In Silico Insight into Nucleosome Repositioning Via Oxygen Delivery and Extracellular Movement

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Research note

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Abstract

OBJECTIVE

Histones and resulting nucleosomes occur within DNA regulating gene expression by slowing, pausing, or halting transcriptional machinery. Positions within the genome have been found with higher affinity for the histone octamer than others. Histone/nucleosome repositioning is adjusted via energy dependent remodeling complexes, and a harmonizing array of constellation proteins and molecules. The energy required to create transcriptional environments is created through oxygen intake, nutrient presence, and extracellular movement. In this paper we aim to help facilitate an *in silico* framework for further experimentation into how partial pressures of oxygen and other gases impact genetic transcription along with extracellular movement and nutrient delivery.

RESULTS

Cell and tissue culture experimentation with biomechanical strain and variable partial pressures of oxygen and other gases can be made into the expression levels of genes such as PH domain leucine-rich repeat-containing protein phosphatase 1 (PHLPP1), and Neuroligin 1 (NLGN1). These genes show *in silico* to have a higher affinity for a histone octamer binding motif, needing adequate cellular energy to be expressed. Extracellular movement and adequate cellular oxygenation are required to properly reposition nucleosome sequences for transcription.

Introduction

Nucleosomes are known to influence transcriptional regulation via presence and affinity of the underlying DNA-histone interactions.[1–3] Nucleosome repositioning is required for gene expression, either by inherent characteristics of RNA Polymerase or ATP-dependent remodeling complexes.[4] *In vivo*, repositioning will occur to occupy high affinity regions, influencing neighboring nucleosomes via linker DNA.[5] Oxygen intake and extracellular muscle movement is required for sufficient levels of intracellular ATP.[6,7] Chromatin structure is achieved by a harmonizing array of DNA-protein interactions and DNA shape.[8–11] The nucleosome core particle has sequence affinities proven *in vitro*.[12,13]

When reintroducing PHLPP protein into tumorigenic cells, tumor growth was reduced by approximately 50%[20]. The identification of NLGN1 expression as part of regulating the dynamic processes of memory consolidation and strengthening[23], along with the anti-tumor properties of PHLPP1 spurred this study. Possible mechanistic insights found from further experimentation may help discover novel therapies and research opportunities.

Methods And Procedures

Simple Python scripts utilizing regular expressions were used to compare the 1998 Lowary and Widom[12] consensus sequence with 82bp segments of hg38 from the National Center for Biotechnology Information. A pattern of 8 “ATC or G” nucleotides between a group of “TA” nucleotide sequences with flanking “ATC or G” nucleotide sequence were discovered and then processed to be scored against the Lowary and Widom consensus sequence. The pattern is noted in Table 1, where bolded and underlined nucleotides are the “TA” discrete pattern used, and all other nucleotides are “\w” (any letter) characters in regular expression notation. Scoring criteria is as follows according to Lowary and Widom[12]: +1 for a match with a higher identity character, + .5 for a match to a lower identity character, and + 0 for all others. Scored sequences were joined into one file and sorted in descending order by score to determine a liturgical highest affinity to the histone octamer ranking. Resulting genes of interest were input into the Interactive Genomics Viewer from the Broad Institute to determine the viewing of genetic locations; gene information was obtained from GeneCards and UniProt.

Table 1.

9 curated genes from the list of scored sequence matches. Simplified annotations were obtained from GeneCards and UniProt. 10bp periodic repeats of TA dinucleotides are underlined and bolded.

