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Gene expression and phenotypic change in *Paramecium tetraurelia* exconjugants

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SUMMARY

A study of the patterns of phenotypic change in exconjugants using the recessive behavioural mutant pawn (*pwA*) and its wild-type allele shows that both cytoplasmic and nuclear factors contribute to phenomic lag. Following loss of the wild-type allele from the macronucleus, phenomic lag lasts for 6–11 cell cycles in various sublines of a single clone. Inherited cytoplasmic material is estimated to be responsible for phenomic lag of no more than 5–6 cell cycles. Longer persistence of the parental phenotype is due to continued gene activity in macronuclear fragments carrying the wild-type allele. Genes in fragments remain active and can result in maintenance of the parental phenotype as long as fragments are present (up to 11 cell cycles).

Phenomic lag in the other direction, from pawn to wild type, varies from 0 to 2 cell cycles. The major cytoplasmic factor involved is the amount of wild-type material acquired from the mate during conjugation. Extensive cytoplasmic exchange often occurs during normal conjugation and can lead to change of phenotype as early as the first meiotic division. Phenotypic change due to gene expression in macronuclear anlagen brings about phenotypic change near the end of the first cell cycle in +/+ cells and about a cell cycle later in heterozygotes.

1. INTRODUCTION

This paper examines the major cytoplasmic and nuclear factors determining the pattern of phenotypic change in *Paramecium tetraurelia* (Sonneborn, 1975, formerly *P. aurelia*, syngen 4) following entry or loss of a dominant gene at conjugation or autogamy. Both filial and parental genomes are present after reorganization. The filial genome is present in the two macronuclear anlagen derived from the synkaryon. These new macronuclei segregate at the first cell division and each reaches the normal adult macronuclear size and DNA content ($\sim$ 860 c) by the start of the third cell cycle after conjugation (Berger, 1973). The parental genome is contained in approximately 35 non-dividing macronuclear fragments (mean DNA content = $24{\cdot}4 \pm 2{\cdot}2$ c) which are passively segregated to the daughter cells

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at subsequent fissions. Fragments persist through at least the sixth cell cycle in well-fed clones, but are eventually autolysed, the parental genome and its phenotypic manifestations are lost and the filial genome gains functional control.

The time elapsed between the origin of the filial genome at fertilization and the establishment of the corresponding phenotype has been referred to as cytoplasmic (DeGaris, 1935; Sonneborn & Lynch, 1934), or phenomic lag (Sonneborn, 1953), and is similar to a maternal effect in higher organisms (Kimball, 1939). The length of this period depends on a number of genetic and physiological factors. This study examines phenomic lag under various conditions in individual cell lines, and assesses the contributions of gene activity in macronuclear anlagen and fragments and the effect of cytoplasmic gene products on the pattern of change from parental to filial phenotype.

A single recessive mutant gene, *pawnA* (Kung, 1971; Chang *et al.* 1974), and its wild-type allele were studied. This mutation blocks active membrane depolarization and, consequently, prevents ciliary reversal (Kung & Eckert, 1972). Its use permits rapid, repetitive, non-destructive testing of phenotypes of single cells by transfer from normal culture fluid to physiological salt solution. The timing of phenotypic change can be precisely monitored; only a fraction of a second elapses between the setting of the test and the reading of the result.

2. MATERIALS AND METHODS

(i) *Stocks and culture of* Paramecium

Paramecium tetraurelia (Sonneborn, 1975) stock 51 (wild type), and two derived stocks, d4-43 and d4-94, carrying mutant genes, were grown in either baked lettuce or Cerophyl medium (Sonneborn, 1970) at 27 °C. The food organism was *Klebsiella aerogenes*. Stock d4-43 carries two recessive mutant genes: *am* (Sonneborn, 1954), which causes mis-segregation of macronuclei or macronuclear anlagen in a variable fraction of cells, and *nd6* (Sonneborn, 1974), which prevents discharge of trichocysts. Stock d4-94, the behavioural pawn mutant, carries the recessive mutant gene *pwA* (Kung, 1971; Chang *et al.* 1974), which prevents backward swimming.

(ii) *Scoring of behavioural phenotypes*

The behavioural phenotype was considered wild type if its forward movement was stopped or the direction of swimming was reversed when a cell was transferred by micropipette from culture medium to Dryl's (1959) physiological salt solution. A cell was scored as pawn if it continued to swim forward. In some experiments a weak pawn, or intermediate, phenotype was distinguished when cells stopped forward swimming, but did not reverse direction.

