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Unique yeast histone sequences influence octamer and nucleosome stability

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Yeast nucleosomes are known to be intrinsically less stable than those from higher eukaryotes. This difference presents significant challenges for the production of yeast nucleosome core particles (NCPs) and chromatin for *in vitro* analyses. Using recombinant yeast, human, and chimeric histone proteins, we demonstrate that three divergent amino acids in histone H3 ($Q_{120}K_{121}K_{125}$) are responsible for the poor reconstitution of yeast histones into octamers. This QKK motif is only found in Fungi, and is located at the nucleosome dyad axis. Yeast-to-human changes at these positions render yeast histones amenable to well-established octamer reconstitution and salt dialysis methods for generating nucleosomal and longer chromatin templates. By contrast, the most divergent yeast core histones, H2A and H2B, affect the biophysical properties of NCP but not their stability. An evolutionary analysis of H3 sequences shows that a gradual divergence in H3 sequences occurred in Fungi to yield QKK in budding yeast. This likely facilitates the highly euchromatic nature of yeast genomes. Our results provide an explanation for the long recognized difference in yeast nucleosome stability and they offer a simple method to generate yeast chromatin templates for *in vitro* studies.

Keywords: chromatin; nucleosome; yeast

The nucleosome core particle (NCP) represents the most elementary structural subunit of chromatin. It consists of about 145–147 bp of DNA wrapped in one and three quarter left-handed superhelical turns about a heterotypic histone core octamer consisting of a histone H3-H4 tetramer flanked by two adjacent histone H2A-H2B dimers [1]. The dimerizing histone fold domain (HFD) [2] plays a critical role in the maintenance of the histone octamer integrity. In the last approximately 20 years since the first detailed crystallization of the NCP [3], a plethora of studies have ensued in the characterization of NCPs containing histone variants from different organisms including yeast [4]. However, in contrast to the NCP from higher eukaryotes, the yeast NCP has been less extensively characterized and studies in solution are lacking.

Early work by Lohr and Hereford had initially found that in the budding yeast *Saccharomyces cerevisiae*, where 40–60% of the genome is transcribed during the logarithmic growth phase [5], chromatin exhibited a uniform overall DNase1 chromatin sensitivity which was similar to that of transcriptionally poised regions in higher eukaryotes [6]. Thus, the budding yeast chromatin seemed to exhibit an

Abbreviations
AUC, analytical ultracentrifuge; AUT, acetic acid-urea-triton; HFD, histone fold domain; NCPs, nucleosome core particles; NJ, neighbor-joining.

uncharacteristic 'transcriptionally" poised state likely as a result of more permissive, less stable yeast NCP organization. In search of such instability, the first attempt to a biophysical characterization of the yeast NCP [7] showed that the particle exhibited a higher elipticity and had a markedly low DNA melting point of 68 °C. As well, yeast NCPs were particularly sensitive to freezing and thawing at low ionic strength [7]. These observations are indicative of the DNA being less tightly constrained by the yeast histones in the NCP [7]. These results were subsequently confirmed by other groups that also showed that in solution, the native yeast NCP exhibited a salt-dependent instability [8].

The native yeast octamer was not isolated until 1994 [9] and a crystallographic structure of the yeast NCP, using purified recombinant histones was published 7 years later [4]. The high-resolution image suggested that the yeast nucleosome was likely to be 'subtly' destabilized mainly as a result of changes in the L1 loop of the HFD of histone H2A. Histone H2A-H2B dimers in the NCP interact at the interface generated by the L1 loops of the adjacent H2A molecules. Two amino acid changes within this region H42Q and V44I result in a loss of the hydrogen bond interactions at this interface [4].

It is important to note that the main component of yeast histone H2A corresponds to histone H2A.X variant [10] and yeast H3 is a histone H3.3 variant [11]. Both variants have been involved in NCP destabilization [12,13]. Histone H3 has been shown to possess a high diversity of variants acquired after species separation which in metazoans plays an important role in the tissue-specific regulation of gene expression [14].

The yeast H3 QKK motif was recently shown to impact nucleosome positioning *in vivo* as well on synthetic nucleosome arrays [15]. Whether this effect is due to changes in nucleosome-nucleosome interactions, or altered NCP-DNA affinity is not clear. In the present article, we further characterize the impact of this motif on yeast histone octamer and NCP stability. We demonstrate that this motif gradually evolved in Fungi, where it likely contributes to the 'open' and mostly euchromatic architecture of these genomes. As yeast-to-human changes at these three positions generate a yeast H3 that readily is incorporated into octamers with yeast H4, H2A, and H2B, our data provide means to generate yeast NCPs and chromatin arrays [15] using standard salt dialysis methods [16]. Such reagents should be useful for the biochemical characterization of chromatin-associated enzymes from Fungi.

