UNIVERSITY OF CALIFORNIA SAN DIEGO

The regulatory function of C-terminal tail in T-cell Protein Tyrosine Phosphatase

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in

Biology

by

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Professor Claire Meaders

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

PTK	Protein Tyrosine Kinase
PTP domain	Phospho-Tyrosine Phosphatase domain
DiFMUP	6,8-Difluoro-4-Methylumbelliferyl Phosphate
ER	Endoplasmic reticulum
*p*NPP	*p*-nitrophenyl phosphate
RCML	Reduced, carboxamidomethylated, and maleylated lysozyme
EGFR	Epidermal growth factor receptor
CFP	Cyan Fluorescent Protein
YFP	Yellow Fluorescent Protein
TROSY	Transverse relaxation optimized spectroscopy
JAK	Janus tyrosine kinase
STAT	Signal transducer and activator of transcription

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LIST OF FIGURES

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ABSTRACT OF THE THESIS

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T-cell protein tyrosine phosphatase (TCPTP) is a classical non-transmembrane PTP that is expressed in every cell, despite its name. In its catalytic domain, it shares 65% similarity identity to PTP1B, which was the first tyrosine phosphatase to be identified, and the most thoroughly researched. Based on its similarity with PTP1B, TCPTP is predicted to have a 280 amino acid catalytic domain followed by the regulatory α helix 7 (α7), and a C-terminal intrinsically disordered region. Allosteric inhibitors that target α7 and the C-terminal tail of

PTP1B have been characterized. Since the active sites of classical PTPs have generally similar features, targeting the active site leads to inhibitors with poor specificity. Therefore, domains that regulate the PTP catalytic activity have been the focus of much recent research. In this study, we will be investigating the role of the C-terminal domain of TCPTP. The regulation of TCPTP by the C-terminal domain are not clearly defined. By knocking out TCPTP and reconstituting the wild-type full length and catalytic domain, we will assess the effect of the C-terminal domain on TCPTP activity in a cellular context. By combining mutagenesis, enzymatic assays, and FRET assays, we will investigate the areas in the protein and mechanisms that are responsible for its inhibition and activation in cells and *in vitro*.

Introduction

Tyrosine phosphorylation is one of the post-translational modifications that play an important role in cell signaling cascades and cellular processes, such as cell growth, survival, and aging. Protein kinases regulate signaling pathways by the addition of a phosphate group to protein, while protein phosphatases counteract their action by its removal[1]. The coordination between kinases and phosphatases plays a vital role in maintaining the cellular functions. When this balance is disrupted, disease such as cancer and autoimmune diseases can emerge.

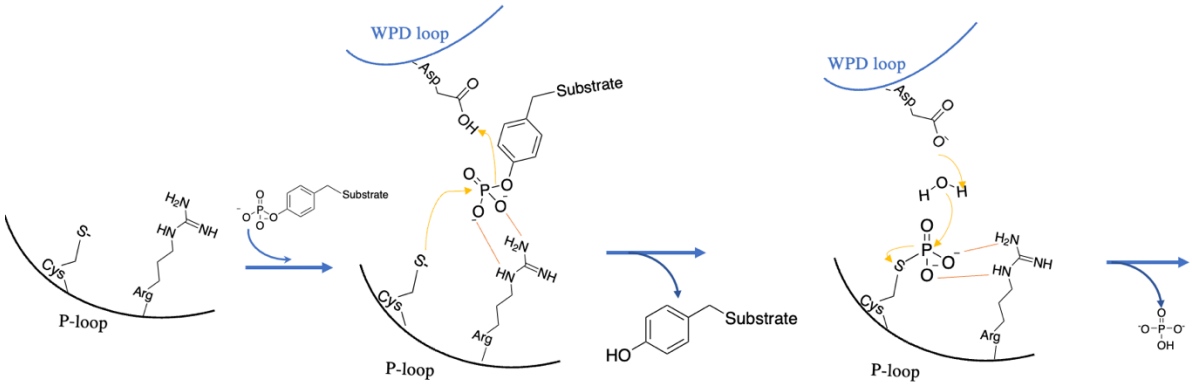


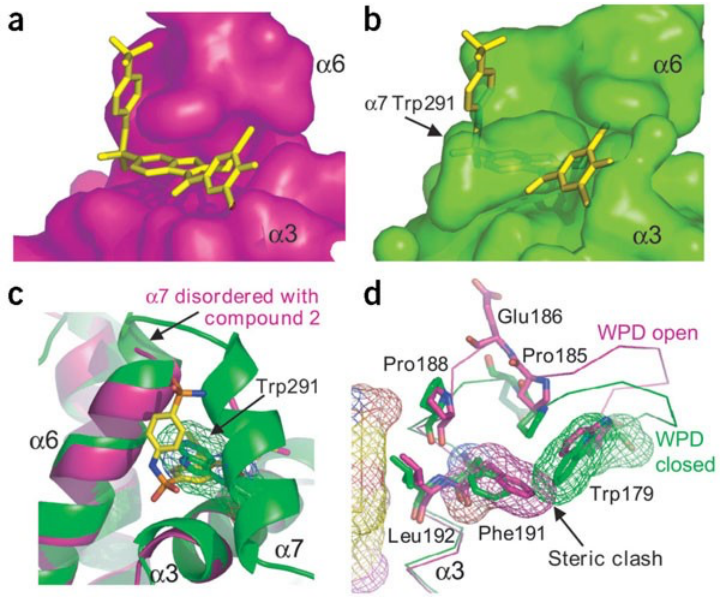
Figure 1: PTP Catalyzed Reaction. In the first step after substrate binding, the active site cysteine attacks the phosphate group, while an arginine in the P-loop neutralizes its charge, coordinates the phosphate oxygens and stabilizes the WPD loop in the closed conformation. The aspartate side chain of the WPD loop facilitates the reaction in both irreversible catalytic steps.

