Measurement of acetylation turnover at distinct lysines in human histones identifies long-lived acetylation sites

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Histone acetylation has long been determined as a highly dynamic modification associated with open chromatin and transcriptional activation. Here we develop a metabolic labelling scheme using stable isotopes to study the kinetics of acetylation turnover at 19 distinct lysines on histones H3, H4 and H2A. Using human HeLa S3 cells, the analysis reveals 12 sites of histone acetylation with fast turnover and 7 sites stable over a 30 h experiment. The sites showing fast turnover (anticipated from classical radioactive measurements of whole histones) have half-lives between \( \sim 1-2 \) h. To support this finding, we use a broad-spectrum deacetylase inhibitor to verify that only fast turnover sites display 2–10-fold increases in acetylation whereas long-lived sites clearly do not. Most of these stable sites lack extensive functional studies or localization within global chromatin, and their role in non-genetic mechanisms of inheritance is as yet unknown.
In eukaryotic cells, 147 bp of DNA is tightly wrapped around a histone octamer, consisting of one copy of H3-H4 tetramer and two copies of H2A-H2B dimer, to form the nucleosome. In the 'beads on a string' model, the repeating nucleosomes and flanking 'linker' DNA form a 10-nm fibre with histone H1 stabilizing the structure. It has been suggested that a chain of nucleosomes is further folded into a 30-nm fibre in the so-called 'solenoid' or 'double start' structure. Many positively charged lysines in histones are believed to have critical roles in this packaging process by neutralizing the negative change of phosphate in the DNA. However, the nucleosome structure occludes DNA from many cellular processes, including transcription, DNA replication and DNA repair. To enable dynamic access to the packaged DNA, cells have evolved a set of specialized chromatin remodelling complexes. One class of remodelers including SWI/SNF, ISWI and CHD alters DNA packaging by sliding, ejecting, or reorganizing nucleosomes in an ATP-dependent manner. Another class achieves their function by adding or removing covalent modifications on histone tails. These histone post-translational modifications include acetylation (ac), monoubiquitylation (ub1) and so on. Acetylation in particular negates the charge on ε-amino groups of lysine residues, therefore changing chromatin folding as the direct consequence of altering the histone–DNA or histone–histone electrostatic interactions. Some histone modifications exert their remodelling effects by recruiting chromatin remodelling factors. Importantly, acetylation can have both effects as it serves as a recognition site for the bromodomain, a motif common in ATP-dependent remodelers.

It has been known for >40 years that there are multiple acetylation sites in the four core histones. The levels of acetylation are regulated by two opposite enzymatic reactions that either transfers or removes the acetyl moiety of acetyl-CoA to the ε-amino group of lysine side chains. These reversible processes are performed by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Acetylation has long been associated with transcriptional activation and HATs have been identified as transcriptional coactivators. In contrast, histone deacetylation is generally thought to have roles in transcriptional repression and HDACs have been identified as transcriptional corepressors. Numerous in vitro acetylation assays and in vivo gene inactivation studies in past decades have suggested the existence of a specific regulatory mechanism based on the substrate specificity of HATs. Based on these results, one would expect diverse genomic distribution patterns for different histone acetylation marks accompanied with preferential binding of each HAT or several HATs as the results of substrate specificity. On the contrary, the genome-wide mapping of 18 acetylation marks showed that they were targeted to enhancers, promoters and actively transcribed gene bodies with only subtle differences. Also, the same approach demonstrated five HATs (p300, CBP, MOF, PCAF and Tip60) and four HDACs (HDAC1, 2, 3 and 6) were recruited to the hyperacetylated regions, contradicting previous findings that HDACs are targeted primarily to transcriptionally inactive regions. In addition to the localization in active genes, HATs and HDACs were found to be transiently recruited to poised genes marked by H3K4 methylation for future activation.

