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Short communication

Neutrophil Elastase in the capacity of the "H2A-specific protease"

M. Dhaenens[a,1], P. Glibert[a,1], S. Lambrecht[b], L. Vossaert[a], K. Van Steendam[a], D. Elewaut[b], D. Deforce[a,*]

[a] Laboratory of Pharmaceutical Biotechnology, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium
[b] Department of Rheumatology, Ghent University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium

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ABSTRACT

The amino-terminal tail of histones and the carboxy-tail of histone H2A protrude from the nucleosome and can become modified by many different posttranslational modifications (PTM). During a mass spectrometric proteome analysis on haematopoietic cells we encountered a histone PTM that has received only little attention since its discovery over 35 years ago: truncation of the histone H2A C-tail at V_{114} which is mediated by the "H2A specific protease" (H2Asp). This enzyme is still referenced today but it was never identified. We first developed a sensitive AQUA approach for specific quantitation of the $H2AV_{114}$ clipping. This clipping was found only in myeloid cells and further cellular fractionation lead to the annotation of the H2Asp as Neutrophil Elastase (NE). Ultimate proof was provided by NE incubation experiments and by studying histone extracts from NE *Null* mice. The annotation of the H2Asp not only is an indispensable first step in elucidating the potential biological role of this enzymatic interaction but equally provides the necessary background to critically revise earlier reports of H2A clipping.

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1. Introduction

The evolutionary conserved histone proteins are intimately associated with DNA to form the chromatin. Their amino-terminal tail and the carboxy-tail of histone H2A protrude from the nucleosome and can become modified by many different PTM. One somewhat dramatic PTM is the enzymatic clipping of histone tails, which was recently shown to play an epigenetic role in differentiation (Duncan et al., 2008; Osley, 2008). Although many previous reports on histone clipping have speculated on its potential to influence transcription (Duncan and Allis, 2011), histone truncation has equally been described in the context of other biological processes, such as neutrophil extracellular trap or NET formation (Papayannopoulos et al., 2010).

During a proteome analysis on haematopoietic cells, we came across a histone clipping event that was first described over 35 years ago in calf thymus histone extracts: truncation of the C-tail of histone H2A at V_{114} (Eickbush et al., 1976). The responsible enzyme was named the 'H2A specific protease' (H2Asp) and its involvement in transcriptional regulation was soon hypothesized but it was never sequenced nor identified (Davie et al., 1986; Eickbush et al., 1988; Eickbush and Moudrianakis, 1978; Elia and Moudrianakis, 1988; Watson and Moudrianakis, 1982). Structurally, this truncation has been suggested to modulate chromatin dynamics and it was shown to be induced during macrophage differentiation (Minami et al., 2007; Vogler et al., 2010). Even today, references to this histone H2A C-tail clipping and the H2Asp still recur, yet no reports have thus far questioned the identity of the enzyme or its involvement in epigenetics (Azad and Tomar, 2014; Mandal et al., 2012; Okawa et al., 2003; Santos-Rosa et al., 2009).

Here we show that the H2Asp actually is Neutrophil Elastase (NE). While we continue to search for the biological potential of this truncation in health or disease, we emphasize that the clipping of the histone H2A C-tail shows remarkable parallels to the more epigenetically established clipping of the H3 N-tail, but could equally be involved in other biological processes such as NET formation. Importantly, we caution that most experimental approaches still do not anticipate on these clipping events while high enzyme kinetics and strong association between nucleosomes could greatly hamper efficient enzyme inhibition in any protocol.

Abbreviations: H2Asp, H2A-specific protease; NE, Neutrophil Elastase; CL, cathepsin L; mESC, mouse embryonic stem cells; cH2A, cleaved histone H2A; PTM, posttranslational modification; MS, mass spectrometry; PBMC, peripheral blood monocytes; AQUA, Absolute Quantitation.

* Corresponding author. Tel.: +32 9 264 8067; fax: +32 9 220 6688.
E-mail address: dieter.deforce@ugent.be (D. Deforce).

[1] These authors contributed equally to this work.

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2. Materials and methods

2.1. Cells and reagents

Phosphate buffered saline (PBS), media, L-glutamine, Foetal bovine serum (FBS), penicillin/streptomycine, Dynabeads and Sypro Ruby were from Invitrogen (San Diego, CA), ammonium bicarbonate (ABC), sodium dodecyl sulfate (SDS), N-cyclohexyl-3-aminopropanesulfonic acid (CAPS) and Tween-20 from Millipore (Billerica, MA). All other reagents were purchased from Sigma Aldrich (St. Louis, MI, USA). Bovine histones (cat. no. 223565) were from Roche (Basel, Switzerland), recombinant human H2A (M2502S) from New England Biolabs (Ipswich, MA) and purified Neutrophil Elastase from Abcam (Ab80475, Cambridge, UK). Raji and Jurkat cells were obtained from ATCC. Total leukocytes were obtained by red blood cell lysis on whole blood (Qiagen, Venlo, Netherlands). Lymphocytes were isolated from healthy volunteers using Ficoll-Paque. T-cells were purified by means of the RosetteSep® Human T Cell Enrichment Cocktail (Stem cell Technologies, Grenoble, France). Cells were cultured in Dulbecco's Modified Eagle medium supplemented with 2% (w/v) L-glutamine, 10% (w/v) FBS and 50 IU/ml penicillin/streptomycine at 37 °C. Apoptosis in Raji's was induced by o/n incubation in 0.2 μg/ml MHCII antibody or 100 ng/ml PMA at 37 °C or by 15 min UV irradiation.

