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against respiratory syncytial virus or the parainfluenza viruses, and genetic-engineering methods should be very valuable in developing effective vaccines against the diseases caused by these viruses. Similar approaches may lead to improved vaccines against influenza virus and measles virus. For influenza viruses, and now for rabies virus, these negative-strand RNA viruses have been shown to be able to express additional protein sequences or transcriptional units. Thus, these viruses may act as vectors to express foreign proteins, which may broaden their use as vaccines for both prophylactic and therapeutic purposes. Finally, these transfectant viruses may be useful in gene therapy. Transient expression of genes may be helpful in therapy of, for example, cystic fibrosis or cancer, and targeting cells using an RNA virus may have advantages over that using viruses with a DNA phase.

Much effort will probably go into the exploitation of these systems, and much will be learned about virus replication, virus–cell interactions and the biological properties of viruses. Furthermore, these systems promise to be important in the development of medically useful biological agents.

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Crystal structure of the *Yersinia* tyrosine phosphatase

James B. Bliska

The crystal structure of the *Yersinia* protein tyrosine phosphatase (PTPase) provides new insights into the function and evolution of an important family of enzymes that regulate signal transduction, cell division and cellular differentiation in eukaryotes[1]. The PTPase family is a structurally diverse collection of enzymes that have two common features: an absolute specificity for the hydrolysis of phosphotyrosine and a conserved signature sequence:

[I/V]HCxAGxxR[S/T]G

(where x is any amino acid) that corresponds to the catalytic site of the enzyme. The carboxy-terminal 262 amino acids of the *Yersinia* enzyme, which include the PTPase domain, have about 20% identity with the catalytic domain of PTP1B (Ref. 2). This region contains all the invariant PTPase residues, including the essential Cys403 in the signature sequence, which forms a thiol-phosphate intermediate during catalysis[3]. Two other PTPases, human PTP1B (Ref. 2) and a low-molecular-mass mammalian PTPase[4] have been crystallized recently and their structures resolved. Together with the *Yersinia* PTPase domain, these structures help to explain how a small number of highly conserved amino acids control PTPase specificity.

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The *Yersinia* PTPase [originally identified as *Yersinia* outer-membrane protein H (YopH)] is essential for the pathogenesis of several diseases, including bubonic plague[5]. YopH is a member of an emerging family of virulence factors that are secreted by type III (Ref. 6) signal-peptide-independent protein-secretion pathways in Gram-negative bacteria. In *Yersinia pestis* and two other pathogenic *Yersinia* species (*Yersinia pseudotuberculosis* and *Yersinia enterocolitica*), YopH is encoded by related virulence plasmids along with other secreted anti-host proteins[5] and the secretion machinery[7]. YopH is secreted by bacteria adhering to host cells and translocated into the host-cell cytoplasm, where it dephosphorylates multiple proteins[8]. It is an essential component of the bacterial strategy

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Fig. 1. The active-site conformational change that occurs on ligand binding to the *Yersinia* protein tyrosine phosphatase (PTPase). When the P loop (gold) binds the phosphate analog tungstate (red), an adjacent loop (unligated form, lavender; tungsten-bound form, white) moves approximately 6 Å into the active site, positioning an invariant aspartic acid for phosphotyrosine hydrolysis. The mechanism of this movement and how it may account for the wide range of measured PTPase activities has been described recently for the structure of a *Yersinia* PTPase–sulfate complex (H.L. Schubert *et al.*, submitted). Figure kindly provided by H.L. Schubert, E.B. Fauman and M.A. Saper, University of Michigan, USA; reproduced with permission from Ref. 16.

for infection[8] through its ability to suppress intracellular signaling in cells of the immune system[9].

Crystal structure of the *Yersinia* PTPase domain

The *Yersinia* PTPase domain consists of an eight-stranded β sheet surrounded by seven α helices that form a prominent substrate-binding cleft[1]. At the base of the cleft lies a phosphate-binding loop (the P loop) that is composed of amino acids 403–410 (CRAGVGRT). The P loop provides a framework of hydrogen bonds that initially stabilize the negatively charged thiolate of the catalytic Cys403 residue. On binding substrate, the hydrogen-bonding array subsequently positions the bound anion for nucleophilic attack by the thiol group. PTP1B and the low-molecular-mass mammalian PTPase have similar active-site clefts and P-loop structures, although their topologies differ significantly outside this central core[2,4]. The specificity of the PTPase anion-binding and transfer reaction may be controlled by the depth of the substrate-binding cleft, which appears to be inaccessible to the shorter side chains of phosphoserine and phosphothreonine.

A substrate-induced conformational change may be an essential step in the catalysis reaction. By analyzing crystals of the protein complexed with the phosphate analog tungstate, Stuckey *et al.*[1] showed that a second loop of amino acids (residues 350–360) shifts towards the catalytic cleft, effectively trapping the bound anion (Fig. 1). The conserved Asp356, an important catalytic residue, moves approximately 6 Å into the active site. This conformational change would place the side chain of the aspartic acid in an ideal position for proton transfer during hydrolysis, assuming that a similar movement occurs when phosphotyrosine binds. In unpublished work, the same conformational change has been observed in crystals of the inactive Cys403→Ser *Yersinia* PTPase domain complexed with sulfate[1]. Thus, this movement may prove to be a general mode of PTPase action as the structures of further PTPase–substrate complexes are resolved. Several observations showing that PTPase substrates are tightly associated with inactive Cys→Ser or Cys→Ala PTPase mutants support the occurrence of this substrate-induced conformational change[10–12].