Beyond studying their modifying enzymes or genomic locations, analysing the kinetics of these modifications has been proven to be very informative. We and others have measured the site-specific kinetics of histone methylation by quantitative mass spectrometry using stable isotope labelled methionine as a tracer. As methionine can be metabolized to S-adenosyl methionine, the direct methyl group donor for the methylation reaction, the labelling is very efficient. By contrast, labelling acetylation is more difficult since cells use multiple metabolic pathways to generate acetyl-CoA. Dozens of studies in the past 30 years have used radioactive acetate as a tracer to estimate the turnover of acetylation on whole histones. As summarized by Waterborg, these studies identified fast (half-life of few min.) and slow turnover rates (half-life of ~30 min.) in all four core histones from yeast to plant and animal cells. However, these results provided an estimation of overall acetylation turnover in each core histone without site-specific information. In addition, these rates appear faster than would be needed to account for the rate observed for transcription.

To address these issues, we developed a new metabolic labelling method to study acetylation kinetics at specific lysines using quantitative target mass spectrometry via selective reaction monitoring (SRM). This first report of acetylation half-lives is selective for pools of old histone by virtue of a double labelling scheme and revealed a subset of sites that are surprisingly long-lived.

**Results**

**Measurement of acetylation turnover in old histone.** Acetyl-CoA is generated from multiple sources, including glucose, pyruvate, glutamine and fatty acids. Using mammalian cells in culture, the main source of acetyl-CoA for histone acetylation was found to be citrate generated during glucose metabolism. Therefore, we used 13C6-glucose (simply referred to here as ‘heavy glucose’) as a source of isotope tracers that via acetyl-CoA would be introduced into the acetyl groups of histones. However, the other sources of acetyl-CoA mean that only some fraction of the acetyl-CoA pool can be labelled by a single source, hence lowering the observed appearance rate of new acetyl groups. However, this effect can be mathematically modelled and the true appearance rate determined for reaching steady-state levels of labelled acetylation (Methods). We also investigated labelling using 2,3 13C2-pyruvate (see below), but only achieved ~70% the labelling level of glucose which itself was near 80% (Supplementary Fig. S1). Both reagents will be converted to acetyl-CoA with a 2 Da 'label' in acetyl groups that is transferred to lysine side chains (Fig. 1b). Our labelling scheme as shown in Fig. 1a includes two labelling steps. First, histones were exhaustively labelled by growing in media containing [13C6]glucose which itself was near 80% in an ideal condition when acetyl-CoA is 100% labelled. Given the rate observed for transcription, all the acetylation being installed before the switch will have light acetyl group (referred as old ac) while the new acetylation will carry a +2 Da acetyl group (referred as new ac) in an ideal condition when acetyl-CoA is 100% labelled. Given the metabolic complexities of new histone synthesis on mixed isotopes, we limited our measurements to old histone that was synthesized before the media switch to track the rate of old acetyl groups being replaced by new (Supplementary Methods).

**Appearance of new acetyl in H3K14ac but not in H3K4ac.** Different from the ‘SILAC’ approach where stable isotopes are used to measure protein expression ratios, we used them here to distinguish old versus new histone while also initiating a temporal pulse of new acetyl-CoA in live cells (Fig. 1a). As shown in Fig. 2a, a fast appearance of new acetyl was observed in H3K4ac from old histone when the K9 in the same peptide was unmodified. The +2 Da mass shift in new acetyl allows discrimination of new from old acetylation even in the 30 h sample with the least intense signals of all the six time points.
Figure 1 | Schematic of experimental workflow for the measurement of histone acetylation turnover. (a) The approach involves two steps of stable isotope labelling to detect old versus new acetylation events specifically on old histones. The workflow begins with exhaustive labelling of cells in heavy arginine media to label the histone backbone (depicted as a dark oval), followed by media exchange to label newly installed acetyl groups in lysine residues.

Fast increase of new acetyl in a subset of acetylation sites. To account for slight variations in absolute loading amounts, two types of normalization schemes, called relative levels and distributive levels, were used in this paper (see formulas in the Methods section). Relative level is the signal for a new acetylation for a particular site normalized against the signals from all the peptides from histone H3 except H3K27-K36 were conserved is similar (Supplementary Fig. S1). It is worth noting that all the acetylation sites. Group I, the 10 sites with fast increases of new acetylation (relative and distributive), had half-lives ranging from 0.8–2.3 h (Table 1; also see Methods and Supplementary Fig. S2 for details of data fitting). It is worth noting that labeled population is only equal to the labelling efficiency of acetyl-CoA pool when all the population of this site is turned over. The fact that the labeled population varied among sites suggests the likely existence of the subpopulation with very low or no turnover in those sites having low levels of labeled population (such as H3K27ac).