Materials and methods

Recombinant histones

Histones were expressed in *E. coli* BL21 (DE3) and purified to homogeneity through acid extraction, ion exchange (Macro-Prep Ion Exchange Media; BioRad, Mississauga, ON, Canada) and reverse phase HPLC (C18-300, 250 mm × 4.6 mm, 5 μm, ACE). Site-directed mutagenesis of histones was performed as per standard protocols.

Preparation of DNA

146 bp mixed-sequence DNA was prepared as previously described [17]. Preparation of 197 bp 601 positioning sequence DNA was as follows: a pWM530 plasmid containing a 25-repeat of 197 bp 601 array DNA [15] was digested with *EcoRV*, and plasmid backbone fragments were separated from array DNA through poly (ethylene glycol) precipitation (6% poly (ethylene glycol) 6000, 0.5 M NaCl, 1× TE, 25 000 ***g*** for 30′ at 4 °C). Separated array DNA was digested with *AvaI* to yield a single 197 bp fragment of the 601 positioning sequence.

Histone octamer and nucleosome reconstitution

Histone octamers were assembled and purified as follows: individual lyophilized histones were combined in a 1.1 : 1.1 : 1 : 1 H2A : H2B : H3 : H4 mole ratio, dissolved in unfolding buffer (7 M guanidine (Gu) HCl, 20 mM Tris pH 7.5, 1 mM DTT) and dialyzed against three changes of refolding buffer (2 M NaCl, 10 mM Tris pH 7.5, 1 mM EDTA). Reconstituted histone octamer was purified by size exclusion chromatography using a GE Akta Pure FPLC and Superdex 200 10/300 GL column (GE Healthcare Life Sciences, Mississauga, ON, Canada) at a flow rate of 0.5 $mL{\cdot}min^{-1}$ refolding buffer. Fractions containing octamer were confirmed by SDS/PAGE, pooled and concentrated. Nucleosome reconstitutions were performed using salt gradient dialysis. Briefly, purified histone octamer was mixed with DNA in RB high buffer (2 M NaCl, 10 mM Tris pH 7.5, 1 mM EDTA, 1 mM DTT) using a 1.1 : 1 octamer: DNA mole ratio, and dialyzed for 30 min at 4 °C. The final [NaCl] was reduced to 10 mM over 24 h through the addition of RB low buffer (10 mM Tris pH 7.5, 1 mM EDTA, 1 mM DTT), and reconstituted NCPs were visualized through 4% native acrylamide gel electrophoresis and ethidium bromide staining.

Micrococcal Nuclease Digestion

NCPs were dialyzed overnight against micrococcal nuclease reaction buffer (10 mM Tris pH 7.5, 50 μM $CaCl_2$) prior to digestion. Dialyzed NCPs were diluted in MNase reaction

buffer to $A_{260} = 1.5$, followed by the addition of 5 U MNase (Worthington Biochemical Corp., Lakewood, NJ, USA) per millilitre of sample. Reactions were quenched through the addition of 1 mM EDTA and subjected to phenol:chloroform extraction. Extracted DNA was visualized through 4% native acrylamide gel electrophoresis and ethidium bromide staining.

Gel electrophoresis

Acetic acid-urea-triton (AUT)-PAGE was performed as described in [18]. (SDS/PAGE) was carried out according to [19]. Native 4–6% PAGE were prepared and run in 1× E buffer (40 mM Tris pH 7.2, 1 mM EDTA, 20 mM NaOAc).

Analytical ultracentrifuge analysis

Sedimentation velocity analytical ultracentrifuge (AUC) analyses were performed in a Beckman XL-I analytical ultracentrifuge (Beckman-Coulter Instruments, Brea, CA, USA) using an An-55 Al aluminum rotor and cells with double sector aluminum-filled Epon centerpieces, then analyzed as described elsewhere [17].

