Phenotypic Assortment in *Tetrahymena thermophila*: Assortment Kinetics of Antibiotic-Resistance Markers, *tsA*, Death, and the Highly Amplified *rDNA* Locus

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ABSTRACT

Phenotypic assortment in *Tetrahymena thermophila* results from random distribution of alleles during amitotic division of the macronucleus. The rate of assortment is dependent on input ratio and the number of assorting units. The assortment of the antibiotic resistance markers *Chx*, *Mpr* and *gal* was determined and is consistent for each with the model of 45 assorting chromosomes. The gene *tsA* (previously ts-1) shows normal assortment, in contrast to previous reports. A mutation in the highly amplified ribosomal locus (*rdn*A2) assorts as if present at only 45 copies. Death of clones occurred at a rate consistent with assortment for a single gene.

TETRAHYMENA thermophila contains two distinctly different nuclei: a diploid germinal micronucleus and a somatic macronucleus containing 45C equivalents of DNA. The macronucleus arises during conjugation from the diploid zygote nucleus [see GOROVSKY (1980) for review]. This differentiation includes: (1) replication of 85–90% of the sequences to 45C of DNA (YAO and GOROVSKY 1974; YAO and GALL 1979); (2) fragmentation of the five germinal chromosomes into several hundred chromosomes (ALTSCHULER and YAO 1985; CONOVER and BRUNK 1986); (3) rearrangements of some of the DNA sequences (YAO 1982; IWAMURA, SAKAI and MURAMATSU 1982; YAO, KIMMELL and GOROVSKY 1974; YAO *et al.* 1984); and (4) the initiation of transcriptional activity.

The macronucleus divides amitotically, with no obvious mechanism to ensure equal partitioning of the genome to the two daughter nuclei. Therefore, although the macronuclei formed from heterozygous micronuclei should initially contain an equal mix of the two alleles, subsequent divisions may lead, by chance, to unequal daughters. Since the macronucleus is responsible for the phenotype of the cell, this vegetative growth produces a variety of phenotypes among the progeny. Cells may arise in which all copies of one of the alleles for a particular gene have been lost. The appearance of subclones pure for one or the other allele has been termed phenotypic assortment [see NANNEY and PREPARATA (1979) and ORIAS and FLACKS (1975) for models].

The generally accepted model for phenotypic assortment assumes (1) about 45 copies of each macronuclear chromosome exist in the mature macronucleus; (2) daughter cells receive about 45 copies of each macronuclear chromosome; (3) there is no control on the specific allele distribution to the daughter macronuclei. The kinetics of assortment during growth of heterozygous lines can be determined by measuring the appearance of cells which stably express the phenotype of one allele. Two factors are known to affect the kinetics of assortment: (1) the relative proportion of the two alleles in the initial macronucleus (the input ratio); and (2) the total number of assorting units in the macronucleus. The input ratio will determine the proportion of each pure type in the fully assorted population; the number of assorting units will determine the time of appearance of assorted cells and the final rate of assortment as the last copy of the other allele is lost (SCHENSTED 1958; DOERDER, LIEF and DOERDER 1975). Assortment behavior of a variety of different genes may provide evidence to test the recent suggestion of BRUNK (1986) that there may be a wide range of copy numbers for different genes, rather than the uniform 45 generally assumed.

Input ratios and numbers of assorting units have been measured for a variety of markers (NANNEY and PREPARATA 1979). All are expected to reflect typical macronuclear organization, and all assort with kinetics which may be interpreted in terms of 45 assorting units as described in the model of SCHENSTED (1958). In this report we extend these observations to three antibiotic resistance markers also expected to reflect typical macronuclear gene organization: *Chx*, *Mpr* and *gal*. The appearance of antibiotic sensitive cells from heterozygous resistant lines facilitates the measurement of cells expressing one allele. Input ratios and

TABLE 1

Genetic description of stocks

Stocks	Micronuclear genotype	Macronuclear phenotype[a]
ts5a	*tsA/tsA*	temp-s IV
JR2	*Chx*A2/*Chx*A2; *Mpr/Mpr*; *tsA/tsA*	cy-s 6mp-s temp-r VII
SB210	*gal/gal*	dgal-s VI
CU336	*Mpr/Mpr*	6-mp-s VII
CU355	*Chx*A2/*Chx*A2	cy-s IV
CU364	*rdn*A2/*rdn*A2	pm-s IV
CU369	*Chx*A2/*Chx*A2; *Mpr rdn*A2/*Mpr rdn*A2	cy-s pm-s dgal-r II
CU401	*Mpr/Mpr*	6 mp-s VII
x683-A23II	*Chx*A2/*Chx*A2; *rdn*A2/*rdn*A2	cy-s, 6mp-s, pm-s IV

