seminars in **CELL BIOLOGY**, Vol 4, 1993 : pp 389-396

Bacterial and viral protein tyrosine phosphatases

Kun-Liang Guan and Jack E. Dixon†*

Unrestricted protein tyrosine phosphatase (PTPase) activity may play a role in pathogenesis. For instance, the virulence determinant gene, yopH, of Yersinia pseudotuberculosis encodes a PTPase. The phosphatase activity of the YopH protein is essential for the pathogenesis of Y. pseudotuberculosis. Yersinia pestis, the bacterium which causes the bubonic plague, also contains a gene closely related to yopH. The action of YopH on host proteins appears to break down signal transduction mechanisms in many cell types including those of the immune system. This may contribute to the ability of the bacterium to escape effective surveillance by the immune system. The vaccinia virus VH1 gene, like yopH in the Yersinia bacteria, encodes a protein phosphatase. The VH1 PTPase defines a new class of phosphatases capable of dephosphorylating both phosphoserine/threonine and tyrosine containing substrates. Proteins sharing sequence identity to this dual-specificity phosphatase have been identified from other viruses, yeast and man. Although a complete understanding of the function of these dual-specificity phosphatases is not presently available, they clearly play important roles in cell cycle regulation, growth control and mitogenic signaling mechanisms. The unique catalytic properties of the dual specificity phosphatases suggest that these catalysts constitute a distinct subfamily of phosphatases.

Key words: bacterial pathogenesis / enzyme catalysis / phosphatases

PROTEIN PHOSPHORYLATION is a widely used mechanism in cellular regulation. The enzymes which control the phosphorylation status of a cell are protein kinases and phosphatases. Protein kinases are typically classified into two families, serine/threonine kinases and tyrosine kinases, based on their substrate specificity.[1] Protein kinases having a dual-specificity for both serine/threonine and tyrosine residues have also recently been described.[1-3] Similar criteria have been used to classify protein phosphatases.[4,5] There are protein phosphatases (PP) which preferentially dephosphorylate phosphoserine and threonine residues (for review see ref 4). Protein tyrosine phosphatases (PTPases) are enzymes which dephosphorylate phosphotyrosine residues.[5-7] The superfamily of protein kinases (i.e. tyrosine as well as Ser/Thr kinases) all share amino acid sequence identity. In contrast, PTPases show no sequence identity to the protein serine/threonine phosphatases.

The PTPases comprise a diverse family of enzymes which can be structurally divided into two groups, receptor-like and non-receptor PTPases (refs 5-7; H. Saito, this issue, pp 379-387). The receptor-like PTPases have an extracellular domain of variable length, a transmembrane domain, and usually two, tandemly repeated intracellular phosphatase domains. The non-receptor PTPases have a single phosphatase domain with divergent N-terminal or C-terminal amino acid extensions.[5-7] These N- and C-terminal extensions may include sequence motifs such as src homology 2 (SH2) domains, intracellular localization signals, signals regulating protein stability, or sequences which have amino acid identity to cytoskeletal proteins.

The purpose of this review is to discuss two unique phosphatases. First we will discuss a PTPase found in the pathogenic bacterial genus *Yersinia* which plays an essential role in the virulence of this microorganism. Then, we will devote our attention to a virus-encoded phosphatase which can efficiently dephosphorylate both phosphoserine/threonine and phosphotyrosine residues. Cellular homologs of the viral phosphatase will also be addressed.

Phosphatase and the bubonic plague

A result which underscores the importance of PTPase regulation in normal cellular function was uncovered by identifying a PTPase in the pathogenic bacterial genus *Yersinia*.[8] The genus *Yersinia* is comprised of three species, *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*. *Yersinia* is a causative agent in human disease ranging from gastrointestinal syndromes to the bubonic plague.[9] Although a complete understanding of the pathogenic process in any of these diseases is not available, several of the bacterial virulence

*From the *Department of Biological Chemistry and Institute of Gerontology, †Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI 48109-0606 and the Walther Cancer Institute, Indianapolis, IN, USA*
©1993 Academic Press Ltd
1043-4682/93/060389 + 08$8.00/0

factors have been located on a naturally occurring 70 kb plasmid.[10] Bacteria without this 70 kb plasmid are avirulent but grow normally *in vitro*. The expression of a set of proteins encoded on the virulence plasmid is correlated with the capacity of the bacterium to avoid host defense mechanisms. These proteins, also known as YOPs (for *Yersinia* outer membrane proteins), are secreted into the bacterial periplasmic space. The *yop51* gene (encoding 51 kDa protein) of *Y. enterocolitica* has been defined as a key virulence determinant, and mutation in this gene alters the virulence of *Yersinia*.[11]

We have demonstrated that the C-terminal region of the *Yersinia* 51 kDa protein shares amino acid sequence identity to the catalytic domain of several PTPases.[8] A number of invariant residues conserved in the eukaryotic PTPases are also conserved in Yop51 (Figure 1). This unexpected observation raised a number of interesting questions: (1) does the Yop51 gene encode a PTPase? (2) is the phosphatase activity required for virulence? (3) what are the substrates of the phosphatases? The latter question is particularly intriguing because bacteria are generally believed to contain no tyrosine phosphorylated protein.

