# Включение H3.3 в центромерный хроматин: Кто, Как и Зачем?

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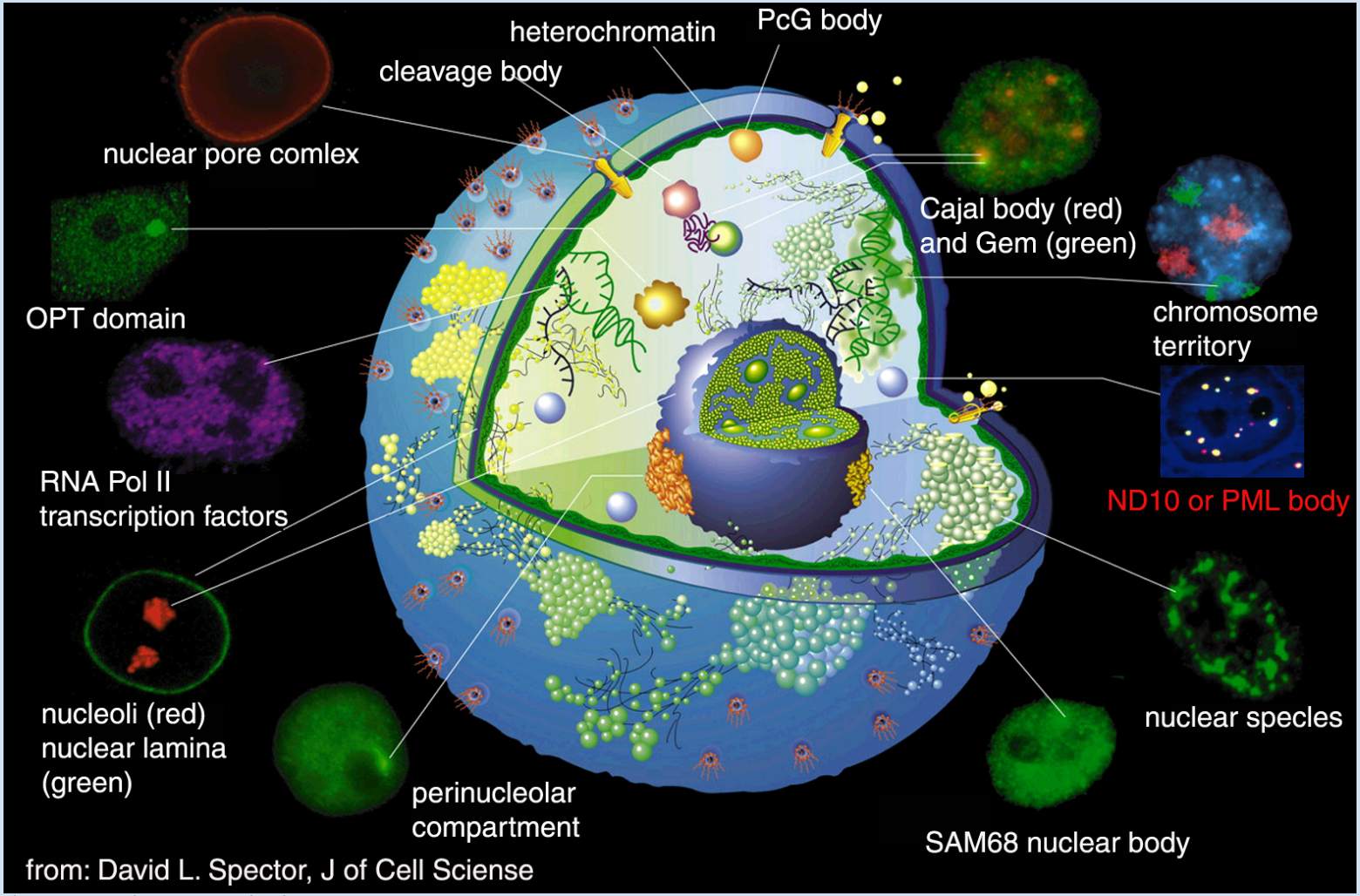
ИНСТИТУТ ЦИТОЛОГИИ
29 апреля 2015

# Nuclear domains (compartments)



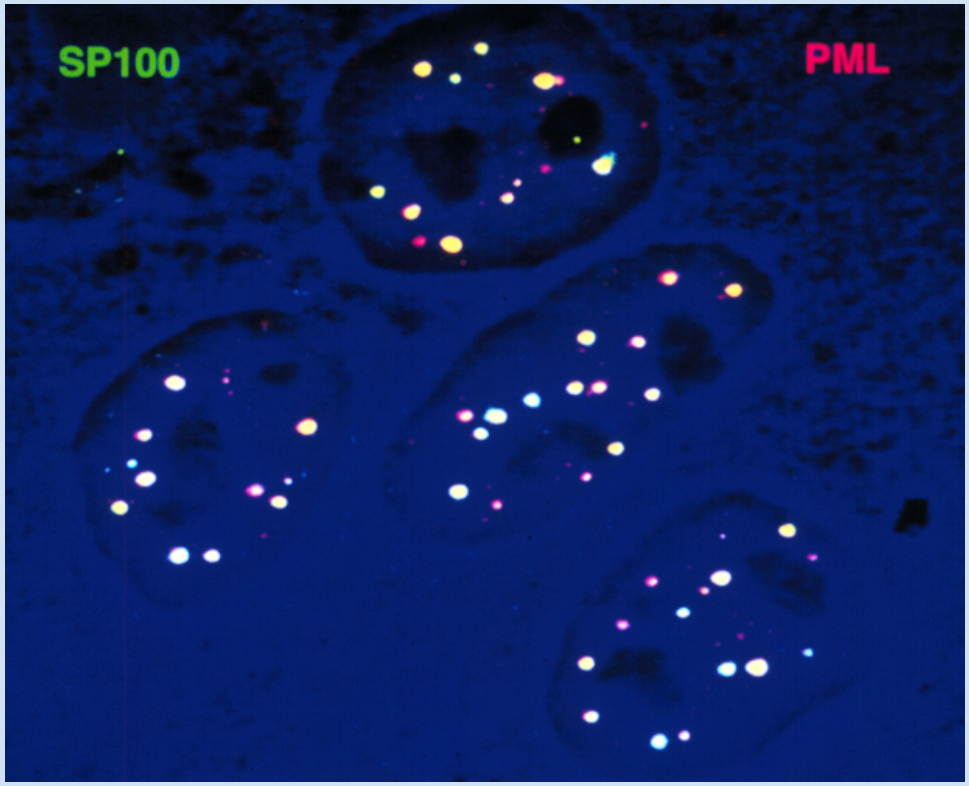

- do not have delineating membranes
-contain defined subset of proteins
-can be identified by light and electron microscopy, and in vivo by GFP fusions
- some of nuclear compartments can be biochemically isolated

From: Spector DL. J Cell Sci. 2001 114(Pt 16):2891-3

# PML Nuclear Bodies/Nuclear Domain 10 (ND10)

Nuclear matrix associated domains with the average frequency of 10 per nucleus and a diameter of 0.1-1.0 μκm in most cells

Assay: microscopy after immunostaining for PML and Sp100

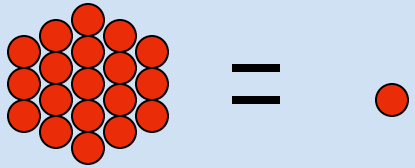



Sp100 and PML co-localize at PML bodies

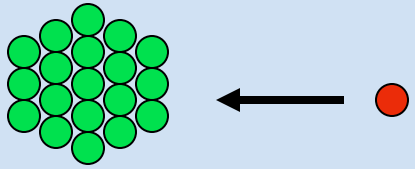
# Functional roles for nuclear domains

Increased accumulation of protein in nuclear domains may indicate:

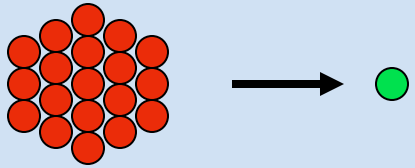
-no functional role



-site of action



-site of inactivation



One of the of PML bodies/ND10 functions:

nuclear depot by controlled recruitment and release of selected proteins

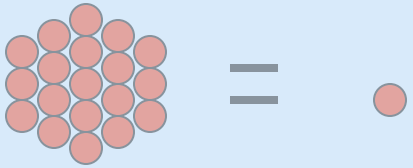
# External insults modify PML NBs/ND10

- Hyperthermia (fever) = Heat Shock (HS)
- Interferon exposure (PML, SP100, Daxx: IFN regulated)
- Heavy metal exposure (Cd++; As+++)
- UV light
- Hormone action (progesteron)
- Viral Infection (Ad5; HSV-1; HCMV; EBV; KSHV; HDV)
- Aging

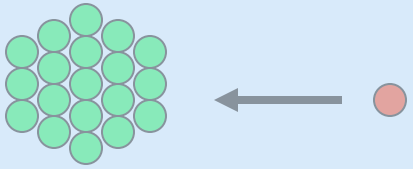
# Functional roles for nuclear domains

Increased accumulation of protein in nuclear domains may indicate:

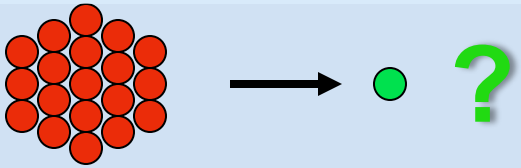
-no functional role



-site of action



-site of inactivation




- PML bodies/ND10 function as a nuclear depot
- PML bodies/ND10 are modified by stress
- What is localization of ND10 proteins upon stress (HS)?

# List of Proteins Found in PML bodies/ ND10

## Constitutive Proteins

- **PML;** promyelocytic leukemia
- **Sp100;** PBC autoantigen
- **SUMO** (Small Ubiquitin-like Modifier)
- **ATRX;** a-thalassemia X-linked mental retardation, SWI/SNF2 chromatin remodeling
- **Daxx**

Facultative Proteins

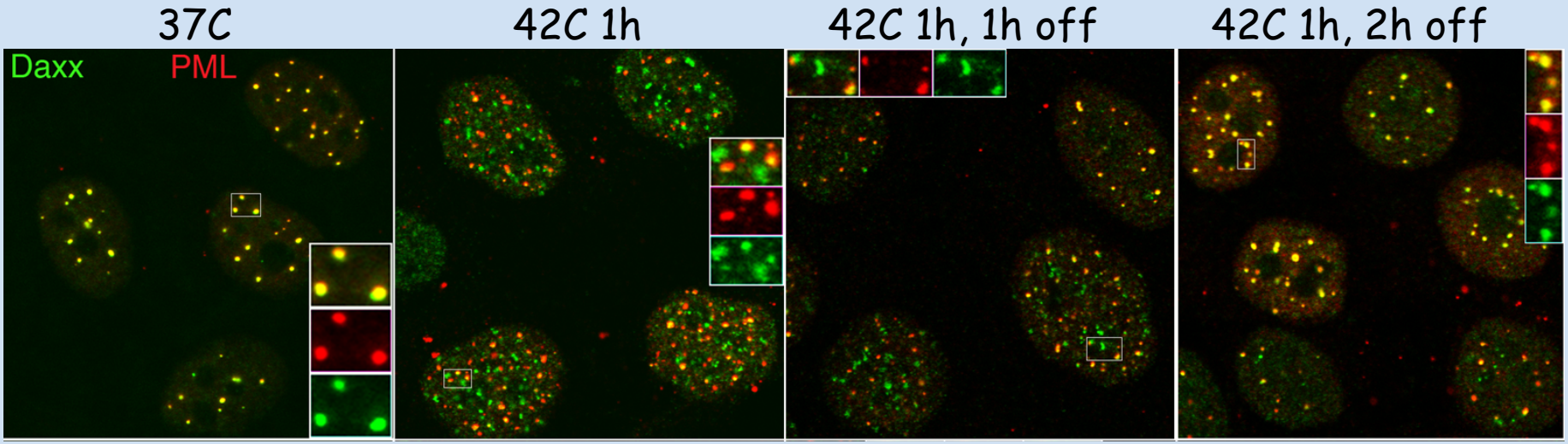
- BLM; helicase mutated in Bloom syndrome
- p53; after interferon exposure
- CBP; histone acetylase
- HDAC2; histone deacetylase
- HP1; heterochromatin
- Pax 5; transcription factor
- pp71; HCMV tegument protein
- ICP0; HSV-1 transactivator
- E4 orf3; Ad5 IE protein
- SENP-1; desumofication enzyme

