Laboratory Exercise

A Computer Lab Exploring Evolutionary Aspects of Chromatin Structure and Dynamics for an Undergraduate Chromatin Course*

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Abstract

The study of chromatin constitutes one of the most active research fields in life sciences, being subject to constant revisions that continuously redefine the state of the art in its knowledge. As every other rapidly changing field, chromatin biology requires clear and straightforward educational strategies able to efficiently translate such a vast body of knowledge to the classroom. With this aim, the present work describes a multidisciplinary computer lab designed to introduce undergraduate students to the dynamic nature of chromatin, within the context of the one semester course "Chromatin: Structure, Function and Evolution." This exercise is organized in three parts including (*a*) molecular evolutionary biology of histone families (using the H1 family as example), (*b*) histone structure and variation across different animal groups, and (*c*) effect of histone diversity on nucleosome structure and chromatin dynamics. By using freely available bioinformatic tools that can be run on common computers, the concept of chromatin dynamics is interactively illustrated from a comparative/evolutionary perspective. At the end of this computer lab, students are able to translate the bioinformatic information into a biochemical context in which the relevance of histone primary structure on chromatin dynamics is exposed. During the last 8 years this exercise has proven to be a powerful approach for teaching chromatin structure and dynamics, allowing students a higher degree of independence during the processes of learning and self-assessment. © 2013 by The International Union of Biochemistry and Molecular Biology, 41(2):95–102, 2013.

Keywords: *Bioinformatics; histones; evolution; electrostatic properties*

Background

In eukaryotes and some archaebacteria, DNA is found associated with histones in a nucleoprotein complex called chromatin, which allows for a high extent of compaction of the genetic material within the limited space of the cell nucleus [1]. The repetitive subunit of chromatin, the nucleosome (Fig. 1*a*), consists of an octamer of core histones (two of each H2A, H2B, H3, and H4) around which two left handed superhelical turns of DNA are wrapped. The nucleosome core particles (NCPs) are joined together in the chromatin fiber by short stretches of linker DNA that interact with linker H1 histones, resulting in an additional folding of the chromatin fiber. Chromatin also provides the support on which most DNA metabolic functions (i.e. replication, transcription, and repair) take place. The interaction of DNA with histones dynamically modulates chromatin structure (chromatin dynamics). This process requires the concerted action of histone-modifying enzymes, ATP-dependent chromatin remodeling complexes as well as histone variants with specialized functions [2]. The resulting histone marks, in combination with the specialized domains imparted by histone variants, dynamically modify the chromatin structure in what it has been referred to as the "histone language" based on a "histone code" [3].

Histone variants constitute a minority group of histones within the major families, usually sharing structural homology with the major types but also having their own features often

