



Distinct structural features & triple substrate specificity



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GT MASIM







Applied Mathematics from Genome to Environment



Head Sophie Schbath

4 Teams StatInfOmics dedicated to Statistics and Bioinformatic of Omics data

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4 Teams

Applied Mathematics from Genome to Environment

StatInfOmics dedicated to Statistics and Bioinformatic of Omics data

Comparative genomic with evolution analysis





Info and options Contextual menu: browse multiple homologies per gene





4 Teams



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- Comparative genomic with evolution analysis
- Meta-Omics









Applied Mathematics from Genome to Environment



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StatInfOmics dedicated to Statistics and Bioinformatic of Omics data

Comparative genomic with evolution analysis

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✤ Transcriptomics











Applied Mathematics from Genome to Environment

- Comparative genomic with evolution analysis
- Meta-Omics
- Transcriptomics
- Methods in statistics and algorithmic
- Structural bioinformatics







1st Research axis: functional metagenomics using 3D-Omics

Developing intensive recognition and prediction of protein folds







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Developing intensive recognition and prediction of protein folds



Computing intensive homology modeling of proteins

MaiAGE













Outline

- Orf virus introduction
- OH1 structural characterization *in silico/in vitro*
- OH1 functional characterization in silico/in vitro
- Conclusions & perspectives











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Orf Poxvirus family Like Vaccinia & Variola virus

Orf = old english for « rough »

dsDNA virus Large 200-300 nm Envelop





Introduction

Orf virus





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dsDNA virus Large 200-300 nm Envelop



Bovine papular stomatitis virus Parapoxvirus of red deer in New Zealand Pseudocowpox virus







- Is responsible for Echtyma disease, primarily in sheep and goats
- Forms contagious pustular dermatitis
- Replicates in keratinocytes and epithelial cells of the oral mucosa.
- Is reported worldwide
- Is purulent for 7 weeks
- Prevents lambs form sucking mother ewes and causes weight loss
- Induces mastitis to mother ewes
- Can lead to death in its severe form on immune compromised animals





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Highly contagious by nature Zoonotic through direct contact

Live vaccine that can cause reinfection



Vaccine: immunity for 6-8 months Secondary infections Economical impact +++





Strain UY1107 was fully sequenced : shows 90 essential and conserved genes



Among them, a putative virulence factor was identified, by analogy with Vaccinia and Variola viruses



Codes for a putative tyrosine phosphatase



Deposited at genbank ID: KY651216

Hypothesis: KY651216 is a Tyr-phosphatase responsible for dephosphorylation of phosphorylated substrates

Signaling mediators: Tyrosine phosphatase/kinase are molecular switches that turns on/off host substrates





Orf virus : new strain isolated in Uruguay in 2015

Signaling initiates at the membrane

- Regulates essential cellular processes
- detects external micro changes
- Adapts the cell to the environment accordingly

Survival of microorganism related to its ability to perceive, decode & transmit signal and to respond consistently







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High 40% similarity with Vaccinia and Variola Tyr-phosphatase

Strict conservation of the active site of a Tyr-phosphatase



BIM 16-17 Nov





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Strict conservation of the active site of a Tyr-phosphatase

Ser to Cys substitution within helix α_1



BIM 16-17 Nov



- Homology modeling of Tyrosine phosphatase with 3D Vaccinia and Variola VH1 as 3D templates: Chains A then B.
- Reconstruction of the dimer by superposition onto the dimer template.
- Minimisation with Charmm to relax the side chains











The main differences are located at the interface

Helix α_1 at the N-terminal end is largely engaged in domain swapping



- Protein purified to homogeneity Histrap and size-exclusion chromatography wt and C112S
- Crystallisation screening using sitting drop vapor diffusion for both









Beamline Proxima1 2 sets of data collection



Crystal grew after 3 days for C112S No crystal after 3 months for wt



Data statistics 5NCR

P2₁ a, b, c (Å) 49.56 63.55 55.39 α, β, γ (°) 90.00, 97.07 90.00 Refinement of the best at 1.89 Å resolution Molecular replacement using Phaser Model building with Coot Refinement cycles within Refmac CCP4 package and Buster Rwork/Rfree 18.72/21.69

Pdb deposition 5NCR





Solved structure reveals:



Solved structure reveals the conserved fold of DUSP – Dual Specificity Phosphatase



Solved structure reveals a dimer organization distinct from Vaccinia phosphatase









Orf OH1

Vaccinia VH1

Solved structure reveals a dimer organization distinct from Vaccinia phosphatase



 α_{1B}

 α_{5B}

α_{6Α})

