

Torriani A. Influence of inorganic phosphate in the formation of phosphatases by *Escherichia coli*. *Biochim. Biophys. Acta* 38:460-9, 1960.
[Biological Laboratories, Harvard University, Cambridge, MA]

In this paper the existence of an alkaline phosphatase (AP) in *E. coli* was demonstrated and evidence was presented for a negative control of its synthesis by inorganic phosphate (Pi). An acid phosphatase was also studied; its synthesis was found to be independent from the level of phosphate in the growth medium. [The SCJ® indicates that this paper has been cited in over 610 publications since 1961.]

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"At the Pasteur Institute in Paris, with Jacques Monod, I was studying the rate of synthesis of enzymes to understand the kinetics of 'adaptation.' We chose some adaptive and some constitutive enzymes. One of these was the acid phosphatase known to be produced by *E. coli*. The cells were grown in a device (the Bactogene or chemostat¹) which allowed continuous exponential growth in conditions limiting the growth rate. When we used phosphate as the limiting factor, I observed that a new phosphatase suddenly appeared. This suggested that inorganic phosphate (Pi) was exerting a negative control on the synthesis of the enzyme. The new phosphatase had an alkaline pH optimum and hydrolyzed all phosphomonoesters.

"Negative control was rather new at that time and was treated with distaste by Monod, who was geared toward positive control. Thus, my observation that Pi inhibited the synthesis of alkaline phosphatase (AP) remained untold for almost two years until I moved from the Pasteur Institute to Harvard University. I was slowly getting the facts organized into a paper which was very thoroughly criticized and corrected by

A. Pappenheimer, when I received a letter from M. Pollock in London telling me that Horiuchi² in Japan had also found this enzyme and was publishing a note in *Nature*. I dashed the paper through! It has been cited often because it represents the initial observation and brings a complete proof of the existence of this enzyme. It also gives the method of limiting phosphate used to induce its synthesis. This finding has been at the basis of the study of the Pi regulon in *E. coli*.

"At that time (1959), Levinthal at the Massachusetts Institute of Technology was interested in demonstrating the colinearity of DNA and the amino acid sequence of protein. AP seemed an ideal protein to use since its activity is easy to measure and colonies, whether producing the enzyme or not, are also easy to screen. So, I moved to MIT. With Levinthal and Garen I started, by brute force, to analyze millions of colonies produced after mutagenesis and a collection of mutants with an altered enzyme was rapidly produced.³ The protein was purified and its properties analyzed. The MW of each of the two subunits turned out to be small enough (43000) to make AP an interesting candidate for sequencing. However, the colinearity was proved by Yanofsky with tryptophan-synthetase and the sequence of AP was completed only recently.⁴ But AP has continued to be an interesting protein. Its regulatory mechanism has been worked out in the last 20 years. It is now part of the phosphate regulon which involves porines, binding proteins, transport systems, proteins excretion (AP is a periplasmic protein), positive and negative control factors, and their corresponding genes. But...no one knows how Pi functions as a repressor yet!

"The enzyme is widely used in DNA analysis. Removal of the 5'-phosphate from DNA cleaved by endonucleases makes AP as very useful to scientists as it is to *E. coli*...under conditions of stress!"

1. Monod J. La technique de culture continue: théorie et applications. *Ann. Inst. Pasteur* 79:390-410, 1950.
2. Horiuchi T, Horiuchi S & Mizuno D. A possible negative feedback phenomenon controlling formation of alkaline phosphomonoesterase in *Escherichia coli*. *Nature* 183:1529-30, 1959.
3. Levinthal C. Genetic and chemical studies with alkaline phosphatase of *E. coli*. *Brookhaven Symp. Biol.* 12:76-85, 1959.
4. Bradshaw R A, Cancedda F, Ericsson L H, Neumann P A, Piccoli S P, Schlesinger M J, Shriefer K & Walsh K A. Amino acid sequence of *Escherichia coli* alkaline phosphatase. *Proc. Nat. Acad. Sci. US* 78:3473-7, 1981.