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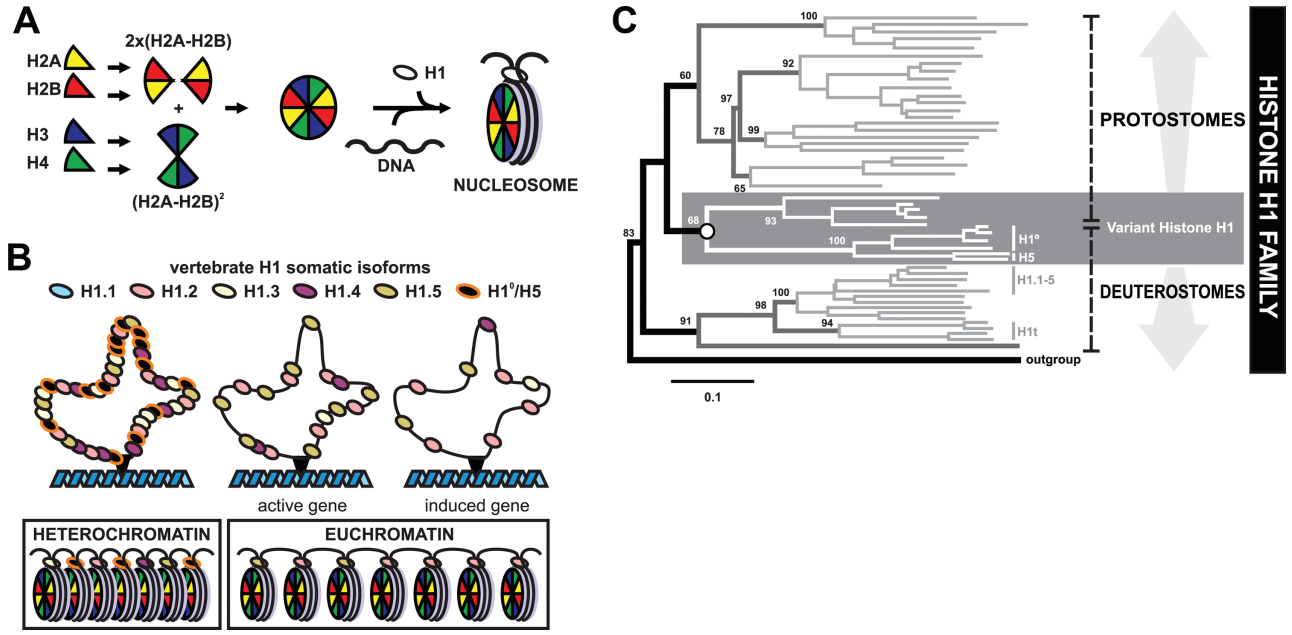

FIG 1

Structure, function, and evolution of histone H1 in chromatin. (a) *Protein and nucleic acid components of chromatin including core histones (H2A, H2B, H3, H4), linker histone (H1), and DNA.* (b) *Model of somatic H1 isoform distribution in chromatin and resulting chromatin states adapted from [5,6]. In this model, H1 variants are selectively depleted upon gene expression, suggesting that the somatic isoforms have different functional roles based on their selective depletion from different classes of DNA sequences.* (c) *Phylogenetic relationships among H1 proteins in metazoan animals using evolutionary distances. Numbers for interior branches represent bootstrap probability values based on 1,000 replicates. The monophyletic origin of "variant" H1 proteins is indicated by an open circle.*

related to specific functions in chromatin dynamics. These variants stand out by displaying specific amino acid changes at conserved positions, which are usually subject to post-translational modifications. Since the stability of chromatin relies on the balance between the electrostatic charges of DNA (acidic) and histones (highly basic proteins), the exchange of canonical histones by specialized variants often results in the modification of the electrostatic equilibrium, affecting the stability of the nucleosome and triggering a dynamic change in chromatin structure (i.e. relaxation or compaction) linked to a specific metabolic function (i.e. replacement of histone H2A by an acetylated H2A.Z variant destabilizes the nucleosome structure facilitating gene expression). Linker H1 proteins display the highest diversity of specialized variants among histones, modulating the local accessibility of chromatin for other proteins which contribute to the functional state of chromatin (Fig. 1*b*). Until now, the term histone code was essentially restricted to core histone modification patterns, but undoubtedly the modification of linker histones adds an important aspect to this code. Moreover, the existence of varied patterns of H1 variants in chromatin and their post-translational modifications allow the assumption that H1 histones contribute to the regulation of gene expression [4–6]. In the present exercise, the H1 family is used to illustrate how histone amino acid variation affects chromatin structure and dynamics.