In matings between pawn and a wild-type cell, each cell behaved as an independent unit, i.e. the pawn cell did not show periods of stopping or reverse swimming while the wild-type cell did; consequently, the pair of cells either swam in tight circles with the pawn cell on the outside or the pair spun laterally. Wholly wild-type or wholly pawn pairs showed the characteristics of single cells of their

phenotype. Pairs gave consistent responses on repeated tests if they were equilibrated between tests for a minute or so in culture fluid.

Trichocyst discharge was tested by addition of picric acid (Pollock, 1974).

(iii) *Heterokaryons*

Heterokaryons containing *pwA/pwA*; *am/am* macronuclei and *pwA/+*; *am/+* micronuclei were obtained through macronuclear regeneration (Sonneborn, 1940, 1947) following mating of *pwa/pwA*; *am/am* and wild-type cells. Macronuclear regeneration occurred in the descendants of the *am/am* parent following mis-segregation of macronuclear anlagen induced by action of the *am* gene (Sonneborn, 1954). Occurrence of macronuclear regeneration was confirmed cytologically and by observation of behavioural phenotype.

(iv) *Determination of the time course of phenotypic change*

(*a*) *In a sample of many clones.* Exconjugants or exautogamonts (100–200) isolated in fresh medium in depression slide well cultures were tested for behavioural phenotype and allowed to grow. After each fission, one daughter cell was discarded, the phenotype of the other was tested and the cell was allowed to perpetuate the line. The phenotype of each cell line was tested at least once during each cell cycle. Each cell line was from a separate clone.

(*b*) *Within a single clone.* The behavioural phenotype of each cell in a *pwA/pwA* clone of *pwA/+* parentage was tested during each cell cycle until phenotypic change to pawn was complete. After each fission daughter cells were placed in separate wells, numbered to indicate lineage.

(v) *Preparation of labelled cells*

Cells containing tritium-labelled cytoplasm were prepared by growing log-phase cells 6 h in 10 μCi/ml tritiated leucine and non-labelled bacteria followed by extensive washing (more than 10^{-6} dilution of activity) then growth for a further 6 h period in non-labelled medium to allow dissipation of soluble labelled material.

(vi) *Preparation of autoradiographs and grain counting*

Whole cell autoradiographs were prepared (Berger, 1971), and grain counts were made over a 200 μm^2 area of cytoplasm.

3. RESULTS

(i) *Phenotypic transition from wild-type to pawn*

Transition from wild-type to pawn phenotype was studied at autogamy in heterozygotes (*pwA/+*), and when such heterozygotes were backcrossed to *pwA/pwA* homozygotes; in each case half of the progeny of wild-type parentage were expected to be *pwA/pwA* homozygotes which would eventually assume the pawn phenotype.

(*a*) *Phenotypic change in well-fed clones.* The median phenomic lag (Methods, (iv) (*a*)) was about 7·6 cell cycles in exconjugants and about 7·0 cell cycles in

exautogamonts (Fig. 1*a*). Much of the variation in the time of phenotypic change in these experiments is variation within clones as shown by analysis of phenotypic change in a single clone (Methods (iv) (*b*)). Phenotypic change occurred as early as the sixth cell cycle in some cell lines, but not until the 12th in others (Fig. 1*a*). The median phenomic lag for this clone was 8·6 cell cycles. In some subclones phenotypic change was consistently earlier than in others, and in some 'trails' of wild-type phenotype persisted for several cell cycles after most cells had become pawn (Fig. 2).

