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Parasexual Cycle in Cultivated Human Somatic Cells

Abstract. *Somatic segregation for three different autosomes was demonstrated in two strains of human diploid fibroblasts derived from subjects known to be heterozygous for chromosomal variants. Recombinant diploid cells appeared within cultures of tetraploid clones isolated from mass cultures. Tetraploid cells regularly occur in mass cultures and within clones of diploid cells. Such a parasexual cycle (2n → 4n → 2n), with recombination of entire linkage groups, could form the basis of a beginning formal genetic analysis in man.*

The discovery of the parasexual cycle of *Aspergillus nidulans* by Pontecorvo and co-workers (*1*) permitted a formal genetic analysis of this and other fungi based entirely on mitotic recombinational events. Pontecorvo suggested that a formal genetic analysis of the human species, based on mitotic recombination within cultivated somatic cells, might also be possible (*1*). The present report (*2*) presents cytogenetic evidence that a parasexual cycle does in fact occur in such cell cultures.

Fibroblast cultures were established from skin biopsy explants derived from a 55-year-old woman, heterozygous for a long submetacentric No. 16 chromosome [the proposita reported in (*3*)], and a 21-month-old girl known to be heterozygous (peripheral blood cultures) for partial deletions of the short arms of chromosome No. 18 and of the short arms of one of the D group chromosomes. Cytogenetic findings in skin fibroblast cultures from the 21-month-old girl were identical to the cytogenetic findings in peripheral blood cultures (Fig. 1). Her father was found to be carrying the D group deletion and her mother, the No. 18 deletion. (Medical genetic aspects of this family will be the subject of a separate report.) The techniques of cell culture, including methods of skin biopsy, cloning, and cytogenectic analysis, have been previously described (*4*).

The frequency of tetraploid cells in mass cultures of these and other strains generally vary from 1 to 4 percent, although in occasional strains frequencies of 10 to 15 percent are observed. One or more tetraploid clones (derived from a single cell as determined by direct microscopy) can readily be isolated from a random sample of 100 cells; therefore, selective techniques were not required to obtain tetraploids. Two such tetraploid clones, one from each of our two chromosomally marked cultures, were examined cytogenetically during repeated "passages" (reseeding of the cultures by trypsinization). The distributions of counts from three such passages gave frequencies of diploid and presumptively diploid cells in the range of 3.7 to 17.1 percent. Comparable proportions of diploid cells have been found in several other tetraploid clones derived from various individuals. There was no evidence of sequential, progressive chromosome loss from tetraploid to diploid. The hypotetraploid counts that were observed (25 out of a total of 171 counts) were interpreted as having resulted from random artifactual losses of chromosomes.

A total of 36 diploid cells were of sufficient quality to permit detailed karyotypes, including group D chromosomes. Of these, 35 were of sufficient quality also to permit complete analysis of the No. 18 chromosome pair. On the basis of visual inspection, four different types of somatic segregants were identified: D/D, Dp−/Dp−, 18/18, and 18p−/18p− (symbol p designates the short arm of the chromosome and the negative sign refers to a deletion) (*5*). These data are summarized in Table 1, and examples of the findings are given in Fig. 1. No instances of double recombinants were found. Twelve recombinants were also found among the 47 diploid cells karyotyped from passages of the other chromosomally marked tetraploid clone (Table 2 and Fig. 2).

Table 1. Cytogenetic characterization of diploid cells from a predominately tetraploid clone of skin fibroblasts from a child heterozygous for partial deletions of two autosomes, one a group D chromosome and the other a group E chromosome (No. 18); p− refers to a short arm deletion.

Karyotypes		No. of cells	Percentage of total cells
Group D karyotypes			
Nonrecombinant	D/Dp−	32	89.0
Recombinant	D/D	2	5.5
Recombinant	Dp−/Dp−	2	5.5
Group E karyotypes			
Nonrecombinant	18/18p−	30	85.7
Recombinant	18/18	4	11.4
Recombinant	18p−/18p−	1	2.9

In order to document our cytogenetic diagnoses, measurements of centromeric indices (ratios of lengths of the short arms to total lengths of chromosomes) (*5*) were determined for all presumed recombinant chromosomes and compared with suitable controls. The results, given in Tables 3 and 4, confirmed the original cytogenetic diagnoses, although the data for recombinants Dp−/Dp− No. 1 and 18/18 No. 1 might be interpreted as having marginal significance.