Classical Protein Tyrosine Phosphatases (PTPs) are an extended family of enzymes characterized by 280-amino acid conserved phosphotyrosine phosphatase (PTP) domains, and they can be divided into two major types, receptor PTPs and non-transmembrane PTPs[2]. Non-transmembrane PTPs generally include one PTP domain and at least one regulatory domain.

Two main structural elements are present at the active site of PTPs: the P loop is responsible for positioning the incoming phosphate substrate for catalysis and additionally contains the cysteine residue that acts as the nucleophile and an arginine residue responsible for switching the WPD loop from an open, inactive form to a close, active form, while at the same time stabilizing the protein-substrate complex. The WPD loop in turn contains an Aspartic acid that acts as an acid to protonate the oxygen of the Tyrosine leaving group, generating the phosphoenzyme and tyrosine peptide. The same Aspartate then acts as a base to deprotonate a water molecule that attacks the phospho-cysteine, which generates a phosphate ion and regenerates the active enzyme[3] (Figure 1). WPD loop motion is the rate-determining step of PTP-catalyzed reactions[3]; as a result, PTPs can regulate their rate of catalysis by controlling the motion of the WPD loop.

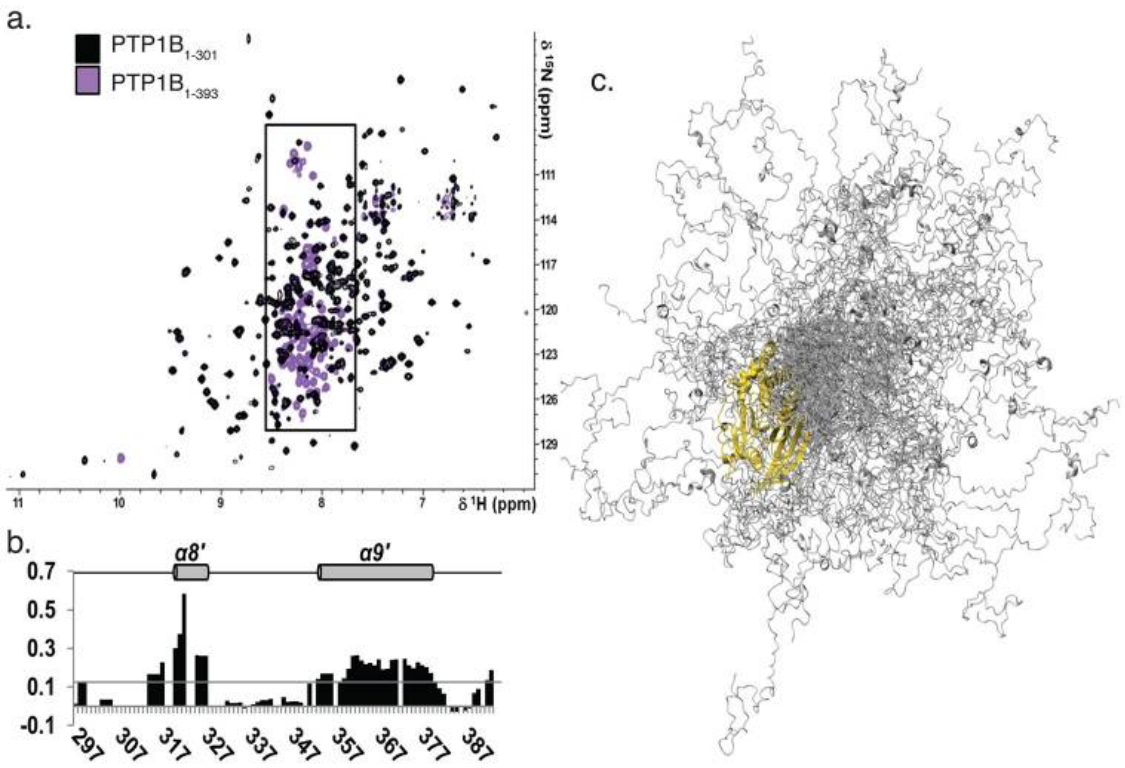
TCPTP (T-cell Protein Tyrosine Phosphatase) is a non-transmembrane PTP belonging to the NT1 sub-class together with PTP1B. Both TCPTP and PTP1B are characterized by a classic N-terminal PTP catalytic domain, made up of both α helices and beta strands, a C-terminal regulatory domain that includes an extra helix, termed α helix 7, specific to these two enzymes, and a disordered region. Alternative splicing of the TCPTP C-terminal tail gives rise to 48 kDa and 45 kDa TCPTP forms. The 48 kDa TCPTP (TC48) is specifically recruited to the ER, while the 45 kDa form (TC45) is localized to the nucleus. The TC45 nuclear localization signal is shown to be bipartite, meaning that it consists of two basic residue stretches: 350–358 and 377–381[15]. Unlike TCPTP, PTP1B has been the topic of intense research for the last 30 years, as it plays an important role in diabetes and cancer[4]. However, interest in TCPTP has been steadily growing due to its importance in inflammatory diseases[5].

The C-terminal domain of PTP1B, particularly α7, is functionally and structurally well-characterized as it has been reported to play a role in PTP1B allostery and its activity. In addition, the C-terminal tail of PTP1B has been characterized as a flexible, intrinsically disordered region, which is sensitive to protease. α helix 7 of PTP1B has been found to be ordered in crystal structures that show a closed WPD loop, indicating higher catalytic activity. Normally, Trp291 mediates the interaction of α7 with α helices 3 and 6 in a closed WPD loop PTP1B. However, the PTP1B inhibitor compound 2 was found to bind at the Trp291 interaction site, resulting in the displacement of helix α7 from the surface of the PTP domain. This ultimately resulted in helix α7 being disordered, stabilizing the open conformation of the WPD loop and decreasing catalytic activity (Figure 2a-d). Thus, compound 2 has been reported to be a potent, non-competitive allosteric inhibitor of PTP1B[6].