The first question was addressed by using recombinant Yop51 protein produced in *Escherichia coli*. The recombinant Yop51 protein was capable of dephosphorylating tyrosine phosphorylated substrates.[8] Like all the other mammalian PTPases, the *Yersinia* phosphatase had an absolute specificity for phosphotyrosine and did not hydrolyze phosphoserine or phosphothreonine-containing substrates. Furthermore, a mutation of the cysteine residue which is absolutely conserved in all PTPases completely abolished the phosphatase activity of Yop51. These experiments unequivocally demonstrated that Yop51 has tyrosine phosphatase activity. Recombinant Yop51 has been purified from *E. coli* in large quantities.[12] Recombinant Yop51 has the highest catalytic activity among all of the PTPases characterized to date,[12] with a k_{cat} value 10^2 to 10^5 higher than any of the other PTPases (Table 1). This has made it possible to define further residues important in binding or catalysis. Crystals of the catalytic domain of Yop51 have been obtained and an X-ray structure of the protein is in progress (M. Saper *et al*, unpublished data). Yop51 serves as a good model for kinetic and functional studies of the PTPase family of proteins.

All three species of *Yersinia* contain the highly related 70 kb virulence plasmid.[10] The *yop51* homolog of *Y. pseudotuberculosis* is the *yopH* gene, which encodes a protein having 99% amino acid sequence identity to Yop51.[13] Genetic evidence has demonstrated that the *yopH* gene is also important for virulence. Bliska *et al* have conducted an elegant experiment to determine the relationship between the phosphatase activity and virulence.[14] The catalytically essential cysteine residue was mutated to an alanine in the *yopH* gene by site-directed mutagenesis. This PTPase deficient *yopH* gene was used to replace the wild-type *yopH* gene by homologous recombination. Two *Y. pseudotuberculosis* strains, one with an active *yopH* protein and the other phosphatase-deficient, were used to infect mice. The mice infected with the active *yopH* gene were moribund 5 days after infection. In contrast, mice infected with the catalytically-inactive *yopH* gene survived after day 7, and had only mild disease symptoms. These observations indicated that the intrinsic PTPase activity of YopH is essential for *Yersinia* to cause disease in the mouse model. The mice infected with the wild-type bacterium had 10 to 100 times more bacteria in their spleen than those infected with the PTPase-deficient bacterium. In culture, *Yersinia* with the catalytically-inactive *yopH* gene had a doubling time identical to bacteria harboring the wild-type gene. This suggested that the growth of PTPase-deficient *Yersinia* in the infected mice was suppressed by the host, while the wild-type bacterium was able to escape the host immune system and grew efficiently.

Y. pestis is the causative agent of the plague or the 'black death'. The bubonic plague has been one of the world's most devastating pandemics. In the 14th century, the eruption of the plague in Europe killed about one quarter of the continental population.[9] The most recent pandemics of the plague occurred in Asia, with more than 10 million deaths. *Y. pestis* also contains a 70 kb plasmid, similar in composition to the plasmid found in the other species of *Yersinia*. The amino acid sequence of the *yop51/yopH* equivalent gene in *Y. pestis* has not been determined, however, Southern hybridization using the *yopH* gene as a probe has demonstrated that a *yopH/yop51*-related gene is highly conserved in *Y. pestis*.[10] It is likely that the PTPase also plays a critical role in this devastating disease which has altered the course of human history.

