Функция вариантов гистонов в гормонорезистентном раке предстательной железы: Что мы знаем и о чём (пока) только догадываемся

Alexander Ishov

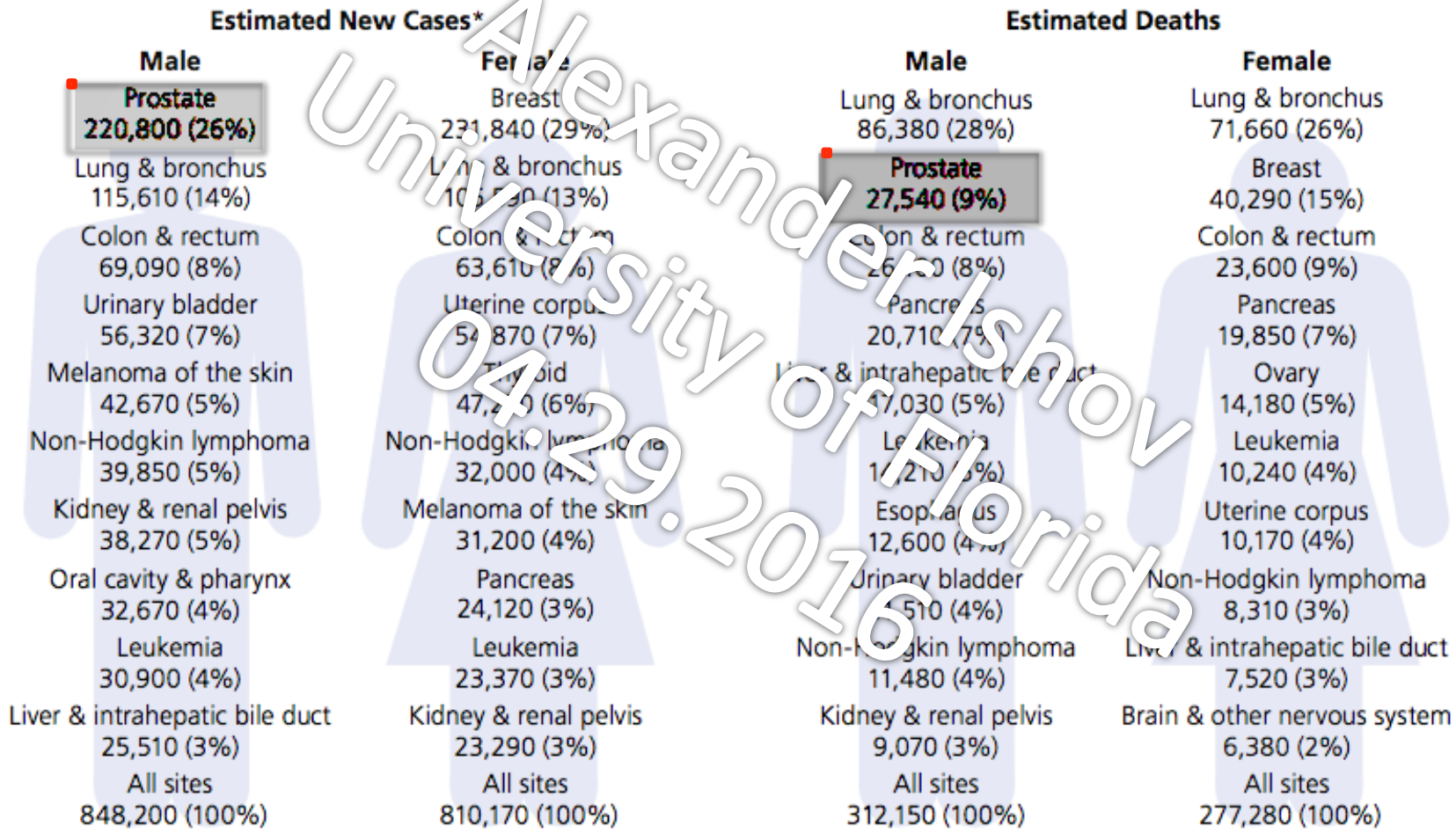
UF Health Cancer Center,
Department of Anatomy & Cell Biology
College of Medicine, University of Florida

Институт Цитологии РАН, 29 апреля 2016

Prostate cancer

- The most common non-skin cancer and the second leading cause of cancer death in men in the United States
- Androgen dependent activation of the androgen receptor (AR) for prostate-specific antigen (PSA) production and survival of normal and malignant prostate epithelial cells
- Standard treatment: androgen deprivation therapy (ADT) via surgical or medical (abiraterone, enzalutamide) castration (prevention of testosterone production)
- Disease progression after initial ADT, despite low levels of testosterone, is termed castration resistant prostate cancer (CRPC)

Leading Sites of New Cancer Cases and Deaths: 2015 Estimates



Excludes basal cell and squamous cell skin cancers and in situ carcinoma except urinary bladder.

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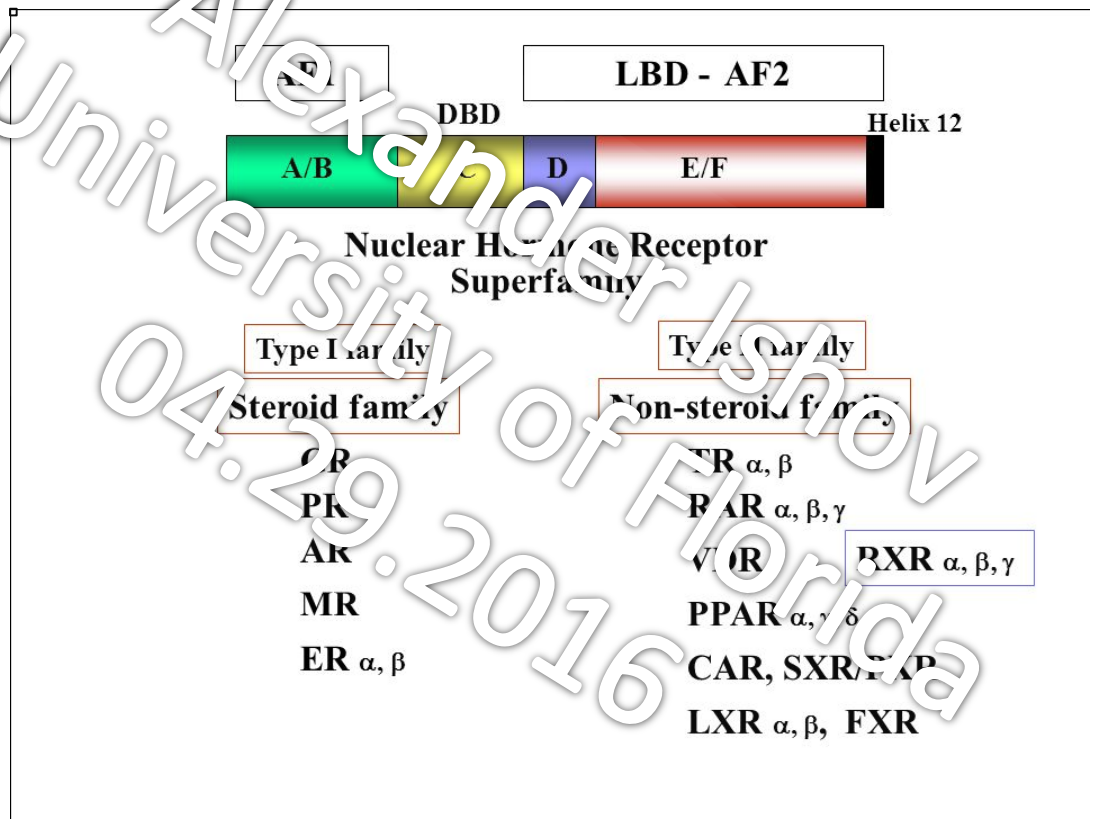
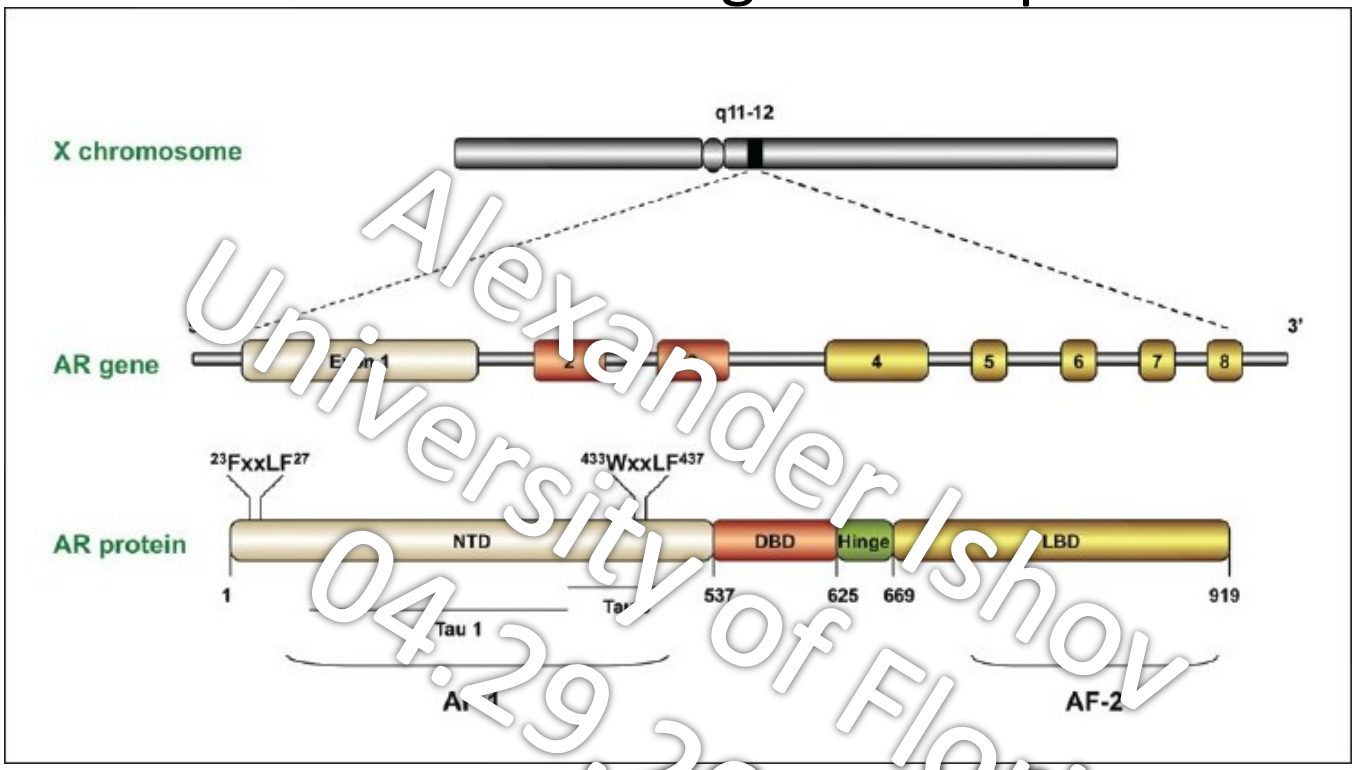
Androgen Receptor (AR)

The androgen receptor (AR) is critical for the normal growth and development of the prostate gland, and also in prostate carcinogenesis and progression to androgen-independent disease



AR is a transcription factor and a member of the **nuclear hormone receptor** superfamily, specifically **steroid hormone receptor** family of genes (that also includes ER, MR, GR, PR)

Structure of the AR gene and protein



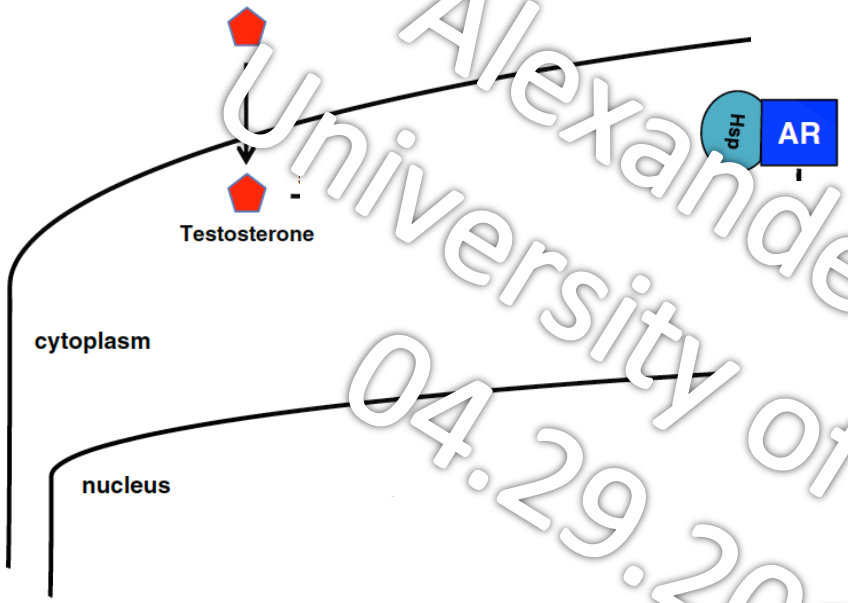
- AR gene is located on the X chromosome (q11-12) and consists of 8 exons. It codes for a protein of 919 amino acids with a mass of 110 kD.

