CHAPTER 5

From the phosphodiesterase autotaxin to dual specificity phosphatases:

A short introduction on dual specificity phosphatases

5.1 Introduction

The previous chapters describe the discovery, optimization and biological validation of autotaxin (ATX) inhibitors. Next to the phosphodiesterase ATX, protein phosphatases are another interesting class of enzymes in drug discovery.[1] Like ATX, protein phosphatases have the ability to hydrolyze phosphate esters, however, their substrates are intracellular phosphorylated proteins rather than lipids. Therefore, ATX and protein phosphatases have different functions in biology. Here a short introduction on protein phosphatases is provided with the main focus on dual specificity phosphatases as an introduction to the next chapter.

After protein synthesis, approximately one-third of mammalian proteins are phosphorylated by protein kinases (Figure 1).[2] Dynamic protein phosphorylation is one of the major mechanisms by which cells regulate transcription, signal transduction, proliferation, differentiation, motility and metabolism.[3-8] The phosphorylation state of cellular proteins is tightly controlled by the concerted and reversible action of protein kinases and protein phosphatases. The importance of controlling the activity of protein kinases in biology has long been recognized and this has resulted in the development of several clinically approved protein kinase inhibitors (e.g. Imatinib). A growing body of evidence now demonstrates that the regulation of protein dephosphorylation by protein phosphatases is equally important, which stimulates the development of protein phosphatase inhibitors.[4]

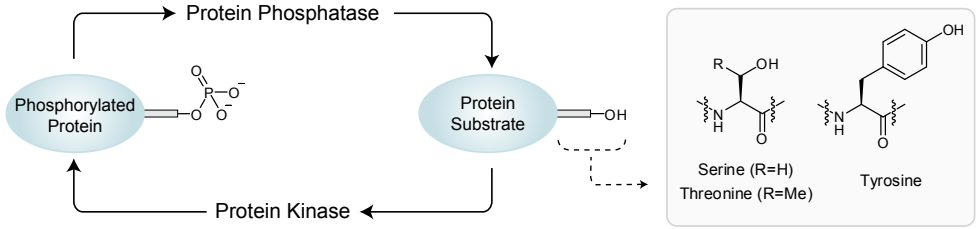

Figure 1: The phosphorylation status of proteins is regulated by both protein phosphatases and protein kinases. Protein phosphorylation occurs predominately on the amino acids serine, threonine and tyrosine.

Protein phosphatases have historically have classified as serine/threonine (PSP),[9] tyrosine (PTP),[10] and dual specificity (DUSP)[11] phosphatases based on their preference for specific phosphorylated hydroxyl amino acids (Figure 1) over others. The unique feature that distinguishes DUSPs from PSPs and PTPs is their ability to dephosphorylate both phosphorylated tyrosine and serine/threonine residues within a protein substrate. Both classical PTPs and DUSPs share a similar mechanism of catalysis for the hydrolysis of phosphorylated substrates that involves the formation of a phosphoryl-enzyme intermediate (Figure 2).[11-13] The highly conserved catalytic domain in DUSPs contains the consensus sequence HCXXGXXRS(T) (Table 1). The cysteine residue (C), which is essential for catalytic