Molecular evolutionary analysis of H3 sequences

Data mining experiments were performed on molecular databases including GenBank [20] and Joint Genome Institute-MycoCosm [21] in order to retrieve nucleotide histone H3 sequences corresponding to organisms representative of diverse eukaryotic groups, with special emphasis on Fungi (319 H3 sequences belonging to 185 species). Nucleotide sequence alignments were performed on the basis of translated amino acid sequences using the BioEdit program [22]. Molecular phylogenetic trees were reconstructed using the neighbor-joining (NJ) method [23] based on the obtained alignments, and the reliability of the groups defined by the topology was contrasted using bootstrap analysis (1000 replicates). All molecular phylogenetic analyses were carried out using the program MEGA version 7 [24]. The analysis of the histone H3 tri-residue configuration across eukaryotes was based on the eukaryotic tree of life described by [25].

Results

Three yeast-specific residues in histone H3 create an intrinsically unstable histone octamer

In an effort to generate recombinant chromatin substrates for yeast and human histone modification enzymes, we set out to assemble histone octamers from bacterially expressed core histones from these species (Fig. 1A, right). As expected, human core histones readily reconstitute into octamers using the standard dialysis reconstitution method [16], and size exclusion chromatography as a readout [1]. About 75–90% of core histone material is found in the octamer species that elutes at 12.5 mL (Fig. 1A left, orange line), with the remainder detected as H2A/H2B heterodimer eluting at ~ 15 mL. However, we consistently find that yeast histones assemble into octamers poorly, with typical yields of < 25% histone octamer, and the remainder eluting in H3/H4 tetramer and H2A/H2B dimer species (Fig. 1A yellow line). Thus, yeast octamers appear much less stable than human octamers. We previously used a protein-chimera approach to determine that three amino acid differences (yH3 $Q_{120}K_{121}K_{125}$; hH3 $M_{120}P_{121}Q_{125}$) near the histone H3 carboxyl-terminus are the major contributor to a divergent chromatin structure in yeast [15]. These features, which are found at the end of the third alpha helix of H3, also mediate histone octamer stability because a 'chimeric yH3' with yeast-to-human changes at these positions stabilize recombinant yeast octamers (Fig. 1A, yH3α3 h purple line). Moreover, the reciprocal is also true: human octamers assembled with yeast H3 are relatively unstable (Fig. 1B yellow line), and this destabilizing effect is lost if yH3 contains the MPQ human sequence in the third alpha helix (Fig. 1B purple). Taken together these results demonstrate that three amino acid differences in histone H3 account for the intrinsic instability of yeast octamers.

Divergent features of H2A and H2B affect hydrodynamic properties of the octamer

Histones H2A and H2B are the most divergent core histones; between yeast and humans they display 72% and 67% identity, respectively, compared to 84% and 92% for H3 and H4 [26]. To determine if differences in these proteins also influence octamer stability, we reconstituted octamers with human H3/H4 and yeast H2A/H2B (Fig. 1C, light blue line). We find that this mixture of histones reconstitutes as efficiently as the all-human complex. Of note, this 'mixed-octamer' can be distinguished from the all-human octamer, as it consistently elutes slightly earlier in preparative SEC (Fig. 1C) indicative of a more relaxed (or open) conformation. Single substitutions of yH2A or yH2B into the human octamer have intermediate effects on the efficiency of dialysis-mediated assembly *in vitro*, suggesting that sequence divergences in each of these histones influence the biophysical properties of the octamer, but not its stability.

