Comparison of the Voltage Sensitive Phosphatases from Vertebrate Species

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August 4, 2016

MONTANA INBRE IDeA Network of Biomedical Research Excellence

MONTANA STATE UNIVERSITY

2016 Cell Biology and Neuroscience Research Experience for Undergraduates (CBN-REU) Program

ABSTRACT

The voltage sensing phosphatase (VSP) is a transmembrane protein which regulates the phosphatidylinositol phosphate (PIP) signaling pathway in a voltage dependent manner. VSP is unique because it is the first example of a voltage regulated enzyme and suggests a direct link between the membrane potential and the PIP signaling pathway. The membrane potential is an important signal in normal cellular processes controlling neuronal signaling, muscle contractions, and immune responses while PIPs regulate many different processes in the cell, including membrane trafficking, promoting cell death, and cell growth[1]. When either pathway is compromised, many serious diseases can occur, including autism[2], epilepsy[3], and cancer[4].

The phosphatase and tensin homolog (PTEN) is a tumor suppressor frequently mutated in cancer[4]and a 3-phosphatase of phosphatidylinositol-3,4,5-trisphosphate, $PI(3,4,5)P_3$. The catalytic domain of VSP shares a 44% identity with PTEN; however, VSP functions as both a 3- and 5-phosphatase[5,6,7]. Interestingly, VSP has been found to be expressed in non-small cell carcinoma and hepatobiliary cancers[8], suggesting it may also play a role in cancer and could indicate an unexplored role of voltage in cancer cell propagation.

The majority of VSP research has focused on the tunicate *Ciona intestinalis* (sea squirt) species of the protein (Ci-VSP) and very little is known about the vertebrate VSPs. I have been studying the vertebrate VSP species *Gallus gallus* (chicken, Gg-VSP) and *Danio rerio* (zebrafish, Dr-VSP) in order to compare the functions of these vertebrate species to Ci-VSP. Dr-VSP has been successfully mutated for voltage clamp fluorometry (VCF) experiments. VCF is a technique that changes the voltage of the VSP, flourescently tagged, expressed cell activating and causeing protein movement which changes the fluorescent read out. Several of the Dr-VSP mutations have expressed and display voltage-dependent fluorescence changes that vary from the equivalent Ci-VSP mutation suggesting that the different species of VSP do not all function simiarily. The rest of the vertebrate species are still being mutated to include labeling sites.

