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Post-Translational Regulation of Protein Function by Acetylation: Many Occurences, Few Rules

M. GIACCA
ICGEB
Trieste
Italy

Mauro Giacca, MD PhD
Director, ICGEB Trieste
giacca@icgeb.org
http://www.icgeb.org

Post-translational protein modification by acetylation

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Regulation of protein function by acetylation

Contributions from the Giacca Lab

Manganaro, L, et al. 2010. Nat Med 16, 329.	HIV-1 Integrase
Terreni, M, et al. 2010. Retrovirology 7, 18.	HIV-1 Integrase
Paolinelli, R, et al. 2009. Nature Struct Mol Biol 16, 412.	CDC6
Mateo, F, et al. 2009. Nucleic Acids Res 37, 7072.	CDK2
Sabo, A, et al. 2008. Mol Cell Biol 28, 2201.	CDK9
Pantano, S, et al. 2006. Proteins 62, 1062.	HIV-1 Tat
Galbiati, L, et al. 2005. Cell Cycle 4, 930.	E2F-1
Cereseto, A, et al. 2005. EMBO J. 24, 3070.	HIV-1 Integrase
Lusic, M, et al. 2003. EMBO J. 22, 6550.	HIV-1 LTR chromatin
Pantano, S, et al. 2002. J. Mol. Biol. 318, 1331.	HIV-1 Tat
Marzio, G, et al. 2000. J. Biol. Chem. 275, 10887.	E2F-1

Sites of acetylation and methylation of yeast histones

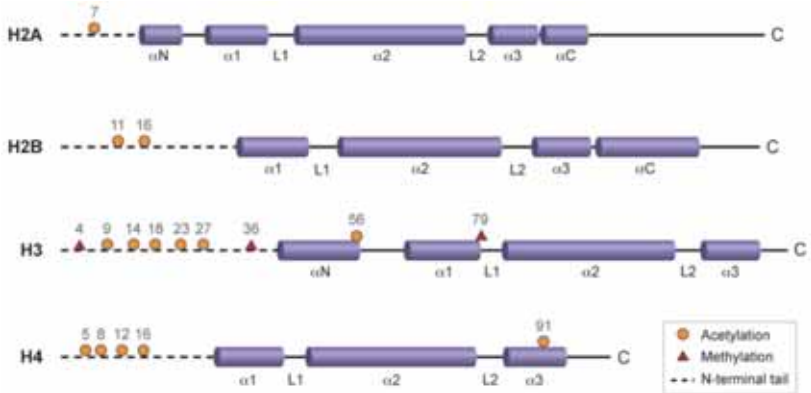

Figure 1
Sites of acetylation and methylation on histones H2A, H2B, H3, and H4 in yeast. Acetylation (*orange circles*) and methylation (*red triangles*) sites in the N-terminal tails (*dashed lines*) and globular domains (α-helices, e.g. αN and α1, and looped regions, L1 and L2) are indicated (2a).

Annu. Rev. Biochem. 2007. 76:75–100

TABLE 1. Summary of Known Histone and Transcription Factor Acetyltransferases and Deacetylases

Protein	Enzyme	Substrates*	Organism	Remarks
Gcn5p	HAT A	H3 (K14), H4 (K8, 16), H2B (free)	Yeast	Co-activator/adaptor
Gcn5p/Ada	HAT A	H3, H2B (nuc)	Yeast	Purified complex (0.8 MD)
SAGA	HAT A	H3, H2B (nuc)	Yeast	Purified complex (1.8 MD)
Gcn5p/Ada3?	HAT A	H4 (nuc), Sin1p	Yeast	Purified complex
Gcn5p?	HAT A	All core (nuc?)	Yeast	Gcn5p overproduced in vivo
p55	HAT A	H3, H4, H2B (free)?	*Tetrahymena*	
hTAF$_{II}$250	HAT A	H3 (K14), H4 (free), TFIEβ	Human	Largest subunit for TFIID
dTAF$_{II}$230	HAT A	H3, H4 (free)	*Drosophila*	
yTAF$_{II}$130	HAT A	Not available	Yeast	Essential for cell cycle progression
p300/CBP	HAT A	All core (nuc), K5, 9, 12, 16 of H4 pep, TFIEβ (K52), TFIIF, p53 (K373, 382, peptide)	Human	Co-activator, interacts with E1A and T Ag, and nuclear receptors
PCAF	HAT A	H3 (nuc)	Human, *Drosophila*	Carboxyl half homologous to yGcn5p; interacts with p300/CBP
ACTR	HAT A	H3, H4 (nuc)	Human	Co-activator, interacts with CBP, PCAF and nuclear receptors
SRC-1	HAT A	H3, H4 (nuc), H3 (K9 and 14) (peptide)	Human	Co-activator; interacts with PCAF
Esa1p	HAT	Free H4, H2A, H3	Yeast	Essential protein; homologous to dMOF
Tip60	HAT A	H4, H2A, H3	Human	Interacts with HIV-1-Tat proteins; nucleosomes inhibit HAT activity; nuclear protein
Hat1p	HAT B	H4 (K12) (free)	Yeast	Associates with Hat1p (RbAp48 family)
Hat1p	HAT B	H4 (K12, K5) (free)	Yeast	Recombinant form
Rpd3p	HDAC	All acetylated histones	Yeast	Transcriptional regulator (>2 MD); HDB complex (0.6 MD); histones hyper-acetylated in *rpd3*Δ cells, esp. H4 K5 and 12
mRpd3	HDAC	Not available	Human	Co-repressor; repression inhibited by deacetylase inhibitors; interacts with mSin3p; recruited by nuclear receptors
Hda1p	HDAC	All acetylated histones	Yeast	HDA complex (350 kD)
HDAC1	HDAC	Not available	Human	Associates with RbAp48

*Free (non-nucleosomal) histones, nucleosomal histones (nuc), or synthetic peptide substrates. Acetylated lysines, when available, are listed as well. When both free and nucleosomal histones are good substrates for a HAT, only nucleosomal specificities are listed.
†J. Brownell, T. Ranalli, C. D. Allis (unpublished observations).

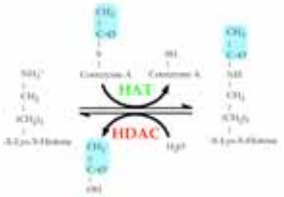

Figure 1. Equilibrium of steady-state histone acetylation is maintained by opposing activities of histone acetyltransferases and deacetylases. Acetyl coenzyme A is the high-energy acetyl moiety donor for histone acetylation. Histone acetyltransferases (HATs) transfer the acetyl moiety to the ε NH_3^+ group of internal lysine residues of histone N-terminal domains. Reversal of this reaction is catalyzed by histone deacetylases (HDACs).

Mammalian HATs and HDACs.

HAT	HDAC
GNAT family PCAF, GCN5L2	**Class I** HDAC1-3, HDAC8
CREBBP/EP300 family CREBBP, EP300	**Class II** HDAC4-7
MYST family HTATIP, ZNF220, HBO1, MORF, MYST1	**Sirtuins** (NAD-dependent HDACs) SIRT1-7
TAFII 250 family TAFII 250	
SRC family ACTR, SRC1, SRC3, NCOA2	
Other HATs TCF2 GTF3C1	

ACTR, activin receptor; CREBBP, CREB-binding protein; EP300, e1a-binding protein p300; GCN5L2, general control of amino-acid synthesis 5-like 2; GNAT, GCN5-related acetyltransferase; GTF3C1, general transcription factor 3c, polypeptide 1; HAT, histone acetyltransferase; HBO, histone acetyltransferase binding to ORC; HDAC, histone deacetylase; HTATIP, HIV Tat interactive protein; HIV tat, human immunodeficiency virus type I *trans*-acting transcription factor; MORF, MOZ-related factor; MOZ, monocytic leukemia zinc finger protein; MYST, MOZ, YBF2/SAS3, SAS2, TIP60 protein family; NCOA2, nuclear receptor coactivator 2; ORC, original recognition complex; PCAF, EP300/CREBBP-associated factor; SIRT, sirtuin; SRC, steroid receptor coactivators; TAF, TATA box-associated factors; TCF2, transcription factor 2; ZNF220, Zinc finger protein 220.

Current Opinion in Genetics & Development 2004, **14**:308–315

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Activation of p53 Sequence-Specific DNA Binding by Acetylation of the p53 C-Terminal Domain

Wei Gu and Robert G. Roeder*
Laboratory of Biochemistry and Molecular Biology
The Rockefeller University
New York, New York 10021

Summary

The tumor suppressor p53 exerts antiproliferation effects through its ability to function as a sequence-specific DNA-binding transcription factor. Here, we demonstrate that p53 can be modified by acetylation both in vivo and in vitro. Remarkably, the site of p53 that is acetylated by its coactivator, p300, resides in a C-terminal domain known to be critical for the regulation of p53 DNA binding. Furthermore, the acetylation of p53 can dramatically stimulate its sequence-specific DNA-binding activity, possibly as a result of an acetylation-induced conformational change. These observations clearly indicate a novel pathway for p53 activation and, importantly, provide an example of an acetylation-mediated change in the function of a nonhistone regulatory protein. These results have significant implications regarding the molecular mechanisms of various acetyltransferase-containing transcriptional coactivators whose primary targets have been presumed to be histones.

Regulation of p53 by post-translational modification

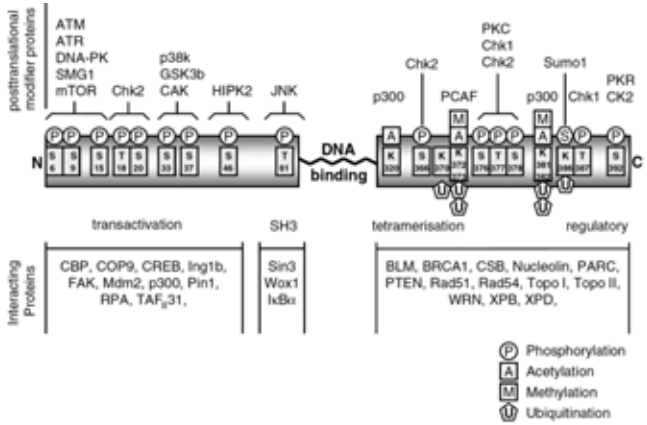

Figure 1 Post-translational modification of p53. In the upper part of the schematic representation of p53, the proteins that phosphorylate, acetylate or methylate the molecule are indicated. Where modification occurs at the same or adjacent sites this is shown, for example, at K381, K382 methylation and acetylation. Ubiquitination also occurs at these sites which is shown under the p53 schematic. The transactivation, SH3, DNA binding, tetramerization and regulatory domains of p53 are outlined, as well as the proteins that interact with these domains

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HAT FAT KAT

HDAC FDAC KDAC

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E2F Family Members Are Differentially Regulated by Reversible Acetylation*

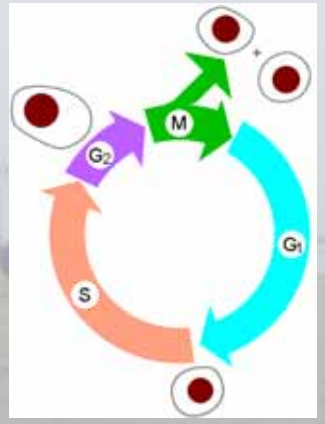
(Received for publication, November 16, 1999, and in revised form, January 13, 2000)

Giuseppe Marzio‡§, Christian Wagener¶, Maria Ines Gutierrez‡, Peter Cartwright¶, Kristian Helin¶, and Mauro Giacca‡**

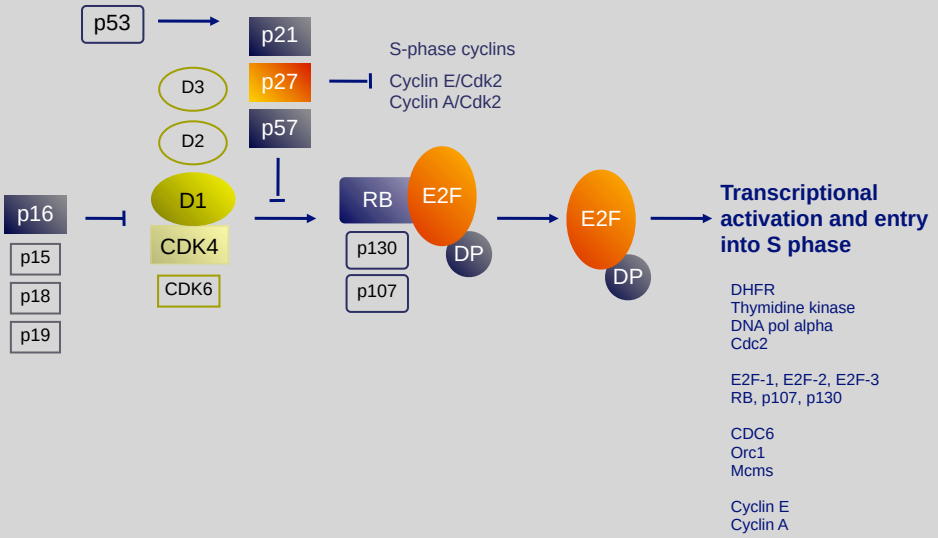
From the ‡Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology, Area Science Park, Padriciano 99, 34012 Trieste, Italy and ¶Department of Experimental Oncology, European Institute of Oncology, via Ripamonti 435, 20141 Milan, Italy

The six members of the E2F family of transcription factors play a key role in the control of cell cycle progression by regulating the expression of genes involved in DNA replication and cell proliferation. E2F-1, -2, and -3 belong to a structural and functional subfamily distinct from those of the other E2F family members. Here we report that E2F-1, -2, and -3, but not E2F-4, -5, and -6, associate with and are acetylated by p300 and cAMP-response element-binding protein acetyltransferases. Acetylation occurs at three conserved lysine residues located at the N-terminal boundary of their DNA binding domains. Acetylation of E2F-1 *in vitro* and *in vivo* markedly increases its binding affinity for a consensus E2F DNA-binding site, which is paralleled by enhanced transactivation of an E2F-responsive promoter. Acetylation of E2F-1 can be reversed by histone deacetylase-1, indicating that reversible acetylation is a mechanism for regulation also of non-histone proteins.

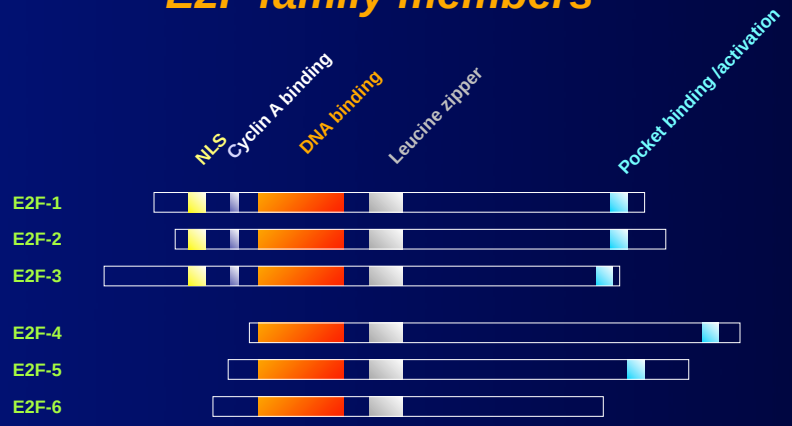
The eukaryotic cell cycle



Regulation of G1-S transition



E2F family members



E2F-1, -2, and -3

- Nuclear
- Associated with pRb
- Cell cycle regulated
- Bind p300/CBP

E2F- 4, -5, and -6

- No NLS
- Associated with p107 and 130
- Constant throughout cell cycle
- No p300/CBP binding

E2F-1 acetylation

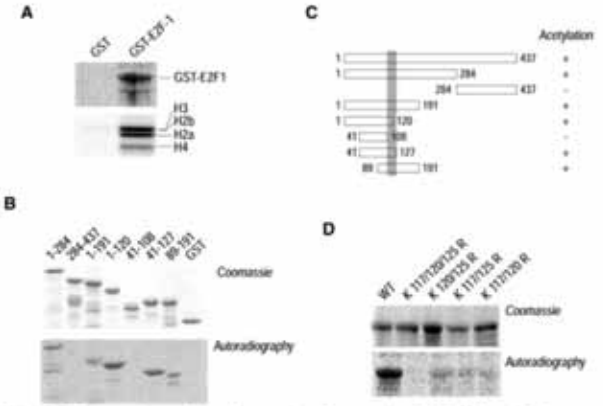


FIG. 1. **Acetylation of E2F-1.** *A*, E2F-1 associates with and is acetylated by a nuclear HAT. HeLa cell Dignam nuclear extract was incubated with GST or GST-E2F-1 on agarose beads, and bound proteins were assayed for HAT activity. Acetylated proteins were resolved by SDS-PAGE (7% acrylamide *upper panel*, 12% *lower panel*) and detected by autoradiography. *Upper panel*, acetylation of E2F-1; *lower panel*, acetylation of histones. *B*, minimal acetylated domain of E2F-1 comprises aa 41–127. GST-E2F-1 fragments on agarose beads were incubated with HeLa Dignam nuclear extract in the presence of [^{14}C]acetyl-CoA. Acetylated proteins were resolved by 10% SDS-PAGE. *Upper panel*, Coomassie-stained gel. *Lower panel*, autoradiography. *C*, schematic representation of the E2F-1 fragments used for the experiments in *B*. The minimal region overlapping all acetylation-positive fragments is *shaded* in *gray*. This region contains lysines at positions 117, 120, and 125. *D*, mutations of Lys-117, Lys-120, and Lys-125 suppress acetylation of E2F-1. Mutations of two out of three lysines decrease the acetylation levels. GST-E2F-1 proteins on agarose beads were treated as in *B*. *Upper panel*, Coomassie-stained gel. *Lower panel*, autoradiography.

