

In Silico docking of Influenza-Interleukin 6 interactions and the role of HDAC-6 in its modelling

Journal	British Journal of Virology
Manuscript ID	SANDF20140225220231
Manuscript Type	Original Research Article
Date Submitted by the Author	Wed, 31 Dec 1969, 06:00 PM
Complete List of Authors	■ Mr sandeep satapathy ■ Mr Rupam Ghosh

In Silico docking of Influenza-Interleukin 6 interactions and the role of HDAC-6 in its modelling

Sandeep Satapathy^{*#}, Rupam Ghosh[#]

Dept. of Biological Sciences,

Indian Institute of Science Education and Research, Bhopal, India

Email Id: satapathys@live.in/rupam@iiserb.ac.in

^{*}Corresponding author

[#]Equal Contribution of both authors.

Abstract: Influenza virus infections have been seen to be persistent throughout the year seasonally claiming several lives. IL-6/IL-2 receptors have been identified as crucial mediators for clearance of influenza by up regulating several intracellular defense mechanisms. Associated with IL-6 signalling is the expression acceleration of sequestrome 1(SQSTM1)/ aggresome and colocalization of HDAC-6 to the cytoplasm. The key player HDAC6 is investigated for modelling several intracellular processes that come into action to bring out the viral clearance. The shuttling of HDAC6 takes place from nucleus to cytoplasm and vice versa controlling the overexpression of IFN γ and ISGs which subsequently triggers virus specific immune response.

Keywords: Histone Deacetylases (HDACs), Influenza virus, Interferon gamma (IFN γ), IFN γ stimulated genes (ISGs), Interleukin 6 (IL-6).

Introduction:

Around the globe, influenza virus induced flu has been the cause of rising mortality in the past years, with India witnessing a total of 8-12% registered cases per year [1]. Studies have proved that of several types of cytokines involved in the immune response against influenza, Interleukin 6 (IL-6) and its receptor (IL-6R) play a crucial role in the fight against the disease and in the efficiency of viral clearance [2]. IL-6 is expressed upon recognition of the viral glycoprotein and RNA strands by pattern recognition receptors like Toll like receptors (TLRs) and Retinoic acid Inducible Gene-1 (RIG) like receptors (RLRs). Downstream to IL-6/IL-6R stabilization, the viral genome undergoes replication and synthesis of several proteins which preferentially layer at the periphery, beneath the plasma membrane [3]. The formation of the sequestrome (SQSTM-1) or aggresome induces the expression of histone deacetylase 6 (HDAC6).

HDAC6, a class II histone deacetylase is one of the least studied HDACS with special reference to its shuffling activity between nucleus and cytoplasm. HDAC6, a transcriptional co-repressor ; deacetylates histone and non histone proteins changing the expression pattern of the genes and their protein product. It contains two catalytic domains in between (DD1 and DD2), a SE14 (serine/ glutamate rich tetradecapeptide repeat) and a zinc finger binding domain (BUZ) at the C terminus as well as a nuclear localization signal (NLS) and nuclear export signal (NES) towards the N terminus [4].

BUZ is known to bind to monoubiquitin of the hitherto polyubiquitinated protein aggresome. Studies have proved that it also co-purifies with ubiquitin specific proteases (USPs) [4]. Further, HDAC catalytic domain (DD1 and DD2) share similarity with BRAP-2 (BRAC-1 associated protein -2) and thus sense any DNA damage induced by integration of viral genome and subsequent replication [4]. So, both aggresome and DNA damage induce HDAC6 expression in flu conditions. HDAC6 known to be predominantly cytoplasmic, is known being understood to efficiently shuttle between the nucleus and cytoplasm [5]. The cytoplasmic anchorage of HDAC6 depends on the SE14 repeats [6] as well as the deacetylation of the lysine residue at the nuclear export signal (NES) at the N-terminus [7].

The two catalytic domains of HDACs share a distinct homology and are placed intermediately in the sequence. The nuclear localization signal is known to be activated upon destabilization of SE14 repeats along with BUZ as well as the acetylation of NES at the N terminal [7]. The C-terminus BUZ (zinc finger binding domain) binds to the ubiquitinated protein aggregates. However, the effect of BUZ upon viral infection is least studied and thus is one of our focus of investigation. HDAC 6 out of all other HDACs has been explicitly central to disease induced cellular modifications, the reason being its importance in α -tubulin dependent deacetylase, that brings about increased cell motility, immune synapse improvisation and engulfment of viral particles and subsequent apoptosis of infected cells by caspases [11] (Fig1). Caspase-3 has been identified as a viral nucleocapsid protein released into the host cell cytosol upon infection [12].

Fig.1. HDAC6 centrality to biological functions,e.g Viral recognition,Immune synapse modification etc.