Gene Symbol	RefSeq Accession	Start Position	End Position	Score	Simplified Annotation
PHLPP1	NC_000018.10	62810938	62811020	31	Regulates Akt, tumor suppressor, Akt regulates the balance between cell survival and apoptosis through a cascade that primarily alters the function of transcription factors that regulate pro and antiapoptotic genes. Dephosphorylation of 'Ser-473' of Akt triggers apoptosis and suppression of tumor growth.
	AATTAGTTAAGATGAGATCA**TA**CTGGATTA**TA**ATGGACCC**TA**AATCTAAT**TA**CTGGTCCT**TA**TAAGAAACAAAGAGGACACA				
KCNH7	NC_000002.12	162529090	162529172	29	Voltage-gated potassium channel subunit, crucial role in returning the depolarized cell to a resting state.
	AATCTTCTAAAGGAGATGTC**TA**GTGGGATA**TA**TGAGGTTG**TA**AGTAAGCC**TA**AAGAAAGA**TA**TGAGCTGGAGATACTGATTT				
GRIK2	NC_000006.12	102071550	102071632	28.5	Subunit of marinate glutamate receptor. Associated with synaptic plasticity, learning and memory, may be involved in transmission of light from retina to hypothalamus. A deletion-inversion mutation is associated with autosomal recessive mental retardation.
	TAGCTGATAACCCTAACCAC**TA**TGGAACAC**TA**AGAGGACA**TA**TATTATTT**TA**CAAATATG**TA**AAAGGGATGATAATATTTTA				
C4orf22	NC_000004.12	80864382	80864464	28.5	Chromosome 4 Open Reading Frame 22.
	AATTGGGTGTGTCTTGTATA**TA**TGTGTGTG**TA**TGTGTATA**TA**TATAGGTA**TA**TATATATA**TA**TATACCTATATAAGTATATA				
NLGN1	NC_000003.12	173840054	173840136	28	Involved in formation and remodeling of CNS synapses
	CGACATATTCAAGGAACACA**TA**TGGCAATA**TA**TGTGACTC**TA**AATGCCAT**TA**TTGCATTT**TA**TTGGACAGAGTCCTGATTTC				
STK39	NC_000002.12	168245953	168246035	27.5	Encodes a serine/threonine kinase that is thought to function in the cellular stress response pathway, activated in response to hypotonic stress, catalytically activated kinase activates p38 MAP kinase pathway, intermediate in response to cellular stress
	TATGTATATATTTATATATA**TA**TTTCCGTC**TA**TGCTGTTT**TA**TTCCCCAA**TA**GCAGGTGA**TA**AAGGCTCATTTAAACACCTA				
NRIP1	NC_000021.9	15050977	15051059	27.5	Modulates transcriptional activity of variety of receptors including estrogen receptor, lipid and glucose metabolism, gene expression in heart, skeletal muscle, liver, block expression of genes involved in energy dissipation, mitochondrial uncoupling, g uncoupling protein 1 and carnitine palmitoyltransferase 1b, suppresses expression of MT proteins succinate dehydrogenase complex b and CoxVb, negative regulator of glucose uptake in mice.
	GTAGTAGTAGCAGTAGCATT**TA**CCTACAGC**TA**ATGCAAAC**TA**GCGAATCC**TA**AACTTGGT**TA**CCCGCCCTGCCTTGCCTTTC				
ICA1	NC_000007.14	8139555	8139637	27.5	This gene encodes a protein with an arfaptin homology domain that is found both in the cytosol and as membrane-bound form on the Golgi complex and immature secretory granules. This protein is believed to be an autoantigen in insulin-dependent diabetes mellitus and primary Sjogren's syndrome.
	AGGGCAGTGAAACTGTTTTG**TA**AGGTCCTA**TA**ATGGTAGA**TA**TATATCAT**TA**TACATTTG**TA**ATGCCCACAAAATGTATAAC				
CASP3	NC_000004.12	184629514	184629596	27	A cysteine-aspartic acid protease that plays a central role in the execution-phase of cell apoptosis. The encoded protein cleaves and inactivates poly(ADP-ribose) polymerase while it cleaves and activates sterol regulatory element binding proteins as well as caspases 6, 7, and 9. This protein itself is processed by caspases 8, 9, and 10. It is the predominant caspase involved in the cleavage of amyloid-beta 4A precursor protein, which is associated with neuronal death in Alzheimer's disease.

TACAAGCAAAATACCATGGA**TA**GCTGATTT**TA**GTTGATTC**TA**TAAATTAT**TA**TGTAGTCA**TA**AAGGCAAAAATAAGCATAAA

STATISTICAL SIGNIFICANCE

Finding one of these sequences, a 5x TA dinucleotide repeat motif with a 20bp flanking sequence for consensus analysis, is 1 in 6,533,600 through standard statistical probability in random chance. This probability results in approximately 460 total probable sequences to be found in the human genome with a purported histone binding motif per the Lowary and Widom experimentations[12]. There are 41,158 sequences like this found, resulting in an NP-hard problem and also a statistically enriched sequence by sheer observation. These results show the top 9, or top 2% most likely to be bound within a histone octamer from the Lowary and Widom[12] experiments, scored as noted.