 $\alpha_{1\Delta}$





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Solved structure reveals a dimer organization distinct from Vaccinia phosphatase





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25 kDa

15 kDa

Solved structure reveals a dimer organization distinct from Vaccinia phosphatase



• Ultra Analytical Centrifugation

C15S shows also a dimer (data not shown)

C15S is not mandatory for a dimerization





Detection of Parapox specific features of OH1

M. Mariadassous

Both Cys 15 and the 6 residues insertion are conserved within Parapoxvirus genus

- Phylogenetic studies explore how parapoxvirus associates N-terminal Ser15 to Cys substitution
- Multiple sequence alignment of 64 phosphatases from Chordopox family
- Tcoffee Alignment
- Position with >50% gaps filtered
- Correlation techniques based on mutual information

Ala40 locates in the vicinity of the active site

Tyr152 could stabilize helix α_1

D1-Bovine-papular-stomatitis-virus tr-D3IZ73-Pseudocowpox-virus tr-D3IZK4-Pseudocowpox-virus tr-Q6TVR3-Orf-virus-SA2000 -A0A7MA86-Parapoxvirus-red-deer · tr-Q6TW43-orf-virus tr-W5U9D4-Orf-virus tr-Q070E0-Nile-crocodilepox-virus tr-Q6VZG4-Canarypox-virus sp-Q9J592-fowlpox-virus tr-Q70H20-Fowlpox-virus tr-A0A068EF29-Penguinpox-virus tr-A0A068EG98-Pigeonpox-virus tr-Q98249-Molluscum-contagiosum-virus-subtype-1 · tr-U3UBD0-Squirrelpox-virus tr-Q77PC3-Babbit-fibroma-virus tr-Q85315-Rabbit-fibroma-virus · tr-O8V3M6-Swinenox-virus tr-Q9IGU7-Tanapox-virus tr-Q9DHP1-Yaba-like-disease-virus tr-H6TA62-Cotia-virus tr-A0A097IVT2-Cotia-virus tr-O91T23-Lumny-skin-disease-virus tr-A0A075CH78-Goatpox-virus tr-Q6TUU3-Yaba-monkey-tumor-virus tr-B2CWH6-Myxoma-virus sp-Q85297-Myxoma-virus tr-Q77GL6-Lumpy-skin-disease-virus tr-Q77GC9-Lumpy-skin-disease-virus tr-Q91MT8-Lumpy-skin-diseaseVIRUS tr-Q9WH06-Sheeppox-virus tr-T2AU07-Myxoma-virus tr-Q08FA1-Deerpox-virus tr-Q08FS1-Deerpox-virus tr-G3EIF7-Yoka-poxvirus sp-P80994-Raccoon-poxvirus tr-Q0NJL8-Variola-virus sp-P33064-Variola-virus tr-Q0N562-Variola-virus tr-Q0NLW9-Variola-virus tr-Q0NCN7-Variola-virus-isolate-Human-South-Africa tr-Q0NG54-Variola-virus · tr-Q76Q11-Variola-minor-virus · tr-Q8V2S5-Camelpox-virus · tr-Q775U6-Camelpox-virus-strain-CMS tr-Q8JLD2-Ectromelia-virus tr-Q5IXT4-Monkeypox-virus tr-O8OMW6-Cowpox-virus tr-I0AZE8-Ectromelia-virus tr-Q8V4Z3-Monkeypox-virus-Zaire · tr-G0XX41-Cowpox-virus · tr-M9WG49-Vaccinia-virus tr-Q80DY2-Cowpox-virus tr-Q0NPB0-Taterapox-virus sp-P20495-Vaccinia-virusstrain-Copenhagen tr-Q77TK5-Vaccinia-virus-strain-Tian-Tan · tr-Q1M1M0-Vaccinia-virus tr-Q6BZK3-Babbitpox-virus · sp-P07239-Vaccinia-virusstrain-Western-Reserve · tr-Q76RC6-Vaccinia-virus tr-B9U1I4-B9U1I4Vaccinia-virus tr-Q0GNY5-Q0GNY5-HorsepoxVirus tr-A9J1R8-A9J1R8-VacciniavirusstrainAnkara -3CM3-Vaccinia ·