- Daxx: conservative protein essential for embryonic development
- Daxx is implicated in apoptosis, transcriptional regulation and mitosis

# Daxx Localization Upon HS and Recovery

To study the effect of hyperthermic stress on Daxx localization, HEp2 (human larynx carcinoma) cells were exposed to 42C for 1h and either fixed immediately or recovered at 37C for 1h/2h

Staining: Daxx/PML




- During HS, Daxx forms additional patterns (20-40 per cell, often in pairs) that do not co-localize with PML NB's

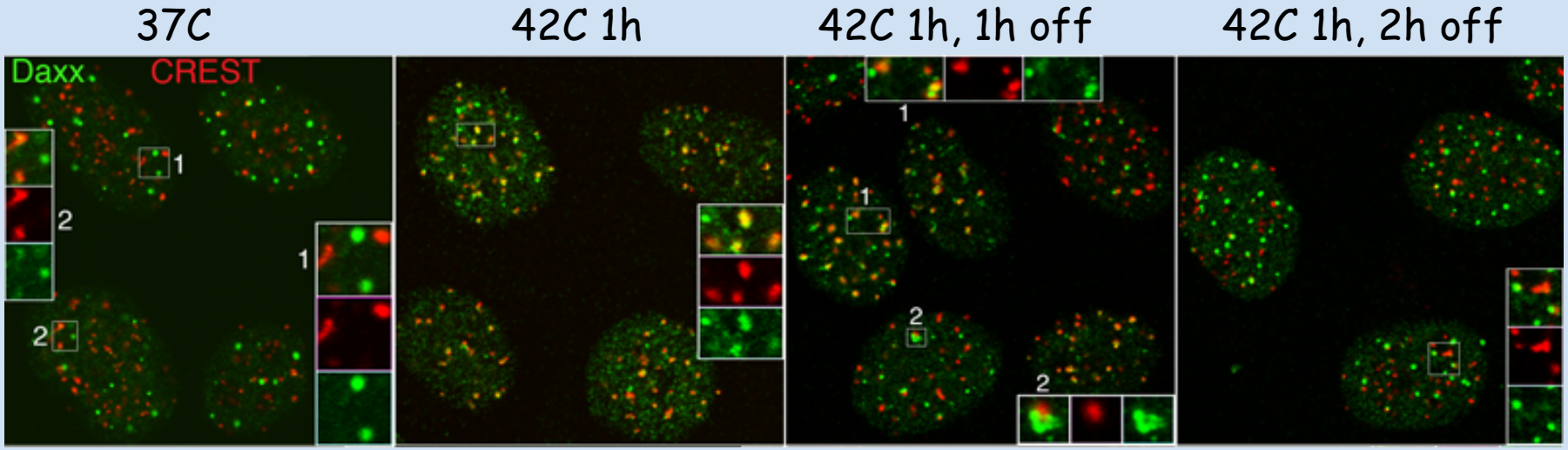
- New patterns mostly disappear at 2h of recovery

What are those new patterns?

Morozov et al., *Nucleus* 2012

# Daxx and Centromeres

Staining: Daxx/CREST (human autoimmune anti-centromere antibody)



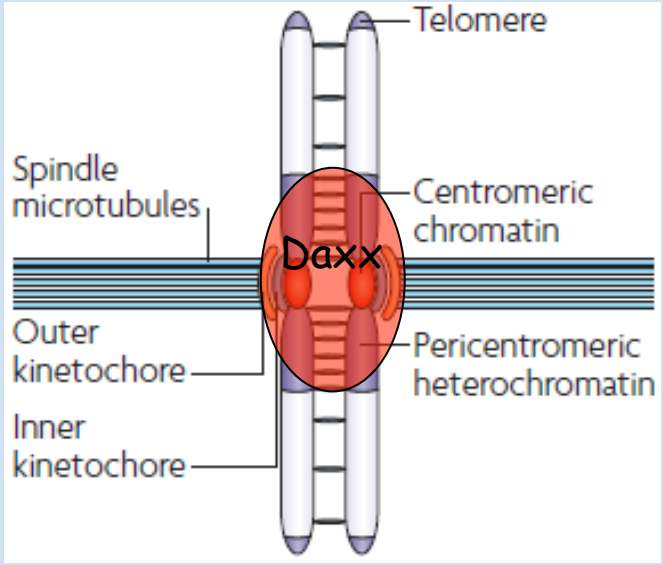

- before HS, Daxx colocalizes with centromeres in some cells
- after HS and 1h of recovery, Daxx colocalizes with centromeres in most cells
- 2h of recovery: no Daxx association with centromeres

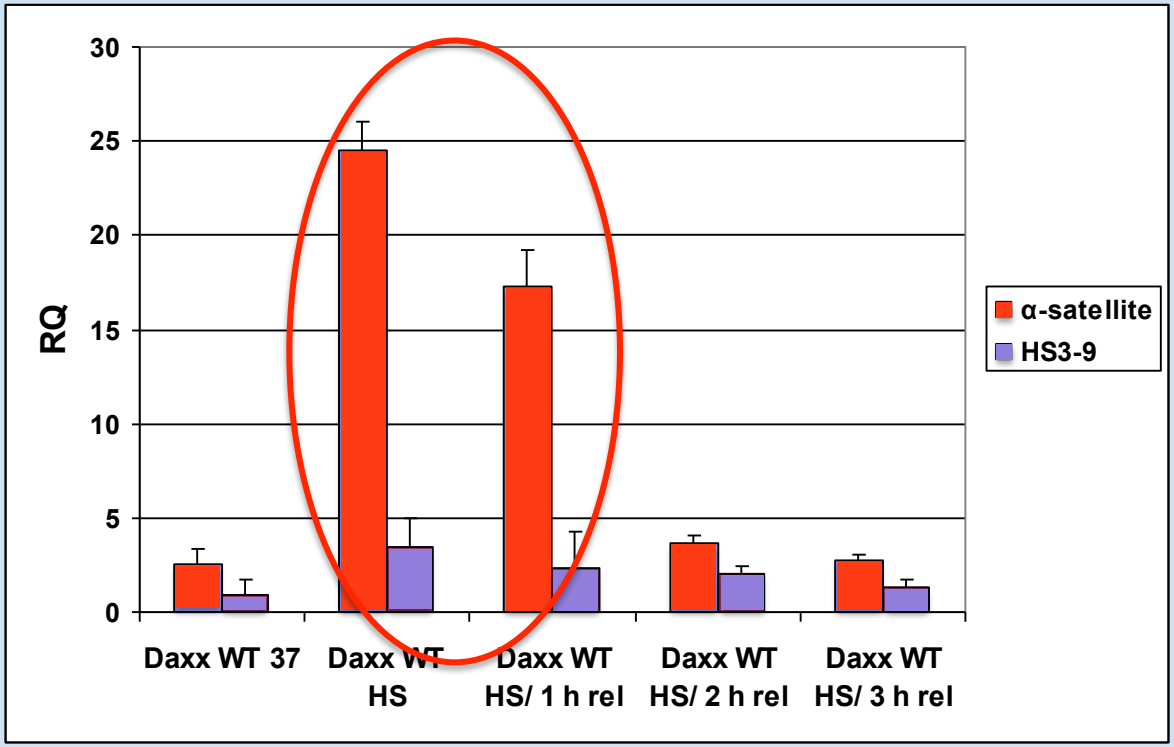
Morozov et al., *Nucleus* 2012

# Daxx association with CEN and periCEN

HEp2 cells expressing FLAG/HA-Daxx were exposed to 42C for 1h and either fixed immediately or recovered at 37C for 1 - 3h.

Daxx ChIP on **CEN (pan-centromeric alpha-satellite)** and **periCEN (satellite 3-9)**



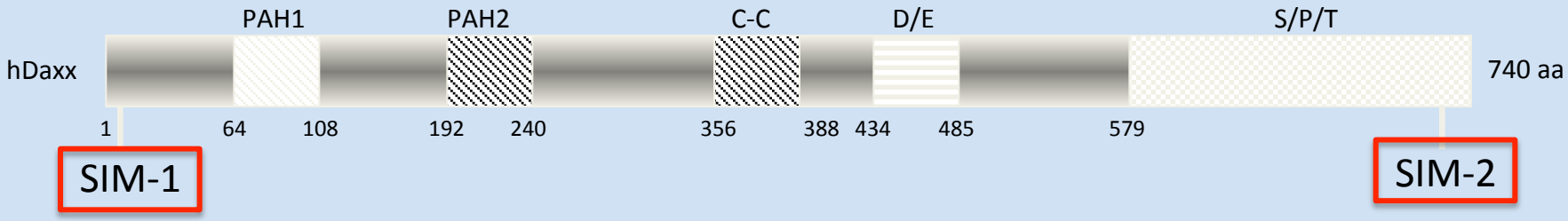




Daxx accumulates at **CEN** and **periCEN** during stress and recovery

Morozov et al., *Nucleus* 2012

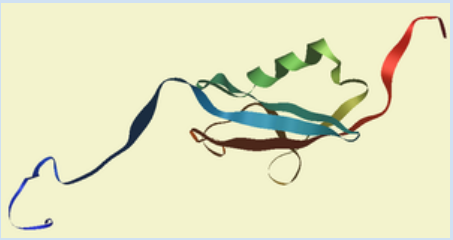
# What attracts Daxx to CEN/periCEN?

Daxx contains two SUMO-interaction domains (SIM) (Santiago A. et al., 2009).




Is SUMOylation important for Daxx localization at CEN/periCEN after stress?