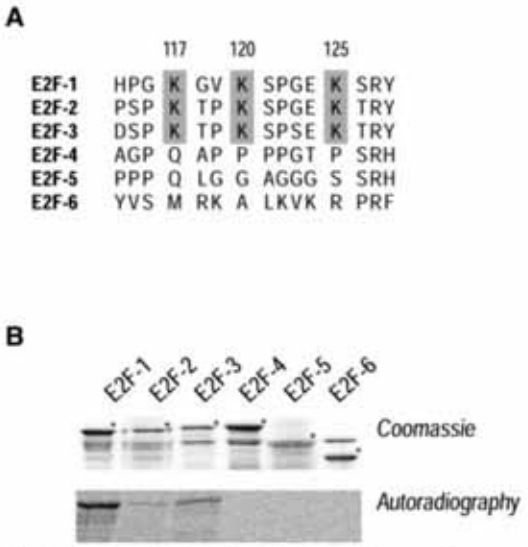
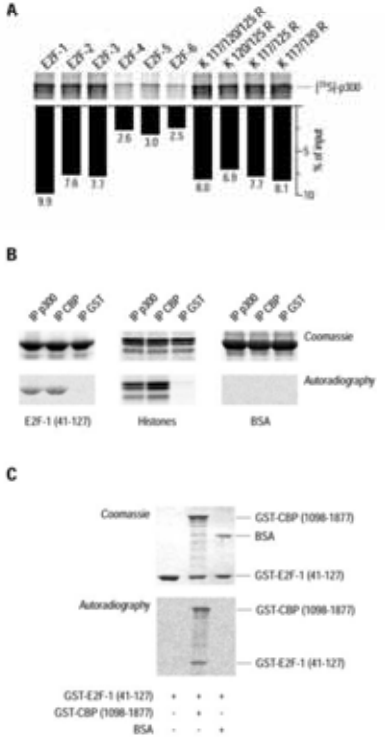


FIG. 2. **Acetylation of E2F family members.** *A*, sequence alignments of the E2F-1 to -6 regions homologous to aa 114–128 of E2F-1. The lysines conserved in E2F-1, E2F-2, and E2F-3 are *shaded* in *gray*. *Numbering* is according to E2F-1. *B*, E2F-1, E2F-2, and E2F-3 are acetylated by a nuclear HAT. GST-E2F proteins (indicated by *asterisks* in the Coomassie panel) were treated as indicated in *B*. *Upper panel*, Coomassie-stained gel. *Lower panel*, autoradiography.

FIG. 3. **E2F binds to and is acetylated by p300/CBP.** *A*, binding of E2F family members and E2F-1 lysine mutants to ^{35}S-labeled p300. *B*, acetylation of E2F-1 by immunoprecipitated (*IP*) p300 and CBP. GST-E2F-1 (aa 41–127), histones, or BSA were incubated in the presence of [^{14}C]acetyl-CoA with the indicated immunoprecipitated proteins. Proteins were resolved by 12% SDS-PAGE. *C*, E2F-1 is acetylated by recombinant CBP. GST-E2F-1 (aa 41–127) was incubated with recombinant GST-CBP (aa 1098–1877) or BSA in the presence of [^{14}C]acetyl-CoA. Proteins were resolved by 10% SDS-PAGE. The *upper band* in the autoradiography corresponds to the autoacetylated CBP fragment (aa 1098–1877; Ref. 37).



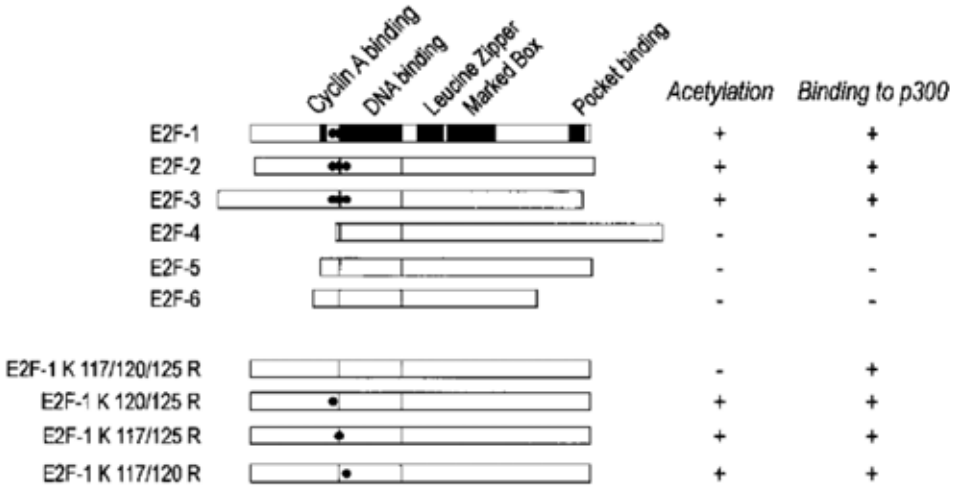
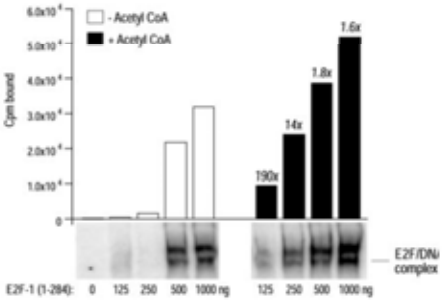


FIG. 4. **Summary of binding and acetylation results.** E2F family members and E2F-1 mutants are aligned according to the position of their DNA binding domain. The locations of Lys-117, Lys-120, and Lys-125 are indicated by *gray dots*.

E2F-1 acetylation increases DNA binding



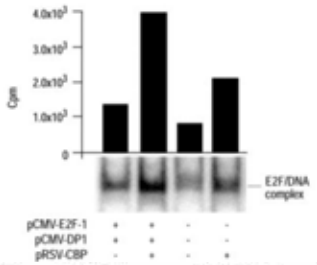


FIG. 6. **Acetylation of E2F-1 increases DNA binding.** *A*, acetylation increases DNA binding *in vitro*. GST-E2F-1 (aa 1–284) was incubated with the enzymatically active GST-CBP (aa 1098–1877) protein, without or with acetyl-CoA, as indicated. After incubation, increasing amounts of the reactions, containing the indicated nanograms of GST-E2F-1 protein, were used for electrophoretic mobility shift assays using an E2F probe. The *numbers* on *top* of each bar on the *right side* of the histogram indicate fold binding of the samples incubated with acetyl-CoA over the respective samples incubated in the absence of acetyl-CoA. *B*, acetylation increases DNA binding *in vivo*. Overexpression of CBP increases DNA affinity of endogenous and transfected E2F. U2OS cells were transfected with expression vectors for E2F-1 and/or CBP, as indicated. Transfections of E2F-1 were always carried out in the presence of a DP1-expression plasmid. Nuclear extracts were analyzed by electrophoretic mobility shift assays using an E2F probe.

E2F-1 acetylation increases transcriptional activation

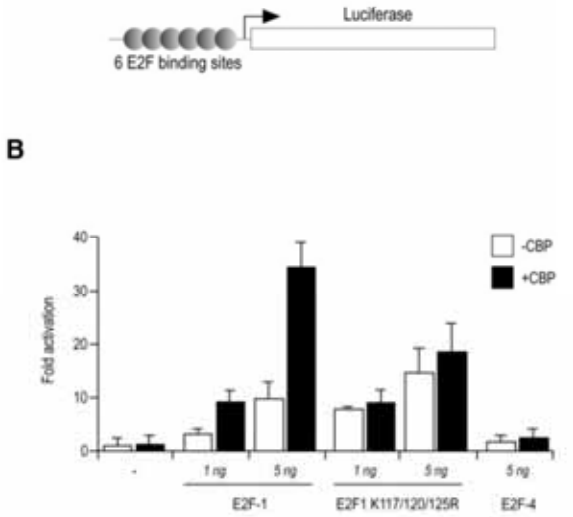


FIG. 7. **Acetylation of E2F-1 increases transcriptional activation.** *A*, schematic representation of the reporter plasmid used in the transfection experiments. The plasmid contains six E2F-binding sites upstream of the luciferase reporter gene (not drawn to scale). *B*, overexpression of CPB HAT enhances wild type E2F-1 transactivation. Transcriptional activation of an E2F-responsive promoter was studied in Saos-2 cells after transfection of different amounts of expression vectors for wild type E2F-1, E2F-1 K117R/K120R/K125R and E2F-4, in the presence or absence of an expression plasmid for CBP, as indicated. All E2F transfections also contained 1 or 5 ng of an expression plasmid for DP1.

ARTICLES

nature structural & molecular biology

Acetylation by GCN5 regulates CDC6 phosphorylation in the S phase of the cell cycle

Roberta Paolinelli[1,2,4,5], Ramiro Mendoza-Maldonado[2,5], Anna Cereseto[1] & Mauro Giacca[2,3]

In eukaryotic cells, the cell-division cycle (CDC)-6 protein is essential to promote the assembly of pre-replicative complexes in the early G1 phase of the cell cycle, a process requiring tight regulation to ensure that proper origin licensing occurs once per cell cycle. Here we show that, in late G1 and early S phase, CDC6 is found in a complex also containing Cyclin A, cyclin-dependent kinase (CDK)-2 and the acetyltransferase general control nonderepressible 5 (GCN5). GCN5 specifically acetylates CDC6 at three lysine residues flanking its cyclin-docking motif, and this modification is crucial for the subsequent phosphorylation of the protein by Cyclin A–CDKs at a specific residue close to the acetylation site. GCN5-mediated acetylation and site-specific phosphorylation of CDC6 are both necessary for the relocalization of the protein to the cell cytoplasm in the S phase, as well as to regulate its stability. This two-step, intramolecular regulatory program by sequential modification of CDC6 seems to be essential for proper S-phase progression.

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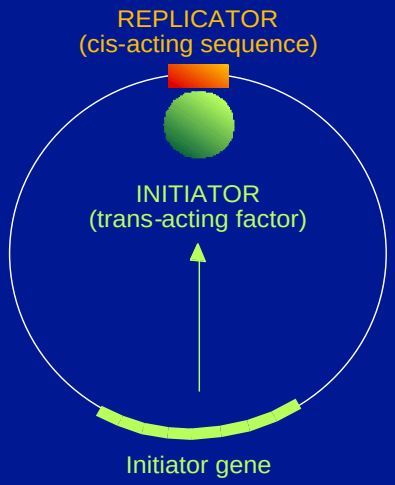
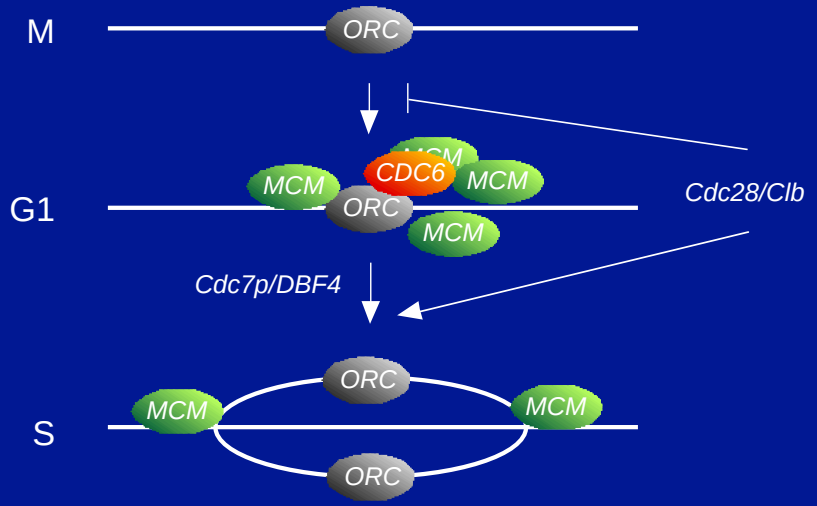
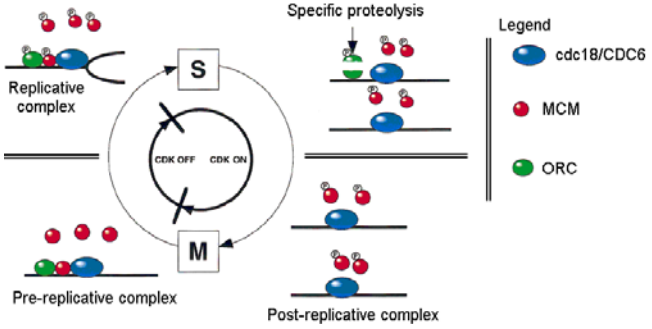
The replicon model
Jacob and Brenner, 1963

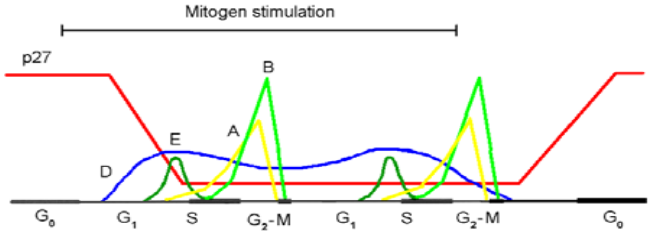


Protein-DNA interactions at S. cerevisiae ARS during the cell cycle



Coupling cyclin/CDK activity and origin activation in the cell cycle





J. Wuarin and P. Nurse, *Cell* **85**, 785 (1996)

Cell, Vol. 122, 915–926, September 23, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.08.013

CDKs Promote DNA Replication Origin Licensing in Human Cells by Protecting Cdc6 from APC/C-Dependent Proteolysis

Niels Mailand* and John F.X. Diffley*
Cancer Research UK London Research Institute
Clare Hall Laboratories
South Mimms
Hertfordshire EN6 3LD
United Kingdom

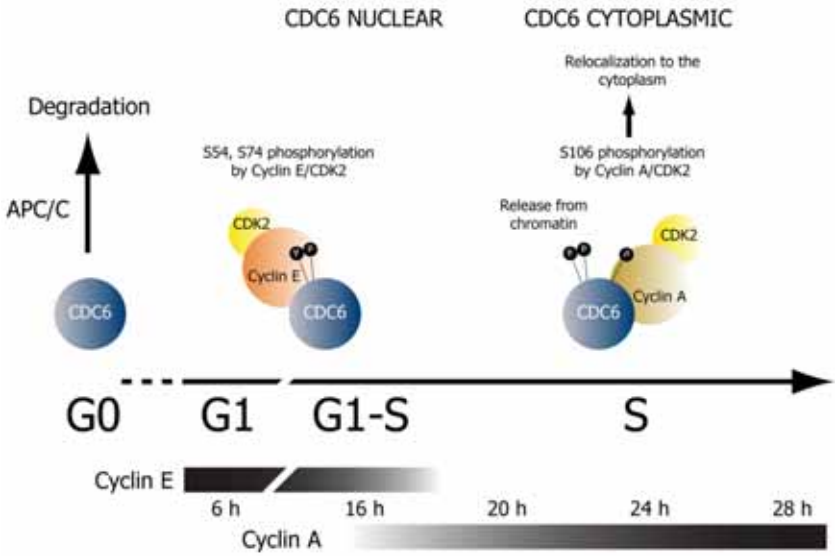
Summary

Cyclin-dependent kinases (CDKs) restrict DNA replication origin firing to once per cell cycle by preventing the assembly of prereplicative complexes (pre-RCs; licensing) outside of G1 phase. Paradoxically, under certain circumstances, CDKs such as cyclin E-cdk2 are also required to promote licensing. Here, we show that CDK phosphorylation of the essential licensing factor Cdc6 stabilizes it by preventing its association with the anaphase promoting complex/cyclosome (APC/C). APC/C-dependent Cdc6 proteolysis prevents pre-RC assembly in quiescent cells and, when cells reenter the cell cycle from quiescence, CDK-dependent Cdc6 stabilization allows Cdc6 to accumulate before the licensing inhibitors geminin and cyclin A which are also APC/C substrates. This novel mechanism for regulating protein stability establishes a window of time prior to S phase when pre-RCs can assemble which we propose represents a critical function of cyclin E.