In the process IL-6, also activates MyD88 dependent pathway, which further renders an active NF- κ B transcription factor that translocates into the nucleus to express the Interferon Stimulated Genes (ISGs) which form IFN γ as product released in the vicinity of the affected cell to inhibit the spread of viral infection [8]. The IFN γ expression is accompanied by a wide range of cytoskeletal rearrangement and lowered expression of global protein content, subjecting it to the attack of Natural killer cells (NK cells) [10].

In this paper, we have tried to illustrate the cascade of events that underlie the IL-6 and HDAC6 interactions and subsequent clearance of the influenza virus infection.

Materials and Methods:

The data for HDAC6, IL-6 and Caspase-3 have been obtained from National Centre of Biotechnology Information (NCBI) database. All these protein molecules have *Homo sapiens* origin. The accession numbers are AAH69243.1 (HDAC6, 1215aa), CAG29292.1 (IL-6, 212aa) , CAC88866.1 (Caspase-3, 277 aa).

The bioinformatics softwares used for the sequence alignment is CLUSTALW Omega (accessible at www.ebi.c.uk/Tools/msa/clustalwo) provided by European Molecular Bioinformatics Laboratory- European Bioinformatics Institute (EMBL-EBI) and blastP (NCBI, accessible at blast.ncbi.nlm.nih.gov).

The interactome pattern has been studied using Protinfo PPC (accessible at protinfo.compbio.washington.edu/PPC).

Results and Discussion:

HDAC-6 sequence analysis: There are two catalytic domains of HDAC6 (DD1-domain1 and DD2- domain2). DD1 spans 286-374 and DD2 spans 485-835 amino acids (Fig.2,3).

The putative active sites of DD-1 in HDAC are – 215,216,224, 225, 253, 255,346 and 384 amino acid(aa) residues. Only one active site 346aa lies within DD-1. The putative active sites for domain 2 are identified as 610,611,614, 620, 649,651,742, 780,622,624, 630,634 aa which all span in the DD2 region. 891-1048 aa in HDAC6 in the viral G glycoprotein recognition domain and thus caspase3 binds to this domain, and cleaves it at 1088 bp at the site of the zinc finger binding domain (BUZ).

Fig.2. Homology between yeast Hda1 and human HDAC6

Fig.3. HDAC6 functional domain

Fig.4. The amino acid residue of the homologous regions and the active sites of HDAC6.

Interaction of IL-6 and HDAC-6: The homology prediction of IL-6 and HDAC6 shows very few conserved regions, but regions of similarity are between 1-131 aa (IL-6) and 621-1066 aa (in HDAC 6). The 485-835 aa of DD-2 of HDAC6 also shows the very few sequence alignment, other than a few active site residues (610, 611, 619,620, 649, 651, 742, 780, 622, 624, 630,634). Further, protein blast (BlastP) available at NCBI, confirmed some common functional product (Fig.4). However, the sequence similarity indicates that IL-6 leads to HDAC-6 overexpression via a mediator molecule as like sequestrome (SQSTM1) or aggresome, instead of direct spatial interaction. The interaction is also temporally defined as the presence of the former, leads to the expression of the latter in the proposed model.

Fig.5. Multi protein alignment of HDAC6 and IL-6 using CLUTALWO.

Interaction of Caspase-3 and HDAC6: Caspase-3 shares homology with 286-374 aa residue of HDAC-6. The most representative homologous aa residues of caspase-3 are 10,11,23-24,28, 37, 40, 48, 51, 53, 54,55, 61,64 to 156. The other region of the similarity is between 157-490 aa of caspase-3 and 527-922 aa of HDAC-6(Fig.5,7). The high similarity of both indicate a potential spatial interaction of both, as well as a possible case of colocalized expression giving a temporal profiling.

Interaction of Caspase-3 and IL-6: Caspase -3 and IL-2 had very few regions of sequence based homology and therefore we examined both for protein blast using (BLASTP) available at NCBI(Fig.6,7). The functional product similarity test also confirmed no direct correlation of both and thus denying the possibility of a spatial interaction of both .

Fig.6. CLUSTALWO between HDAC-6 and Caspase 3.

Fig.7. CLUSTALWO between IL-6 and Caspase 3.

Fig.8. Color code used in the CLUTALWO

Modelling the cascade of events: We designed an algorithm based on due interaction patterns and the known literature on the behavior of each of the key proteins (HDAC-6, IL-6). The cascade has been represented (Fig.10,11).