In order to seek evidence for somatic segregation involving sex chromosomes, 49 diploid cells from two tetraploid clones (derived from a culture made from the foreskin of a newborn) were analyzed. No XX or YY recombinants were found. This is in keeping with Ohno's failure to find examples of somatic segregation involving sex chromosomes in his detailed studies of this phenomenon in deer mice and fish (*6*).

Extensive cytogenetic studies of possible mechanisms of somatic segregation could not be carried out in tetraploid clones from our strains with the autosomal markers because of their eventual

Table 2. Cytogenetic characterization of diploid cells from a predominately tetraploid clone of skin fibroblasts from a woman heterozygous for a morphologic variant of chromosome No. 16; 16?q+ represents a chromosome No. 16 with an apparent increase in length of its long arm.

Group E karyotypes		No. of cells	Percentage of total cells
Nonrecombinant	16/16?q+	35	74.5
Recombinant	16/16	5	10.6
Recombinant	16?q+/16?q+	7	14.9

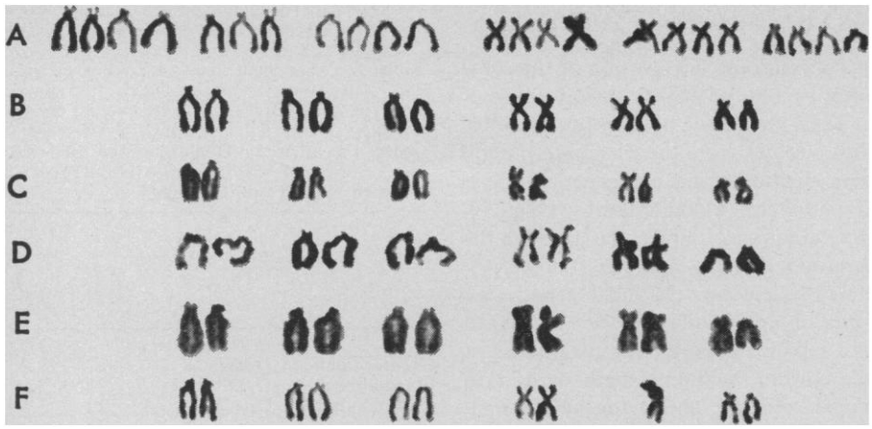

Fig. 1. Partial karyotypes (group D, or chromosomes Nos. 13 to 15, and group E, or chromosomes Nos. 16 to 18) of representative cells from a clone of tetraploid skin fibroblasts from a child heterozygous for two autosomal short arm partial deletions, symbolized by p—. (A) Tetraploid (missing a D chromosome); note the pair of D group chromosomes with short arm deletions (10th and 11th from left) and the pair of No. 18 chromosomes with short arm deletions (extreme right); (B) diploid nonrecombinant; (C) diploid recombinant, 18/18; (D) diploid recombinant, 18p—/18p—; (E) diploid recombinant, D/D; and (F) diploid recombinant, Dp—/Dp—.

Table 3. Centromeric indices (ratios of lengths of short arms to total lengths) of chromosomes from recombinant and nonrecombinant karyotypes of diploid cells from a predominately tetraploid clone of skin fibroblasts from a child with partial deletions of two autosomes, one a group D chromosome and the other a group E chromosome (No. 18). Controls are karyotypes from mass cultures of randomly selected strains of human skin fibroblasts. Numbers in parentheses refer to ranges of centromeric indices (R) or to total number of cells measured (N). Underlined figures refer to those two of the six members of the group D set of chromosomes which were diagnosed as bearing short arm deletions; p— refers to a short arm deletion.

Group D karyotypes		Centromeric indices	
		D	Dp—
Controls	($N = 59$)	18.3(R, 13–25)	
Nonrecombinant	D($N = 74$)	17.3(R, 14–25)	
Nonrecombinant	Dp—($N = 15$)		7.1(R, 1–9)
Recombinant	D/D No. 1 cell	21, 21, 18, 18, 18, 18	
Recombinant	D/D No. 2 cell	14, 14, 17, 15, 16, 16	
Recombinant	Dp—/Dp— No. 1 cell	16, 17, 17, 19, 5, 11	
Recombinant	Dp—/Dp— No. 2 cell	19, 19, 21, 19, 9, 7	