- The AR consists of four structurally and functionally distinct domains:

✓ a poorly conserved **N-terminal domain (NTD)**
✓ a highly conserved **DNA-binding domain (DBD)**
✓ and a moderately conserved **ligand-binding domain (LBD)**
✓ The **'Hinge region'** separates the LBD from the DBD and also contains part of a bipartite ligand-dependent NLS for AR nuclear transport

Lonergan and Tindall, J Carcinog 2011

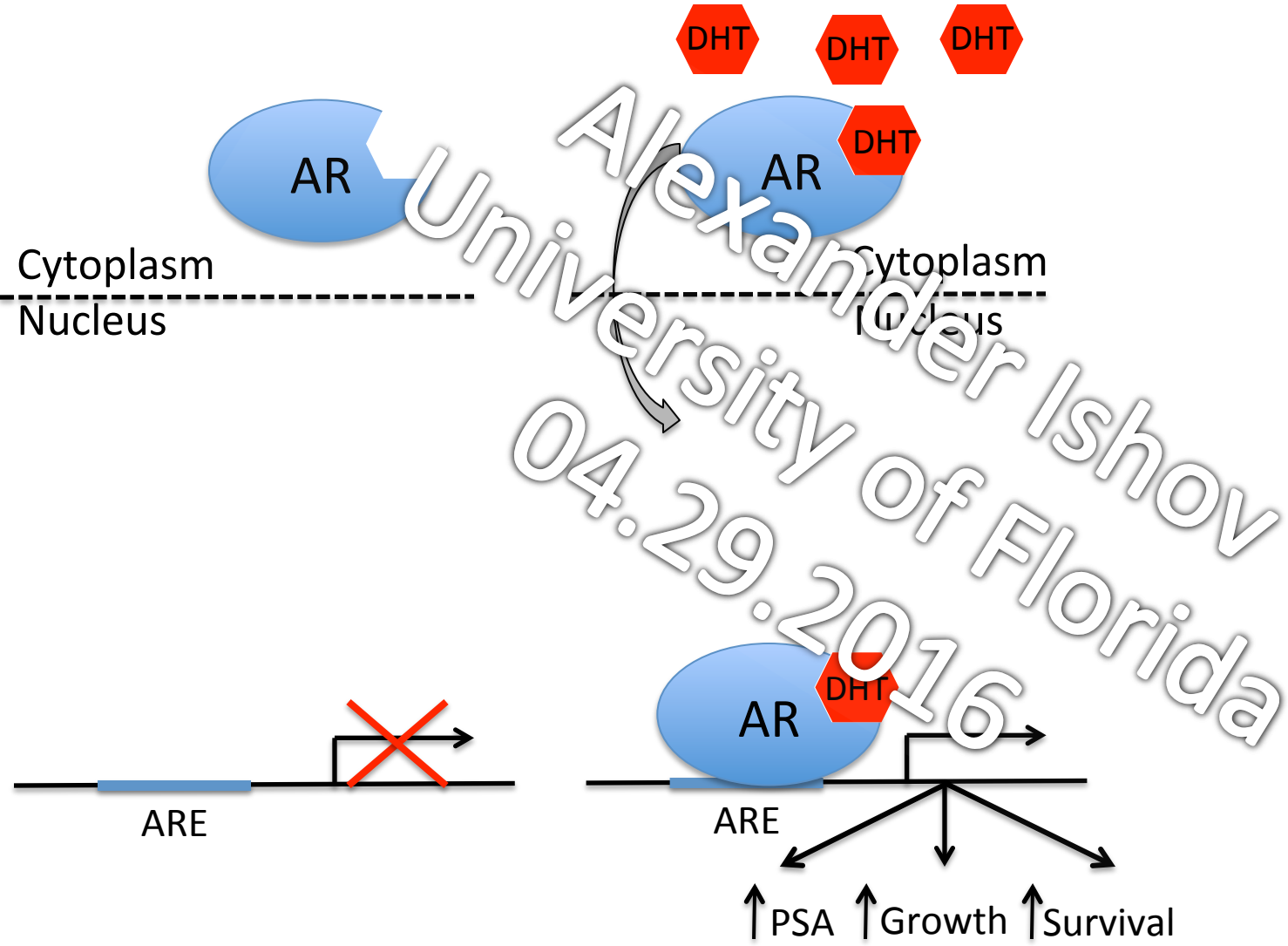
Mechanism of AR activation



- In the absence of hormone, AR is bound by chaperone heat shock proteins (Hsp's) in cytoplasm
- In the prostate, testosterone is converted to dihydrotestosterone (5α-DHT), ligand for AR
- 5α-DHT binding induces Hsp's dissociates from AR allowing AR to dimerize, translocate to the nucleus, bind to AREs (androgen response elements) and regulate gene transcription

Pharmacology & Therapeutics 140 (2013) 223–238

WT Androgen Receptor Activation



Omitted for simplicity: HSP-based cytoplasm retention, phosphorylation, dimerization of AR.

DHT: dihydrotestosterone

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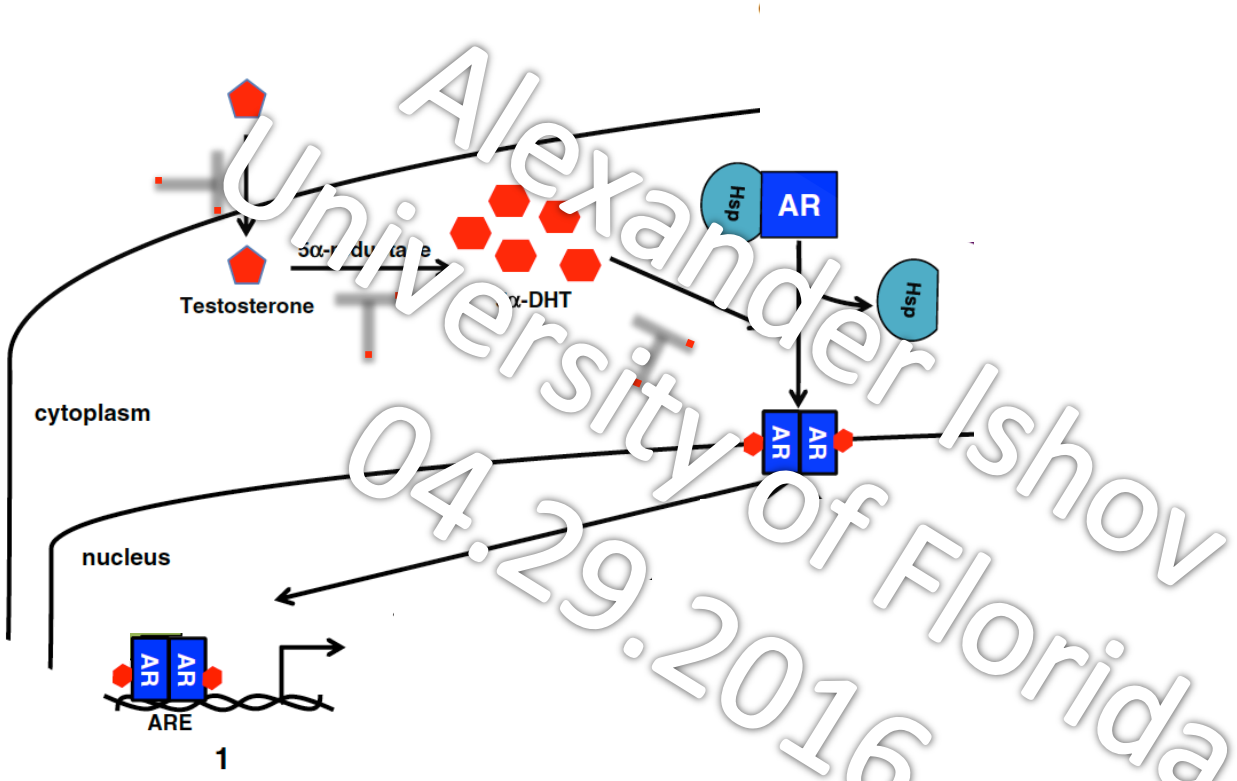

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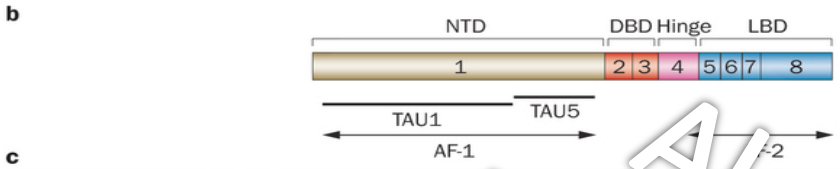
Mechanisms of persistent AR signaling activity in castration resistance prostate cancer (CRPC)

- Intracranial synthesis of androgens
- Polymorphism and/or overexpression of steroid transport
- Changes in co-regulatory molecules including co-activators and co-repressors that modulate AR stability and ligand sensitivity
- Activation of the AR complex via "cross-talk" with other signaling pathways
- Amplification and/or overexpression of AR
- AR mutations that can broaden ligand specificity
- Post-translational modifications of AR
- Constitutively active AR mutants and splice variants

Alternatively spliced AR isoforms identified in prostate cancer



c

AR variants	Alternative names	Transcripts	Tissue expression	Cell line expression	References
AR23		1 2 I2 3 4 5 6 7 8	CRPC	NA	23
ARQ640X		1 2 3 4	CRPC	NA	24
AR-V1	AR4	1 2 3 CE1	Benign, hormone-naive, CRPC	CWR-R1, 22Rv1, VCaP	16, 17
AR-V2		1 2 3 3 CE1	NA	22Rv1	17
AR-V3	AR1/2/2b, AR6	1 2 CE4 3 CE1	NA	22Rv1	15, 17
AR-V4	AR1/2/3/2b, AR5	1 2 3 CE4 3 CE1	NA	CWR-R1, 22Rv1	17
AR-V5		1 2 3 CE1	CRPC	22Rv1	17
AR-V6		1 2 3 CE2	CRPC	22Rv1	17
AR-V7	AR3	1 2 3 CE3	Benign, hormone-naive, CRPC	LNCaP, CWR-R1, C4-2, C4-2B, LNCaP 95, VCaP, CWR-22, 22Rv1	16, 17, 19
AR-V8		1 2 3 I3	NA	VCaP	19
AR-V9		1 2 3 CE5	CRPC	VCaP, 22Rv1	19, 21
AR-V10		1 2 3 I3	NA	VCaP	19
AR-V11		1 2 3 I3	NA	VCaP	19
AR-V12	AR^{V567es}	1 2 3 4 8	Benign, hormone-naive, CRPC	LuCaP86.2, LuCaP136, 22Rv1, VCaP	18, 21
AR-V13		1 2 3 4 5 6 9	CRPC	22Rv1	24
AR-V14		1 2 3 4 5 6 7 9	CRPC	22Rv1	24
AR-V15		1 2 3 4 5 6 9	NA	VCaP	25
AR-V16		1 2 3 4 5 6 7 8 9	NA	VCaP	25
AR-V18		1 2 3 4 5 6 9	NA	VCaP	25
AR8		1 I2 3 CE3	Benign, malignant	CWR-R1, C4-2, C4-2B, CWR22	22

a | A schematics of the AR gene

b | Wild-type AR structure; NTD (brown), DBD (red), hinge (pink) and the LBD (blue).

c | Alternatively spliced AR (AR-V) in prostate cancer. Transcripts of AR-V functional domains are shown in the same colors as for AR-WT. Novel exons are shown in yellow.