[a] Abbreviations: sensitivity (s), or resistance (r) to temperature (temp), cycloheximide (cy), 6-methyl purine (6mp), 2-deoxygalactose (dgal), and paromomycin (pm). Mating type is indicated by roman numerals.

numbers of assorting units are consistent with those found for other markers.

We also examined two genes expected to have atypical macronuclear organization. The ribosomal DNA is present at 200 copies per haploid equivalent for 10,000 copies for macronucleus (ENGBERG *et al.* 1976; KARRER and GALL 1974; YAO, KIMMEL and GOROVSKY 1974; GOROVSKY 1980). The allele *rdn*A2, a mutation in the gene for ribosomal DNA, confers resistance to the aminoglycoside antibiotic paromomycin (BRUNS *et al.* 1985; SPANGLER and BLACKBURN 1985). Assortment for such an allele should be very slow or nonmeasurable if the phenomenon is sensitive to the number of units. We also reexamined the allele ts-1 (now designated *tsA* in BRUNS 1984); MCCOY (1973) reported that this allele fails to assort. We found the assortment behavior of *tsA* and *rdn*A2 to be indistinguishable from that of other measured genes.

Finally, we analyzed the kinetics of death of clones to test the assumption that the total number of any macronuclear chromosome is maintained. Cells are generated during vegetative growth which have more or less DNA than the expected amount (DOERDER 1979; DOERDER and DEBAULT 1975), and macronuclear division is quite often unequal (DOERDER and DEBAULT 1975). This suggests that the total number of macronuclear chromosomes is not conserved during division. If the distribution of these chromosomes during division is totally random, all copies of a particular chromosome might be lost, by chance, leading to a high frequency of dead cell lines. We found that clonal death did occur, but at a rate that suggests that some level of regulation exists.

MATERIALS AND METHODS

Strains: The strains of *Tetrahymena thermophila*, inbred strain B, their micronuclear genotypes and macronuclear phenotypes appear in Table 1.

Markers: *Chx*A2, a dominant mutation providing resistance to cycloheximide at 25 μg/ml (ARES and BRUNS 1978; BLEYMAN and BRUNS 1977); *Mpr*, a dominant mutation providing resistance to 6-methylpurine at 15 μg/ml (BYRNE, BRUSSARD and BRUNS 1978); *gal*, a recessive mutation providing resistance to 2-deoxygalactose at 2.5 mg/ml (ROBERTS and MORSE 1980); *tsA* (previously ts-1), a recessive mutation leading to temperature sensitivity (40°) (MCCOY 1973); *rdn*A2, a dominant mutation in the structural gene for ribosomal RNA (BRUNS *et al.* 1985; SPANGLER and BLACKBURN 1985) providing resistance to paromomycin at 100 μg/ml.

Culture methods: As described previously (BRUNS, BRUSSARD and MERRIAM 1983), cells were grown in PPYS [1% proteose peptone (Difco), 0.1% yeast extract (Difco) and 0.003% Sequestrine (Geigy)] at 30° to 1–2 × 10^5, washed once with 0.01 M Tris pH 7.4, and starved for at least 2 hr at 1–2 × 10^5. Equal numbers of appropriately marked strains of unlike mating types were mixed and allowed to mate without shaking at 30°. After 6–8 hr, single pairs were isolated into drops of PPYS with penicillin and streptomycin (each at 250 μg/ml) in petri dishes. Petri dishes were incubated for 3 days at 30°. The resultant clones were then replicated to microtiter plates containing 1% peptone for a master plate and to plates containing appropriate antibiotics in PPYS. Cross-fertilizers were identified by resistance to the appropriate drugs.

A master plate of 72 clones was constructed from identified cross-fertilizers. When possible, these represented 72 independent synclones (progeny of single mating pairs); where this was not possible, several drops were inoculated from a single synclone. When progeny of a single cross-fertilizer are expanded for analysis, as done previously (*e.g.*, ALLEN and NANNEY 1958), the final assortment values reflect the actual input ratio in a single macronucleus. Analysis of a large number of independent cross-fertilizers should give a closer approximation to the random (1:1) input expected for the population of macronuclei.