The acetylation sites H4K20 and H3.3-K36 exhibited a distinct behaviour and were therefore classified separately as Group II (Fig. 3). At these sites, there is a sharp reduction in the overall acetylation level, yet no appreciable new acetylation detected (see Fig. 3 panels for H4K20 and H3.3-K36). Also at these sites, increasing methylation contributed in part to the observed decrease of overall acetylation signal (Supplementary Methods). For Group III containing seven sites (Table 1), no change of distributive levels of new acetylation was observed (that is, no evidence for acetylation turnover in the 30 h experiment; see Fig. 3b). To further confirm our findings, we performed the same experiment using heavy pyruvate as the tracer. Despite dropping a few sites in the assay due to a lower labelling efficiency (~60% versus 80% for glucose) and higher noise levels, the overall trend is similar (Supplementary Fig. S1). It is worth noting that all the peptides from histone H3 except H3K27-K36 were conserved among the three H3 variants (H3.1, H3.2, and H3.3); therefore results reported here represent the average turnover for these three variants. The levels determined are shown in Table 1 and ranked according to their turnover rate within Group I.
Butyrate-induced acetylation is restricted to Group I sites. To confirm our findings, we measured histone acetylation levels for all 19 sites in response to broad-spectrum HDAC inhibition. Both relative levels and fold changes were reported and the results shown in Supplementary Fig. S3 for the time course data obtained from cells treated with sodium butyrate. To avoid secondary effects from cell cycle arrest caused by butyrate treatment, the fold change of acetylation levels in 6 h of treatment was used in Table 1 for comparison to rates of turnover. Strikingly, only Group I sites showed between 2 and 10-fold increases in acetylation stoichiometry. In addition, the response to butyrate treatment showed a similar trend to the half-lives of fast turnover sites (Fig. 4a). All the Group III sites with no turnover showed <1.1 fold or lower response to butyrate. This strongly corroborates our kinetic findings that turnover of these sites is in fact extremely low.

Discussion

An overall summary of the rates of acetylation turnover mapped onto sites on histone tails is provided in the Fig. 5 diagram. No correlation between turnover rate and the normal levels of acetylation was found except that the three most abundant acetylation sites (H3K14ac, 28%; H3K23ac, 25%; H4K16ac, 36%) all had fast turnover. Other than that, turnover rates for sites with low acetylation stoichiometry could be found in all three different groups (Table 1 and Fig. 4b).

Genome-wide mapping of HATs and HDACs by ChIP-seq have shown both of them are enriched in active genes and appear to positively correlate with transcription levels9. This suggests the removal of acetylation in active genes to reset chromatin modification after gene transcription. Such observations are consistent with a well-established model in Saccharomyces cerevisiae where the Rpd3S histone deacetylase complex is recruited to H3K36me2/3-decorated chromatin (methylated by Set2) to deacetylate nucleosomes behind elongating RNA Pol II to prevent cryptic initiation of transcription within the coding region23. In addition to deacetylation of nucleosomes within the gene body, the Set3 histone deacetylase complex can also be recruited by H3K4me2 (methylated by Set1) to deacetylate 5’ transcribed regions24. Different from yeast HDAC complex, the mammalian counterpart lacks the chromodomains for the direct targeting to H3K36me2/3 chromatin. However, both Tip60 and HDAC6 can be targeted to active genes through the interaction with Pol II (ref. 9).