2.2. Mice

Null mice *stock# 006112 strain# B6.129X1-Elanetm1Sds/J* and wild type control mice *stock# 005304 Strain# C57BL/6NJ* were purchased from The Jackson Laboratory (Bar Harbour, MA, USA) and bred at the animal facility of Ghent University Hospital. All experimental procedures were approved by the local ethics committee according to national animal welfare legislation. All mice were genotyped as instructed by the supplier. Blood samples were obtained by cardiac puncture of mice under terminal anaesthesia. Leukocytes were recovered by red blood cell lysis and histones were isolated as described below.

2.3. Histone isolation

All steps were done at 4 °C. Harvested cells were washed twice in PBS containing 1 mM PMSF and protease inhibitor cocktail. 10^7 cells/ml were resuspended in Triton extraction buffer (PBS containing 0.5% (v/v) Triton X 100, 1 mM PMSF and protease inhibitor cocktail) and lysed by gentle stirring. Pelleted nuclei were washed in PBS containing 1 mM PMSF and proteinase inhibitor cocktail. Histones were extracted overnight after benzonase treatment of the sonicated nuclei by acid extraction: incubation in 250 μl 0.2 M HCl at 4 °C with gentle stirring. Precipitated proteins were pelleted and the supernatant containing the histones was dried and stored at −80 °C until further use. Protein quantitation was done by Bradford Coomassie Assay from Thermo (St. Waltham, MA, USA).

2.4. Western blot analysis

3 μg of dried histone extract was resuspended in Laemmli buffer, incubated at 99 °C and run on a 15% Tris–HCl gel (Biorad, Hercules, CA) and transferred to nitrocellulose membrane in a 10 mM CAPS buffer with 20% MeOH (Merck) in 100 min at 120 V. Histone H2A (LS-C24265, LifeSpan BioScience, Seattle, WA) was used at a 1:1000 dilution in 0.3% Tween20, 1%BSA. For the detection of biotinylated H2A 1:10 000 Avidin-HRP (18-4100-94) (eBioscience, San Diego, CA) was used.

2.5. Mass spectrometry analysis

All tryptic digests were performed after reduction in freshly prepared 50 mM TCEP 25 mM TEABC solution for 2 h at 56 °C and alkylation in 200 mM MMTS solution for 1 h at room temperature. The samples were separated on an U3000 (Dionex) in a 70 min organic gradient from 4% to 100% buffer B (80% (v/v) ACN in 0.1% (v/v) FA) and analyzed on an ESI Q-TOF Premier (Waters, Wilmslow, UK). Data was searched against SwissProt database using Mascot 2.3 (Matrix Science, London, UK). For the specific analysis of the H2A V_{144} clipping, the isobaric peptides (AQUA1; AQUA2) were combined in a stock solution in a ten (AQUA1, Thermo) to one (AQUA2, Sigma Aldrich) ratio and added to 1 μg of histone extract right before MS analysis.

2.6. Sucrose gradient ultracentrifugation

Mechanical isolation of nuclei was done by lysing the cells in a cell plunger after 5 min of incubation in 10 mM Hepes pH 8, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM DTT at 4 °C. After 10 strokes, nuclear isolation efficiency was found to be at least 90% by microscopy. The pelleted nuclei were resuspended in 3 ml buffer S1 (0.25 M sucrose, 10 mM $MgCl_2$) and brought on top of 3 ml buffer S2 (0.35 M sucrose, 0.5 mM $MgCl_2$). After 5 min at 572 × *g* at 4 °C, the pellet was resuspended in S2 for a second wash and the nuclei were lysed by sonication in 50 mM Tris–HCl pH 7.5 supplemented with 250 U of benzonases for 10 min for DNA degradation. The linear sucrose gradient (5 ml) 10–50% was prepared in 0.01 M Tris–HCl pH 7.5 with 0.001 M Na_2EDTA. The gradient was placed at 4 °C for 2 h. 200 μl of the nuclear extract was applied on top of the gradients and they were spun at 190,000 × *g* for 1040 min at 4 °C in a Centrikon T1080 ultracentrifuge. After centrifugation, 10 fractions of 500 μl were collected manually.