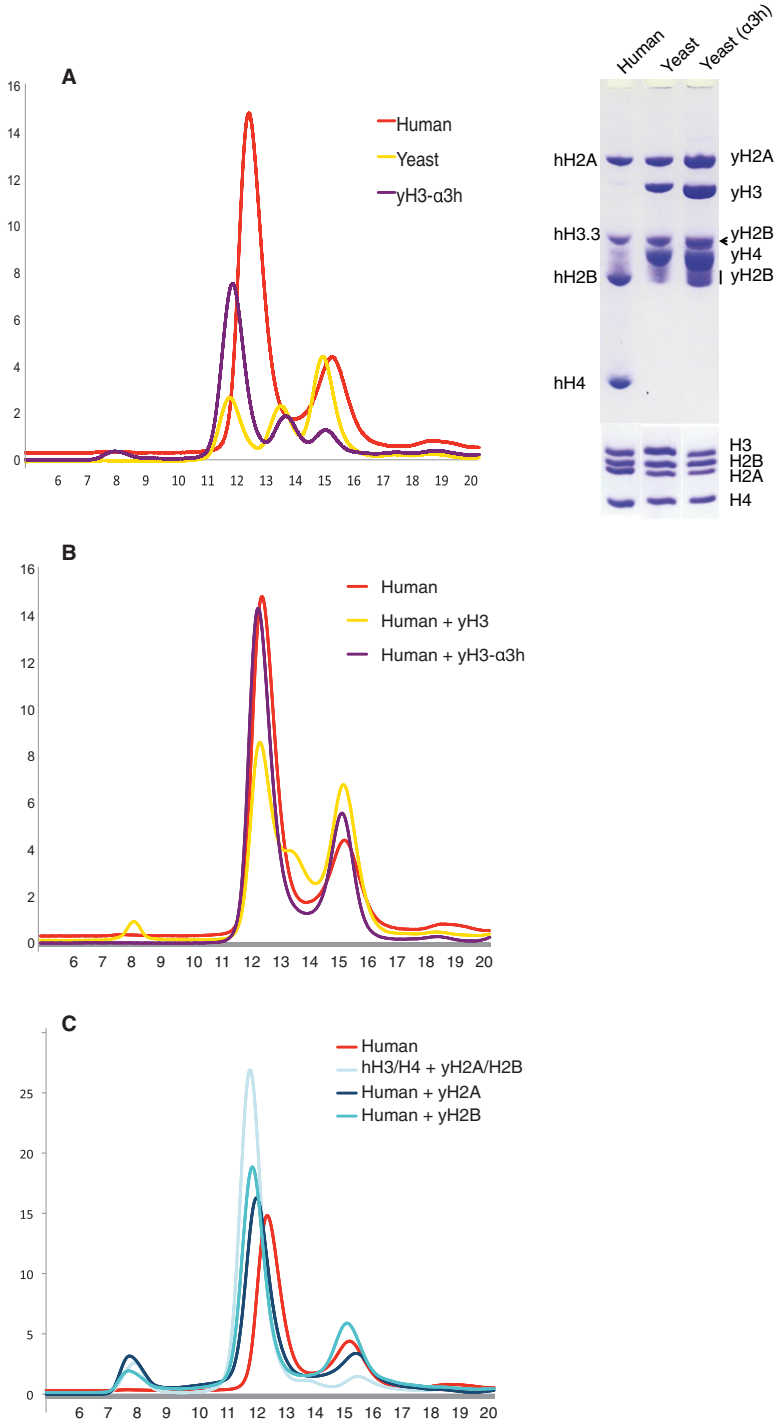

Fig. 1. The QKK motif of yeast H3 impedes octamer assembly. Comparative gel filtration chromatography of reconstituted histone octamers. (A) *Left*: Chromatograms from SEC of octamer reconstitutions containing canonical human histones (red), yeast histones (yellow), or yeast histones H4, H2A, H2B, and a chimeric yH3α3 h (purple). The *x*-axis corresponds to volume of refolding buffer (mL) and the *y*-axis represents the absorbance at 280 nm, measured in mAUs. *Right*: Acid-Urea-Triton (AUT; upper panel) and SDS/PAGE (lower panel) separation of bacterially expressed human and yeast core histones. (B) Chromatogram of human octamer reconstitutions containing either all human histones (red), human H4, H2A, H2B, and yH3 (yellow), or human H4, H2A, H2B, and a chimeric yH3α3 h (purple). (C) Chromatogram of human octamer reconstitutions containing all human histones (red); human histones H4, H3, H2B, and yH2A (dark blue); human histones H4, H3, H2A, and yH2B (turquoise), or human H4/H3 and yH2A/H2B (cyan). All traces are from a single experiment that is a representative of at least three separate reconstitutions. The reduction in protein signal in yeast and chimeric-yeast octamers is due to the removal of insoluble aggregates prior to injection in FPLC.

Differences in yeast histone sequences influence NCP properties

We next determined if divergent sequences between yeast and human histones influence the biophysical properties of mononucleosomes. Histone octamers were assembled on 197 bp 601 Widom DNA [27] by salt dialysis [16]. As expected, native PAGE separation of human, yeast, and mixed nucleosomes illustrates that each of these complexes forms a stable NCP. However, nucleosomes harboring yH2A/H2B migrate noticeably faster than human counterparts (Fig. 2A). As these nucleosomes show a more relaxed