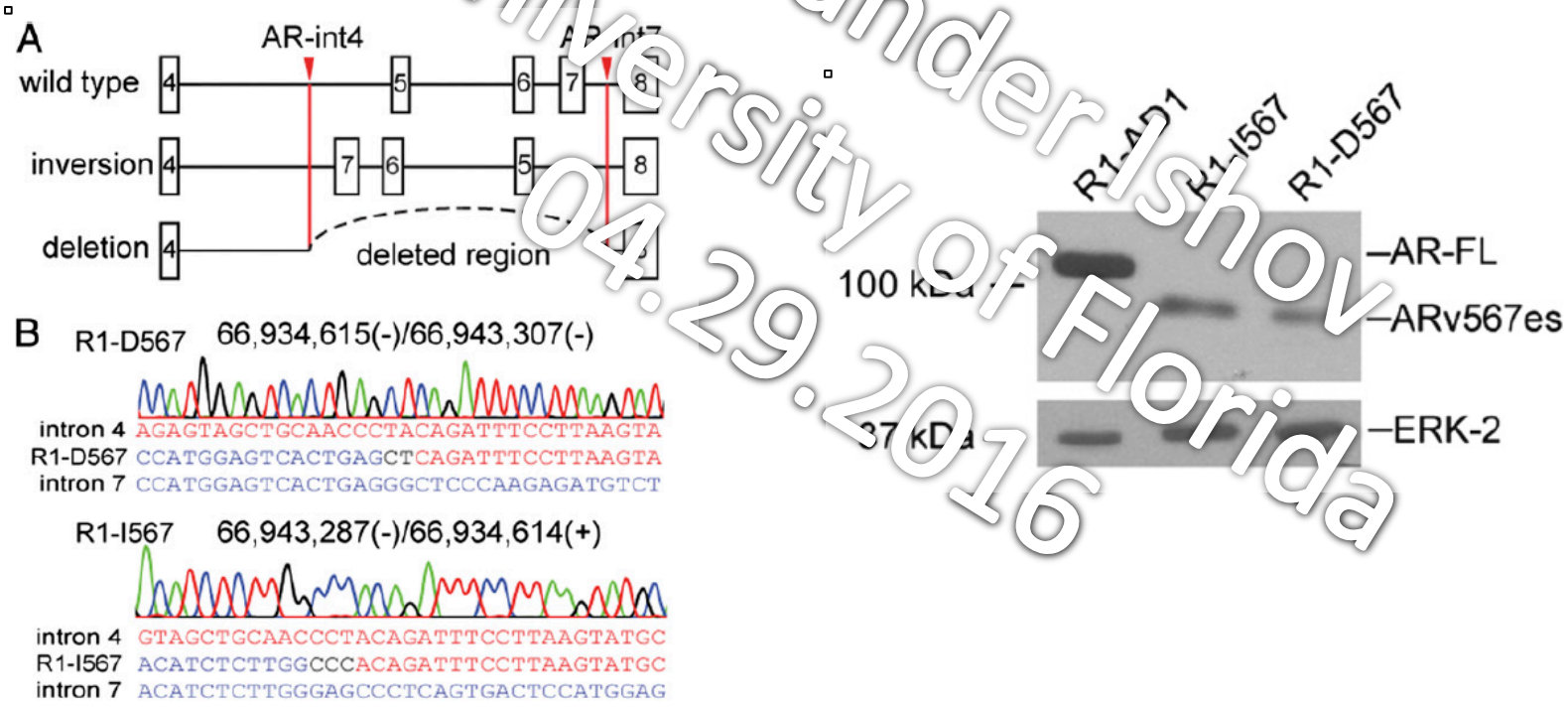
Abbreviations: AF, activation function; AR, androgen receptor; AR-FL, full-length androgen receptor; AR-V, androgen receptor variant; CE, cryptic exon; CRPC, castration-resistant prostate cancer; DBD, DNA-binding domain; I, intron; LBD, ligand-binding domain; NTD, N-terminal domain; TAU, transcription activation unit

(2015 *Nat. Rev. Urol.* doi:10.1038/nrurol.2015.13

Cell Line Model for CRPC?

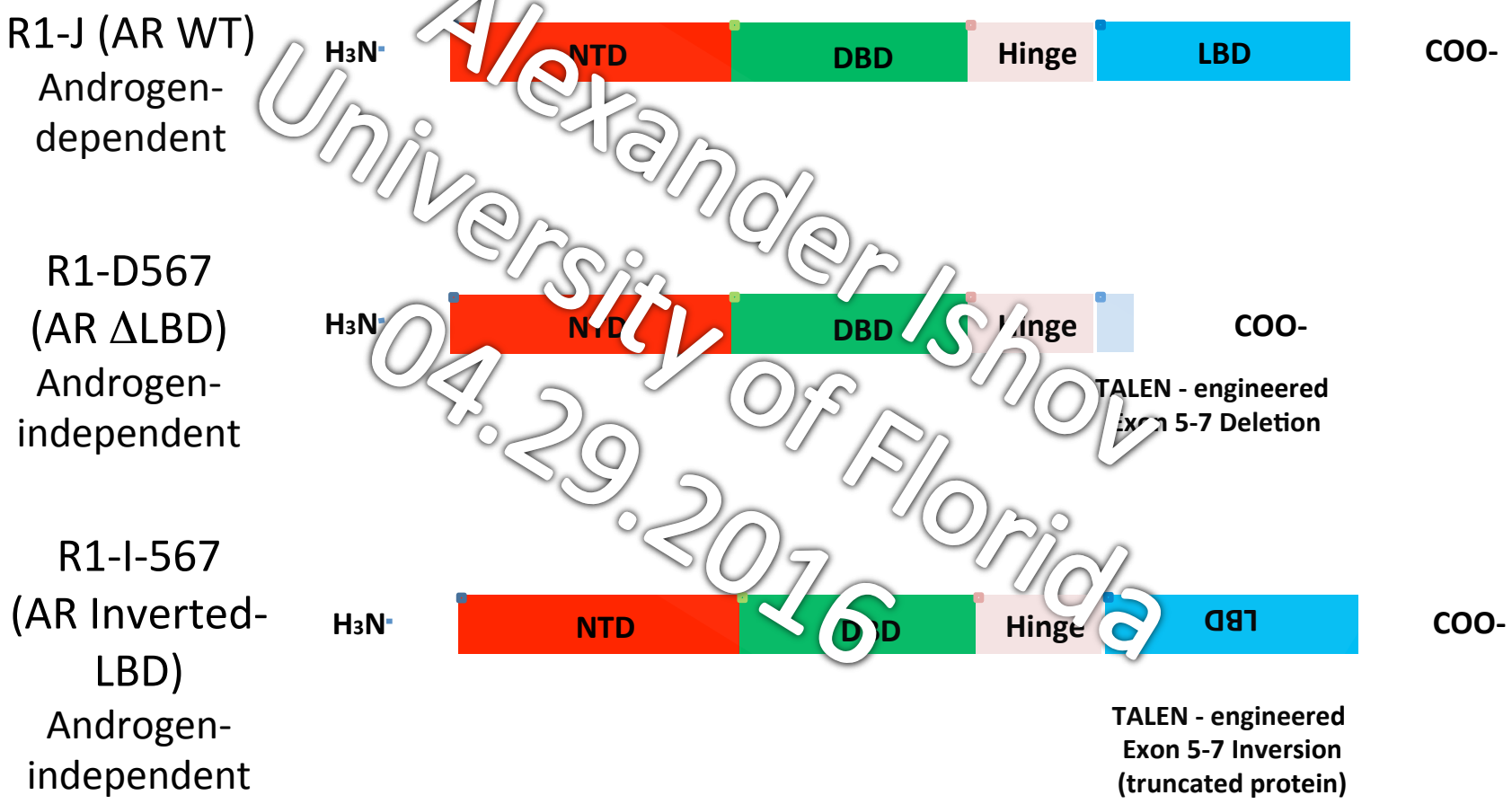
TALEN-engineered AR gene rearrangements reveal endocrine uncoupling of androgen receptor in prostate cancer

Michael D. Nyquist[a,b], Yinoming Li[a], Tae Hyun Hwang[c,1], Luke S. Manlove[a,b], Robert L. Vessella[d,e], Kevin A. T. Silverstein[a,c,f], Daniel F. Voytas[g,h], and Scott M. Dehm[a,i,2]



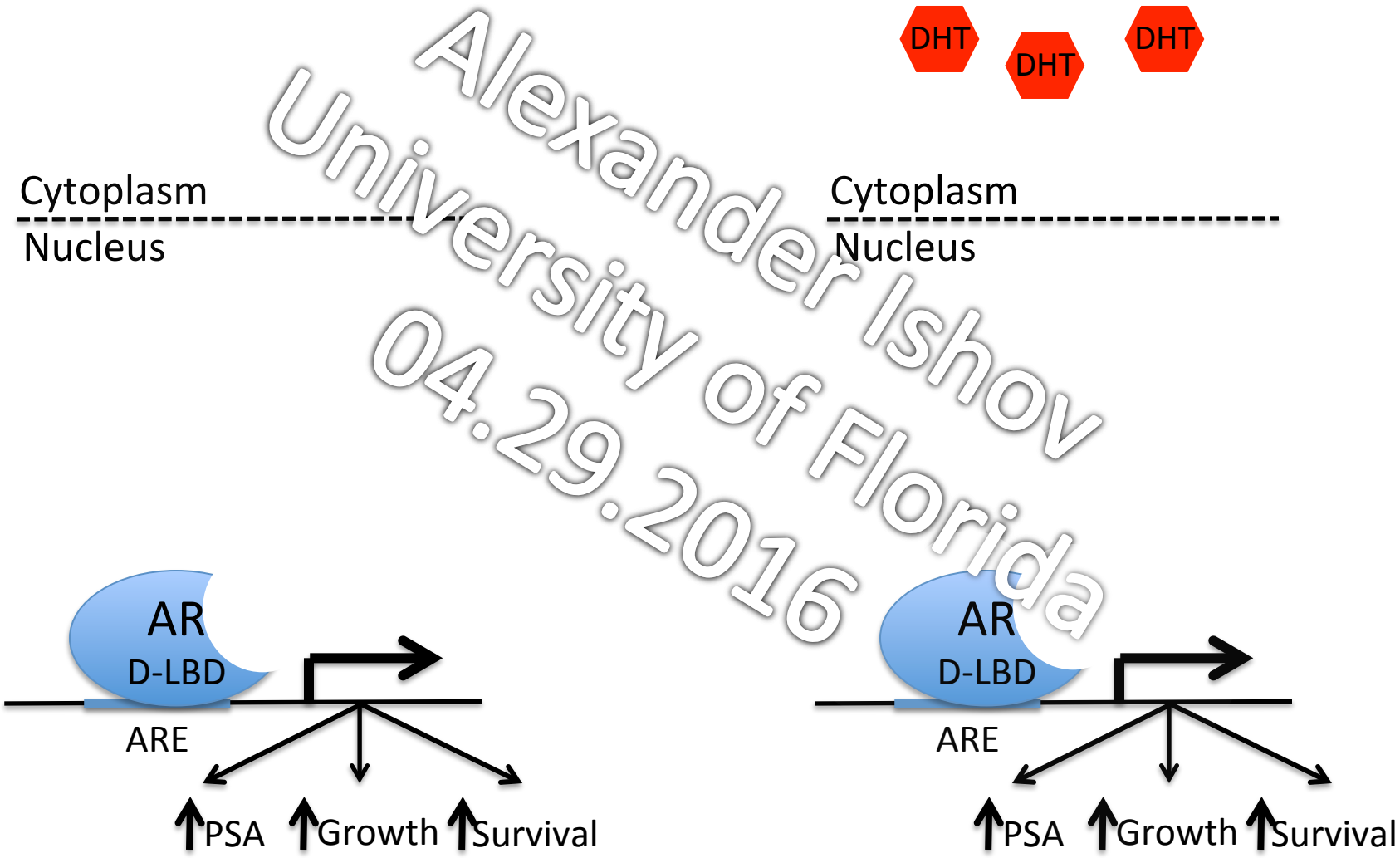
Characterization of parental R1-AD1 cells (single cell sub clone recurrent prostate cancer cell line CWR-R1 (AR wt) and genome-engineered R1-I567 and R1-D567 cells.

Prostate Cancer Cell Lines

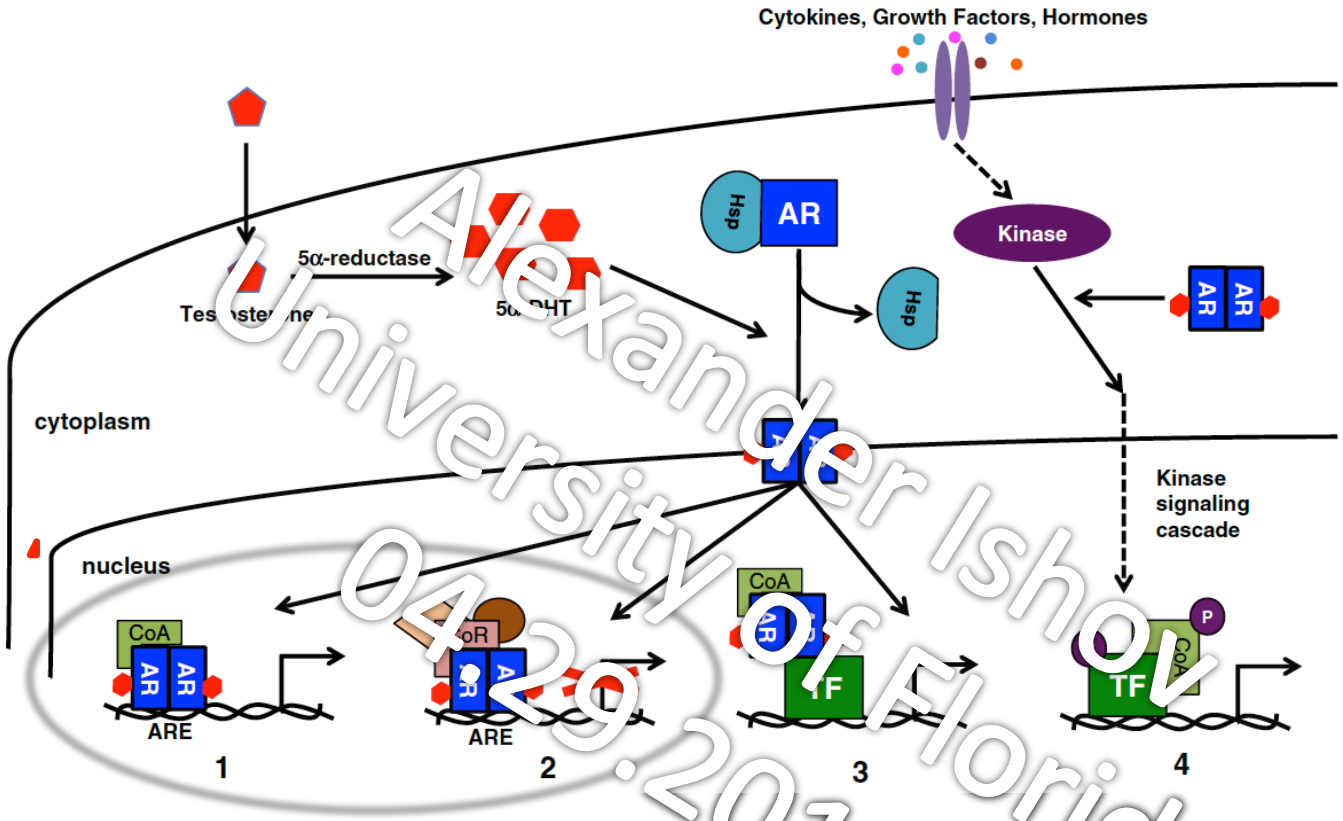


Lallous N et al. International journal of molecular sciences, 2013.
Nyquist M D et al. Proceedings of the National Academy of Sciences, 2013.
Dagvadorj A et al. Clinical Cancer Research, 2008.