Group E karyotypes		Centromeric indices	
		18	18p—
Controls	($N = 98$)	31.0(R, 24–36)	
Nonrecombinant	18($N = 33$)	30.8(R, 25–36)	
Nonrecombinant	18p—($N = 30$)		19.5(R, 10–25)
Recombinant	18/18 No. 1 cell	32 and 26	
Recombinant	18/18 No. 2 cell	29 and 31	
Recombinant	18/18 No. 3 cell	34 and 33	
Recombinant	18/18 No. 4 cell	29 and 29	
Recombinant	18p—/18p— No. 1 cell	16 and 17	

Table 4. Centromeric indices of chromosomes from recombinant and nonrecombinant karyotypes of diploid cells from a predominately tetraploid clone of skin fibroblasts from a woman heterozygous for a morphologic variant of chromosome No. 16. Controls are normal No. 16 chromosomes from the E group karyotypes prepared for Table 3. Numbers in parentheses refer to ranges of centromeric indices (R) or to total number of cells measured (N); 16?q+ represents a chromosome No. 16 with an apparent increase in length of its long arm.

Group E karyotypes		Centromeric indices	
		16	16?q+
Controls	($N = 30$)	43.3(R, 41–49)	
Nonrecombinant	16($N = 51$)	43.7(R, 40–50)	
Nonrecombinant	16?q+($N = 53$)		34.0(R, 29–39)
Recombinant	16/16 No. 1 cell	41 and 49	
Recombinant	16/16 No. 2 cell	41 and 41	
Recombinant	16/16 No. 3 cell	44 and 45	
Recombinant	16/16 No. 4 cell	44 and 48	
Recombinant	16/16 No. 5 cell	40 and 40	
Recombinant	16?q+/16?q+ No. 1 cell	36 and 37	
Recombinant	16?q+/16?q+ No. 2 cell	33 and 32	
Recombinant	16?q+/16?q+ No. 3 cell	38 and 33	
Recombinant	16?q+/16?q+ No. 4 cell	31 and 35	
Recombinant	16?q+/16?q+ No. 5 cell	35 and 35	
Recombinant	16?q+/16?q+ No. 6 cell	31 and 30	
Recombinant	16?q+/16?q+ No. 7 cell	35 and 34	

senescence (*7*). Instead, an early passage of a vigorous tetraploid clone of fibroblasts from the foreskin of a newborn was chosen. Of 20,000 cells analyzed in metaphase, ten examples of "genomic segregation" were found (*8*), involving the apparent segregation of complete or nearly complete haploid sets. Of 2000 cells analyzed in anaphase and telophase, there were six tripolar and four tetrapolar mitoses. None of these events appear to be sufficiently frequent to account for the comparatively high frequency of diploid cells present in our tetraploid clones. Selective replication of diploids probably does not occur to any substantial degree, since there is usually a relatively constant proportion of tetraploid cells in mass cultures and there is a comparable cloning efficiency of tetraploid and diploid cells. At least one important cytogenetic mechanism appears to be tetrapolar mitosis, since each such event could result in four diploid cells. As Ohno (*6*) has emphasized, somatic segregation (chromosome recombination) follows tetrapolar mitoses in only two of four daughter cells having a consecutive (as opposed to alternative) alignment of chromosomes. The frequency of a specific type of chromosomal recombination would therefore be 12.5 percent (two cells, each with complementary products of somatic segregation, out of a total of eight possible daughter cells, or one cell with a specific type of recombination out of a total of eight possible daughter cells). In the case of the chromosome No. 16 marker (Table 2), where differential selection for segregants is not likely to occur and where 47 diploid cells are available for analysis, the chi-square test is consistent with the hypothesis of a ratio of 1:1:6 for recombinant 16/16:recombinant 16?q+/16?q+:parental 16/16?q+ ($P = .85$).

Little information is available concerning the mechanism responsible for tetraploidy in such cultures. It is possible that cell fusion with subsequent homosynkaryosis (*9*, *10*) may be responsible for a proportion of tetraploid cells. However, tetraploidy may also result from failure of cytoplasmic cleavage with subsequent fusion of two daughter nuclei or from endoreduplication (*6*).

