of the thyroid gland of vertebrates, but in the opinion of others (*8*) the endostyle does not have special importance as a synthetic center for thyroactive materials.

Balanoglossus gigas, like other enteropneusts (*9*), does not have such an organ, but according to Gorbman, Clements, and O'Brien (*10*) iodine is present in some groups of cells in the epidermis of *Saccoglossus kowalewsky;* Thomas (*5*) found the same to be true for *S. apantesis.*

Barrington and Thorpe (*11*), using chromatographic methods and I^{131}, have shown that the whole body of another enteropneust—*Saccoglossus horsti*—concentrates some amount of 3-monoiodotyrosine. Histological preparations of the hepatic region of *Balanoglossus gigas* show several intraepithelial glands with homogenous cytoplasm that looks like the colloid substance of thyroid follicles. The hepatic region of *Balanoglossus* is a rather difficult material for histological preparations; the tissues disintegrate rapidly when out of sea water. We expect, however, that autoradiography and chromatography with I^{131} may result in separation of the iodine bound to protein.

F. B. De Jorge, Paulo Sawaya
J. A. Petersen, A. S. F. Ditadi
Department of General and Animal Physiology, Institute of Marine Biology, University of São Paulo, São Paulo, Brazil

References

1. P. Sawaya, *Nature* **167**, 730 (1951).
2. R. Bird and D. J. Jackson, *Clin. Chem.* **8**, 389 (1962).
3. C. J. Van der Horst, "Hemichordata," in *"Bronn's Klassen u. Ord. d. Tierreichs"* (Akademie Verlag, Leipzig, 1939), vol. 4, pt. 4, p. 10.
4. L. H. Hyman, *The Invertebrates*, vol. 5, *Smaller Coelomate Groups* (McGraw-Hill, New York, 1959), p. 201.
5. I. M. Thomas, "Some aspects of the evolution of thyroid structure and function in the evolution of living organisms," *Symp. Royal. Soc. Victoria* (Melbourne Univ. Press, Victoria, 1959), p. 171.
6. E. W. J. Barrington, "Some endocrinological aspects of the Protochordata," in *Comparative Endocrinology*, A. Gorbman, Ed. (Wiley, New York, 1959), pp. 250–265.
7. J. Roche, *Bull. Soc. Chim. Biol.* **42**, 1155 (1960).
8. H. A. Bern and J. Nandi, "Endocrinology of poikilothermic vertebrates," in *Hormones*, G. Pincus, K. V. Thimann, E. B. Astwood, Eds. (Academic Press, New York, 1964), p. 228.
9. J. Leloup, "Biochemie comparée des hormones thyroidiennes," in *Coloquio sobre a Tireoide*, C. Chagas and L. C. Lobo, Eds. (Inst. Biofisica, Univ. of Rio de Janeiro, 1963), pp. 58 and 62.
10. A. Gorbman, M. Clements, R. O'Brien, *J. Exptl. Zool.* **127**, 75 (1954).
11. E. W. J. Barrington and A. Thorpe, *Gen. Comp. Endocrinol.* **3**, 166 (1963).

12 August 1965

Phosphatase Mutants in Aspergillus nidulans

Abstract. *Three mutants at three different phosphatase loci produce inactive enzymes at 35°C but partially or fully active enzymes at 25°C. Furthermore, a suppressor mutant* (su5pal*A*1) *which restores alkaline phosphatase activity in the* pal*A*1 *mutant is an allele of* palc*C*4, *a mutant with a simultaneous reduction in alkaline and acid phosphatase activity. These data suggest that the phosphatase proteins may be made up of two or more different polypeptide chains, and that some of the polypeptide chains are common to two or more of these enzymes.*

Electrophoretic and genetic studies indicate that the phosphatase system in *Aspergillus nidulans* has several features which distinguish it from the same system in *Escherichia coli.* Among these are (i) the large variety of phosphatase mutants and (ii) the large number of genetic loci affecting enzyme activity. Mutants have been isolated which affect either separately or together two or more of the four electrophoretically distinct phosphatases found in crude extracts of the wild type (*1*, *2*). Furthermore, one genetic suppressor (*su*B2*pal*B7) of a mutant lacking alkaline phosphatase (*pal*B7) restores alkaline phosphatase activity with a concomitant loss of an acid phosphatase component. Another suppressor (*su*A1*pal*B7) produces a partial restoration of alkaline phosphatase activity with an electrophoretic change in a different acid phosphatase component.

On the basis of these results I proposed a hypothesis that monomers interact in four distinct heteromultimeric proteins (*2*). This hypothesis would gain support if it could be shown that at least one alkaline phosphatase locus, one acid phosphatase locus, and one locus with no phosphatase at all control, indeed, the structure of the corresponding proteins. Furthermore, since it has been proposed that both a multiple loss mutant and a suppressor mutant may in some instances affect a common monomer, they should fall into the same cistron (*2*).