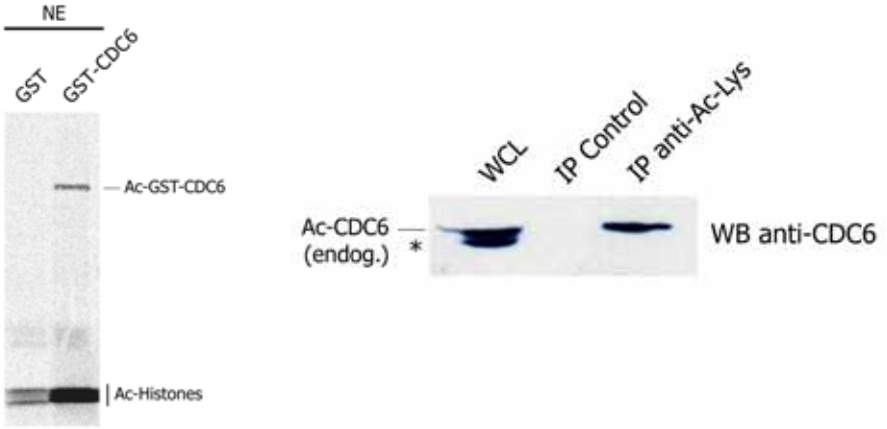
in metazoans: inactivation of the mitotic CDK, cdc2, causes endoreduplication (Itzhaki et al., 1997), and treatment of postreplicative cells with a CDK inhibitor induces relicensing (Ballabeni et al., 2004; Coverley et al., 1996, 1998). However, metazoans also contain an additional licensing inhibitor called geminin (Blow and Dutta, 2005; McGarry and Kirschner, 1998). In human cells, geminin is targeted for proteolysis by the APC/C E3 ubiquitin ligase (McGarry and Kirschner, 1998), ensuring that the licensing inhibitors geminin and cyclin A are degraded from late mitosis through G1 phase, allowing pre-RCs to assemble during this period.

In addition to targeting licensing inhibitors for proteolysis, the APC/C also targets the essential pre-RC assembly factor, Cdc6, for proteolysis (Petersen et al., 2000). Coregulation of licensing inhibitors and activators, however, would make it impossible for cells to achieve a state where pre-RC components are all present but licensing inhibitors are absent. Cyclin E can either promote or inhibit pre-RC assembly, depending on context (see Diffley [2004] for discussion). Here we show that CDKs, including those containing cyclin E, promote licensing by phosphorylating a regulatory domain of Cdc6, thus preventing its degradation by the APC/C. This allows Cdc6 to accumulate before the licensing inhibitors geminin and cyclin A when quiescent cells re-enter the cell cycle. We propose a simple model to explain the positive role of cyclin E in licensing and discuss how context might also allow cyclin E to be a licensing inhibitor.

Regulation of origin licencing by CDC6 phosphorylation and subcellular localization

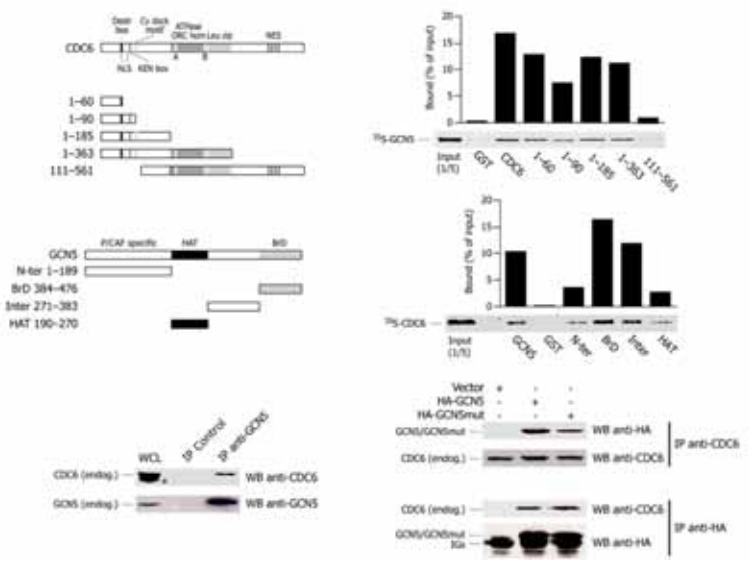


hCDC6 is acetylated in vitro and in vivo

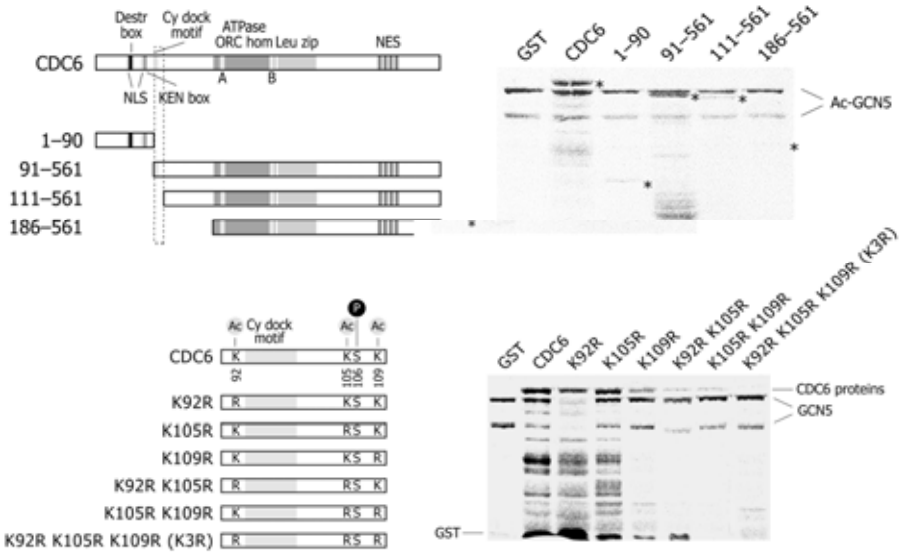


Paolinelli, R, et al. 2009. Nature Struct Mol Biol 16, 412.

hCDC6 is acetylated by and binds GCN5 both in vitro and in vivo



GCN5 acetylates hCDC6 lysines 92, 105 and 109



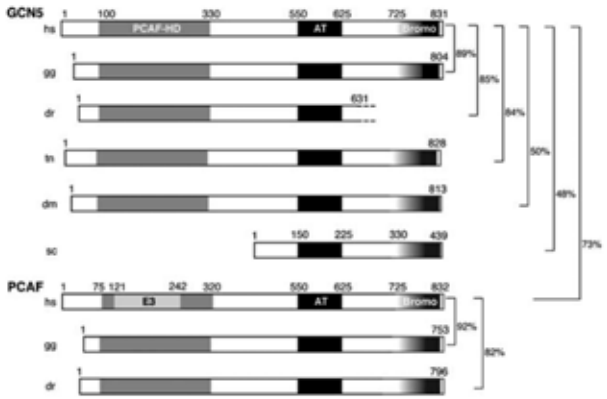


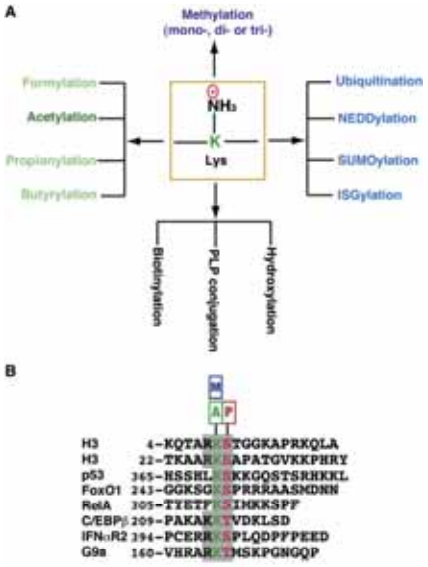
Figure 1 The overall structure of the GCN5 and PCAF enzymes in vertebrates, *Drosophila* and yeast. Schematic representation and domain organization of the GCN5 and PCAF proteins from human (hs; *Homo sapiens*), chicken (gg; *Gallus gallus*), zebrafish (dr; *Danio rerio*), pufferfish (tn; Tetraodon nigroviridis), *Drosophila melanogaster* (dm) and yeast (sc; *Saccharomyces cerevisiae*) are shown. The PCAF homology domain (PCAF-HD) is shown in grey, the AT domain is shown in black and the bromo domain (Bromo) is shaded. The recently described ubiquitin E3 ligase domain (E3) of PCAF (Linares *et al*., 2007) is also indicated. The numbers over the boxes indicate amino-acid positions. The identity between the different factors is indicated in % on the right of the horizontal lines, representing the pair wise comparisons. AT, acetyl transferase.

Table 1 Composition of the 2 MDa and 700 kDa GCN5- and PCAF-containing multiprotein complexes

	2 MDa complexes					*700 kDa complexes*		
Complex	*ySAGA*	*dSAGA/TFTC*	*hTFTC*	*hSTAGA*	*hPCAF*	*ADA*	*ATAC*	*ATAC*
Organism	*Saccharomyces cerevisiae*	*Drosophila melanogaster*	*Homo sapiens*	*H. sapiens*	*H. sapiens*	*S. cerevisiae*	*D. melanogaster*	*H. sapiens*
HAT subunit	yGcn5	dGCN5	hGCN5	hGCN5	hPCAF	yGCN5	dGCN5	hGCN5
	yAda1	dADA1	hADA1[a]	hSTAF42	?	—	—	
	yAda2	dADA2b	hADA2b[a]	hADA2b	?			
	—	—	—	—	hADA2a	yAda2	dADA2a	hADA2a
	yAda3	dADA3?	hADA3	STAF54	hADA3	yAda3	dADA3	?
	yAda5/Spt20	?	?	?	?	—		
	ySpt3	dSPT3	hSPT3	hSPT3	hSPT3	—	—	
	ySpt7	?	hSPT7L	STAF65g	?	—	—	
	ySpt8	?	?	?	?	—	—	
			hTAF2[c]					
			hTAF4[c]					
	yTAF5	—	hTAF5[c]	—	—	—	—	
		dTAF5L/WDA	hTAF5L	hTAF5L	hTAF5L	—		
	yTAF6		hTAF6[c]	—	—	—		
		?	hTAF6L	hTAF6L	hTAF6L	—		
	yTAF9	dTAF9	hTAF9	hTAF9	hTAF9	—		
			hTAF9b			—		
	yTAF10	dTAF10	hTAF10	hTAF10	hTAF10	—		
	yTAF12	?	hTAF12	hTAF12	hTAF12	—		
	Tra1	dTra1/dTRRAP	hTRRAP	hTRRAP	hPAF400	—		
	ySgf11	?	?	hSTAF46?	?	—		
	ySgf29	?	hSGF29[a]	STAF36	?	—		
	ySgf73	?	HATXN7	hATXN7	?	—	—	
	yUbp8	?	?	hSTAF60?	?	—	—	
	ySus1	dEny2	?	?	?	—	—	
				hSTAF55				
	—	?	hSAP130	hSAP130	?			
	—	—	—	—	—	yAhc1	—	?
							dATAC	?
							dHCF	?

The factors, described in the different complexes (Grant *et al*., 1997, 1998a; Martinez *et al*., 1998, 2001; Wieczorek *et al*., 1998; Brand *et al*., 1999b; Eberharter *et al*., 1999; Georgieva *et al*., 2001; Kusch *et al*., 2003; Muratoglu *et al*., 2003; Helmlinger *et al*., 2004; Rodriguez-Navarro *et al*., 2004; Pallan *et al*., 2005; Guelman *et al*., 2006a; Demeny *et al*., 2007; Kuraibe *et al*., 2007), are represented on a horizontal line as homologues from different species. Different names on a horizontal line mean that these homologues are known under different names in different species. The novel TAF nomenclature has been used (Tora, 2002). '?' means that cDNAs encoding homologue factors to the yeast proteins in the given organism have been identified, but the presence of the factor in the corresponding complex has not yet been demonstrated. '—' means that in the given complex the corresponding factor is absent. Factors shown with '[a]' have been recently identified in TFTC by mass spectrometry (our unpublished results). Factors in TFTC shown with '[c]' copurify with TFTC, but seem to be present in a separate complex (Demeny *et al*., 2007) and our unpublished results). STAFs in green were positioned as homologues of the yeast proteins, when cDNAs encoding putative human proteins with approximately the STAF size were found.

Post-translational modification of lysines



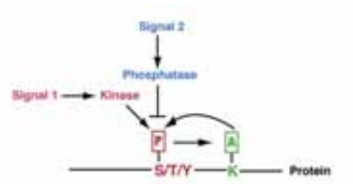
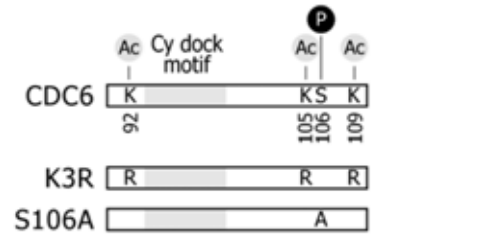


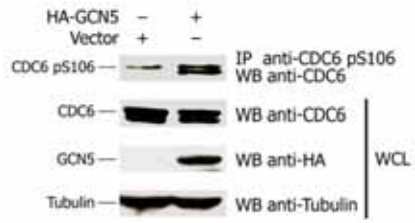
Figure 1. Multiplicity of Lysine Modifications and Signal Conversion from Phosphorylation to Acetylation

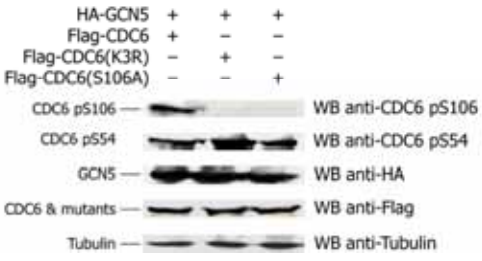
(A) The side chain of a lysine (K) residue can be modified by acetylation and other covalent modifications. Except for hydroxylation, all listed modifications target the ε-amino group. Formylation, propionylation, and butyrylation are three recently discovered modifications that are structurally similar to acetylation; however, it is not known if formylation occurs enzymatically. Aside from formylation, hydroxylation, and pyridoxal 5′-phosphate (PLP, a vitamin B6 derivative) conjugation, all listed modifications are reversible. Acetylation of a lysine residue precludes further modifications by others and vice versa.

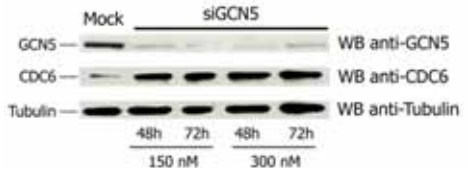
(B) Alignment of sequences surrounding KS dipeptides from histone H3, p53, and several other proteins. The KS dipeptides are highlighted in color. Small rectangles with the letters A, P, and M denote acetylation, phosphorylation, and methylation, respectively. Interferon α receptor 2 (IFNαR2) acetylation at Lys399 and phosphorylation at Ser400 might crosstalk with one another (Tang et al., 2007). It remains to be established whether similar interplay occurs in p53, FoxO1, RelA (NF-κB subunit) (Perkins, 2006), and CCAAT/enhancer binding protein β (C/EBPβ) (Hasselgren, 2007). Methylation of the highlighted lysine residues has been documented only for histone H3, p53 (Huang et al., 2006b), and G9a (Sampath et al., 2007). It is unclear whether the serine in p53 is phosphorylated or the lysine in G9a is acetylated.