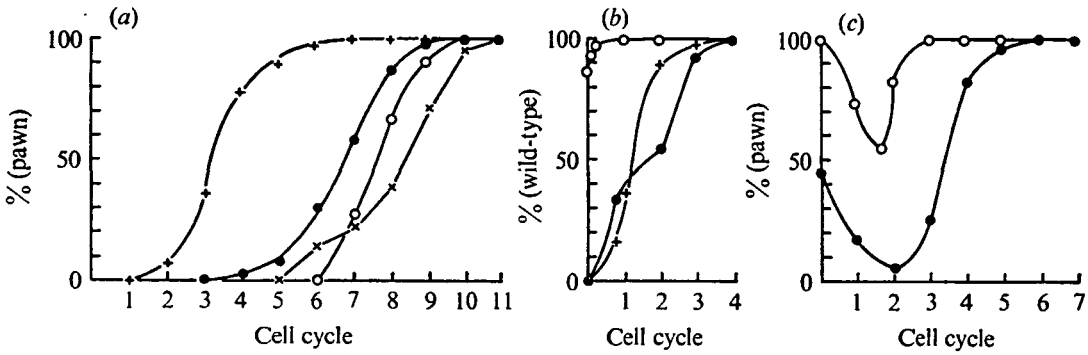

Fig. 1. Phenotype change in *Paramecium*.

(*a*) Phenotypic change from wild type to pawn. *y*-axis: percentage of all lines of *pwA/pwA* genotype expressing pawn phenotype during various cell cycles following conjugation or autogamy. *X*-axis: number of cell cycles following conjugation or autogamy. ○, Exconjugants, well-fed cells; sample size = 52 lines. ●, Exautogamonts, well-fed cells; sample size = 59 lines. +, Exautogamonts, cells starved for 4 days after autogamy before being transferred to excess food; sample size = 92 lines. ×, exautogamonts, well fed, all lines from a single clone.

(*b*) Phenotypic change from pawn to wild type. *y*-axis: percentage of all lines derived from *pwA/pwA* parents expressing the wild-type phenotype during various cell cycles following entrance of the wild-type allele at conjugation or autogamy. *X*-axis: number of cell cycles after conjugation or autogamy. ○, Pattern of phenotypic change in *pwA/+* cells following conjugation with substantial cytoplasmic exchange; sample size = 36 lines. ●, Pattern of phenotypic change in *pwA/+* cells following conjugation with little cytoplasmic exchange; sample size = 52 lines. +, Pattern of phenotypic change in +/+ cells following introduction of the wild-type gene at autogamy; sample size = 56 lines.

(*c*) Phenotypic change in *pwA/pwA* cells derived from pawn parents which had mated with *pwA/+* cells without change in genotype. *y*-axis: percentage of all lines of *pwA/pwA* genotype which were expressing pawn phenotype. *x*-axis: number of cell cycles after conjugation. ○, Experiment no. 1; sample size = 51 lines. ●, Experiment no. 2; sample size = 22 lines.

(*b*) *Effect of starvation.* The median phenomic lag was 3·3 cell cycles in *pwA/+* heterozygotes allowed to undergo autogamy, then starved 4 days in exhausted medium before transfer to fresh medium in depression well cultures (Fig. 1*a*).

(ii) *Association of macronuclear fragments with parental phenotype*

Previous observations (Berger, 1973) suggest that genes in macronuclear fragments remain active in RNA synthesis throughout their life in the cell and may

contribute to persistence of the wild-type phenotype following loss of the dominant allele from the macronucleus. A significant association would be expected between possession of a fragment carrying the wild-type gene, and manifestation of the wild-type phenotype.

Cells with *pwA/pwA* macronuclei and *pwA/+* fragments were obtained by allowing heterozygotes to undergo autogamy. The phenotypes of cells from an exautogamous clone were repeatedly tested and when almost all cells had become pawn (8th to 9th cell cycle after autogamy, as judged by the total number of cells) the phenotype of each cell was tested and the cell was stained with azure A (Berger,