Substrates for *Yersinia* PTPase

In bacteria phosphotyrosine-containing proteins are rare, suggesting that the substrates of the *yopH* gene

```
Yop51   QACGGEKLHR FRDIQCCRQT AW........ .......... ..........
YPTP1   IEPRNDARHR YVNIMPYERN RVHL...... .......... ..........
YPTP2   EGIQSFTKHR YSNILPYEHS RVKLPHSPKP PAVSEASTTE TKTDKSYPMC
hTcell  KFPENRNRHR YRDVSPYDHS RVKL...... .......... ..........
rPTP1   KLPKNKNRHR YRDVSPFDHS RIKL...... .......... ..........
hPTP1C  QRPENKGKHR YKNILPFDHS RVIL...... .......... ..........
rLAR    NSEVNKPKHR YANVIAYDHS RVLL...... .......... ..........
Mega1   KLPQNISKHR YRDISPYDAT RVIL...... .......... ..........
PEP     QRPKNIKKHR YKDILPYDHS LVEL...... .......... ..........

Yop51   .......... RAD.LNANYI QVGNTRT... ....IACQYP LQSQLESHFR
YPTP1   ......KTLS GNDYINASYV KVNVPGQSIE PGYYIATQGP TRKTWDQFWQ
YPTP2   PVDAKNHSCK PNDYINANYI NVTQINPDFK ...YIATQAR LPSTMDDFWK
hTcell  .......QNA ENDYINASLV .....DIEEA QRSYILTQGP LPNTCCHFWL
rPTP1   .......HQE DNDYINASLI .....KMEEA QRSYILTQGP LPNTCGHFWE
hPTP1C  ..QGRDSNIP GSDYINANYI KNQLLGPDEN AKTYIASQGC LEATVNDFWQ
rLAR    ...TSIDGVP GSDYINANYI .....DGYRK QNAYIATQGP LPETMGDFWR
Mega1   ........KG NEDYINANYI .NMEIPSSSI INQYIACQGP LPHTCTDFWQ
PEP     ...SLLTSDE DSYYINASFI .....KGVYG PKAYIATQGP LSTTLLDFWR

Yop51   MLAEN...RT PVVAVLASSS EIANQKFGMP DYFRQSGTYG SITVESKMTQ
YPTP1   MCYHNCPLDN IVVVMVTPLV EYNREKCYQ. .YWPRGG.VD DTVRIASKWE
YPTP2   MI...TLNKV KVVISLNSDD ELNLRKWDI. .YWNNLSYSN HTIKLQNTWE
hTcell  MVWQQ...KT KAVVMLNRIV EKESVKCAQ. .YWP.TD.DQ EMLFKETGFS
rPTP1   MVWEQ...KS RGVVMLNRIM EKGSLKCAQ. .YWPQKE.EK EMVFDDTNLK
hPTP1C  MAWQE...NS RVIVMTTREV EKGRNKCVP. .YWPEVG.MQ RAY...GPYS
rLAR    MVWEQ...RT ATVVMMTRLE EKSRVKCDQ. .YWPARG.TE .TY...GLIQ
Mega1   MTWEQ...GS SMVVMLTTQV ERGRVKCHQ. .YWPEPT.GS SSY...GCYQ
PEP     MLWEY...RI LVIVMACMEF EMGKKKCER. .YWAEPG.ET QLQF..GPFS

Yop51   QVGLGDG... .......... .........I M.ADMYTLTI REAGQKTISV
YPTP1   SPGGANDMTQ FPSDLKIEFV NVHKVKDYYT V.TDIKLTPT DPLVGPVKTV
YPTP2   NICNINGCVL RVFQVKKTAP QNDNISQDCD LPHNGDLTSI TMAVSEPFIV
hTcell  VKLLSED... .......... ....VKSYYT V.HLLQLENI NS.GE.TRTI
rPTP1   LTLISED... .......... ....VKSYYT V.RQLELENL AT.QE.AREI
hPTP1C  VTNCGEH... .......... ....DTTEYK L.RTLQVSPL DN.GDLIREI
rLAR    VTLVDTV... .......... ....ELATYT M.RTFALHKS GS.SE.KREL
Mega1   VTCHSEE... .......... ....GNTAYI F.RKMTLFNQ EK.NE.SRPI
PEP     ISCEAEK... .......... ....KKSDYK I.RTLKAKFN ...NE.TRII

Yop51   PVVHVGNWPD QTAVSSEV.. .......... ..TKALASLV DQTAETKRNM
YPTP1   HHFYFDLWKD .MNKPE.... .......... .EVVPIMEL. ..CAHSHSLN
YPTP2   YQLQYKNWLD SCGVDMNDII KLHKVKNSLL FNPQSFITSL EKDVCKPDLI
hTcell  SHFHYTTWPD .FGVPE.... .......... .SPASFLNFL FKVRESGSLN
rPTP1   LHFHYTTWPD .FGVPE.... .......... .SPASFLNFL FKVRESGSLS
hPTP1C  WHYQYLSWPD .HGVPS.... .......... .EPGGVLSFL DQINQRQESL
rLAR    RQFQFMAWPD .HGVPE.... .......... .YPTPILAFL RRVKACNP..
Mega1   TQIQYIAWPD .HGVPD.... .......... .DSSDFLDFV CHVRNKRAG.
PEP     YQFHYKNWPD .HDVPS.... .......... .SIDPILQLI WDMRCYQE..

Yop51   YESKGSSAVA DDSKLRP.VV HCRAGVGRTA QLIGAMCM.. ..........
YPTP1   SRGN...... ......P[illegible] HCRAGVGRTG TFIALDHLMH DTLDFKNITE
YPTP2   DDNNSELHLD TANSS.PLLV HCSAGCGRTG VFVTLDFLLS ILSPTTNHSN
hTcell  PDHG...... ......PAVI HCSAGIGRSG TFSLVDTCLV LM........
rPTP1   PEHG...... ......PIVV HCSAGIGRSG TFCLADTCLL LM........
hPTP1C  PHAG...... ......PIIV HCSAGIGRTG TIIVIDMLME NI........
rLAR    LDAG...... ......PMVV HCSAGVGRTG CFIVIDAMLE RM........
Mega1   .KEE...... ......PVVV HCSAGIGRTG VLITMETAMC LI........
PEP     DDCV...... ......PICI HCSAGCGRTG VICAVDYTWM LL........

Yop51   .......NDS RNSQLSVEDM VSQMRVQRNG IMVQKDEQLD VLIKLAEGQG
YPTP1   RSRHSDRATE EYTRDLIEQI VLQLRSQRMK .MVQTKDQFL FIYHAAKYLN
YPTP2   KIDVWNMTQ. ....DLIFII MNELRKQRIS .MVQNLTQYI ACYEALLNYF
hTcell  .....EKGDD ...IN.IKQV LLNMRKYRMG .LIQTPDQLR FSYMAIIEGA
rPTP1   .....DKRKD PSSVD.IKKV LLEMRRFRMG .LIQTADQLR FSYLAVIEGA
hPTP1C  .....STKGL DCDID.IQKT IQMVRAQRSG .MVQTEAQYK FIYVAIAQFI
rLAR    .....KH... EKTVD.IYGH VTCMRSQRNY .MVQTEDQYV FIHEAL..LE
Mega1   .....ECNQP VYPLD.I... MRTMRDQRAM .MIQTPSQYR FVCEAILKVY
PEP     .....KDGII PKNFS.VFNL IQEMRTQRPS .LVQTQEQYE LVYSAVLELF
```