3. Results and discussion

3.1. High throughput quantitation of H2AV_{114} clipping by AQUA

During a mass spectrometric proteome analysis on haematopietic cells, we detected only one semi-tryptic peptide out of >7400 annotated MSMS spectra that recurred in all 6 replicate runs: VTIAQGGVLPNIQAV, a fragment derived from histone H2A ending at V_{114} (clipped H2A or cH2A, Fig. 1A). Remarkably, this specific fragment was already described in calf thymus 1976 but the responsible enzyme, named H2A specific protease (H2Asp) was never identified (Eickbush et al., 1976). To be able to specifically quantify the V_{114} clipping, a sensitive mass spectrometry approach based on the AQUA (Absolute Quantitation) principle was developed using two isotopically labelled synthetic peptides (Fig. 1A and B). To our knowledge, this is the first description of a technique that can specifically quantify a histone clipping event in high throughput. To validate the efficiency of this approach, we quantified the amount of clipped H2A (cH2A) in 0.1–2.5 μg of bovine histones and consistently found it to be between 3% and 9%, despite the 25-fold loading difference (Fig. 1C and D). Note that this cH2A was detected in commercial calf thymus histones, the source from which the H2A specific protease was first isolated (Eickbush et al., 1976).

3.2. The H2A specific protease is Neutrophil Elastase

Screening larger cohorts of histone extracts from different haematopoietic origins indicated that cH2A prevalence was strongly related to myeloid content since PBMCs, leukocytes and the pellet from Ficoll-Paque separated haematopoietic cells showed the presence of cleaved H2A (Fig. 2A, left panel). In T-cells, Raji's and Jurkat cells only intact H2A was detected. Lymphoid

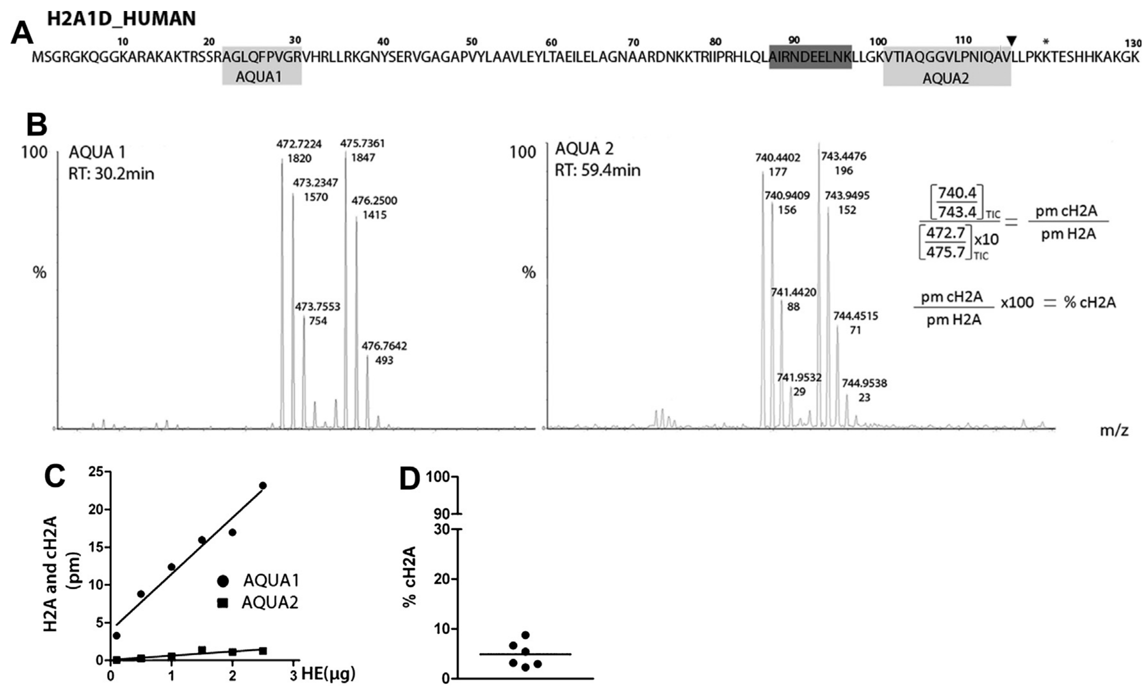

Fig. 1. Accurate quantitation of the H2AV$_{114}$ clipping event. (A) Histone H2A sequence. Epitope for Western blot detection is highlighted in dark grey (cH2A = 12 kDa). Sequences boxed in light grey are used for the AQUA approach. Asterisk indicates K$_{119}$ ubiquitination site. Arrow head: H2AV$_{114}$ clipping. (B) MS spectrum of a histone extract spiked with both AQUA peptide sequences indicated in light grey in (A). Both synthetic peptides are isotopically labelled with ^{13}C and ^{15}N at the proline increasing their molecular weight with 6 Dalton which results in a 3 *m/z* (2+) shift. 10 pm of the N-terminal tryptic AQUA1 peptide AGLQF**P**VGR (*m/z* 475.7) and 1 pm of the specific semi-tryptic C-terminal AQUA2 peptide VTIAQGGVL**P**NIQAV$_{114}$ (*m/z* 743.4), allow to quantify the relative amount of H2A that is clipped (cH2A) in a given sample according to the formula given on the right. This 10-fold difference in loading compensates for differences in ionization efficiency and in-source decay. Peaks are annotated with the m/z on the top and the TIC (Total Ion Count) below. RT, retention time. (C) The AQUA1 peptide quantifies the total amount of H2A present and can thus be used to eliminate all sample variation due to differential protein composition of the histone extract. To illustrate the need for the AQUA1 peptide 0.1–2.5 μg of bovine histone extract was loaded with 10 pm of AQUA1 and 1 pm AQUA2. The quantity of both total H2A (determined by AQUA1) and cH2A (determined by AQUA2) can be absolutely determined (left axis, absolute quantity). (D) The formula was used to calculate the % cH2A in all samples analyzed in (B) and predicted the clipping within a 6% range despite the 25-fold loading difference.