CDC6 phosphorylation on Ser106 depends on GCN5-mediated CDC6 acetylation

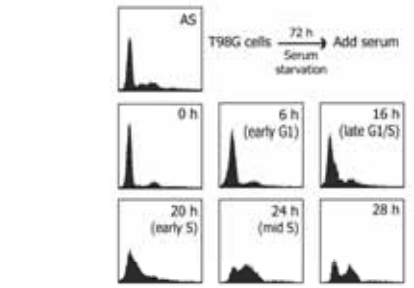


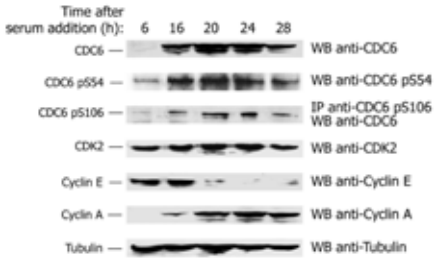


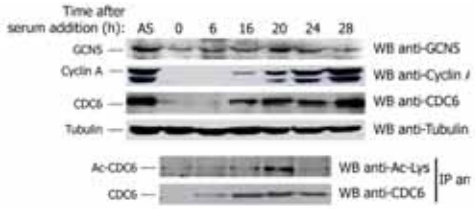


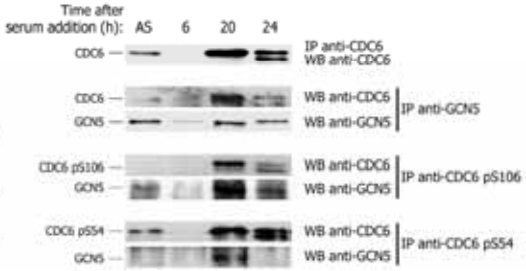


Cell cycle-dependent CDC6 acetylation

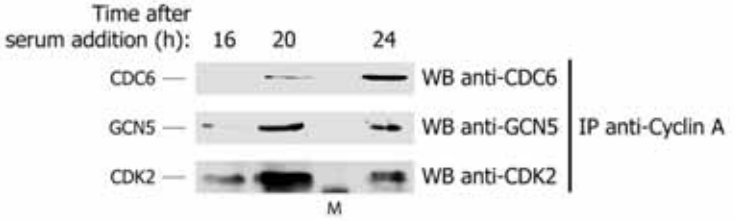


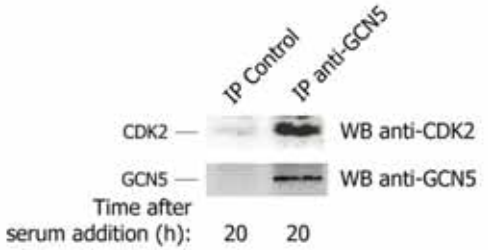




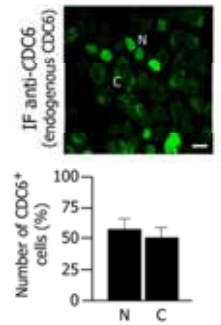


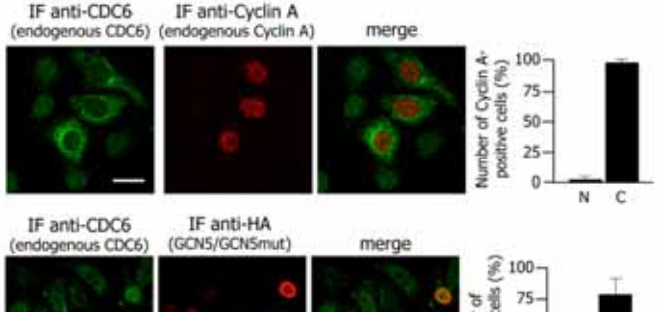
GCN5 HAT forms a complex with CyclinA/CDK2

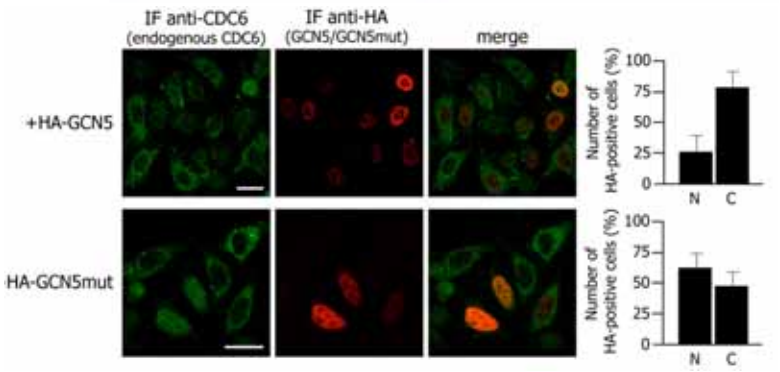




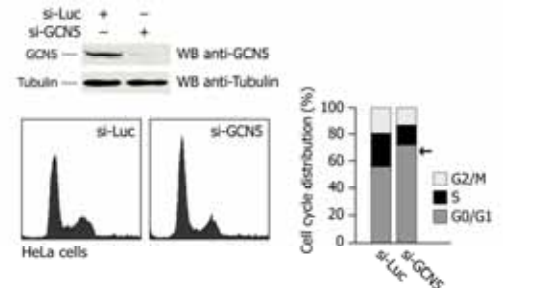
Acetylation by GCN5 induces cytoplasmic localization of CDC6

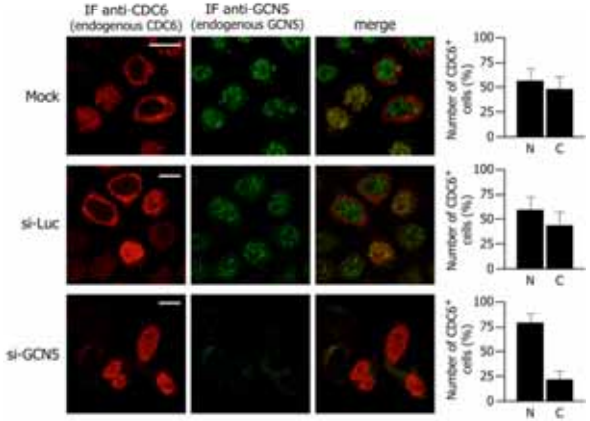




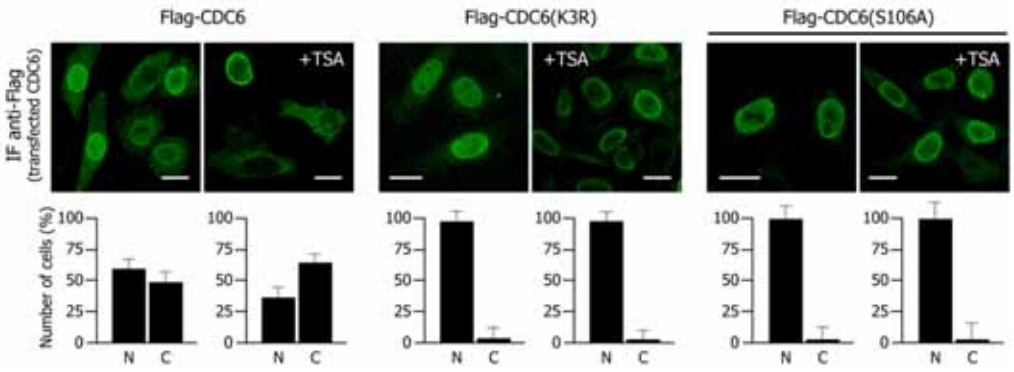


Nuclear accumulation of CDC6 after GCN5 knock down

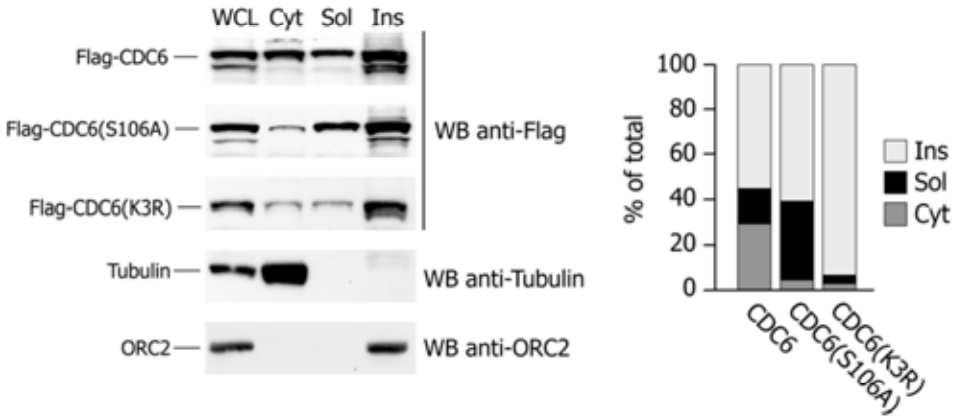




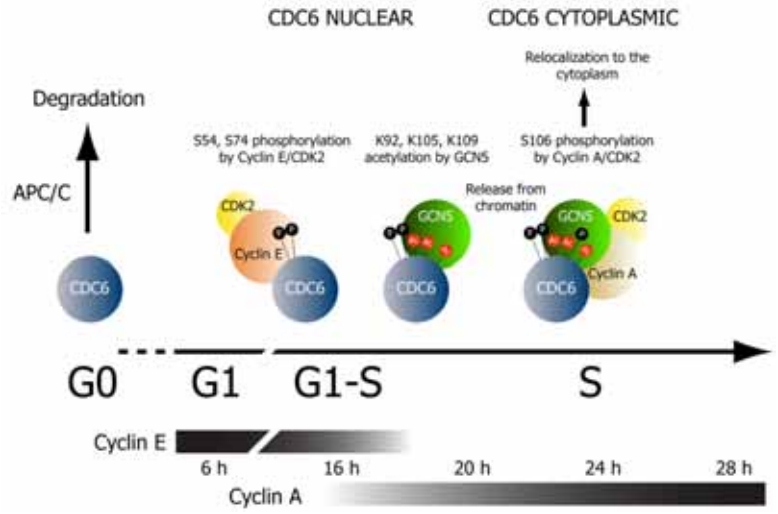
Exclusive nuclear localization of the CDC6 K3R and S106A mutants



CDC6 K3R and S106A mutants are localized in the insoluble nuclear compartment



Sequential modification of CDC6 by acetylation and phosphorylation in early S-phase regulates cell cycle progression



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Regulation of HIV-1 gene expression by histone acetylation and factor recruitment at the LTR promoter

Marina Lusic[1], Alessandro Marcello[1], Anna Cereseto[1,2] and Mauro Giacca[1,2,3]

[1]Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste and [2]Scuola Normale Superiore and Istituto di Fisiologia Clinica, CNR, Pisa, Italy

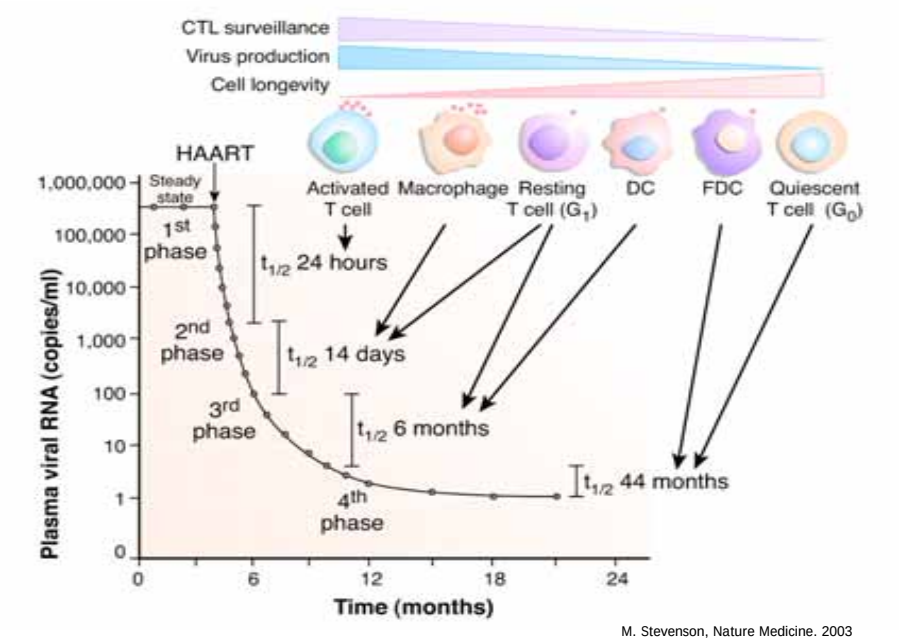
[3]Corresponding author
e-mail: giacca@icgeb.org

In HIV-1 infected cells, the LTR promoter, once organized into chromatin, is transcriptionally inactive in the absence of stimulation. To examine the chromosomal events involved in transcriptional activation, we analyzed histone acetylation and factor recruitment at contiguous LTR regions by a quantitative chromatin immunoprecipitation assay. In chronically infected cells treated with a phorbol ester, we found that acetylation of both histones H3 and H4 occurs at discrete nucleosomal regions before the onset of viral mRNA transcription. Concomitantly, we observed the recruitment of known cellular acetyl-transferases to the promoter, including CBP, P/CAF and GCN5, as well as that of the p65 subunit of NF-κB. The specific contribution of the viral Tat transactivator was assayed in cells harboring the sole LTR. We again observed nucleosomal acetylation and the recruitment of specific co-factors to the viral LTR upon activation by either recombinant Tat or a phorbol ester. Strikingly, P/CAF was found associated with the promoter only in response to Tat. Taken together, these results contribute to the elucidation of the molecular events underlying HIV-1 transcriptional activation.

Keywords: chromatin immunoprecipitation/histone acetyl-transferases/histones/HIV-1/long terminal repeat/nucleosomes/Tat

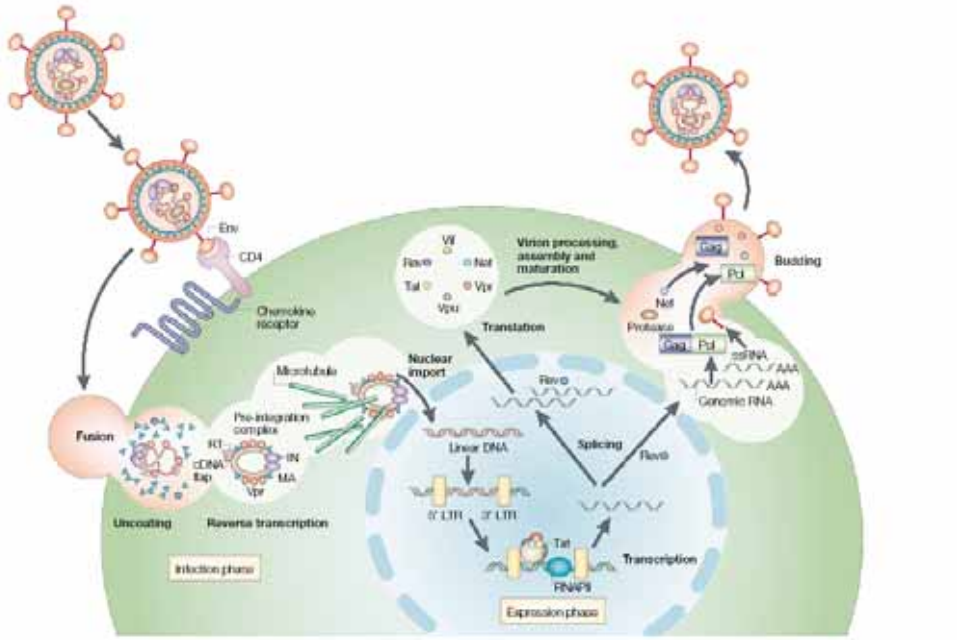
Paolinelli, R, et al. 2009. Nature Struct Mol Biol 16, 412.

Cellular Reservoirs of HIV-1 Replication and Persistence

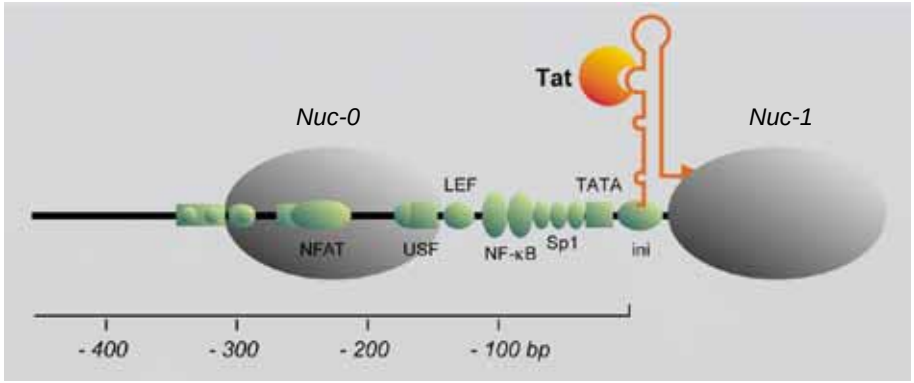


M. Stevenson, Nature Medicine. 2003

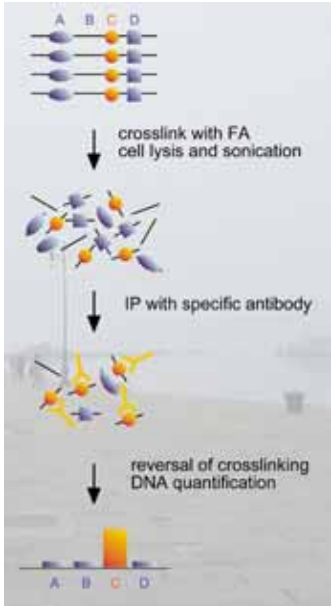
HIV-1 replication cycle

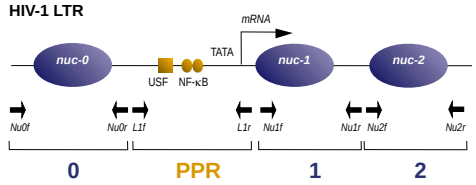


Chromatin conformation at the HIV-1 LTR promoter



Quantitative ChIP and genomic footprinting at the HIV-1 LTR promoter: Summary





- Most transcription factor bind the LTR irrespective of transcriptional state
- Transcriptional activation determines the recruitment of NF-kB p65 to the promoter.
- Histone acetylation occurs at the integrated HIV LTR upon activation with TPA or Tat; Acetylation precedes the onset of transcription.
- Transcriptional activation is coupled to the recruitment of specific HATs to the LTR.