The aggresome included HDAC-6 expression co-localized with α -tubulin in cytosol, leads to increased acetylation of polymerized α -tubulin. This causes increased motility and endocytosis, which helps in higher infiltration of viral particles into the same cell

due to ease of access (Fig.9). The NES at N-terminal is simultaneously deacetylated leading to the activation of NLS and repression of NES. Prior to this the monoubiquitin bound BUZ gets cleaved at 1088bp by USPs (ubiquitin specific protease) upon caspase-3 binding at metaviral in glycoprotein recognition site of domain 2 (Fig.10). The C-terminal truncated version of HDAC, translocates into the nucleus where it acetylates the histones for upregulated expression of most of the housekeeping proteins and deacetylates for downregulation of accessory protein and major histocompatibility protein class I (MHC I). This makes the infected cell subject to programmed cell death by apoptosis via Natural killer cell (NK) action. The HDAC-6 simultaneously gets activated at the same NES domain of N-terminus, causing its shuttling back to the cytosol and repetition of the same process till viral clearance of the infected cell is achieved. The IL-6 mediated activation of MyD88 dependent pathway upregulates Interferon Stimulated genes (ISGs) via active NF- κ B transcription factor, leading to expression of Interferon ($\text{IFN}\gamma$), which limits the spread of viral pathogens to the cell in vicinity (Fig.11).

Fig.9. Sequence comparison of different tubulin and HDAC 6 action site.

Fig. 10.Cascade of events post Viral infection at the cytosol- nucleus interface and in the cytosolic periphery.

Fig. 11.Cascade of events post Viral infection inside the nucleus and production of $\text{IFN}\gamma$.

Interaction of HDAC6 and other proteins: Using Protinfo software, we deciphered a range of different proteins either colocalizing or spatially interacting with HDAC6. To list, a few of them are Tubg1, Axin 1, Mapre 1, APC, Clip 2, SS 18, Clasp which mainly regulate cell cytoskeletal rearrangement, cell growth and survivability (Fig.12).

Fig.12. Interaction of HDAC6 with other putative proteins in stressed condition using Protinfo.

Phylogenetic relation of HDAC-6/IL-6/Caspase3: The bootstrap analysis method of phylogenetic relation between the three key protein molecule also shows significant similarity of HDAC-6 and caspase-3, that in contrast to IL-6. However IL-6 and HDAC-6 have few regions of homology. (Fig.13)

Fig.13. Phylogenetic relation between IL-6, HDAC6 and caspase3 in chronological order.

Conclusion:

The proposed model is based on the interaction patterns of the key protein molecules like HDAC-6 and IL-6 in influenza infection condition. The in-silico docking gives an early insight to the possible pathway taken up by the immune system to evade the

pathogen. However, sufficient experimental evidences can establish the proposed model. The future outcomes of this model promises for epigenetic modulation of HDACs for treating diseases of different virulence.

Conflict of Interest:

This is an independent project carried out in collaboration of both the authors. All softwares used for the project work are open sourced and reference has been quoted wherever applicable.

References:

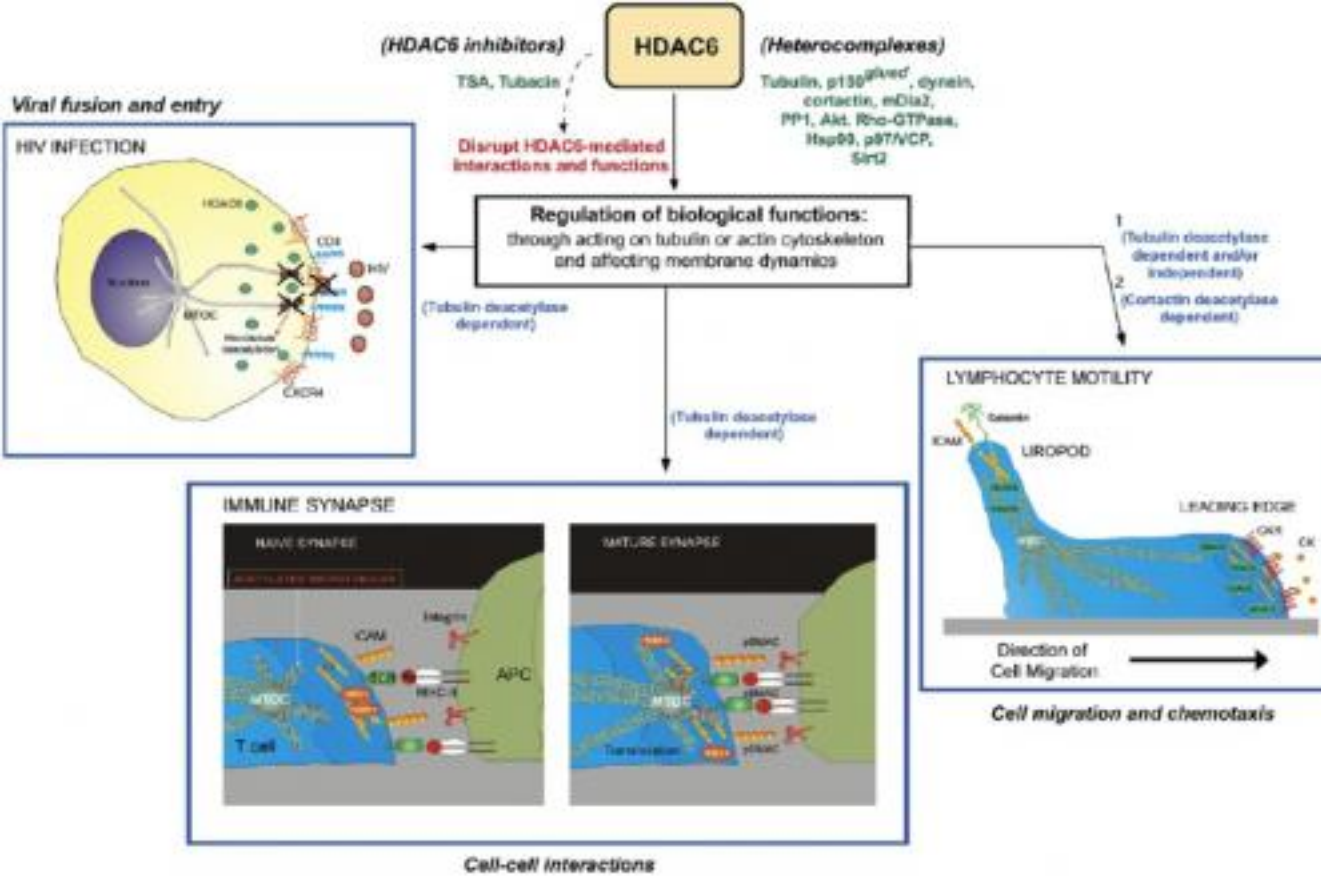
1. CDC global health report (www.phac.aspc.gc.ca).
2. Dienz O, Rud JG, Eaton SM, Lanthier PA, Burg E, Drew A, Bunn J, Suratt BT, Haynes L, Rincon M. Essential role of IL-6 in protection against H1N1 influenza virus by promoting neutrophil survival in the lung. *Mucosal Immunol.* 2012 May;5(3):258-66. doi: 10.1038/mi.2012.2. Epub 2012 Feb 1.
3. Matloob Hussain, Kevin S Harrod. Influenza A virus-induced caspase-3 cleaves the histone deacetylase 6 in infected epithelial cells. *FEBS Letters* 583 (2009) 2517–2520.
4. Sara S. Hook, Amir Orian, Shaun M. Cowley, and Robert N. Eisenman*. Histone deacetylase 6 binds polyubiquitin through its zinc finger (PAZ domain) and copurifies with deubiquitinating enzymes. *PNAS.* October 2012, vol. 99 no. 21.

5. Hiroyuki Kayamuro, Yasuo Yoshioka, Yasuhiro Abe, Shuhei Arita et.al. Interleukin-1 Family Cytokines as Mucosal Vaccine Adjuvants for Induction of Protective Immunity against Influenza Virus. *J. Virol.* December 2010 vol. 84 no. 24 12703-12712. (Myd88).
6. Nicholas R. Bertos, Benoit Gilquin, Gordon K. T. Cha et.al. Role of the Tetradecapeptide Repeat Domain of Human Histone Deacetylase 6 in Cytoplasmic Retention. *The Journal of Biological Chemistry.* Vol. 279, No. 46, November 12, 2004 .
7. Yuanjing Liu, Lirong Peng, Edward Seto, Suming Huang, and Yi Qiu. Modulation of Histone Deacetylase 6 (HDAC6) Nuclear Import and Tubulin Deacetylase Activity through Acetylation. *J Biol Chem.* 2012 August 17; 287(34): 29168–29174.
8. C Boyault, K Sadoul, M Pabion_ and S Khochbin_HDAC6, at the crossroads between cytoskeleton and cell signaling by acetylation and ubiquitination. *Oncogene* (2007) **26**, 5468–5476.
9. Young-Sang Koh, Jung-Eun Koo, Amlan Biswas, □Koichi S. Kobayash. MyD88-Dependent Signaling Contributes to Host Defense against Ehrlichial Infection. *Plos One*, July 2013.
10. Kayla A. Holder, Staci N. Stapleton, Maureen E. Gallant, Rodney S. Russell and Michael D. Gran. Hepatitis C Virus–Infected Cells Downregulate NKp30 and Inhibit Ex Vivo NK Cell Functions. *The Journal of Immunology*, August 2013, 191: 000–000.
11. Charlotte Hubbert, Amaris Guardiola, Rong Shao, Yoshiharu Kawaguchi et.al. HDAC6 is a microtubule-associated deacetylase. *Nature* **417**, 455-458 ,23

May 2002.