Raji cells were subjected to different forms of stress to exclude any aspecific degradation event (Fig. 2A, middle panel). No cH2A could be detected after overnight incubation with MHC II antibodies or PMA, nor after 15 min of UV irradiation, while all these treatments induced cell death as seen by trypan blue staining. For all abovementioned samples the presence or absence of the specific V$_{114}$ clipping site was analyzed by AQUA. Eickbush et al. (1976) described that the H2Asp itself is co-extracted during an acid histone extract. Histones were thus isolated from samples with low myeloid content (PBMC) without protease inhibitors to obtain samples with active H2Asp. Incubation for 30 min at 37 °C corroborated the presence of active H2Asp, which also clipped K$_{119}$ ubiquitinated H2A (Fig. 2A, right panel). Next, a constant level of bovine histones (10 μg) was incubated by an increasing amount of this extract (5 ng to 1 μg) for 2 h at 37 °C (Fig. 2B). Bovine histone H2A was the histone being clipped most at the lowest amount of extract, confirming that H2AV$_{114}$ is a very sensitive clipping site.

When these histone extracts containing H2Asp activity were analyzed by mass spectrometry only one serine protease was identified in these histone extracts: Neutrophil Elastase (NE), previously known as medullasin. This finding agrees perfectly with all previous reports on the H2Asp (Davie et al., 1986; Eickbush et al., 1988; Eickbush and Moudrianakis, 1978; Eickbush et al., 1976; Elia and Moudrianakis, 1988; Watson and Moudrianakis, 1982): (i) NE is a serine protease (Korkmaz et al., 2008), (ii) which is inactivated at low pH, (iii) activated by high salt content (Lestienne and Bieth, 1980), (iv) is inactivated by (calf thymus) DNA (Lestienne and Bieth, 1983), (v) it is transcribed in the thymus, one of the few tissues where NE mRNE can be found (Hs.99863 in EST Profile Viewer, NCBI), (vi) is extremely basic (pI 9.9) making it very susceptible to co-extraction during acid histone isolation, (vii) it has known valine preference (Schilling et al., 2010) and a predicted cleavage site at H2AV$_{114}$ with >99% specificity in SitePrediction (Verspurten et al., 2009), (viii) ubiquitinated H2A is a substrate, and finally (ix) we too found it to associate strongly with nucleosomes in our sucrose gradient ultracentrifugation experiments on proteins extracted from isolated nuclei (see Section 3.4).

3.3. Confirmation of NE in its capacity of the H2Asp

Next, both histones from Raji cells and bovine histones were incubated with commercially available purified NE. This results in cleavage of all histones in a dose dependent manner (Fig. 2C). The specific V$_{114}$ fragment was the only detected fragment in the lowest concentration range (0.00001 U), illustrating the susceptibility of the substrate. Ultimate proof was generated by comparing isolated leukocytes from WT, heterozygous NE$^{+/-}$ and NE *Null* mice using the AQUA approach (Fig. 2D). The only mutated amino acid compared to human is indicated by the box. Both K and R are tryptic cleavage sites and when samples are prepared by this enzymatic digestion, the peptide sequences corresponding to the AQUA peptides will be formed in both. As in human leukocytes, the clipping was present in control and heterozygous mouse leukocytes. However, the clipping of H2A at V$_{114}$ could not be detected in the NE *Null* mice.

3.4. NE strongly interacts with nucleosomes in vitro

For histone H2A clipping to occur *in vivo*, requires both enzyme and substrate to physically interact. A strong association of the H2Asp to chromatin and nucleosomes was reported during