Androgen Receptor deleted for Ligand Binding Domain (AR D-LBD)



Mechanism of AR action



(1) AR recruits co-activator complexes (i.e. CBP/p300) to remodel chromatin for transcription.

(2) AR, through a poorly defined mechanism that may also recruit co-repressors, also can inhibit transcription.

(3) AR also regulates transcription through a tethering mechanism by binding to other transcription factors (TFs) and additional coactivators to target genes.

Search for AR co-activators and co-repressors

(4) In addition, AR can initiate extranuclear signaling. Activated, hormone bound AR can interact with kinases (i.e. AKT, SRC) activating a kinase cascade. The signaling cascade can activate other TFs in the absence of direct AR binding to the gene.

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AR and Protein Daxx

MOLECULAR AND CELLULAR BIOLOGY, Dec. 2004, p. 10529–10541 Vol. 24, No. 24
0270-7306/04/$08.00+0 DOI: 10.1128/MCB.24.24.10529–10541.2004

Negative Modulation of Androgen Receptor Transcriptional Activity by Daxx

Ding-Yen Lin,[1,2] Hsin-I Fang,[1] Ai-Hong Ma,[3] Yu-Sung Huang,[1,2] Yeong-Shiau Pu,[4] Guido Jenster,[5] Hsing-Jien Kung,[3] and Hsiu-Ming Shih[1,2]*

ELSEVIER

Cell Biology International 28 (2004) 709–714

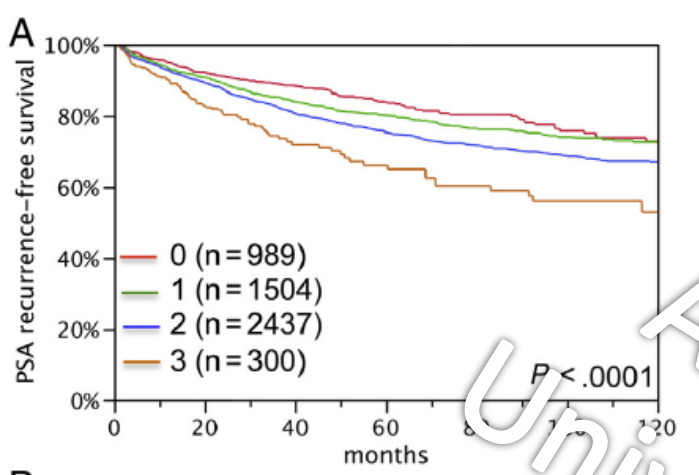
Cell Biology International

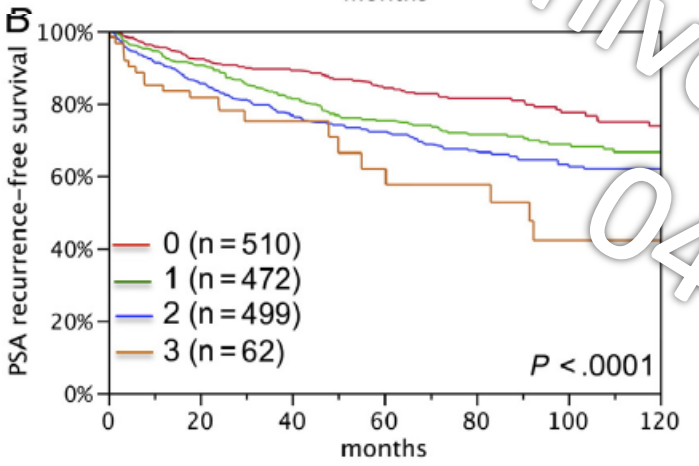
www.elsevier.com/locate/cellbi

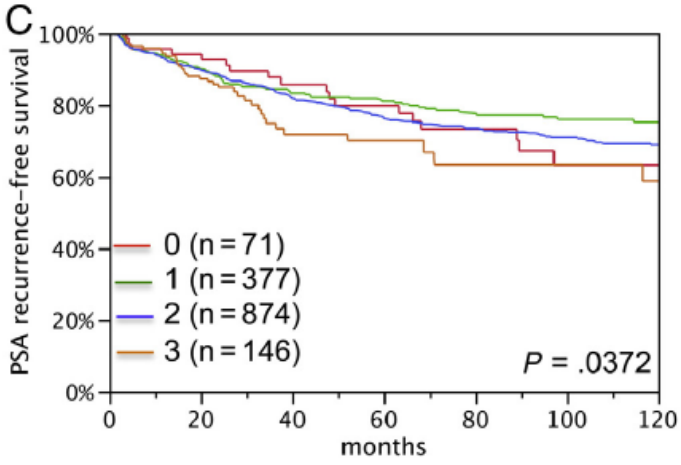
Cloning and functional characterization of a rat Daxx that functions as a corepressor for the androgen receptor

Haruo Mizuta*, Yasuo Kuroda

Daxx expression in prostate cancer







Daxx expression was analyzed by immunohistochemistry on a tissue microarray containing 7478 prostate cancer specimens.

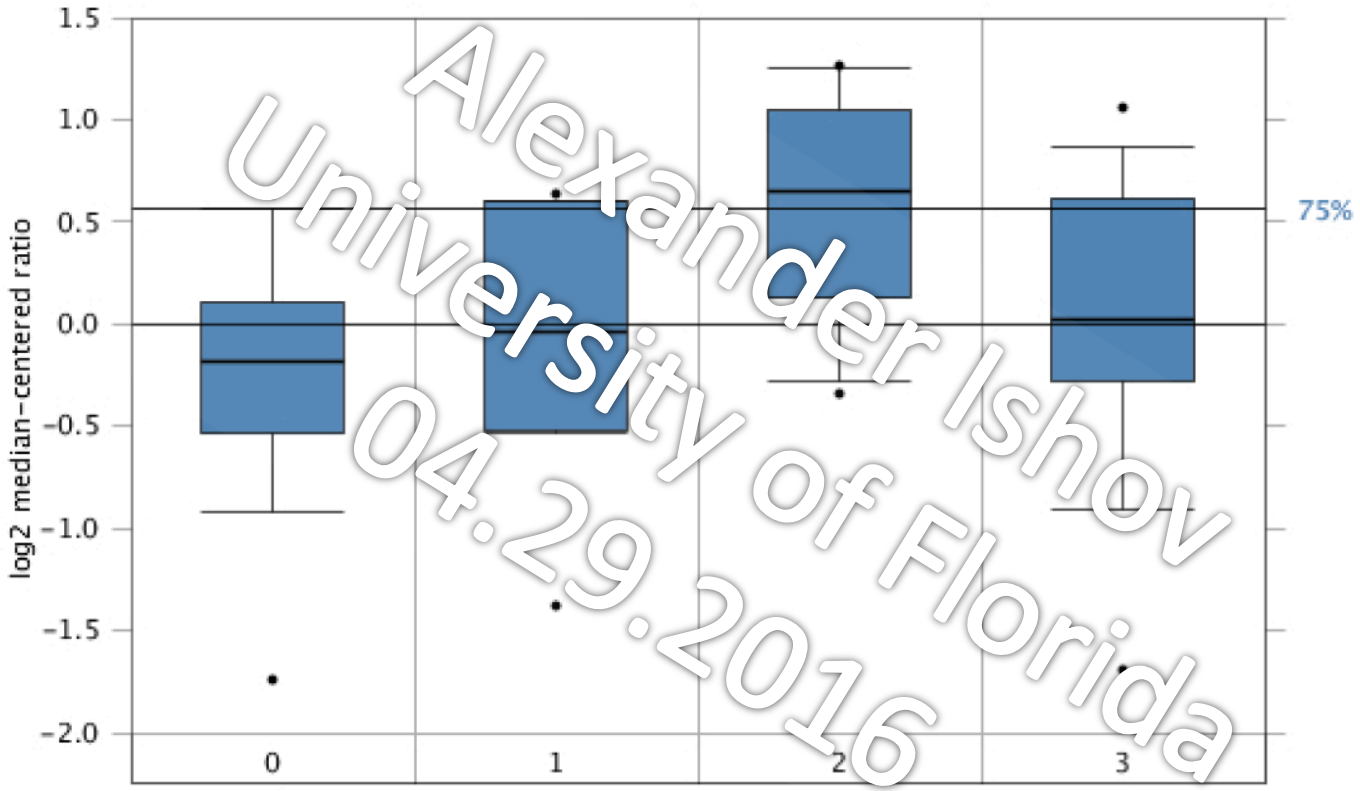
Association of Daxx expression with biochemical recurrence.
A, All prostate cancers (n = 5230).
B, ERG-negative prostate cancers (n = 1543).
C, ERG-positive prostate cancers (n = 1468).

Overexpression of Daxx in prostate cancer is an independent predictor of early prostate-specific antigen recurrence, mostly in ERG-negative prostate cancer.

Hum Pathol. 2013 Sep;44(9)

Daxx Expression in Prostate Cancer

(from https://www.oncomine.org)



0 = No value (27)
1 = Benign Prostatic Hyperplasia (11)
2 = Prostatic Intraepithelial Neoplasia (13)
3 = Prostate Carcinoma (49)

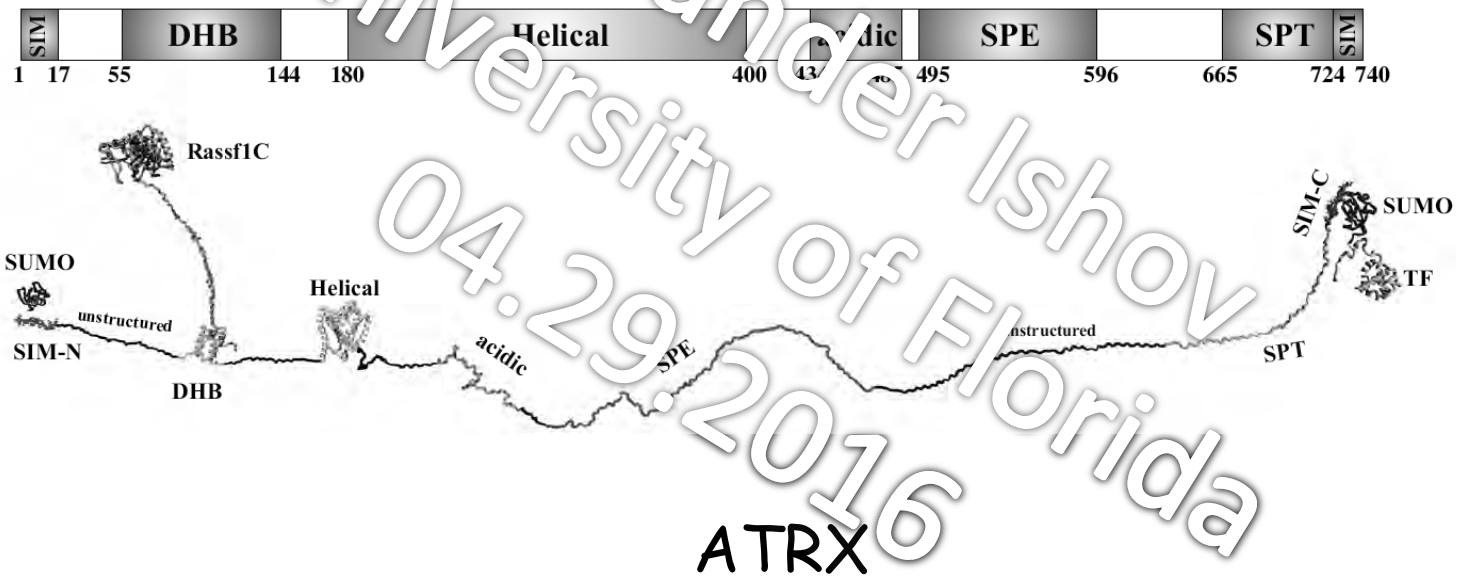
From: Nat Genet. 2007 39(1):41-5
101 samples
10,656 measured genes