INTRODUCTION

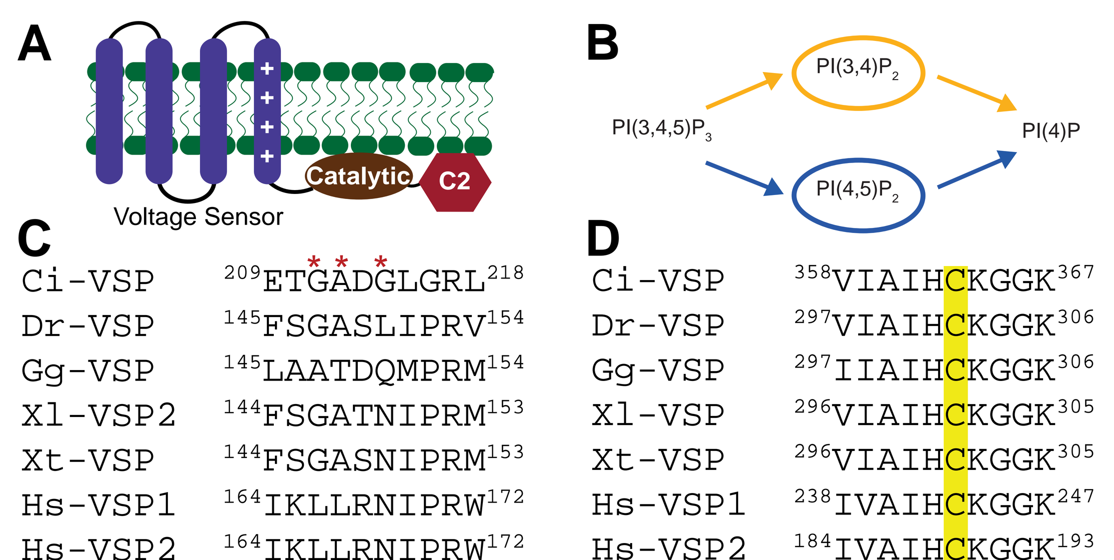

Figure 1. A) Cartoon of the voltage sensing phosphatase. B) Cartoon of VSP's enzymatic activity with PIPs. VSP functions as both a 3- and a 5-phosphatase of PIPs. C) Alignment of the S3-S4 linker. Astericks indicate amino acids that have been the focus of this study. D) Alignment of the active site. The active site of VSP has a highly conserved sequence. When the highlighted cysteine is mutated in Ci-VSP all enzymatic activiy is stopped.

PHYSIOLOGICAL IMPORTANCE

Animal	Adult	Juvenile
Human H-VSP1 & 2	brain, stomach, testes[10,11]	unknown
Sea Squirt Ci-VSP	neural complex, blood, testes[12,13]	stomach, intestine, blood[12,13]
Chicken Gg-VSP	testes[14,15]	kidney, brain, stomach[14,15]
Zebrafish Dr-VSP	ovaries, testes[16,17]	kidney, eye[16,17]
Frog Xl-VSP1&2; Xt-VSP	liver, kidneys, ovaries, testes[18]	unknown

Table 1. Comparision of the VSP expression patterns in the organs of species. VSP expression is highly variable not only between speecies but also between adult and juvenile stages of the same species.