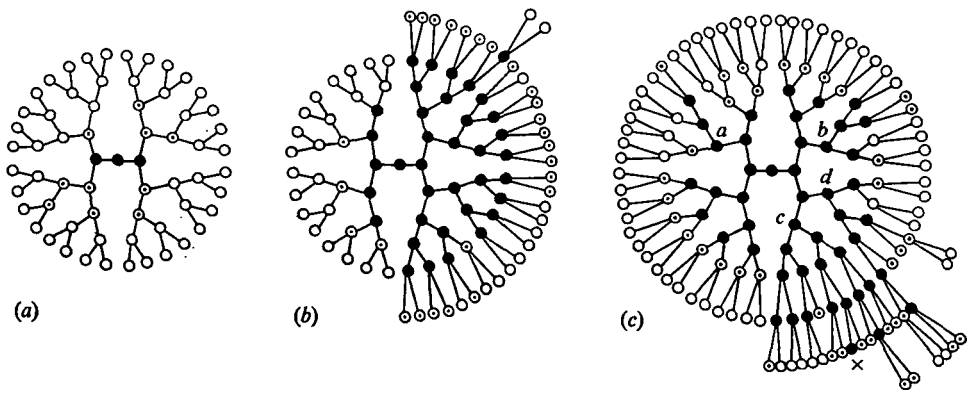

Fig. 2. Phenotypic change from wild type to pawn in selected segments of a single *pwA/pwA* clone of *pwA/+* parentage. Each diagram shows phenotypes of descendants from a single cell of the 4th cell cycle produced at the third fission after autogamy. The phenotype of the progenitor cell is represented by the circle at the centre of each diagram, and the phenotypes of its progeny in successive cell cycles are indicated as follows: ●, wild-type; ○, pawn; ⊙, weak pawn. Cells at the periphery of each diagram were fixed and stained. (*a*) Subclone 5, an example of early phenotypic change, wild-type phenotype was lost by the sixth cell cycle. (*b*) Subclone 6, derived from the sister cell of the progenitor of subclone 5, showing marked differences in the persistence of wild-type phenotype in two halves of the subclone. (*c*) Subclone 7, showing a number of 'trails' of wild-type phenotype in cell lines starting at positions marked by *a*, *b*, *c*, and *d*. The cell indicated by × contained a macronuclear fragment.

Table 1. *Test of association of fragments carrying the wild-type allele with possession of the wild-type phenotype in a well-fed exautogamous* pwA/pwA *clone, nine to ten cell cycles after autogamy*

	Cell phenotype				
	Pawn		Wild-type		
	Observed	Expected	Observed	Expected	Total
Cells without fragments	349	(341·51)	39	(46·48)	388
Cells with a fragment	11	(18·48)	10	(2·52)	21
Total	360		49		409

Degrees of freedom = 1. H_0 = homogeneity.
G = 17·62 by G test procedure for 2 × 2 tables, Sokal & Rohlf (1969).
$P_\alpha < 0{\cdot}0005$.

1973) for detection of macronuclear fragments. There was a strong, but not complete, association between presence of the wild-type phenotype and possession of a macronuclear fragment carrying the wild-type allele (Table 1).

Some cells may have multiplied more slowly than others, and may thus have retained both fragments and the wild-type phenotype for a longer period of time than cells which multiplied rapidly. However, a strong negative correlation between possession of fragments and presence of the pawn phenotype was observed when phenotypic change was followed in a single clone (section (i) (*a*)). Cells were fixed during the cell cycle following that in which the pawn phenotype first occurred, and were searched for macronuclear fragments. In more than 500 pawn cells, only two macronuclear fragments were found, and these were in advanced stages of autolysis, while a single medium-sized fragment was found in the last remaining wild-type cell (11th cell cycle). This cell was from a 'trail' of wild-type phenotype (Fig. 2*c*).

(iii) *Phenotypic transition from pawn to wild type*

Heterozygous exconjugants of pawn parentage were isolated into fresh medium and the pattern of phenotypic change was determined. Typically, cells showed wild-type phenotype within 2 h of the end of conjugation, and many showed the wild-type phenotype at the end of conjugation (Fig. 1*b*). This was universally so when there was delayed separation of conjugants. In some experiments phenotypic change occurred before fertilization. In one experiment 30/53 (57 %) of the cells of pawn parentage showed wild-type phenotype by metaphase of the first meiotic division (determined by staining mating pairs when wild-type phenotype appeared), and more than 95 % had become wild-type by the end of conjugation, about 30 min after fertilization. Typically more than half of all cells of pawn parentage showed wild-type phenotype by the end of conjugation (three experiments with 50–100 pairs each).

Thus gene action in macronuclear anlagen could not be responsible for the early phenotypic transition. The effect may be the result of cytoplasmic exchange during mating.

(iv) *Cytoplasmic exchange during conjugation*

^{3}H-leucine-labelled +/+ cells were mated to non-labelled *pwA*/*pwA* cells. Samples of mating cells and newly separated exconjugants were selected at various times during and after conjugation as shown in Fig. 3, with non-mating cells from the mating culture serving as controls. The cells were dried on slides, fixed and autoradiographs were prepared. There was significant transfer of label from the highly labelled wild-type cell to the non-labelled pawn mate before the end of the first meiotic prophase (3 h after mixing cells of complementary mating types) in these relatively young clones (less than 20 cell cycles old). The significant regression of the activity of the recipient cell on that of the donor shows that label in the recipient came from the donor and not from a non-specific source such as the medium – a conclusion supported by the lack of label in non-labelled control cells.