# SUMO Intro



-Small ubiquitin-related modifier (SUMO ) is ~ 100 amino acid polypeptide that can be covalently conjugated to a lysine of substrate protein

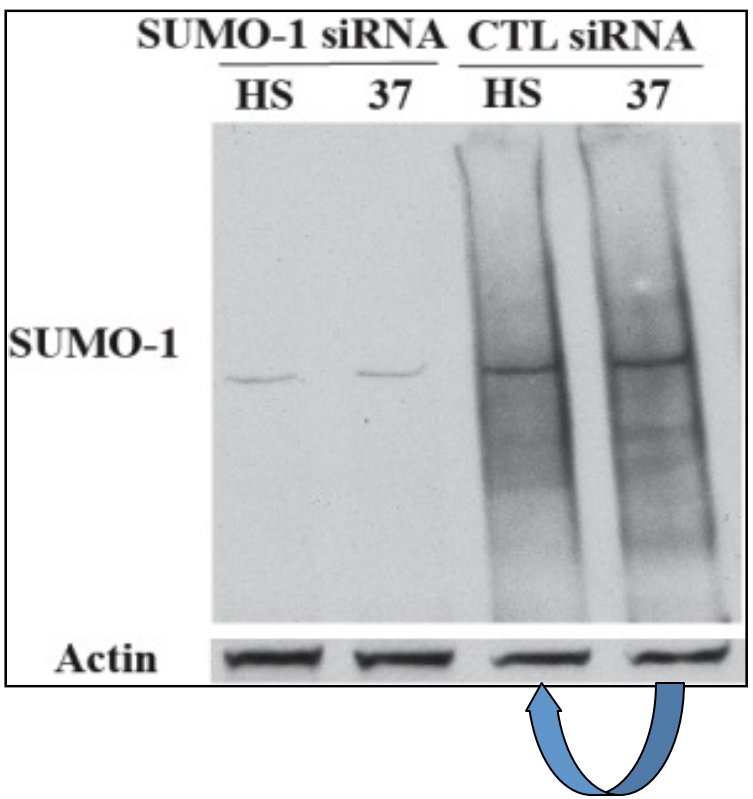
-SUMO can add binding interfaces or remove them

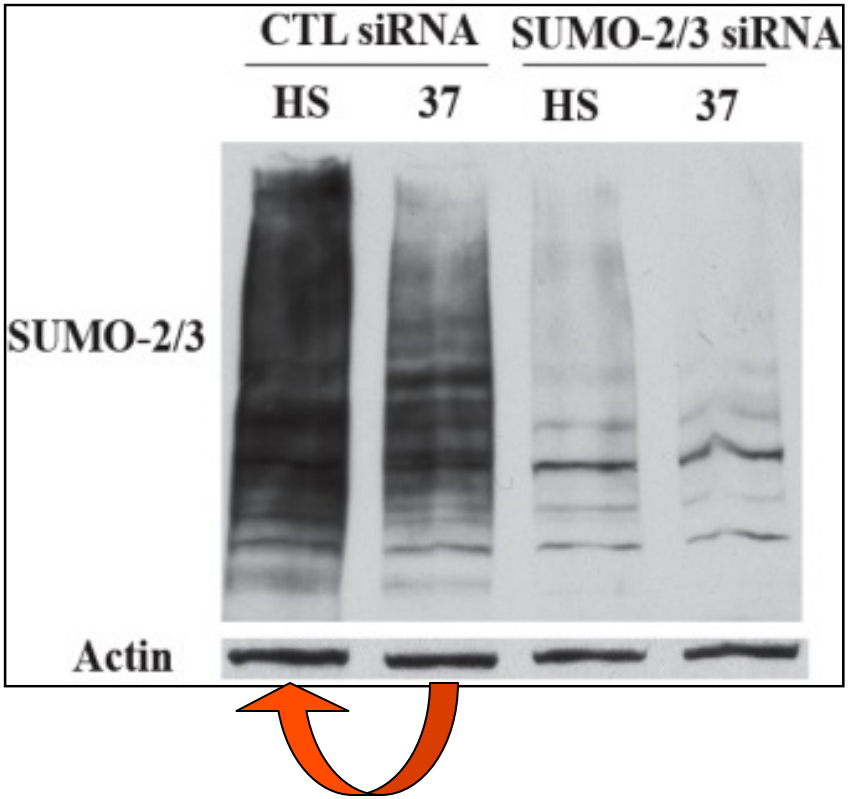
-SUMOylation involves in intra-nuclear protein targeting, formation of subnuclear structure, regulation of transcription, DNA replication/repair, chromosome segregation, and protein stability

-Four SUMO family proteins in mammals: SUMO-1/4; SUMO-2, -3 are 96% identical, only about 46% identity with SUMO-1/4

**-SUMO-1 and SUMO2/3 are differentially regulated in vivo:**
**SUMO-1 is conjugated to substrates under normal condition,**
**SUMO-2/3 is mostly conjugated to target proteins in response to stress**

# Depletion of SUMO-1 and SUMO-2/3 by siRNA

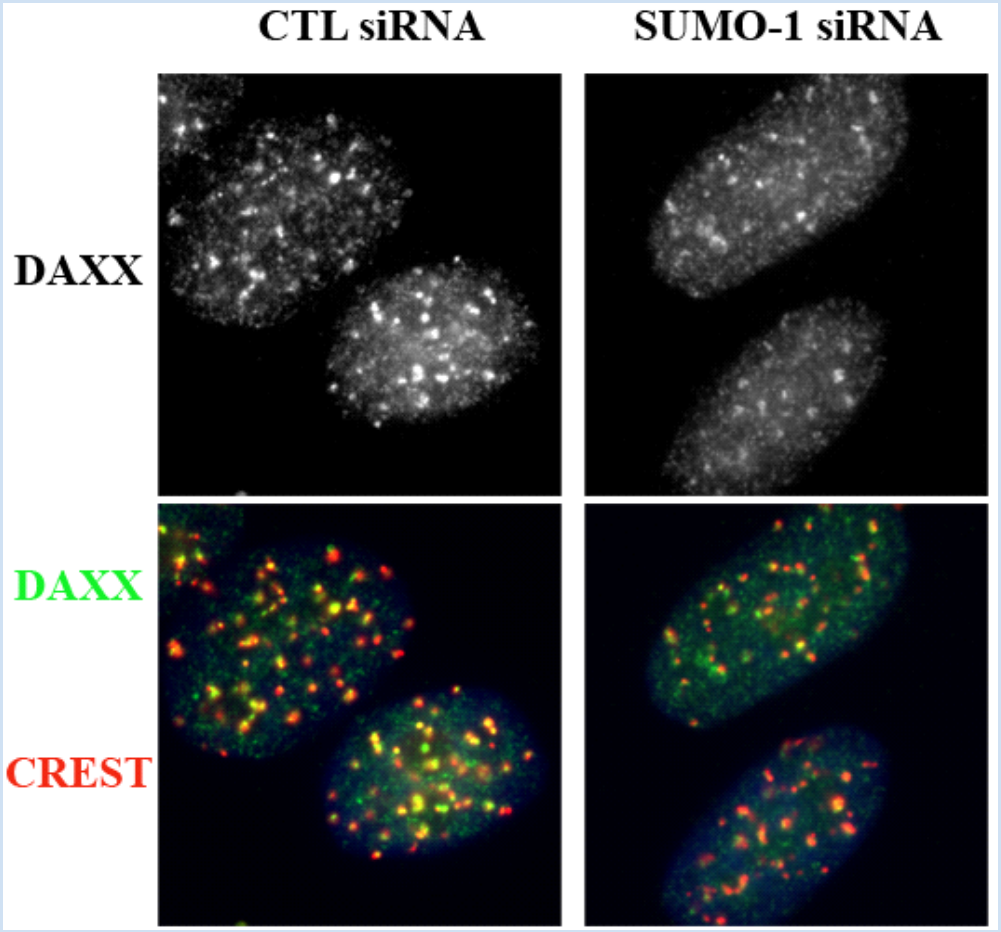



Sumoylation by SUMO-2/3 is elevated in response to HS

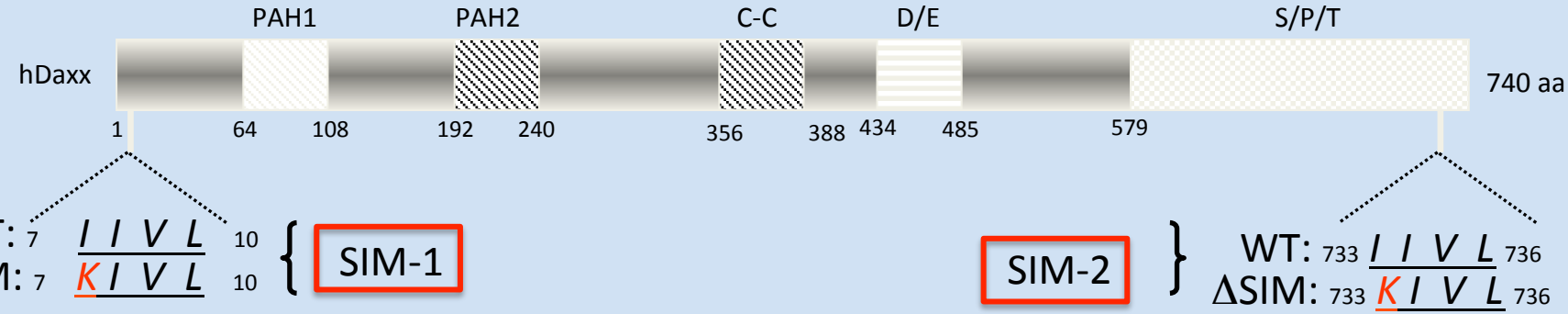
# Is SUMOylation important for Daxx localization at CEN/periCEN after stress?

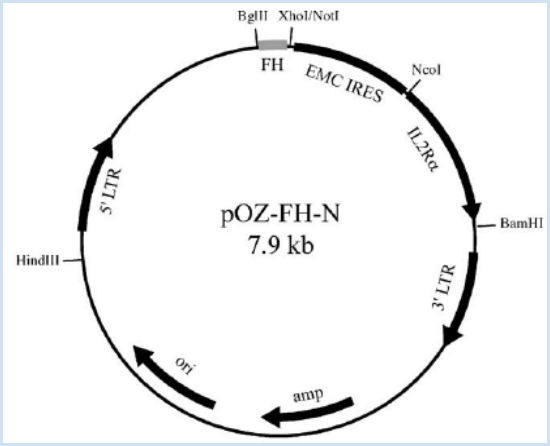
HEp2 cells were transfected with control (CTL), SUMO-1 or SUMO-2/3 siRNA. 72h later cells were exposed to heat shock (1h, 42C).
Staining: **Daxx** and **CREST (centromeres)**



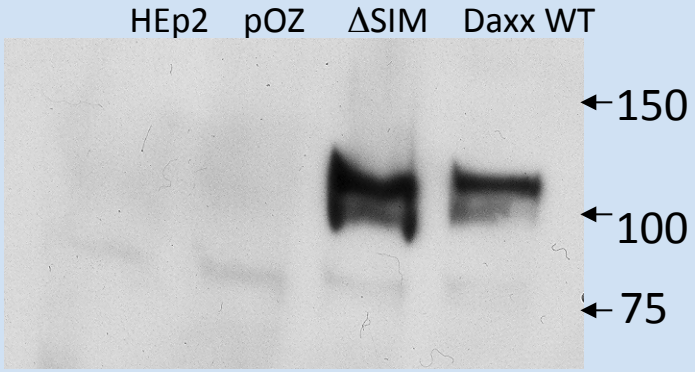

Daxx deposition at centromeres upon HS requires SUMO-2/3, but not SUMO-1

# Complimentary approach: SIM inactivation of Daxx




From: Nakatani Y, Ogryzko V. Methods Enzymol. 2003;370:430-44.