12. Karlberg H1, Tan YJ, Mirazimi A. Induction of caspase activation and cleavage of the viral nucleocapsid protein in different cell types during Crimean-Congo hemorrhagic fever virus infection.
13. Goldberg AL (2003) Protein degradation and protection against misfolded or damaged proteins. *Nature* 426: 895–899. doi: 10.1038/nature02263.
14. Yao TP (2010) The role of ubiquitin in autophagy-dependent protein aggregate processing. *Genes Cancer* 1: 779–786. doi: 10.1177/1947601910383277.
15. Watanabe Y, Tanaka M (2011) p62/SQSTM1 in autophagic clearance of a non-ubiquitylated substrate. *J Cell Sci* 124: 2692–2701. doi: 10.1242/jcs.081232.
16. Kawaguchi Y, Kovacs JJ, McLaurin A, Vance JM, Ito A, et al. (2003) The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. *Cell* 115: 727–738. doi: 10.1016/S0092-8674(03)00939-5.
17. Jiang, Q., Ren, Y. and Feng, J. (2008). Direct binding with histone deacetylase 6 mediates the reversible recruitment of parkin to the centrosome. *J. Neurosci.* 28, 12993-13002.
18. Jordan, M. A. and Wilson, L. (2004). Microtubules as a target for anticancer drugs. *Nat. Rev. Cancer* 4, 253-265.
19. Joshi, H. C. (1998). Microtubule dynamics in living cells. *Curr. Opin. Cell Biol.* 10, 35- 44.

20. Kawaguchi, Y., Kovacs, J. J., McLaurin, A., Vance, J. M., Ito, A. and Yao, T. P. (2003). The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. *Cell* 115, 727-738.
21. Kirschner, M. W. (1987). Biological implications of microtubule dynamics. *Harvey Lect.* 83, 1-20.
22. Komarova, Y. A., Akhmanova, A. S., Kojima, S., Galjart, N. and Borisy, G. G. (2002). Cytoplasmic linker proteins promote microtubule rescue in vivo. *J. Cell Biol.* 159, 589- 599.