Cys15 Ala40 Tyr152

	EAKNDWYAKLLLR	TRAGAPIYLRLTEYV	YLGS <mark>A</mark> EDARAVVMASGVD	FKCVVNMTTAAP-ESI	AVYHIPLRDDNVT	SIEGVIPPLVKLLE	RLEAEKKPTLVH	ICVAG I NIRSGAAAMAY	MHRWEVFFLKTY	ELRDSRGAFLEN	NNFRYQL I KM	VTGA
	GNNNEWYARLLLR	TRAGTPLTFRLTEHV	YLGSAEDARSVLRESG I D	FKCLVNMTMSTP-SGL	TAYHIPLRDDDET	NI TA IMPALVKLLE	RLEAERKPTLVH	ICVAG I NIRSGAAAMAY	MHKRFVYFLKTY	EIRDLRGAFLEN/	ANFRMQL I KM	VCDSSS
	GNNSEWYARLLLR	TRAGTPLTFRLTEHV	YLGSAEDARSVLRESGVD	FKCLVNMTMSTP-SGL	TAYHIPLRDDDET	NITAIMPALVKLLE	RLEAERKPTLVH	ICVAG I NIRSGAAAMAY	MHKREVYELKTY	EIRDLRGAFLEN/	ANFRMQL I KM	VCDSSS
	GDKSEWYARLLLRC	TRAGPPLAMRLTDHV	YLGSAEDARAVLRDSGVD	FKCLVNMTMSTP-AGI	TAYHIPLRDDDKT	NITSIMPALVKLLA	RLEAEKKPTLVH	CVAGVNRSGAAAMGY	MHKREVYELKTY	EIRDLRGAFLEN	TNFRYQL I KM	VCDSPS
	DDKNDWYARLLLRC	TOGGTPVVMRLTEYV	YLGNAEDARRVVRETGVF	FQCVVNMTTSTP-SGI	TAYHI PLRDDDV1	DISAIMPPLVKLLE	RLEAERRPTLVH	ICVAG I NRSGAAAMAY	MHRREVYELKTY	ELRDLRGAFLEN	ONFRLQL I KL	VV
	GDKSEWYARLLLRC	TRAGPPLALRLTDHV	YLGSAEDARAVLRDSGVD	FKCLVNMTMSTP-AGT	TAYHIPLRDDDTT	NIASIMPALVKLLA	RLEAEQKPTLVH	CVAGVNRSGAAAMGY	WHKREVYELKTY	EIRDLRGAFLEN	ANFRYQL I KM	VCDSPS
	GDKSEWYARLLLRC	TRAGPPLALRLTDHV	YLGSAEDARAVLRDSGVD	FKCLVNMTMSTP-AGI	TAYHIPLRODOTT	NITSIMPALVKLLA	RLEAEKKPTLVH	CVAGVNRSGAAAMGY	WHKREVYELKTY	E IRDLRGAFLEN	ANFRYQL I KM	VCDSPS
	GGKSNLYRDLTLMS	TD-THAAFLQVTKNT	YLGGYDNVSSGEF-HHHG	HAY I LNLSYHAEGV	KVISLNVEDSPH	NISQYFKHENALLL	HCEKSDKKTLVH	CVAGVNHSGAAVLSY	ISKKLIYFLFIY	LLKHKHGAFVEN	AFHEQIVSY	VA
	MDEKQLYKYTTNKS		YLGNYKNVSQLPEKTF	FKYTINVSTLLNHSDV		DISKHINAVVGILK	KODLEKVPVLVH	CMAGINHSAAMIMSY	MEVALIYELYVY	ELKSTHGAFLEN	KSFLNQTVDK	[]
	MDEKQLYKHTTKS	TN-TOVKFTKTTDYV	VIGNTHINVIELPNKIP	FKTIVNVSMLLKHTDI		SISKHIDAVIYVLK	KCESLKIPVLVH	CWAG INRSSAMINGY		ELKY INGAFTEN	CSFLINQ I IDKI	
	MDEKQL TKHI I TK			EKY INNUSWELKHIDI			KCESLKIPVLVH	CMAC INDECAMINGT		ELKSTHGAFTEN	CELICI IDK	
				EKY IVAVSMLLKATOT			KCESIKIDVIVH	CMACINDSSAMINSY		ELKSTROAFTEN	SELKOLIDK	/
			VI CONNINAL ES SOVE	EDV/I NMSM/I DOSSA				CLACINESCAMINAY				/
	MOGKNEVEGLILKS	TOOL PRAPSPITEN	VI GNYKDAMG LAA-SGVA	FRYVI NI TTELKOPHV	TWHIPI VODESS		REPOCSEVI VH	CVAGVNRSGVMIMAY		GI REORGAEVENI	SEBBOL LEH	