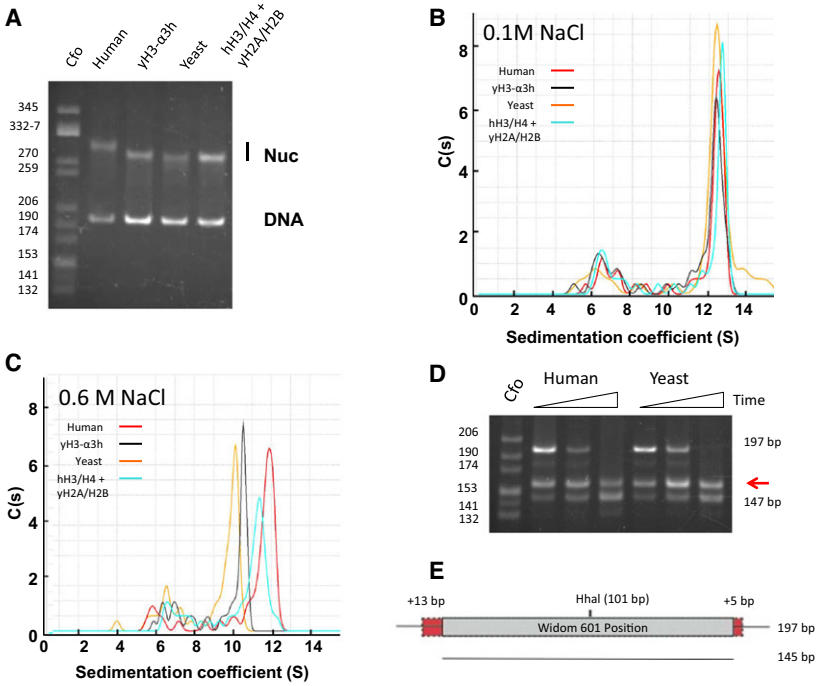

Fig. 2. Divergences in yeast H2A and H2B are the major determinants of altered yeast NCP properties. (A) Native PAGE analysis of human, chimeric yH3α3 h, yeast, and mixed human H3/H4:yeast H2A/H2B mononucleosomes. (B, C) AUC sedimentation velocity plot of human (red), yeast (orange), chimeric yeast yH3α3 h (black), and mixed human H3/H4:yeast H2A/H2B mononucleosomes (cyan) at 0.1 M NaCl and 0.6 M NaCl, respectively. (D) MNase time-course digest of human and yeast mononucleosomes. (E) Schematic diagram of 197 bp 601 DNA after MNase digestion highlighting the 163 bp NCP intermediate formed by the 145 bp Widom core DNA and a 5 bp- and 13 bp-protected region (red). Cfo marker is generated from a restriction digest of pBR322 with CfoI.

conformation in the analytical ultracentrifuge (see below), this faster electrophoretic mobility likely reflects an increase in negative charge of the NCPs that is contributed by DNA released from interaction with the histone octamer. This increased electrophoretic mobility is largely due to the presence of yeast H2A (results not shown). As yH2A is functionally an H2A.X protein [10], a previous report showing that H2A.X-containing nucleosomes release NCP-flanking DNA corroborates our findings [12].

To gain insight into the nature of this difference, we subjected nucleosomes to sedimentation velocity in the analytical ultracentrifuge (AUC). At a low ionic strength of 0.1 M NaCl (near physiological conditions), human, yeast, chimeric yeast (yH3α3 h), and mixed (hH3/H4: yH2A/H2B) octamers all sediment with identical properties (Fig. 2B). However, at 0.6 M NaCl conditions known to disrupt ionic interactions between histones and flanking nucleosomal DNA without compromising its compositional integrity [28], we observe significant differences in the sedimentation coefficients of these mononucleosome species. While human 197 bp NCP particles sediment at 12*S*, yeast particles sediment at ~ 10*S* (Fig. 2C). This reveals that human NCP is significantly more compact than the yeast counterpart. It is likely that both features of H2A/H2B and H3 contribute to this difference because chimeric yH3α3 h-containing yeast nucleosomes (Fig. 2C black line), and human nucleosomes with yeast H2A/H2B (Fig. 2C, cyan line) exhibit intermediate sedimentation coefficients. The fact that distinct sedimentation properties of these mononucleosomes are only observed at this ionic strength is likely explained by an alteration of the histone-linker DNA interactions in the regions at the flanking ends of the nucleosome core particle (NCP) comprising 145 bp. At low ionic strength (0.1 M NaCl), these would play a much more significant role in promoting a compact nucleosome structure, and indeed we find that yeast and chimeric-yeast particles that are more relaxed at 0.6 M NaCl (Fig. 2C, S = 10–10.5) appear more compact at 0.1 M (Fig. 2B; S = 12). We tested this hypothesis in two ways. First, we confirmed that both yeast and human nucleosomes have strong interactions with the DNA flanking the NCP by performing a time-course MNase digestion on these particles (Fig. 2D). These assays are performed at low ionic strength and show a defined MNase pause yielding an intermediate 163 bp species before the established 145 bp Widom DNA protected by the NCP (Fig. 2D,E). This intermediate is generated in the digestion of yeast and human 197 bp mononucleosomes confirming an interaction between histone octamers and a defined linker DNA flanking the 601-NCP. Sequencing of this fragment revealed that MNase pauses at 5 and 13 base pairs from the boundaries of the canonical 601–145 bp nucleosome (Fig. 2E). Secondly, to confirm that histone-DNA interactions beyond the NCP explain the effect of ionic