Western Blot for HEp2-DaxxWT and HEp2-DaxxΔSIM (anti-HA ab's)

Daxx contains two SUMO-interacting motifs (SIMs)

Daxx ΔSIM, in which both SIMs are mutated, does not interact with SUMO

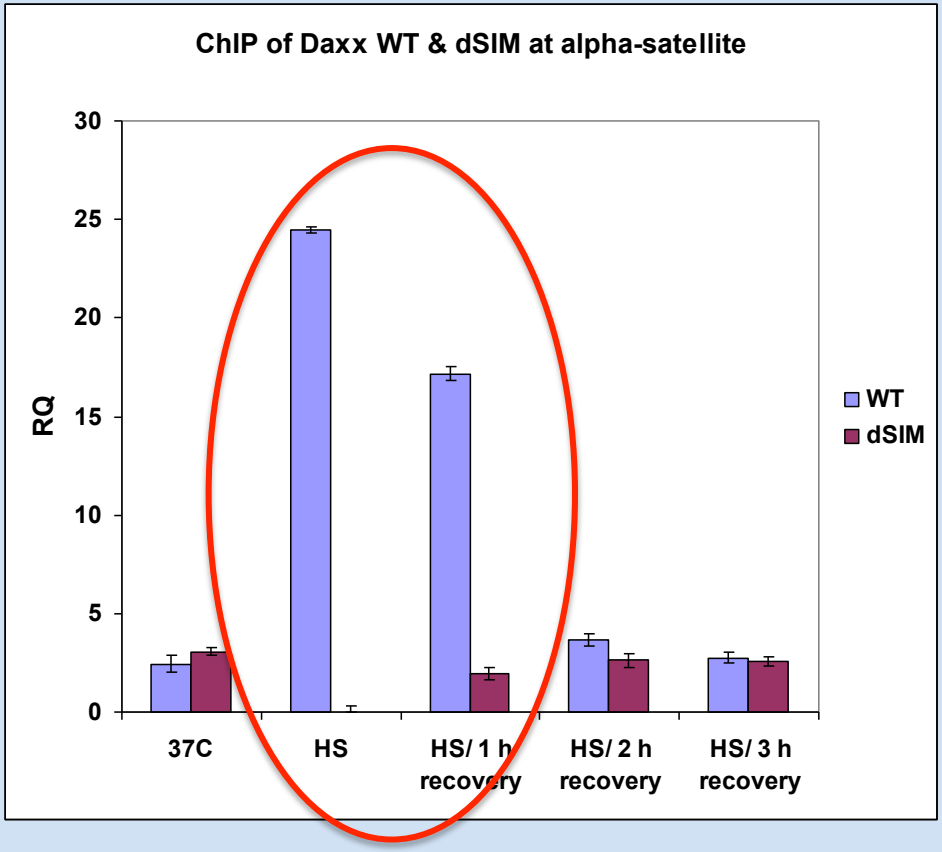
Daxx WT and Daxx ΔSIM were cloned in pOZ (left);

HEp2 cells created for stable expression of corresponding constructs (right)

# How SIM mutations affect Daxx localization upon stress?

HEp2 cells expressing FLAG/HA-Daxx and FLAG/HA-DaxxD-SIM

ChIP for Daxx and DaxxD-SIM on CEN (pan-centromeric alpha-satellite)




Daxx association with CEN is SIM-dependent

# How SIM mutations affect Daxx localization upon stress?

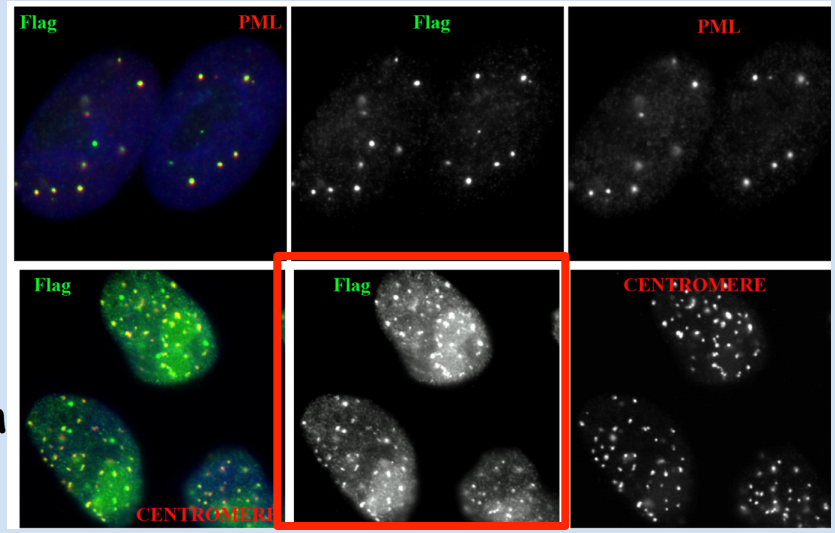
HEp2 cells stable expressing of Daxx WT and Daxx Delta-SIM in frame with FLAG/HA were fixed at 37C or after exposure to 42C for 1h

Staining: FLAG (Daxx)/PML (top) and FLAG (Daxx)/CREST (bottom)

**Daxx WT**

**37C**

**Heat Shock 42C, 1h**




SIM mutations block Daxx localization at CEN/periCEN upon HS

Sumoylation (2/3) of protein(s) X at CEN/periCEN upon HS

➡

Daxx recruitment via SIM's

# Accumulation of Daxx to centromeres under different stress condition

| Heat shock, 42C, 1h | UV 10 $mJ/cm^2$ | Gamma irradiation | Proteosome inhibition MG132 | TSA | $Cd^{2+}$ | $As_2O_3$ | $H_2O_2$ | 17-AAG |
|---|---|---|---|---|---|---|---|---|
| YES | NO | NO | YES | YES | YES | NO | NO | YES |

# Daxx at CEN/periCEN:

- *Daxx accumulates at CEN/periCEN upon application of mild HS (42C).
  This accumulation is:
  rapid (30' of HS),
  reversible (1 h after HS release),
  does not change Daxx stability.

- *Daxx deposition is mediated by SUMO-2/3, but not by SUMO-1 and requires functional Daxx SIM's, suggesting for potential interaction with SUMOylated partner(s).

- *Among PML NB's associated proteins, only SNF2 helicase/ATPase ATRX is associated with Daxx upon stress.

Sumoylation (2/3) of protein(s) X at CEN/periCEN upon HS ➡ Daxx recruitment via SIM's ➡ ATRX recruitment

FUNCTION?

## Potential functions of Daxx at CEN/periCEN:

- Set up the cell cycle checkpoint for the stress recovery?
- Changes in the follow-up kinetochore assembly?
- Genome stability?
- Changes in chromatin structure and epigenetic modifications?
- Regulation of transcription at CEN/periCEN repeats?

## Potential functions of Daxx at CEN/periCEN:

- Set up the cell cycle checkpoint for the stress recovery?
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# CEN and periCEN transcription in mouse and human cells

Transcripts: non-coding RNA

Transcription from CEN/ periCEN is implicated in:

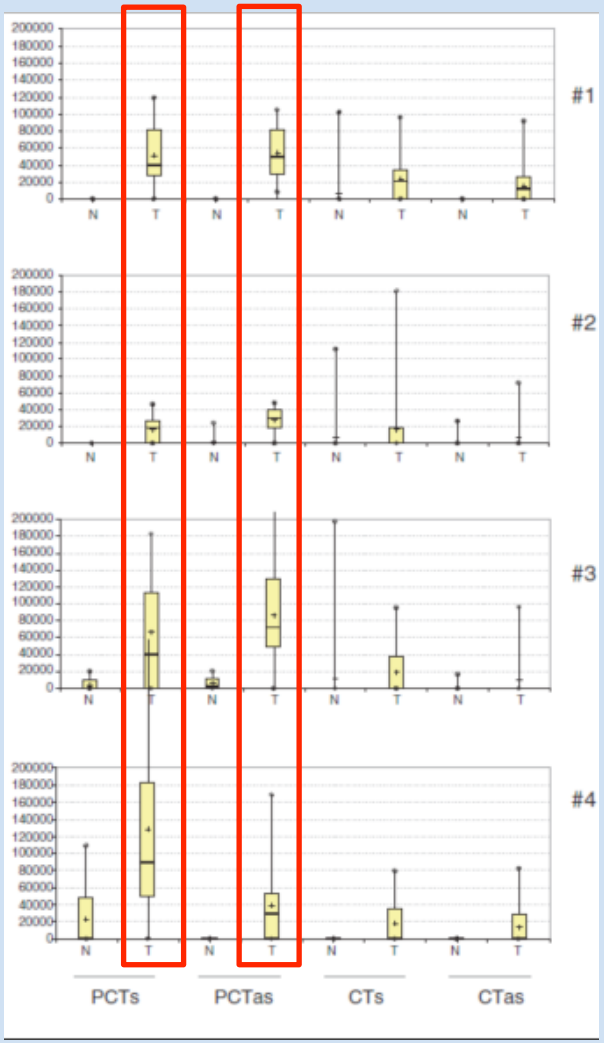
-stress

-development

-proliferation

-carcinogenesis

Expression of CEN and periCEN is elevated in lung cancer specimens

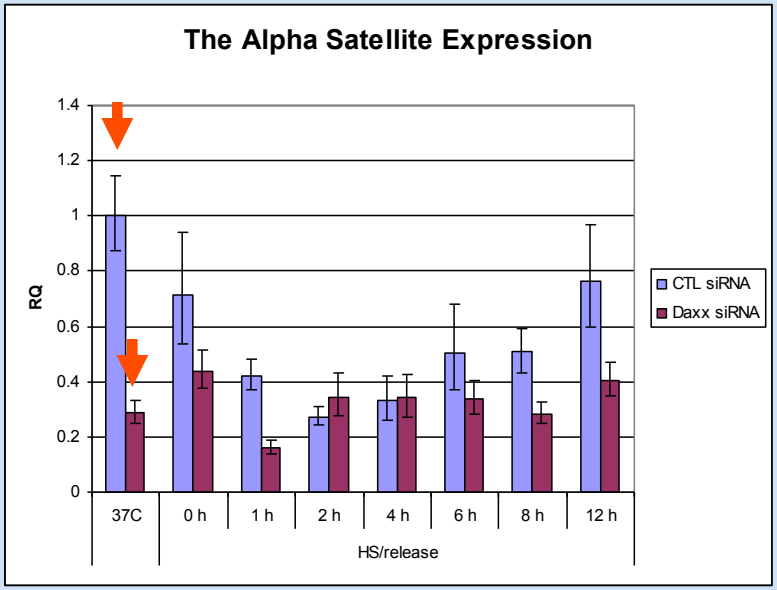



Eymery et al., *Nucleic Acids Res*. 2009

# Transcription of centromeric (CEN) and pericentromeric (periCEN) repeats during stress and release

HEp2 cells expressing **Daxx shRNA** and **CTL shRNA** were exposed to 42C for 1h and either fixed immediately or recovered at 37C for 1 -12h