As mating proceeded, there were increases in both the amount and concentration of label in the recipient (less-labelled) cells, and in the slope of the regression. Concentration of label in the recipient cells was inversely related to concentration of label in the donors.

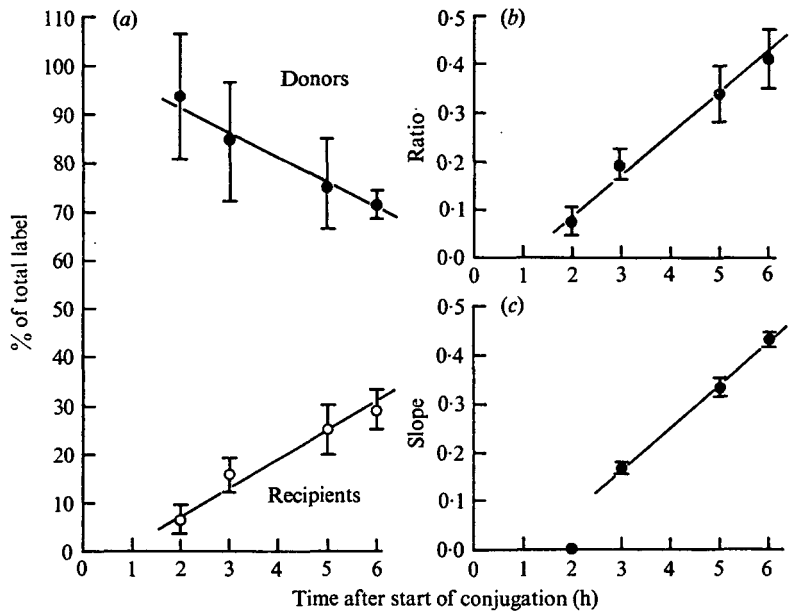

Fig. 3. Exchange of labelled material during conjugation. x-axis for all figures: time (h) after start of conjugation (mixing of complementary mating types). (*a*) y-axis: percentage of the total label per pair in donors (●, heavily labelled cells) and recipients (○, lightly labelled cells). Activity per pair was estimated by the sum of the concentration of label in the two cells. (*b*) y-axis: ratio of recipient activity (grain count) to donor activity. Vertical bars are 95% confidence intervals of the mean. Errors of ratios determined by procedure of Beers (1953). (*c*) y-axis: slope of regression of recipient grain counts on donor grain counts within each sample. Correlation coefficients ranged between 0·62 and 0·66 for the points with significant slope.

Most pairs of exconjugants did not show delayed separation. Cytoplasmic bridges were noted in only 5 of 77 pairs. In bridged pairs, the concentration of label in the two cells was nearly equal. The frequency of highly labelled recipient cells rose during conjugation. By the end of conjugation (6 h group) 73% (56/77) of recipient cells showed at least 30% of the label concentration of the donors. Most (62/77) cells in this group showed wild-type phenotype.

(v) *Analysis of early phenotypic change*

(*a*) *Effect of cytoplasmic exchange.* The time-course of phenotypic change was examined when *pwA*/+ heterozygotes were mated to *pwA*/*pwA*; *nd6*/*nd6* homozygotes. Half of the cells derived from the pawn parent (identifiable after conjugation by the dischargeless trichocysts produced by the *nd6* gene in the parent) would

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experience no genotypic change because they received a *pwA* bearing pronucleus from their mate.

At the conclusion of the experiment the trichocyst phenotype of each pawn line was again determined and all lines with dischargeless trichocysts were discarded because they arose either as non-conjugants or through macronuclear regeneration. Fig. 1 (*c*) shows the fraction of the lines of pawn parentage and *pwA/pwA* genotype which were expressing the pawn phenotype during various cell cycles. Exconjugants from control matings of pawn homozygotes showed no change of phenotype. In one experiment the amount of cytoplasmic exchange was presumably quite small (based on correlation with phenotypic change in labelling experiments, section (iv)). Yet there was a significant shift of phenotype from pawn toward wild type, at about the first fission after conjugation. The transient wild-type phenotype shown by about half of the lines subsequently disappeared. In a second experiment, with a younger clone (5–9 cell cycles old *v.* more than 20 in the first experiment), the median duration of wild-type phenotype was 3·4 cell cycles. However, by the sixth cell cycle, all cells had reverted to pawn.