strength on yeast and human nucleosome properties, we assembled these octamers onto random sequence ~ 147 bp that lacks this DNA (Fig. 3A). Both yeast and human octamers form compacted and characteristic mononucleosome of 12S at low ionic strength (Fig. 3B solid lines). However, at high ionic strength human nucleosomes are unable to maintain a compacted state, and migrate similarly to relaxed yeast nucleosomes (Fig. 3B, dashed lines). This result, taken together with our chimeric nucleosome data, confirms that the increased compacted nature of human nucleosomes is mediated by interactions between human-specific features in the H3 C-terminus, H2A/H2B, and the regions of DNA adjacent to the NCP.

The octamer destabilizing features near the H3 C-terminus likely evolved in Fungi

In order to analyze the configuration of the yeast QKK tri-residue in a broader evolutionary context, histone H3 protein sequences were retrieved from major eukaryotic groups including Opisthokonts, Amoebozoa, Archaeplastida, Stramenopiles, Alveolates, Rhizaria, and Excavates [25]. These results showed the presence of a human MPQ tri-residue in all major eukaryotic groups with noted exceptions for the cases of Fungi and some excavate representatives, where different tri-residue configurations were found (Fig. 4A). Interestingly, the H3 phylogeny reconstructed for Fungi representatives (Fig. 4B) revealed that the tri-residue configuration in early diverging lineages has already departed from the archetypal MPQ configuration displayed by other eukaryotes into the QPQ configuration. This latter configuration is widespread across basidiomycetes and gives rise to an additional configuration (QPA) through the modification of the third position of the tri-residue. On the contrary, the diversity of tri-residue configurations is much greater in the case of ascomycetes, where the basal QPQ configuration seems to have undergone changes in second (QSQ, QNQ, QKQ) and third positions (QKK) of the tri-residue. Taken together, these comparisons which reveal deviations from the human MPQ motif are completely restricted to Fungi and that there is variability in this epitope within this group.

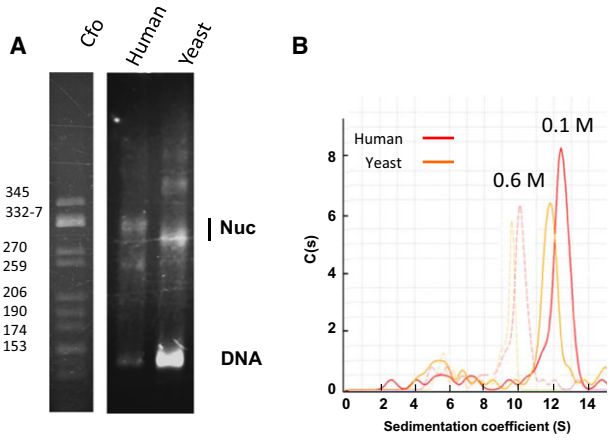

Fig. 3. Analysis of human and yeast NCPs reconstituted with mixed random sequence 146 bp DNA. (A) Human and yeast mononucleosomes reconstituted with mixed random sequence 146 bp DNA and analyzed by native PAGE. Nuc, nucleosome. The sizes of the Cfo DNA ladder are given. (B) AUC sedimentation velocity of human and yeast 146 bp NCPs. Plot of human (red) and yeast (orange) mononucleosomes at 0.1 M NaCl (solid line) and 0.6 M NaCl (dashed line) of panel A).

Discussion

Divergent sequences in yeast histones influence octamer stability and nucleosome properties

The relative instability of yeast nucleosomes, compared to those of higher eukaryotes, has been observed for some time. Lewis and colleagues first described that purified yeast histones reconstitute into mononucleosomes with only an ~ 16% efficiency compared to ~ 80% efficiency for calf or chicken histones [7]. In fact, after using nucleosome chimeras and excluding any instability contribution from yeast H4, Lewis and coworkers predicted that sequences in histone H3 of yeast might mediate yeast NCP instability. Our experiments reconstituting recombinant bacterially expressed yeast histones into octamers (Fig. 1) show a remarkably similar phenomenon, suggesting that failure to assemble yeast histones into nucleosomes is due to poor octamer assembly. We demonstrate that only three divergent H3 residues ($Q_{120}K_{121}K_{125}$) near the nucleosome dyad axis mediate this effect. Yeast-to-human changes at these residues rescue the intrinsic instability of yeast octamers (Fig. 1A), and human-to-yeast sequence swapping imparts instability to octamers comprised of human histones (Fig. 1B). Thus, it appears that subtle chemical changes at the dyad axis can influence the formation of octamers.

