragment of a homologous chromosome. Most of the triradial interchanges seen in Fanconi's anemia (FA) and Bloom's syndrome (BS) involve one chromosome and a centric fragment of another chromosome, which can easily be distinguished



a b

Fig. 6. Triradial chromatid interchanges in metaphases of a patient with Fanconi's anemia, reunion of a whole chromosome with acentric fragments, which undoubtedly are nonhomologous parts of other chromosomes

even after conventional staining. However, we have found examples of triradial interchanges in FA, which show the reunion of an acentric fragment with a broken chromosome (Fig. 6a and b).

At least these findings demonstrate that a homologous triradial interchange is a possibility. Considering the possibility suggested by the chromosome in Figure 5f, it seems more probable that many of these configurations that are interpreted as PPE fall into the third class, although the origin—nondisjunction of a homologous fragment or homologous triradial interchange—can by no means be proven, at least not in the rare instances reported here. Noël et al. (1977) were able to experiment with one case in which PPEs of chromosome 2 were repeatedly found in metaphases from blood cultures. They used sister-chromatid exchanges to demonstrate that mitotic malsegregation occurred and that the triradial configurations did not arise from true partial endoreduplication.

A prerequisite for the formation of such a triradial figure is a break in the whole chromosome. We did not always see an interruption of the chromatids at the site of a possible reunion in the aberrations under discussion. However, in the

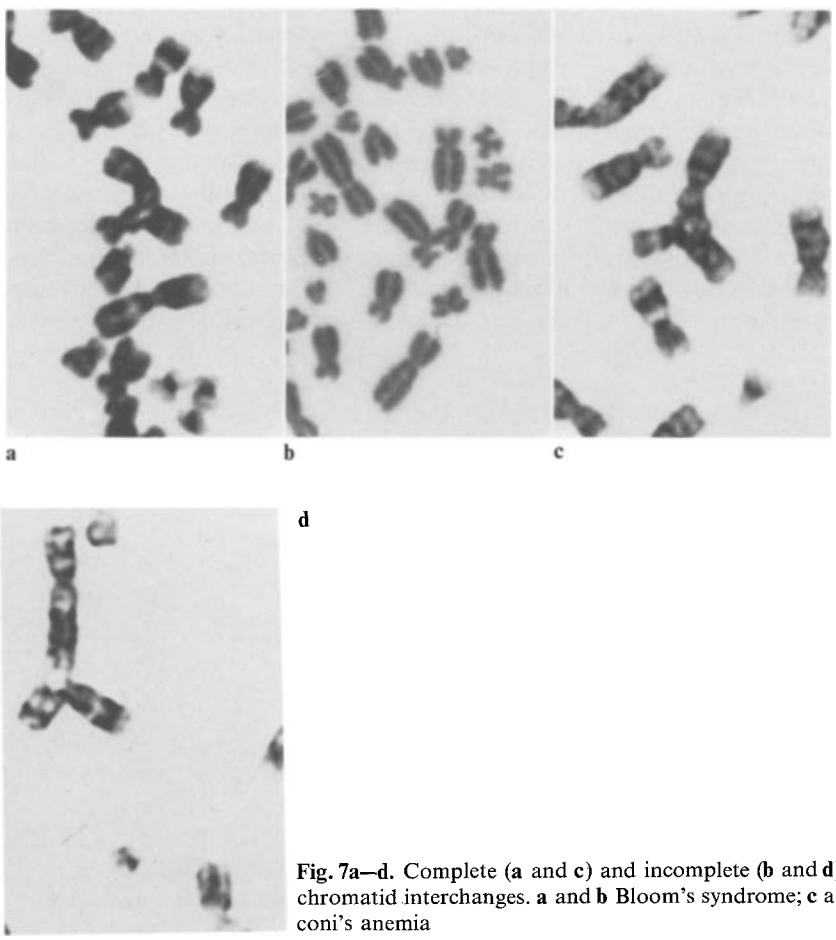


Fig. 7a–d. Complete (**a** and **c**) and incomplete (**b** and **d**) triradial chromatid interchanges. **a** and **b** Bloom's syndrome; **c** and **d** Fanconi's anemia

triradial interchanges, composed of nonhomologous chromosomes and fragments, the break often remained visible. The difference mentioned here should not be overemphasized, because in FA and BS many interchanges are complete while others are incomplete (Fig. 7). Hence, completeness (Fig. 7a and c) or incompleteness (Fig. 7b and d) seems to be no argument for either origin of the configurations.

Since, in cases with increased chromosome instability or cells treated with chromosome-breaking agents, double fragments are never found close to a homologous chromosome, it can be concluded that double fragments lying between the homologous chromatid arms (Fig. 5c) are stronger and more stably positioned than those in a side-by-side adhesion. However, this would be extremely difficult to prove.

Figure 8 demonstrates a duplicated fragment of a chromosome 2 (Fanconi's anemia). This can derive from three different aberrations:

1. Nondisjunction of a homologous fragment, formed after a PPE (Fig. 5c) with sister-chromatid reunion;
2. Homologous triradial interchange, shown in Figure 5e; and
3. Asymmetrical interchange of homologous chromosomes (type II, Schroeder and German, 1974).

Hence, finding fragments like that shown in Figure 8 does not help to differentiate between PPEs, homologous triradial interchanges, and asymmetrical interchanges type II, which are extremely rare in FA.

The two origins of PPEs and homologous triradial interchanges have one prerequisite in common: in both instances the fragments have to be adjacent to the whole chromosome during interphase. Somatic pairing of parts of homologous chromosomes in human cells actually does occur, while somatic pairing of the whole homologous chromosome is still an open question. This consideration is important for investigations of the internal order of the chromosomes in the interphase nucleus (Vogel and Schroeder, 1974).

From the literature concerning partial endoreduplications, it seems that specific loci of certain chromosomes, e.g., chromosome 2, are more often involved than others in a PPE or a homologous triradial interchange. It could very well be that accumulated data on such configurations will help to resolve the problem as to how the chromosomes are arranged in the interphase nucleus. Perhaps certain

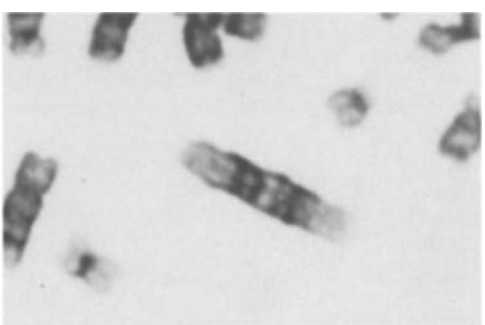


Fig. 8. End-to-end duplication of an acentric fragment, Giemsa banded, Fanconi's anemia

loci containing heterochromatin stick closely together, while other parts of the homologous chromosomes deviate more or less from a pairing position. In this case, associated fragments in a PPE or a homologous triradial interchange may be indicative for more adjacent points along the chromatids (of certain chromosomes?). Especially for clearly distinguishable incomplete homologous triradial interchanges, the loci of reunion demonstrate necessarily interphase pairing and thus add useful information on the construction of an order of the chromosomes in the interphase nucleus.

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