Transfer and assortment analysis: Single cells were incubated in individual drops on Petri dishes at 30° in humid boxes and transferred daily with a micropipet. Each drop thus represents a unique clone. A drop contained *ca.* 1000 cells after 24 hr, representing *ca.* 10 cell divisions. A single cell was transferred to a fresh drop of medium; the remainder of the drop was transferred to a microtiter plate for analysis. For multiple analyses, replicates were made to appropriate drug plates; for single analysis, the original plate was overlaid with the desired drug. Replicates were incubated at 30° for 3 days for drug sensitivity or for 1 day at 40° for temperature sensitivity.

Clones (individual wells) were scored for drug or temperature sensitivity. Clones were scored as sensitive only when three successive tests showed sensitivity. Sensitive clones

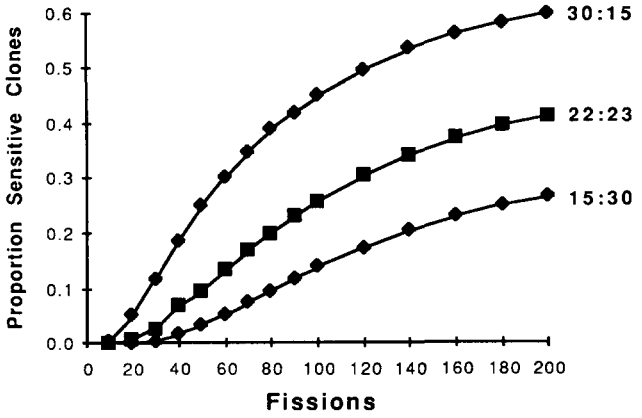

FIGURE 1.—Theoretical curves for accumulation of sensitive clones by phenotypic assortment. The cumulative proportion of cells assorted to one pure type as a function of input ratio by fission numbers. The data are taken from the computer simulation by DOERDER, LIEF and DOERDER (1975) for a model according to SCHENSTED (1958) assuming 45 assorting units.

were carried throughout the experiment to determine stability of the sensitive phenotype; clones scored sensitive for three successive passages remained sensitive throughout the remainder of the transfers.

In some instances a well would contain a few or no live cells. When this occurred, a cell was recovered from the previous transfer in an attempt to rescue the clone; generally such attempts failed. When a clone in the transfer series could not be revived, it was scored as dead, and a cell from a non-assorted clone was used to replace the clone in the transfers, to maintain the total number.

Data analysis: Previously, assortment data have been presented as instantaneous rates (*e.g.*, ALLEN and NANNEY 1958). Here we also plot the cumulative proportion of sensitive cells as a function of total cell generations. Such a plot may be readily compared to the curves from the computer simulation for various input ratios (DOERDER, LIEF and DOERDER 1975).

RESULTS

DOERDER, LIEF and DOERDER (1975) have generated a computer simulation for assortment based on the model of SCHENSTED (1958). This simulation assumes 45 assorting units in the macronucleus, and provides the relative proportions of cell lines expressing pure types for each allele as a function of the number of fissions. Tabular data are provided for a variety of input ratios (the proportion of the two alleles in the starting macronucleus). Figure 1 is a graphic representation of the cumulative proportion of cells expressing one pure phenotype at three input ratios. The initial time of assortment, the initial rates and the final plateau value are all sensitive to input ratio. In this form, the input ratios can be estimated roughly from the plateau value approached. In addition, the terminal slopes, which represent the distribution of the final copy of the dominant allele (ORIAS and FLACKS 1975), and may be used to estimate the number of assorting units, are strikingly similar for different ratios.

TABLE 2

Crosses used to generate heterozygotes

Cross No.	Stocks	Assortment for[a] cy	mp	ts	dgal	pm
1	CU355 × CU336	cy	mp			
2	ts5a × CU336		mp	ts		
3	JR2 × SB210	cy	mp	ts	dgal	
4	CU336 × CU369		mp			pm
5	CU401 × CU364		mp			pm
6	CU401 × CU364					pm
7	CU401 × CU364					pm
8	CU401 × CU364					pm

[a] Abbreviations as in Table 1.