Figure 1. Amino acid sequence alignment of the catalytic domain of the PTPases. Amino acid identities are shown in black. The positions of 'conserved' amino acid substitutions are shown in gray. The figure was reproduced from a paper by Zhang and Dixon.[34]

are most likely host proteins. A clue regarding potential substrates of YopH came from studies with macrophages.[14,15] By examining the phosphotyrosine content of cellular proteins in macrophages infected with *Yersinia*, two prominent proteins (120 kDa and a 55 kDa) were found to be dephosphorylated upon infection. Dephosphorylation of these proteins was due to the activity of the *yopH* gene because infection by *Yersinia* with a catalytically-inactive *yopH* gene did not cause tyrosine dephosphorylation. When *Yersinia*-containing the catalytically-inactive *yopH* gene was used to infect the J774A.1 line of macrophage cells, the inactive YopH protein tightly associated with the 120 kDa and 55 kDa proteins. These two proteins

Table 1. Comparison of the k_{cat} of various PTPases* (p-nitrophenyl phosphatase was the substrate used in each of the studies)

PTPase	k_{cat} (sec^{-1})
Yop51	1234
PTPU323	48
rLar	6.1
YPTP1	1.6
VH1	0.3
cdc25	0.75

*Data are from Zhang *et al.*[12]

could be isolated by immunoprecipitation with a YopH-specific antibody. Interestingly, an *in vitro* kinase assay demonstrated that the 120 and 55 kDa proteins possessed protein tyrosine kinase activity.[15] Therefore, YopH phosphatase may regulate host protein tyrosine kinase activity by dephosphorylation. Activation of protein tyrosine kinases is an essential signaling mechanism in many cells, including macrophages. The ability of *Yersinia* to suppress the immune system appears to be directly related to the ability of the YopH PTPase to inactivate the host defense system by destroying its ability to carry out effective signal transduction. Interaction of YopH with p120 and p55 may underly this process.

Microscopic examination of infected macrophages has indicated that *Yersinia* physically resides inside the host cells. Entry of *Yersinia* into macrophages is probably mediated by phagocytosis.[14] A model has been proposed which incorporates the pathogenesis and intracellular location of the bacterium. In the model, the bacteria secrete the phosphatase following phagocytosis. The phosphatase, in turn, crosses a eukaryotic cell membrane and enters the cytoplasm where it dephosphorylates important regulatory proteins. This model suggests that the bacterium must first enter the cell before the phosphatase can dephosphorylate intracellular proteins. However, recent experiments suggest that the bacterium may not have to enter the cell. Entry of *Yersinia* into epithelial cells requires the function of invasin, a cell surface bacterial protein.[16] Experiments with invasin-negative *Yersinia* have demonstrated that significant tyrosine dephosphorylation occurs even though more than 90% of *Yersinia* entry into the cell is blocked.[14] These data suggest that YopH protein can be functionally expressed by bacteria residing outside of the host cell. The PTPase then finds its way into the host cells. It appears that bacterial-macrophage cell-cell contact is not necessary for entry of the PTPase into the mammalian cell. It should also be recalled that the YopH phosphatase has an extremely high PTPase activity. A small number of bacteria entering epithelial cells may produce enough PTPase to dephosphorylate host proteins.

A viral dual specificity phosphatase which dephosphorylates Ser/Thr and Tyr phosphoproteins

Protein phosphatases have generally been divided into serine/threonine or tyrosine-specific subfamilies based upon their substrate specificity. Some of the serine/threonine phosphatases appear to have low levels of tyrosine phosphatase activity.[4] The significance of the tyrosine phosphatase activity is unclear. Recently, an open reading frame, H1, encoded by the vaccinia virus genome, was shown to have a low but significant amino acid sequence identity with the PTPases.[17] VH1 (for vaccinia virus H1 open reading frame) encodes a 171 residue polypeptide which is smaller than the catalytic domain of most PTPases (PTPase catalytic domains are usually approximately 250 amino acid residues in length).[5-7] The sequence identity between the PTPases and VH1 is restricted to the active-site region (Figure 2) raising the question: is VH1 a tyrosine phosphatase?