These data show that cytoplasmic exchange and/or contact during mating is sufficient to cause a change of phenotype which lasted for up to five cell cycles, and that maximum expression of the wild-type phenotype did not occur until at least the latter part of the first cell cycle after conjugation.

(*b*) *Effect of gene activity in macronuclear anlagen.* The time course of phenotypic change was followed when the wild-type allele was brought into expression by allowing heterokaryons containing a *pwA/pwA* macronucleus and *+/pwA* micronuclei to undergo autogamy. This resulted in each cell containing both micronuclei and anlagen of either *+/+* or *pwA/pwA* genotype and macronuclear fragments of the *pwA/pwA* genotype. Transition from pawn to wild-type phenotype apparently occurred late in the first cell cycle or during the first part of the second cell cycle in most lines and was virtually complete by the end of the second cell cycle (Fig. 1*b*).

The time course of phenotypic change from pawn to wild type was also followed in heterozygous exconjugants derived from backcrossing *pwA/+* cells to *pwA/pwA* homozygotes. In experiments in which there was little early phenotypic change (attributed to cytoplasmic exchange) transition from pawn to wild-type phenotype occurred during the latter part of the second cell cycle. However, in some lines the pattern of phenotypic change was more complex. Near the end of the first cell cycle some cells became wild type, maintained the wild-type phenotype for several hours and then reverted to pawn phenotype, only to become permanently wild type toward the end of the second cell cycle. The transient period of wild-type phenotype was attributed to the effect of small amounts of cytoplasmic exchange during mating, and the later permanent change to gene action.

4. DISCUSSION

Factors contributing to the duration of phenomic lag in *Paramecium* include: direction of phenotypic change, patterns of gene activity in macronuclear anlagen and fragments (containing the filial and parental genomes respectively), their relative gene dosages, and the amount of cytoplasmic material containing wild-type RNA and protein gene products, whether inherited from the parent cell, or acquired from a wild-type mate by cytoplasmic exchange.

In the *transition from dominant to recessive phenotype* phenomic lag is long (6–11 cell cycles) and has both cytoplasmic and nuclear components. The major cytoplasmic component of phenomic lag following loss of a dominant gene from the macronucleus is persistence of RNA and protein products of the dominant parental gene in the cytoplasm. The maximum contribution of parental wild-type cytoplasmic products can be estimated from the cytoplasmic exchange experiments (section (v)). Cells which retained the homozygous recessive genotype but which received wild-type cytoplasm assumed the wild-type phenotype for up to five cell cycles after mating. If maximum persistence of the wild-type phenotype was the consequence of maximal cytoplasmic exchange as suggested by the labelling experiments (section (iv)), then the concentration of wild-type gene products would be approximately half that in a *pwA*/+ cell. Thus, if the gene products are stable, as they seem to be, the maximum expected phenomic lag due to parental cytoplasm would be five to six cell cycles, or about one cell cycle longer than that observed in the cytoplasmic exchange experiments.

Persistent gene activity in macronuclear fragments carrying the wild-type allele is a major factor in the long persistence of wild-type phenotype following loss of the corresponding gene from the macronucleus, and in the substantial variation in persistence of wild-type phenotype among sublines within the same clone. The persistent association of fragments with wild-type phenotype (section (ii)) suggests that genes in macronuclear fragments can remain active as long as the fragments persist (up to 11 cell cycles in well-fed cells), and that possession of a single fragment carrying the wild-type gene can be sufficient to maintain the wild-type phenotype against a homozygous mutant macronucleus.

These conclusions are consistent with observations that the rate of RNA synthesis per unit volume of macronuclear fragments and macronucleus are the same, and that the rate of RNA synthesis per unit volume of macronuclear fragment does not significantly decrease as fragments age (Berger, 1973), supporting the original suggestion of Sonneborn & Lynch (1934) that continued genic activity in macronuclear fragments is one of the bases of phenomic lag.