Histochemical techniques are available for demonstrating the presence of alkaline or acid phosphatase activity in colonies of *Aspergillus* (*2*). A number

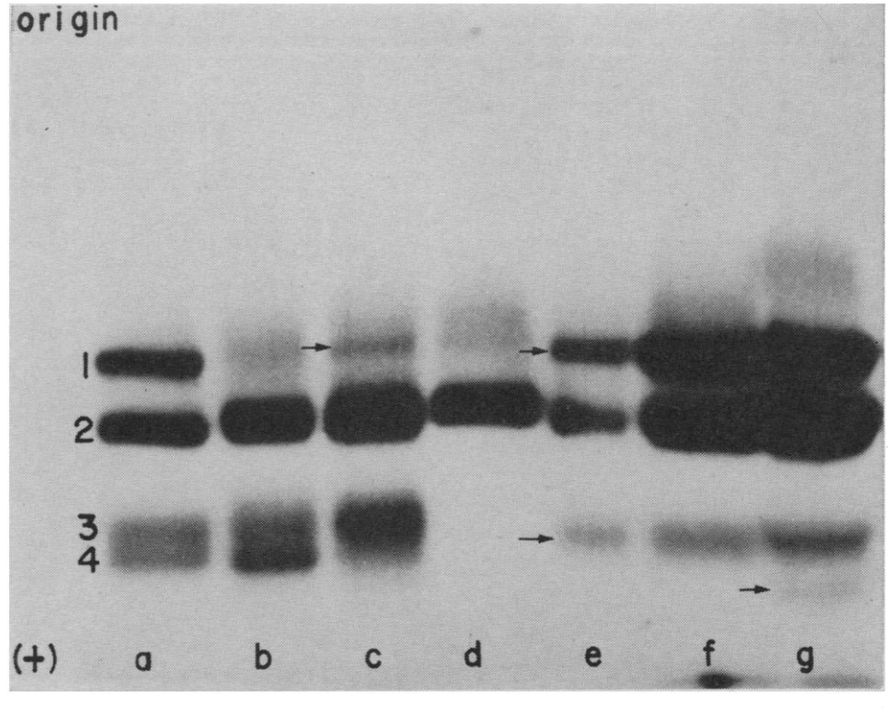

Fig. 1. Electrophoresis patterns of the alkaline (bands 1 and 2) and acid (bands 3 and 4) phosphatases in crude extracts of the wild type and several strains lacking phosphatase grown at 35° and 25°C; (*a*) wild type at 35° and 25°C; (*b*) *pal*B7 at 35°C; (*c*) *pal*B7 at 25°C; (*d*) *palc*A1 at 35°C; (*e*) *palc*A1 at 25°C; (*f*) *pac*C5 at 35°C; (*g*) *pac*C5 at 25°C. Arrows indicate temperature-sensitive phosphatases produced by the mutant strains. The gel was run at room temperature at a potential difference of 5 volt/cm.

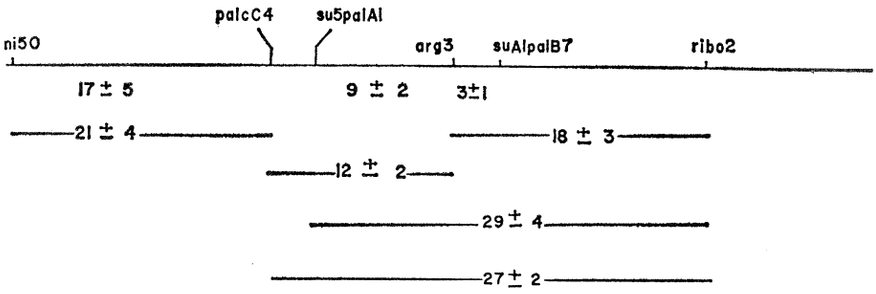

Fig. 2. Partial map of linkage group VIII.

of mutants lacking phosphatase were grown at 25°C and 35°C; they were tested for alkaline (*p*H 8.2) and acid (*p*H 4.8) phosphatase activity (Table 1). The mutants indicated by the dagger show partial or complete restoration of enzyme activity at 25°C. The data suggest that these mutants might be synthesizing a temperature-sensitive and, hence, an altered form of the normal enzyme. To check this possibility the mutants which showed partial or complete restoration of enzyme activity at 25°C were grown in low phosphate liquid medium at both 35° and 25°C. Crude extracts were made, and the enzyme patterns were analyzed by horizontal starch-gel electrophoresis (*3*, *4*). The results for the *pal*B7, *palc*A1, and *pac*C5 mutants are given in Fig. 1. In the wild type, bands 1 and 2 have *p*H optima greater than 7.0, whereas bands 3 and 4 have *p*H optima less than 7.0. At 25°C the mutant *pal*B7 forms an altered alkaline phosphatase, *palc*A1 an altered alkaline and acid phosphatase, and *pac*C5 an altered acid phosphatase. The electrophoretic pattern of *pal*D8 was identical at both temperatures; *pac*B4 was not analyzed since it proved difficult to grow in liquid culture.