Cells heterozygous for various markers were produced by crossing homozygous cells of desired genotypes as listed in Table 2. Progeny were identified by their resistance to the appropriate antibiotics. The appearance of sensitive cells arising from phenotypic assortment in the heterozygotes was followed as described in MATERIALS AND METHODS. Figure 2a shows the assortment behavior for the three antibiotic markers *Chx*, *Mpr* and *gal*. As noted in the legend, data from one to five experiments were pooled for this figure.

Kinetics for assortment of these three alleles were similar. Though the initial time, initial rates, and final levels approached through 180 generations vary somewhat with the particular gene, the terminal rates are again similar and consistent with the theoretical curves (Table 3). All data are thus consistent with the model of 45 assorting units, with input ratios between 22:23 and 15:30.

Figure 2b shows assortment behavior of the atypical markers *tsA* and *rdn*A2. The *tsA* allele does assort, albeit late and to a lesser degree than the antibiotic resistance markers. This may reflect some selection against the sensitive allele during routine growth at 30°. In view of the report that this gene does not assort (MCCOY 1973), heterozygotes were generated from different *tsA* stocks, kindly provided by D. L. NANNEY. The lines were expanded, and examined for temperature sensitive clones. Sensitive assorted cells appeared in all lines (data not shown).

Figure 2b also shows the assortment kinetics for *rdn*A2, a mutation in the structural gene for the ribosomal RNA (BRUNS *et al.* 1985; SPANGLER and BLACKBURN 1985) which confers resistance to paromomycin. Remarkably, the assortment is similar to the others (Figure 2a), although this locus is present in about 10,000 copies per macronucleus.

Death of clones was noted in all crosses. The pooled data are plotted in Figure 2b. Again the kinetics are consistent with the assortment of a single gene, rather than with random loss of all copies of any chromosome.

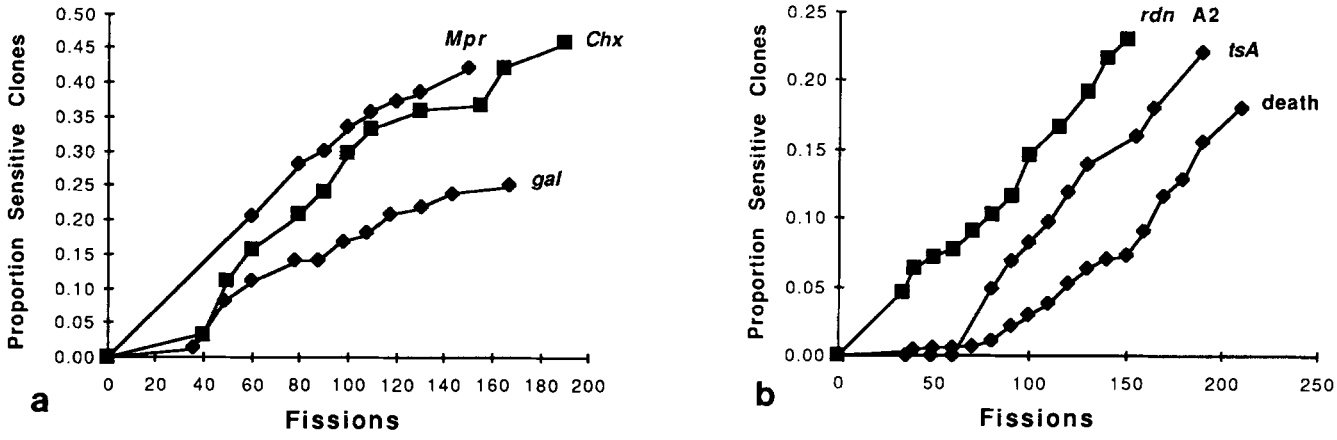

FIGURE 2.—Phenotypic assortment to the stable recessive form. (a) Genes with expected typical macronuclear organization, *Mpr*, *Chx*, and *gal*. Data are plotted as the cumulative total number of drug sensitive clones as a function of the number of cell divisions from zygote formation. Curves represent pooled data from several experiments (*cf.* Tables 1 and 2): *Mpr* (experiments 1–5 for 360 lines analyzed); *Chx* (experiments 1 and 3 for 144 lines); *gal* (experiment 3 for 72 lines). (b) Genes with expected atypical macronuclear organization, *tsA* and *rdn*A2, and death. Data are plotted as the cumulative total number of temperature sensitive (*tsA*) or drug sensitive (paromomycin for *rdn*A2) clones or clonal death as a function of the number of cell divisions from zygote formation. Curves represent pooled data from several experiments (*cf.* Tables 1 and 2): *rdn*A2 (experiments 4–8 for 360 lines), *tsA* (experiments 2 and 3, for 144 lines) death (experiments 1–8 for 576 lines).