CEN transcription




Depletion of Daxx REPRESSES transcription: CEN (at 37C) periCEN (HS)

Surprise: mostly Daxx is transcription repressor via attraction of HDAC1/2 (c-met)

**What is the mechanism of Daxx-dependent repression of CEN and periCEN?**

Morozov et al., *Nucleus* 2012

# Daxx and Histone 3.3

**Distinct Factors Control Histone Variant H3.3 Localization at Spec**

Aaron D. Goldberg,
Scott Dewell,[2] Mar
Jeffrey C. Miller,[9] Y
Shahin Rafii,[5,6,7] C
Ileana M. Cristea,[1

ATRX interacts with H3.3 in maintaining telomere structural integrity in pluripotent embryonic stem c

Lee H. Wo
Ross D. Ha

[1]Chromosome
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20:351–360 © 2010

**The death-associated protein DAXX is a novel histone chaperone involved in the replication-independent deposition of H3**

Pascal Drané,

IGMBC (Institut
F-67404, France;

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Daxx is an H3.3-specific histone chaperone and cooperates with ATRX in replication-independent chromatin assembly at telomeres

Peter W. Lewis[1], Simon J. Elsaesser[1], Kyung-Min Noh, Sonja C. Stadler, and C. David Allis[2]

Laboratory of Chromatin Biology and Epigenetics, Rockefeller University, 1230 York Ave, New York, NY 10065

Contributed by C. David Allis, June 23, 2010 (sent for review June 14, 2010)

The histone variant H3.3 is implicated in the formation and maintenance of specialized chromatin structure in metazoan cells. H3.3-containing nucleosomes are assembled in a replication-independent manner by means of dedicated chaperone proteins. We previously identified the death domain associated protein (Daxx) and the ·-thalassemia X-linked mental retardation protein (ATRX) as H3.3-associated proteins. Here, we report that the highly conserved N terminus of Daxx interacts directly with variant-specific residues in the H3.3 core. Recombinant Daxx assembles H3.3/H4 tetramers on DNA templates, and the ATRX–Daxx complex catalyzes the deposition and remodeling of H3.3-containing nucleosomes. We find that the ATRX–Daxx complex is bound to telomeric chromatin, and that both components of this complex are required for H3.3 deposition at telomeres in murine embryonic stem cells (ESCs). These data demonstrate that Daxx functions as an H3.3-specific chaperone and facilitates the deposition of H3.3 at heterochromatin loci in the context of the ATRX–Daxx complex.

essential for H3.3 incorporation at telomeres. Apart from ATRX, we also identified Daxx in H3.3 immunoprecipitations (IP) (9). Daxx and ATRX have been shown to form a complex (13) and colocalize at pericentric heterochromatin and promyelocytic leukemia bodies (PML bodies) (14, 15). Loss of ATRX leads to epigenetic alterations, including abnormal levels of DNA methylation at repetitive elements such as telomeres in murine cells (16). Moreover, ATRX and H3.3 have essential roles in maintaining telomere chromatin (9, 17).

To gain further insight into H3.3-specific deposition pathways, we sought to identify the direct binding partner of H3.3. Here we show that Daxx binds directly to H3.3, and importantly, this binding is specific for H3.3 and not H3.1. We find that Daxx alone, or when present in the ATRX–Daxx complex, can effectively assemble H3.3-containing nucleosomes. Additionally, we show that ATRX recruits Daxx to telomeres, and both complex subunits are required for H3.3 deposition at telomeric chromatin in murine ESCs.

Daxx/ATRX complex as a new chaperone of histone H3.3

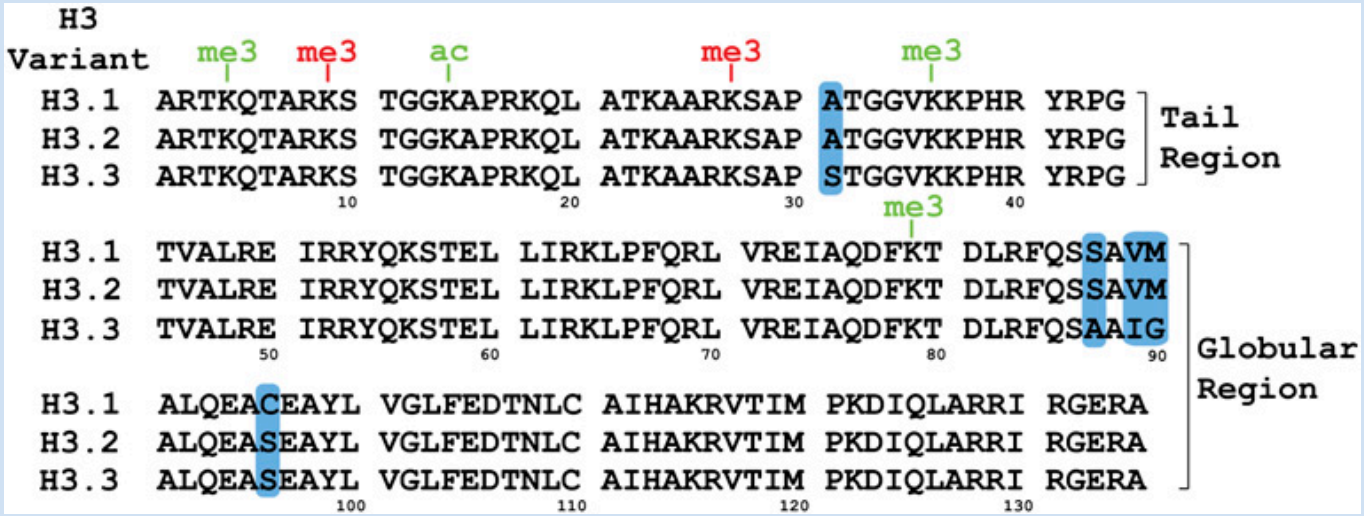
# Histone H3.3 summary

-Histone H3 variant, is associated with actively transcribed genes

-Non-S-phase (replication-independent) loading

-Previously reported chaperone: HIRA

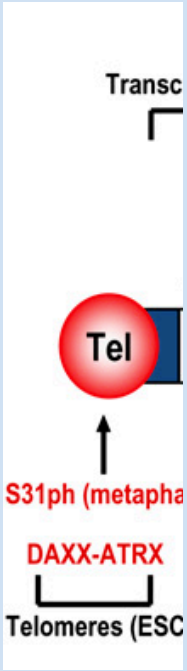
-Daxx/ATRX complex is a new chaperone of histone H3.3

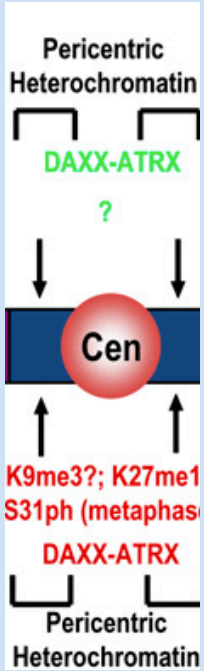



Sequence alignment of human H3 variants. Changes in amino acid composition are highlighted in blue

Campos and Reinberg, *Genes Dev.* 2010

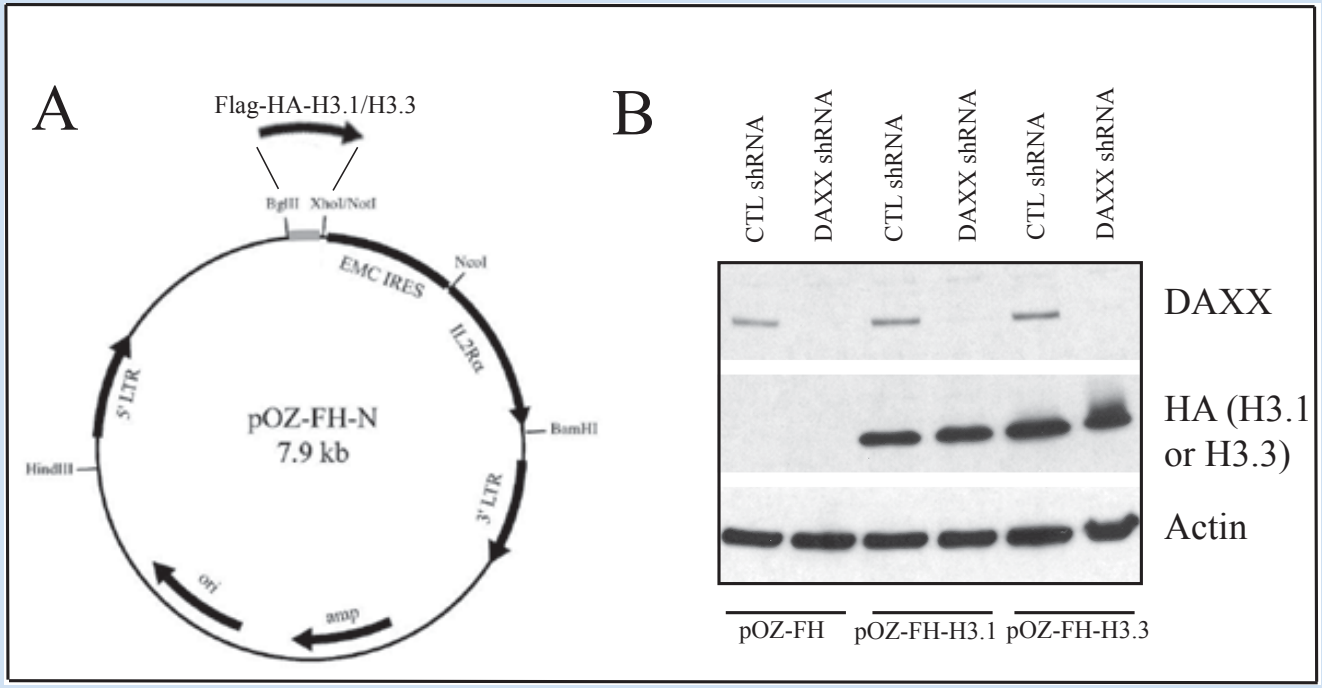
# Model of H3.3 deposition by histone chaperones HIRA and DAXX




Campos and Reinberg, *Genes Dev.* 2010

# Production and characterization of H3.3/H3.1 cell lines




A: Histones H3.3 and H3.1 were cloned in frame with FLAG/HA in pOZ vector (Nakatani Y, Ogryzko V. Methods Enzymol. 2003;370:430-44.)

B: HEp2 cells expressing H3.3 and H3.1 on the background of control shRNA or Daxx shRNA.

Western blot analysis for: Daxx (top), H3.3/H3.1 (middle); loading control: actin (bottom).