Fission yeast

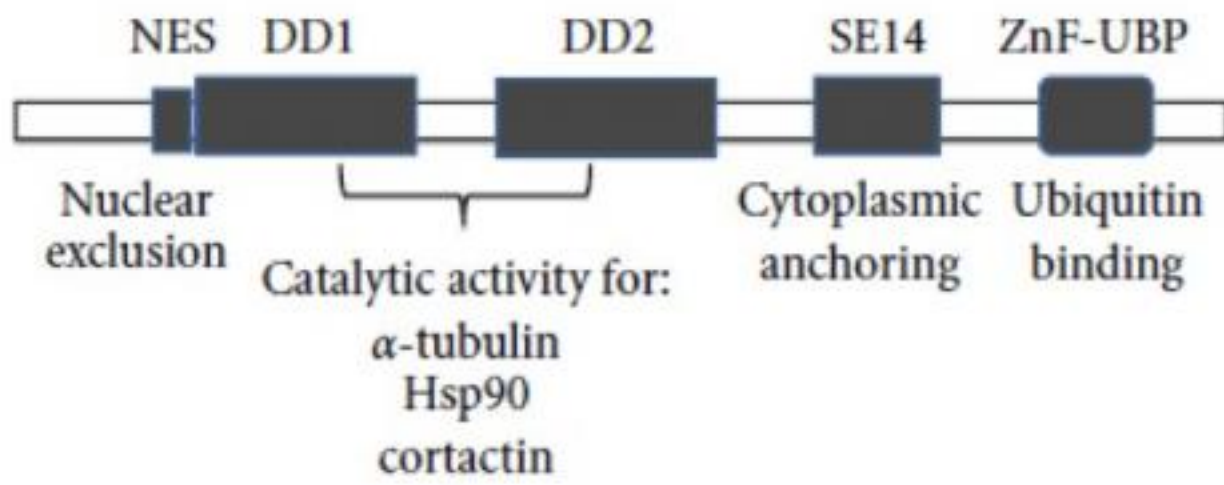
Hda1

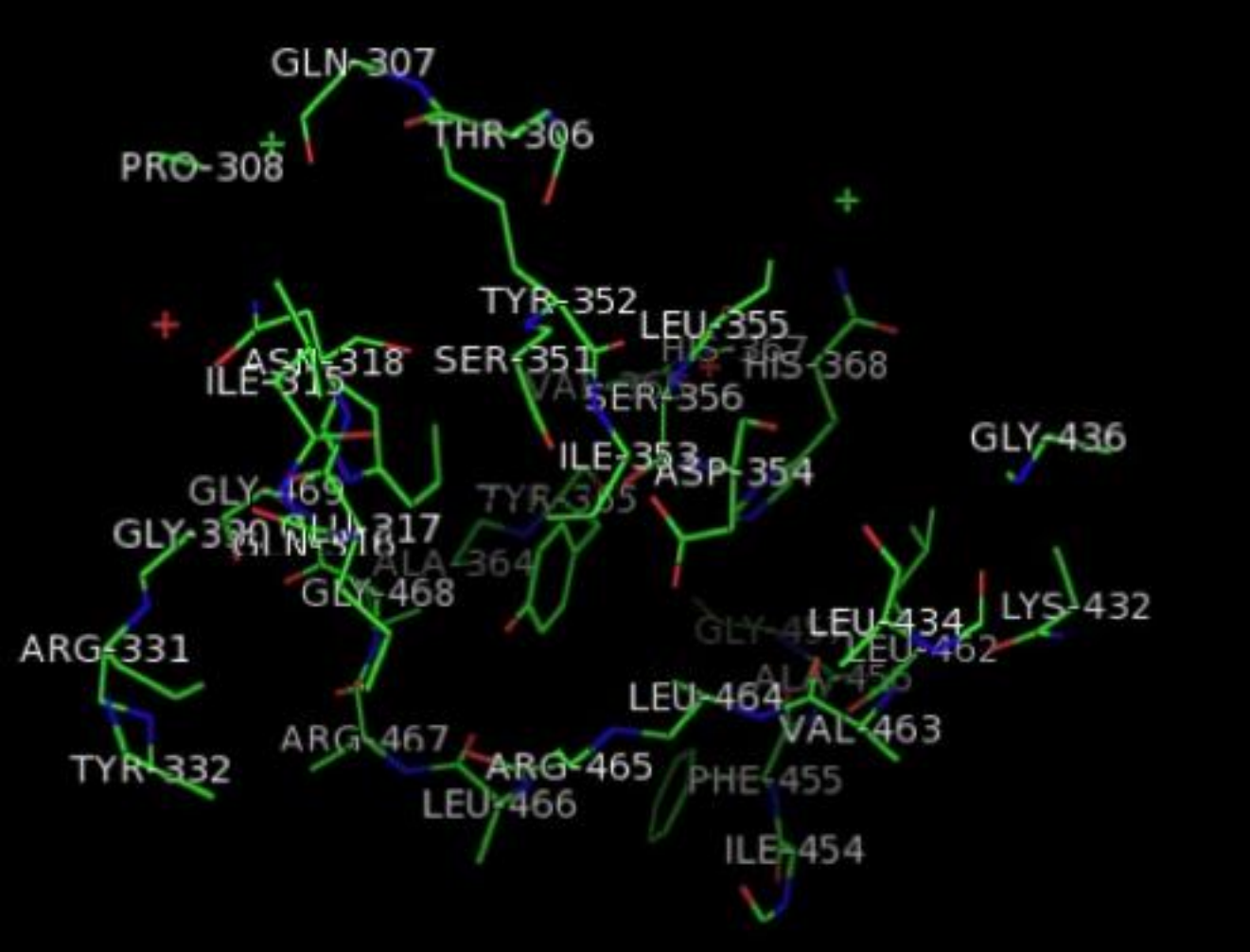


H. sapiens HDAC6



TRENDS in Cell Biology





☒	10001	1	-----MYRM	4
☒	10002	1	MTSTGQOSTITRQRRSRQNPQSPQDSSVTSKRNIRKGAVERSIPLAEVKKKGKMKGLQAMEEDLIVGLQGMOLMLEA	80
☒	10001	5	QLLSCLALSALVINSAPTSSSTKNTQLQLERLLDLQMIINGINNYKPKLIRMLTPKYMPKATELKHLCLEEEK	84
☒	10002	81	EALAGTGLVLDEQLNEFHCLWDSFFEGPERLHAIKEQLIQEGLLDRCVSFQARFAEKELMLVHSLEYIDLNETTQYMG	160
☒	10001	85	PLEEVLNLAQSKNFHLRFRDLISHINIVIVLELKGSETTFMCEYADETAII-----VEFLNRWITFCQSIISTLT---	153
☒	10002	161	EGELRVLADTYDSVYLHFNYSYSCACLASGSVLRRLVDVLGAEIRKMGMAIIRFPGRHAQSSLMDSGYCMFHEVAVAAARYAQ	240
☒	10001		-----	
☒	10002	241	KHRIHRVLIVDMOVHBSQGTQFTFDQDPSVLYFSIRYEQGRFWPHLKASNWSITGFGQGQSYTINVFWNQVGRDADYI	320

[illegible]