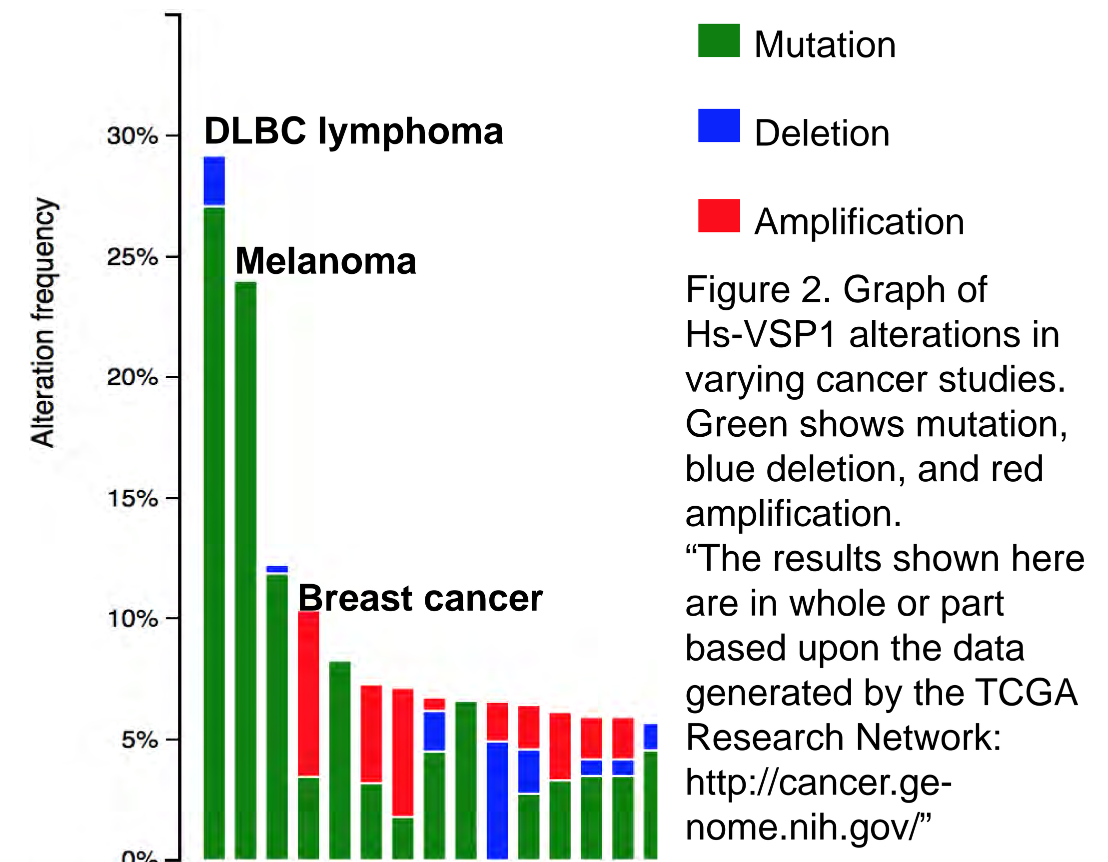

Figure 2. Graph of Hs-VSP1 alterations in varying cancer studies. Green shows mutation, blue deletion, and red amplification. "The results shown here are in whole or part based upon the data generated by the TCGA Research Network: http://cancer.genome.nih.gov/"