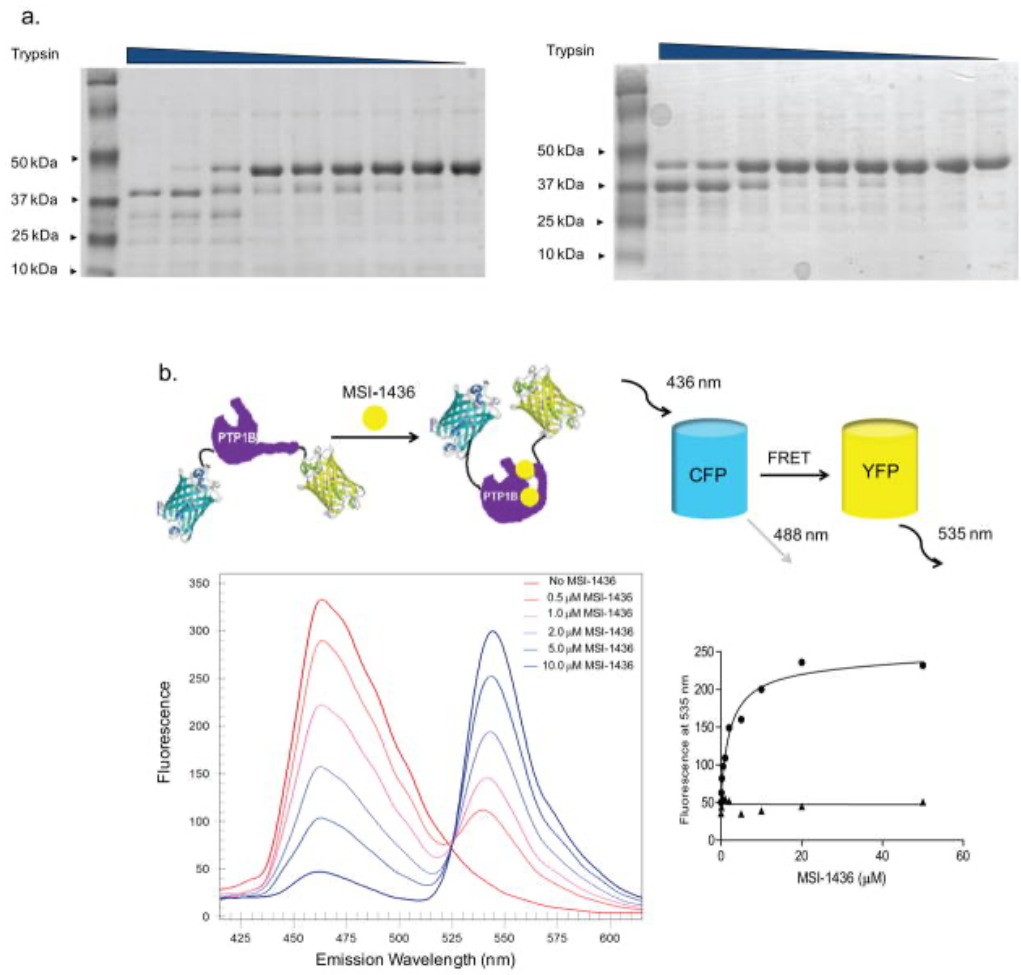


Weismann et al., 2014
Figure 2: Comparison of crystal structures. (A) Binding of compound 2 between α helices 3 and 6. (B) Location of α7 and Trp291 superposed to bound compound 2. (C) Superposition of the binding sites of compound 2 and Trp291 explains why α helix 7 becomes disordered upon displacement by compound 2. (D) WPD loop opens when compound 2 displaces Trp291.

The C-terminal tail of PTP1B has also been studied by NMR (Nuclear Magnetic Resonance) and was shown to be intrinsically disordered. Overlay of the 2D [^{1}H,^{15}N] TROSY (Transverse Relaxation Optimized Spectroscopy) NMR spectra of PTP1B full-length and catalytic domain shows that residues 300–393 are solvent-exposed and flexible. In this 2D NMR, the peaks are spread out in the spectrum as each peak's ^{15}N and ^{1}H frequencies reflect the unique chemical environment around the residue. So, when a part of the protein is exposed to solvent and flexible, the chemical environment of different nuclei is similar and averaged over several conformations. As a result, the corresponding peaks are clustered in the middle (Figure 3a). Despite the region being disordered, the secondary structure propensity (SSP) scores for PTP1B 300–393 residues show two partially populated helices, α8′ and α9′. α8′ is about 50% partially populated, while α9' is 20% partially populated (Figure 3b). Figure 3c is a representation of PTP1B full-length, and it demonstrates that the flexible PTP1B C-terminal tail has different conformations.



Krishnan et al., 2014
Figure 3: NMR studies of PTP1B C-terminal tail. (A) Overlay of 2D [^{1}H,^{15}N] TROSY spectra of PTP1B Full Length (purple), and PTP1B Catalytic Domain (black). (B) Secondary structure propensity (SSP) scores of residues 297-287, indicating that there are two partially populated helices. (C) 100 representative conformers of PTP1B C-terminal tail superimposed based on NMR constraints: PTP1B catalytic domain (yellow) and C-terminal tail (gray)



Krishnan et al., 2014
Figure 4: FRET studies of MSI1436 binding to the PTP1B C-terminal tail. (A) Left: In the absence of MSI-1436, C-terminal tail of PTP1B is trypsin-sensitive. Right: If the full length PTP1B is incubated with MSI-1436, PTP1B becomes more trypsin-resistant. Amount of trypsin decreases from left to right lanes. (B) Schematic representation MSI-1436 binding to full-length PTP1B fused to CFP and YFP, showing an increase of emission at 535nm as MSI-1436 concentration increases.