	MOKKSI YENVI I KS	TGAL PKAR I BVTDYV	VI GNYNDAKAVPT-SGVG	EKYLI NI TTE IKNSSV			NCEDKHYPVI VH	CMAGVNBSGALIMAY	MSBKEMYELYLY	SIBEOBGAELEN	SEBBOLIEK	LINET-
	MDKKSLYENVLLKS	TGAL PKAR I BVTDYV	YI GNYNDAKAVPT-SGVG	EKYLINITTEIKNSSV		DI TKYEDYTTTELS		CMAGVNRSGA LIMAY	MSBKEMYELYLY	S I BEOBGAELEN	SEBBOLLEK	LINET-
	MORKSLYENVLLKS	TGSI TKAKARVTEYV	YI GNYNDA INI CS-SELE	EKYLLNI TTELKNSS I			KODKNEHA LL VH	CLAGVNRSGAMIMAY	MHRBELYELYVY	IMBEKBGAFLEN	SEBKOLIDK	LINEST
	MDKKCLYENVLLKS	TGLLPKAKARVTDYV	YLONYNNALSINE-YGIC	FKYLLNLTTELONSSV	NUMPLIONETT	DLTKHEDYVTNELS	KOKNHYPVLVH	CLAGVNRSGAMIMAY	MTKREMEEL YVY	SMREQRGAFLEN	SERKOLLEK	VIKN
	MOKKCIYENVLLKS	TGLLPKAKARVTDYV	YLGNYNNALSINE-YGIC	FKYILNLTTEICNSSV	NIIHMPLLONETT	DLTKHEDYVTNELS	KCDKHHYPVLVH	CLAGVNRSGAMIMAY	MTKREMEELYVY	SWREQRGAFLEN	SFRKQLLEK	VIKN
	MOKKSLYENVLLKS	TGYLEKAKARVTEYV	YLGNYNDA I NAPY-SDVK	FKYILNLTPEIVNSPI	NI IHLPL IDDECT	DLSKYFDYTTNLLT	KCEIEHYPILVH	CLAGVNRSGA LIMAY	MSRRFMYFLYIY	AMREKRGAFVEN	SFRKQLINK	IVDT
	MDKKSLYENVLLKS	TGYLEKAKARVTEYV	YLGNYNDA I NAPY-SDVK	FKYILNLTPEIVNSPI	NI IHLPL IDDECT	DLSKYFDYTTNLLT	KCEIEHYPILVH	CIAGVNRSGAI IMAY	MSRRFMYFLYIY	AMREKRGAFVEN	SFRKQIINK	IVDT
	MOKKSLYENVLLKS	TGSLPKAKARVTDYV	YLGNYDDA I NA I S-SNVN	FKYILNLTTEFNDSRI	NIIHMPLIDDEKT	NUNDHEDYVTNELS	KCDEEHYPVLVH	CVAGVNRSGAMIMAY	MSKRFIYFLYIY	SMREKRGAF I EN	PSFRKQL I DK	IINELK
	MDKKSLYENVLLK	TGSLPKAKARVTDYV	YLGNYDDA I NA I S-SNVN	FKYILNLTTEFNDSRI	NIIHMPLIDDEKT	NLNDHFDYVTKFLS	KCDEEHYPVLVH	CVAGVNRSGAMIMAY	MSKRFIYFLYIY	SMREKRGAF I EN	PSFRKQL I DK	IINE
	MOKNEMYKNVLLKS	TGLLPKAKARVTDYV	YLGNYNDAISIDE-SGVF	FKYILNLTTEICNSSV	NITHMPLLONETT	DLTKHEDYVTNELS	KCDEHHYPVLVH	CVAGVNRSGAMIMAY	MTKREVYELHVY	SWREQRGAFLEN	PSFRKQVIEK	VT
	MDKKSLYENVLLK	TGALPKARVRVTDYV	YLGNYNDAKAAPT-SGIG	FKYILNLTTEIKNSSI	TIIHMPLVDDEYT	DLTKYFDYATTFLS	NCEDKHYPVLVH	CMAGVNRSGA I IMAY	MSRKFMYFLYIY	SIRECRGAFLEN	PSFRRQIIEK	IINET-
	MDKKSLYENVLLKS	TGALPKARVRVTDYV	YLGNYNDAKAAPT-SGIG	FKYILNLTTEIKNSSI	TIIHMPLVDDEYT	DLTKYFDYATTFLS	NCEDKHYPVLVH	CMAGVNRSGA I IMAY	MSRKFMYFLYIY	SIRECRGAFLEN	PSFRRQIIEK	(I I NEHK
