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Essay

Memories: Discoveries of Histone Deacetylase and RBM10 and Serendipity

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Since the discovery of histone deacetylase (HDAC) in 1969, I have been studying the molecular and cellular biology of the cell nucleus, where I have encountered unexpected results and several serendipitous chances. I would like to describe some of these memories.

Discovery of histone deacetylase : Epigenetics

In molecular biology, "epigenetics" refers to the study or mechanisms that regulate gene expression by changing the structure of chromatin without altering DNA sequence. The main mechanisms include methylation modification of DNA bases and modification reactions of histones that bind to DNA to make up chromatin. In 1964 - 1968, Vincent G. Allfrey and his colleagues reported acetylation of histones f2a1 (now H4) and f3 (H3) at the ε-amino group of lysine residues and suggested that histone acetylation is involved in altering DNA-histone interactions at times of gene activation [1, 2]. Based on their findings, we began our research assuming the existence of histone deacetylase (HDAC), which removes acetyl groups from histones, and were able to discover HDAC, a universal suppressor of gene expression in epigenetics [3,4].

To show HDAC, an assay method for the enzyme had to be devised. This was done according to the basics of organic chemistry: after incubating enzyme preparations (cell extracts) with substrate histones labeled with radioactive acetic acid, the acetic acid released by HDAC was extracted with an organic solvent from acidified reaction mixtures, and the radioactivity of the extracts was measured. I remember it worked well.

Serendipity

Serendipity refers to the accidental discovery of an unexpected phenomenon that is different from the original purpose. For this to happen, as Louis Pasteur said in 1854 at the ceremony of the university that later became the University of Lille, it is important to have a prepared mind, that is, to have the knowledge, the skills, and the mind to be able to notice the phenomenon that is about to pass by.

There are 18 types of HDACs in humans, which are classified into four classes based on differences in structure and functional characteristics. They are Zn^{2+}-dependent class I (HDAC 1, 2, 3, 8), class II (HDAC 4 - 7, 9, 10), and class IV (HDAC 11), and NAD^{+}-dependent class III [sirtuin (SIRT) 1 – 7]. In our original 1970 paper, the molecular diversity of HDACs was apparent from multiple overlapping broad peaks of enzyme activity in DEAE-cellulose ion exchange chromatography and in Sephadex G-200 gel filtration of cell extracts [4]. However, the significance of this HDAC diversity was not pursued further because of the possibility that HDAC might form complexes with various molecules, probably making analysis difficult. Thus, we missed a serendipitous opportunity to demonstrate the molecular diversity of HDAC.

In addition, when product inhibition of HDAC was examined, acetic acid (two-carbon fatty acid) that is released by the reaction and propionic acid (the three-carbon fatty acid similar to acetic acid) showed no significant effect. However, 8 years later, several papers reported that a four-carbon fatty acid (butyric acid) is a strong inhibitor of HDACs. Thus, in our study, we did not go one step further and did not examine butyric acid.

As of May 2024, PubMed lists 47,009 articles on HDACs; HDACs are a group of molecules that will continue to be studied [5] not only in the fundamental field of gene regulation, but also in related diseases and in various areas such as stem cell differentiation and pluripotency [6,7], brain function [8,9], SIRTs that delay ageing [10] and so on.

Thyroid Hormone Receptor: Regulation by Fatty Acids and Fatty Acyl-CoAs, and Hibernation

The thyroid hormone (T3)-bound receptor (TR) binds to specific genes in the nucleus and regulates their transcriptional activity. In 1989, we found that lipase treatment of nuclear TR extracts significantly inhibited the T3-binding activity of the extracts. This inhibition was due to fatty acids (FAs) released from lipids in the extracts by the treatment: FAs competitively inhibited T3 binding to TR [11]. It was also shown that CoA esters of fatty acids (fatty acyl-CoAs, FA-CoAs) inhibit TRs more strongly than free FAs [12,13] and that administration of norepinephrine to rats significantly reduces T3-binding in tissues, particularly the heart, in fatty acid-mobilized rats [14].

The physiological action of thyroid hormones is to increase basal metabolic rate/ energy expenditure. During hibernation, hibernating animals gradually break down the fat stored in the fall and use the resulting fatty acids and glycerol for energy and glucose production, respectively, to sustain hibernation. Fatty acids transported into tissue cells are esterified with coenzyme A (CoA) to form fatty acyl CoAs

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(FA-CoAs), which are then converted to acetyl CoA (Ac-CoA) by β-oxidation in the mitochondria. Ac-CoA is used to produce ATP (energy provider for various cellular reactions) and heat. Our findings suggest that a fraction of FFA and FA-CoA reaches the cell nucleus and binds to TR, suppressing its activity, and that in hibernating animals, this suppression of TR by FA-CoA/free FA reduces basal metabolism, preventing wasteful or excessive consumption of stored fat to allow stable and sustained hibernation. However, this intriguing hypothesis has not been tested experimentally and remains to be verified.