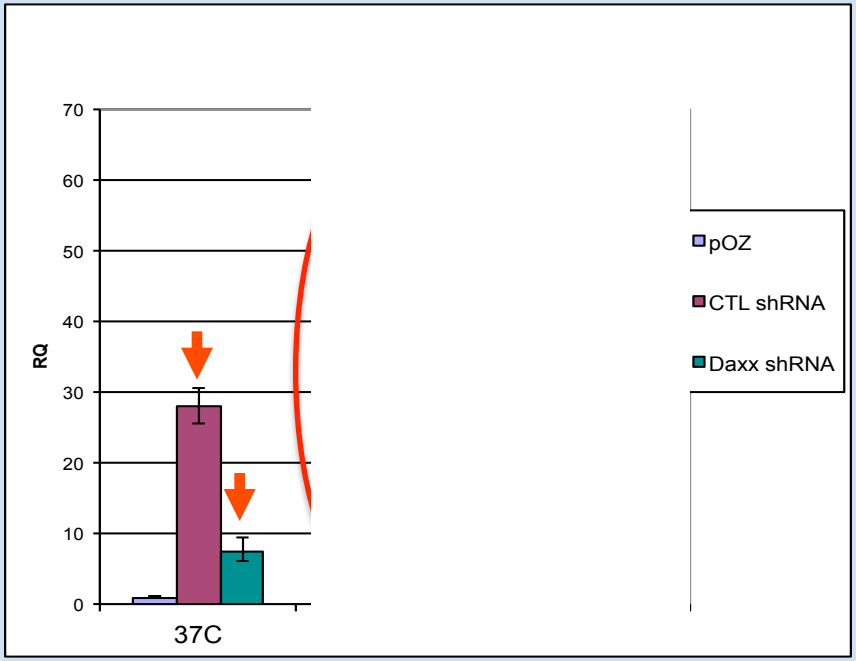
# Histone H3.3 association with CEN and periCEN

HEp2 cells expressing FLAG/HA-H3.3 and FLAG/HA-H3.1 on the sh-Daxx (Daxx shRNA) and sh-control (CTL shRNA) background

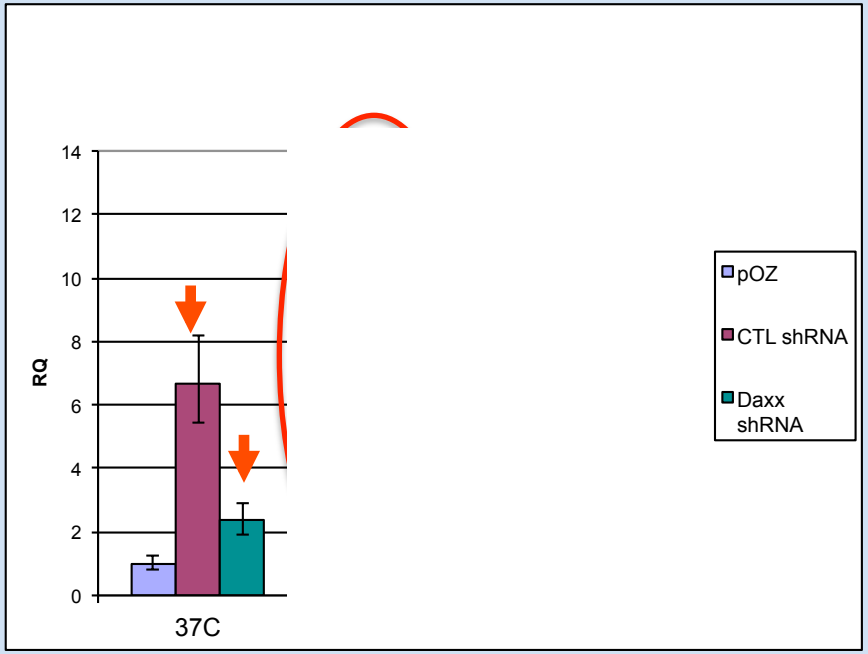
ChIP for H3.3 on CEN (pan-centromeric alpha-satellite) and periCEN (satellite 3-9)

pOZ: negative control for ChIP

## H3.3 at CEN




## H3.3 at periCEN




*H3.3 loading at CEN and periCEN is Daxx-dependent
*No obvious Daxx-dependent changes after HS…

Morozov et al., *Nucleus* 2012

# Function of Daxx at CEN/periCEN

1. Loading of H3.3 on repeats (both CEN/periCEN) in control conditions

2. As a result of this loading, increased transcription: at CEN in control, at periCEN in stress-induced condition

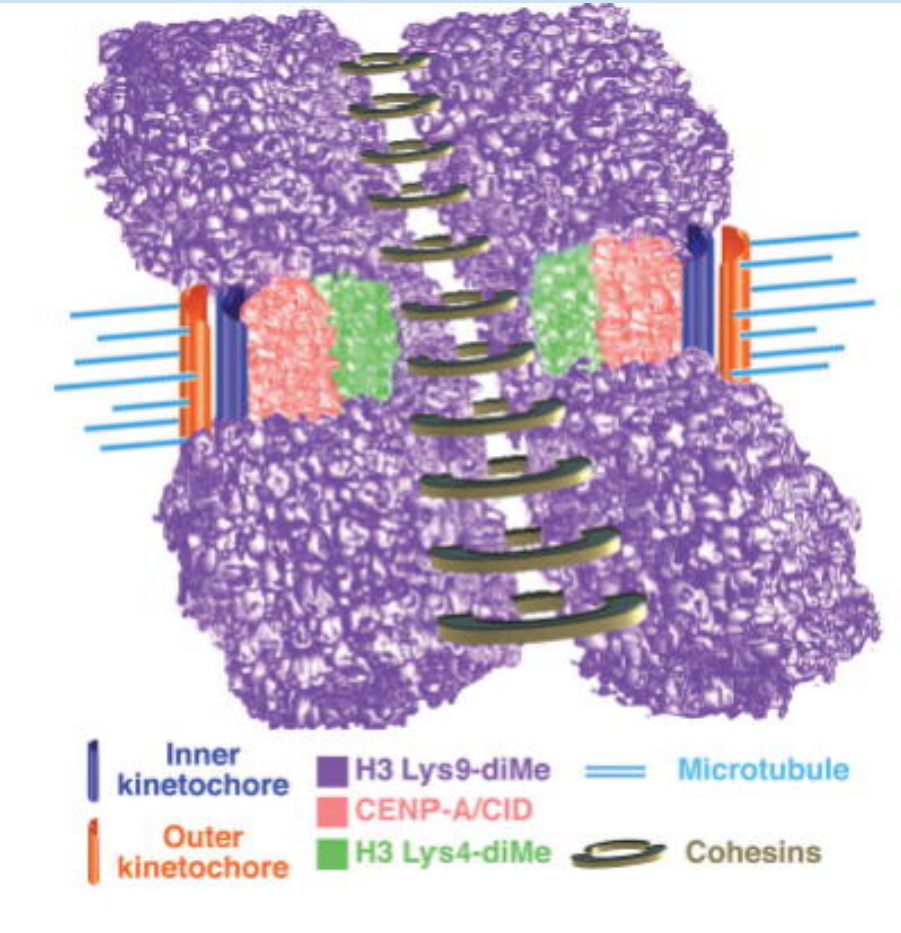
But: Daxx is mostly associated with CEN/periCEN UPON/AFTER stress, when levels of H3.3 does not change….

What is the function of Daxx hyper-accumulation at CEN/periCEN upon stress?

## Potential functions of Daxx at CEN/periCEN:

- Set up the cell cycle checkpoint for the stress recovery?
- Changes in the follow-up kinetochore assembly?
- Genome stability?
- Changes in chromatin structure and epigenetic modifications?
- Regulation of transcription at CEN/periCEN repeats?

# Model of 3-Dorganization of centromeric (CEN) and pericentromeric (periCEN) chromatin




CEN: interspersed CENP-A and H3 Lys4Me2 nucleosome may mediate formation of the 'cylindrical' three-dimensional centromere structures observed in metaphase chromosomes

periCEN: H3 Lys9Me2/3 chromatin, which recruits heterochromatin proteins (HP1) and cohesion proteins (RAD21/ SCC1)

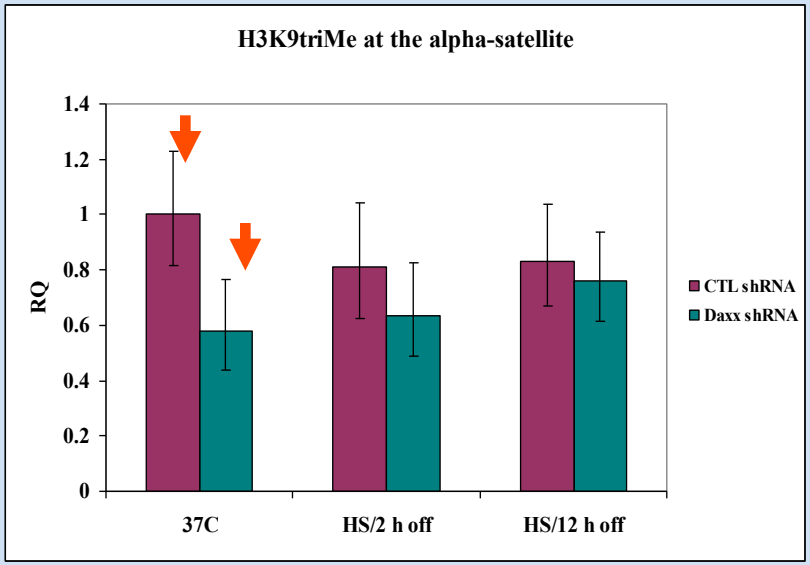
Sullivan and Karpen, *Nat Struct Mol Biol* 2004

# Epigenetic Modifications of chromatin: CEN

HEp2 cells expressing sh-Daxx (Daxx shRNA) and sh-control (CTL shRNA)

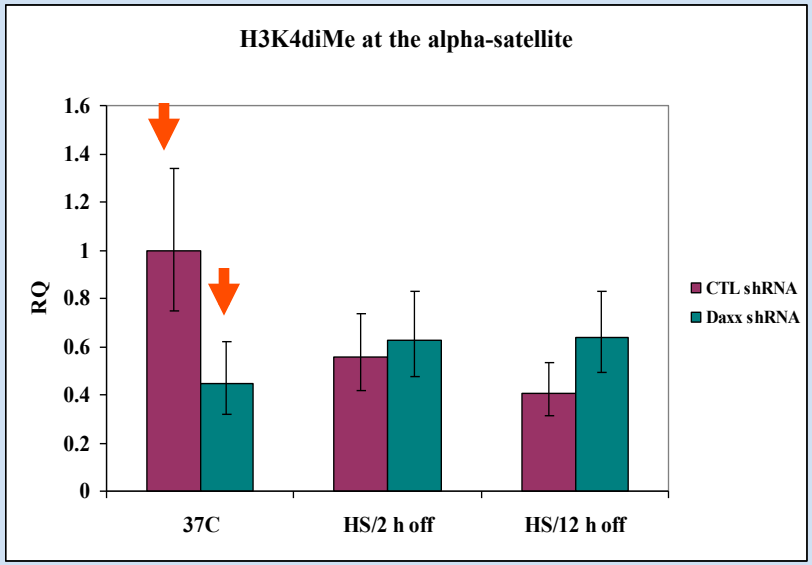
ChIP for H3K9tri-Me (repressive) and H3K4di-Me (active) on CEN