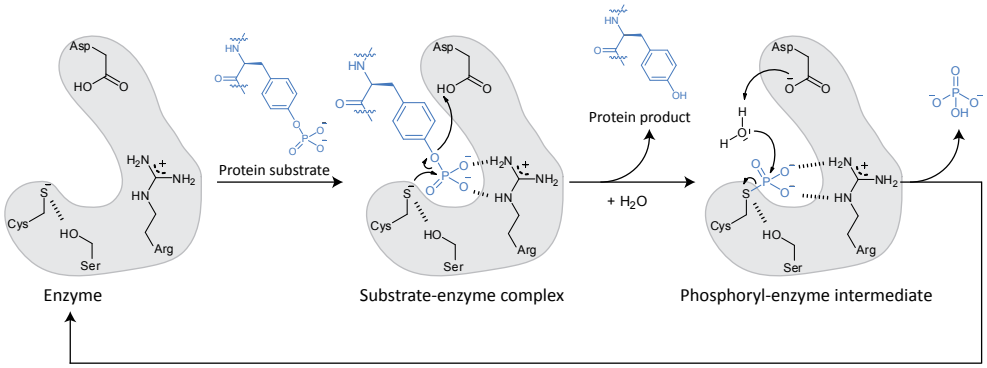

Figure 2: Proposed catalytic mechanism for DUSPs and classical PTPs.

activity, is positioned at the centre of the catalytic pocket.[11] The arginine (R) of the consensus sequence coordinates to the phosphate group of the protein substrate to assist with catalysis. There is also a conserved aspartic acid present upstream of this conserved motif that acts as a general acid/base catalyst. The serine (S) of the consensus sequence, threonine (T) in some other protein phosphatases (i.e. DUSP23), most likely plays a role in stabilizing the cysteinyl anion through hydrogen bonding.[14-16] The catalytic pocket of a DUSP is shallow, but broader than that of classical PTPs, which is thought to be the mechanism how DUSPs can simultaneously accommodate more than one phosphorylated residue.[13]

Although a number of potent and selective inhibitors of PSPs isolated from natural sources are known, like tautomycin and fostriecin,[17] selective PTP or DUSP inhibitors are still rare.[18] Genetic approaches using small interfering RNA (siRNA) can provide some insight into the biological function of these protein phosphatases in cells. However, some PTPs and DUSPs interact with other proteins and regulate their function in a manner that is independent of their phosphatase activity.[19] In addition, wrong gene annotations in commercial siRNA libraries and the difficulty of reproducible transfections of siRNA ask for independent controls. Therefore, potent and selective small molecule inhibitors of protein phosphatases are valuable to

Table 1: Conserved Catalytic Domain for DUSPs and PTPs.

DUSP3	H	C	R	E	G	Y	S	R	S
DUSP13	H	C	A	M	G	V	S	R	S
DUSP2	H	C	Q	A	G	I	S	R	S
DUSP4	H	C	Q	A	G	I	S	R	S
DUSP5	H	C	E	A	G	I	S	R	S
DUSP26	H	C	A	V	G	V	S	R	S
DUSP1	H	C	Q	A	G	I	S	R	S
DUSP27	H	C	V	M	G	R	S	R	S
DUSP22	H	C	L	A	G	V	S	R	S
DUSP14	H	C	A	A	G	V	S	R	S
DUSP12	H	C	H	A	G	V	S	R	S
DUSP16	H	C	L	A	G	I	S	R	S
DUSP10	H	C	Q	A	G	V	S	R	S
DUSP5	H	C	Q	A	G	V	S	R	S
PTPMT1	H	C	K	A	G	R	S	R	S
SSH3	H	C	K	M	G	V	S	R	S
DUSP15	H	C	F	A	G	I	S	R	S
DUSP6	H	C	L	A	G	I	S	R	S
DUSP7	H	C	L	A	G	I	S	R	S
DUSP13	H	C	V	V	G	V	S	R	S

[a] Table displays the PTP signature sequence in DUSP3, a well studied DUSP, compared with the 20 closest human homologs. Conserved amino acids are depicted in blue. Serine (S) is partially conserved (purple).

study the function of protein phosphatases, allowing immediate and reversible inhibition. The discovery of small molecule inhibitors of protein phosphatases has been challenging, especially the search for selective inhibitors. Selective inhibitors are difficult to obtain due to the highly conserved nature of the PTPs and DUSPs active site (Table 1).

In the next chapter we study the involvement of protein phosphatases in *Salmonella typhimurium* infection of human cells and we describe the discovery and development of inhibitors that target protein phosphatases that are essential for this infection process.

5.2 References

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