It should be added that the inhibition of TR by free FAs had been reported the previous year by Wiersinga et al [15]. We were not aware of this.

Discovery of RBM10 and serendipity

The S1 proteins B2, C1, and D1,which we discovered in 1983 [16], are isoforms produced by alternative splicing of the primary transcript (pre-mRNA) of the gene currently designated *AUF1*, *hnRNPD*, or *hnRNPD0*. In 1996, with the aim of revealing the gene(s) of S1 proteins, cDNA cloning of these proteins was performed using E. coli, λgt11 phage with incorporated cDNAs of rat liver mRNAs, and a rabbit polyclonal antibody that recognized B2, C1, and D1. No clear E. coli plaques were obtained that reacted with the antibody, probably because the S1 proteins expressed from the cDNAs are toxic to E. coli, but one plaque was found that showed weak reactivity. Since the reactivity was too weak, it was discarded. However, after reconsideration, the sample tube was recovered from the trash can the next day and its cDNA was examined, which turned out to be a clone encoding a novel protein. The gene for this protein was named S1-1 (now known as RBM10), and studies of RBM10 began [17].

The discovery of RBM10 was the result of the very weak cross-reactivity of RBM10 to the antibody for S1 proteins, and in this case, serendipity was fortunately at work. RBM10 was one of the first to be discovered among the dozens of RBM proteins currently known, and as shown in the next section, research has recently become more active because of its biological importance in the cells.

Function of RBM10

RBM10 is an RNA-binding protein found primarily in the cell nucleus. It acts on primary transcripts (pre-mRNAs) of various genes to induce an alternative splicing called exon skipping, in which certain exons are not included in the generated mRNAs, thereby leading to diversity in gene products [18]. Research on RBM10 has recently become more active because of its clinical importance, as shown, for example, by the studies of RBM10 mutations causing TARP syndrome (an X-linked pleiotropic anomaly such as cleft palate and atrial septal defect) in fetal life and various cancers of the lung, ovary, pancreas, prostate, liver, etc. in adults (see review [19]). This review describes the molecular biology of RBM10 and its significance in medicine, focusing on the gene and protein structures of RBM10, its cell biology, its molecular functions and regulation, and the mutations of RBM10 and associated diseases.

S1 proteins and RBM10: unexpected results and serendipity

The 1976 paper by Weintraub and Groudine showed that when isolated nuclei are mildly treated with DNase I or staphylococcal nuclease, transcriptionally active chromatin is readily fragmented and released from the chromatin [20]. Inspired by this, we began research to understand the structure of transcriptionally active chromatin. This was the research that preceded RBM10.

Nuclei isolated from various rat tissues were washed to remove the soluble fraction, and gently treated with DNase I or staphylococcal nuclease. The reaction was stopped with EDTA 2Na (5 mM), and the proteins (S1 proteins) were collected in the centrifuged supernatant (first supernatant: S1). Contrary to expectations, the S1 proteins consisted of a very simple group of proteins [16]. They are HSP (heat shock protein), three isoforms of AUF1, and two isoforms of CBF-A (also known as CArG box motif binding proteins, and as hnRNPAB). AUF1 and CBF-A are hnRNP proteins that associate with pre-mRNAs on chromatin during transcription [21, 22]. Indeed, treatment with RNase A instead of DNase I released the same set of S1 proteins from isolated nuclei [23]. It is now known that AUF1 mainly controls mRNA degradation [24] and that CBF-A regulates not only splicing but also transcription [25].

S1 proteins were isolated as a simple group of proteins. As a matter of fact, their isolation was an accidental result. That is, EDTA 2Na, which was added to stop the reaction, lowered the pH of the reaction solution to 4.9, precipitating 80% of the proteins (protein-nucleic acid complexes) such as histones and non-histone proteins in the S1 fraction and selectively leaving only the S1 proteins in the supernatant [26]. Before the experiment, I could not predict the pH-lowering effect of added EDTA 2Na; therefore, the isolation of S1 proteins was an unexpected result obtained by chance.

At that time, isolation of simple group proteins from cell nuclei was known with histones, which are extracted with 0.25 N hydrochloric acid, and HMG proteins, which are re-extracted with 2% trichloroacetic acid from 0.35 M NaCl-soluble nuclear fractions [27]. The S1 proteins were also isolated as a group of proteins with a simple composition, and what is interesting is that the S1 proteins were isolated unexpectedly, and RBM10 was discovered by serendipity during the study of these S1 proteins.

I have described some miscellaneous thoughts that come to mind as I recall my research. In research, we sometimes come across serendipity or unexpected results. It would be nice if this led to the development of new research.

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