



Effect of Histone H3 E73D Mutation on *in vitro* Chromatin Silencing

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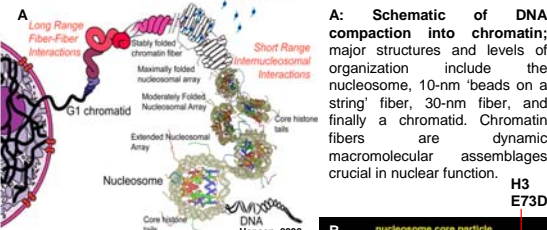
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Abstract

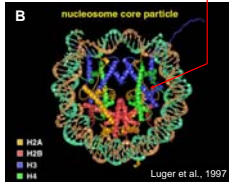
Organization of DNA into chromatin requires the presence of small basic proteins called histones. Core histones are highly conserved across various species. In addition to their function as DNA packing material, histones play an important role in the regulation of transcription, replication, and gene silencing. In particular, histone-mediated silencing is achieved via histone modifications and through interactions with suppressor proteins. In the yeast *Saccharomyces cerevisiae*, a single amino acid substitution (E73D) found within the conserved region of histone H3, has been shown to de-repress silencing at the telomeres and mating type loci in yeast (Thompson et al., 2003). It has been proposed that the substitution may shorten the amino acid side chain length enough to disrupt a necessary interaction at the H3-H4 interface within the nucleosome. Alternatively, the E73D mutation may affect the binding affinity of Sir3 for histone H3. Sir3 and Sir4, chromatin-associated repressor proteins, are known to mediate telomeric and mating loci silencing in *S. cerevisiae* (Grunstein et al., 1997). In order to test the effect of the E73D mutation on nucleosome stability *in vitro*, the crystal structure of nucleosomes containing the H3 mutant will be determined to ascertain the charge interactions between the amino acid residues within the vicinity of the substituted residue. In addition, binding studies will be carried out to investigate Sir protein binding to nucleosomes containing H3 E73D histones. Finally, analytical ultracentrifugation experiments will provide quantitative data on the *in vitro* dynamics of nucleosomal arrays comprised of nucleosomes containing H3 E73D.

H3 E73D Mutation in Context



A: Schematic of DNA compaction into chromatin; major structures and levels of organization include the nucleosome, 10-nm 'beads on a string' fiber, 30-nm fiber, and finally a chromatid. Chromatin fibers are dynamic macromolecular assemblages crucial in nuclear function.

B: Position 73 on histone H3 (blue) in the nucleosomal context; wild type glutamate at position 73 is solvent-exposed, and potentially interacts with residues on histone H4 (green) and/or with DNA in the vicinity. The E73D mutation may disrupt hydrogen bonding interactions, affecting nucleosome and/or higher order chromatin stability.



Significance of E73D Mutation

In *S. cerevisiae*, regions of heterochromatin have been thoroughly defined. These loci include the silent mating type loci (HML and HMJR) and the sub-telomeric regions. Transcriptional silencing at these loci is achieved through interactions between histones and chromatin-associated Sir proteins. Sir3 associates with the N-terminal tail of H3 to yield a condensed chromatin structure, effectively inhibiting transcription. Mutations within H3 N-terminus disrupt histone-Sir interactions, facilitating expression within normally silenced regions.

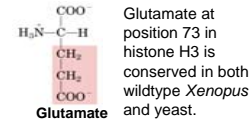
The E73D mutation displays the strongest effect on HM silencing for any known H3 mutation. The mutant strain mated more than 4 orders of magnitude less efficiently than the wildtype strain, indicative of strong de-repression of HML. Mating deficiencies caused by E73D were restored to near-wildtype levels by *sir3* repressor alleles, suggestive of a direct interaction between Sir3 and both the H4 N terminus and the H3 core domain.