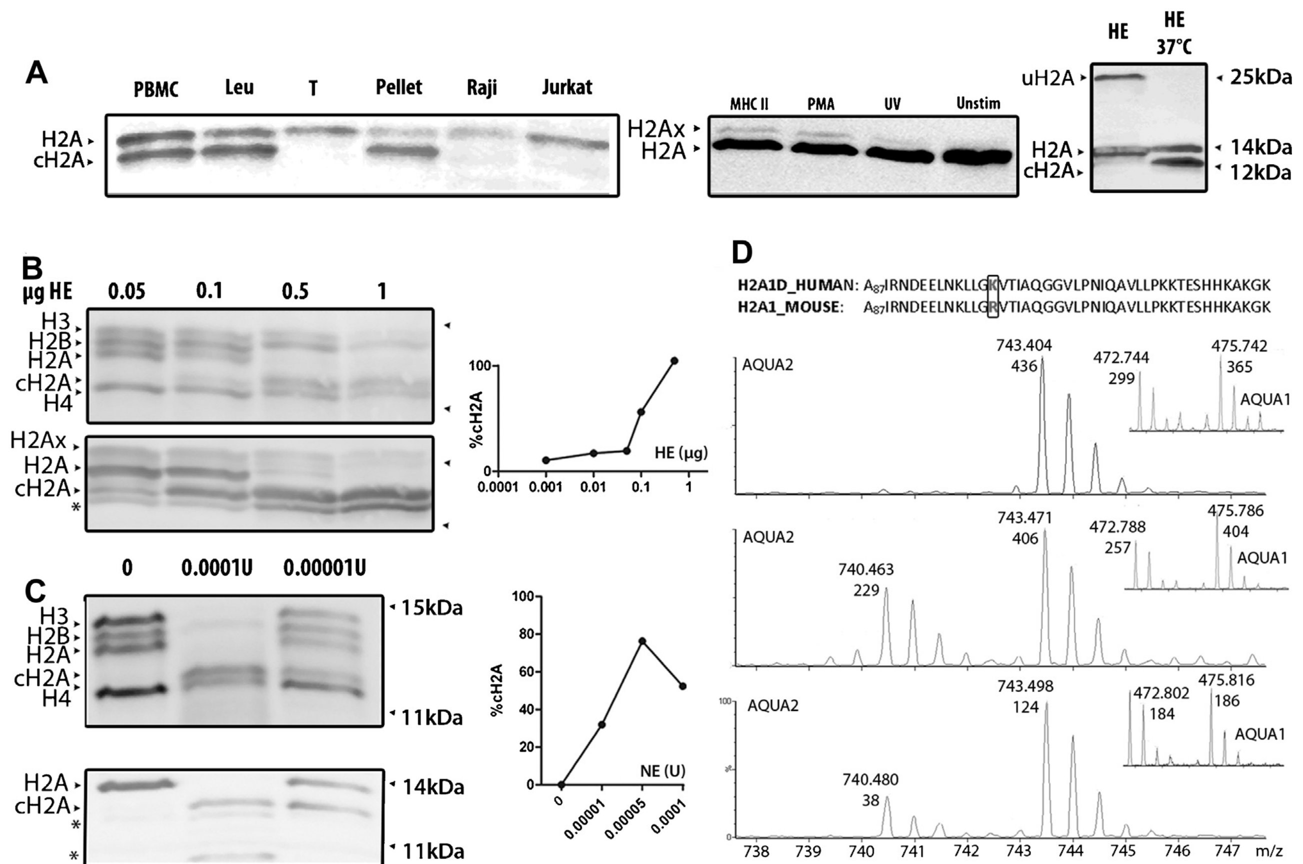

Fig. 2. Neutrophil Elastase in the capacity of the H2A specific protease. (A) cH2A is present in the total leukocytes (Leu), buffy coat (PBMCs) and pellet from Ficoll-Paque separated haematopoietic cells (left panel). Purified T-cells (T), nor lymphoid Raji or Jurkat cell lines showed any cH2A. Raji cells incubated overnight with MHCII antibodies or PMA, or irradiated for 15 min with UV lacked cH2A, excluding aspecific degradation (middle panel). Histones extracted in the absence of inhibition show H2Asp activity coordinately clipping ubiquitinated H2A (uH2A, identified by MS) (right panel). (B) 1 ng to 1 μg of leucocyte histone extract was incubated with 10 μg of buffered bovine histones for 2 h at 37 °C (0.05–1 μg is shown). Sypro stain (upper panel) and H2A western blot (lower panel) illustrate the presence of active H2Asp in these histone extracts and shows the susceptibility of histone H2A. AQUA quantitation confirms the V_{114} clipping site (1 ng to 1 μg is shown in right panel). Asterisks: additional degradation fragment. Molecular weight markers as in (C). (C) Commercially purified NE clips all bovine histones when incubated at only 0.0001 U (upper panel: Sypro-stain). Histone H2A is the only substrate at 0.00001 U (lower panel: H2A blot). cH2A AQUA quantitation of Raji histones (RH) incubated with 0–0.005 U of NE. High NE concentrations impairs accurate quantitation by AQUA (right panel). (D) Histone extracts from leukocytes from five mice per phenotype were pooled and analyzed by AQUA. The only mutated amino acid compared to human is indicated by the box. Both K and R are tryptic cleavage sites. cH2A is found in histone extracts from leukocytes from both wild type and heterozygous mice (lower two panels) but is undetectable in NE *Null* mice (upper panel). Upper right corner: AQUA 1 peptide (475.7 *m*/*z*) used to quantify the total amount of H2A.