TABLE 3

Terminal rates of assortment

Category	Terminal rate[a]
Theoretical 30:15	0.0015
Theoretical 22:23	0.0016
Theoretical 15:30	0.0013
Chx	0.0016
Mpr	0.0017
gal	0.0012
tsA	0.0015
*rdn*A2	0.0017
Death	0.0014

[a] The terminal rate is calculated from the slope of the straight line fit by least squares approximation for values over 100 fissions in Figures 1, 2 and 3. The fractions represent the proportion of accumulated sensitive (or dead) clones per fission.

Assortment data are generally reported as R_F values, the proportion of cells assorting to stable genotype per generation; the apparent number of assorting units N may be calculated directly from this value, using the equation $R_F = 1/(2N\text{-}1)$ (SCHENSTED 1958). Table 4 shows such calculations for each gene examined. Since only the assortment of the sensitive allele may be determined directly, it is necessary to adjust the data as described in the legend to make this calculation. An input ratio of 1:1 is assumed, with one cell assorting to stable resistance for each cell assorting to stable sensitivity. Figure 3 shows the R_F values and apparent number of assorting units. The number of apparent assorting units ranges from *ca.* 20 (*tsA*, *gal*) to 60 (*Mpr*, *Chx*). If input ratios are estimated from plateau values, both *tsA* and *gal* would appear to reflect a 1:2 ratio. Estimates of N made using this ratio (data not shown) hover near 60 for these genes. A new method of calculation by F. P. DOERDER (personal communication) shows close similarity for R_F for all of the genes, with estimates of N in the range of 36–44 for *Mpr*, *Chx* and *gal*, of 60 for *tsA*, and of 56 for *rdn*A2 (data not shown).

All five genes described here have been mapped syntenically (BRUNS 1984): *Chx*A and *gal* lie on chromosome *1*, *Mpr* and *rdnA* on chromosome arm 2L, and *tsA* on chromosome *3*. Examination for patterns of assortment indicated no co-assortment for the pair *Chx* and *gal* or for *Mpr* and *rdnA*. This is consistent with the demonstrated fragmentation of chromosomes during macronuclear differentiation, and with previous studies of assortment of meiotically linked loci (ALLEN 1964; DOERDER 1973; MCCOY 1977).

DISCUSSION

The ciliate macronucleus is a novel eukaryotic nucleus. The five chromosomes in the germinal nucleus of *Tetrahymena thermophila* are fragmented during conjugation to form several hundred chromosomes in the somatic macronucleus. The macronucleus divides amitotically during vegetative growth, with no apparent mechanism to ensure equal partitioning of the genome to the two daughter nuclei. As a result, allelic makeup may vary within members of the same clone, resulting in phenotypic heterogeneity. For any allelic pair in a heterozygote, the end point of this process is a macronucleus containing only one of the two alleles. This may result in a change in phenotype, a phenomenon termed phenotypic assortment. The kinetics of appearance of subclones expressing altered phenotypes are affected by the initial proportion of each allele at polyploidization (the input ratio) and by the

TABLE 4

Stabilization rates and estimated number of assorting units for the genes *Chx*, *Mpr gal*, *tsA*, *rdn*A2 and death

Alleles	Transfer generation	Fraction stabilizing[a]	Fissions/transfer	Mean R_F sensitive allele	Assorting units[b]
Mpr$^+$/*Mpr*	1–3	48/360	10	.0037 ± .0026	68
(1:1)	4–5	53/264	15	.0081 ± .0024	31
	6–8	28/158	10	.0068 ± .0017	37
	9–10	10/102	10	.0052 ± .0011	48
	11–13	22/82	20	.0055 ± .0032	46
Chx$^+$/*Chx*	1–3	23/144	10	.0060 ± .0024	42
(1:1)	4–6	20/98	13	.0069 ± .0038	37
	7–8	9/58	15	.0064 ± .0031	39
	9–11	14/49	20	.0104 ± .0096	24
tsA/*tsA*$^+$	1–3	12/144	10	.0030 ± .0016	83
(1:2)	4–6	8/102	10	.0027 ± .0007	92
	7–8	6/75	15	.0029 ± .0016	86
	9	6/48	30	.0030	83
gal$^+$/*gal*	1–3	8/72	10	.0041 ± .003	61
(1:2)	4–6	4/48	13	.0023 ± .0002	109
	7–8	3/36	10	.0045 ± .0023	56
	9–11	2/27	25	.0016 ± .0003	156
*rdn*A2/*rdn*$^+$	1–3	28/360	10	.0015 ± .0005	167
(1:2)	4–6	11/282	10	.0013 ± .0006	193
	7–9	23/249	10	.0034 ± .0013	74
	10–12	23/180	13	.0041 ± .0017	61