Xenopus H3 Position 73 Amino Acid Substitutions

Selecting amino acids for substitution at position 73

Amino Acid	Mutation	Characteristics
Aspartate	E73D	Side chain length is one carbon shorter; charged conserved
Glutamine	E73Q	Side chain length is conserved; polar uncharged
Serine	E73S	Shorter side chain length; polar uncharged
Alanine	E73A	Shorter side chain length; non-polar
Arginine	E73R	Longer side chain; positively charged

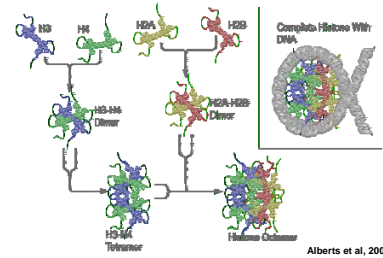
Position 73 in wild type H3:



Xenopus and Yeast H3 and H4 histones are highly conserved



Histone Octamer Refolding



Equimolar amounts of *Xen.* histones H2A, H2B, and H4 combined with each H3 mutant, E73D and E73Q, in unfolding buffer (6M Guan. HCl)

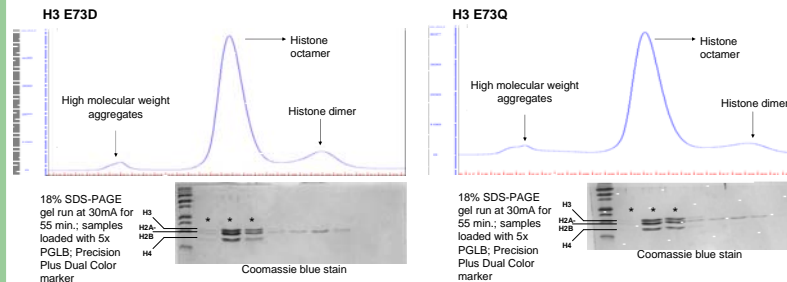
Histones dialyzed against refolding buffer (2M NaCl) at 4°C over an 18-hr. period

Histone octamer purified via gel filtration (native conditions)

Histone	Molecular Weight
H2A	13,960 Da
H2B	13,774 Da
H3	15,273 Da
H4	11,236 Da

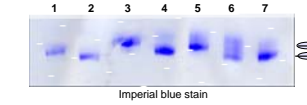
Octamer Purification

Octamers purified on a Superdex 200 size exclusion column



Nucleosome Reconstitutions

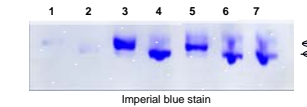
wt octamer + α -sat. DNA (control)



Lane DNA:octamer

1	0.7:1 US
2	0.7:1 S
3	1.2:1 US
4	1.2:1 S
5	1.7:1 US
6	1.7:1 S
7	2.0:1 S

H3 E73D octamer + α -sat. DNA

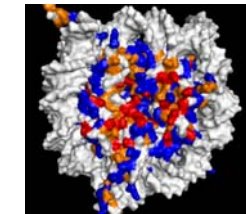


5% Native gels run at 150 V for 55 min. @ 4° C; samples loaded with 50% sucrose

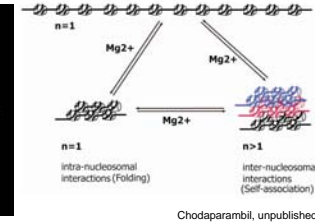
Conclusions

- Able to purify recombinant *Xenopus* histone H3 E73D and E73Q mutants using standard histone purification protocols
- Successfully reconstituted nucleosomes with histone H3 mutants
- H3 mutants do not significantly disturb octamer and nucleosome stability

H3 E73 Is Solvent Exposed



Nucleosomal Arrays



Future Directions

- Screen crystallography buffer conditions to determine ideal conditions for protein crystallization
- Construct nucleosomal arrays containing histone H3 mutants to test extent of folding and self-association using analytical ultracentrifugation
- Design and carry out binding assays for chromatin-associated silencing protein Sir3 with histone H3 mutants in nucleosomes and arrays

References

- Thompson, J.S., Snow, M.L., Giles, S., McPherson, L.E8833., and Grunstein, M. (2003). Genetics 163, 447-452
- Grunstein, M. (1997). Current Biology 9, 383-387

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