METHODS

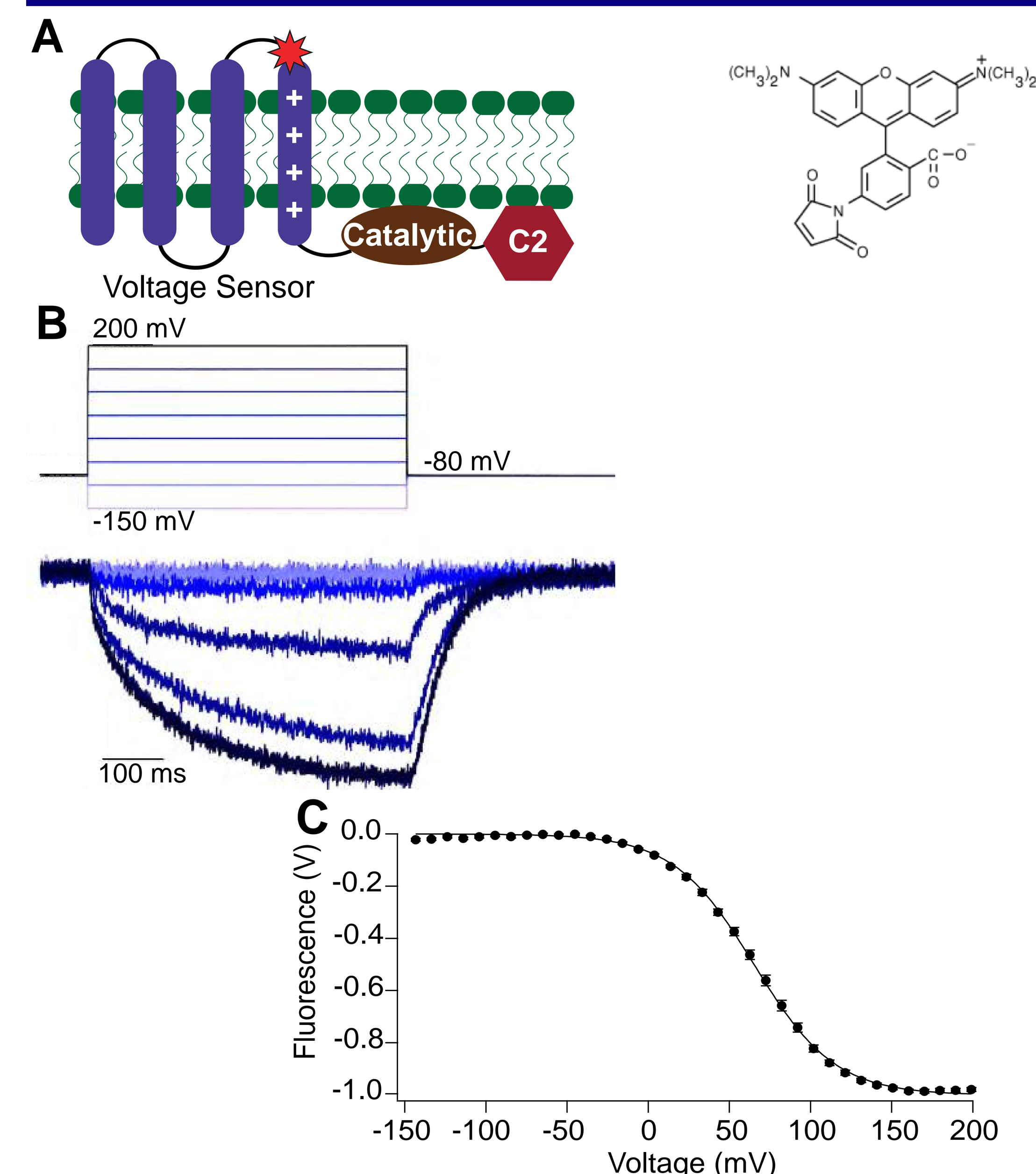

Figure 3. A) Cartoon showing the location of the labeling site for TMRM. TMRM structure shown at right. B) Voltage protocol and corresponding fluorescence traces of wild type Ci-VSP G214C. 50mV steps. Representative cell. C) Fluorescence to voltage graph of Ci-VSP G214C. Error bars are S.E.M. n = 10

REPRESENTATIVE DATA