The likely outcome of targeting both HDACs and HATs to actively transcribed genes will be a high turnover rate of histone acetylation in these regions. Indeed, we observed very fast turnover in 12 acetylation sites (Group I and II, in Table 1) with half-lives ranging from 0.8 to 2.3 h. In addition, all these marks except H4K20ac have been shown to be localized in promoters/enhancers, and/or active transcribed gene bodies with positive correlations to transcriptional activity4. As mentioned in the Introduction, previous pulse/chase studies using radioactive acetate have determined overall acetylation of H3 and H4 to be as fast as 2–3 min. in the fast phase and ~30 min. in the slow phase. Considering the transcriptional elongation rate of 0.3–0.8 kb min$^{-1}$ determined in mammalian cells by imaging the transcription of a single gene25, it takes ~6–15 min to transcribe through a 5 kb small gene. With the additional time required for transcription initiation, it is improbable for one cycle of removal of acetylation in the gene bodies for all the genes to be as fast as several minutes. Therefore, it is possible that previous studies using radioactive acetate selectively labelled histones in highly transcribed genes during the short pulse due to a
limited pool of labelled acetyl-CoA. This contrasts with our half-lives, determined with steady-state levels of acetyl-CoA as indicated by the stable population of labelled histones at each lysine in Group I. As shown in Fig. 3b, the highest labelling efficiency is ~80%, implying that at least 80% of acetyl-CoA pool is labelled by the glucose labelling scheme reported here.

Figure 3 | Appearance of new acetyl group in 19 acetylated lysines using $^{13}$C$_6$-glucose as a tracer. (a) Relative levels of both new acetylation (occurred after the media switch; blue diamonds) and all acetylation (both old and new; red squares) in 19 acetylated sites. These levels were determined by normalizing against overall signal from all measured modified species (see Methods). (b) Distributive levels of new acetylation for 19 acetylated sites were determined by dividing the peak area of new acetylation by that of total acetylation present at each particular site. The ten fast sites reaching a steady plateau are shown in upper panel, while the sites with no detectable new acetylation are displayed in the lower panel. Data from two biological replicates with three technical replicates of each were reported with s.e.m. values shown as error bars.
Table 1 | Site-specific acetylation turnover and half-lives for 19 acetylation sites in core histones.

| Group | Rank | Acetylation site | Turnover rate (h⁻¹) | T₁/₂ (h) | Labelled population Acetylation stoichiometry | Acetylation overall methylation stoichiometry * | Response to Butyrate (fold change) |
|-------|------|------------------|---------------------|---------|-----------------------------------------------|-----------------------------------------------|-------------------------------|
| I     | 1    | H2AK9            | 0.82 (± 0.09)       | 0.8 (± 0.1) | 0.67 (± 0.02)                                | 0.9%                                          | 10.8                          |
|       | 2    | H3K14            | 0.67 (± 0.08)       | 1.0 (± 0.1) | 0.591 (± 0.006)                              | 27.8%                                         | 2.49                          |
|       | 3    | H3K18            | 0.65 (± 0.07)       | 1.1 (± 0.1) | 0.698 (± 0.005)                              | 4.1%                                          | 5.6                           |
|       | 4    | H3K23            | 0.64 (± 0.09)       | 1.1 (± 0.1) | 0.67 (± 0.01)                                | 25.1%                                         | 2.4                           |
|       | 5    | H3.3-K27         | 0.61 (± 0.02)       | 1.1 (± 0.1) | 0.187 (± 0.008)                              | 2.7%                                          | 58%                           |
|       | 6    | H2AK5            | 0.6 (± 0.2)         | 1.3 (± 0.4) | 0.497 (± 0.006)                              | 9.9%                                          | 3.4                           |
|       | 7    | H4K16            | 0.4 (± 0.2)         | 1.7 (± 0.8) | 0.6 (± 0.2)                                 | 35.7%                                         | 2.14                          |
|       | 8    | H3K23            | 0.4 (± 0.1)         | 2.0 (± 0.5) | 0.4927 (± 0.008)                            | 2.2%                                          | 60%                           |
|       | 9    | H4K5             | 0.36 (± 0.07)       | 2.0 (± 0.4) | 0.42735 (± 0.00007)                          | 12.7%                                         | 4.5                           |
|       | 10   | H3.1/2-K27       | 0.32 (± 0.08)       | 2.3 (± 0.6) | 0.308 (± 0.009)                              | 1.5%                                          | 70%                           |
| II    | 1    | H4K20            | 1.1 (± 0.9)         | 1.0 (± 0.8) | N/A                                           | 0.2%                                          | 95%                           |
|       | 2    | H3.3-K36         | 0.6 (± 0.2)         | 1.2 (± 0.4) | N/A                                           | 6.9%                                          | 81%                           |
| III   | 1    | H2AK13           | Undetectable       | Undetectable | Undetectable | 6.4%                                          | N/A                           |
|       | 2    | H2AK36           | Undetectable       | Undetectable | Undetectable | 3.7%                                          | N/A                           |
|       | 3    | H3K4             | Undetectable       | Undetectable | Undetectable | 3.6%                                          | N/A                           |
|       | 4    | H2AK15           | Undetectable       | Undetectable | Undetectable | 3.2%                                          | N/A                           |
|       | 5    | H3K79            | Undetectable       | Undetectable | Undetectable | 3.1%                                          | N/A                           |
|       | 6    | H3K56            | Undetectable       | Undetectable | Undetectable | 2.5%                                          | N/A                           |
|       | 7    | H3K122           | Undetectable       | Undetectable | Undetectable | 1.9%                                          | N/A                           |