During the last 15 years, the study of chromatin has experienced a rebirth due to the discovery of its highly dynamic nature. More recently, the interest in chromatin has grown even more due to its potential to carry epigenetic information. Such an exciting scenario encouraged the idea of designing an undergraduate course focused exclusively on chromatin, aiming to keep students up with the state of the art in such a rapidly changing field. This project finally came true in 2005 as a one semester course referred to as "Chromatin: Structure, Function and Evolution," within the M.Sc. program in Cellular Biology, Molecular Biology, and Genetics of the University of A Coruña (Spain). Since then, this course has been taught once a year for an average audience of 20 students per semester. Among the different units constituting this course, chromatin dynamics is perhaps the most challenging topic to teach in the classroom, given that (with very few exceptions) the dynamic nature of chromatin is totally unknown to students at that level. Chromatin dynamics constitute a complete unit of the course (~10 hours) in which the different mechanisms responsible for chromatin remodeling are introduced and discussed. Special attention is devoted here to chromatin specialization mediated by exchange of histone variants and histone post-translational modifications due to two major reasons: both mechanisms involve amino acid variation in histones and both trigger chromatin remodeling by modifying the ionic properties of the nucleosome core particle (and consequently its affinity for DNA).

The exercise described in the present work aims to introduce students to chromatin dynamics from a comparative standpoint, using bioinformatic resources that provide a set of

TABLE I *Description of the computational tools used across the current computer lab*

Tool	*Type*	*Purpose within this exercise*	*Availability*
GenBank	Online server	Genetic sequence database	http://www.ncbi.nlm.nih.gov/genbank/
BLAST	Online server	Alignment search tool	http://blast.ncbi.nlm.nih.gov/
BIOEDIT	Local program	Sequence alignment/editing	http://www.mbio.ncsu.edu/bioedit/bioedit.html
MEGA	Local program	Molecular evolutionary genetics analyses	http://www.megasoftware.net/
PDBe	Online server	3D structure protein data bank	http://www.ebi.ac.uk/pdbe/
DEEPVIEW	Local program	Protein modeling	http://spdbv.vital-it.ch/
SWISS-MODEL	Online server	Protein structure homology/modeling	http://swissmodel.expasy.org/
PYMOL	Local program	3D structure rendering/editing	http://www.pymol.org/
PIPSA	Online server	Comparison electrostatic interaction properties of proteins	http://pipsa.eml.org/
VMD	Local program	Visualization of electrostatic potentials	http://www.ks.uiuc.edu/Research/vmd/
Histone Database	Online server	Histone sequence database	http://research.nhgri.nih.gov/histones/

powerful research tools for analyzing molecular information [7] as well as a very important field of training [8,9]. To this end, it is presented as an multidisciplinary computer lab (6 hour) organized in three parts: (*a*) Molecular evolutionary biology of histone families, (*b*) histone structure and variation across different animal phyla, and (*c*) functional consequences of histone diversity on nucleosome structure and chromatin dynamics. Students are challenged to independently explore molecular databases, get acquainted with sequence alignments and evolutionary analyses, perform protein structure homology modeling, and infer/compare the electrostatic interaction properties among the modeled proteins. All results and graphical representations collected in the present work were generated by students during the class of 2011–2012. At the end of the unit, students were able to visualize the importance of histone diversity and its contribution to chromatin dynamics. Overall, this exercise emphasizes laboratory skills deemed critical for success of students within their scientific careers [10].

Laboratory Exercise

Molecular Data Mining and Evolutionary Inference

In the first part of the unit, students were provided with a text file including a problem protein sequence of unknown nature to them. The protein chosen to kickstart this exercise was the human histone $H1^0$ (GenBank accession number: NP_005316, see Table I for information on the different bioinformatic tools and resources used throughout this computer lab), a protein generally expressed by terminally differentiated cells and involved in chromatin condensation/gene silencing [5]. This sequence is used as reference in data mining routines using the BLAST tool, both in the general GenBank database as well as in the specific Histone Database [11]. By this homology mining students are expected to retrieve different histone H1 sequences (~100–120 nonredundant protein sequences) belonging to a wide range of organisms. However, the focus of the current exercise was circumscribed to eukaryotes and more specifically to metazoan animals, Illustrating the great diversity of the histone H1 protein family in this group of organisms.