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Lusic, M, *et al.* 2003. EMBO J. 22, 6550.
Demarchi, F, *et al.* 1996. J. Virol. 70, 4427.
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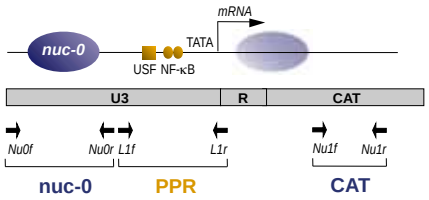
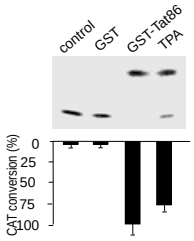
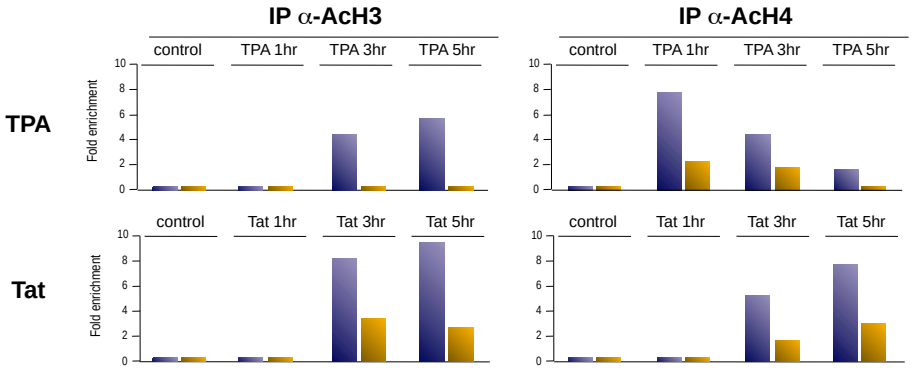
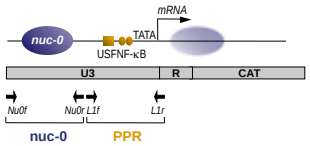
Histone acetylation at the HIV-1 promoter after treatment of HL3T1 cells with TPA or rTat

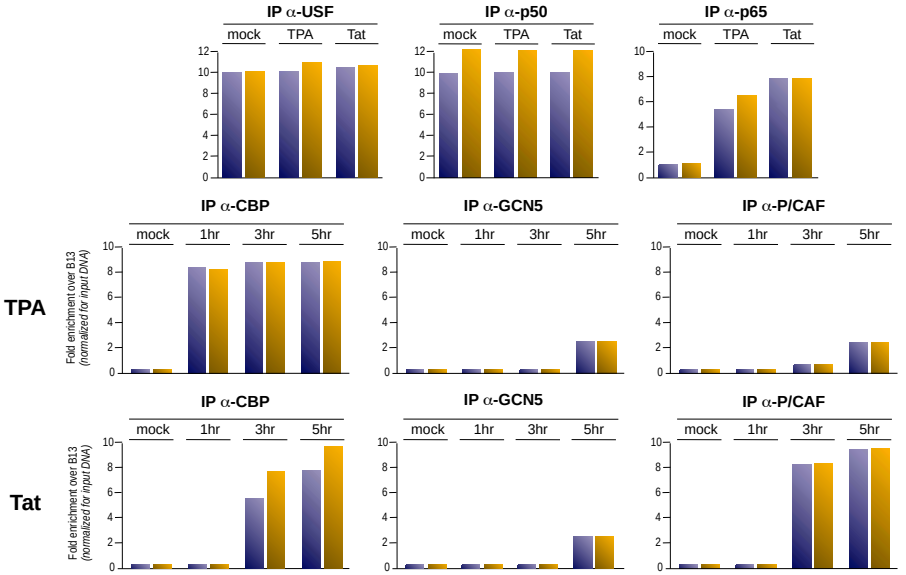




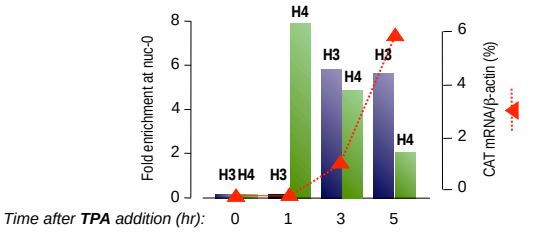


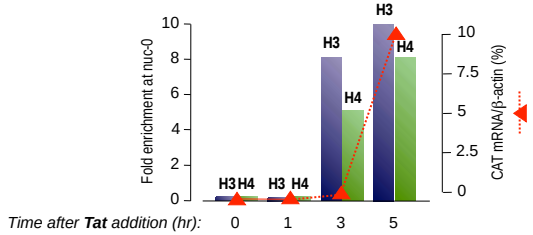
Factor and HAT recruitment at the HIV-1 promoter after treatment of HL3T1 cells with TPA or recombinant Tat

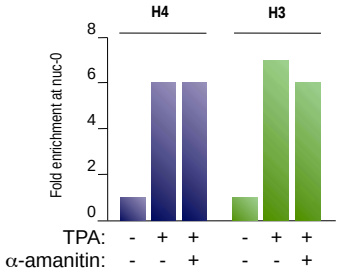




Histone acetylation at the HIV-1 promoter precedes the onset of transcription







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0270-7306/08/$08.00+0 doi:10.1128/MCB.01557-07

Acetylation of Conserved Lysines in the Catalytic Core of Cyclin-Dependent Kinase 9 Inhibits Kinase Activity and Regulates Transcription▽†

Arianna Sabò,[1] Marina Lusic,[2] Anna Cereseto,[1] and Mauro Giacca[1,2]*

Molecular Biology Laboratory, Scuola Normale Superiore, AREA della Ricerca, Via Moruzzi 1, Pisa, Italy,[1] and Molecular Medicine Laboratory, International Center for Genetic Engineering and Biotechnology, Padriciano 99, Trieste, Italy[2]

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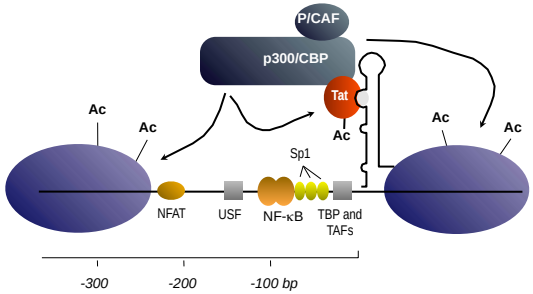
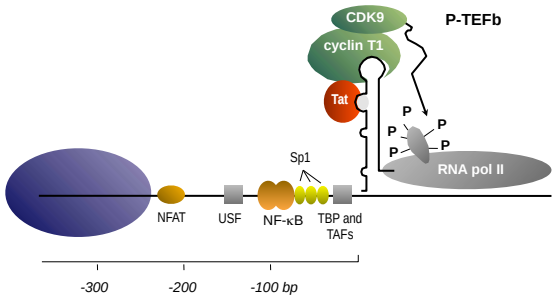
Promoter clearance and transcriptional processivity in eukaryotic cells are fundamentally regulated by the phosphorylation of the carboxy-terminal domain of RNA polymerase II (RNAPII). One of the kinases that essentially performs this function is P-TEFb (positive transcription elongation factor b), which is composed of cyclin-dependent kinase 9 (CDK9) associated with members of the cyclin T family. Here we show that cellular GCN5 and P/CAF, members of the GCN5-related *N*-acetyltransferase family of histone acetyltransferases, regulate CDK9 function by specifically acetylating the catalytic core of the enzyme and, in particular, a lysine that is essential for ATP coordination and the phosphotransfer reaction. Acetylation markedly reduces both the kinase function and transcriptional activity of P-TEFb. In contrast to unmodified CDK9, the acetylated fraction of the enzyme is specifically found in the insoluble nuclear matrix compartment. Acetylated CDK9 associates with the transcriptionally silent human immunodeficiency virus type 1 provirus; upon transcriptional activation, it is replaced by the unmodified form, which is involved in the elongating phase of transcription marked by Ser2-phosphorylated RNAPII. Given the conservation of the CDK9 acetylated residues in the catalytic task of virtually all CDK proteins, we anticipate that this mechanism of regulation might play a broader role in controlling the function of other members of this kinase family.

Transcriptional activation of the HIV-1 LTR



Recruitment of co-activators and histone acetyl-transferases



Recruitment of P-TEFb to promote polymerase processivity

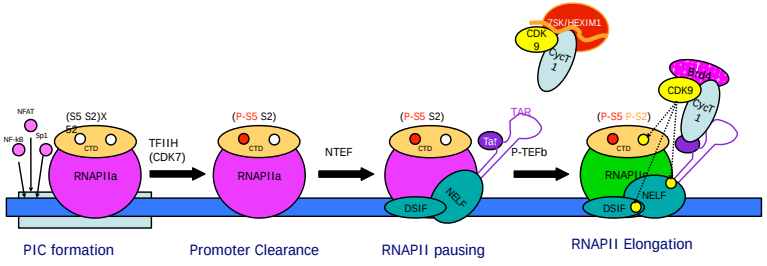
Positive Elongation Factor b (P-TEFb)

Plays a critical role in the transition from abortive to productive elongation

Contains CDK9 and its regulatory Cyclin T1 subunit (or the minor forms Cyclin T2 or K)

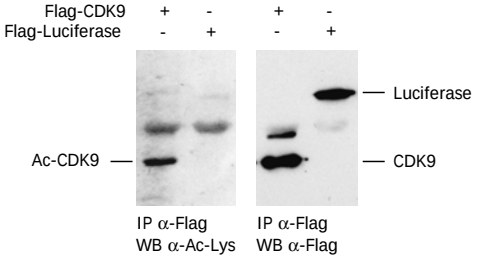
CDK9 phosphorylates both the C-terminal domain (CTD) of the largest subunit of RNAPII and the inhibitory factor Spt5.

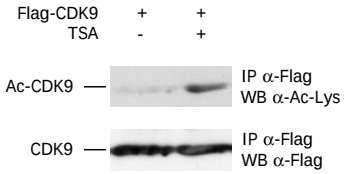
About 50% of Cyclin T1/CDK9 heterodimer in the nucleus is complexed with the inhibitory factors 7SK RNA and HEXIM1 protein; an additional regulatory component is Brd4, which binds P-TEFb through its bromodomain and promotes P-TEFb activity



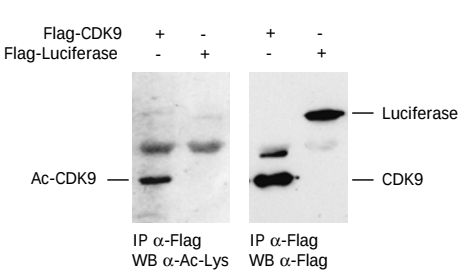
Adapted from M.Barboric, B.M.Peterlin
PLoS Biology 2005 Vol. 3, No. 2

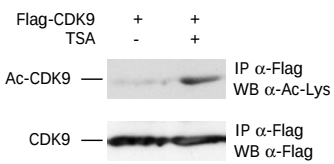
CDK9 is acetylated inside the cells

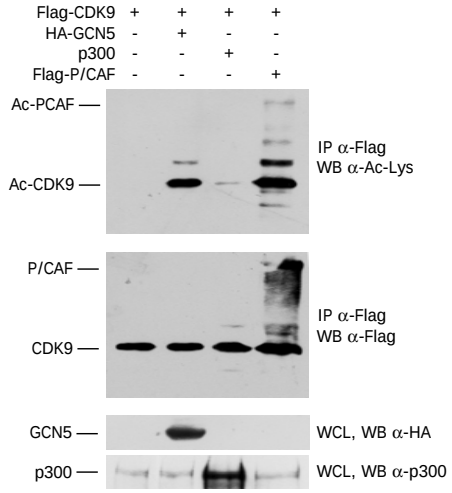




GCN5 and P/CAF HATs acetylate CKD9 inside the cells

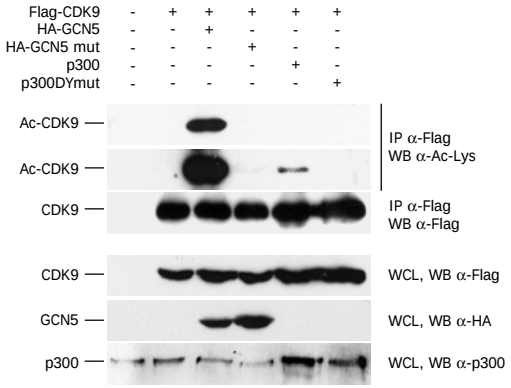




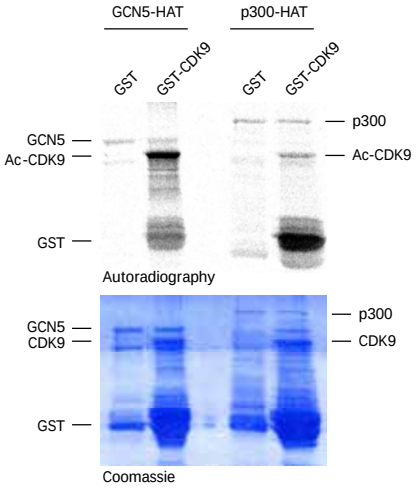


GCN5 acetylates CDK9

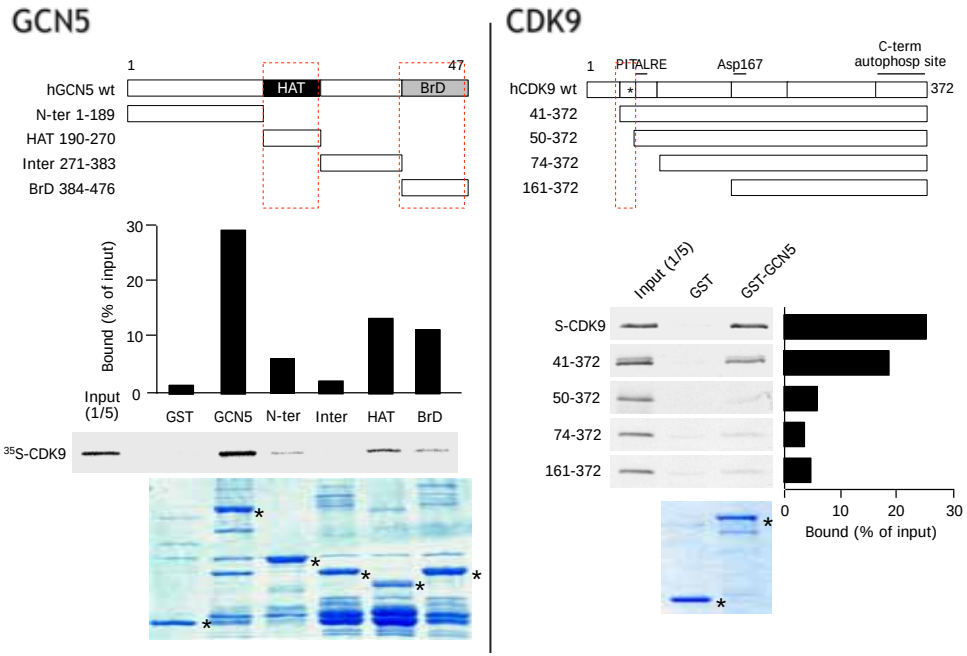
in vivo



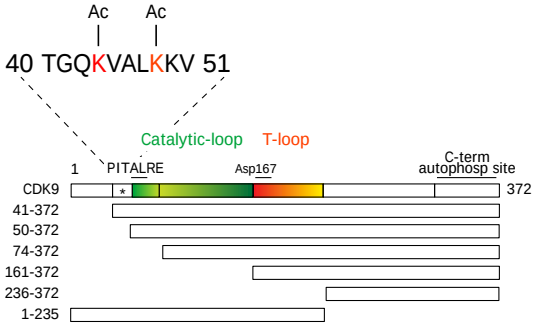
in vitro

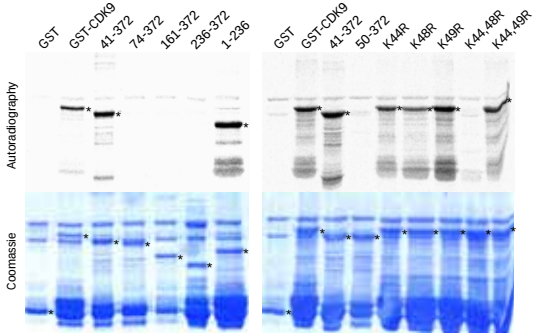


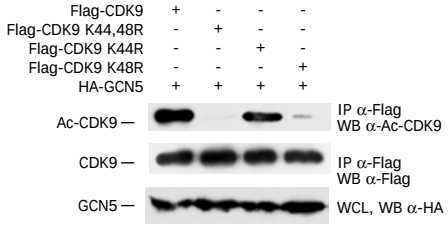
The N-terminus of CDK9 binds the bromo and HAT domains of GCN5 in vitro



GCN5 acetylates CDK9 K48 (and K44)





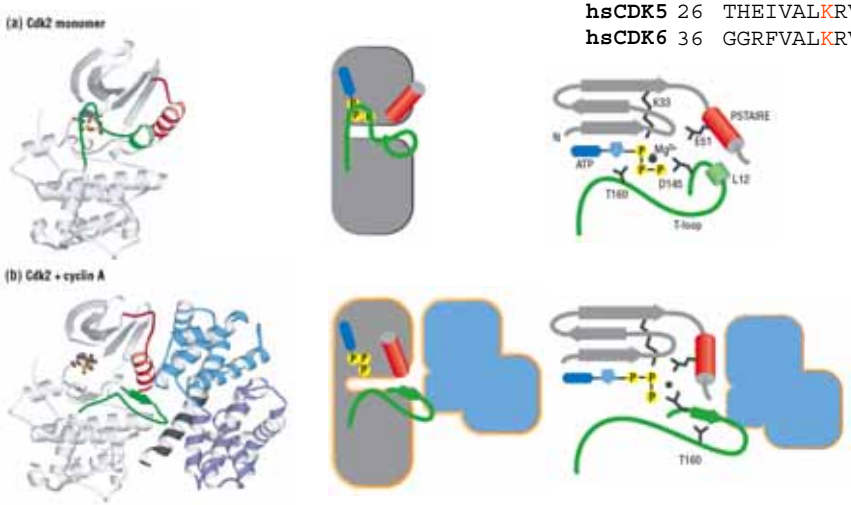


CDK9 K48 corresponds to the invariant lysine positioned in the catalytic site of most CDKs

```
                    44   48
hsCDK9  41  TGQKVALKKV  50
hsCDK7  34  TNQIVAIKKI  43
hsCDK8  45  DDKDYALKQI  54
ScBur1  82  TQRQVAMKKI  91

hsCDK1  26  TGQVVAMKKI  35
hsCDK2  26  TGEVVALKKI  35
hsCDK4  28  SGHFVALKSV  37
hsCDK5  26  THEIVALKRV  35
hsCDK6  36  GGRFVALKRV  45
```