Daxx

Daxx is ND10/PML body associated protein (Ishov et al., 1999) essential for embryonic development (Ishov et al., 2004)

Daxx is implicated in apoptosis, transcriptional regulation (Morozov et al 2008) and mitosis/ PTX resistance (Lindsay et al., 2007, Gi[illegible]zi[illegible] et al., 2012 Giovinazzi et al., 2013)

Daxx contains several conserved domains including two SUMO-interacting motifs (SIMs) (Santiago A. et al., 2009)



ATRX

ATRX: alpha thalassemia/mental retardation syndrome X-linked mutated

SWI/SNF2 family of helicase/ATPases with chromatin remodeling activity

ATRX interacts with Daxx and is targeted to PML-NB's by Daxx

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
Royal Children'
Centre, East M
Victoria 3052,
Melbourne, Roy

20:351–360 © 201

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

Pascal Drané,

IGMBC (Institut
F-67404, France;

The histone va
investigate the
associated prot
protein) are spe
binding prefere
DAXX is a H3.
replication-dep
in these cells c
associates with
H3.3 nucleoso

DNA repeat ch
chaperone invo

GENES & DE

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-M[illegible] Noh, Samuel 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)

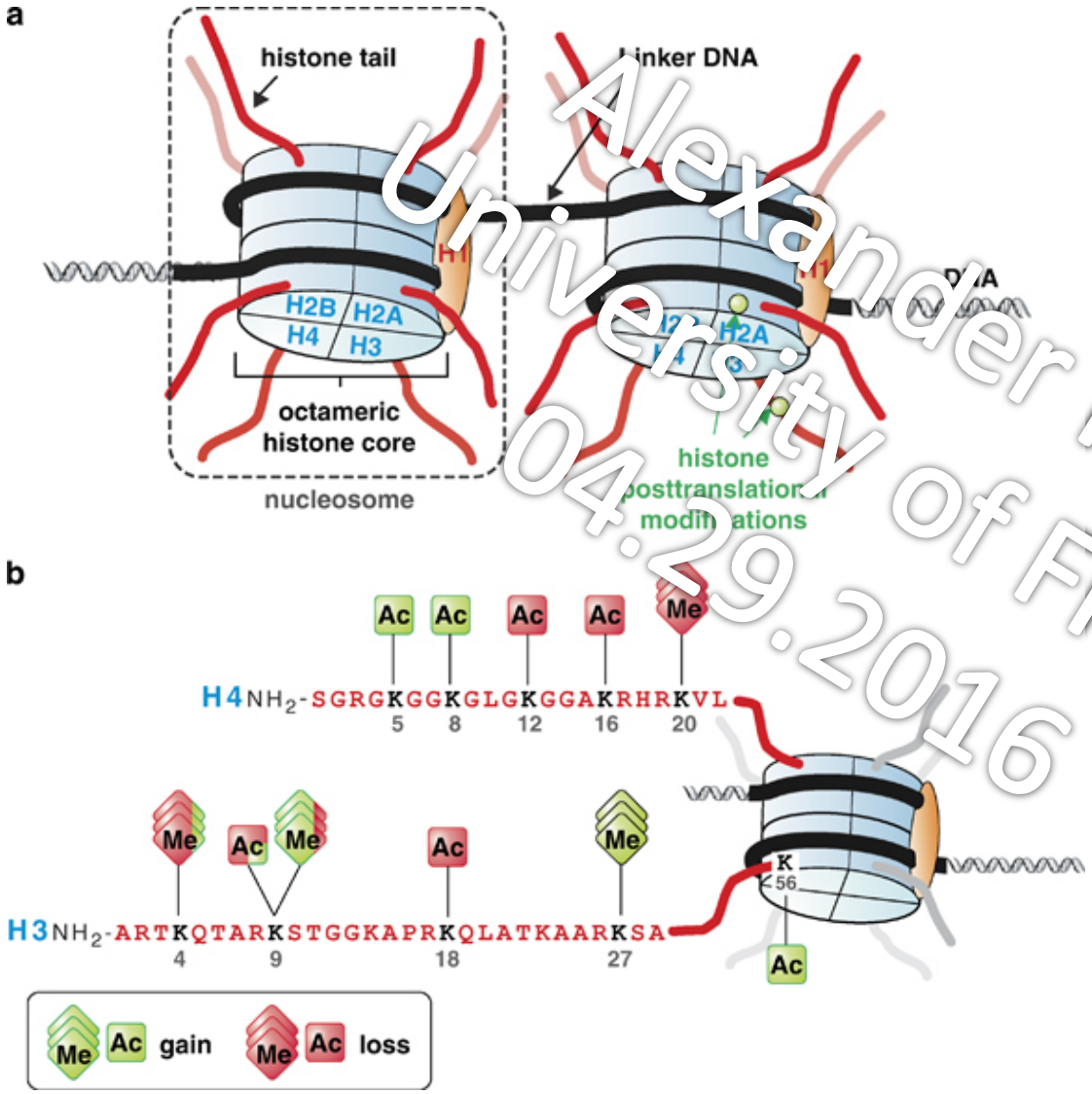
The histone variant H3.3 is implicated in the formation and maintenance of specialized chromatin structure in embryonic stem cells. H3.3-containing nucleosomes are assembled in a replication-independent manner by means of dedicated chaperone proteins. We previously identified the death domain associated protein (Daxx) and the α-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

Chromatin Structure and Histone Modifications

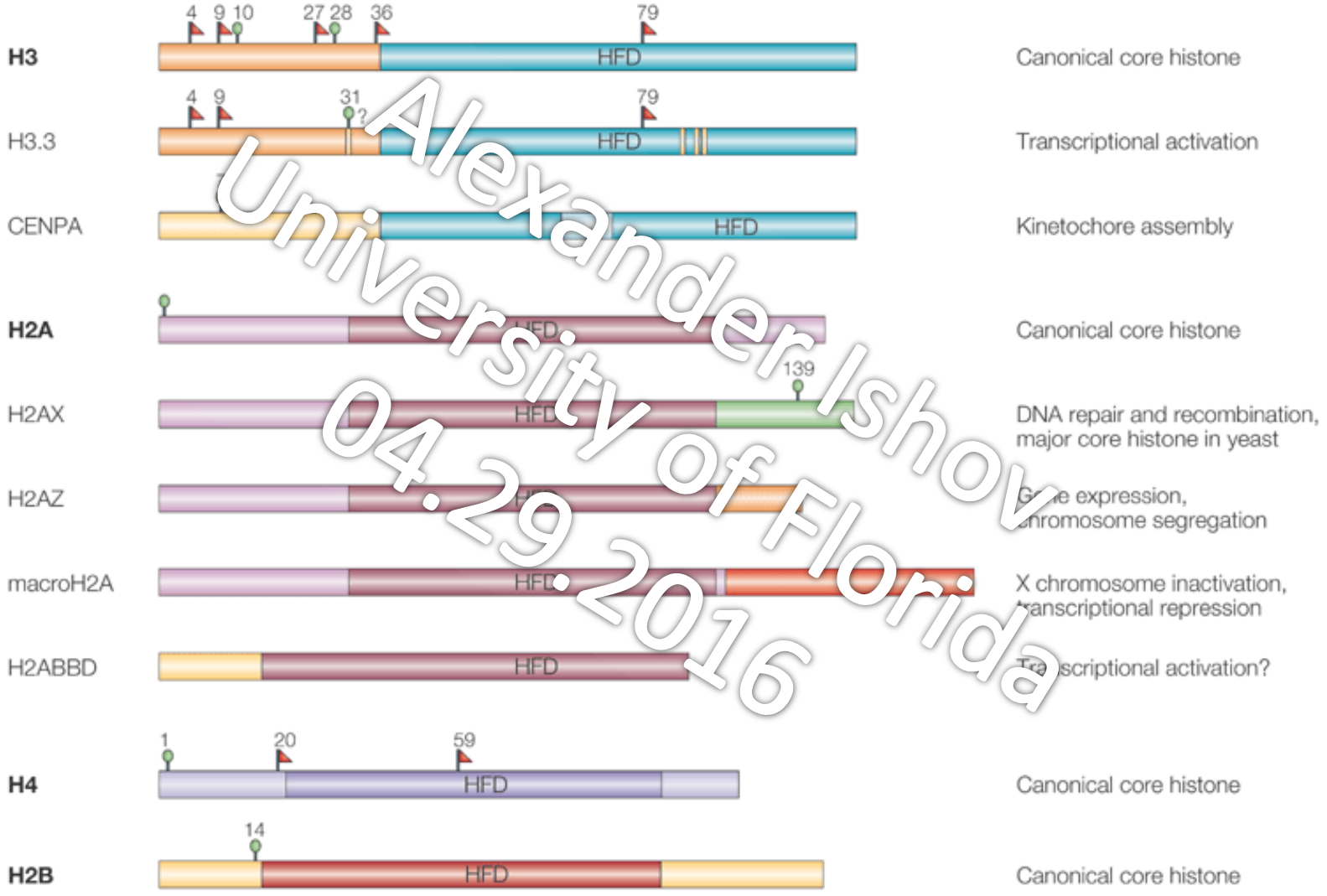


(a) Chromatin is made of repeating units of nucleosomes, which consist of ~147 base pairs of DNA wrapped around a histone octamer consisting of two copies each of the core histones H2A, H2B, H3 and H4. Linker histone H1 is stabilizing higher order chromatin structure.

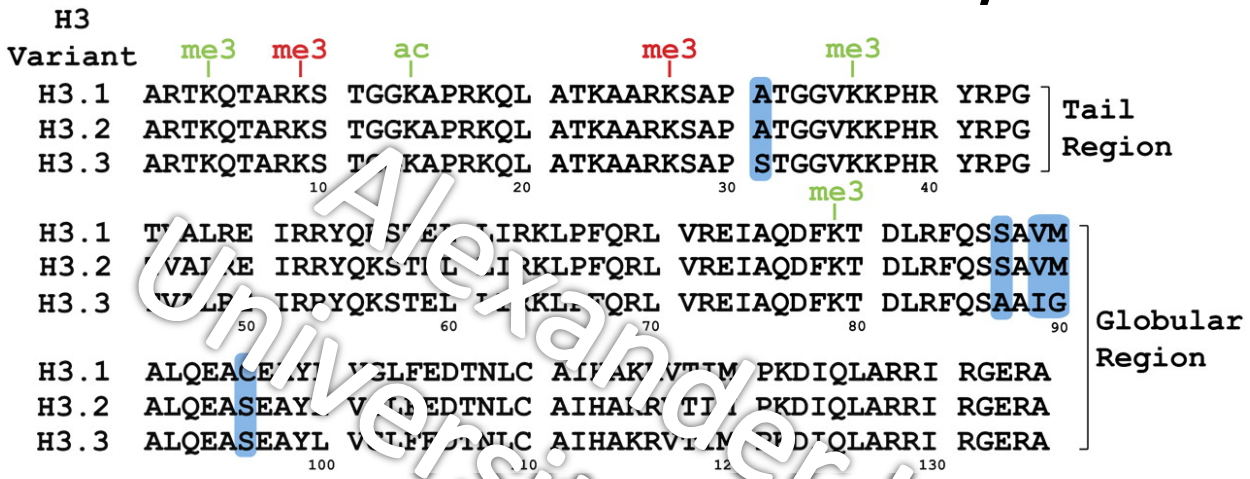
(b) The histones are subject to a variety of post-translational modifications, primarily on their N-terminal tails.