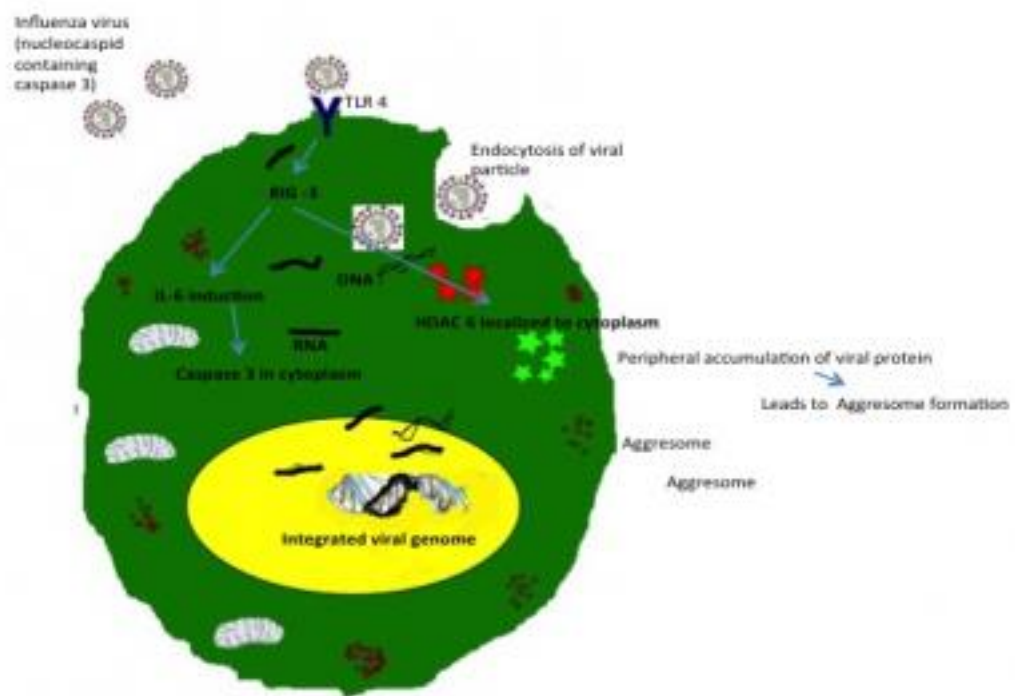
gi 16516817 emb CAC88866.1	MENTENSVDSKSIKNLEPKIIHGSESMDSGMSWDIGYKMDYFEMGLCIII	50
gi 512412 emb CAA01199.1	----MYRMQLLSICIALSLAIVTNSAPTSS-STWKTQLQLHLLLDLQMIL	45
	: : * * : : * . . * : . * : : : : : . * : :	
gi 16516817 emb CAC88866.1	NNKMFHKSTGMTSRSGTDVDAANLRETFNNLKYEVRNKNDLTREEIVELM	100
gi 512412 emb CAA01199.1	NGINNYKNPKLTR-----MLTFKPYMPKWKATELKHLQCLEKELKPL	86
	* . * : * . . : *	
gi 16516817 emb CAC88866.1	RDVSKEDHSKRSSFVVCVLLSHGEEGIIIFGTNGFVDLKKITNFFRGRCRS	150
gi 512412 emb CAA01199.1	EEVLNLAQSKN-----FHLRPRDLISNIN-----	110
	. : * : : * * . : . : * . *	
gi 16516817 emb CAC88866.1	LIGKPKLFIQACRGTELDGCIETDSGVDDDMACHKIPVDADFLYAYSTA	200
gi 512412 emb CAA01199.1	-----VIVLELKGSETTF-----	123
	. * : : * : *	
gi 16516817 emb CAC88866.1	PGYYSWRNSKDGSWFIQSLCAMLKQYADKLEFMHILIRVNRKVKATEFESF	250
gi 512412 emb CAA01199.1	-----MCEYADETATIVEFLNRWITFCQSIISTLT--	153
	: * . : * : * : : : . : : : :	
gi 16516817 emb CAC88866.1	SFDAIFHAKWQIPCIIVSMLIKELYFYH	277
gi 512412 emb CAA01199.1	-----	

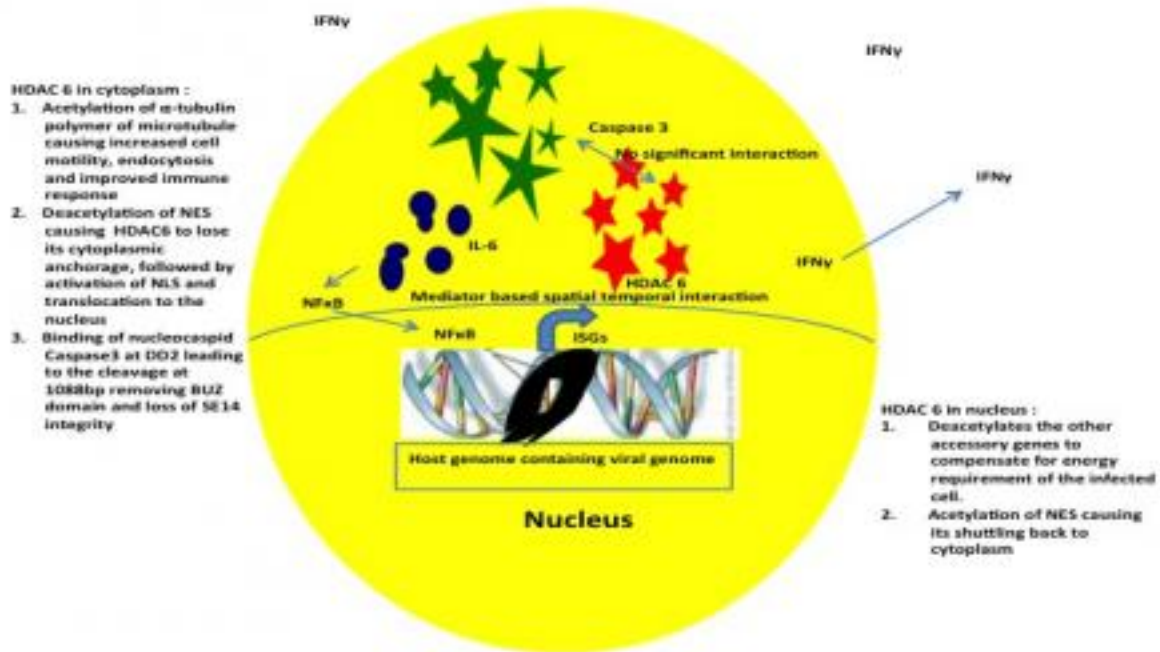
Residue	Colour	Property
AVFPMLIV	RED	Small (small+ hydrophobic (incl.aromatic -I))
DE	BLUE	Acids
RK	MAGENTA	Basic - H
STYHONGQ	GREEN	Hydroxyl + sulphydryl + amine + G
Others	Grey	Unusual amino/imino acids etc.

