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Alternative Interpretation of the Molecular Structure and Somatic Genetics of Acid Phosphatase-1 in *Tetrahymena pyriformis*

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Acid phosphatase-1 isozymes with different electrophoretic mobilities were discovered in syngen 1 of *Tetrahymena pyriformis* (Allen *et al.*, 1963*a*, *b*). The careful and intensive studies of Allen and collaborators (Allen, 1967, 1971; Allen *et al.*, 1963*a b*,) have established the following main picture concerning the isozymes and their genetic determination: (1) The electrophoretic mobility differences are due to the presence of two different alleles of a single gene, assumed to be the structural gene for the acid phosphatase monomer. The homozygote for each allele has a single main molecular species of acid phosphatase-1 (cell types P_1 and P_5, respectively). (2) A "young" heterozygote (less than 40 binary fissions old) produces three molecular species, called isozymes 1, 3, and 5, respectively (cell type $P_{1,3,5}$). Isozymes 1 and 5 correspond to the molecular species present in the respective homozygotes. Isozyme 3, which has an intermediate electrophoretic mobility, is characteristic of the heterozygote. (3) Beginning at about 50 fissions after sexual reorganization, stable subclones begin to segregate out from the heterozygotes; these express only isozyme 1 or 5, respectively. (4) If an unsegregated heterozygote is grown for over 100 fissions, new molecular species (isozymes 2 and 4) appear, interspersed in electrophoretic mobility between isozymes 1, 3, and 5. This cell possessing all five isozymes is designated $P_{1,2,3,4,5}$. (5) From this novel cell type, *three* stable classes of segregants can be obtained by clonal selection, expressing exclusively either isozyme 1, or isozyme 3, or isozyme 5, respectively.

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The appearance of these various cell types is ascribed to somatic (macronuclear) genetic events that are not yet understood and that ultimately result in the expression of only one of the two alleles present in the germline of a heterozygote. This is a phenomenon of general occurrence in syngen 1 of *T. pyriformis* and is known as *allelic exclusion* (Nanney, 1964).

Allen (1967, 1968, 1971) has interpreted these findings by proposing the following model: (1) The existence of a maximum of five isozymes in heterozygotes is explained by assuming that the acid phosphatase is a tetramer. The polypeptide monomers specified by the two different alleles are called A and B, respectively (see Fig. 1). Five different tetramers are possible, depending on the proportions of A and B monomers: A_4, A_3B, A_2B_2, AB_3, and B_4 (see Table I). (2) The presence of only three of the five possible types of tetramers in a young heterozygote is explained by making two *ad hoc* assumptions: (a) dimers are the obligatory intermediates in the assembly of a tetramer; (b) only dimers composed of like subunits are synthesized in the young heterozygote. (3) The appearance of the $P_{1,2,3,4,5}$ cell type is explained by the further *ad hoc* assumption that the restriction postulated in (2b) above is relaxed after a number of fissions, and all three dimers are synthesized.

The main objection which can be raised to Allen's tetramer model is that it provides no explanation for the stable type P_3 produced late in the

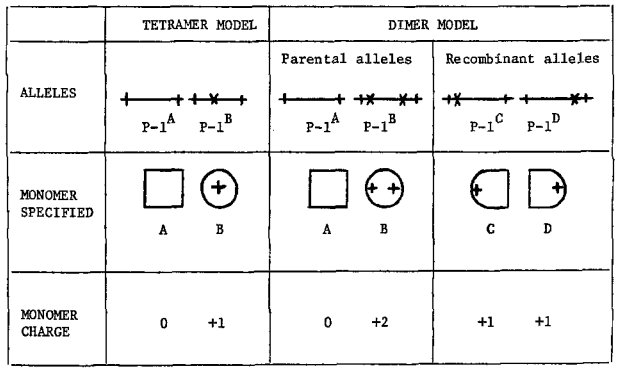

Fig. 1. Diagramatic representation of the alleles of the acid phosphatase-1 gene and of the monomers which they specify under each model. The X in the allele representation indicates a base pair difference with respect to the homologous allele. The X specifies an amino acid differing by a charge of +1 from the corresponding amino acid in the homologous monomer. The amino acid with the more positive charge has been indicated with a + sign in the monomer representations. The A monomer has been arbitrarily assigned a charge of 0 merely to facilitate the analysis. The absolute charge is unimportant; only the *charge differences* between the monomers is relevant here.