The intrinsically disordered C-terminal tail of PTP1B is sensitive to protease. However, when full-length of PTP1B is incubated with MSI-1436, the C-terminal tail becomes more trypsin-resistant (Figure 4a). The aminosterol natural product MSI-1436, or Trodusquemine is a non-competitive reversible PTP1B inhibitor, and is shown to have two binding sites in the C-terminal tail of PTP1B[7]. Förster Resonance Energy Transfer (FRET)

assays, in which full-length PTP1B is fused to CFP (Cyan Fluorescent Protein) and YFP (Yellow Fluorescent Protein) on the N- and C-termini, show that there is an increase in FRET emission at 535nm in the presence of MSI-1436. This indicates that N-terminally fused CFP is close enough to C-terminally fused YFP to transfer energy. In the absence of MSI-1436, there is a higher peak of emission at 488nm, suggesting that YFP is nowhere near CFP. These sets of experiments demonstrate that MSI-1436 achieves PTP1B inhibition by bringing the C-terminal domain closer to the catalytic domain (Figure 4b). This results in a more compact structure of PTP1B, restricting substrates access to the active site of PT1B[7]. In the same paper, the authors also tested the therapeutic potential of MSI-1436 in mice models of HER2 (Human epidermal growth factor receptor 2) -dependent breast cancer. MSI-1436 treated animals displayed a decrease in both tumor size and lung metastasis[7]. These experiments all highlight the effect that the C-terminal domain has in the enzyme, and that targeting the C-terminal tail, which is unique to this protein, can offer a new approach to potent and specific drug development for cancer.

The role of the C-terminal domain of TCPTP, including α helix 7 and the remaining protein tail, remains poorly defined, though one has been suggested based on comparisons with PTP1B. However, while the catalytic domain of TCPTP is 65% similar to PTP1B, the C-terminal domains have only 11% similarity (Figure 5). Despite the high similarity of their catalytic domains, the biological functions of TCPTP are different from PTP1B, as for example, TCPTP null mice die at 3-5 weeks of age[8] and PTP1B null mice displayed resistance to diet-induced diabetes and obesity[9]. In addition, the tail of the PTP1B is proline-rich, while the tail of TCPTP is positively charged. These differences highlight the importance of the

study of the TCPTP C-terminal domain as a separate subject as it can give rise to new drugs and therapeutics development, which can be potent and specific to each enzyme.

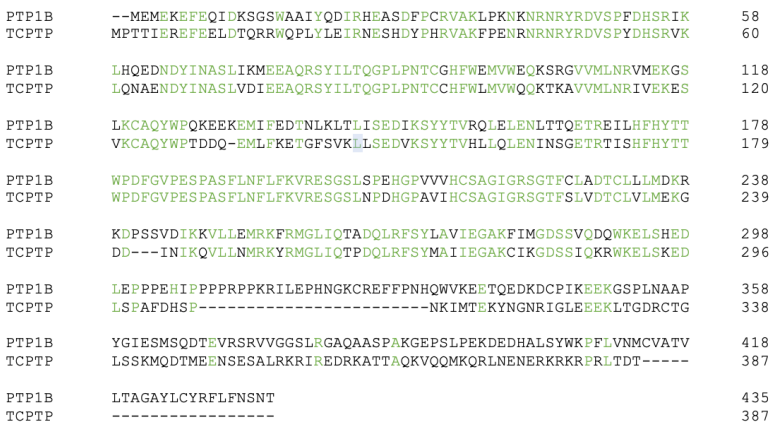
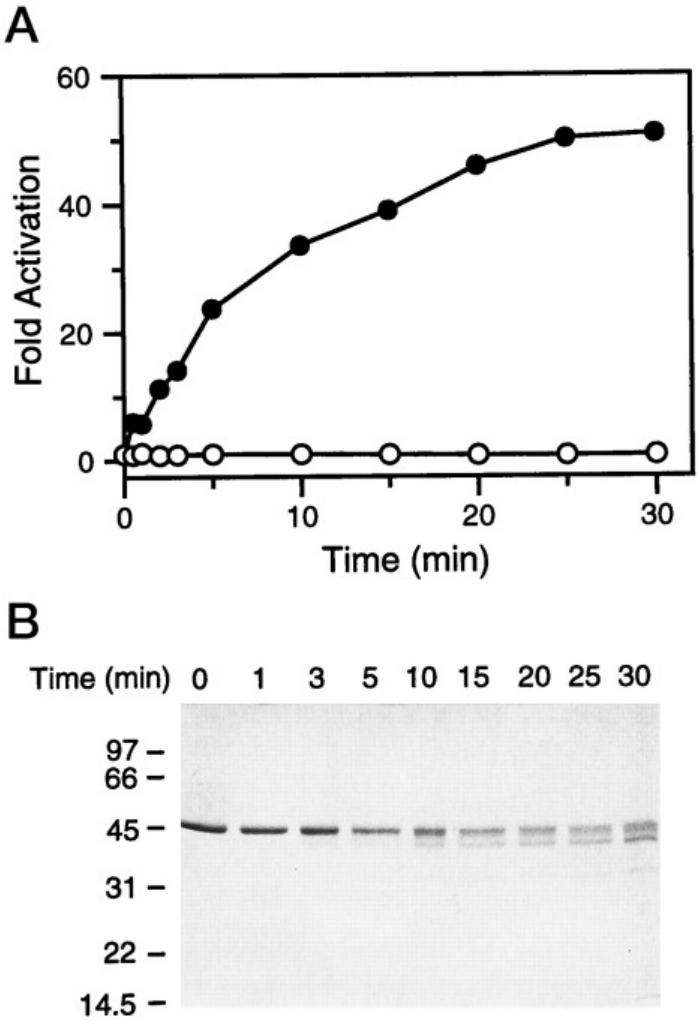


Figure 5: Sequence alignment of PTP1B and TCPTP. Identical residues are in green, different residues are in black. PTP1B full sequence and TC45 sequence are used.