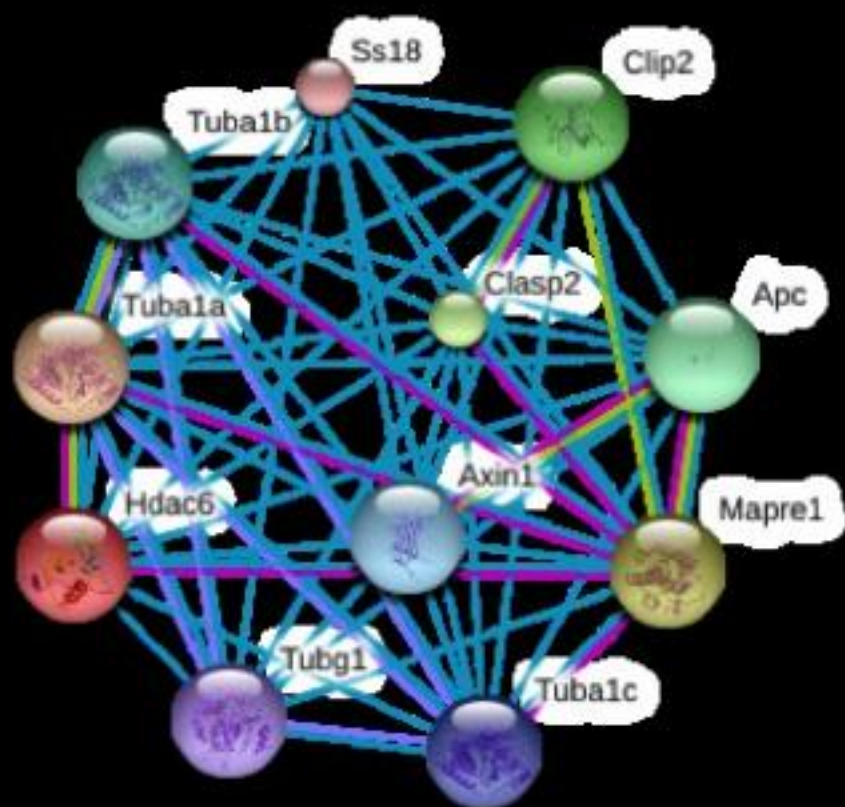
Acetylation



Human α1	24-	YCLEHGI	QPDGQMPSDK	TI-GGGDDSFN	TFFSETG-57 (P05209)
Mouse α1	24-	YCLEHGI	QPDGQMPSDK	TI-GGGDDSFN	TFFSETG-57 (NP_035783)
<i>D. melanogaster</i> α1	24-	YCLEHGI	QPDGQMPSDK	TV-GGGDDSFN	TFFSETG-57 (P06503)
<i>C. elegans</i> α3	24-	YCLEHGI	QPDGQMPSDK	SL-GGSDDSFN	TFFSETG-57 (BAA22203)
<i>A. thaliana</i> α6	24-	YCLEHGI	QPDGQMPSDK	TV-GGGDDAFN	TFFSETG-57 (NP_849388)
<i>S. cerevisiae</i> α1	24-	YSLEHGI	KPDGHLEDGL	SKPKGGEEGFS	TFFHETG-58 (P09733)
<i>S. cerevisiae</i> α3	24-	YSLEHGI	KPDGHLEDGL	SKPKGGEEGFS	TFFHETG-58 (P09734)







gi|512412|emb|CAA01199.1| 0.35163

gi|16516817|emb|CAC88866.1| 0.45883

gi|46623327|gb|AAH09243.1| 0.46536