Turnover in Group I sites were determined by fitting distributive levels of new acetylation with an exponential model and ranked according to the half-life (from small to large). Mean from two independent fittings of two biological replicates was determined and the s.d. shown inside parenthesis. Acetylation and methylation (if present) stoichiometry were determined from unlabelled asynchronous HeLa cells. For H3 variant-specific sites (for example, H3.3-K36), stoichiometry was calculated within H3.1/2 or H3.3 variants. Turnover values in Group II sites were determined by fitting relative levels of overall acetylation with an exponential model. No detectable turnover was found for sites in Group III, and they were ranked according to their acetylation stoichiometry.

*Overall methylation stoichiometry: the percentages shown for each lysine site were calculated by summing all forms of methylation together (me1 + me2 + me3).

Fold change of acetylation stoichiometry was determined in response to sodium butyrate at 0 versus 6 h (full data set shown in Supplementary Fig. S3c).

Figure 4 | Correlation analyses of acetylation half-lives. (a) Half-lives of acetylation sites with fast turnover correlated with their response to treatment with sodium butyrate. (b) Correlation of T₁/₂ values with the absolute stoichiometry of acetylation for each site. Note that half-lives for sites with undetectable turnover were all set to > 30 h (x-axis, lower right). Both vertical and horizontal error bars indicate the s.d. from their respective analyses.

Figure 5 | Summary of acetylation turnover rates at 19 distinct lysine residues within core histones. Illustrations showing known lysine acetylation sites in four core histones as ‘K’ followed by amino acid residue number. The globular domains are marked by grey bars. Fast turnover sites are overlaid with green and blue arrows for Group I and II sites, respectively. Stable sites (Group III) are indicated by red stop signs. Sites not measured in this study are labelled as ND.
Seven sites showed no significant increase of new acetylation or decrease of old acetylation and were categorized as Group III as ‘no detectable turnover’. Except H3K4ac and H3K56ac, there are precious few functional studies to draw from for these Group III sites. Genome-wide mapping studies of H3K4ac in both human and S. cerevisiae have determined H3K4-ac is localized at active gene promoters6,26. A genetic screen in yeast has determined deacetylation of H3K4 is mainly performed by Hst1 and Sir2 (to lesser extent) in euchromatin, and only dependent on Sir2 in heterochromatin25,26. Importantly, both Hst1 and Sir2 are NAD-dependent deacetylases belonging to the class III HDAC family. Acetylation within the globular core domain of histone H3 on K56 has been shown to have a critical role in packaging DNA into chromatin following DNA replication and repair in budding yeast27. Similar to H3K4ac, deacetylases of H3K56ac are also known to be NAD-dependent class III HDACs, namely Hst3 and Hst4 (refs 28,29). Very interestingly, a puzzling result has been reported recently that levels of K56ac were not affected by all the tested HDAC inhibitor targeting class I/II or class III (ref. 30). This result can be explained by our observation of extremely slow turnover of H3K56ac. In addition to Group III sites, Group I sites might also have stable subpopulations. For example, H3K27ac from both canonical H3.1/2 and the H3.3 variant showed low labelling levels (30 and 20%, respectively), suggesting more than half of K27ac could be stable.