	MDKKSLYENVLLKS	TGSLPKAKARVTDYV	YLGNYDDA I NA I S-SNVN	FKYILNLTTEFNDSRI	NIIHMPLIDDEKT	NUNDHEDYVTNELS	KCDEEHYPVLVH	CVAGVNRSGAMIMAY	MSKRFIYFLYIY	SMREKRGAF I EN	PSFRKQL I DK	IINE
	MDKKSLYENVLLKS	TGSLPKAKARVTDYV	YLGNYDDA I NA I S-SNVN	IFKY I LNLTTEFNDSR I	NIIHMPLIDDEKT	NUNDHEDYVTNELS	KCDEEHYPVLVH	CVAGVNRSGAMIMAY	MSKRFIYFLYIY	SMREKRGAF I EN	PSFRKQL I DK	IINE
	MDKKSLYENVLLK	TGSLPKAKARVTDYV	YLGNYDDA I NA I S-SNVN	IFKY I LNLTTEFNDSR II	NIIHMPLIDDEKT	NLNDHFDYVTNFLS	K <mark>C</mark> DEEHYPVLVH	CVAGVNRSGAMIMAY	MSKRFIYFLYIY	SMREKRGAF I EN	PSFRKQL I DK	IINE
	MDKKSLYENVLLK	TGSLPKAKARVTDYV	YLGNYDDA I NA I S-SNVK	FKYILNLTTEFNDSRI	NIIHMPLIDDEKT	NLNDHFDYVTNFLS	KCDEEHYPVLVH	CVAGVNRSGAMIMAY	MSKRFIYFLYIY	SMREKRGAF I EN	PSFRKQL I DK	IINE
	MDKKSLYENVLLKS	TGALPKARVRVTDYV	YLGNYNDAKAAPT-SDIG	FKYILNLTTEIKNSSI	TIIHMPLVDDEYT	DLTKYFDYATTFLS	NCODKHYPVLVH	ICMAGVNRSGA I IMAY	MSRKFMYFLYIY	SIREORGAFLEN	PSFRRQIIEK	IINET-
	MDKKSLYENVLLKS	TGSLPKAKARVTNYV	YLGNYNDALNAPY-SDIC	FKYILNLTTELKNSHI	NI IHMPLIDDEQT	DLSKHFDYVTDFLS	KCDAQQYPVLVH	CVAGVNRSGAMIMAY	LMTKRFMYFLY I Y	ISMREQRGAFLEN	PSFRKQIIEK	IINER-
	MDKKSLYENVLLKS	TGSLPKAKARVTNYV	YLGNYNDALNAPY-SDIC	FKYILNLTTELKNSHI	NI IHMPLIDDEQT	DLSKHFDYVTDFLS	KCDAQQYPVLVH	CVAGVNRSGAMIMAY	MTKRFIYFLYIY	SWREQRGAFLEN	PSFRKQIIEK	IINER-
	MDKKSLYKYLLLR	TGDMQKAKSRVTKNV	YMGNYKNAMEAPC-SIVE	FKYILNLTMEFCDSNI	NITHIPLIDDMST	DISKYFDYVTDFLT	KCDERNEPVLVH	ICVAGVNRSGSMI LAY	MSRNMVYFLYVY	TLRDLRGAFVEN	SSFRRQIIER	I I NNY-
	MDKKSLYKYLLLRS	TGDIHRAKSRVTNNV	YLGNYKNAMEAPS-SEVK	FKYILNLTMDFTNSNI	NI IHVPMVDDTST	DISIYFDDITAFLS	KCDORNEPVLVH	ICAAGVNRSGAMI LAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKRQIIEK	VIDKN-
	MDKKSLYKYLLLRS	TGDMRRAKSRVTNNV	YLGNYKNA I NAPS-SEVK	FKYVLNLTMDLPNSNI	NITHIPLVDDTT	DISKYFDDVTAFLS	KCDORNEPVLVH	CVAGVNRSGAMILAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKRQIIEK	VIDKN-
	MDKKSLYKYLLLRS	TGDMRRAKSRVTNNV	YLGNYKNAMNAPS-SEVK	FKYVLNLTMDLPNSNT	NTHIPLVDDTT	DISKYFDDVTAFLS	KCDQHNEPVLVH	CVAGVNRSGAMILAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKHQITEK	VIDKN-
	MDKKSLYKYLLLRS	TGDMRRAKSRVTNNV	YLGNYKNAMNAPS-SEVK	FKYVLNLTMDLPNSNI	NITHIPLVDDTT	DISKYFDDVTAFLS	KCDQRNEPVLVH	CVAGVNRSGAMILAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKRQIIEK	VIDKN-