Discussion

Histones, or nucleosomal formations must be changed in different cellular environments for different environmental outputs. Optimally, genes involved with tumor suppression would necessitate tumor suppressing genes to be expressed in a cancerous state. These data show that tumor suppressing genes may have an affinity for histone binding; needing adequate levels of ATP or other metabolic aids to functionally remodel or encode through the nucleosome complexes potentially present, either through added oxygenation of the cell, or other functional metabolic constituents added through biomechanical strain and adequate blood flow.

These data can predict that each cellular environment requires different chromatin structures, most likely dependent on local ATP availability. This differential in ATP availability may be a strong influencing factor into the nature and general expression of different cell or tissue types. The aim of these data and research is to hopefully inspire new findings and possible experimentation into the properties of ATP and other metabolic molecular availability for various cell and tissue types relating to histones and transcription.

Different types of tissue and cell types have varying levels of extracellular movement and resulting blood flow. Bone marrow and fingertip cell types would undoubtedly have different levels of biomechanical stress, resulting blood flow, and extracellular movement. A hypothesized difference in chromatin structure and a resulting different genetic expression profile would likely follow, as most would understand is unanimous in the literature and *in vivo*.

Standard air is comprised of Oxygen, Nitrogen, and Argon among other trace gases. Once standard air is deposited within the body, various biological processes would alter the concentrations of these compounds to be available to different cell types. Notably, oxygen through the properties of red blood cells, will oxygenate cells and tissues through various levels of extracellular movement. In standard cell and tissue cultures, pH is a dominant factor in the proliferation and differentiation of cell types, which is a main perspective of this research with other gas types. Different partial pressures and concentrations of standard air gases may change individual cell type environments. How does intracellular ATP affect transcription and translation?

Results

PH domain leucine-rich repeat-containing protein phosphatase 1 (PHLPP1), a gene which is established to suppress tumors[14–20] contains the highest computationally scored sequence match to the Lowary and Widom histone octamer binding consensus sequence[12], shown in Table 1 and Fig. 1. This is a sequence proposed to have the highest affinity for the histone octamer within the genome. ATP has been shown to impact mRNA transcription *in vitro*.[21,22] Sufficient levels of ATP may allow greater expression of PHLPP1 and other genes in Table 1.

Critical Discussion

Researchers may look at the genes in Table 1 to analyze in the field of cellular stretching, biomechanical strain, oxygenation, aspiration, and supplemented blood flow to analyze the hypothesized increase in expression from the highlighted association with affinity for histone binding.

Many experiments can be made from these observations, providing cell cultures of different types to different levels of cell stress, along with partial pressures of oxygen and other gases. One can infer from these *in silico* experiments that both higher levels of extracellular movement, and partial pressures of oxygen or other atmospheric gases would induce higher levels of expression for the genes in Table 1, and others with similar binding motifs. These higher levels of expression can potentially elucidate novel treatments for varying states of the cell.

Extracellular movement, experimentally provided by cellular stretching, is a proposed mechanism for enhancing the blood flow, oxygenation, and nutritional delivery to different types of cell. This research proposes that increased overall absorption of available extracellularly available molecules would impact genetic expression, and potentially be a target for novel methods of therapy for varying cellular phenomena.

Conclusion

In silico Genome analysis, along with RNA-Seq is undoubtedly a useful tool in proposing further *in vitro* and *in vivo* experimentation into these results. Computational histone binding motif searches of the human genome as shown in this research provides an adequate base to begin projects examining expression levels from discovered genes of interest, notably PHLPP1, a tumor suppressing gene. The enumeration of these expression levels may lay a foundation for future treatments for various cellular environments that require extracellular nutrient delivery *in vivo*.

Limitations

Cell Incubators may need oxygen and other gas certified connections through local EHS certifications.

Abbreviations

ATP - Adenosine triphosphate

DNA - Deoxyribonucleic acid

RNA - Ribonucleic acid

EHS - Environmental, Health, and Safety

bp - Base pairs

NCBI - National Center for Biotechnology Information

hg38 - Human Genome 38 (https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.26/)

Genome - hg38

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data Availability

The datasets generated and/or analyzed during the current study are available in the NCBI RefSeq repository, https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.26/.