earlier studies (Davie et al., 1986; Eickbush et al., 1988). Equally, nuclear substrates of NE have been reported after NE migrates to the nucleus *in vivo* (Lane and Ley, 2003). To verify if NE from leukocytes is present in the nucleus and associates with nucleosomes, we isolated nuclei using mechanical disruption after red blood cell lysis and fractionated the proteins by differential ultracentrifugation on a sucrose gradient from 10% to 50%. Ten different fractions were collected and partially loaded onto a 1D PAGE to visualize the fractionation (Fig. 3). The remaining part of each fraction was digested with trypsin to identify the main proteins present. Remarkably, the only protein that was identified solely in all fractions containing the nucleosomes in the gradient was NE. This illustrates that NE strongly associates with and is being "pulled down" by the nucleosomes.

Although this finding supports a potential physical interaction in the nucleus, we cannot exclude that the presence of the NE, and H2Asp in other reports, is in fact a cytoplasmic contaminant, as we could also detect other cytoplasmic proteins in the nuclear extracts. On the other hand, it can be concluded that NE will strongly attach to the nucleosomes when they are colocalized, since the other proteins did not co-migrate with the nucleosomes in the gradient. Because the DNA was enzymatically degraded in these extracts, this finding is in line with the notion that NE might directly bind the histone H3 N-tail as was suggested for the H2Asp earlier (Elia and Moudrianakis, 1988). Very high enzyme activity to specific substrate sequences, as seen here for NE-mediated $H2AV_{114}$ clipping, has also been suggested to be a possible indicator of functional relationship (Timmer et al., 2009). Taken together however, the very strong association with the nucleosomes and the high enzyme kinetics towards histone H2A make complete inactivation of this enzymatic reaction extremely difficult. Notably, by spiking biotinylated recombinant H2A during the histone extraction procedure and staining the subsequent western blot with avidin we found that not all *in vitro* activity could be inhibited in isolated cell populations with high myeloid content in our hands (data not shown). Because this is not the first report on pitfalls during *in vitro* inhibition of NE (Groth and Alban, 2008), we caution that unintentional histone clipping during cell disruption can strongly influence many epigenetic experimental approaches such as ChIP seq and Western blot.

The annotation of the H2Asp as NE thus is not only an essential first step towards elucidating any potential biological role of this enzymatic interaction, but equally provides the necessary background to critically revise earlier reports of $H2AV_{114}$ clipping and coordinately increases awareness on histone clipping.