TCPTP regulates multiple signaling pathways by binding to and dephosphorylating Janus-activated kinases, JAK1 and JAK3[10], and the transcriptional activators STAT3 and STAT5[8]. JAK-STAT pathways are responsible for inflammatory responses as they promote cytokine and interleukin signaling. TCPTP null mice have been observed to have splenomegaly and lymphadenopathy[9], which are signs of abnormal cytokine signaling. In addition, TCPTP has been associated to pathways that control diseases such as rheumatoid arthritis[4] and Crohn's disease[12]. Thus, modulating the activity of TCPTP is a promising strategy to tackle autoimmune diseases.

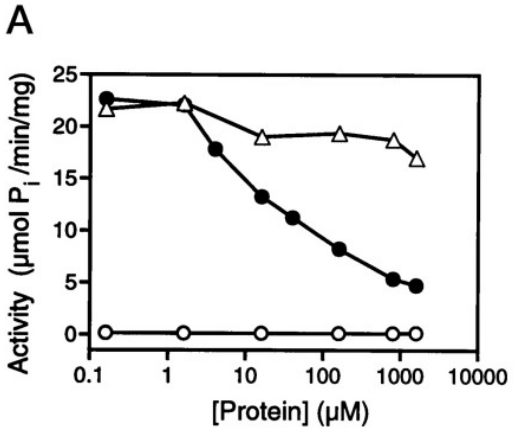
Previous studies suggested that TC45 is sensitive to protease, and when treated with trypsin, the 45 kDa protein generates a 33 kDa fragment with a transient intermediate 42 kDa fragment. However, the protease-sensitive region in TC45 remains unknown. By analyzing TC45 proteolytic fragments with mass spectrometry and N-terminal sequencing, it was observed that the C-terminal region (residues 352- 387) is protease-sensitive[12]. When TC45 is

subjected to limited proteolysis, in which the protein is incubated with low concentrations of trypsin in a controlled way, activation of TCPTP is observed over time, maxing out after 30 minutes (Figure 6). Even though the C-terminal tail of TCPTP is positively charged compared to the PTP1B proline-rich C-terminal tail, TCPTP C-terminal tail being sensitive to protease suggests that it might be an intrinsically disordered region, similar to PTP1B.



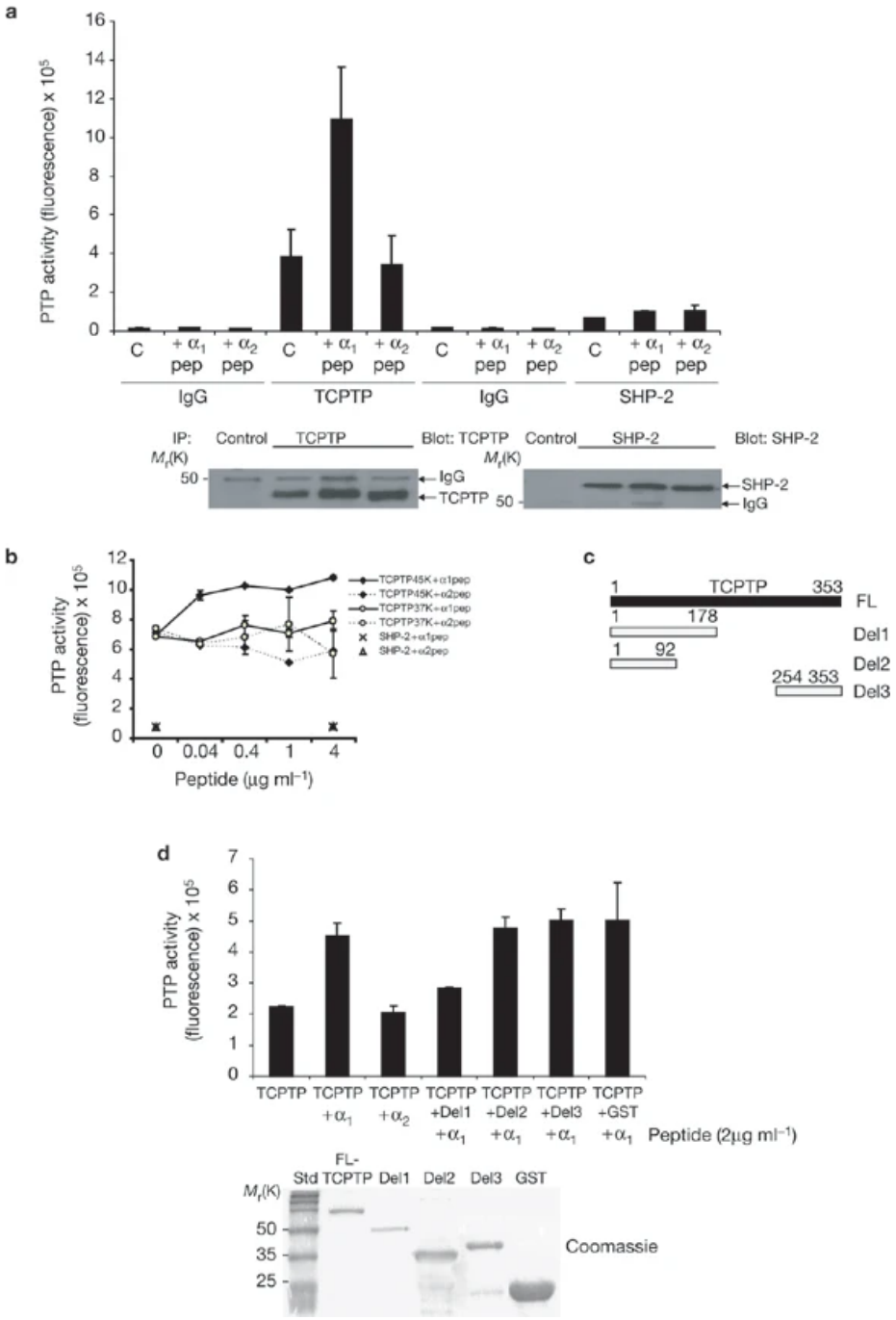
Hao et al., 1997
Figure 6: Limited proteolysis of TCPTP results in activation of the enzyme. (A) Enzymatic assays with the TCPTP limited proteolysis samples and 10uM reduced, carboxamidomethylated, and maleylated lysozyme (RCML) as substrate. Open circles: TCPTP without trypsin; Close circles: TCPTP with trypsin (B) Time course of proteolysis with 1:100 trypsin.