The evolutionary history of the H1 family was reconstructed on the basis of the collected proteins by using two computational molecular evolutionary resources. First, the program BIOEDIT [12] helps students to identify homologous amino acid positions across H1 proteins by performing multiple sequence alignments. Second, the program MEGA 5 [13] was used by students to estimate the evolutionary distances among H1 proteins. This latter part of the exercise introduces students to the most widely used tree-building methods as well as to how to test the reliability of the obtained topologies. Students were encouraged to analyze and evaluate the obtained tree topologies in order to identify different histone H1 lineages, posing different hypotheses which may account for their evolutionary origin and differentiation. Figure 1*c* shows the reconstruction of the H1 phylogeny obtained by students, depicting the evolutionary history of these protein families in animals.

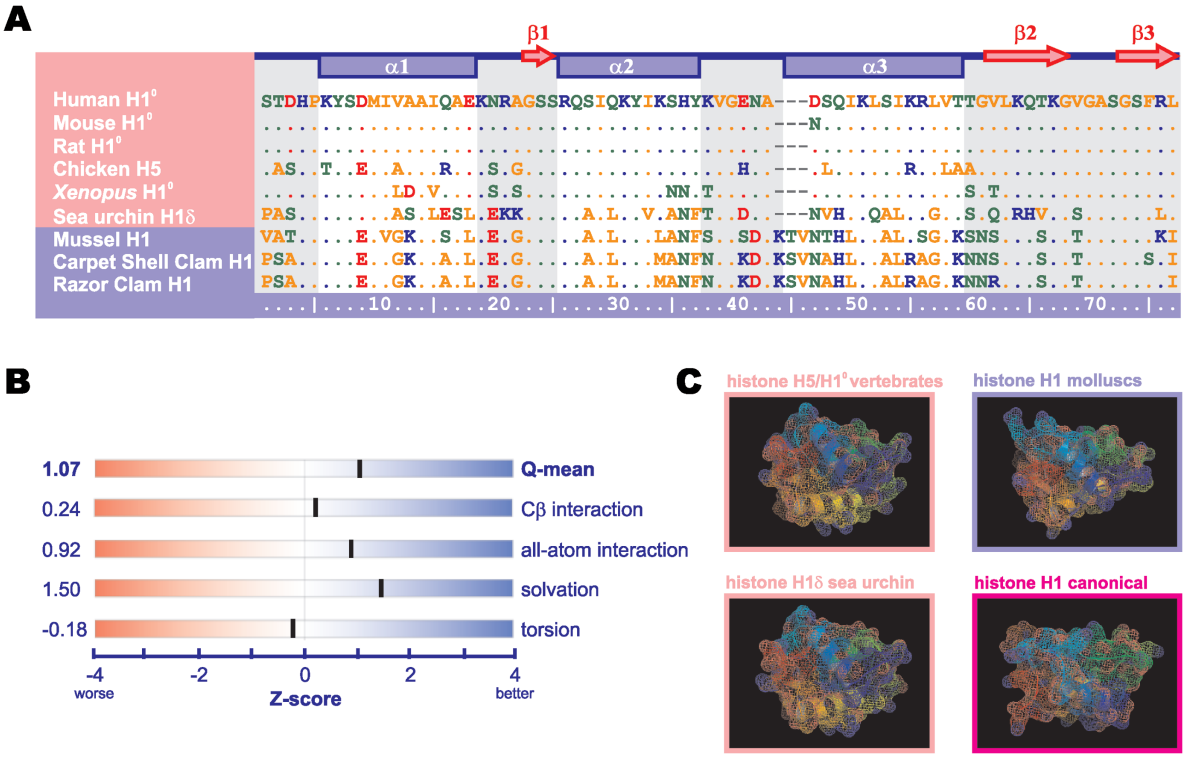

FIG 2

Different steps in homology modeling of histone H1. (a) *Amino acid alignment corresponding to the region of H1 modeled in different animals (the secondary structure is indicated above the alignment). H1 sequences from deuterostome and protostome animals are indicated in pink and blue backgrounds, respectively. Residues are colored based on their side chain properties as: basic (blue), non-polar hydrophobic (purple), acid (red), and polar uncharged (green). Matching residues with the reference sequence (human H1^0) are indicated by dots, whereas gaps are indicated by dashes.* (b) *Z-score of the individual components of QMEAN4 obtained by students in the modeling of human histone H1^0. This score is used to evaluate the generated models based on the linear combination of four structural descriptors using statistical potentials.* (c) *Structures of four representative H1 proteins modeled by students as part of this exercise using the coordinates of histone H5 from chicken as reference (PDB accession 1hst). Structures were rendered using the educational version of the MacPyMol program [14] combining cartoon and mesh representations.*