## Repressive: H3K9tri-Me




Daxx depletion: Reduction before stress

## Active: H3K4di-Me




Daxx depletion: Reduction before stress

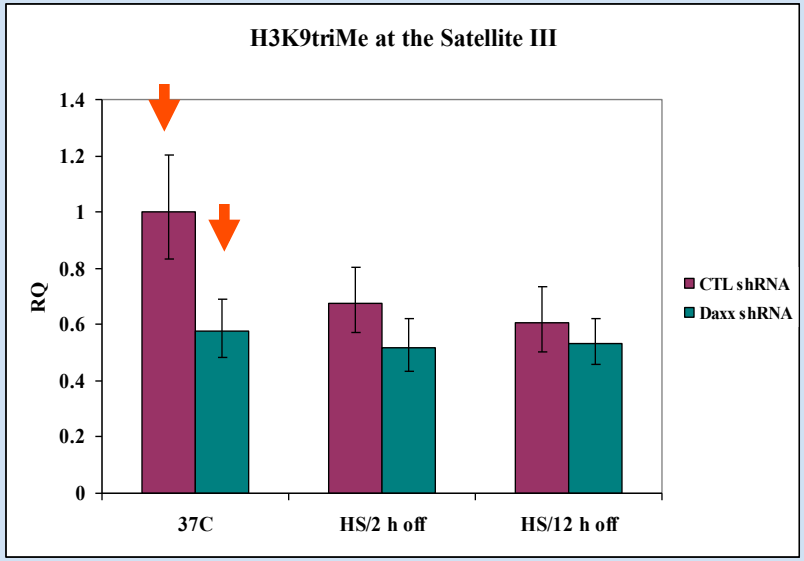
Morozov et al., *Nucleus* 2012

# Epigenetic Modifications of chromatin: periCEN

HEp2 cells expressing sh-Daxx (Daxx shRNA) and sh-control (CTL shRNA)

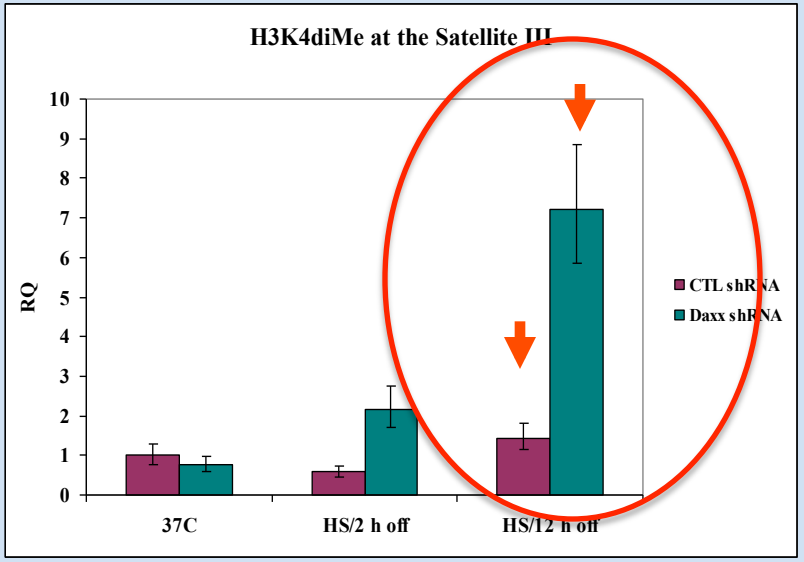
ChIP for H3K9tri-Me (repressive) and H3K4di-Me (active) on periCEN

## Repressive: H3K9 Me2/3




Daxx depletion: Reduction before stress

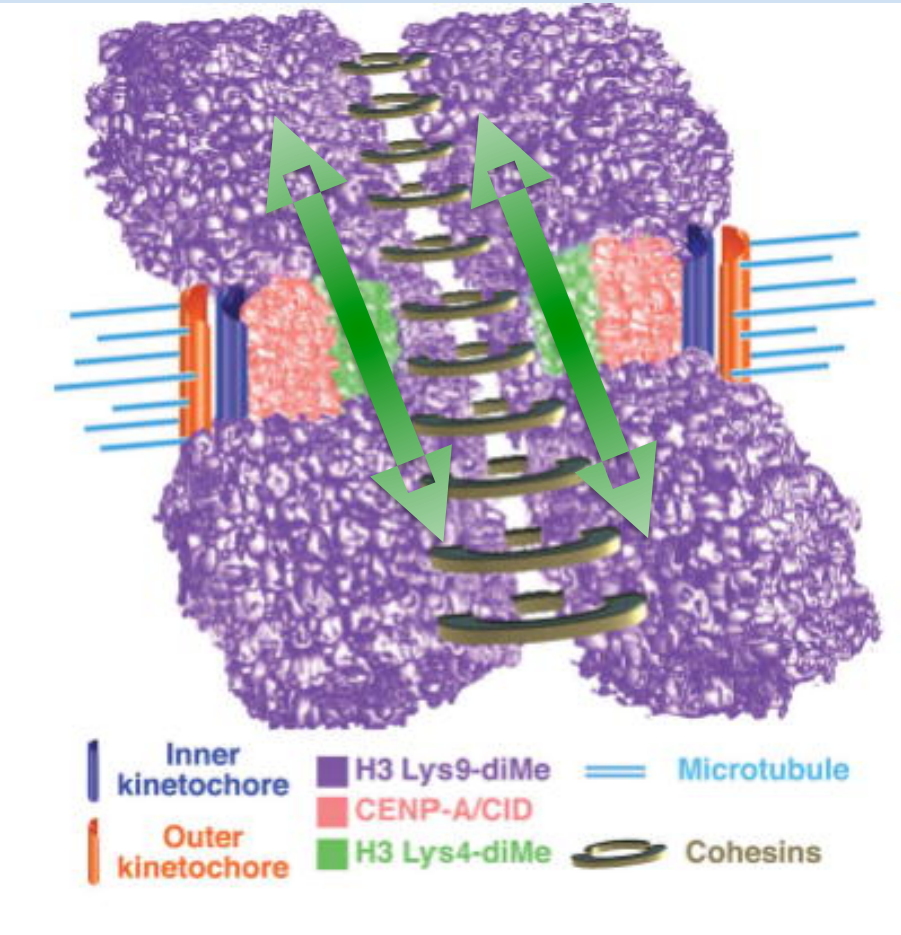
## Active: H3K4 Me2




Daxx depletion: Elevation after stress

Morozov et al., *Nucleus* 2012

# Model of 3-Dorganization of centromeric (CEN) and pericentromeric (periCEN) chromatin




From: Sullivan and Karpen, Nat Struct Mol Biol 2004

CEN: interspersed CENP-A and H3 Lys4Me2 nucleosome mediate formation of the 'cylindrical' three-dimensional centromere structures observed in metaphase chromosomes

periCEN: H3 Lys9Me2/3 chromatin, which recruits heterochromatin proteins (HP1) and cohesion proteins (RAD21/ SCC1)

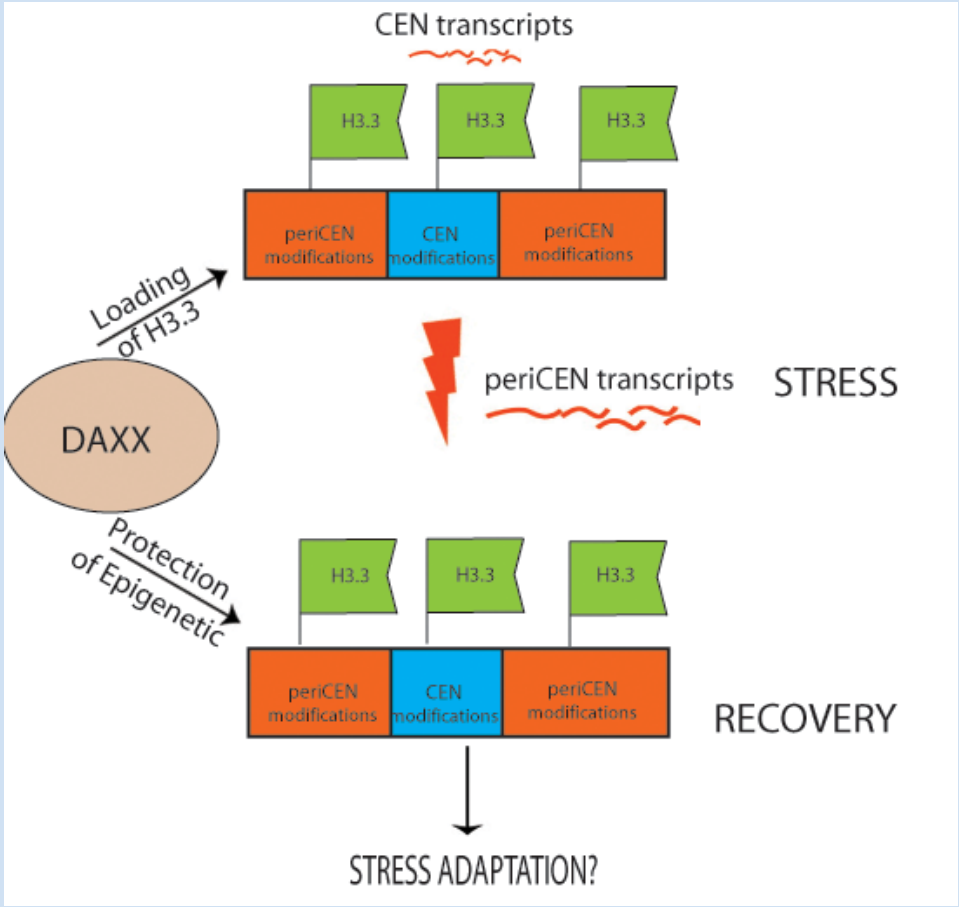
**No Daxx = increase of "active" chromatin modifications at periCEN after stress**

# Summary: Daxx in chromatin epigenetic signature maintenance at CEN/periCEN

1. Loading of H3.3 on repeats (both CEN/periCEN) in control conditions

2. As a result of this loading, increased transcription: at CEN in control, at periCEN in stress-induced condition

3. After stress, "epigenetic signature" protection via Daxx complex assembly at CEN/ periCEN. No Daxx = increase of "active" chromatin modifications at periCEN, changes in chromatin borders!