Initially sufficient inherited gene products or gene activity in the ensemble of macronuclear fragments is present to ensure the production of the wild-type phenotype. As a clone expands, the number of macronuclear fragments per cell is reduced, as is the concentration of inherited gene products. By the fourth or fifth cell cycle some cells contain no macronuclear fragments, the concentration of the wild-type gene products subsequently (1–2 cell cycles later) drops below a critical

level and the mutant phenotype appears. In cells with one or more macronuclear fragments, the concentration of wild-type gene products remains higher and the wild-type phenotype persists. 'Trails' of wild-type phenotype observed in expanding clones presumably correspond to the segregation of genically active macronuclear fragments in a manner formally analogous to abortive transduction in bacteria (Stocker, 1956).

Starvation drastically reduces phenomic lag of recessive traits (Sonneborn, 1953). This effect is, however, difficult to interpret, for starvation leads to marked reduction of the number of macronuclear fragments and autolysis of cytoplasmic material, but also to concomitant growth of macronuclear anlagen (Berger, 1974). Thus the gene dosage of macronuclear anlagen containing the filial genome is increased, while that of the paternal genome (in macronuclear fragments) is reduced. All of these factors presumably shorten phenomic lag.

In the *transition from recessive to dominant phenotype* phenomic lag is short (0–2 cell cycles) as would be expected from the dominance relationship. The major determinants of phenomic lag in this direction are: the amount of cytoplasmic material exchanged with the wild-type mate at conjugation, and the pattern of gene activity in macronuclear anlagen. The former could be important for early phenotypic changes (in some cases during the first meiotic prophase) because the dominant gene products could be acquired before the filial genome was present.

It has long been known that cytoplasmic exchange occurs after fertilization in *P. tetraurelia* conjugants which show delayed pair separation and the presence of a cytoplasmic bridge (Sonneborn, 1944), but the occurrence of extensive cytoplasmic exchange before fertilization during normal conjugation has not been previously documented, although Cullis (1972) has reported exchange of labelled material between mates in *P. bursaria* and McDonald (1964) has reported transfer in *Tetrahymena*.

A physical basis for early exchange of cytoplasmic material is also known. During conjugation, union first develops at the anterior end (anterior hold-fast union, Metz, 1954). In this area small ($< 1\ \mu m$) cytoplasmic connexions between the two cells have been observed (Schneider, 1963; Jurand & Selman, 1969). Following exchange of pronuclei, there is a larger opening between the cells which persists for some time (Schneider, 1963).

Early phenotypic change is attributed to the result of transfer of cytoplasm containing the wild-type gene products to the recessive recipient cell, although transfer of normal membrane sites through membrane fluidity cannot be ruled out. The physiological basis of the pawn trait suggests that cell contact or transfer of ions between cells is unimportant. The plasma membrane of pawns does not undergo active depolarization following mechanical, ionic or electrical stimulation (Kung & Eckert, 1972).

The rapidity with which the phenotype can change from pawn to wild type suggests that the normal product of the pawn locus might possibly be integrated into the membrane without extensive membrane growth, or that it might modify defective membrane sites. The fact that maximum function of wild-type material

acquired during conjugation does not occur until at least the latter part of the first cell cycle or later, suggests that membrane growth may be important in emplacement of the wild-type product. Earlier phenotypic expression presumably results from acquisition of a dose of normal gene product sufficient to change phenotype even if only a smaller fraction is functional.

When cytoplasmic exchange is absent, phenotypic change from pawn to wild type occurs near the end of the first cell cycle in +/+ cells and somewhat later in heterozygotes (Fig. 1*b*). Even if genes in anlagen were active from the start, some phenomic lag would be expected as sufficient wild-type gene product would have to accumulate and displace mutant gene product. Accumulation of wild-type gene product is presumably not rapid, for even though macronuclear anlagen incorporate [^{3}H]uridine at least as early as 2 h after the end of conjugation, they contribute less than 7% of all RNA synthesized during the first cell cycle (Berger, 1973). Displacement of the mutant gene product also requires some time. In the light of these considerations the pattern of phenotypic change observed seems to be consistent with early gene activity in macronuclear anlagen.

Thus the typical pattern of early phenotypic change observed in younger *P. tetraurelia* clones following entry of a dominant gene is the result of two components: a rapidly acting component, attributable to wild-type gene products transferred to the recessive recipient cell from its mate during conjugation, and a later permanent component, attributable to gene activity in macronuclear anlagen.

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