Expression and purification of the VH1 protein allowed demonstration that it can dephosphorylate p-nitrophenyl phosphate as well as tyrosine phosphorylated peptides and proteins.[17] Serine phosphorylated casein was also rapidly dephosphorylated by the vaccinia phosphatase. When serine phosphatase and tyrosine phosphatase activities were compared using serine or tyrosine phosphorylated casein as artificial substrates, VH1 hydrolyzed phosphoserine approximately seven times more efficiently than phosphotyrosine. The phosphatase activity of VH1 did not require divalent cations and was not inhibited by okadaic acid, a type 1 and 2A serine/threonine phosphatase inhibitor. In contrast, the VH1 phosphatase was very sensitive to vanadate, a potent PTPase inhibitor. Mutation of the conserved cysteine residue completely abolished both tyrosine and serine/threonine phosphatase activity of VH1, suggesting the same catalytic mechanism was being employed for both substrates.

VH1 related phosphatases are highly conserved in the viruses of the orthopoxvirus family.[18] This observation suggests that the phosphatase is important

B

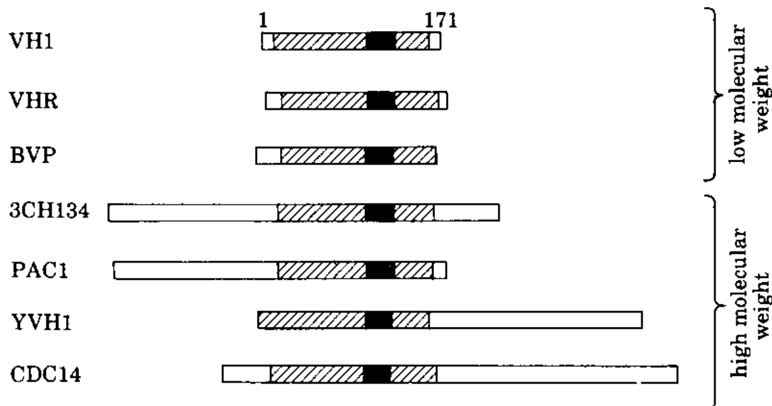
```
    Bvp  FKTPLRPELFAYVTSEED    VWTAEQIVKQNPSIGAIIDLTN
  Cdc14  ETSMYSPQILLHLRSPQEDHPKGYLATKSSHLNQPFKSVLNEFANN
   Pac1  TSRSDSRAPVYDQGGPVEILPYLFLGSCSHSSDLQGLQACGITAVL
 3ch134  SGCSSCSTPLYDQGGPVEILSFLYLGSAYHASRKDMLDALGITALI
    Vhr  LLSDGSGCYSVPSQPCNEVTPRIYVGNASVAQDIPKLQKLGITHVL
   Yvh1           MAGNANSVDEEVTRIFLGGIYLGGIRPIIDHRPLGAEF
    Vh1  LRSTGDMHKAKSPTIMTRVTNNVYLGNYKNAMDAPSSE.VKFKYVL

    Bvp          TSKYYDGVHFLRAGLLYKKIQVPGQTLPPESIVQEFID
  Cdc14  HVQLVVRLNSHLYNKKHFEDIGIQHLDLIFEDGTCPDLSIVKDFVG
   Pac1  N          VSASCPNHFEGLHRYKSIPVEDNQMVDIS    AWFQ
 3ch134  N          VSANCPNHFEGHYQYKSIPVEDNHKADIS    SWFN
    Vhr  NAAEGRSFMHVNTNANFYKDSGITYLGIKANDTQEFNLS    AYFE
   Yvh1  NITHILSVIKFQVIPEYLIRKGVTFKNIPIDDDDVTAVL    QYFD
    Vh1  NLTMDKYTLP              NSNINVIHIPLVDDTTTDLS    KYFD

    Bvp  TVKEFTEKC                     PGMLVGVHCTHGINRTGY
  Cdc14  AAETFIKR                          GGKVAVHCKAGLGRTGC
   Pac1  EAIGFID WV                  KNSGGRVLVHCQAGISRSAT
 3ch134  EAIDFID SI                  KDAGGRVFVHCQAGISRSAT
    Vhr  RAADFIDQAL                  AQKNGRVLVHCREGYSRSPT
   Yvh1  ETNRFIDQCLFPNEVEYSPRLVDFKKKPQRGAVLAHCQAGVSRSVT
    Vh1  DVTAFFSKC                   DQRNEPVLVHCAAGVNRSGA

    Bvp  MVCRYLMHTLGIAPQEAIDR      FEKAR GHKIERQNYVQDLF
  Cdc14  LIGAHLIYTYGFTANECIGF      LRFIRPGMVVGPQQHWLYLH
   Pac1  ACLAYLMQSRRVRLDE      AFDIVKQRRGVISPNFSFMGQLL
 3ch134  ACLAYLMRTNRVKLDE      AFEFVKQRRSIISPNFSFMGQLL
    Vhr  AVIAYLMMRQKMDVKS      ALSIVRQNRE.IGPNDGFLAQLC
   Yvh1  AIVAYLMYRYGLSLSM      AMHAVKRKRPSVEPNEAFMEQLH
    Vh1  AILAYLMSKNKESLPMLYFLYVVHSMRDLRGAFVENPSFKRQII
```