Model: Daxx as a guardian of epigenome (and, probably, genome?) stability

Morozov et al., *Nucleus* 2012

# Deposition of histone variant H3.3 at centromeres: Who, How, and Why do we care?

# Cancer connection: 2012+

Acta Neuropathol (2012) 124:439–447
DOI 10.1007/s00401-012-0998-0

ORIGINAL PAPER

**K27M mutation in histone H3.3 defines clinically and biologically**
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Cancer Cell
Article

Cell PRESS

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LETTER

doi:10.1038/nature10833

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Somatic histone H3 alterations in pediatric diffuse intrinsic pontine

**Anatomic Pathology** / *H3.3* Mutations in Pediatric Diffuse H

***H3F3A* K27M Mutation in Pediatric CNS Tumors**

A M

nature genetics

Distinct *H3F3A* and *H3F3B* driver mutations define chondroblastoma and giant cell tumor of bone

***DAXX/ATRX*, *MEN1*, and mTOR Pathway Genes Are Frequently Altered in Pancreatic Neuroendocrine Tumors**
Yuchen Jiao, *et al.*
*Science* **331**, 1199 (2011);
DOI: 10.1126/science.1200609

Cancer Cell
Review

# Histone H3.3 Mutations: A Variant Path to Cancer

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[2]Genome Center, University of California Davis School of Medicine, 451 Health Sciences Drive, Davis, CA 95616, USA
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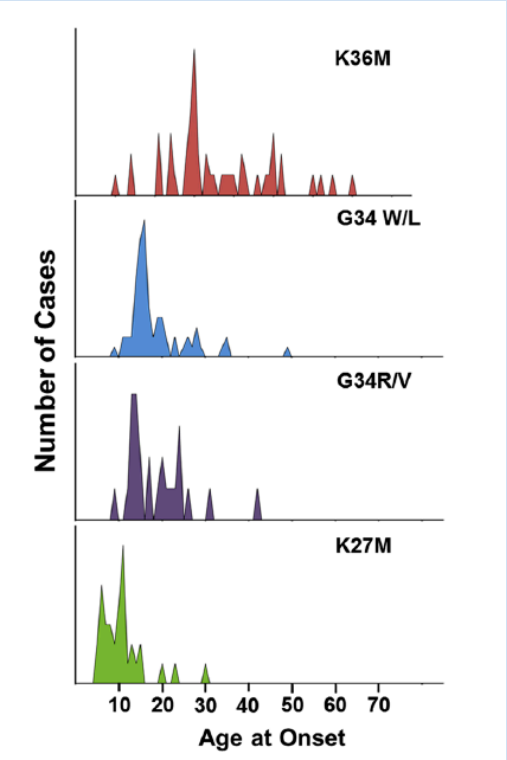
A host of cancer types exhibit aberrant histone modifications. Recently, distinct and recurrent mutations in a specific histone variant, histone H3.3, have been implicated in a high proportion of malignant pediatric brain cancers. The presence of mutant H3.3 histone disrupts epigenetic posttranslational modifications near genes involved in cancer processes and in brain function. Here, we review possible mechanisms by which mutant H3.3 histones may act to promote tumorigenesis. Furthermore, we discuss how perturbations in normal H3.3 chromatin-related and epigenetic functions may more broadly contribute to the formation of human cancers.

# H3.3 mutations and Cancer

| | | K27 | K36 | | G34 |
|---|---|---|---|---|---|
| H3.3 | *H3F3A* | AAG | AAG | | GGG |
| | *H3F3B* | AAA | AAG | | GGG |
| H3.1 | | AAG (6/10)<br>AAA (4/10) | AAG (8/10)<br>AAA (2/10) | | GGC (8/10)<br>GCT (2/10) |
| Mutation | Met | ATG | ATG | Val | GTG<br>GTC<br>GTT |
| | | | | Arg | CGG<br>CGA<br>CGT<br>CGC |
| | | | | Trp | TGG |
| | | | | Leu | TTG<br>TTA<br>CTT<br>CTC<br>CTA<br>CTG |

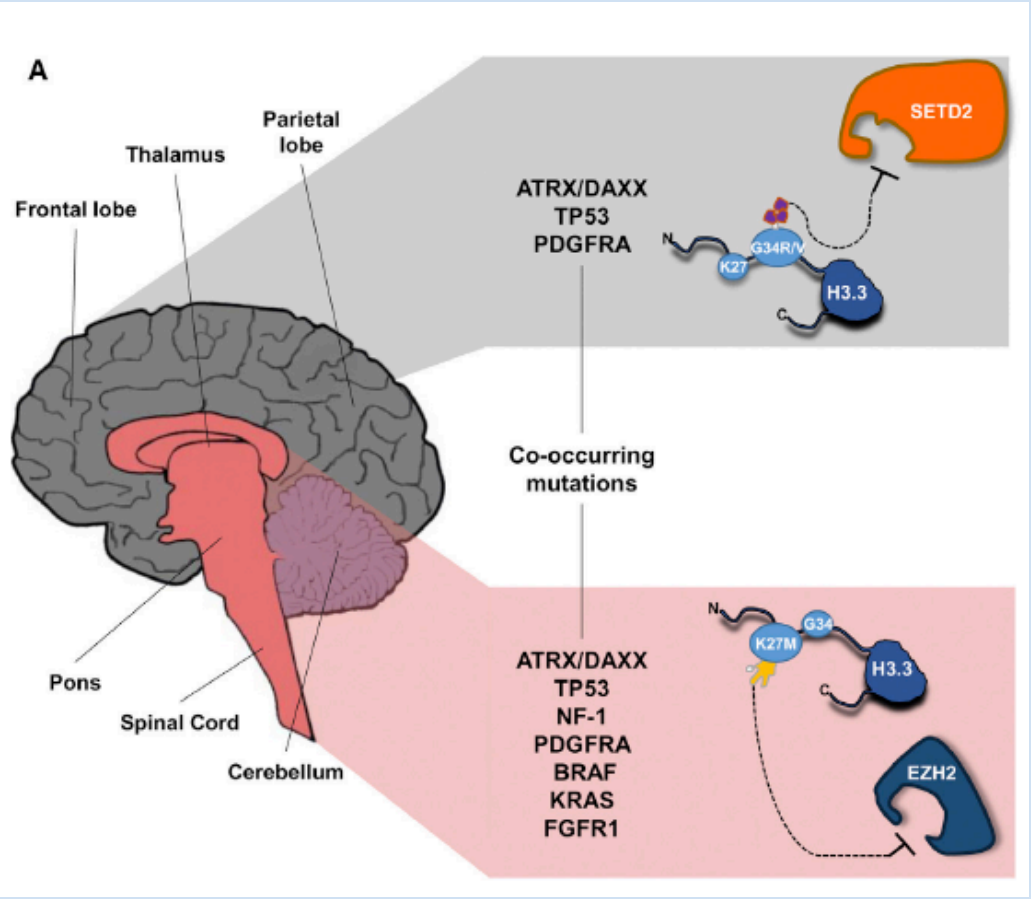
Codon usage in histone H3.3 and H3.1 genes at the sites of histone mutation




Age of presentation for histone H3 mutant tumors

Kallappagoudar et al., *Chromosoma* 2015

# Distribution and Characteristics of H3.3-Mutated Gliomas

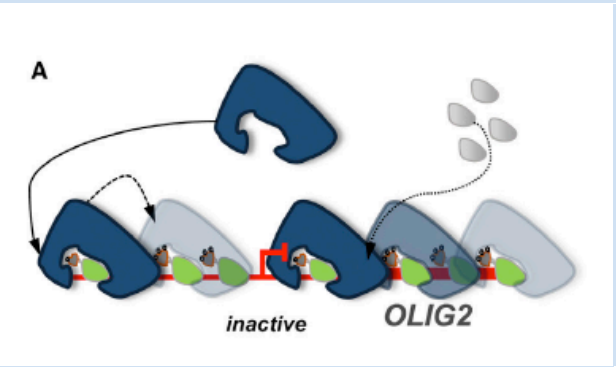


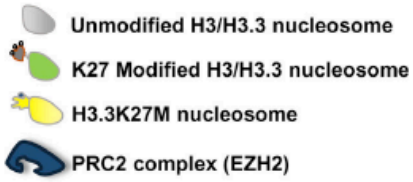

- G34R/V mutations (gray, top) localize to cerebral/cortical hemispheres, specifically in frontal, parietal, occipital, and temporal lobes.
- K27M mutations (pink, bottom) localize primarily to midline locations, including the spinal cord, thalamus, pons, and brainstem.
- G34R/V and K27M mutations overlap with mutations in TP53 and ATRX/DAXX.

Mutations in H3.3 directly (K27M) or indirectly (G34R/V) alter posttranslationally modified residues. G34R/V mutations appear to affect K36me3 levels, possibly through inhibition of the methyltransferase SETD2, while K27M mutations attenuate EZH2 methyltransferase function, decreasing global K27me3 levels.

Yuen and Knopfler *Cancer Cell* 2013

# H3.3 mutants: The Polycomb Connection




(A) "Wild type": H3.3K27M is not incorporated into the chromatin and thus does not inhibit PRC2 function. PRC2 trimethylates H3K27, spreading H3K27me3 marks throughout the promoter region and gene body, effectively repressing transcription.

Mutant H3.3K27M can interact with PRC2 to alter transcription in a number of possible scenarios:

(B) "Sequestered": H3.3K27M mimics K27 methylation and allosterically inhibits EZH2 methyltransferase function outside of chromatin, sequestering it in the nucleoplasm.

(C) "Trapped": In this scenario, H3.3K27M is incorporated into the promoter region. Binding of EZH2 to H3.3K27M blocks EZH2 on the same and nearby nucleosomes. Thus, this region is not properly silenced and does not recruit additional silencing factors to spread H3K27me3.

Yuen and Knopfler *Cancer Cell* 2013

# Histone variants barcoding: 2006

## Histone H3 variants and their potential role in indexing mammalian genomes: The "H3 barcode hypothesis"

Sandra B. Hake and C. David Allis*

…Here, we present a hypothesis, the "H3 barcode hypothesis."

…Our hypothesis rests on the central concept that mammalian histone H3 variants (H3.1, H3.2, and H3.3), although remarkably similar in amino acid sequence, exhibit distinct posttranslational "signatures" that create different chromosomal domains or territories, which, in turn, influence epigenetic states during cellular differentiation and development.

…Although we restrict our comments to H3 variants in mammals, we expect that the more general concepts presented here will apply to other histone variant families in organisms that employ them…

# Core histone variants, their functions, and features

| Histone | Biological function and features | Conservation |
|---|---|---|
| H3 variants | | |
| H3.3 | Gene activation, silencing, and chromosome segregation. Can be deposited in replication-independent way | Yes, but in yeast, it is the only noncentromeric H3 variant |
| CenpA | Epigenetic marker of centromere | Present in most of eukaryotes, but less conserved than other H3 histones |
| H3.X | Euchromatin in primates | Primate specific |
| H3.4/H3t | Sperm genome and nucleolus of somatic cells | Mammalian specific |
| H3.Y | Euchromatin in primates | Primate specific |
| H3.5/H3.3c | Euchromatin in hominid testis | Hominid specific |
| H2A variants | | |
| H2AZ | Poising genes for activation. Gene activation, gene silencing, and chromosome segregation | Present in most of eukaryotes |
| macroH2A | Association with repressed/silenced chromatin, large size due to an additional C-terminal domain | Vertebrate specific |
| H2A.BBD | Splicing, replication Active transcription | Mammalian specific |
| H2AX | Double-strand break repair/meiotic remodeling of sex chromosomes and genome integrity. The function is mediated by the phosphorylated form γH2A.X | Present in most of eukaryotes |
| H2B variants | | |
| TH2B | Chromatin to nucleoprotamine transition | |
| H2BFWT | Sperm telomere binding | Primate specific |
| H2BE | Transcription regulation in olfactory neurons | |

Evelyne et al., Aging Cell 2015

# Summary of human core histone variants and associated factors

| Histone | Chaperones and associated factors |
|---|---|
| H2A variants | |
| H2A.Z | CHZ1/NAP1/SWR1/INO80 |
| H2A.X | FACT |
| macroH2A | ATRX |
| H2A.B forms* | N.D. |
| H2B variants | |
| (TS) H2B.1* | N.D. |
| H2B.W* | N.D. |
| H3 variants | |
| CENP-A | HJURP |
| H3.3 | HIRA/ATRX/DAXX |
| (TS) H3.4* | N.D. |

Skene and Henikoff, *Development* 2013