Throughout this initial step in the exercise, students get acquainted with molecular databases, data mining routines, sequence classification, and organization. This approach encompasses a great educational value, allowing students to independently explore different computational tools very useful in extracting information from huge amounts of molecular data. In the age of Next Generation Sequence technologies such approach is mandatory for the analysis of biologically relevant information in molecular databases. Additionally, this part of the unit helps students realize about the great potential of molecular evolutionary analyses in identifying the structural and functional constraints guiding the evolution of protein families.

Protein Structure Homology Modeling of H1 Histones

The reconstructed phylogeny sets an evolutionary context for studying the role of histone H1 in the dynamic chromatin of different organisms. By scrutinizing the obtained topologies, students were able to identify an independent group of approximately 20–25 "variant" H1 proteins sharing a common evolutionary origin. The differentiation of this lineage probably underlies a functional specialization across its members, playing a role slightly different from that of their "canonical" H1 counterparts (Fig. 1*c*). More interestingly, students realized that representatives of this variant lineage are found in the two major clades of metazoan animals: protostomes (i.e. molluscs) and deuterostomes (i.e. mammals). Consequently, the objective of the second part of the unit pursues the analysis of the macromolecular variation across variant H1s in different organisms. This strategy was chosen to help students correlate the variation observed in protein primary structures with the resulting heterogeneity in protein secondary and tertiary structures, especially as it pertains to the functional specialization imparted by variant H1 proteins to local chromatin regions.

With this in mind, students were directed to the Protein Data Bank to search for 3D structures corresponding to the identified variant H1 proteins. The results obtained revealed the presence of a single hit (the tertiary structure of the H5