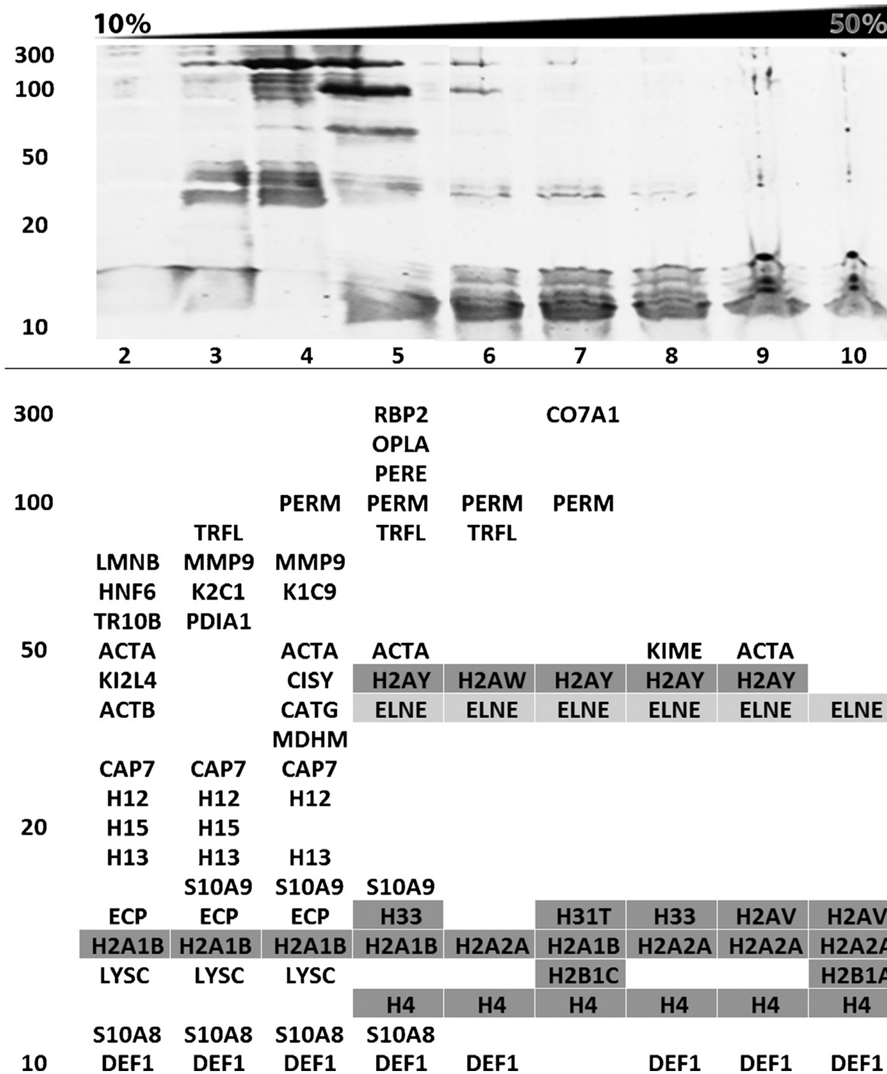

Fig. 3. Strong association between NE and nucleosomes in differential sucrose gradient ultracentrifugation. Protein extracts from mechanically isolated nuclei were fractionated on a sucrose gradient to simultaneously verify direct associations between nucleosomes and NE. Ten different fractions were collected from 10% to 50% linear sucrose gradient after overnight ultra-centrifugation at 190,000 × g. These fractions were loaded onto a 1D PAGE and stained with Sypro staining to visualize the efficiency of the fractionation (upper panel, image vertically compressed). Note that the increasing sucrose content of these samples greatly reduces band pattern resolution towards the 50% sucrose fractions. All fractions were coordinately analyzed by mass spectrometry (lower panel). Proteins are annotated by their Uniprot accession name where "*_HUMAN" is discarded. Core histone isoforms are not indicated. Proteins are roughly organized by their molecular weight (MW) for comparison to the gel image in the upper panel. All higher density lanes (5–10) are enriched for nucleosomes, as seen by the presence of the core histones highlighted in dark grey and as seen by their characteristic band pattern in the gel. NE (ELNE) was the only protein to have specifically co-migrated with the nucleosomes towards higher densities.

3.5. *The H2Asp is Neutrophil Elastase: broadening the biological perspective*

Nearly all previous reports on $H2AV_{114}$ clipping and the H2Asp have speculated on the potential transcriptional implications of such reaction (Azad and Tomar, 2014; Davie et al., 1986; Eickbush et al., 1988; Eickbush and Moudrianakis, 1978; Eickbush et al., 1976; Elia and Moudrianakis, 1988; Minami et al., 2007; Okawa et al., 2003). Although we do not provide evidence of epigenetic implications here, by expressing the V_{114} truncated cH2A directly, Vogler et al. have at least demonstrated the viability of cells with this truncated H2A (Vogler et al., 2010). Even more, they also showed that this led to increased histone exchange kinetics and nucleosome mobility *in vivo* and *in vitro*, in line with what was found for H2Asp-mediated clipping of H2A *in vitro* (Eickbush et al., 1988). An interesting parallel thus emerges when considering that retinoic acid (RA)-induced differentiation of THP-1 promonocytes into macrophages is briefly accompanied by histone H2AV114 clipping (Minami et al., 2007), just as H3 clipping by cathepsin L (CL) is induced while mouse embryonic stem cells (mESC) are differentiated with RA (Duncan et al., 2008). Both the H2A carboxy-tail (V_{114}) and the H3 amino-tail (A_{21}) clipping sites are located adjacent at the entry and exit points of the DNA. Of note, while the clipping of histone H3 is considered essential for early mESC differentiation, Duncan et al. argued that the viability of CL *Null* mice might be due to functional redundancy (Duncan et al., 2008). We found that histone H2A isolated from NE *Null* mice showed additional bands on western blot that are similar in molecular weight, but are not derived from V_{114} clipping as seen by our specific AQUA approach (data not shown). Just as for CL *Null* mice, NE *Null* mice are viable and do not show intrinsic deficiencies in hematopoietic differentiation (Belaaouaj et al., 1998). Yet, this could equally be seen as an argument against its involvement in epigenetics. Notably, the NE *Null* mice do show a reduced capacity to form immunological structures called neutrophil extracellular traps or NET (Papayannopoulos et al., 2010). This process involves the active migration of NE and myeloperoxidase to the nucleus in neutrophils followed by chromatin decondensation and externalization of the

DNA to entrap microbial pathogens. It would thus be of great interest to verify the prevalence of the $H2AV_{114}$ clipping site in NET.

Taken together, the fact that the H2Asp actually is NE now surfaces an alternative biological process apart from the epigenetic context in which it has been primarily described up to now. However, it will remain challenging to uncouple any histone clipping event from continuous histone turnover and degradation (Deal et al., 2010; Ivanov et al., 2013; Qian et al., 2013).

References

Azad GK, Tomar RS. Proteolytic clipping of histone tails: the emerging role of histone proteases in regulation of various biological processes. Mol Biol Rep 2014 [epub ahead of print].

Belaaouaj A, McCarthy R, Baumann M, Gao Z, Ley TJ, Abraham SN, et al. Mice lacking neutrophil elastase reveal impaired host defense against gram negative bacterial sepsis. Nat Med 1998;4:615–8.