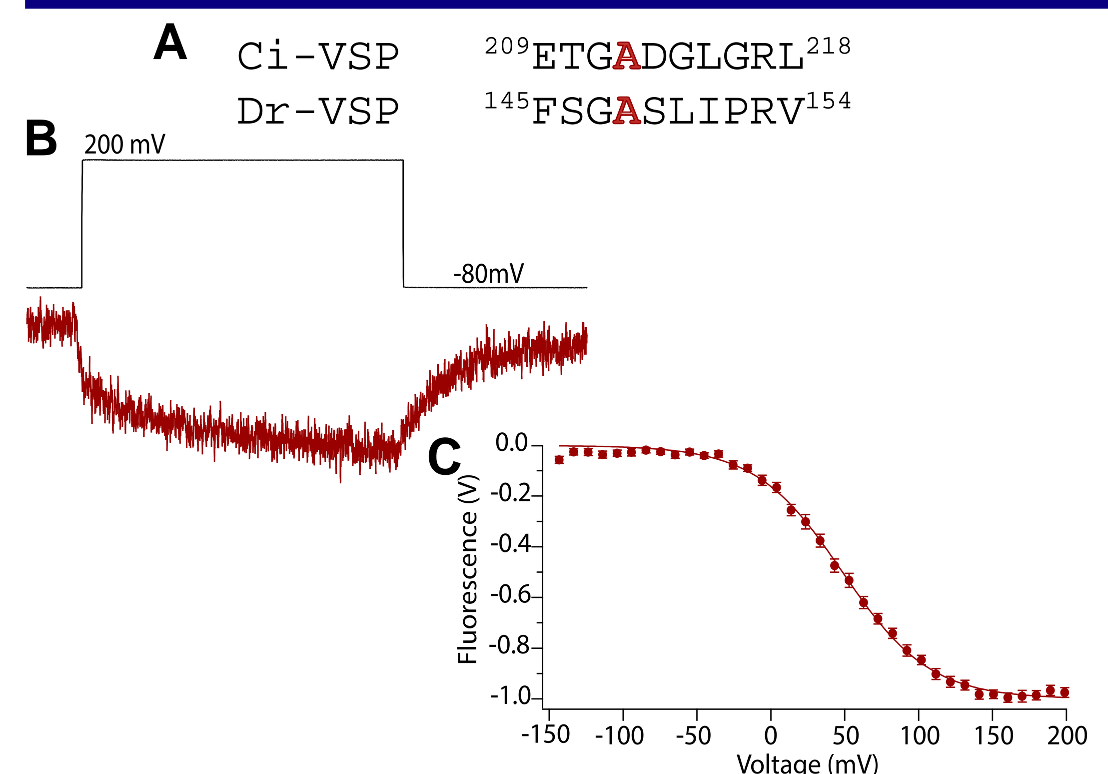

Figure 3. Dr-VSP A148C. A) Alignment of Ci-VSP and Dr-VSP labeling sites. Dr-VSP A148C and Ci-VSP equivalent site in red B) Fluorescence change corresponding to a voltage pulse to 200mV. A148C also only shows a two part decrease. Representative cell. C) Fluorescence to voltage graph. Dr-VSP A148C moves beginning around -10mV. Error bars are S.E.M. n = 13