It would be of interest to carry out similar studies with a variety of chromosomally marked strains to determine the frequency of somatic segregation as a function of specific genomes and autosomes. Should it prove to be of widespread occurrence, it could form the basis of a beginning formal genetic analysis in man. Cytogenetic studies would have to be combined with biochemical or antigenic analysis of clones from individuals heterozygous for markers that are expressed in cell culture. In view of the widespread occurrence of enzyme polymorphism in man (*10*), it is probable that a variety of suitable markers could be exploited for such a genetic analysis. The zymogram technique (*10*) should permit the identification of heterozygous clones and each of the two types of homozygous diploid segregants. Replicate plating of such segregants could permit the assignment of other loci to the relevant linkage groups, since all loci linked to the segregated chromosomes would become homozygous. Such an analysis cannot be carried out efficiently, however, without methods for the selection of recombinant diploid clones. Selective techniques are especially essential in view of the eventual "senescence" of such cultures (*7*); in our laboratory, more than 100 mass cultures and over 200 clones from a variety of human diploid cultures have ceased to replicate, with a maximum life-span of about 65 cell doublings (*11*).

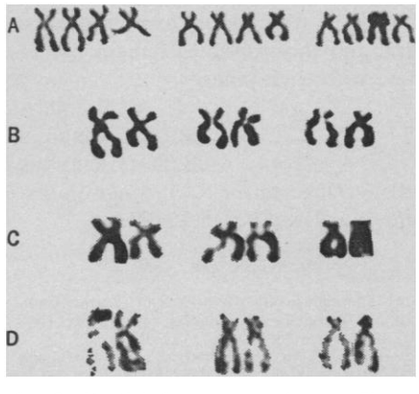

Fig. 2. Partial karyotypes (group E, or chromosomes Nos. 16 to 18) of representative cells from a clone of tetraploid skin fibroblasts from a woman heterozygous for a morphologic variant of chromosome No. 16. (A) Tetraploid; (B) diploid nonrecombinant; (C) diploid recombinant 16/16; and (D) diploid recombinant, 16?q+/16?q+.

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Fungal Endogenous Rhythms Expressed by Spiral Figures

Abstract. *Culture zonations in two fungi,* Nectria cinnabarina *and* Penicillium diversum, *are expressions of endogenous rhythms. These culture zonations may take the form of either concentric rings or Archimedes' spirals. The rhythm in* N. cinnabarina *is noncircadian. The rhythm in* P. diversum *is relatively insensitive to temperature and has a period of approximately 24 hours. The lack of a demonstrable mechanism for phase shifting suggests that this rhythm may also be noncircadian.*

Spirals of biological origin are common and almost always logarithmic (*1*). A notable exception is the web of some spiders which is often an Archimedes' spiral. Archimedes' spirals occur in the fungi *Nectria cinnabarina* and *Penicillium diversum*. These spirals are the visible expression of endogenous rhythms.

Nectria cinnabarina was cultured on media containing 48 g/liter of Bacto-potato dextrose broth (PDB) and 20 g/liter of Bacto-agar (Difco). *Penicillium diversum* was cultured on 12 g/liter of PDB and 20 g/liter of Bacto-agar. Cultures were initiated from a single germinated spore placed in the center of a plate. Sporulation of *N. cinnabarina* was induced by growing the cultures under continuous illumination from a Growlux fluorescent lamp at 4.8×10^3 erg cm^{-2} sec^{-1}. Since growth of the *P. diversum* isolate was somewhat inhibited by light, the cultures were maintained in continuous darkness, where sporulation occurred. All cultures were incubated in constant temperature chambers.

Both *N. cinnabarina* and *P. diversum*, under the conditions described above, produce concentric rings of spores similar to many fungi that exhibit zonation phenomena (*2*). Culture zonations of both fungi are produced with stable periodicities under constant environmental conditions. The rhythm of *N. cinnabarina* is noncircadian. The period of the rhythm ranges from 6 to 16 hours, depending upon the temperature. A Q_{10} of 2.73 has been determined for the period of the *N. cinnabarina*

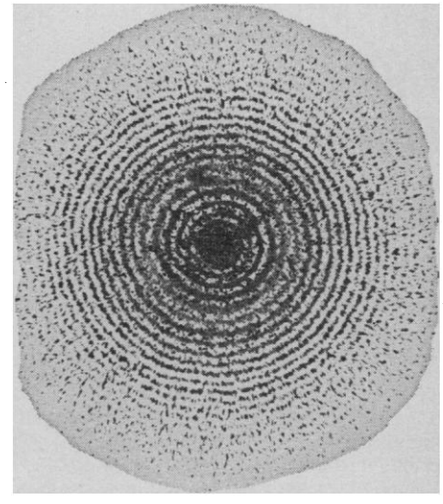

Fig. 1. Culture zonation in *Nectria cinnabarina* in the form of a single Archimedean spiral.