Füllgrabe et al., Oncogene 2011

Histone Variants



Histone H3 variants in eukaryotic cells



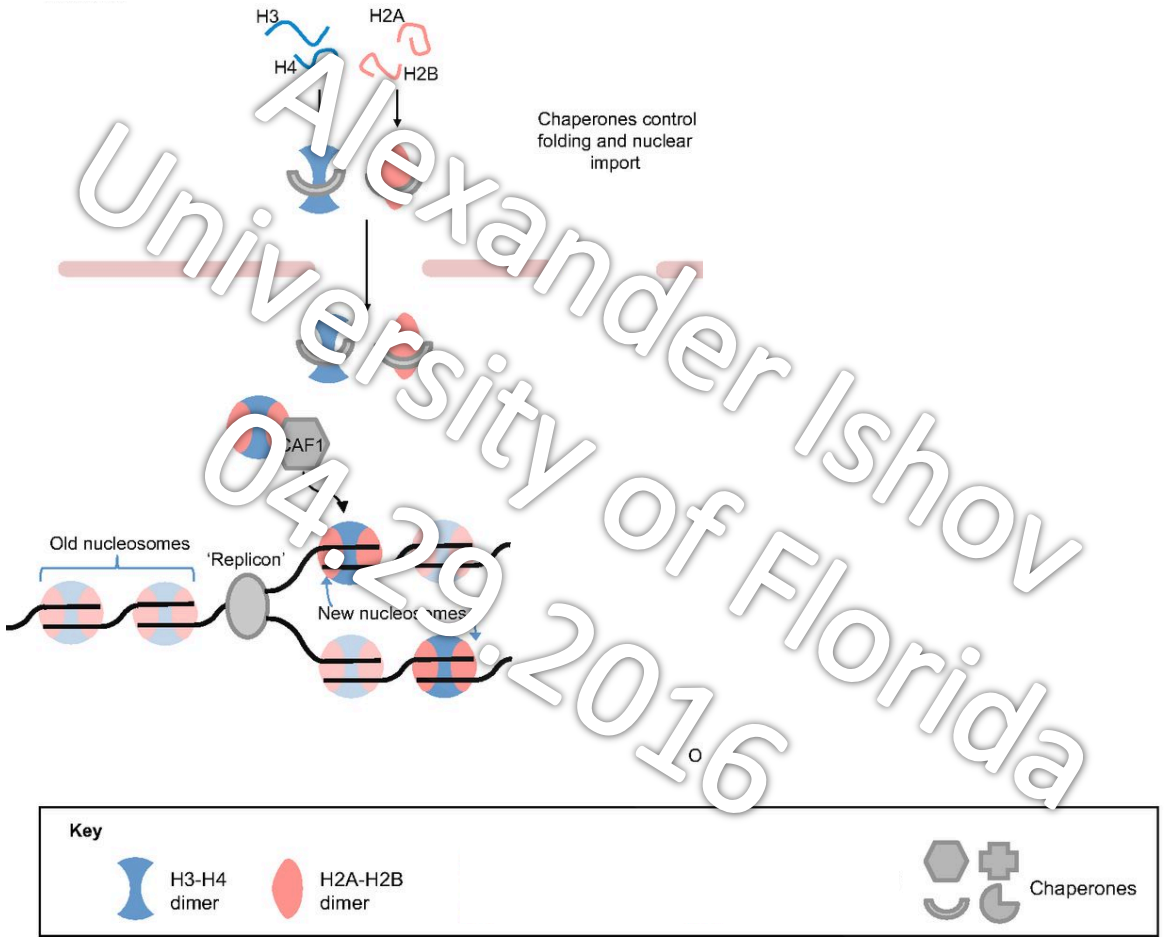
- In mammals there are three major types of histone H3 variants, H3.1, H3.2, and H3.3
- Deposition of H3.1 and H3.2 is cell cycle- and replication-dependent (S phase)

Campos and Reinberg 2010, Genes Dev.; Schwartzentruber et al., 2012, Nature

The deposition of canonical histones and histone variants

Replication-dependent deposition of "canonical" histones

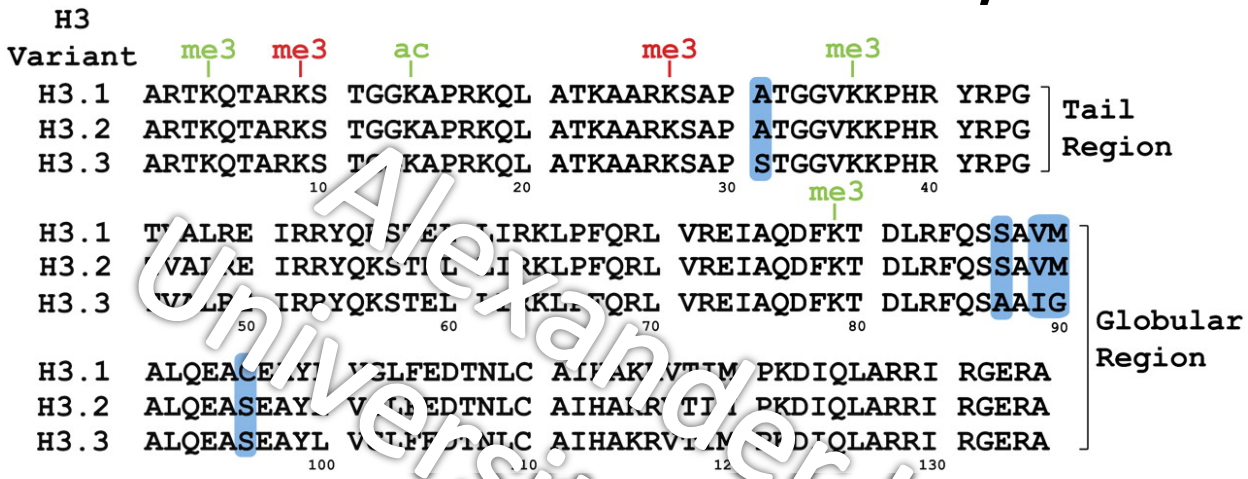
Replication-independent deposition of histone variants



(A) During replication, the chaperone CAF1 incorporates histone octamers behind the replication fork to regenerate chromatin.

Modified from: Skene, and Henikoff 2013

Histone H3 variants in eukaryotic cells



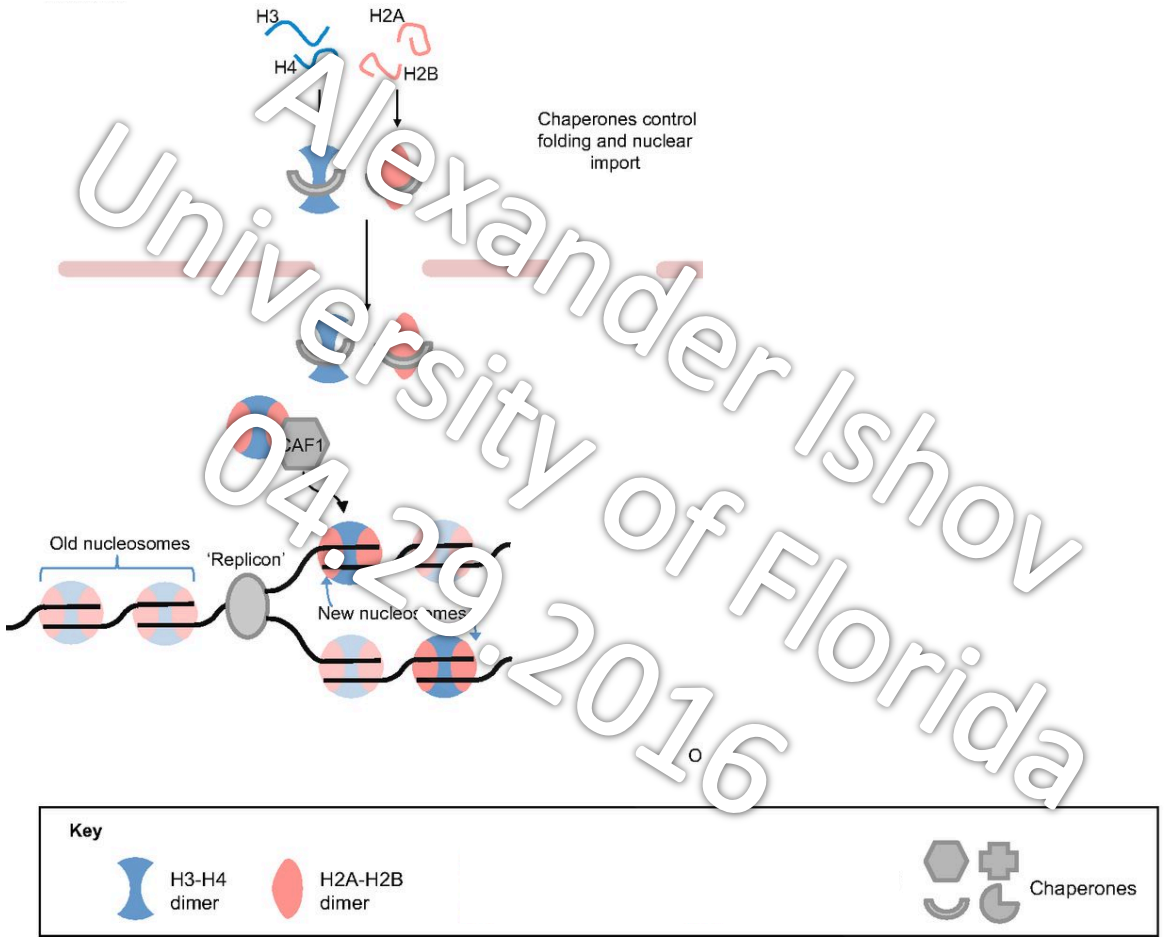
- In mammals there are three major types of histone H3 variants, H3.1, H3.2, and H3.3
- Deposition of H3.1 and H3.2 is cell cycle- and replication-dependent (S phase)
- H3.3 is expressed throughout the cell cycle and assembled into chromatin co-transcriptionally

Campos and Reinberg 2010, Genes Dev.; Schwartzentruber et al., 2012, Nature

The deposition of canonical histones and histone variants

Replication-dependent deposition of "canonical" histones

Replication-independent deposition of histone variants

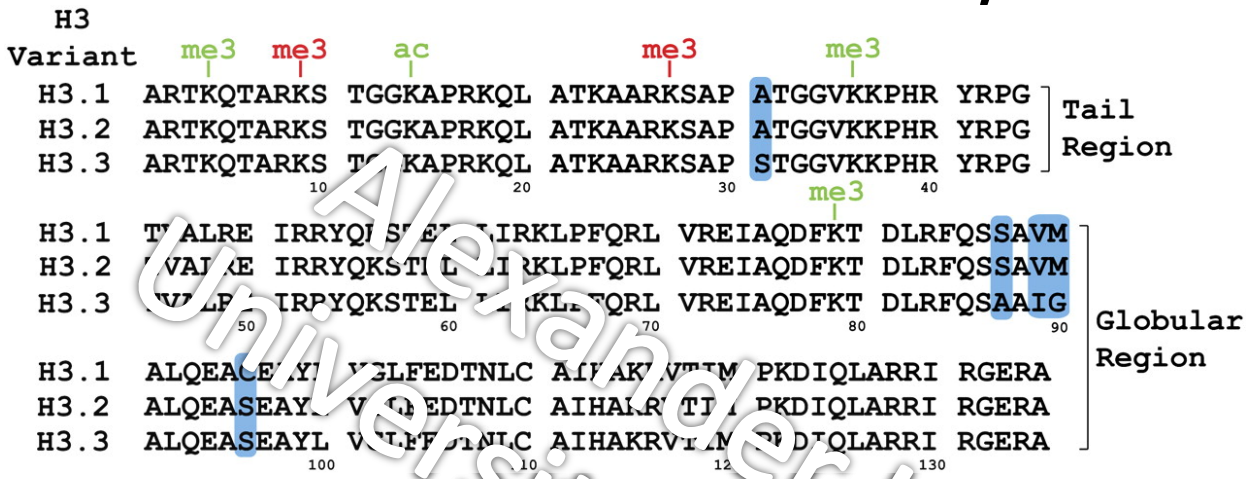


(A) During replication, the chaperone CAF1 incorporates histone octamers behind the replication fork to regenerate chromatin.

(B) Outside of replication, the incorporation of histone variants by chaperones as Hira and Daxx maintains nucleosome density following eviction by processes as transcription.

Modified from: Skene, and Henikoff 2013

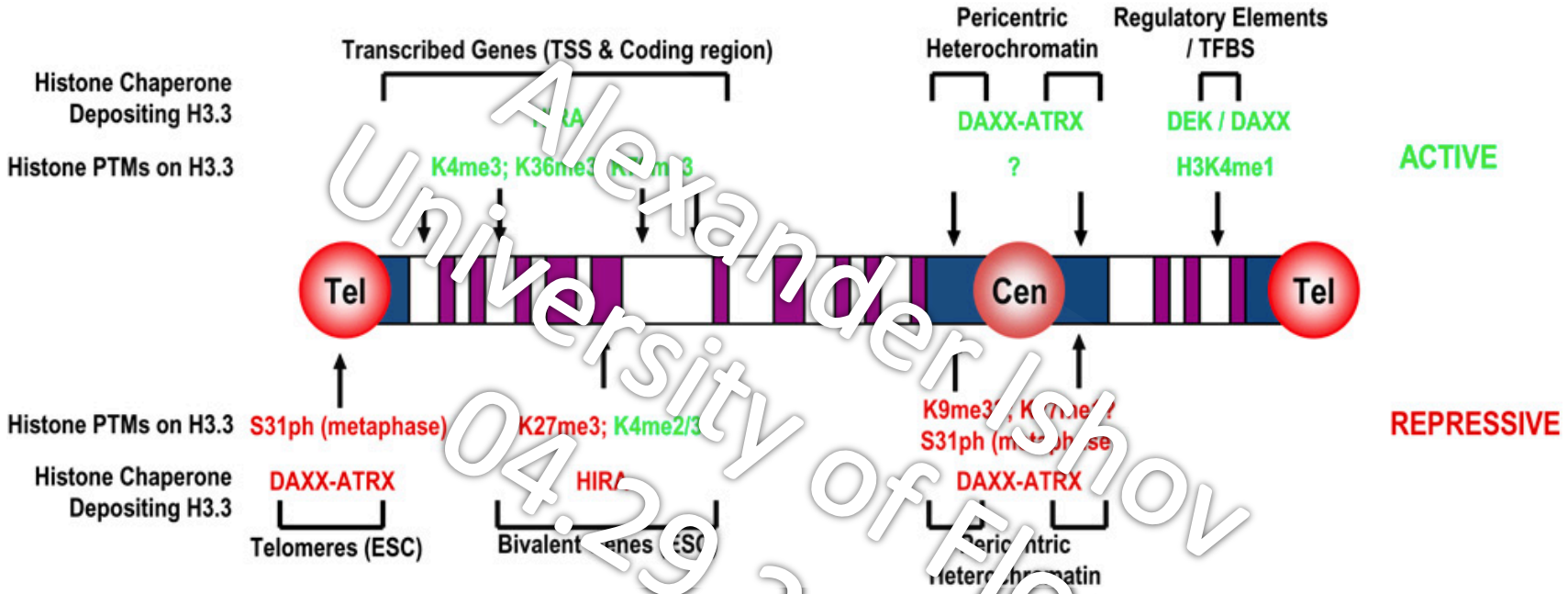
Histone H3 variants in eukaryotic cells



- In mammals there are three major types of histone H3 variants, H3.1, H3.2, and H3.3
- Deposition of H3.1 and H3.2 is cell cycle- and replication-dependent (S phase)
- H3.3 is expressed throughout the cell cycle and assembled into chromatin co-transcriptionally
- Chaperones for H3.1 and H3.3 are different
  - H3.1: CAF-1, Replication-dependent
  - H3.3: HIRA or Daxx-dependent assembly pathways, Replication-independent
- Nucleosome assembly of histone variants confers additional epigenetic control:
  - H3.3 is associated with elevated transcription