Yersinia YopH	C	R	A	G	V	G	R	T
Vaccinia VH1	C	A	A	G	V	M	R	S
Nostoc IphP	C	T	A	G	K	D	R	T
Rhodanese	C	R	K	G	V	T	-	A

Fig. 2. Comparison of P-loop motifs in the active sites of microbial phosphatases and mitochondrial rhodanese.

P-loop motifs: lateral gene transfer or convergent evolution?

YopH is unique in that it is the only tyrosine-specific phosphatase that has been identified in a prokaryote. However, a distinct class of protein phosphatases with dual specificity has been found in a baculovirus and in several orthopoxviruses[13]. The prototype of these enzymes is the vaccinia-virus VH1 protein, which hydrolyzes both phosphotyrosine and phosphoserine[14]. Dual-specificity phosphatases contain the conserved P-loop motif (Fig. 2), but otherwise have little sequence similarity with PTPases.

The dual-specificity viral phosphatases and the *Yersinia* PTPase all appear to act on targets within infected eukaryotic cells. It has been suggested that these virulence determinants were acquired by lateral gene transfer from eukaryotic organisms[3,9,15]. However, two recent observations challenge the notion that these types of enzyme evolved initially in eukaryotes. Stuckey *et al.*[1] have found that the P-loop motif is conserved in rhodanese, a mitochondrial sulfur transferase (Fig. 2). As it is generally accepted that mitochondria evolved from prokaryotes that were internalized by primitive eukaryotic cells, the P loop may be an evolutionarily conserved motif for anion binding and hydrolysis. Furthermore, Potts

et al.[15] have genetically and biochemically characterized a dual-specificity phosphatase (IphP) in the cyanobacterium *Nostoc commune*. As *N. commune* is free living, IphP probably evolved directly from prokaryotic ancestry. In addition, a potential target for IphP has been found in *N. commune*, a rare example of protein tyrosine phosphorylation in a prokaryote.

These results raise the possibility that tyrosine phosphorylation and its associated enzyme functions arose in evolution before the divergence of prokaryotes and eukaryotes. As eukaryotic organisms evolved and began to use protein tyrosine phosphorylation as a major mechanism to activate cellular responses in the immune system, microorganisms such as *Yersinia* seem to have acquired new genetic traits to subvert this process.

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State of the art: coronaviruses

Pierre Talbot and Gary Levy

The 6th International Symposium on Corona- and Related Viruses discussed progress in the understanding of the molecular biology, immunology and pathogenesis of corona-, toro- and arterivirus infections. These large, enveloped animal viruses are responsible for a variety of common acute and chronic diseases in birds and mammals, including humans[1], and mainly cause infections of the respiratory, gastrointestinal and nervous systems[2]. In humans, coronaviruses cause 10–35% of common colds, have been implicated in some diarrheal diseases, and may be involved in multiple sclerosis, an inflammatory, autoimmune neurological disorder of multifactorial etiology[3]. In the veterinary field, corona- and related viruses cause economically very important losses in cattle, pigs and chickens[2].

Coronaviruses have the longest known RNA genome (27–31 kb), which is of positive polarity. Replication of the viral genome occurs by the production of a characteristic 3′-coterminal nested set of several subgenomic RNAs[4]. This replication strategy is also characteristic of the toroviruses and arteriviruses, although the latter have a smaller RNA genome[1].

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Pathogenesis, immune responses and vaccines

Throughout the meeting, state-of-the-art speakers reviewed themes in the current research on corona- and related viruses. The opening lecture was an inspiring outside view of studies aiming to define host genes involved in susceptibility and resistance to various infections. The applications of this technology to coronaviruses have so far been undeservedly limited, although a good example has been the identification of a murine-hepatitis susceptibility gene of the fibrinogen family (Emil Skamene, Montreal General Hospital, Quebec, Canada).

The pathogenesis of coronavirus infections has been studied mainly with the murine coronavirus mouse hepatitis virus (MHV), a common mouse pathogen. Neurotropic strains of MHV cause demyelinating diseases of rodents that provide an animal model of human central nervous system (CNS) disorders, such as multiple sclerosis. The JHM strain of MHV has been used to identify determinants of tropism on both the virus and the target cells (Samuel Dales, University of Western Ontario, London, Ontario, Canada).

Several cellular receptors used by coronaviruses to enter target cells are now known. These include members of the carcinoembryonic antigen family for MHV, the aminopeptidase N for the 229E strain of human coronavirus and porcine transmissible gastroenteritis virus, and 9-O-acetylated neuraminic acid for bovine coronavirus. Binding domains on viral proteins are now starting to be identified, and it is

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