Davie JR, Numerow L, Delcuve GP. The nonhistone chromosomal protein, H2A-specific protease, is selectively associated with nucleosomes containing histone H1. J Biol Chem 1986;261:10410–6.

Deal RB, Henikoff JG, Henikoff S. Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. Science 2010;328:1161–4.

Duncan EM, Allis CD. Errors in erasure: links between histone lysine methylation removal and disease. Prog Drug Res/Fortschritte der Arzneimittelforschung/Progres des recherches pharmaceutiques 2011;67:69–90.

Duncan EM, Muratore-Schroeder TL, Cook RG, Garcia BA, Shabanowitz J, Hunt DF, et al. Cathepsin L proteolytically processes histone H3 during mouse embryonic stem cell differentiation. Cell 2008;135:284–94.

Eickbush TH, Moudrianakis EN. The histone core complex: an octamer assembled by two sets of protein–protein interactions. Biochemistry 1978;17:4955–64.

Eickbush TH, Watson DK, Moudrianakis EN. A chromatin-bound proteolytic activity with unique specificity for histone H2A. Cell 1976;9:785–92.

Eickbush TH, Godfrey JE, Elia MC, Moudrianakis EN. H2a-specific proteolysis as a unique probe in the analysis of the histone octamer. J Biol Chem 1988;263:18972–8.

Elia MC, Moudrianakis EN. Regulation of H2a-specific proteolysis by the histone H3:H4 tetramer. J Biol Chem 1988;263:9958–64.

Groth I, Alban S. Elastase inhibition assay with peptide substrates – an example for the limited comparability of in vitro results. Planta Med 2008;74:852–8.

Ivanov A, Pawlikowski J, Manoharan I, van Tuyn J, Nelson DM, Rai TS, et al. Lysosome-mediated processing of chromatin in senescence. J Cell Biol 2013;202:129–43.

Korkmaz B, Moreau T, Gauthier F. Neutrophil elastase, proteinase 3 and cathepsin G: physicochemical properties, activity and physiopathological functions. Biochimie 2008;90:227–42.

Lane AA, Ley TJ. Neutrophil elastase cleaves PML-RARalpha and is important for the development of acute promyelocytic leukemia in mice. Cell 2003;115:305–18.

Lestienne P, Bieth JG. Activation of human leukocyte elastase activity by excess substrate, hydrophobic solvents, and ionic strength. J Biol Chem 1980;255:9289–94.

Lestienne P, Bieth JG. Inhibition of human leucocyte elastase by polynucleotides. Biochimie 1983;65:49–52.

Mandal P, Azad GK, Tomar RS. Identification of a novel histone H3 specific protease activity in nuclei of chicken liver. Biochem Biophys Res Commun 2012;421:261–7.

Minami J, Takada K, Aoki K, Shimada Y, Okawa Y, Usui N, et al. Purification and characterization of C-terminal truncated forms of histone H2A in monocytic THP-1 cells. Int J Biochem Cell Biol 2007;39:171–80.

Okawa Y, Takada K, Minami J, Aoki K, Shibayama H, Ohkawa K. Purification of N-terminally truncated histone H2A-monoubiquitin conjugates from leukemic cell nuclei: probable proteolytic products of ubiquitinated H2A. Int J Biochem Cell Biol 2003;35:1588–600.

Osley MA. Epigenetics: how to lose a tail. Nature 2008;456:885–6.

Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. J Cell Biol 2010;191:677–91.

Qian MX, Pang Y, Liu CH, Haratake K, Du BY, Ji DY, et al. Acetylation-mediated proteasomal degradation of core histones during DNA repair and spermatogenesis. Cell 2013;153:1012–24.

Santos-Rosa H, Kirmizis A, Nelson C, Bartke T, Saksouk N, Cote J, et al. Histone H3 tail clipping regulates gene expression. Nat Struct Mol Biol 2009;16:17–22.

Schilling O, Barre O, Huesgen PF, Overall CM. Proteome-wide analysis of protein carboxy termini: C terminomics. Nat Methods 2010;7:508–11.

Timmer JC, Zhu W, Pop C, Regan T, Snipas SJ, Eroshkin AM, et al. Structural and kinetic determinants of protease substrates. Nat Struct Mol Biol 2009;16:1101–8.

Verspurten J, Gevaert K, Declercq W, Vandenabeele P. SitePredicting the cleavage of proteinase substrates. Trends Biochem Sci 2009;34:319–23.

Vogler C, Huber C, Waldmann T, Ettig R, Braun L, Izzo A, et al. Histone H2A C-terminus regulates chromatin dynamics, remodeling, and histone H1 binding. PLoS Genet 2010;6:e1001234.

Watson DK, Moudrianakis EN. Histone-dependent reconstitution and nucleosomal localization of a nonhistone chromosomal protein: the H2A-specific protease. Biochemistry 1982;21:248–56.

EST Profile viewer for NE: http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Hs.99863