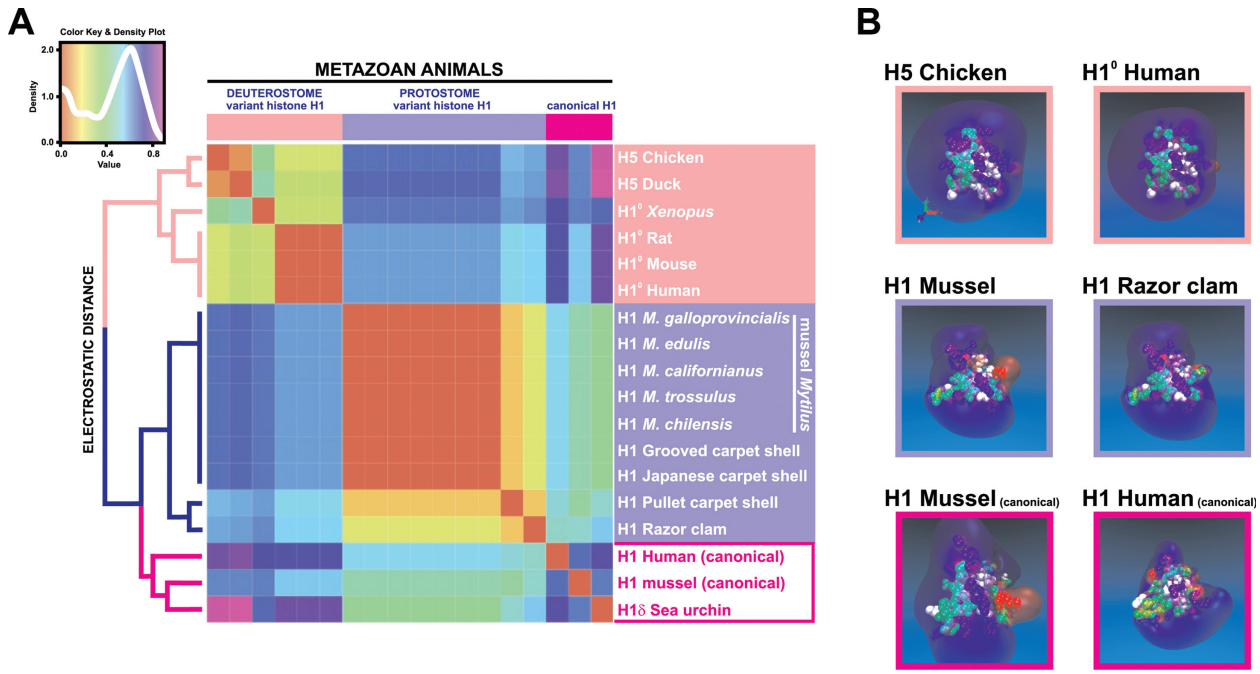

FIG 3

Analysis of electrostatic properties in modeled histone H1 proteins. *(a) Electrostatic distances calculated from the similarity indices for the electrostatic potentials of variant H1 proteins represented in a color-coded matrix (heat map). The color code and the number of comparisons for each distance interval are indicated in the color key/density plot. The tree along the left side of the image assembles the proteins into groups with similar electrostatic potentials (epogram). Variant H1 sequences from deuterostome and protostome animals are indicated in pink and blue backgrounds. (b) Spatial representation of electrostatic potentials in 6 H1 proteins representing the different groups defined by the epogram. Positively and negatively charged surfaces are indicated in blue and red, respectively; colors were assigned to amino acids according to their physical and chemical structural characteristics as in Fig. 2.*

protein from chicken, PDB accession: 1hst). Consequently, students are encouraged to find alternative approaches in order to perform comparative structural studies among these proteins. At this point, the value of protein structure homology modeling is introduced to students, as it represents the only feasible alternative to carry out comparative studies in those cases where crystal structure information is lacking. Consequently, the tertiary structure of the histone H5 and its coordinates were used as reference to perform homology modeling of the variant H1 proteins previously retrieved from GenBank.

Histone H1 proteins ("problem" proteins) were modeled by students by aligning their raw amino acid sequences against the "reference" 3D histone H5 protein, in the region indicated in Fig. 2*a*. Students performed this analysis with the help of two specific bioinformatic tools: firstly, they used the program DEEPVIEW-SWISS PDB VIEWER [15] to generate preliminary models for each H1 variant, and secondly, the SWISS-MODEL Server [16] helped students evaluate the quality of the obtained models (Fig. 2*b*). After saving each model as an independent file in pdb format, students were able to render the modeled H1 proteins (Fig. 2*c*) and compare them with their corresponding amino acid sequences, unveiling the consequences of amino acid variation on H1 structure. This part of the exercise provides students with a valuable insight into protein homology modeling methods and their intrinsic value for comparative studies. Most importantly, they learned how to evaluate the quality of the obtained models based on different quality parameters and how to decide whether those models are eligible or not for further analyses.

Relationships Among H1 Proteins Based on Electrostatic Functional Properties

The relevance of amino acid variation and its consequences for protein structure has been brought to the attention of students throughout this exercise. However, in the specific case of histones there is another critical protein feature that must be taken into account. Given that the association of histones and DNA in chromatin is mediated by ionic interactions, the electrostatic equilibrium between these highly basic proteins and nucleic acids is of outstanding relevance for chromatin structure and function. Indeed, dynamic changes in overall histone charges will stabilize/destabilize the nucleosome particle, resulting in a phenomenon known as chromatin "breathing." To address this problem, students were encouraged to analyze the electrostatic properties of the H1 proteins modeled, trying to find evidences supporting their functional specialization in chromatin.