Lewis *et al.* also demonstrated that purified yeast nucleosomes are relatively unstable [7]. To overcome this problem, we used the 601 nucleosome positioning sequence to compare yeast and human NCPs. While the use of this strong nucleosome positioning sequence almost certainly will mask nucleosome stability

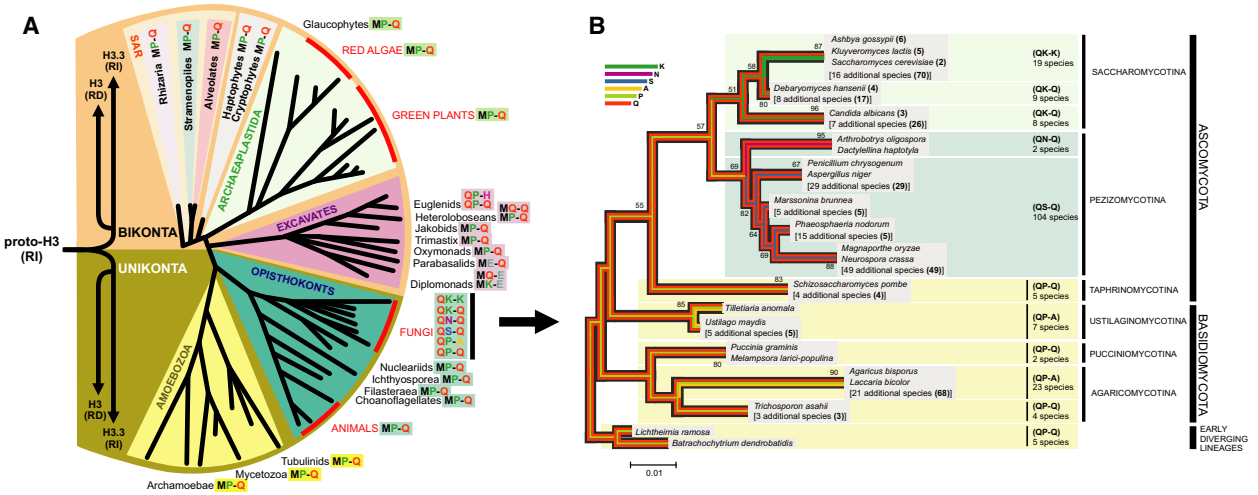

Fig. 4. The QKK motif of Histone H3 evolved in fungi. (A) Analysis of the H3 tri-residue configuration across the eukaryotic tree of life [tree adapted from [25]]. The C-terminal characteristic MP-sequence is present in all major eukaryotic groups except for Excavates (Bikonta) and Fungi (Unikonta), where different alternatives to this archetypal configuration can be found [see (B)]. The origin and evolution of actual replication-dependent (RD) and replication-independent (RI) eukaryotic H3 histones is represented in left part of the figure, probably originating from an ancestral RI proto-H3 containing the MPQ configuration [11]. (B) Phylogeny of H3 proteins along with the different configurations of the H3 tri-residue sequence within the Fungi kingdom. Different residues were assigned specific colors to illustrate their evolution in the phylogenetic tree. The numbers for internal branches represent bootstrap confidence values for the groups defined by the tree (only shown when BS ≥ 50%). The number of sequences analyzed for each taxa are indicated in parentheses and boldface.

differences to some degree, we still observe that mononucleosomes built with yeast histones differ from those built with human histones. Yeast H2A and H2B make major contributions to this effect. The yeast 197 bp nucleosome appears less compact than human at high ionic strength (Fig. 2C) reflecting a lower affinity for DNA that flanks the NCP. Thus, the lower affinity of the yeast nucleosome for the 601 DNA is partially compensated by the interactions with the linker DNA. This is supported by our previous report that yeast octamers position onto arrays of 197 bp × 601 sequence in a fashion that occludes linker DNA [15]. As this *in vitro* effect is dependent on the QKK motif, and humanization of these residues changes nucleosome positioning in living yeast [15], it appears that species-specific features near the dyad axis influence the positioning and/or dynamics of nucleosomes in a chromatin context. A large pool of literature linking biochemical changes at the nucleosome dyad axis to nucleosome stability supports this conclusion. For example, nucleosome destabilizing *sin* mutations are located in this region [29,30] and numerous post-translational modifications or remodeling activities target this area to regulate nucleosome dynamics and chromatin structure/stability [30–35]. It is therefore not surprising that genetically encoded differences in this area similarly influence chromatin properties.