Table I. Monomer Composition of the Five Isozymes of the Acid Phosphatase-1 Under Each Model[a]

Isozyme		Monomer composition	
Name	Charge	Tetramer model	Dimer model
1	+4	B_4	B_2
2	+3	AB_3	BC or BD
3	+2	A_2B_2	AB, C_2, D_2, or CD
4	+1	A_3B	AC or AD
5	0[b]	A_4	A_2

[a] Isozyme charges result from the charge assigned to each type of monomer (see Fig. 1).

[b] At *p*H 7.5, isozyme 1 is known to have a net positive charge (Allen *et al.*, 1963*a*, *b*), but as explained in Fig. 1 only *charge differences* are relevant here.

vegetative life of a heterozygote. Indeed, the existence of this stable type is difficult to reconcile with the model on independent molecular and genetic grounds. To account for the absence of isozymes 1 and 5 in the P_3 stable cell, it is necessary to introduce further *ad hoc* assumptions that imply very specific changes in the structure of the dimers or monomers in different cell types, at different times. Consequently, the model also implies complex enzymatic and regulatory mechanisms to accomplish the required specific structural changes in the phosphatase monomers or dimers. The tetramer model also requires that the stable P_3 cell type synthesize both the A and B monomers. This in turn implies the persistence of some subclones which are capable of expressing both alleles in a *stable, permanently undetermined* state. This, as Allen has emphasized, constitutes the only exception to the observation of allelic exclusion made in all syngen 1 heterozygotes studied.

The observations on stable type P_3, which are so difficult to explain on the tetramer model, become a simple and necessary consequence of the following model (see also Table I and Fig. 1): (1) The acid phosphatase is a dimer. (2) The monomers specified by the two different alleles (A and B) differ by 2 units of charge (Fig. 1). (3) The charge differences depend on at least two DNA base-pair differences. (4) Vegetative (macronuclear) intragenic recombination can occur. The two recombinant alleles specify monomers (C and D) which differ from one another in primary structure but not in charge. This charge is intermediate between that of the "parental" monomers (Fig. 1).

The dimer model readily explains the following observations: (1) the absence of significant amounts of isozymes 2 and 4 in young heterozygotes, (2) the sequence of cell types which lead to the stable P_3 type [$P_{1,3,5}$ to

$P_{1,2,3,4,5}$ (rare recombination), to $P_{1,3,5}$ or $P_{3,4,5}$ (loss of rarer parental allele), to finally P_3 (loss of other parental allele)], and (3) the delayed appearance of the P_3 stable type. The last two observations are left unexplained by the tetramer model.

The dimer model makes experimentally testable predictions, which include (1) the subunit structure of the phosphatase, (2) macronuclear (genetic) and molecular differences between stable P_3 types, (3) the existence of a pseudostable P_3 type (possessing CD isozyme), and (4) the possibility of detecting germline recombinants from a $P\text{-}1^A/P\text{-}1^B$ heterozygote. The mathematics developed by Schensted (1958) and Allen and Nanney (1958) are quantitatively inadequate to predict the kinetics of vegetative segregation of stable types from the $P_{1,2,3,4,5}$ cell type because three or four functional alleles can be present in the macronucleus.

The occurrence of minor isozyme species in $P\text{-}1^B$ homozygotes and in some heterozygous cell types can be readily explained on the basis of known phenomena in other systems. Even though the number of minor bands superficially favors the dimer model, the data are not quantitatively adequate to rigorously discriminate between it and the tetramer model.

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