Moreover, when the 33 kDa fragment TCPTP encompassing the catalytic domain, which is more active than full length, is incubated with the tail fragment of TCPTP, but not that of PTP1B, in trans, its activity decreases, indicating that the C-terminal tail of TCPTP has the specific property of inhibiting the catalytic domain (Figure 7). Conversely, the activity of full length TCPTP when incubated with C-terminal tail remains the same, likely because it is already inhibited by the C-terminal tail in cis. This suggests that C-terminal tail inhibition requires a binding site within full length TCPTP. When the protein is truncated at residues 363 and 367, the activity increases compared to full-length TCPTP while the deletion of residues 350–358 and 353–371 inside the tail could only partially activate the protein[13]. This suggests that simply reducing the length of the protein is insufficient to activate the protein, highlighting that the specific C-terminal amino acid sequence of the tail plays a role in the auto-inhibition mechanism. However, the residues that play a role in auto-inhibition and how this mechanism affects TTCPTP behavior in cellular contexts have not yet been elucidated.



Hao et al., 1997
Figure 7: The C-terminal tail inhibits the 35 kDa catalytic domain TCPTP fragment. Phosphatase activity with RCML reveals that the activity of a 35 kDa TCPTP fragment decreases with increasing concentration of added C-terminal tail (closed circles). No significant inhibition is seen with isolated PTP1B C-terminal tail (triangle). There is no change in activity in TC45 full length protein with addition of the C-terminal tail (open circles).

The integrin α1 tail and the small molecule mitoxantrone have also been reported to activate full length TCPTP, but not the isolated PTP domain, by binding to the N-terminal domain[14,15]. Integrins are cells-adhesion receptors that are involved in the recognition of ligands in extracellular matrix. α1β1 integrin is responsible for cell proliferation and survival, immune functions, and embryonic developments. However, the function of integrins is dependent on the interaction with cytoplasmic proteins, as they do not have intrinsic catalytic activity in signaling pathways[13]. An interaction between TCPTP and endogenous α1β1 integrin was observed in HeLa Cells in response to collagen binding or mitogenic stimulation[13]. Furthermore, in response to cell adhesion to collagen, TCPTP catalytic activity increases. When HeLa cells lysates were treated with a synthetic α1 cytoplasmic tail peptide (α1 peptide: N-YGRKKRRQRRRWKLGFFKRPLKKKMEK-C) an increased was observed in the immunoprecipitated TCPTP activity. However, the treatment with an α2-derived peptide (N-KLGFFKRKYEKMTKNPDEIDETTELSS-C) led to no change in TCPTP catalytic activity (Figure 8a). The difference between α1 and α2 peptide is in the charge: α1 is more positively charges than α2, suggesting that positively charged amino acid residues might be responsible for activating TCPTP.



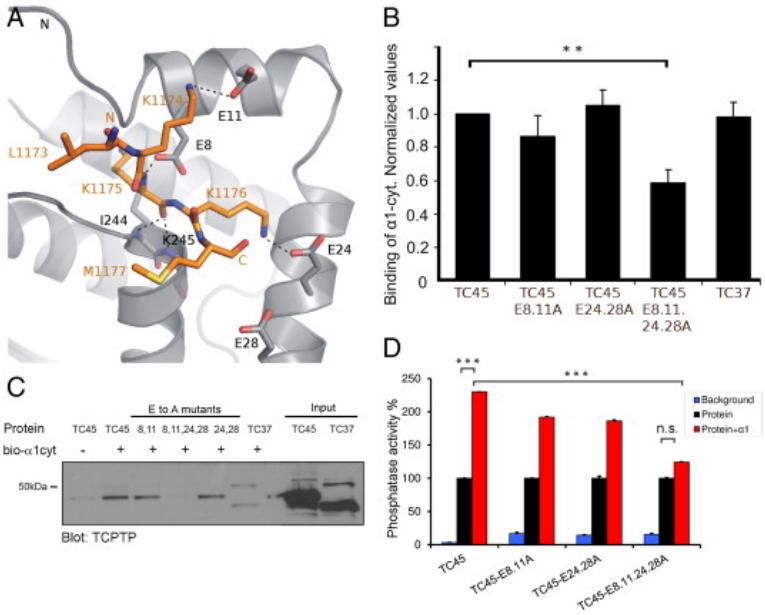
Mattila et.al, 2004

Figure 8: Integrin α1β1 activates TCPTP. (A) HeLa cell lysates were treated with synthetic α1 peptide and subjected to immunoprecipitation with anti-TCPTP antibody. SHP-2 was used as a control. (B) Treatment of recombinant TCPTP with synthetic peptides also activates TCPTP. (C) Representative scheme of deletion mutants to investigate the binding site of α1 peptides. (D) Phosphatase Activity with DiFMUP as substrate and TCPTP, deletion mutants, and α1 peptides.