The asymmetric binding of DNA regions adjacent to the NCP provide support to the asymmetrical unwrapping of the 197 bp 601 DNA from the nucleosome

The existence of a 163 bp NCP in the absence of histone H1 (Fig. 2D,E) reveals the ability of the synthetic 197 bp 601 DNA to form additional strong binding beyond the 146 bp limits of the NCP. This observation is similar to a recent report describing intermediate NCPs of 154 and 161 bp [36]. Moreover, the asymmetric protection of flanking DNA (with respect to the 146 bp NCP DNA) most likely has some bearing on the controversially proposed asymmetric unwrapping of nucleosomal DNA, which was based on DNA-pulling FRET analysis of nucleosomes reconstituted onto this DNA [37]. Our asymmetric protection (Fig. 2D,E) agrees well with the results of Ngo *et al.* [37], and we recognize that the asymmetric unwrapping observed is most likely the result of the sequence peculiarities of the 601 DNA. Indeed, this should not be surprising as DNA sequence plays a critical role in nucleosome dynamics [38].

Evolution of histone H3 in Fungi

The presence of QPQ in early diverging lineages (Fig. 4B) suggests that this configuration constituted

the ancestral state from which additional fungal H3 tri-residue configurations evolved. Accordingly, QPQ led to QPA in some Basidiomycete groups. Similarly, QPQ is present in the subphylum Taphrinomycotina from Ascomycetes (which includes the fission yeast *Schizosaccharomyces pombe*). However, the QPQ configuration changes completely in the remainder of the Ascomycetes, with the tri-residue evolving into QSQ and QNQ in Pezizomycotina, and into QKQ and finally QKK in Saccharomycotina. Such transition involves changes not only in the third position of the tri-residue (as observed in Basidiomycetes), but also in the second position. We speculate that the QKK configuration present in Saccharomycotina might have facilitated an active transcriptional status via general destabilization of chromatin in *Saccharomyces*. However, it is still to be demonstrated if this also applies to other genera displaying the QKK tri-residue configuration, like Kluyveromyces and Ashbya.

Practical considerations for generation of yeast chromatin substrates

The assessment of chromatin-modifying enzyme activity is ideally performed on a species-matched substrate. While the yeast *Saccharomyces cerevisiae* has proven extremely powerful for molecular genetics and *in vivo* analyses of chromatin, the generation of yeast chromatin substrates has proven to be surprisingly difficult. For this reason, most investigators have assayed yeast enzymes on chromatin substrates generated from *Xenopus*, chicken or calf thymus histones. In many cases, this will pose no confounding issues. However, given that histones in yeast are between 62% (H2B) and 92% (H4) identical to human, there is certainly potential for significant differences in yeast chromatin that influence the interaction of chromatin with modifying enzymes. Indeed, we show that NCPs with yeast H2A/H2B have distinct properties from those with human H2A/H2B, migrating faster in PAGE and appear more relaxed in AUC (Fig. 2A,C). Others have recognized this potential issue and generated chromatin from yeast histones using recombinant Nap1, a histone chaperone, to load and position histones onto a DNA template [7,39–41] The work here provides an alternate practical solution to the generation of recombinant yeast octamers that can be used for the generation of mononucleosomes (Fig. 2A), or chromatin arrays [15]. Using modified yeast H3 molecule that has been 'humanized' at the $Q_{120}K_{121}K_{125}$ motif, one can generate a yeast-like chromatin substrate via the salt dialysis method that is modified at only 3 of 498 amino acid positions of the octamer. *In vitro* analysis of yeast chromatin modification factors on such a template will better recapitulate the native chromatin environment encountered by these factors.

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Author contributions

CJN designed the research plan. CJN and JA discussed the different strategies and contributed to the writing of the manuscript. AL performed the experimental section and contributed to writing the manuscript. MC performed all AUC runs. JME-L and RGR worked on the phylogenetic analyses aspects of the manuscript and also contributed to the writing of the manuscript.

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