A



C

VH1 **HCXXGXXR**
PTP **HCXAGXGR**

Figure 2. A. Schematic alignment of VH1 related phosphatases. Hatched bars represent the phosphatase domains whose sequences are aligned in 2B. Filled bars denote the highly conserved active site region of the protein surrounding the active site Cys residue. Sequences unrelated to the phosphatase domain are indicated by open bars. The numbers above VH1 denote the first and last residues of the protein. All other proteins are drawn to scale. B. Sequence alignment of the phosphatase domain of VH1 related proteins. Identical residues (5 out of 7) are indicated by black. Conserved residues are shown in gray. C. Comparison of the conserved residues near the active site of VH1 related phosphatases (VH1) and PTPases (PTP). The letters X represent conserved but not invariant residues.

in viral replication or pathogenesis. VH1 is expressed in the late stages of viral infection,[19] yet the biological role of VH1 in vaccinia virus pathogenesis is unclear. In addition to the 'VH1' protein of the pox viruses, a dual specificity phosphatase, BVH (for baculoviral VH1 homolog) or BVP (for baculoviral PTPase), sharing sequence identity to VH1 has recently been described in baculovirus.[18,20] A sequence alignment of VH1-related phosphatases is shown in Figure 2. Direct biochemical evidence has demonstrated that BVP dephosphorylates phosphoserine/threonine and phosphotyrosine-containing substrates. VH1 and BVP are both small proteins compared with other PTPases and share limited sequence identity (Figure 2). The dual specificity of the VH1-related phosphatases and the low level of sequence identity between VH1-related phosphatases and the tyrosine specific PTPases suggests that the VH1 family is likely to represent a distinct group of phosphatases.

Cellular homologs of dual specificity phosphatases

Some viral genes appear to have originated from host genomes. The most famous case is the v-*src* oncogene which has a closely related cellular homolog, c-*src*. We anticipated that closely related cellular homologs of VH1 might also exist. Attempts to isolate cellular equivalents of VH1 using a VH1 cDNA probe proved to be unsuccessful. However, computer-based search of amino acid sequence data bases for VH1-related cellular phosphatases identified the cell cycle regulator cdc25. Both VH1 and cdc25 share sequence identity at their putative active sites.[21] The cdc25 protein was subsequently demonstrated to be a cdc2-specific phosphatase (22,25). This work is reviewed in this issue by S. Atherton-Fessler *et al* (pp 433-442).

Distantly related cellular proteins homologous to VH1 have been isolated from yeast and human sources. A *Saccharomyces cerevisiae* cell division cycle (cdc) gene, *CDC14*, was recently isolated and cloned.[26] The predicted amino acid sequence contains the conserved PTPase active site sequence (Figure 2). However, no significant sequence identity outside of the active site exists between PTPase and CDC14. Sequence comparison shows that CDC14 is more closely related to BVP than to VH1. The sequence identity between CDC14 and BVP suggests that CDC14 may be a dual specificity phosphatase, although this has not been demonstrated. *CDC14* is an essential gene for cell growth as determined by gene deletion experiments.[26] A possible function of *CDC14* in the cell cycle was suggested by studies using a temperature sensitive mutant. At restrictive temperature, *CDC14*^{ts} arrested cells at late nuclear division. The CDC14-arrested cells were single-budded, unnucleated and contained G2 amounts of DNA. The arrested cells also had long spindles, suggesting that *CDC14* may be involved in chromosomal segregation. Furthermore, the expression of *CDC14* mRNA is cell cycle dependent.

Another yeast VH1-related gene, *YVH1* (for yeast VH1 homolog), has also been isolated and characterized. *YVH1* is closely linked to a yeast gene, *DAL1*, which encodes the yeast nitrogen metabolism enzyme[27,28] allantoinase. *YVH1* encodes a 41 kDa protein in which the putative phosphatase domain is located in the N-terminal half of the polypeptide (Figure 2). Expression of *YVH1* demonstrated that YVH1 protein can hydrolyze p-nitrophenyl phosphate with a specific activity similar to that of cdc25. Interestingly, the recombinant YVH1 hydrolyzed tyrosine-phosphorylated casein but not serine-phosphorylated casein. We believe that YVH1 may be substrate specific and the lack of serine phosphatase activity could result from using 'artificial substrates'. Deletion of *YVH1* significantly affected the growth rate of yeast. The slow growth phenotype was even more dramatic at elevated temperature. Identification of YVH1 substrates will help us to understand its biological function. The mRNA encoding YVH1 is significantly induced upon nitrogen starvation, suggesting that *YVH1* may have a role in yeast nutrient regulation. It is worth noting that the translation initiation site of *YVH1* is only 355 nucleotides distant from the stop codon of *DAL1*.