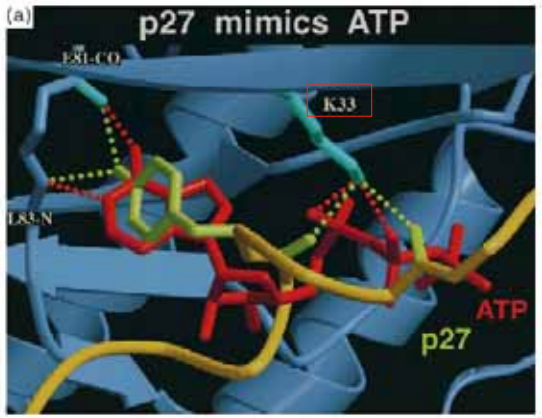
Structural basis of CDK activation
(Pavletich 1995)



CDK9 K48 is positioned in the ATP-binding pocket of CDK9

```
                  15                  30                  45                  60                  75
CDK2 MENFQKVEKIGEGTYGVVYKARNKLTGEVVALKKIRLDTETEGVPSTAIREISLLKELNHPNIVKLLDVIHTEN--------KLYLVFEF    82
CDK9 VSKYEKLAKIGQGTFGEVFKARHRKTGQKVALKKVLMENEKEGFPITALREIKILQLLKHENVVNLIEICRTKASPYNRCKGSIYLVFDF    90
       *  *** ** * * ***   **  *****     * ** * ** ***  *  * * * * *     *           **** *
```

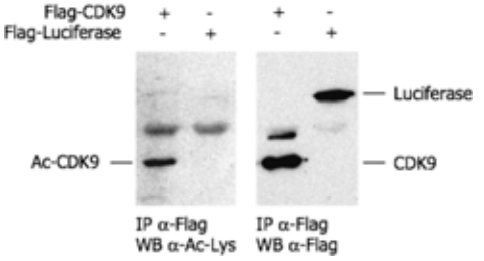
(Filgueira de Azevedo et al. 2002)



CDK2 catalytic pocket

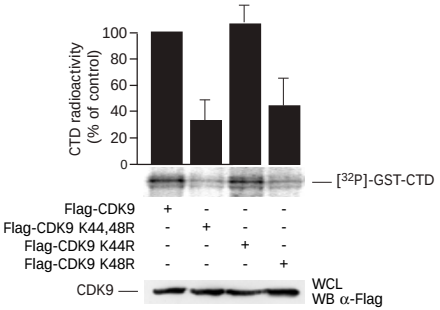
Sequence similarities define two major units in the family of protein kinases: a conserved catalytic core and a nonconserved flanking region.
Moreover, in the catalytic core a conserved lysine is essential for the catalytic activity of the enzyme because it is involved in the process of phosphate transfer.
(Carrera et al. 1993 PNAS).
For Cdk2 this conserved lysine has been proved to be K33 and sequence alignment reveals that Cdk2 K33 is the homolog of Cdk9 K48.

Acetylation of the catalytic domain of CDK9 inactivates kinase activity

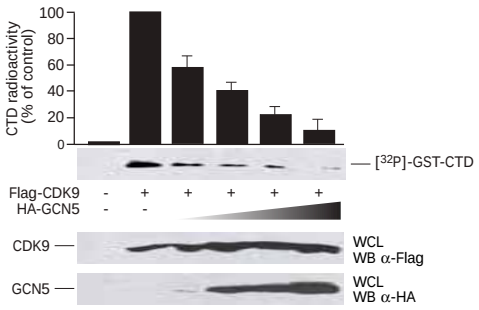


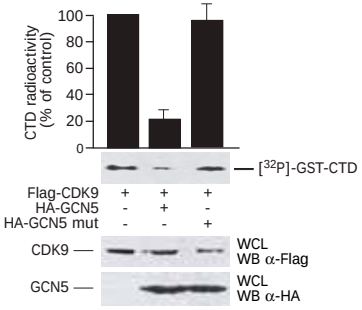
```
                 44   48
hsCDK9 41  TGQKVALKKV 50
hsCDK7 34  TNQIVAIKKI 43
hsCDK8 45  DDKDYALKQI 54
ScBur1 82  TQRQVAMKKI 91

hsCDK1 26  TGQVVAMKKI 35
hsCDK2 26  TGEVVALKKI 35
hsCDK4 28  SGHFVALKSV 37
hsCDK5 26  THEIVALKRV 35
hsCDK6 36  GGRFVALKRV 45
```

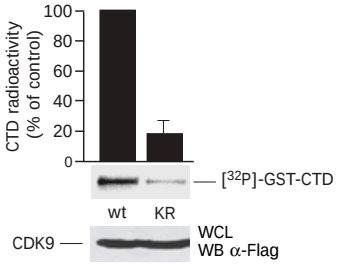


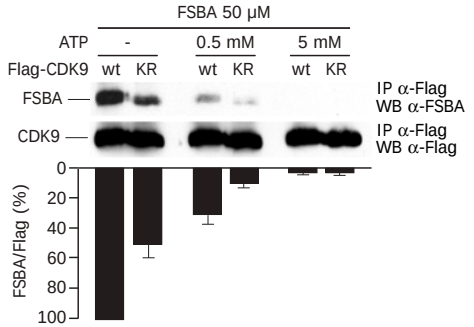
GCN5 mediated acetylation inhibits CDK9 kinase activity



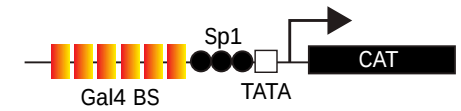


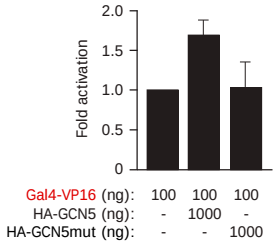
CDK9 K44,48R mutant has reduced kinase and ATP binding activity

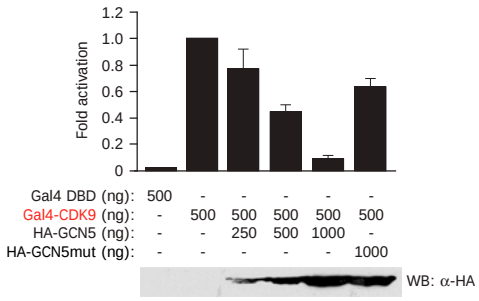


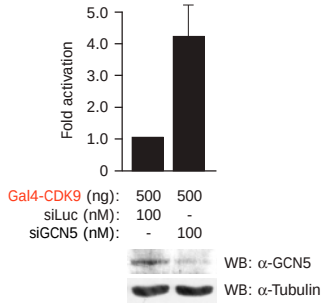


Acetylation inhibits CDK9-activated transcription









Conclusions II

```
                 44   48
CDK9   41  TGQKVALKKV  50
BUR1   82  TQRQVAMKKI  91
CDK5   26  THEIVALKRV  35
CDC2   26  TGQVVAMKKI  35
CDK2   26  TGEVVALKKI  35
CDK4   28  SGHFVALKSV  37
```

Given the conservation of one of the CDK9 acetylated residues in the catalytic task of virtually all CDK proteins, we anticipate that this mechanism of regulation might play a broader role in controlling the function of other members of this kinase family.

Nucleic Acids Research Advance Access published September 22, 2009

The transcriptional co-activator PCAF regulates cdk2 activity

Francesca Mateo[1,2], Miriam Vidal-Laliena[1,2], Núria Canela[1,2], Annalisa Zecchin[3], Marian Martínez-Balbás[3,4], Neus Agell[1,2], Mauro Giacca[3,4], María Jesús Pujol[1,2] and Oriol Bachs[1,2,*]

[1]Department of Cell Biology, Immunology and Neuroscience, Faculty of Medicine, University of Barcelona, [2]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, [3]Molecular Medicine Laboratory, International Centre for Engineering and Biotechnology (ICGEB), [4]Department of Biomedicine, Faculty of Medicine, University of Trieste, Italy, [5]Instituto de Biología Molecular de Barcelona, Consejo Superior de Investigaciones Científicas (CSIC) and [6]Parc Científic de Barcelona, Barcelona, Spain

Received March 31, 2009; Revised and Accepted September 3, 2009

Cyclin dependent kinases (cdks) regulate cell cycle progression and transcription. We report here that the transcriptional co-activator PCAF directly interacts with cdk2. This interaction is mainly produced during S and G_2/M phases of the cell cycle. As a consequence of this association, PCAF inhibits the activity of cyclin/cdk2 complexes. This effect is specific for cdk2 because PCAF does not inhibit either cyclin D3/cdk6 or cyclin B/cdk1 activities. The inhibition is neither competitive with ATP, nor with the substrate histone H1 suggesting that somehow PCAF disturbs cyclin/cdk2 complexes. We also demonstrate that overexpression of PCAF in the cells inhibits cdk2 activity and arrests cell cycle progression at S and G_2/M. This blockade is dependent on cdk2 because it is rescued by the simultaneous overexpression of this kinase. Moreover, we also observed that PCAF acetylates cdk2 at lysine 33. As this lysine is essential for the interaction with ATP, acetylation of this residue inhibits cdk2 activity. Thus, we report here that PCAF inhibits cyclin/cdk2 activity by two different mechanisms: (i) by somehow affecting cyclin/cdk2 interaction and (ii) by acetylating K33 at the catalytic pocket of cdk2. These findings identify a previously unknown mechanism that regulates cdk2 activity.

The EMBO Journal (2005), 1–12 | © 2005 European Molecular Biology Organization | All Rights Reserved 0261-4189/05
www.embojournal.org

THE EMBO JOURNAL

Acetylation of HIV-1 integrase by p300 regulates viral integration

Anna Cereseto[1,2,*], Lara Manganaro[1,2], Maria Ines Gutierrez[1], Mariaelena Terreni[2], Antonio Fittipaldi[2], Marina Lusic[1], Alessandro Marcello[3] and Mauro Giacca[1,2]

[1]Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy, [2]Molecular Biology Laboratory, Scuola Normale Superiore, Pisa, Italy and [3]Molecular Virology Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

Integration of HIV-1 into the human genome, which is catalyzed by the viral protein integrase (IN), preferentially occurs near transcriptionally active genes. Here we show that p300, a cellular acetyltransferase that regulates chromatin conformation through the acetylation of histones, also acetylates IN and controls its activity. We have found that p300 directly binds IN both *in vitro* and in the cells, as also specifically demonstrated by fluorescence resonance energy transfer technique analysis. This interaction results in the acetylation of three specific lysines (K264, K266, K273) in the carboxy-terminus of IN, a region that is required for DNA binding. Acetylation increases IN affinity to DNA, and promotes the DNA strand transfer activity of the protein. In the context of the viral replication cycle, point mutations in the IN acetylation sites abolish virus replication by specifically impairing its integration capacity. This is the first demonstration that HIV-1 IN activity is specifically regulated by post-translational modification.

The EMBO Journal advance online publication, 11 August 2005; doi:10.1038/sj.emboj.7600770
Subject Categories: proteins; microbiology and pathogens
Keywords: acetylation; HIV-1; integrase; p300; viral integration

Terreni et al. Retrovirology 2010, 7:18
http://www.retrovirology.com/content/7/1/18

RETROVIROLOGY

RESEARCH Open Access

GCN5-dependent acetylation of HIV-1 integrase enhances viral integration

Marialena Terreni[1], Paola Valentini[1], Vania Liverani[1], Maria Ines Gutierrez[2], Cristina Di Primio[1], Armida Di Fenza[3], Valentina Tozzini[3], Awatef Allouch[1], Alberto Albanese[3], Mauro Giacca[2], Anna Cereseto[1*]

Abstract

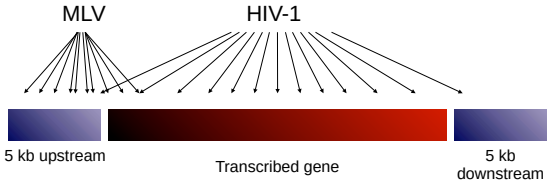
Background: An essential event during the replication cycle of HIV-1 is the integration of the reverse transcribed viral DNA into the host cellular genome. Our former report revealed that HIV-1 integrase (IN), the enzyme that catalyzes the integration reaction, is positively regulated by acetylation mediated by the histone acetyltransferase (HAT) p300.

Results: In this study we demonstrate that another cellular HAT, GCN5, acetylates IN leading to enhanced 3'-end processing and strand transfer activities. GCN5 participates in the integration step of HIV-1 replication cycle as demonstrated by the reduced infectivity, due to inefficient provirus formation, in GCN5 knockdown cells. Within the C-terminal domain of IN, four lysines (K258, K264, K266, and K273) are targeted by GCN5 acetylation, three of which (K264, K266, and K273) are also modified by p300. Replication analysis of HIV-1 clones carrying substitutions at the IN lysines acetylated by both GCN5 and p300, or exclusively by GCN5, demonstrated that these residues are required for efficient viral integration. In addition, a comparative analysis of the replication efficiencies of the IN triple- and quadruple-mutant viruses revealed that even though the lysines targeted by both GCN5 and p300 are required for efficient virus integration, the residue exclusively modified by GCN5 (K258) does not affect this process.

Conclusions: The results presented here further demonstrate the relevance of IN post-translational modification by acetylation, which results from the catalytic activities of multiple HATs during the viral replication cycle. Finally, this study contributes to clarifying the recent debate raised on the role of IN acetylated lysines during HIV-1 infection.

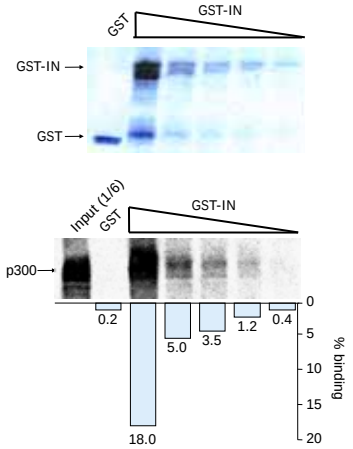
Retroviruses integrate near transcriptionally active regions of DNA

- Acceptor sites for retroviral integrations map near DNase I hypersensitive sites in chromatin *(S. Vijaya et al. J. Virol. 1986)*
- Retrovirus integration and chromatin structure: Moloney murine leukemia proviral integration sites map near DNase I-hypersensitive sites *(H. Rohdewohld et al. J. Virol. 1987)*
- Chromosome structure and human immunodeficiency type 1 cDNA integration: centromeric alphoid repeats are a disfavored target *(S. Carteu et al. J. Virol. 1998)*
- HIV-1 integration in the human genome favors active genes and local hotspots *(A.R.W. Schroder et al. Cell 2002)*

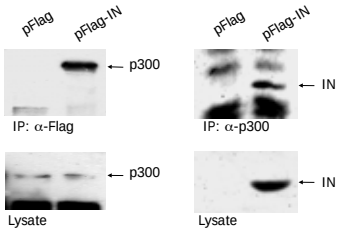


HIV-1 integrase binds p300 HAT

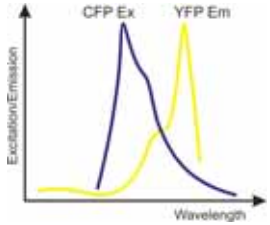
In vitro:



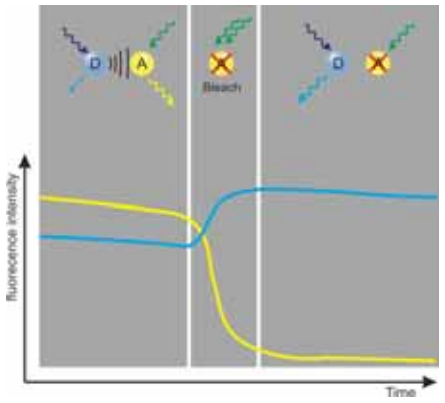
In vivo:

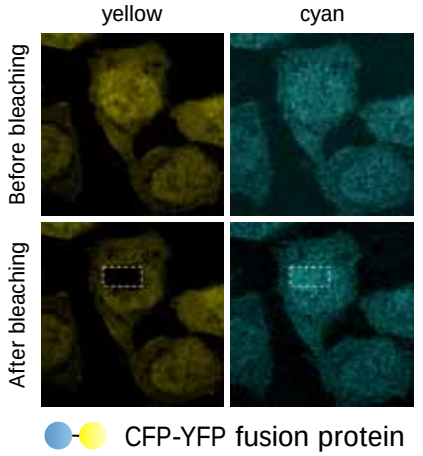


Measurement of FRET between CFP and YFP by acceptor photobleaching

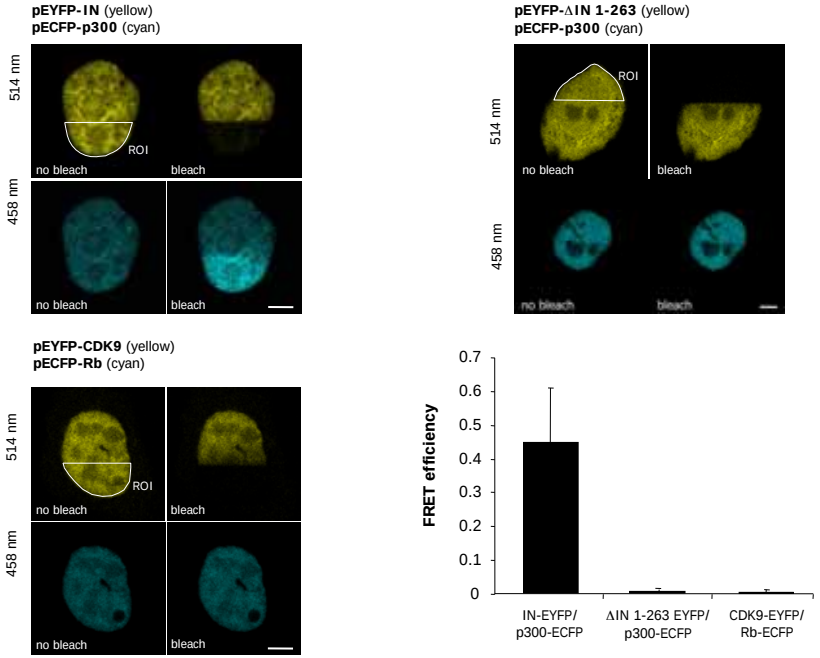


FRET efficiency (K_t):
$K_t=(1/t_0)(R/r)^6$



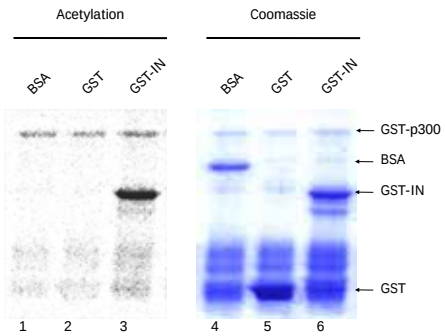


FRET between HIV-1 integrase and cellular p300

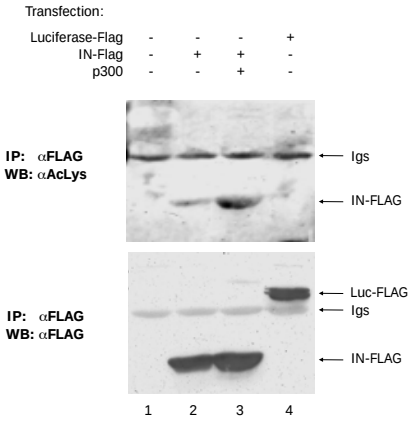


p300 acetylates HIV-1 integrase

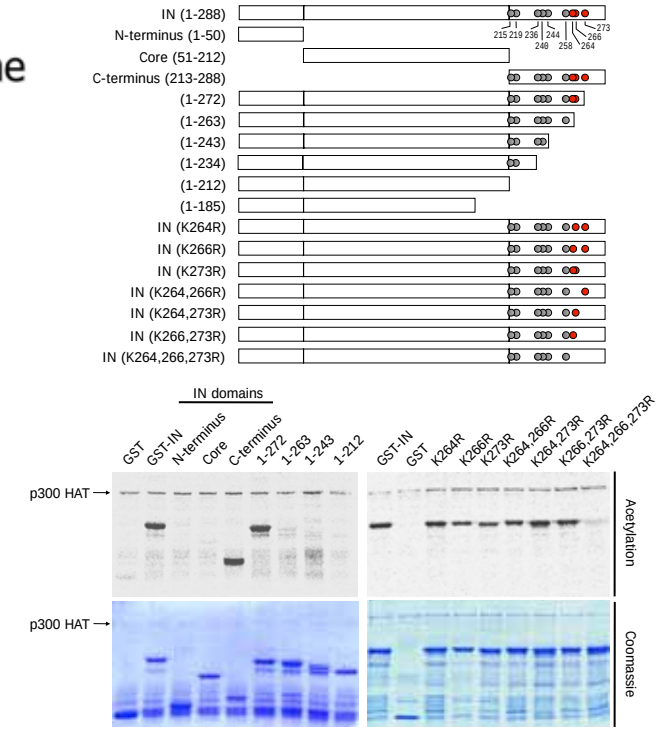
In vitro
(HAT assay with GST-p300 HAT)



In vivo
(IP and Western using anti-acLys Ab)



p300 acetylates three lysines in the IN C-terminal domain



Cereseto A. et al. 2005. EMBO J. 24, 3070

Conserved sequences in the carboxy terminus of integrase

LENTIVIRUS

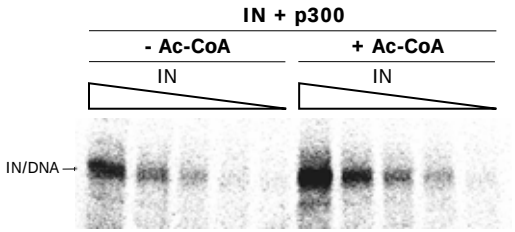
						264		266
HIV1	V	V	P	R	R	K	A	K
HIV2	I	I	P	R	R	K	A	K
SIV	V	V	P	R	R	K	A	K
VISNA	V	I	A	N	K	D	V	K
CAEV	V	I	P	Y	K	D	A	K
OLV	V	I	A	K	K	D	V	K
EIAV	A	V	P	L	T	R	T	K

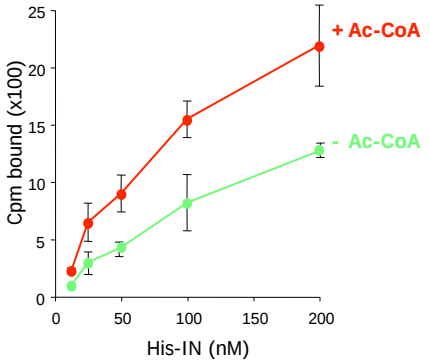
NON LENTIVIRUS

								266
RSV	W	V	P	S	R	K	V	K
BLV	W	V	P	W	R	L	L	K
HTLV-II	W	I	P	W	R	F	L	K
HTLV-I	W	I	P	W	R	L	L	K
REV-A	W	I	H	Y	S	R	L	K
FeLV	W	I	H	A	S	H	V	K
MoMLV	W	I	H	A	A	H	V	K

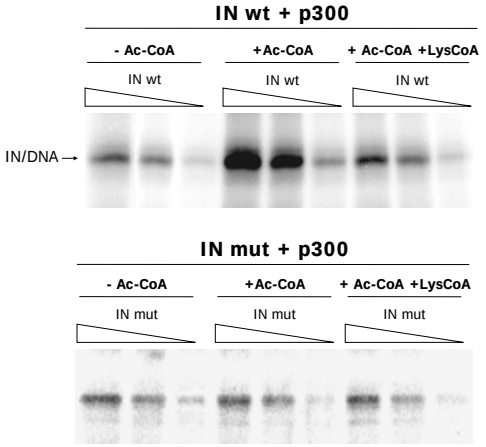
P.M. Cannon et al. J. Virol 1996
R.A. Puras Lutzke et al. Nucl Acids Res 1994

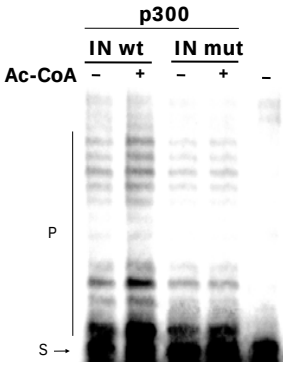
Acetylation enhances integrase affinity to DNA





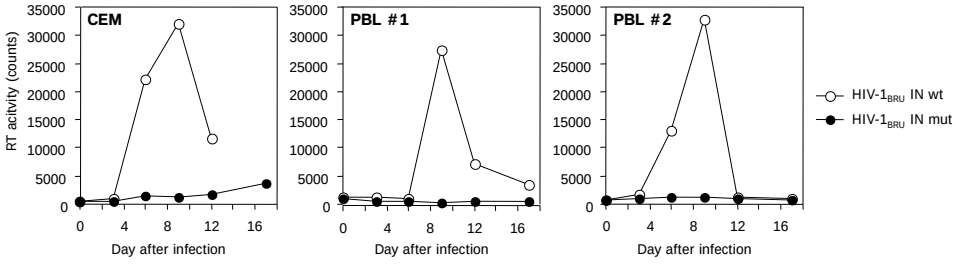
Acetylation enhances IN DNA binding and enzymatic activity

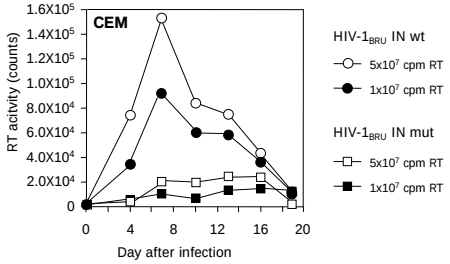


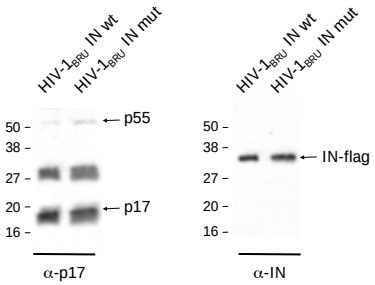


Cereseto A. et al. 2005. EMBO J. 24, 3070

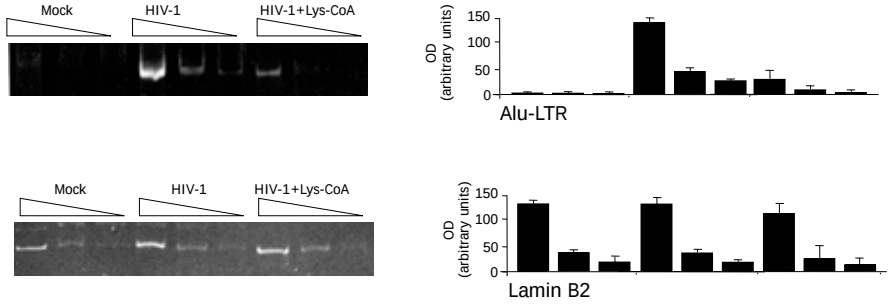
The IN triple lysine mutant is impaired in viral replication

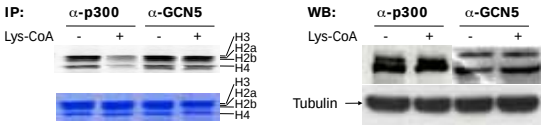






Cellular treatments with a specific inhibitor for p300 (Lys-CoA) impairs HIV-1 integration





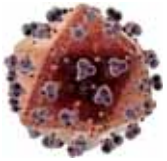
LETTERS

nature medicine

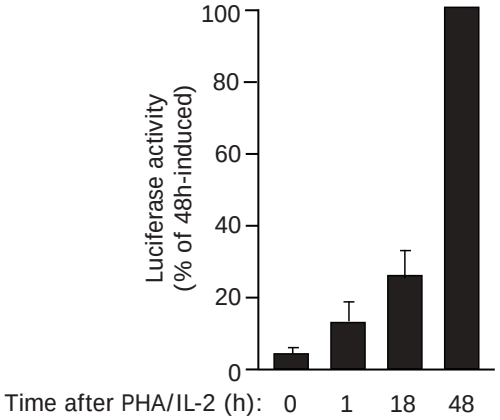
Concerted action of cellular JNK and Pin1 restricts HIV-1 genome integration to activated CD4+ T lymphocytes

Lara Manganaro[1], Marina Lusic[1], Maria Ines Gutierrez[2], Anna Cereseto[2], Giannino Del Sal[3,4] & Mauro Giacca[1]

Long-standing evidence indicates that quiescent human peripheral blood T lymphocytes (PBLs) do not support efficient HIV infection. In resting PBLs, reverse transcription of viral RNA takes longer than in activated cells[1], partially because formation of the late products of reverse transcription is decreased by RNA binding by apolipoprotein B mRNA–editing enzyme, catalytic polypeptide-like 3G (APOBEC3G)[2]. In a subsequent step, integration of the viral complementary DNA that is eventually formed is markedly impaired[3,4]. Here we show that cellular c-Jun N-terminal kinase (JNK), an enzyme that is not expressed in resting CD4+ T cells, regulates permissiveness to HIV-1 infection, and we unravel a new, sequential post-translational pathway of protein modification that regulates viral DNA integration. We found that, in activated T lymphocytes, viral integrase, which mediates HIV-1 cDNA integration into the host cell genome, is phosphorylated by JNK on a highly conserved serine residue in its core domain. Phosphorylated integrase, in turn, becomes a substrate for the cellular peptidyl prolyl-isomerase enzyme Pin1, which catalyzes a conformational modification of integrase. These concerted activities increase integrase stability and are required for efficient HIV-1 integration and infection. Lack of these modifications restricts viral infection in nonactivated, primary CD4+ T lymphocytes.



HIV-1 infection of quiescent peripheral blood CD4+ T-lymphocytes is highly inefficient



Zack, J.A., Arrigo, S.J., Weitsman, S.R., Go, A.S., Haislip, A., Chen, I.S. 1990. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. **Cell** 61, 213-22.

Stevenson, M., Stanwick, T.L., Dempsey, M.P., Lamonica, C.A. 1990. HIV-1 replication is controlled at the level of T cell activation and proviral integration. **EMBO J** 9, 1551-60.

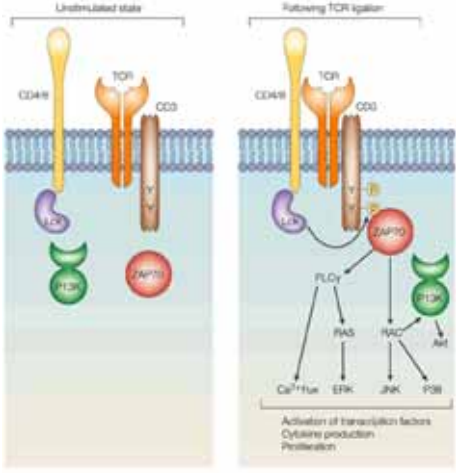
Proposed mechanisms:

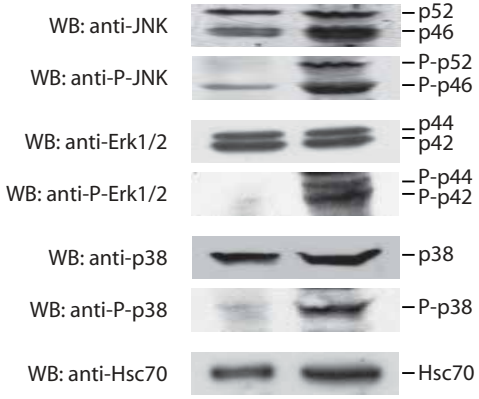
Slow-down of RT
Zhou, Y, et al. J Virol 79, 2199-210.
Pierson, TC, et al. 2002. J Virol 76, 8518.
Spina, CA, et al. 1995. J Virol 69, 2977.
Chiu, YL, et al. 2005. Nature 435, 108.

Inefficient nuclear transport
Bukrinsky, MI, et al. 1992. Proc Natl Acad Sci U S A 89, 6580.

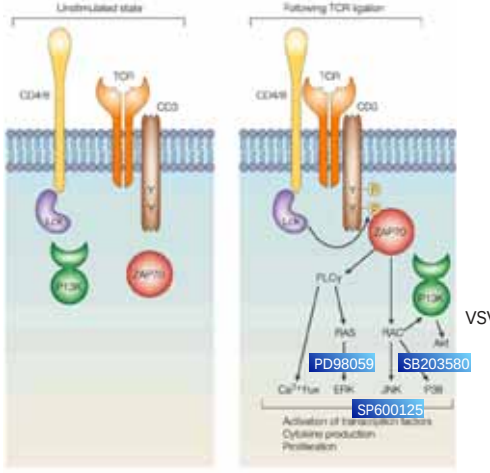
Impaired integration
Polacino, PS, et al. 1995. J Exp Med 182, 617.
Stevenson, M, et al. 1990. EMBO J 9, 1551.