Competing Interests

The authors declare that they have no competing interests.

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Authors' contributions

JM conceived, completed, and wrote the entire work.

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References

1. Hodges C, Bintu L, Lubkowska L, Kashlev M, Bustamante C. Nucleosomal Fluctuations Govern the Transcription Dynamics of RNA Polymerase II. Science. 2009 Jul 31;325(5940):626–8.
2. Bondarenko VA, Steele LM, Újvári A, Gaykalova DA, Kulaeva OI, Polikanov YS, et al. Nucleosomes Can Form a Polar Barrier to Transcript Elongation by RNA Polymerase II. Molecular Cell. 2006 Nov;24(3):469–79.
3. Kireeva ML, Walter W, Tchernajenko V, Bondarenko V, Kashlev M, Studitsky VM. Nucleosome Remodeling Induced by RNA Polymerase II. Molecular Cell. 2002 Mar;9(3):541–52.
4. Corless S, Gilbert N. Effects of DNA supercoiling on chromatin architecture. Biophys Rev. 2016 Sep;8(3):245–58.
5. Struhl K, Segal E. Determinants of nucleosome positioning. Nat Struct Mol Biol. 2013 Mar;20(3):267–73.
6. Wilson DF, Harrison DK, Vinogradov SA. Oxygen, pH, and mitochondrial oxidative phosphorylation. Journal of Applied Physiology. 2012 Dec 15;113(12):1838–45.
7. Golub AS, Pittman RN. Bang-Bang Model for Regulation of Local Blood Flow. Microcirculation. 2013 Aug;20(6):455–83.
8. Segal E, Widom J. What controls nucleosome positions? Trends in Genetics. 2009 Aug;25(8):335–43.
9. Freeman GS, Lequieu JP, Hinckley DM, Whitmer JK, de Pablo JJ. DNA Shape Dominates Sequence Affinity in Nucleosome Formation. Phys Rev Lett. 2014 Oct 14;113(16):168101.
10. Zhang Y, Moqtaderi Z, Rattner BP, Euskirchen G, Snyder M, Kadonaga JT, et al. Evidence against a genomic code for nucleosome positioning Reply to “Nucleosome sequence preferences influence in vivo nucleosome organization.” Nat Struct Mol Biol. 2010 Aug;17(8):920–3.
11. Segal E, Fondufe-Mittendorf Y, Chen L, Thåström A, Field Y, Moore IK, et al. A genomic code for nucleosome positioning. Nature. 2006 Aug;442(7104):772–8.
12. Lowary PT, Widom J. New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. Journal of Molecular Biology. 1998 Feb;276(1):19–42.
13. Zhang Y, Moqtaderi Z, Rattner BP, Euskirchen G, Snyder M, Kadonaga JT, et al. Intrinsic histone-DNA interactions are not the major determinant of nucleosome positions in vivo. Nat Struct Mol Biol. 2009 Aug;16(8):847–52.
14. Liu J, Weiss HL, Rychahou P, Jackson LN, Evers BM, Gao T. Loss of PHLPP expression in colon cancer: role in proliferation and tumorigenesis. Oncogene. 2009 Feb;28(7):994–1004.
15. Hou Y, Deng J, Zhang L, Xie X, Guo X, Sun C, et al. Lower expression of PH domain leucine-rich repeat protein phosphatase 1 (PHLPP1) association with poor prognosis of gastric cancer. Int J Clin Exp Med. 2015;8(11):20481–9.
16. Chen M, Pratt CP, Zeeman ME, Schultz N, Taylor BS, O’Neill A, et al. Identification of PHLPP1 as a Tumor Suppressor Reveals the Role of Feedback Activation in PTEN-Mutant Prostate Cancer Progression. Cancer Cell. 2011 Aug;20(2):173–86.
17. Gangula NR, Maddika S. WD Repeat Protein WDR48 in Complex with Deubiquitinase USP12 Suppresses Akt-dependent Cell Survival Signaling by Stabilizing PH Domain Leucine-rich Repeat Protein Phosphatase 1 (PHLPP1). J Biol Chem. 2013 Nov

29;288(48):34545–54.