Campos and Reinberg 2010, Genes Dev.; Schwartzentruber et al., 2012, Nature

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

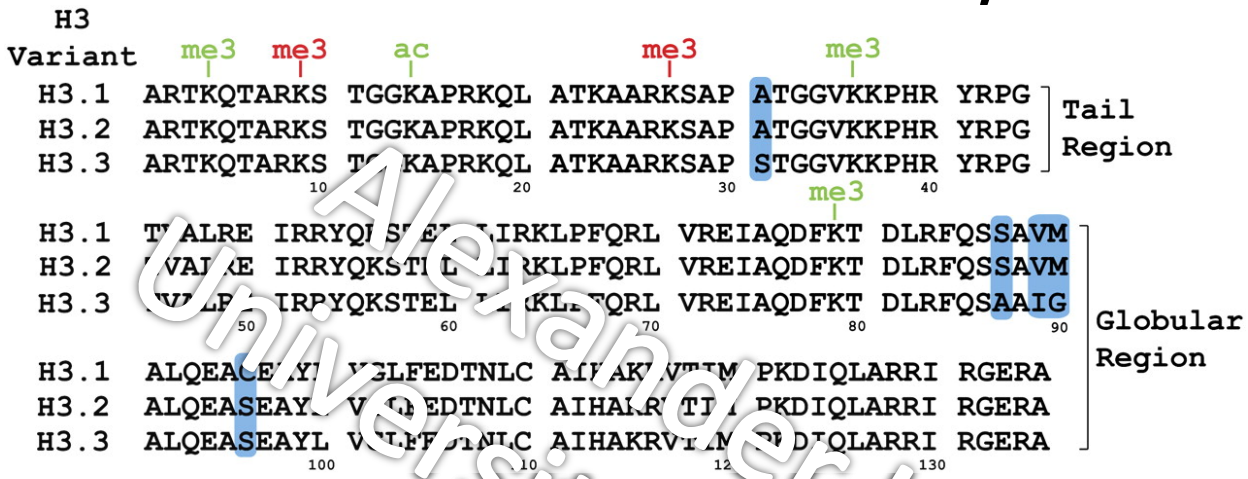


From: Eric I. Campos and Danny Reinberg, *Genes Dev. 2010 24: 1334-1338*

HIRA deposits H3.3 within the coding region of transcribed genes, but also at the transcription start site (TSS) of both active and repressed high-CpG content genes in ESCs.

Daxx/ATRX mediated H3.3 deposition to telomeres, pericentromere/centromere heterochromatin and (potentially) at the coding regions of transcribed genes

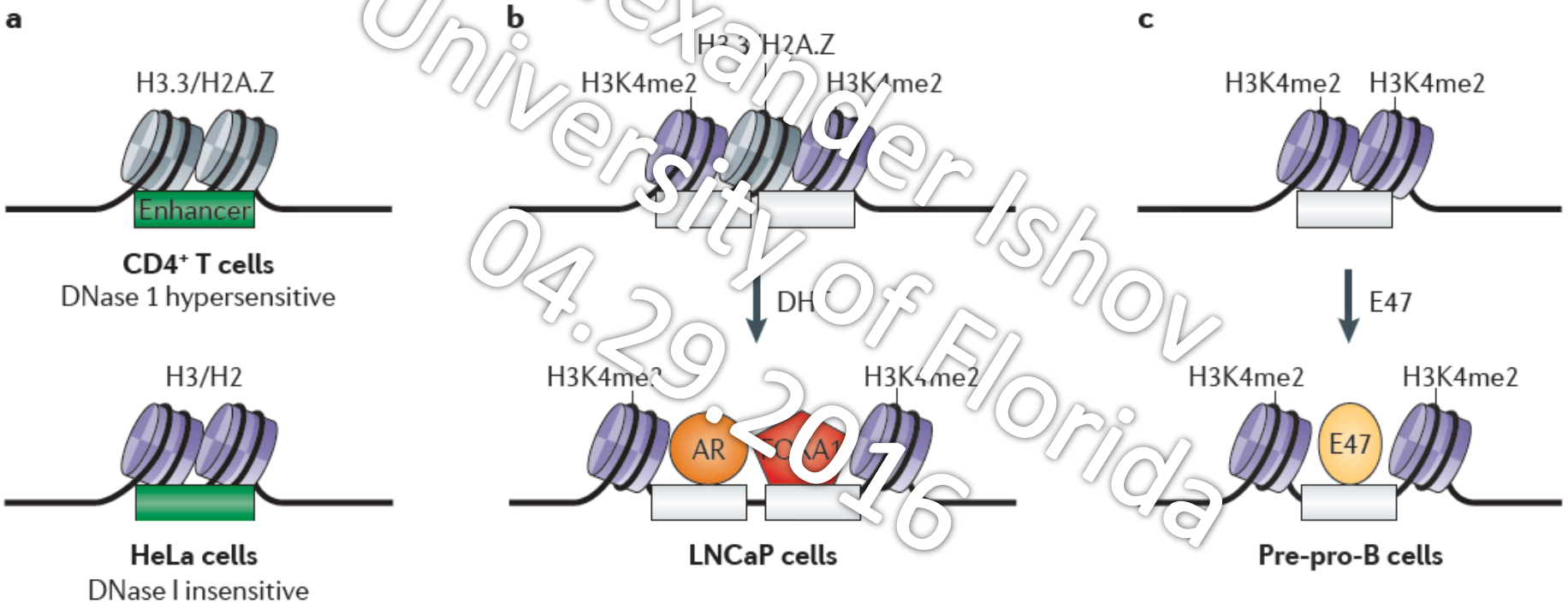
Histone H3 variants in eukaryotic cells



- In mammals there are three major types of histone H3 variants, H3.1, H3.2, and H3.3
- Deposition of H3.1 and H3.2 is cell cycle- and replication-dependent (S phase)
- H3.3 is expressed throughout the cell cycle and assembled into chromatin co-transcriptionally
- Chaperones for H3.1 and H3.3 are different
  - H3.1 – CAF-1 Replication-dependent
  - H3.3 – HIRA or Daxx-dependent assembly pathways
- Nucleosome assembly of histone variants confers additional epigenetic control:
  - H3.3 is associated with elevated transcription

Campos and Reinberg 2010, Genes Dev.; Schwartzentruber et al., 2012, Nature

Many functional promoters and enhancers contain H3.3/H2A.Z dynamic nucleosomes



Nature Reviews Genetics 12, 283-293 (2011)

Histone variants barcoding: 2006

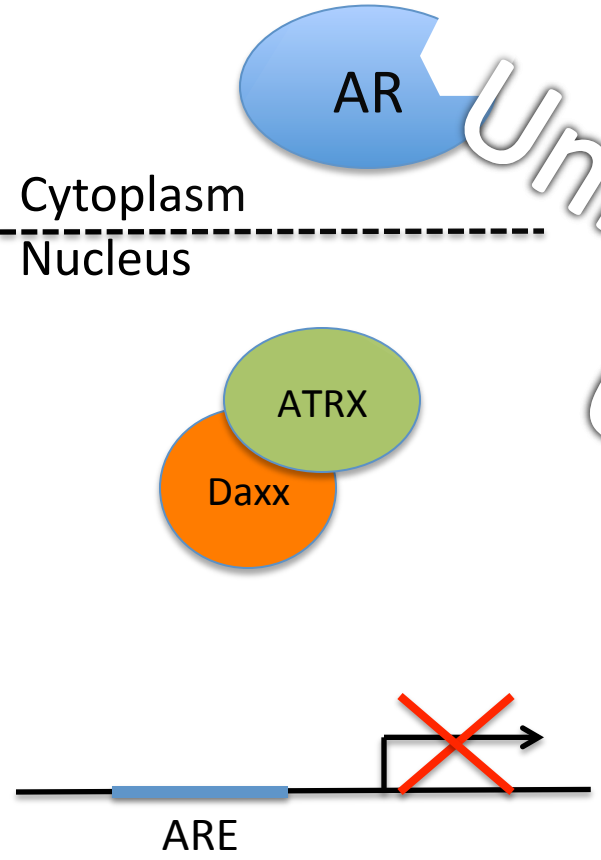
Histone H3 variants and their potential role in indexing mammalian genomes: The "H3 barcode hypothesis"

Sandra B. Hake and C. David Allis

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

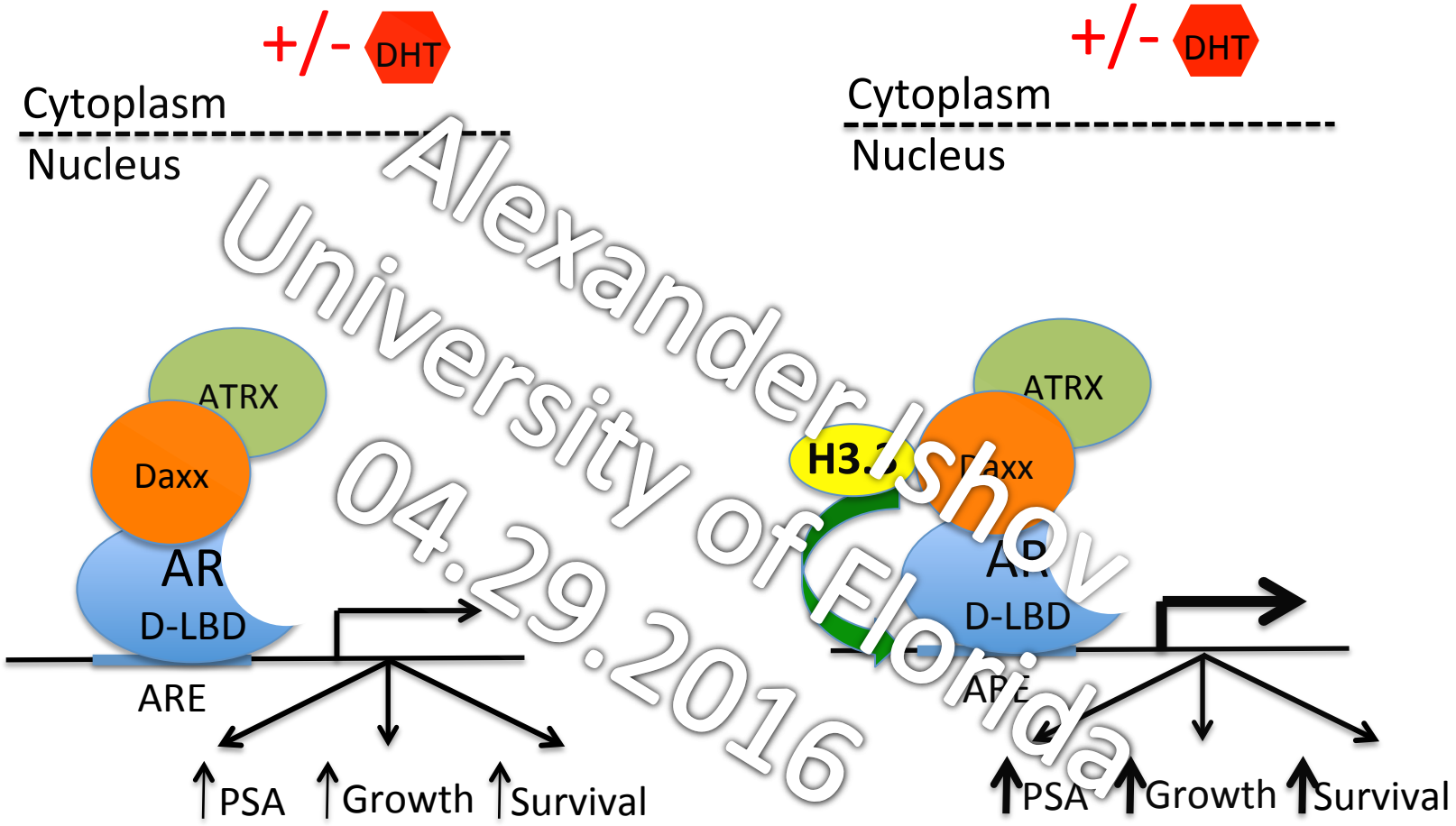
…Our hypothesis rests on the central concept that **mammalian histone H3 variants (H3.1, H3.2, and H3.3)**, although remarkably similar in amino acid sequence, **exhibit distinct posttranslational "signatures" that create different chromosomal domains or territories** which, in turn, influence epigenetic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

Working model: WT Androgen Receptor (AR)



During hormone treatment in AR-WT expressing cells, AR tethers Daxx chaperone activity to ARE, thus elevating hormone-dependent transcription

Working model: AR D-LBD (delta-ligand binding domain)



In CRPC cells, AR-ΔLBD is constantly located in the nucleus
AR-ΔLBD interacts with Daxx and tethers Daxx chaperone activity to ARE, thus elevating hormone-independent transcription

Hypothesis:

Hormone-independent interaction between ARΔLBD and Daxx recruits chaperone complexes and contributes to loading of H3.3 at AREs, thus activating AR-driven transcription and CRPC progression.