TABLE II ***Students responses to tests before and at the end of the course***

Before course test	Rating
I am familiar with advanced biochemistry/molecular biology concepts	1.1
I am familiar with molecular evolutionary concepts	1.9
I am familiar with bioinformatics	0.4
I have previous experience working with molecular databases	0.8
I have previous experience working with phylogeny programs	0.8
I have previous experience working with computational modeling programs	0.2
After course test	**Rating**
I acquired good conceptual understanding of molecular evolution and phylogenies	3.2
I acquired good conceptual understanding of chromatin structure	3.2
I acquired good conceptual understanding of chromatin dynamics	3.7
I feel comfortable performing data mining in DNA/protein databases	3.6
I feel comfortable performing phylogenetic inference using DNA and proteins	3.0
I feel comfortable performing protein homology modeling	3.2
This exercise helped me understand why histone primary structure is important for chromatin structure	3.6
This exercise helped me understand how the interaction of DNA with histone variants dynamically modulates chromatin structure	3.7

Students rated how well they agreed with each statement using the following scale: 0, completely disagree; 1, disagree; 2, neither agree nor disagree; 3, agree; 4, completely agree. Values are averaged for all students attending the course during the last 7 years (2005–2011, approximately 100 students).

Protein electrostatic potentials were calculated by students and compared among modeled variant H1s using the PIPSA pipeline [17]. This online resource allows for the classification of proteins according to their interaction properties, based on the estimation and comparison of their electrostatic potentials. By using this pipeline students evaluated how amino acid changes in histone primary structures modify their corresponding electrostatic properties. Given that the role of histones in chromatin is largely dependent on their interaction properties, the groups defined by PIPSA will pinpoint histone variants sharing a high degree of functional identity. PIPSA introduces students to an array of parameters necessary for such calculations, including the type of rigid-body superposition, the methods by which electrostatic potentials can be computed, as well as the importance of different environmental constants (temperature, ionic strength) and the region of the protein selected (whole protein by default).

Students used PIPSA to calculate electrostatic potentials and to estimate similarity indices for all pairs of proteins, which were subsequently converted to electrostatic "distances" and represented as a color coded distance matrix (heat map) and as a tree (epogram), as shown in Fig. 3*a*. PIPSA also generates several intermediate results, most notably those describing the organization of the electrostatic charges on the surface of each one of the molecules analyzed. During this computer lab, students learned how to render such potentials onto the corresponding H1 3D structures by using the program Visual Molecular Dynamics [18]. By using this approach, they were able to identify differences not only in histone H1 modeled structures, but also in the spatial organization of their electrostatic potentials, resulting from the amino acid variation displayed by H1 proteins in their primary structure. The results obtained by students are illustrated in Fig. 3*b*, representing the electrostatic potentials of different H1 molecules as blue (positive) and red (negative) "clouds."

In a final stage of the exercise, students are encouraged to compare the electrostatic relationships obtained among H1 histones with the evolutionary relationships previously inferred among these proteins (Fig. 1*c*). These results show students that the evolutionary history of H1 proteins is mirrored by their electrostatic relationships, with H1 variants constituting an independent lineage in both cases. Such parallelism illustrates the key concept of this exercise: amino acid changes alter the electrostatic properties of histone variants. Given that chromatin stability is sustained on the electrostatic balance between histones and DNA, replacement of canonical histones by slightly divergent variants will trigger dynamic modifications in chromatin configuration. In the present case, the amino acid changes presented by H1 variants provide them with highly basic potentials, displaying higher affinity for DNA (compared with their canonical counterparts). Consequently, the exchange of canonical H1s by highly basic H1 variants promotes chromatin condensation (structure), resulting in gene inactivation/silencing (function). The amount and specific location of these amino acids constitute a target for selection, resulting in the evolutionary differentiation of H1 variants in protostome and deuterostome animals, probably as a consequence of different functional demands in the chromatin of both groups of organisms.