In addition, similar findings of increased catalytic activity of TCPTP upon incubation with integrin peptides are observed with recombinant TCPTP. Synthetic α1 cytoplasmic tail peptide activates TCPTP, while a synthetic α2 cytoplasmic tail peptide does not (Figure 8b). To investigate the binding site of α1 cytoplasmic tail peptide to TCPTP, TCPTP fragments with different truncations were produced. Del1 mutation consists of 1-178 residues; Del2 mutation consists of 1-92 residues; Del3 mutation consists of C-terminal tail, 254-353 residues (Figure 8c). It was observed that TCPTP is not activated when the protein when deletion 1 mutant (N-terminal residue 1-178 fragment) is co-incubated with TCPTP full length and α1 peptide. This interference of del1 mutant to TCPTP activation indicates that del1 mutant contains the binding site for α1 peptide (Figure 8d). This suggests that the binding site of α1 peptide to TCPTP is the N-terminal domain of the enzyme, and the α1 peptide ultimately displaces the C-terminal tail binding to the catalytic domain, which results in the activation of the enzyme.

In addition to the activation of TCPTP by integrin α1β1, the small molecule mitoxantrone is also found to activate full length TC45. Mitoxantrone is an antitumor antibiotic, used to treat breast cancer, non-Hodgkin's lymphoma, and acute myelogenous leukemia[14]. This compound has a hydrophobic core and flexible arms carrying secondary amine groups, thus making it positively charged. Isothermal Titration Calorimetry (ITC) reveals that mitoxantrone binds to TCPTP 37kDa form, with a 1:1 stoichiometry suggesting that the C-terminal tail of TCPTP does not play a role in binding of mitoxantrone[14]. In addition, phosphatase assays with DiFMUP demonstrate that both mitoxantrone and integrin α1 tail activate TC45. Docking calculations reveal mitoxantrone binding to a surface area of

TC45 around the N-terminal residues, where the area of the protein is hydrophobic (L12, L21, I25, I244, L248, and L265), and surrounded by negatively charged glutamate residues (E8, 11, 24, and 28). When those glutamate residues are mutated to alanine, the binding of mitoxantrone assessed by surface plasmon resonance assay decreases to 41%, and the integrin α1 tail can longer activate TCPTP (Figure 8 b-d). Yet, there is no abolishment of binding with these glutamate to alanine mutations, indicating that there may be more areas in TCPTP catalytic domain that are important for binding of mitoxantrone or integrin α1 tail. These experiments suggest that both mitoxantrone and integrin α1 tail activate TC45 by potentially displacing the C-terminal tail from the catalytic domain. However, the mechanism of such activation, and whether this mechanism is intramolecular or intermolecular remains unknown.



Ylilauri et al., 2013

Figure 9: Binding of Mitoxantrone to TC37. (A) Scheme of the location where mitoxantrone binds to N-terminal glutamate residues of TCPTP. (B) Glutamate to Alanine substitutions reduce the binding affinity. (C) Recombinant TCPTP proteins were incubated with biotin-α1-peptide and coupled to streptavidin beads. The pull down was run on SDS-PAGE and blotted with anti-TCPTP. (D) Phosphatase Assays with DiFMUP showed that integrin α-peptide can no longer activate the mutant TCPTP.

DISCUSSION

Despite suggestions that full-length TCPTP has low phosphatase activity in vitro due to inhibition by the C-terminal domain, there are no studies confirming that TCPTP is kept in an autoinhibited state in cells or whether the autoinhibition is due to an intramolecular or intermolecular interaction. In order to confirm that TCPTP autoinhibition exists in cells, we will be performing IL-6 stimulation assays since TCPTP is known to dephosphorylate STAT3. The experiments can be performed in HEK293T cells with TCPTP overexpression. Western blot with anti-phospho-STAT3(Y705) or STAT3-Luciferase reporter assays will establish that TCPTP plays a role in dephosphorylation of STAT3 in our system. This can be confirmed by treating the cells with a TCPTP specific inhibitor, such as the reversible inhibitor compound 8, and observe hyperphosphorylation of STAT3 by western blotting. Then wildtype TCPTP and the C-terminal truncation at aa 304 (CD304) can be overexpressed followed by comparison of the phosphorylation levels of STAT3 with the expectation that CD304 is a gain-of-function mutant. In addition, TCPTP knock-out cell lines will be made by CRISPR followed by re-expression of TCPTP to confirm the phenotype.

TCPTP catalytic domain lacks the nuclear localization signal (NLS), introducing some uncertainty as to whether catalytic domain of TCPTP is more active than the full length (which is nuclear localized) even if lower STAT3 phosphorylation is observed. Therefore, we will generate a construct with TCPTP catalytic domain carrying a NLS, allowing translocation to the nucleus, and a more straightforward comparison between the two forms of the enzyme. To confirm that TCPTP catalytic domain is in the nucleus, we will also be generating mEGFP (monomeric enhanced green fluorescent protein)- TCPTP (full length and catalytic domain) fusion constructs. By using fluorescence microscopy, we can confirm their nuclear

localization and show that the differences in STAT3 dephosphorylation level is likely due to the enzymatic activity, and thus demonstrate that the C-terminal tail of TCPTP inhibits the catalytic activity of TCPTP in cells.