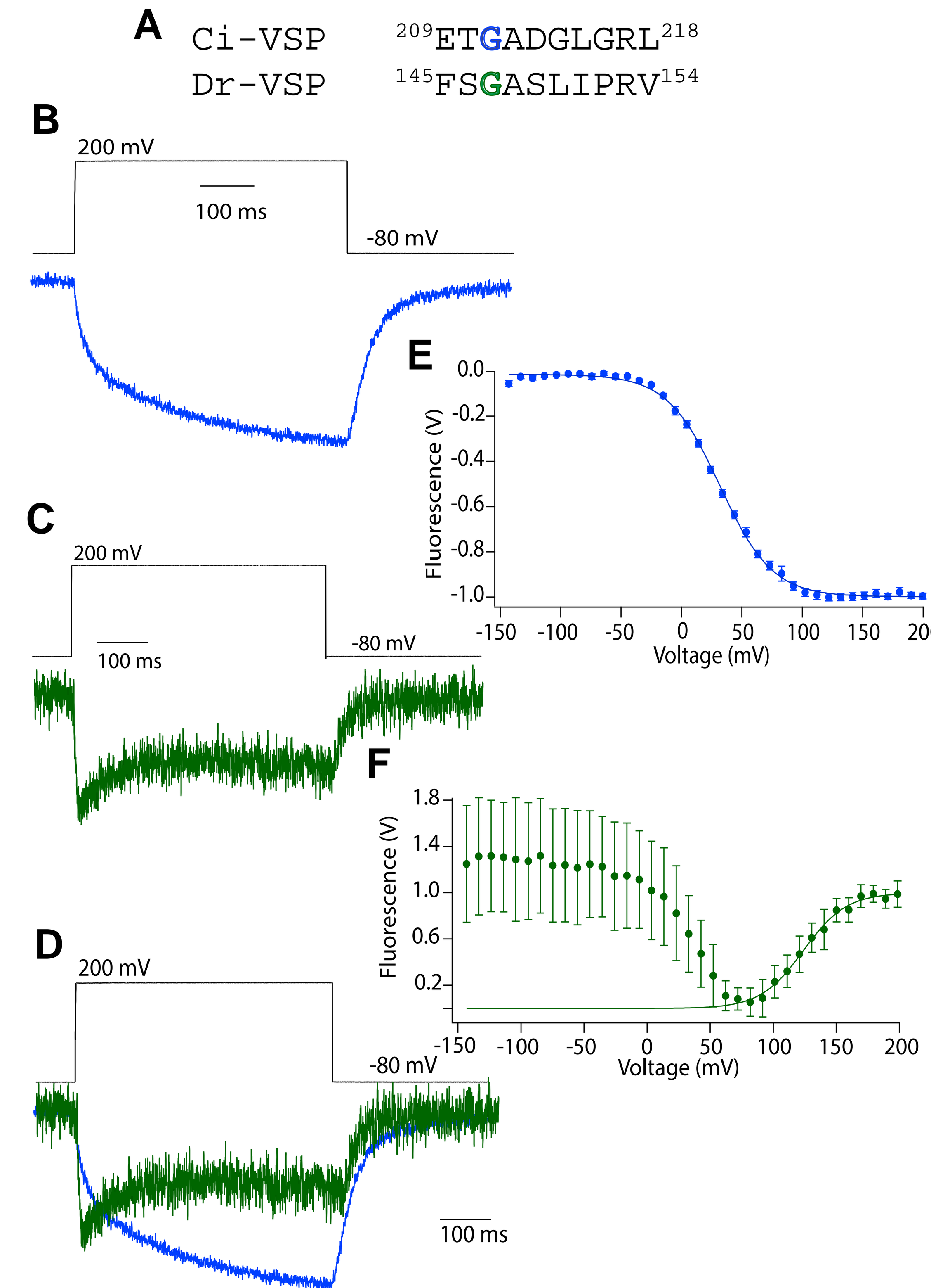

Figure 4. Ci-VSP G211C compared to Dr-VSP G147C A) Alignment of Ci-VSP and Dr-VSP labeling sites. Ci-VSP G211C and Dr-VSP G147C in blue and green respectively. B, C) Fluorescence change corresponding to a voltage pulse to 200mV. Representative cells. B) Ci-VSP G211C exhibits a two part decreasing movement. C) Dr-VSP G147C exhibits a three part decreasing-increasing-decreasing movement. D) Overlay of fluorescence traces. E, F) Fluorescence to voltage graphs. E) Ci-VSP G211C moves beginning around -20mV. n=14 F) Dr-VSP G147C moves above 0mV. n=3 All error bars are S.E.M.

NEW DIRECTION

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Ci-VSP    209ETGADGLGRL218
Dr-VSP    145FSGASLIPRV154
Gg-VSP    145LAATDQMPRM154
Xl-VSP2   144FSGATNIPRM153
Xt-VSP    144FSGASNIPRM153
Hs-VSP1   164IKLLRNIPRW172
Hs-VSP2   164IKLLRNIPRW172
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Figure 5. Current and future labeling sites based off of previous Ci-VSP labeling sites and Ci-VSP and Dr-VSP comparisions with VCF and amino acid alignments.

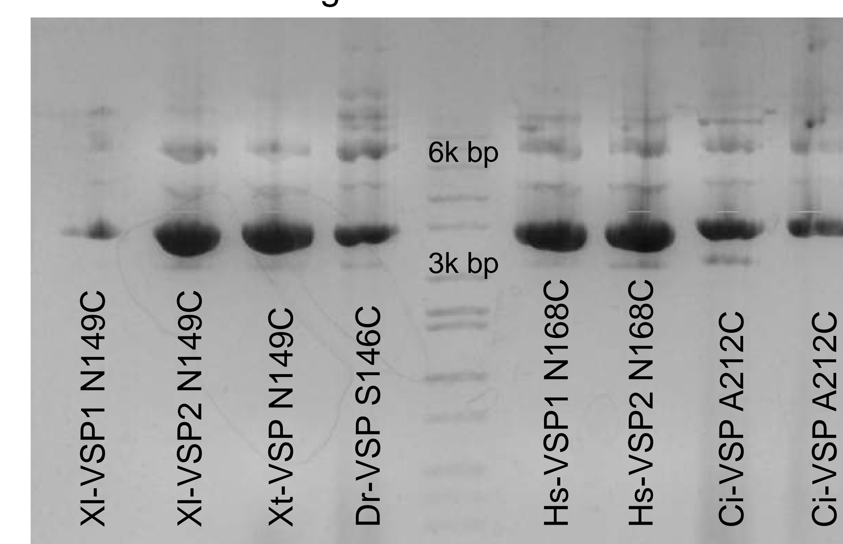

Figure 6. Gel of uncut miniprep labeling site DNA. Gel order from left to right: XI-VSP1 N149C, XI-VSP2 N149C, Xt-VSP N149C, Dr-VSP S146C, 1000kbp ladder, Hs-VSP1 N168C, Hs-VSP2 N168C, Ci-VSP A212C, and Ci-VSP T210C. All labeling sites except for the Ci-VSP A212C and T210C mutations are Ci-VSP G214C equivalents.

CONCLUSIONS

Different species have different movements even at equivalent amino acids and also exhibit different voltage dependencies.