1	MUKKSLYKYLLLHS	TODAPPAKSHVINNV	YLGNYKNAWNAPS-SEVK	FKYVLNL IMDLPNSNI		DISKYFDDVTAFLS	KODQHNEPVLVH	CVAGVINHSGAMTLAY	MSKNWLYFLYVY	SWHULHGAF VEN	SFKHQITEK	VIDKN-
		TOWNHAKSHV I NNV	VICNYKNAWNAPS-SEVK	FKYVLNL IMDLPNSNI		DISKYFDDVIAFLS	KCDQHNEPVLVH	CVAGVNHSGAMTLAY		SWHDLHGAF VEN	SEKROLLEK	VIDKN-
			VI CNYKNAWNAPS-SEVK	EKYVLINE IMULPINSINI							SEKROLLEK	
		TGDWHHAKSHVTNNV	VI CNYKNAMDAPS-SEVK	EKYVI NI TMDI PNSNII			KODORNERVI VH	CAAGVNRSGAMULAY	MSKNML VEL VVV	SWRDL RGAEVEN	SEKROLLEK	
	MOKKSLYKYLLLBS	TGDMYRAKSRVTNNV	YI GNYKNAMDAPS-SEVK	EKYVI NI TMDI PNSNII		DISKYEDDVTAFLS	KODOBNERVI VH	CAAGVNBSGAMILAY	MSKNMLYELYVY	SWRDI BGAEVEN	SEKBOLLEK	VIDKN-
	MOKKSLYKYLLLBS	TODMHRAKSBUTNNI	VI GNYKNAMDAPS-SEVK	EKYVI NI TMDI PNSNII		DI SKYEDDVTAELS	KODONERVI VH	CAAGVNBSGAMLLAY	MSKNML VEL VVV	SMEDI BGAEVEN	PSEKBOLLEK	AVIDKN_
		TGDMHRAKSBVTNNV	VI GNYKNAMDAPS-SEVK	EKYVI NI TMDI PNSNII		DI SKYEDDVTAFI S	KODOBNERVI VH	CVAGVNBSGAMIL AY	MSKNMLYELYVY	SMRDI BGAEVENI	PSEKBOLLEK	VIDKN-
	MOKKSLYKYLLLBS	TGDMHRAKSRVTNNV	YI GNYKNAMDAPS-SEVK	EKYVI NI TMDEPNSNI		DISKYEDDVTAFLS	KCDOBNERVI VH	CAAGVNBSGAMILAY	MSKNMLYELYVY	SMRDI BGAEVENI	SEKBOLLEK	VIDKN-
	MDKKSLYKYLLLBS	TGDMHRAKSRVTNNV	YLGNYKNAMDAPS-SEVK	EKYVI NI TMDI PNSNI		DISKYEDDVTAELS	KCDCONEPVI VH	CAAGVNRSGAMILAY	MSKNMLYELYVY	SMBDL BGAEVEN	SEKROLLEK	VIDKN-
	MOKKSLYKYLLLB	TGDMHBAKSRVTNNV	YLGNYKNAMDAPS-SEVK	FKYVLNLTMDLPNSNI		DISKYEDDVTAFLS	KCDORNEPVLVH	CVAGVNRSGAMILAY	MSKNMLYFLYVY	SMRDLRGAFVEN	SEKROLLEK	VIDKN-
	MOKKSLYKYLLLRS	TGDMHRAKSRVTNNV	YLGNYKNAMDAPS-SDVK	FKYVLNLTMDLPNSNI	NI IHI PLVDDTTT	DISKYFDDVTAFLS	KCDORNEPVLVH	CAAGVNRSGAMILAY	MSKNMLYFLYVY	SMRDLRGAFVEN	SFKRQIIEK	VIDKN-
	MOKKSLYKYLLLRS	TGDMRRAKSRVTNNV	YLGNYKNAMDAPS-SEVK	FKYVLNLTMDLPNSNI	NI IHI PLVDDTTT	DISKYFDDVTAFLS	KCDORNEPVLVH	CAAGVNRSGAMILAY	MSKNMLYFLYVY	SMRDLRGAFVEN	SFKRQIIEK	VIDKN-
	MOKKSLYKYLLLRS	TGDMHRAKSRVTNNV	YLGNYKNAMDAPS-SEVK	FKYVLNLTMDLPNSNI	NITHIPLVDDTTT	DISKYFDDVTAFLS	KCOORNEPVLVH	CAAGVNRSGAMILAY	MSKNMLYFLYVY	SMRDLRGAFVEN	SFKRQIIEK	VIDKN-