A family of mammalian, VH1-related proteins can be induced by serum and mitogenic growth factors.[29] Charles *et al* have isolated an immediate early gene, *3CH134*, from serum-stimulated mouse fibroblast cells.[29] The mRNA of 3CH134 is transiently induced by growth factors. The 3CH134 mRNA encodes a 367 residue polypeptide in which the C-terminus has sequence identity to VH1 (Figure 2). Inducers of 3CH134 include serum, growth factors and phorbol esters. Interestingly, the human homolog of *3CH134*, *CL100*, is an immediate early gene which appears to function in oxidative stress and heat shock.[30] The tightly regulated expression of immediate early response genes which have phosphatase activity is likely to be important in growth regulation. In fact

both 3CH134 and CL100 possess intrinsic activity dephosphorylating both tyrosyl and threonyl residues in MAP kinase.[35-38] In addition evidence has been presented to indicate that 3CH134 dephosphorylates MAP kinase specifically *in vivo*.[38]

Activation of quiescent T cells by mitogens induces IL-2 production and cell proliferation.[31] An mRNA encoding PAC-1 (for phosphatase of activated cells) was isolated as an immediate early gene in this process.[32] Amino acid sequence comparison revealed that PAC-1 is highly related to 3CH134/CL100. PAC1 is mainly expressed in hematopoietic tissues, with the highest mRNA levels seen in spleen and thymus. Indirect immunofluorescence has shown that PAC1 is predominantly localized in the nucleus.[32] No direct biochemical data has been presented to demonstrate the dual specificity of PAC1 phosphatase activity.

Ishibashi *et al* have isolated a human dual specificity phosphatase using an elegant expression screening strategy.[33] VHR (for VH1-related) encodes a 185 amino acid protein which has limited sequence identity to VH1. It is worth noting that VHR and VH1 have similar sizes, 22 kDa and 21 kDa respectively. VHR effectively dephosphorylates both tyrosine and serine phosphorylated substrates. *In vitro* VHR has been shown to dephosphorylate tyrosine kinase receptors including EGF receptor, PDGF receptor, insulin receptor and keratinocyte growth factor receptor.[33]

VH1-related phosphatases appear to fall into two groups, based on their molecular size. The low molecular weight phosphatases include VH1, BVP and VHR.[17,18,20,33] They contain only the catalytic phosphatase domain (Figure 2). All three phosphatases dephosphorylate artificial substrates and display dual specificity *in vitro*. The high molecular weight group of phosphatases includes *CDC14*, cdc25, *YVH1*, 3CH134 and PAC1.[23-26,28-30,31] Each of these enzymes has a catalytic domain with unique sequences flanking the phosphatase domain. The cdc25 phosphatase is extremely substrate-specific. Interestingly, the only known substrate is cdc2. cdc25 appears to dephosphorylate both threonine and tyrosine residues of cdc2 (S. Atherton-Fessler *et al*, this issue, pp 433-442). 3CH134 is also highly substrate specific. It is a dual specificity phosphatase that dephosphorylates both tyrosyl and threonyl residues in MAP kinase.[35-38] These data suggest that the low molecular weight, VH1 related phosphatases may have a broader substrate specificity than the corresponding high molecular weight phosphatases.

Acknowledgements

We would like to thank Randy Stone for his helpful comments. This work was supported by grants from the National Institutes of Health (NIDDKD 18849; J.E.D.), the Walther Cancer Institute and the American Cancer Society (BE-171; K.G.). We would like to acknowledge the General Clinical Research Center at the University of Michigan grant M01-RR00042, which supports computer-assisted DNA/protein analysis.

References

1. Hunter T (1991) Protein kinases classification. Meth Enzymol 200:3-37
2. Featherstone C, Russell P (1991) Fission yeast p107[wee1] mitotic inhibitor is a tyrosine/serine kinase. Nature 349:808-811
3. Crews CM, Alessandrini A, Erikson RL (1992) The primary structure of MEK, a protein kinase that phosphorylates the ERK gene product. Science 258:478-480
4. Cohen P (1989) The structure and regulation of protein phosphatases. Annu Rev Biochem 58:453-508
5. Fischer EH, Charbonneau H, Tonks NK (1991) Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. Science 253:401-406
6. Charbonneau H, Tonks NK (1992) 1002 protein phosphatases? Annu Rev Cell Biol 8:463-493
7. Pot DA, Dixon JE (1992) A thousand and two protein tyrosine phosphatases. Biochem Biophys Acta 1136:35-43
8. Guan KL, Dixon JE (1990) Protein tyrosine phosphatase activity of an essential virulence determinant in *Yersinia*. Science 249:553-556
9. Butler T (1985) *Yersinia* infections, in Textbook of Medicine (Wyngaarden JB, Smith LH, eds), pp 1600-1603. WB Saunders, Philadelphia
10. Portnoy DA, Wolf-Watz H, Bolin I, Beeder AB, Falkow S (1984) Characterization of common virulence plasmids in *Yersinia* species and their role in the expression of outer membrane proteins. Infect Immun 43:108-114
11. Michielis T, Cornelis G (1988) Nucleotide sequence and transcription analysis of Yop51 from *Yersinia enterocolitica*. Microb Path 5:449-459
12. Zhang Z, Clemens JC, Schubert HL, Stuckey JA, Fischer MW, Hume DM, Saper MA, Dixon JE (1992) Expression, purification, and physicochemical characterization of a recombinant *Yersinia* protein tyrosine phosphatase. J Biol Chem 267:23759-23766
13. Bolin I, Wolf-Watz H (1988) The plasmid-encoded Yop2a protein of *Yersinia pseudotuberculosis* is a virulence determinant regulated by calcium and temperature at the level of transcription. Mol Microbiol 2:237-245
14. Bliska JB, Guan KL, Dixon JE, Falkow S (1991) Tyrosine phosphate hydrolysis of host proteins by an essential *Yersinia* virulence determinant. Proc Natl Acad Sci USA 88: 1187-1191
15. Bliska JB, Clemens JC, Dixon JE, Falkow S (1992) the *Yersinia* tyrosine phosphatase: specificity of a bacterial virulence determinant for phosphoproteins in the J774A.1 macrophage. J Exp Med 176:1625-1630
16. Isberg RR, Voorhis DL, Falkow S (1987) Identification of invasin: a protein that allows enteric bacteria to penetrate cultured mammalian cells. Cell 50:769-778