Table 1. Alkaline and acid phosphatase activity of various mutants, lacking phosphatase of *Aspergillus* grown at 25° and 35°C. The colonies were grown on limiting phosphate agar medium at either of the two temperatures indicated. Enzyme activity was determined by flooding the dishes at room temperature with a buffered solution (*p*H 8.2 or 4.8) containing α-naphthyl phosphate and a diazonium salt. Deposit of a colored complex was indicative of activity.

Strain	Phosphatase activity*			
	*p*H 8.2		*p*H 4.8	
	35°C	25°C	35°C	25°C
bil (wild type)	+	+	+	+
†*pal*A1	−	−	+++	+++
*pal*A14	−	−	+++	+++
†*pal*B7	−	±	+++	+++
*pal*B9	−	−	+++	+++
*pal*B13	−	−	+++	+++
*pal*C4	−	−	+++	+++
†*pal*D8	−	±	+	+
*pal*E11	−	−	+++	+++
*pal*F15	−	−	+++	+++
†*pac*B4	+	+	±	+
†*pac*C5	+	+	−	+
†*palc*A1	−	+	−	+

* Key to symbols: + = wild type activity; ± = partial enzyme activity; − = no enzyme activity. +++ = enzyme activity much greater than wild type. † These mutants show at least partial restoration of the missing enzyme activity or activities at 25°C.

One of the multiple-loss loci (*palcC4*) is located in linkage group VIII. Among several suppressors of the seven known loci affecting alkaline phosphatase, two fall into linkage group VIII, namely, *suA1palB7* and *su5palA1*. Unfortunately, there is no known method to perform complementation tests of each of these suppressors with *palcC4*. Using outside markers, however, one can see whether either of these suppressors maps in the vicinity of *palcC4*. Figure 2 indicates that while *suA1palB7* defines a separate locus, *su5palA1* is very close to *palcC4* and may actually fall into the same cistron.

In an attempt to interpret the data presented here, only two phosphatases, an alkaline and an acid, will be considered. The alkaline phophatase might be composed of the chains *a* and *b*, and the acid phosphatase of the chains *b* and *c*. A structural mutation in the *a* or *c* chain could result in the loss of alkaline or acid phosphatase activity, respectively, while a mutation in the common *b* chain could result in the simultaneous loss of both phosphatases. The capacity of certain suppressor mutations to restore alkaline phosphatase activity and simultaneously cause a loss or electrophoretic change of an acid phosphatase can be explained in a similar manner. In such a scheme *pal*B7 would be assumed to affect the structure of the *a* chain and produce a temperature-sensitive alkaline phosphatase. The suppressor *suA1palB7* is assumed to affect the common *b* chain in such a way that it compensates the effect of the mutation in the *a* chain with respect to alkaline phophatase activity, but the altered *b* chain now causes a loss or electrophoretic change of the acid phosphatase. Furthermore, since both the mutant *palcC4*, and the suppressor locus *su5palA1* may affect a common monomer, they should be in the same cistron (*2*). The fact that *suA1palB7* defines a locus separate from *su5palA1* indicates that there exists a minimum of two common monomers.

The foregoing data support the hypothesis that the four electrophoretically distinct phosphatases represent four heteromultimeric (*5*) proteins, each made up of two or more different polypeptide chains, and that some of the polypeptide chains are common to two or more of these enzymes. The concept of a limited number of monomers being assembled into different enzymes offers a promising approach to the further understanding of complex enzyme systems. In fact, one may even expect that certain monomers may be shared by more than one such enzyme system, for example, the phosphatases and esterases. A single monomer might carry the active site for the ester linkage in general, while the addition of one or more other monomers could result in changes in *p*H optimum, substrate specificity, and other enzyme characteristics.

G. DORN

Department of Genetics,
Albert Einstein College of Medicine,
New York

References and Notes

1. Notations: The following symbols—followed by the isolation number—are employed to designate the various phosphatase mutants: *pal*, loss or reduction in activity at alkaline *p*H but normal or increased activity at acid *p*H; *pac*, loss or reduction at acid but not alkaline *p*H; *palc*, simultaneous loss or reduction in activity at both alkaline and acid *p*H; *su pal*, recessive suppressor of a *pal* mutant. The assignment of a mutant to a particular locus is indicated by the addition of a capital letter between the mutant symbol and the isolation number. For example, after *pal7* was shown (by genetic analysis) to be one of the mutants at the *pal*B locus, its complete symbol became *pal*B7.
2. G. Dorn, *Genet. Res.* **6**, 13 (1965).
3. M. D. Poulik, *Nature* **180**, 1477 (1957).
4. O. Smithies, *Biochem. J.* **61**, 629 (1955).
5. As suggested by Crick and Orgel, the term "multimer" is used to denote the aggregation of several identical monomers without any implication as to their geometrical structure. F. H. C. Crick and L. E. Orgel, *J. Mol. Biol.* **8**, 161 (1964).
6. Support from NSF grant GB-1920 and NIH grant RO1 GM 12711-01. I thank S. Waelsch and C. Roberts for criticism and suggestions.

30 June 1965