18. Nitsche C, Edderkaoui M, Moore RM, Eibl G, Kasahara N, Treger J, et al. The Phosphatase PHLPP1 Regulates Akt2, Promotes Pancreatic Cancer Cell Death, and Inhibits Tumor Formation. Gastroenterology. 2012 Feb;142(2):377-387.e5.
19. Hassan B, Akcakanat A, Holder AM, Meric-Bernstam F. Targeting the PI3-kinase/Akt/mTOR signaling pathway. Surg Oncol Clin N Am. 2013 Oct;22(4):641–64.
20. Gao T, Furnari F, Newton AC. PHLPP: A Phosphatase that Directly Dephosphorylates Akt, Promotes Apoptosis, and Suppresses Tumor Growth. Molecular Cell. 2005 Apr;18(1):13–24.
21. das Neves RP, Jones NS, Andreu L, Gupta R, Enver T, Iborra FJ. Connecting Variability in Global Transcription Rate to Mitochondrial Variability. Weissman JS, editor. PLoS Biol. 2010 Dec 14;8(12):e1000560.
22. Márquez-Jurado S, Díaz-Colunga J, das Neves RP, Martinez-Lorente A, Almazán F, Guantes R, et al. Mitochondrial levels determine variability in cell death by modulating apoptotic gene expression. Nat Commun. 2018 Dec;9(1):389.
23. Katzman A, Alberini CM. NLGN1 and NLGN2 in the prefrontal cortex: their role in memory consolidation and strengthening. Curr Opin Neurobiol. 2018 Feb;48:122–30

Figures

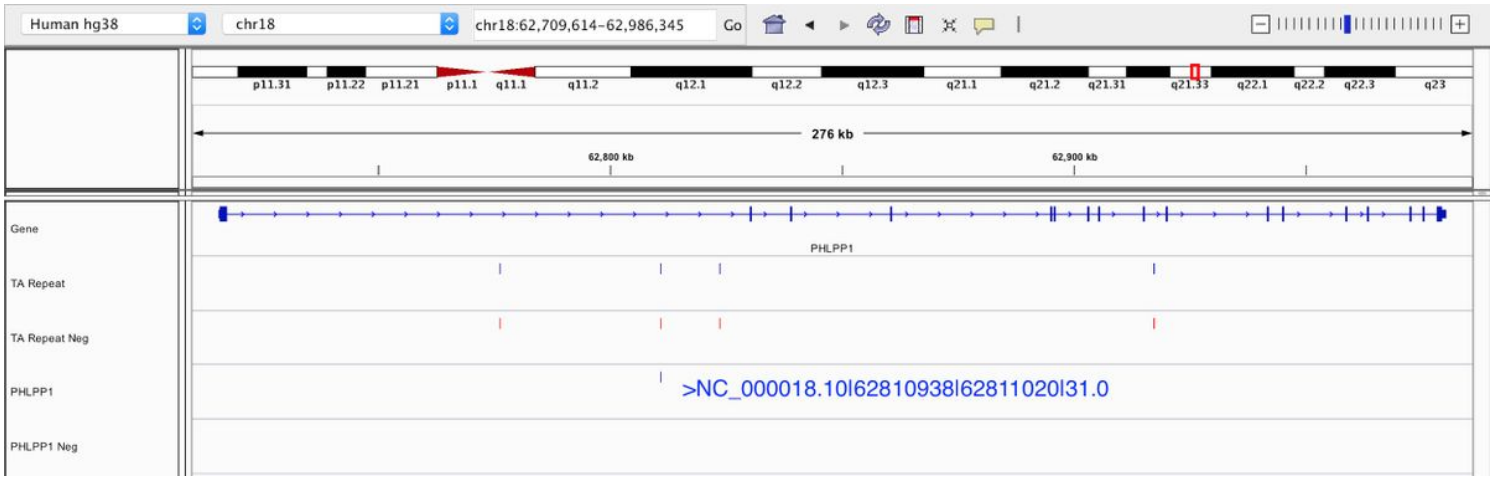

Figure 1

PHLPP1 (PH Domain and Leucine Rich Repeat Protein Phosphatase 1) set with tracks 2 and 3 denoting 5x 10bp TA repeats and tracks 4 and 5 showing positive and negative strand searches for the gene's high scoring match to the consensus.