FUTURE WORK

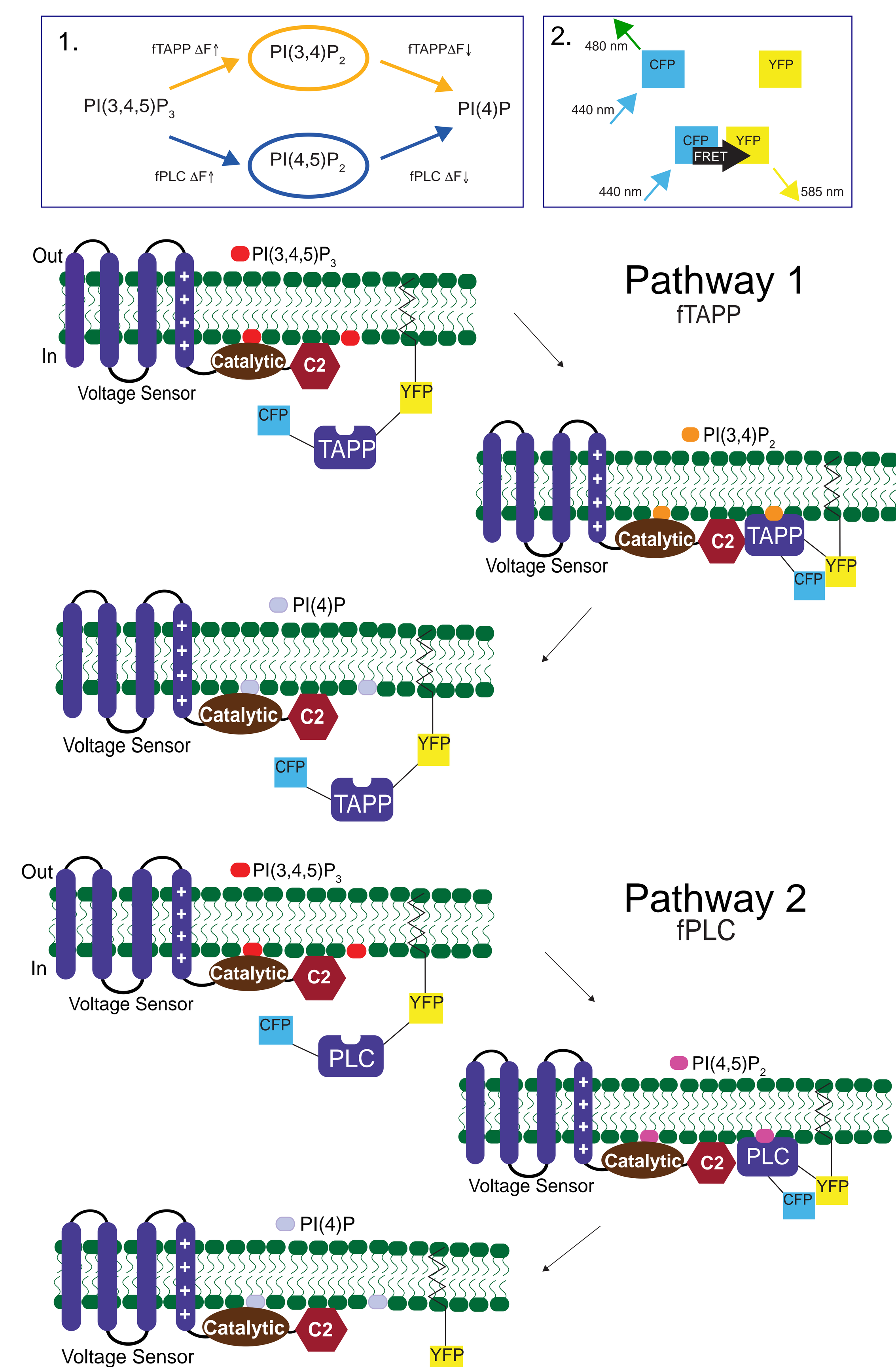

Figure 7. Cartoon showing how the FRET/PH-domains[19] interact with PIPs. Pathway 1 shows the fTAPP-PH domain binding with $PI(3,4)P_2$ then releasing when $PI(3,4)P_2$ is dephosphorylated into PI(4)P. Pathway 2 shows the fPLC-PH domain binding with $PI(4,5)P_2$ then releasing when $PI(4,5)P_2$ is dephosphorylated into PI(4)P. Insert 1 shows fluorescence changes from fPH-domains as PIPs are dephosphorylated. Insert 2 is a FRET diagram. FRET only occurs when the CFP and YFP are close enough together.

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ACKNOWLEDGEMENTS

The REU in Cell Biology Program is supported by NSF Research Experiences for Undergraduates grant # DBI-0453021 to Montana State University.
Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM103474. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
NIH R01GM111685 to Dr. Susy Kohout