[a] Since only the appearance of the sensitive assorters is followed, it is necessary to make some assumptions to make these data comparable with previously published results. Examination of the plateau values approached in Figure 2, a and b, may be compared with the theoretical curves in Figure 1 to estimate an allele ratio. This may be used to calculate the number of unstable clones remaining at any time. Thus, for a 1:1 allele ratio, as suggested for *Chx* and *Mpr*, an interval which produces 4 clones assorting to sensitivity is calculated to have reduced the number of unstable clones by 8, the other 4 assorting to stable resistance; for a 1:2 ratio, as suggested for *gal*, *tsA* and *rdn*A2, 4 clones assorting to sensitivity will reduce the number of unstable clones by 12, 8 having assorted to stable resistance in the same interval. These rates are pooled over approximately 30 generations to give an R_F value for the assortment of the sensitive allele.

[b] The equation $R_f = 1/2N\text{-}1$ (SCHENSTED 1958) is used to calculate the effective number of assorting units N. The R_F calculated for the sensitive allele is doubled to account for concomitant assortment of the resistant allele.

total number of copies of that gene. The final plateau value for an allele reflects the input ratio; the terminal slope is affected by the number of units assorting. Figure 1 shows that if there is equal input, the final proportion of cells expressing a recessive phenotype should approach 50%, and such cells should appear in significant numbers (*ca.* 10%) after only 50 generations. This is of practical utility in generating heterocaryons for genetic studies (BRUNS and BRUSSARD 1974).

It is generally assumed that the two alleles are amplified equally, yielding an approximately 1:1 input in the newly formed macronucleus, and that assortment is the result of the random physical loss of all copies of one of the alleles. Although no allelic restriction endonuclease fragment length polymorphisms

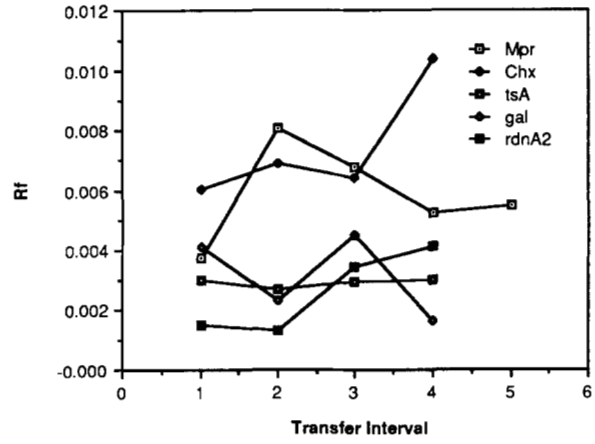

FIGURE 3.—Stabilization rates R_F for typical markers *Chx*, *Mpr*, *gal*, atypical markers *tsA* and *rdn*A2, and death. Adjusted R_F values are averaged over approximately 30 fission intervals, including 2 or 3 transfers. The R_F values for the sensitive allele is doubled to include the concomitant assortment of the resistant allele.

have been reported, HOWARD and BLACKBURN (1985) did follow the vegetative fate of a processing polymorphism, presumably created during macronuclear differentiation. One clone showed equal amounts of the two forms after 25 generations, but a significant difference after 100 generations. Thus, the two forms were created and amplified equally during macronuclear development, but one was lost during vegetative growth. WHITE and ALLEN (1986) also report alternate processing forms and assortment of molecular species. ALLEN *et al.* (1985) noted that polymorphisms of the 5s genes continue both to be generated during vegetative growth, and to assort during subsequent vegetative growth. On the other hand, there are examples of predictable bias during amplification. These include loci for surface antigens (DOERDER 1973) and an allele for the rDNA (PAN *et al.* 1982).