	MDKKSLYKYLLLRS	TGDMHRAKSRVTNNV	YLGNYKNAMDAPS-SEVK	FKYVLNLTMDLPNSNI		DISKYFDDVTAFLS	KCOORNEPVLVH	CAAGVNRSGAMILAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKRQIIEK	VIDKN-
	MDKKSLYKYLLLRS	TGDMHKAKSRVTNNV	YLGNYKNAMDAPS-SEVK	FKYVLNLTMDLPNSNI	NI IHI PLVDDTTT	DISKYFDDVTAFLS	KCDQRNEPVLVH	CAAGVNRSGAMI LAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKRQIIEK	VIDKN-
	MDKKSLYKYLLLRS	TGDMHKAKSRVTNNV	YLGNYKNAMDAPS-SEVK	FKYVLNLTMDLPNSNI	NI IHI PLVDDTTT	DISKYFDDVTAFLS	KCDORNEPVLVH	CAAGVNRSGAMI LAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKRQIIEK	VIDKN-
	MDKKSLYKYLLLRS	TGDMHRAKSRVTNNV	YLGNYKNAMDAPS-SEVK	FKYVLNLTMDLPNSNI	NITHIPLVDDTTT	DISKYFDDVTAFLS	KCDORNEPVLVH	CAAGVNRSGAMI LAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKRQIIEK	VIDKN-
	MDKKSLYKYLLLRS	TGDMHRAKSRVTNNV	YLGNYKNAMDAPS-SEVK	FKYVLNLTMDLPNSNI	NIIHIPLVDDTTT	DISKYFDDVTAFLS	KCDORNEPVLVH	CAAGVNRSGAMILAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKRQIIEK	VIDKN-
	MDKKSLYKYLLLRS	TGDMHKAKSRVTNNV	YLGNYKNAMDAPS-SEVK	FKYVLNLTMDLPNSNI	NI IHI PLVDDTTT	DISKYFDDVTAFLS	KCDORNEPVLVH	CAAGVNRSGAMILAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKRQIIEK	VIDKN-
	MDKKSLYKYLLLR	TGDMHKAKSRVTNNV	YLGNYKNAMDAPS-SEVK	FKYVLNLTMDLPNSNI	NI IHI PLVDDTTT	DISKYFDDVTAFLS	KCDORNEPVLVH	CAAGVNRSGAMILAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKRQIIEK	VIDKN-
	MOKKSLYKYLLLR	TGDMHKAKSRVTNNV	YLGNYKNAMDAPS-SEVK	FKYVLNLTMDLPNSNI	NIIHIPLVDDTTT	DISKYFDDVTAFLS	KCDORNEPVLVH	ICAAGVNRSGAMI LAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKRQIIEK	VIDKN-
	MDKKSLYKYLLLR	TGDMHKAKSRVTNNV	YLGNYKNAMDAPS-SEVK	FKYVLNLTMDLPNSNI	NITHIPLVDDTTT	DISKYFDDVTAFLS	KODORNEPVLVH	ICAAGVNRSGAMI LAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKRQIIEK	VIDKN-
	MDKKSLYKYLLLRS	TGDMHKAKSRVTNNV	YLGNYKNAMDAPS-SEVK	FKYVLNLTMDLPNSNI	NIIHIPLVDDTTT	DISKYFDDVTAFLS	KCDQRNEPVLVH	CAAGVNRSGAMILAY	MSKNMLYFLYVY	SMRDLRGAFVEN	PSFKRQIIEK	VIDKN-
		TGDMHKAKSRVTNNV	YLGNYKNAMDAPS-SEVK	EKYVLNLTMOLPNSNI	VITHTPLVDOTTI	DISKYFDDVTAFLS	KCDORNEPVLVH	CAAGVNRSGAMILAY		ISMRDLRGAFVEN	SEKROITEK	
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Highlights