The observation that acetylation turnover could be twofold faster when adjacent sites are both acetylated (that is, for K9/K14 on H3) but ~50% slower when the neighbouring site was methylated (Supplementary Fig. S4) highlights the regulatory effects of nearby modifications. Such observations can be explained by either promiscuous behaviour of HATs and HDACs, or intentional recruitment or occlusion of such enzymes at specific genomic locations. The higher turnover of dual-acetylated H3 at both K9 and K14 could be caused by higher local HAT/HDAC concentrations in the chromatin region where they are present. In addition, the observation of H3.3 variant-specific K36ac suggested that its cognate HAT/HDAC pair(s) have superior substrate specificity capable of discriminating the single amino acid changes among the H3 variants in this region of sequence (localized five residues away from K36; see Fig. 5, Supplementary Fig. S5a and Supplementary Methods). With more genetic alterations of HATs being identified in cancers and the use acetylation modulators (for example, HDACi, HAT inhibitors, bromodomain inhibitors) increasing in cancer research31, the results described here provide a new context to understand and measure the pharmacokinetic behaviour of different acetylation sites in vivo.

The importance of acetylation is not limited to histones, as it appears as widespread as phosphorylation in intracellular signalling22. It should not escape our attention that high efficiency metabolic labelling of acetyl-CoA provides a useful tool for functional studies of protein acetylation generally. For example, what is the turnover rate of acetylation in p53 when DNA damage is induced?

Methods

Stable isotope tracer labelling and sample preparation. HeLa S3 cells were grown in adherent for 7 days at 37 °C in heavy DMEM media (omission of arginine, AthenaES) supplemented with [13C6]arginine (99% enriched, Cambridge Isotope Laboratories) and 10% dialyzed fetal bovine serum before release into glucose and sodium pyruvate free DMEM plus 10% dialyzed fetal bovine serum supplemented with [13C6]glucose (2 g l−1 99% enriched, Cambridge Isotope Laboratories) or [2,3-13C2]pyruvate (10 mM, 99% enriched, Cambridge Isotope Laboratories). Cells were washed twice with phosphate buffered saline to remove old media before releasing cells into new media. Stable isotope labelled cells were harvested at time points of 0, 0.25, 1.5, 3, 6 and 24 h after the media switch and 5 mM sodium butyrate treated cells at 0, 1, 5, 3, 6 and 24 h before collection and flash freezing in liquid N2. Histones from acid extraction of nuclei were derivatized using propionic anhydride and digested with trypsin, as described previously13,14.

Nano-capillary liquid chromatography triple quadrupole mass spectrometry (nLC-QqQ MS). The digested and derivatized histone peptides were diluted in 0.1% TFA and injected to nLC-QqQ MS (Dionex nanoLC and a ThermoFisher Scientific TSQ Quantum). Peptides were first loaded to a trapping column (2 cm × 150 μm) and then separated with an analytical capillary column (10 cm × 75 μm). Both were packed with Magic C18 resin (Michrom). The chromatograph gradient was achieved by increasing percentage of buffer B from 2–35% at a flow rate of 0.35 μl min−1 (A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile) over 40 min. The peptides were then introduced into QqQ MS by electrospray from an emitter with 10 μm tip (New Objective) as they were eluted from capillary column. The QqQ settings were as follows: collision gas pressure of 1.5 mTorr; Q1 peak width of 0.7 (FWHM); cycle time of 3.5 s; skimmer offset of 10 V; electrospray voltage of 2.6 kV.