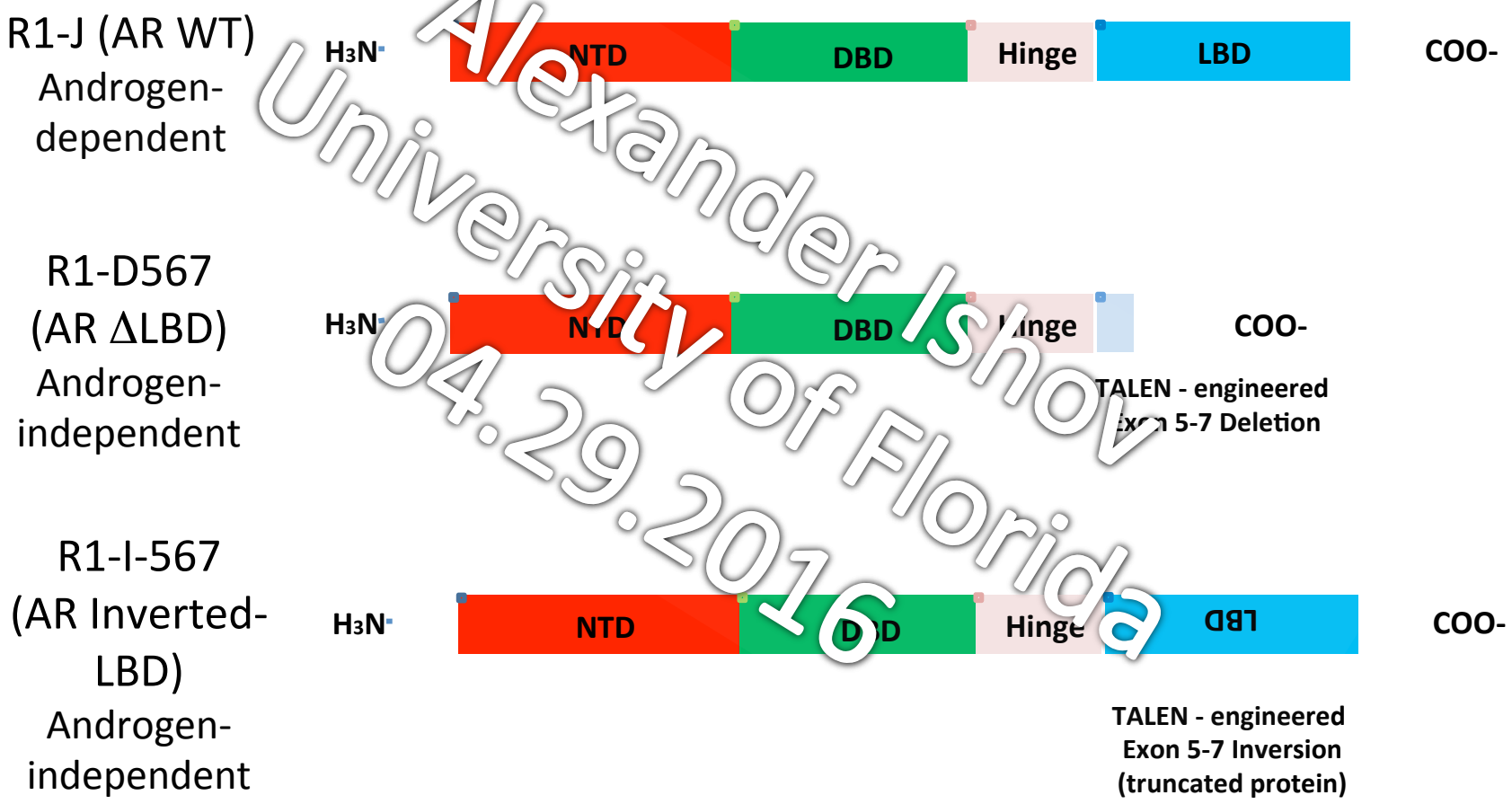
Questions:

- Do histone variants contribute to CRPC biology?
- Does the interaction between histone chaperone Daxx and AR play a role in this process?

Directions:

- Evaluate functions of histone variant H3.3 in the initiation and progression of CRPC
- Explore mechanisms underlying histone variant H3.3 profiling in CRPC
- Investigate mechanisms of AR-dependent recruitment of histone chaperones for identification of new therapeutic targets

Prostate Cancer Cell Lines



Lallous N et al. International journal of molecular sciences, 2013.
Nyquist M D et al. Proceedings of the National Academy of Sciences, 2013.
Dagvadorj A et al. Clinical Cancer Research, 2008.

Characterization of prostate cancer cell lines with AR wt and AR rearrangements

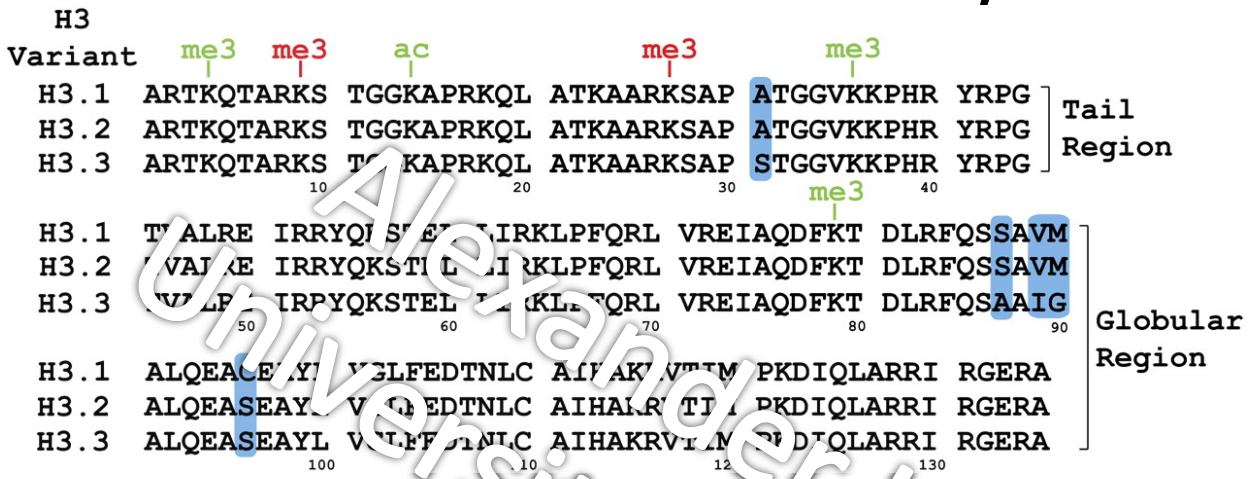
CWR-R1 sub-clone, deletion negative **AR -WT** clone 1 (R1-AD1), AR exons 5-7 deletion (**AR-ΔLBD**, R1-D567), AR exons 5-7 inversion (R1-I567) were characterized for expression of AR and Daxx. Actin: loading control.

Does AR-ΔLBD interact with Daxx?

Directions:

- Evaluate functions of histone variant H3.3 in the initiation and progression of CRPC:
  - Determine H3.3 function in CRPC analyzing proliferation, invasion and expression profiles in AR-WT and ARΔLBD models
  - **Evaluate chromatin association profiles of H3.3**

Histone H3 variants in eukaryotic cells

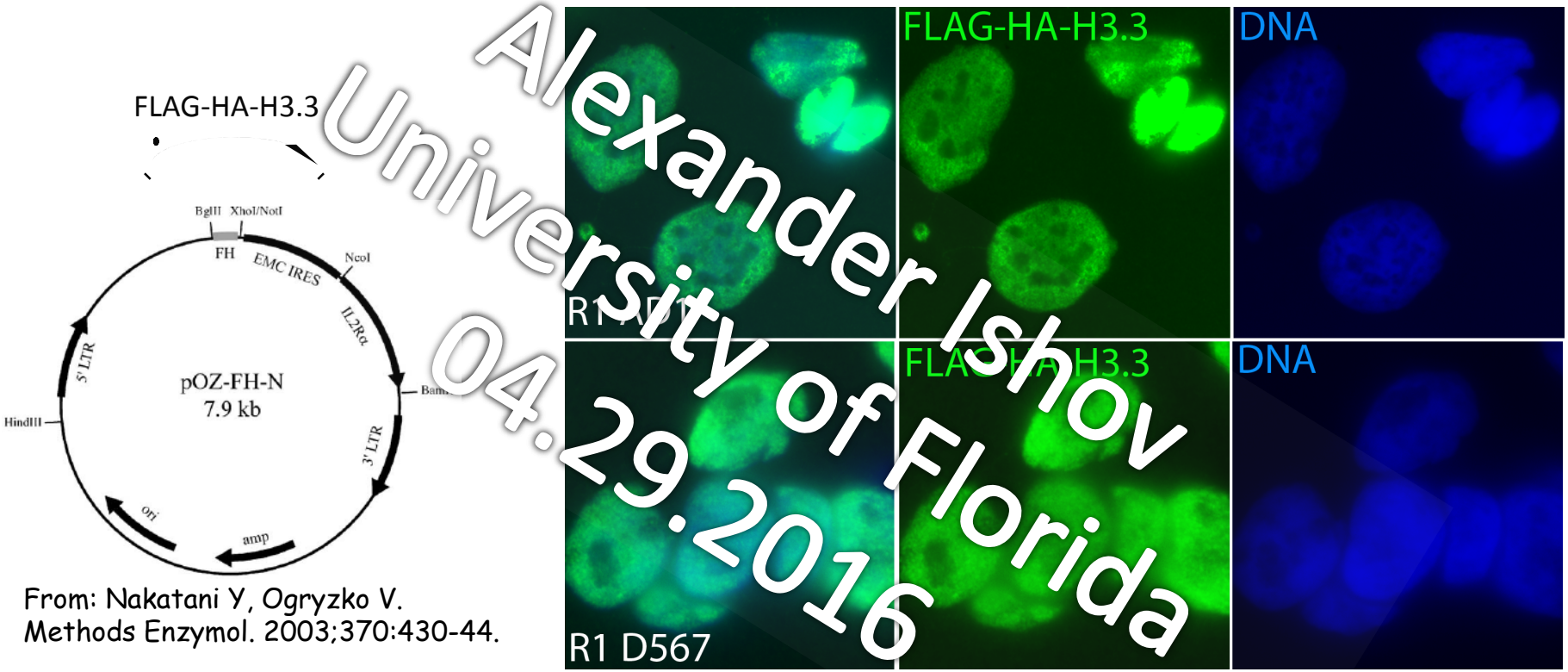


Difference between H3.1/2 and H3.3 is just 4 amino acids:

No Specific Antibodies Available for endogenous H3.3…

Campos and Reinberg 2010, Genes Dev.; Schwartzentruber et al., 2012, Nature

Creation of H3.3 expressing cell lines



- FLAG-HA-H3.3 cloned in pOZ (left)
- AR-WT (R1 AD1) and AR-ΔLBD (R1 D567) cells modified for stable expression of FLAG-HA-H3.3 (right)

Directions:

- Evaluate functions of histone variant H3.3 in the initiation and progression of CRPC:
  - Determine H3.3 function in CRPC analyzing proliferation, invasion and expression profiles in AR-WT and ARΔLBD models
  - **Evaluate chromatin association profiles of H3.3: production of FLAG/HA knock-in cells to study endogenous H3.3**

Conclusions

- ARΔLBD accumulates in nuclei in both untreated and hormone-treated cells
- ARΔLBD interacts with Daxx, mostly in the nucleus
- Proliferation of CRPC cells is reduced by AR, Daxx and H3.3 depletion
- Several genes are co-regulated by ARΔLBD and Daxx, but not by another H3.3 chaperone HIRA; Daxx regulates H3.3 deposition at promoters
- Depletion of Daxx and AR reduces H3.3 association with AR-induced promoter

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