TABLE III ***Proteins successfully used in this computer lab***

Reference Protein Sequence	*Organism*	*Protein Family*	*GenBank Accession*	*Chromatin Context*	*PDB Reference Structure*
$H1^0$	Human	Histone H1	NP_005316	Linker histone	1hst
H2A.Z.1[a]	Mouse	Histone H2A	NP_058030	Core histone	1f66
H2B	Drosophila	Histone H2B	NP_724342	Core histone	1aoi
Nucleoplasmin	Chimpanzee	Nucleophosmin/ Nucleoplasmin	XP_519642	Histone chaperone	1k5j
NASP1	Zebrafish	Nuclear Autoantigenic Sperm Protein	NP_956076	Histone chaperone	*in silico*[b]

[a] *Previously H2A.Z-1, new nomenclature according to Ref. 19.*
[b] *Predicted* in silico *from NASP1 primary structure.*

Student Reaction and Evaluation

Students come to this course with uneven backgrounds, especially as it pertains to advanced molecular biology concepts and bioinformatics (Table II). Since previous teaching experience has revealed that many of them feel lost when starting using these computational resources, students were provided with a detailed step-by-step guide of the exercise. These guide, together with the user-friendly interfaces of the programs and online resources used, helped students find their way through the different parts of the computer lab. The teaching experience also revealed that a key element in the success of this exercise is to distribute students in working groups of 2–3 people, including at least one student with previous knowledge in molecular biology and other student with computational skills. This organization allows students to join forces in solving the proposed problems, increasing their learning experience and achieving a higher degree of success in the evaluation of their knowledge.

Overall, students responded very positively to the computer lab. By analyzing and comparing different histone variants, they are able to assess the effect of histone amino acid variation on the stability of the nucleosome core particle and the dynamic remodeling of chromatin. Consequently, this exercise helps them translate the bioinformatic information into a biochemical context. On an end of course evaluation, students expressed that they had become quite comfortable working with molecular evolutionary genetics software, homology modeling software as well as molecular dynamics programs (Table II). Furthermore, they clearly acknowledged the teaching potential of the computer lab for this part of the chromatin course.

For the evaluation of the present exercise, students craft a paper summarizing their findings with the guidance of a short questionnaire, specifically addressing the following:

1. Explain how molecular databases provide a framework for the comparative study of chromatin structure/function.
2. Describe the different types of parameters used to evaluate the quality of the variant H1 structures modeled throughout the exercise.
3. Indicate how amino acid variation in histone primary structure influences chromatin structure.
4. Is there a correlation between the evolutionary history of amino acid substitutions in H1 proteins and the variation observed in their electrostatic potentials?
5. Could this agreement/disagreement be reconciled with the functional role of linker histones in dynamic chromatin?

Conclusion

The computer lab exercise described in this work constitutes a powerful approach for teaching of chromatin dynamics to undergraduate students. Its organization decreases costs and allows students a higher degree of independence during the processes of learning and self-assessment. By using bioinformatic tools, students are challenged to explore by themselves the protein components of the fundamental subunit of chromatin (the nucleosome) and the link between histone sequence variation and chromatin dynamics. During this process they handle molecular and structural data from a comparative perspective in a broad evolutionary context. Since the beginning of this course 8 years ago, different modifications and improvements have been progressively introduced. Although histone proteins constitute very good examples to illustrate the link between structure variation and functional diversity in chromatin, other histones and chromatin-related proteins have been used as examples in this exercise in the past (Table III). Among them, those proteins relying on ionic interactions in order to fulfill their roles have produced the best results, most notably histone variants and histone chaperones. In addition, the present exercise has been reorganized to keep up with the new discoveries in the field, combining different key concepts

in evolutionary biology, biochemistry, and molecular biology in a computational framework.

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