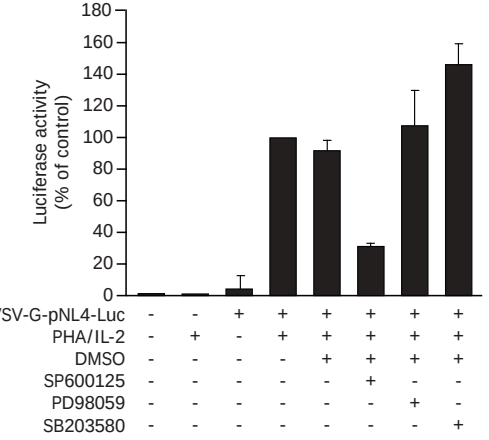
MAPK activation following TCR ligation





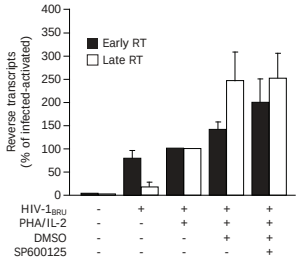
Cellular JNK activity is required for efficient HIV-1 infection of primary human T lymphocytes



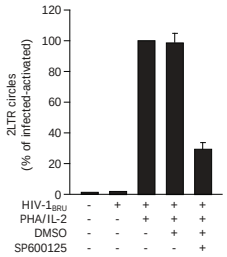


Cellular JNK activity is required for efficient HIV-1 integration in primary human T lymphocytes

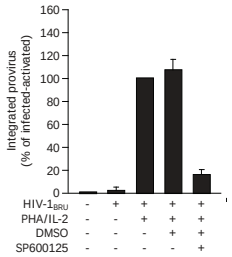
JNK inhibition does not affect RT early steps



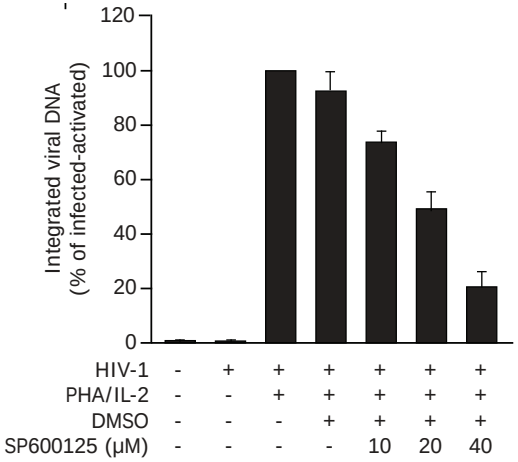
JNK inhibition impairs HIV-1 nuclear entry



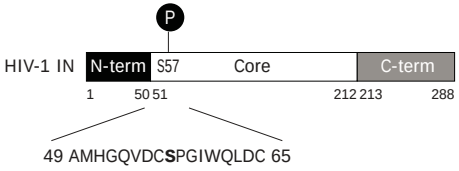
JNK inhibition blocks HIV-1 integration

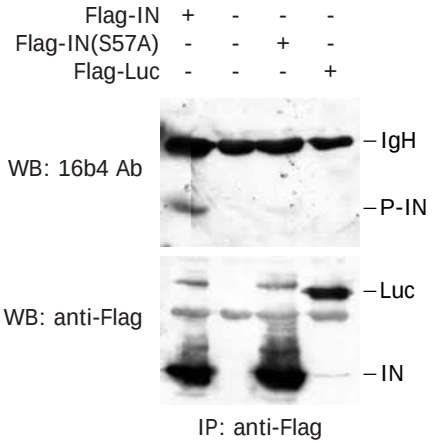


Dose-dependent JNK inhibition blocks HIV-1 integration



HIV-1 integrase is phosphorylated on serine 57

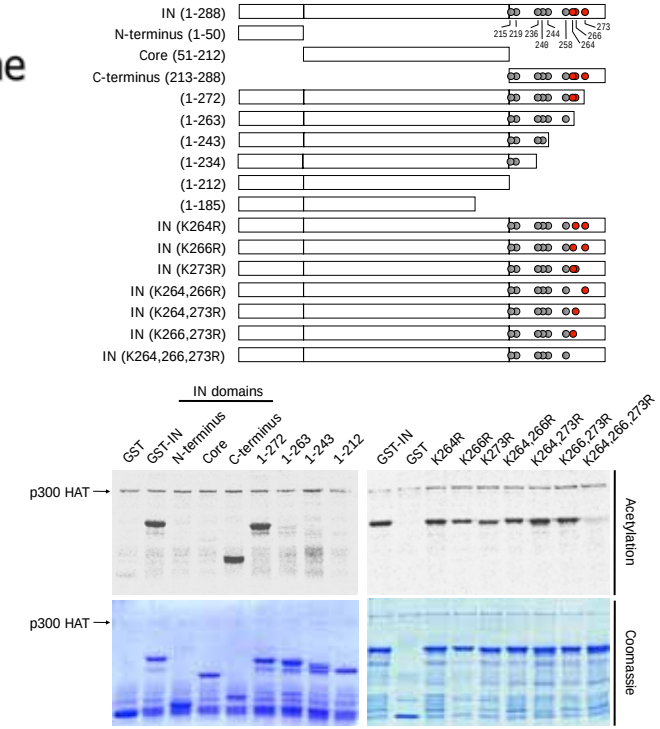




Conservation of the $S^{57}P^{58}$ motif in the IN proteins of different HIV-1 subtypes and other lentiviruses

HGQVDCSPGIWQLD	HIV-1 Subtype A
HGQVDCSPGIWQLD	HIV-1 Subtype B
HGQVDCSPGIWQLD	HIV-1 Subtype C
HGQVDCSPGIWQLD	HIV-1 Subtype D
HGQVDCSPGIWQLD	HIV-1 Subtype F
HGQVDCSPGIWQLD	HIV-1 Subtype G
HGQVDCSPGIWQLD	HIV-1 Subtype H
HGQVDCSPGIWQLD	HIV-1 Subtype K
HGQVDCSPGIWQLD	HIV-1 Subtype 01
HGQVDCGPGIWQLD	HIV-1 Subtype 02
HGQVNSDLGTWQMD	HIV-2
HGQVDCSPGIWQVD	SIV(CPZ.CD.90.ANT)
HGQVDASPGVWQMD	SIV(VER.DE.x.AGM3)
HGQVNAELGTWQMD	SIV(MAC.US.x.251_1A11)
HGQVNAELGTWQMD	SIV(SMM.US.x.PGM3)
AGCVMRSPNHWQAD	EIAV
GGQLKIGPGIWQMD	FIV

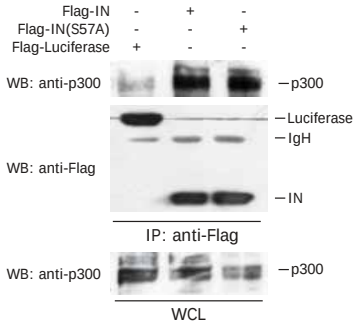
p300 acetylates three lysines in the IN C-terminal domain



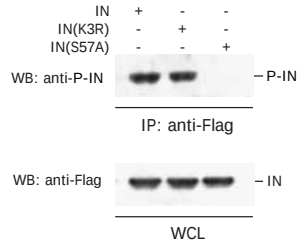
Cereseto A. et al. 2005. EMBO J. 24, 3070

Lack of correlation between IN acetylation and phosphorylation

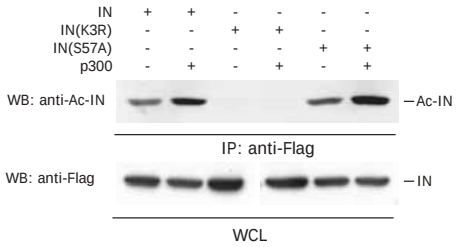
IN (S57A) mutant interacts with p300 in vivo



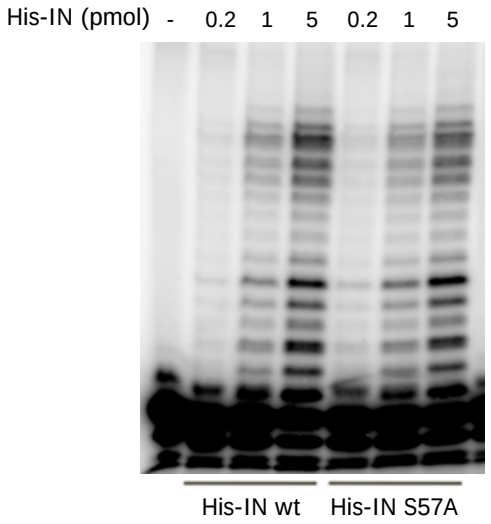
IN mutant defective for acetylation can still be phosphorylated



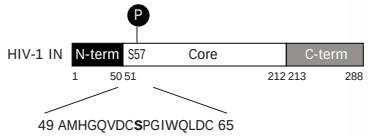
IN (S57A) mutant is still acetylated on lysines 264,266,273



Enzymatic activity of IN is not affected by the S57A mutation



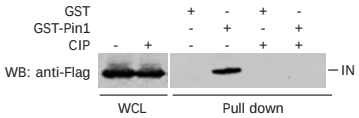
The cellular prolyl-isomerase Pin1 specifically binds phosphorylated serines/threonines followed by a proline (S-P), and isomerizes the peptidyl bond between the phosphorylated aminoacid and the proline residue



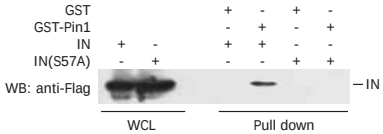
K.P. Lu et al., Nat.Rev. Mol. Cell. Biol. 2007

Phosphorylation-dependent binding of HIV-1 integrase to cellular prolyl-isomerase Pin1

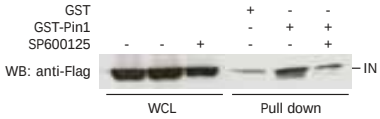
Pin1 binds HIV-1 phospho-IN



Integrity of IN S57 is essential for binding to Pin1



Binding of Pin 1 to IN depends on the catalytic activity of JNK



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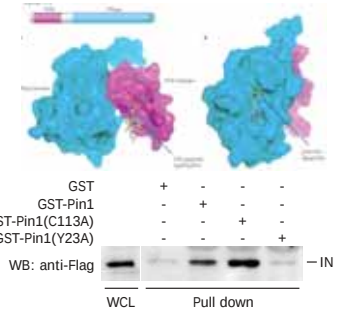
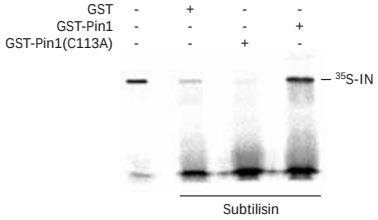


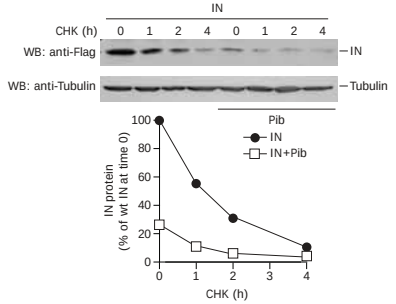
WW domain of Pin1 is required for its binding to IN



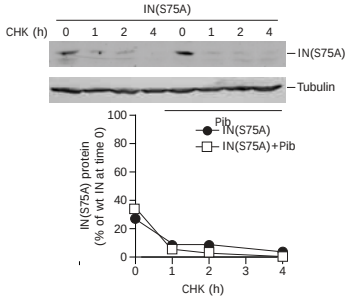
Pin1 catalyzes a conformational change in IN

Pin1 regulates HIV-1 integrase stability

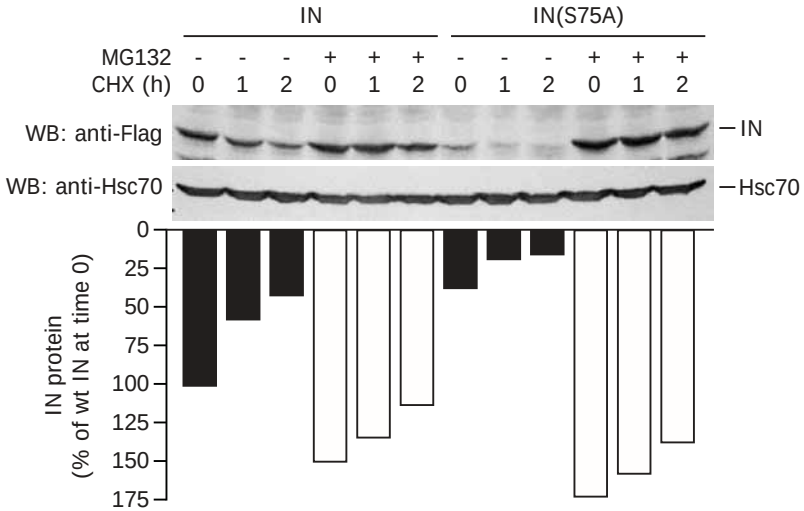
Pin1 inhibition decreases IN stability



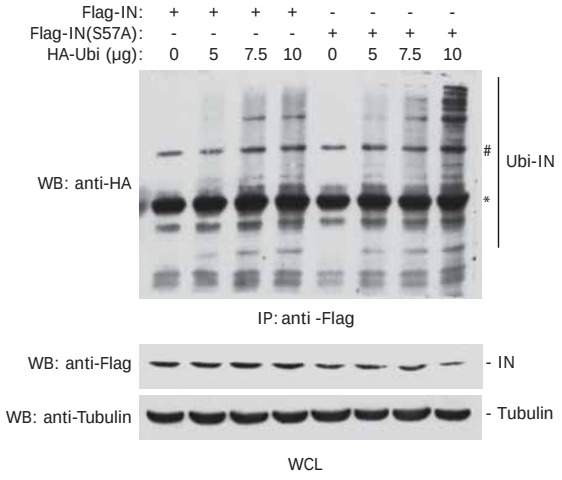
IN(S57A) mutant is less stable than wild type IN and insensitive to Pin inhibition

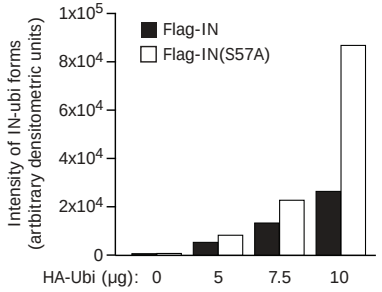


Proteasome inhibition restores IN and IN(S75) stability

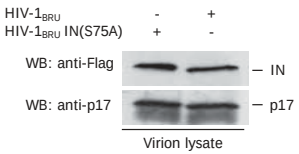


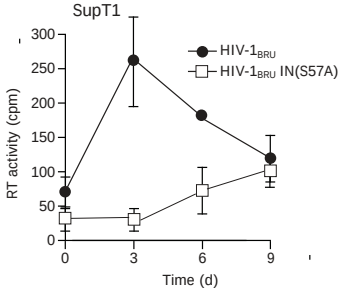
IN(S57A) mutant is more ubiquitinated than wt IN in vivo

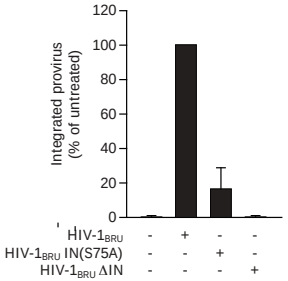


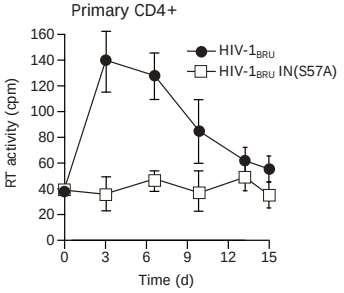


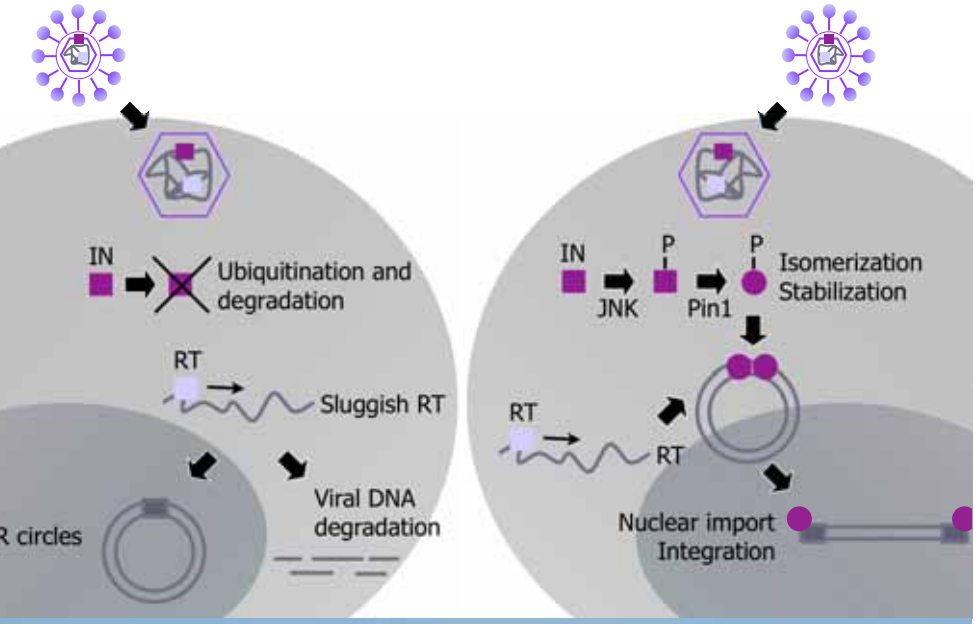
Replication and integration of HIV-1$_{BRU}$ IN(S57A) are impaired











International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

Molecular Medicine Laboratory

Serena Zacchigna
Sabrina Tafuro
Lorena Zentilin
Chiara Collesi
Lucio Barile
Lucia Pattarini
Antero Macedo
Jasmina Lovric
Miguel Mano
Alessandro Carrer
Silvia Moimas
Uday Puligadda
Giulia Ruozi
Giulia Felician
Maryam Kazemi
Milena Sinigaglia
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Fabrizio Pirozzi
Marina Dapas
Barbara Boziglav
Michela Zotti

Lara Manganaro
Marina Lusic
Chiara Vardabasso
Annalisa Zecchin
Maria Ines Gutierrez

University of Trieste and Laboratorio Nazionale CIB, Trieste, Italy
Giannino Del Sal

Scuola Normale Superiore, Pisa, Italy
Anna Cereseto
Silvia Agostini

Thanks!

giacca@icgeb.org