17. Guan KL, Broyles SS, Dixon JE (1991) A Try/Ser protein phosphatase encoded by vaccinia virus. Nature 350: 359-262
18. Hakes DJ, Martell KJ, Zhao W, Massung RF, Esposito JJ, Dixon JE (1993) A protein phosphatase related to the vaccinia virus VH1 is encoded in the genomes of several orthopoxviruses and a baculovirus. Proc Natl Acad Sci USA 90:4017-4021
19. Rosel JL, Earl PL, Weir JP, Moss B (1986) Conserved TAAATG sequence at the transcriptional and translational initiation sites of vaccinia virus late genes deduced by structural and functional analysis of the HindIII H genome fragment. J Virol 60:436-449
20. Sheng Z, Charbonneau H (1993) The baculovirus autographa californica encodes a protein tyrosine phosphatase. J Biol Chem 268:4728-4733
21. Moreno S, Nurse P (1991) Clues to the action of cdc25 protein. Nature 351:194
22. Strausfel U, Labbe JC, Fesquet D, Cavadore JC, Picard A, Sadhu K, Russell P, Doree M (1991) Dephosphorylation and activation of a $p34^{cdc2}$/cyclin B complex by human cdc25 protein. Nature 351:242-244
23. Dunphy WG, Kumagai A (1991) The cdc25 protein contains an intrinsic phosphatase activity. Cell 67:189-196
24. Gautier J, Solomon MJ, Booher RN, Bazan JF, Kirschner MW (1991) cdc25 is a specific tyrosine phosphatase that directly activates $p34^{cdc2}$. Cell 67: 197-211
25. Millar JBA, McGowan CH, Lenaers G, Johns R, Russell P (1991) $p80^{cdc25}$ mitotic inducer is the tyrosine phosphatase that activates $p34^{cdc2}$ kinase in fission yeast. EMBO J 10:4301-4309
26. Wan J, Xu H, Grunstein M (1992) *CDC14* of *Saccharomyces cerevisiae*. J Biol Chem 267:11274-11280
27. Buckholz RG, Cooper TG (1991) The allantoinase (*DAL1*) gene of *Saccharomyces cerevisiae*. Yeast 7:913-923
28. Guan KL, Hakes DJ, Wang Y, Park HD, Cooper TG, Dixon JE (1992) A yeast protein phosphatase related to the vaccinia virus VH1 phosphatase is induced by nitrogen starvation. Proc Natl Acad Sci USA 89:12175-12179
29. Charles CH, Abler AS, Lau LF (1992) cDNA sequence of a growth factor-inducible immediate early gene and characterization of its encoded protein. Oncogene 7:187-190
30. Keyse SM, Emslie EA (1992) Oxidative stress and heat shock induce a human gene encoding a protein-tyrosine phosphatase. Nature 359:644-647
31. Ullman KS, Northrop JP, Cornelis LV, Crabtree GR (1990) Transmission of signals from the T lymphocyte antigen receptor to the gene responsible for cell proliferation and immune function. Annu Rev Immunol 8:421-452
32. Rohan PJ, Davis P, Moskaluk CA, Kearns M, Krutzsh H, Siebenlist U, Kelly K (1993) PAC-1: A mitogen-induced nuclear protein tyrosine phosphatase. Science 259:1763-1766
33. Ishibashi T, Bottaro DP, Chan A, Miki T, Aaronson SA (1992) Expression cloning of a human dual-specificity phosphatase. Proc Natl Acad Sci USA 89:12170-12174
34. Zhang ZY, Dixon JE (1993) Protein tyrosine phosphatases: mechanism of catalysis and substrate specificity. Adv Enzymol, in press
35. Charles CH, Sun H, Lau LF, Tonks NK (1993) The growth factor-inducible immediate-early gene 3CH134 encodes a protein tyrosine phosphatase. Proc Natl Acad Sci USA 90:5292-5296
36. Alessi DR, Smythe C, Keyse SM (1993) The human CL100 gene encodes a Tyr/Thr-protein phosphatase which potently and specifically inactivates MAP kinase and suppresses its activation by oncogenic ras in *Xenopus* oocyte extracts. Oncogene 8:2015-2020
37. Zheng CF, Guan KL (1993) Dephosphorylation and inactivation of the mitogen activated protein kinase by a mitogen induced Thr/Tyr protein phosphatase. J Biol Chem 268:16116-16119
38. Sun H, Charles CH, Lau LF, Tonks NK (1993) MKP-1 (3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylates MAP kinase *in vivo*. Cell, in press