SRM method development and mass spectral data analysis. SRMs capable of discriminating 19 acetylation sites from 13 different histone peptides were developed with two to four fragment ions for each species. Various histone peptides with different modifications were purchased or custom synthesized from AnaSpec or Genescript to assist the method development. Data were analysed in Skyline with Savitzky–Golay smoothing32. All labelled and unlabelled peaks were grouped as a whole for manual peak determination to avoid bias. Total peak areas from SRMs were used for quantification (as listed in Supplementary Table S1).

Peptide nomenclature and data handling. All the peptides with their modifications that were quantified in this study are listed in Supplementary Table S1. In this paper, we simply used the position of lysine that can be acetylated in the protein to refer the peptide. For example, T3KQTAR8 was referred to as the ‘H3K4 peptide’ and K9STGGKAPR17 as the ‘H3K9-K14 peptide’. For the purpose of determining acetylation turnover, three forms were measured for the peptide targeted by mono-acetylation (H3K56, H3K122 and H2A26): unmodified (chemical derivatized by propionylation, +56 Da); acetylated (light and heavy, +42 and +44 Da); six forms were measured for the peptide targeted by both acetylation (ac) and methylation (me) to the single lysine: unmodified, acetylated (light and heavy), methylated and acetylated (light and heavy), me1, me2 and me3 (H3K4, H3K9, and H4K20); nine forms were measured for the peptide targeted by di-acetylation to two lysines (H3K18-K23, H2AK5-K9 and H2AK13-K15); 18 forms were measured for H3K9-K14 (K9 is methylated and acetylated, K14 is acetylated). Multiple channels of measurement were aggregated together for each site for those peptides with many modification states. For example, five channels of K14ac with K9 carrying un, me1, me2, me3 or ac, were summed together to represent the total level of K14ac. Finally, for histone H4 we only measured H4K5ac and H4K6ac because the two internal acetylation sites (H4K8ac and H4K12ac) were difficult to measure with SRM.

Distributive level = new acetylation/(new + old acetylation) (1) Relative levels = new acetylation/all modifications on peptide (2) Two quantification schemes were used in this study, differed simply by which pool of peptides was used to normalize the signal for any given acetyl mark10,14. Distributive level is the term used to refer to the normalized signal of heavy acetylation within each acetylation site (that is, the signal of heavy ac is divided by the combined signal of heavy plus light ac). Relative level is used to indicate signals from all the modification species found at a particular site are summed as total (ac site: unmodified + light ac + heavy ac; ac + me site: unmodified + me1 + me2 + me3 + light ac + heavy ac). Recall that heavy acetylation is new and light acetylation is old.

Determination of acetylation turnover rates and half-lives. An exponential decay model with three parameters was used to fit the data in Group I using the distributive levels of new acetylation (with all six time points). In the equation, new acetylation P* (1-exp(-k*t)) + n, P represents the labelled pool of acetylation in each site, k for the turnover rate, and n for the noise (initial value is not zero; see Supplementary Fig. S2a). The curve fitting was performed by the ecolot software package in Matlab (version 7.10). A different model (total acetylation = a*exp(-k*t) + c) was used to fit the data in Group II using relative levels of total acetylation, where parameter a is the initial level of acetylation in old histone, c for stable level of acetylation and k for the turnover rate of deacetylation (Supplementary Fig. S2b). The half-life of turnover is calculated by ln(2)/k.

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Acknowledgements
This work was supported by Northwestern University, the Chicago Biomedical Consortium with support from the Searle Funds at The Chicago Community Trust, a gift from the Zell Family to the Robert H. Lurie Comprehensive Cancer Center, NIH R01 GM 067193-09 (to N.L.K.), and the Northwestern University Physical Sciences Oncology Center (U54 CA 143869).

Author Contributions
Y.Z. and N.L.K. designed research; Y.Z. performed research; Y.Z., P.M.T and N.L.K. analyzed data; and Y.Z. and N.L.K. wrote the paper.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications
Competing financial interests: The authors declare no competing financial interests.
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How to cite this article: Zheng, Y. et al. Measurement of acetylation turnover at distinct lysines in human histones identifies long-lived acetylation sites. Nat. Commun. 4:2203 doi: 10.1038/ncomms3203 (2013).