The relative amounts of the two alleles, the input ratio, may be estimated from the final proportion of sensitive cells when studying heterozygotes for antibiotic resistance markers. For *Chx* and *Mpr* the final value approaches 50%, as expected for equal input for the two alleles; for *gal*, the final value is more suggestive of a 1:2 input ratio. The terminal slope in each case is essentially the same, and consistent with the computer simulation for 45 assorting units.

We expected that two of the alleles here would be anomalous: *tsA* and *rdn*A2. MCCOY (1973) reported that the allele ts-1 of *tsA* failed to assort. We find essentially normal assortment, though it begins late and goes to a lower final level. The lower assortment rate may be an artifact resulting from selection against the recessive temperature sensitive phenotype even at 30°.

The paromomycin resistance allele, being an allele of the structural gene for the ribosomal RNA, has a unique macronuclear organization. It is present in 10,000 copies as a palindrome [see GOROVSKY (1980)

for review]. The heterozygote would be expected to have 5–6000 copies of each allele present initially. Assortment with this number of units should be very slow, and there would probably be no detectable clones assorted to sensitivity after 180 generations (F. P. DOERDER, personal communication). Surprisingly, the assortment behavior cannot be distinguished from that of other genes: plateau values correspond to an input ratio of 1:2, and the terminal rate is similar to that for 45 assorting units. Thus, this case seems to violate the dependence of assortment kinetics on the number of molecules involved. We suggest that a higher level of organization may be involved, and that the nucleolus is the assorting unit rather than the individual rDNA molecule. This model would require that each nucleolus be derived from a single type of rDNA molecule. NIELSON and ENGBERG (1985) have demonstrated physical assortment of rDNA molecules in a *T. pigmentosa* strain heterozygous for an intron in the rDNA, intron$^+$/intron$^-$. These authors also suggest that nucleoli are the assorting units and that a nucleolus contains a single type of rDNA molecule.

In spite of an amitotic division in the macronucleus, large numbers of dead cells do not appear as a regular consequence of division and vegetative growth. This suggests that some form of control must operate to maintain complete sets of the genome, particularly in view of the variability of macronuclear size and the existence of chromatin extrusion bodies reported by DOERDER (1979). Since the balance of macronuclear chromosomes seems to remain fairly fixed (CONOVER and BRUNK 1986), a mechanism to control copy numbers of each macronuclear chromosomes has been suggested. The remarkable viability of multiple monosonics (BRUNS, BRUSSARD and MERRIAM 1983) supports such a model.

Several previous studies shed light on the possibility of phenotypic assortment as the cause of vegetative death. PREER and PREER (1979) have provided a calculation which predicts loss of all copies of one of the five chromosomes at 1.7 per 100 generations, if there is random elimination during division. Larger numbers of assorting units [the several hundred macronuclear chromosomes reported by ALTSCHULER and YAO (1985) and CONOVER and BRUNK (1986)] would lead to more rapid death in such a model (PREER and PREER 1979). Actual values ranging from 0.6 to 2.5 per 100 generations have been reported in different lines (NANNEY 1959). ORIAS and NEWBY (1975) reported death or severe growth retardation of subclones after 180 fissions, but show no kinetics for the phenomenon. Since all clones here were carried throughout the experiments, those which "died," *i.e.*, lost the ability to divide, could be scored and the rate of appearance calculated. Figure 2b includes a curve showing this assortment to death. Surprisingly, this curve is not strikingly different from any of the standard assortment curves. This rate is much slower than would be predicted if random loss of any of the several hundred macronuclear chromosomes led to death. Alternatively, death could result from assortment for a lethal gene in the genomes; this would necessitate invoking a single recessive lethal heterozygotes in most lines, since all lines followed here behaved similarly. The low value argues for strong control on the composition of the macronucleus, and may suggest a counting mechanism for "functional units."

Assortment may be used to estimate the number of copies of a particular gene in the macronucleus, and to provide some estimate of the numbers of particular macronuclear chromosomes. It is clear that a wide range of values for N may be derived using various "appropriate" adjustments when only one allele of a pair is analyzed. Nonetheless, in any particular manipulation here, the range of values calculated is only approximately twofold. While the number of genes analyzed in this way is admittedly still small, these data do not support the suggestion (BRUNK 1986) that there might be a wide range of copy numbers among the many macronuclear chromosomes, but rather that there is control at least to a factor of two for individual macronuclear chromosomes.

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