- OH1 displays distinct structural features compared to VH1 phosphatases
- Orf virus OH1 is a covalent dimer involving the N-terminal Cys15
- Orf virus OH1 possibly depicts the structure of Parapoxvirus genus phosphatases







Outline

- Orf virus introduction
- OH1 structural characterization in silico/in vitro
- OH1 functional characterization in silico/in vitro
- Conclusions & perspectives





Tyrosine phosphatase

• OH1 shows a phosphatase activity *in vitro*



Phosphatase activity assayed at 37°C using the artificial substrate pNPP

Michaelis-Menten Kinetics constants







Monitoring of dephosphorylation by absorbance at 405nm of pNP

Tyrosine phosphatase



Phosphatase activity assayed at 37°C using the artificial substrate pNPP

Michaelis-Menten Kinetics constants







Tyrosine phosphatase



A new tyrosine phosphatase



- Phosphoinositides can be dephosphorylated
- PtdIns3P and PtdIns5 (3,5)P2 can be dephosphorylated

- ✤ The phospho-sugar moiety can be accommodated
- The phospho-group in 5' binds into the active pocket

Relevant physiologically?





A new tyrosine phosphatase



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Highlights

Segovia D et al, JMB 2017

- OH1 displays distinct structural features compared to VH1 phosphatases
- Orf virus OH1 is a covalent dimer involving the N-terminal Cys15
- Orf virus OH1 possibly depicts the structure of Parapoxvirus genus phosphatases
- Orf virus OH1 is a dual specifity phosphatase that presents activity towards PInsP in vitro







Outline

- Orf virus introduction
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- OH1 functional characterization in silico/in vitro
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Interaction with STAT1



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OrfV dephosphorylates STAT1

JAK1

STAT1

Stat 1_Stat 1

Stat 1 Stat 1

GAS

IFN₂

JAK2

Interaction with STAT1

Send to -

Virus Res. 2015 Oct 2;208:180-8. doi: 10.1016/j.virusres.2015.06.014. Epub 2015 Jun 22.

Orf virus inhibits interferon stimulated gene expression and modulates the JAK/STAT signalling pathway.

Harvey R¹, McCaughan C², Wise LM³, Mercer AA⁴, Fleming SB⁵.

Author information

Format: Abstract -

Abstract

Interferons (IFNs) play a critical role as a first line of defence against viral infection. Activation of the Janus kinase/signal transducer and activation of transcription (JAK/STAT) pathway by IFNs leads to the production of IFN stimulated genes (ISGs) that block viral replication. The Parapoxvirus, Orf virus (ORFV) induces acute pustular skin lesions of sheep and goats and is transmissible to man. The virus replicates in keratinocytes that are the immune sentinels of skin. We investigated whether or not ORFV could block the expression of ISGs. The human gene GBP1 is stimulated exclusively by type II IFN while MxA is stimulated exclusively in response to type I IFNs. We found that GBP1 and MxA were strongly inhibited in ORFV infected HeLa cells stimulated with IFN-γ or IFN-α respectively. Furthermore we showed that ORFV inhibition of ISG expression was not affected by cells pretreated with adenosine N1-oxide (ANO), a molecule that inhibits poxvirus mRNA translation. This suggested that new viral gene synthesis was not required and that a virion structural protein was involved. We next investigated whether ORFV infection affected STAT1 phosphorylation in IFN-γ or IFN-α treated HeLa cells. We found that ORFV reduced the levels of phosphorylated STAT1 in a dose-dependent manner and was specific for Tyr701 but not Ser727. Treatment of cells with sodium vanadate suggested that a tyrosine phosphatase was responsible for dephosphorylating STAT1-p. ORFV encodes a factor, ORFV057, with homology to the vaccinia virus structural protein VH1 that impairs the JAK/STAT pathway by dephosphorylating STAT1. Our findings show that ORFV has the capability to block ISG expression and modulate the JAK/STAT signalling pathway.

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JAK1/STAT1 pathway



Nucleus



Interaction with STAT1







Interaction with STAT1

Running hypothesis: OH1 dephosphorylates STAT1 by a direct interaction

- Modeling of the missing loop and phosphorylation at Tyr 701
- Docking of Protein/Protein interaction using protein/protein docking server



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Perspectives

- OH1 /STAT1 needs further investigations *in silico*
- Pull-down *in vitro* experiments show that OH1 interacts directly with STAT1
- Pull-down *in vitro* experiments show that OH1 interacts with GAPDH Glyceraldehyde-3-phosphate dehydrogenase
- GAPDH is a house-keeping protein involved in glycolysis and modulation of the organization of the cytoskeleton
- GAPDH could not only be a metabolic enzyme but could possibly be involved in immunity









Acknowledgements





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Virology

Dario Porley <u>Mabel Berois</u>

Crystallization



Danilo Segovia
Frederik Saul
Patrick Weber
<u>Ahmed Haouz</u>





Distinct structural features & triple substrate specificity



Thank you very much for your kind attention



