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Grunberg-Manago M, Ortiz P J & Ochoa S. Enzymatic synthesis of polynucleotides. I. Polynucleotide phosphorylase of *Azotobacter vinelandii*. *Biochim. Biophys. Acta* 20:269-85, 1956.
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The isolation, partial purification, and some properties of polynucleotide phosphorylase of *Azotobacter vinelandii* are described. The enzyme catalyzes the synthesis of highly polymerized ribonucleic acid-like polynucleotides from 5'-nucleoside diphosphates with release of orthophosphate. The reaction requires magnesium ions and is reversible. [The *SCI*® indicates that this paper has been cited in over 365 publications.]

Pleasure from PNPase

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Polynucleotide phosphorylase (PNPase) was discovered during my studies with Severo Ochoa on oxidative phosphorylation.

I had observed in soluble *Azotobacter vinelandii* extracts an exchange between inorganic phosphate P^{32} and adenosine triphosphate (ATP), and we were hoping that this exchange corresponded to ATP synthesis linked to electron transport. *Az. vinelandii* was chosen because I thought there would be a better chance of finding an active oxidative phosphorylation system in an obligatory aerobe.

I had started purifying the exchange system when Sigma sent us some pure crystalline ATP just put on the market. The crystalline ATP was inactive on the exchange, and, to my surprise, I determined that the real substrate was the adenosine diphosphate (ADP) contaminating the previous ATP. No reaction was known that could lead to such an exchange, and we were hoping that in some way or another the exchange was still related to electron-linked phosphorylation.

After the excitement of discovering a new reaction, I had a difficult time as the following few months were very frustrating. I could not ascribe the observed exchange to a specific reaction. I was in the same position as one who by chance obtains crystals in a crude mixture of proteins and tries to ascribe to the crystals a specific protein without knowing its function. There was always a slight phosphate liberation during exchange, but the reaction was complicated due to traces of adenylate kinase and ATPase. Severo also started to doubt the meaning of this exchange and encouraged me to try with *Az. vinelandii* the reconstitution system of G.B. Pinchot[1] from *Alcaligenes faecalis* leading to phosphate uptake.

However, before starting a new system I decided to study in detail the reaction responsible for phosphate liberation as I had the feeling that it was related to this exchange. First, I replaced ADP by inosine diphosphate (IDP) (adenylate kinase is inactive with inosine derivative), and I thereby avoided the complication of mono- and triderivatives formed by the adenylate kinase. And then, I performed an IDP saturation curve. This was not so straightforward as at that time diphosphates were not easily available and were expensive, and I had to use quite large amounts of them (the enzyme having a low affinity for the nucleotides) for an experiment that looked trivial. However, I found out that under saturation conditions, I observed a large amount of phosphate liberated. I was relieved as I understood that the inorganic phosphate was a true product of the reaction. At this point it might have been a rather uninteresting reaction, namely, a hydrolysis of diphosphates to monophosphates; however, the reaction was reversible and the reversibility of a hydrolytic reaction seemed very unlikely. So, with enthusiasm I started identifying the other product of the reaction by column chromatography. To my satisfaction, no mononucleotide was found but a product was formed that was not eluted from the column under my experimental conditions. Hoping but not daring to believe entirely that the product was at high molecular weight, I tried paper chromatography and saw a nice ultraviolet spot that didn't move from the origin of the chromatogram. At that point I knew instinctively that I discovered an important new reaction: I had synthesized an RNA-like polymer.

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to obtain blood samples as needed and to undergo repeated testing, always of course in the hope that we would make a breakthrough discovery of some means of treatment. In fact, it was thanks to the willingness of two of these patients to go far beyond what would ordinarily be asked of them that we eventually secured proof for a receptor defect. In 1983 we were able to obtain open liver biopsies, following informed agreement by the parents and the patients and approval by the Ethical Committee of the hospital, and these showed conclusively that there was no binding of ^{125}I-hGH by their liver membranes[2] compared to liver membranes from healthy transplant donors.

In the meantime many more patients with this syndrome had been described in various populations not only around the Mediterranean, but also in Argentina, Denmark, Japan, and, recently, Brazil; and in the literature we found that it had been coined "Laron type dwarfism (LTD)" (which in the beginning always felt somewhat embarrassing). The syndrome aroused much interest since it was a model in nature of a feedback mechanism defect for a peptide hormone.

The recent characterization of the hGH receptor gene has opened the way for still further elucidation of the nature of the receptor defect. In collaboration with W.I. Wood and associates from Genentech as well as J.S. Parks of Emory University, Atlanta, Georgia, it was found that LTD patients have deletions of certain exons of the extracytoplasmatic portions of the hGH receptor,[3] confirming that the pathogenesis in this syndrome resides in defects in the structural gene for the GH receptor. Almost simultaneously a French group reached the same conclusions.[4] Another recent finding, that the extracytoplasmatic portion of the hGH receptor is identical with the circulating GH binding proteins (confirmed by the absence of this protein in the serum of LTD patients),[5] has made it possible for us to identify the heterozygote carriers for this disease[6] and thus may be of considerable practical importance in genetic counselling.

It now appears that the only possible hope for therapy for this disease is the missing hormone IGF-I. The recent biosynthesis of IGF-I by recombinant DNA technology made it possible for us to initiate the first clinical therapeutic trials in patients with LTD.[7]

This syndrome, in addition to presenting a new disease entity, serves as a unique human model to learn about the physiology of hGH and IGF-I and their interaction. By monitoring the 24-hour hGH secretion in LTD patients,[8] we found that they secreted values similar to those observed in acromegalics, however, with opposite metabolic effects. This demonstrates that hGH exerts its main actions not directly, but via IGF-I. On the other hand, optimal IGF-I action may depend on hGH: in LTD patients in whom the GH-dependent binding protein (IGF-I BP3) is missing, the biological half-life of injected IGF-I is shorter than in healthy subjects.[9,10]

It took 20 years from the description of the disease to the elucidation of its pathogenesis. It is hoped that it will take much less time to find the correct way to use IGF-I to make possible the achievement of normal growth and height for these unfortunate children.

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