Secondly, the mechanism of how TCPTP achieves the inhibition remains a question. Analysis of available literature leads to the hypothesis that the C-terminal tail might inhibit substrate access to the active site by binding to the catalytic domain of TCPTP in an ionic strength-dependent fashion. Therefore, we will do mutagenesis of amino acids in both N- and C-terminal domains and perform enzymatic assays to determine which part of the protein is responsible for the auto-inhibition. For example, the binding and activation of mitoxantrone and integrin peptide reveals an area in N-terminal of TCPTP, where the glutamate residues (E8, 11, 24, and 28) and hydrophobic areas reside (L12, L21, I25, I244, L248, and L265). Since the TCPTP C-terminal tail is enriched with positive charge residues (377- 384), the negative charges of TCPTP catalytic domain suggest that the positive charges of C-terminal tail might bind to the catalytic domain, inhibiting the substrates from access to the catalytic site. To confirm that those two sites are responsible for the autoinhibition mechanism, we can do mutagenesis of all those sites, and perform enzymatic assays. As substrates in the enzymatic assays, we will be using both DiFMUP, an artificial small-molecule phosphatase substrate, and an EGFR-derived 12-mer peptide. We will use at least two different substrates since previous report showed that, unlike with RCML, no auto-inhibitory mechanism occurs when using the small-molecule substrate pNPP. These experiments can reveal the areas of the protein responsible for the inhibition mechanism.

Lastly, the question of whether this auto-inhibition mechanism is intramolecular or intermolecular remains unknown. Förster resonance energy transfer can be used to distinguish

between these two possibilities. We will generate a construct in which the amino and carboxy termini of TCPTP are fused to the FRET pair CFP/YFP. After excitation at 430 nm, the expected CFP emission in the absence of FRET is at 475 nm; however, if the two ends of TCPTP are brought together due to the compact state of the autoinhibited form, the energy from CFP is transferred to the YFP, and the expected emission will be at around 530 nm. Enzymatic assays will be performed and slower rate of dephosphorylation observed along with strong FRET will be an indication of intramolecular inhibition. These sets of experiments will help us clarify the inhibition mechanism by C-terminal tail and give us insights on how TCPTP controls its own catalysis rate, which can help design new potentials for drug developments for cancer and autoimmune diseases.

REFERENCES

1. Ardito, F., Giuliani, M., Perrone, D., Troiano, G., Lo Muzio, L."The crucial role of protein phosphorylation in cell signaling and its use as targeted therapy (Review)". International Journal of Molecular Medicine. 2017

2. Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J, Mustelin T. Protein tyrosine phosphatases in the human genome. Cell. 2004

3. Tautz L, Critton DA, Grotegut S. Protein tyrosine phosphatases: structure, function, and implication in human disease. Methods Mol Biol. 2013

4. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML, Kennedy BP. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science. 1999

5. Svensson MN, Doody KM, Schmiedel BJ, Bhattacharyya S, Panwar B, Wiede F, Yang S, Santelli E, Wu DJ, Sacchetti C, Gujar R, Seumois G, Kiosses WB, Aubry I, Kim G, Mydel P, Sakaguchi S, Kronenberg M, Tiganis T, Tremblay ML, Ay F, Vijayanand P, Bottini N. Reduced expression of phosphatase PTPN2 promotes pathogenic conversion of Tregs in autoimmunity. J Clin Invest. 2019

6. Wiesmann C, Barr KJ, Kung J, Zhu J, Erlanson DA, Shen W, Fahr BJ, Zhong M, Taylor L, Randal M, McDowell RS, Hansen SK. Allosteric inhibition of protein tyrosine phosphatase 1B. Nat Struct Mol Biol. 2004

7. Krishnan, N., Koveal, D., Miller, D. H., Xue, B., Akshinthala, S. D., Kragelj, J., Jensen, M. R., Gauss, C. M., Page, R., Blackledge, M., Muthuswamy, S. K., Peti, W., & Tonks, N. K. Targeting the disordered C terminus of PTP1B with an allosteric inhibitor. Nature chemical biology. 2014

8. You-Ten KE, Muise ES, Itié A, Michaliszyn E, Wagner J, Jothy S, Lapp WS, Tremblay ML. Impaired bone marrow microenvironment and immune function in T cell protein tyrosine phosphatase-deficient mice. J Exp Med. 1997

9. Simoncic PD, Lee-Loy A, Barber DL, Tremblay ML, McGlade CJ. The T cell protein tyrosine phosphatase is a negative regulator of janus family kinases 1 and 3. Curr Biol. 2002

10. Yamamoto T, Sekine Y, Kashima K, Kubota A, Sato N, Aoki N, Matsuda T. The nuclear isoform of protein-tyrosine phosphatase TC-PTP regulates interleukin-6-mediated signaling pathways through STAT3 dephosphorylation. Biochem Biophys Res Commun. 2002

11. Hassan SW, Doody KM, Hardy S, Uetani N, Cournoyer D, Tremblay ML. Increased susceptibility to dextran sulfate sodium induced colitis in the T cell protein tyrosine phosphatase heterozygous mouse. PLoS One. 2010

12. Hao L, Tiganis T, Tonks NK, Charbonneau H. The noncatalytic C-terminal segment of the T cell protein tyrosine phosphatase regulates activity via an intramolecular mechanism. J Biol Chem. 1997

13. Mattila E, Pellinen T, Nevo J, Vuoriluoto K, Arjonen A, Ivaska J. Negative regulation of EGFR signalling through integrin-alpha1beta1-mediated activation of protein tyrosine phosphatase TCPTP. Nat Cell Biol. 2005

14. Ylilauri M, Mattila E, Nurminen EM, Käpylä J, Niinivehmas SP, Määttä JA, Pentikäinen U, Ivaska J, Pentikäinen OT. Molecular mechanism of T-cell protein tyrosine phosphatase (TCPTP) activation by mitoxantrone. Biochim Biophys Acta. 2013

15. Tiganis T, Flint AJ, Adam SA, Tonks NK. Association of the T-cell protein tyrosine phosphatase with nuclear import factor p97. J Biol Chem. 1997