ISSN 0025-7680

International Symposium
NEW DIRECTIONS IN CANCER MANAGEMENT
Academia Nacional de Medicina
Buenos Aires, 7-8 August 2000

MEDICINA (Buenos Aires) 2000; 60 (Supl. II): 21-26

MAMMALIAN HISTONE ACETYLTRANSFERASE COMPLEXES

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Abstract Over the last decade, great progress has been made in elucidating how the human genome operates in the chromatin context. This paper describes our work on two human acetyltransferases, PCAF and TIP60, and their interaction partners. This study provides new clues on the function of these enzymes. In a striking parallel with the general transcription factor TFIID, PCAF complex contains proteins that have histone-like domains. We speculate that these subunits can presumably form a nucleosome-like structure on DNA, which would allow PCAF to contribute to the maintenance of an active state of chromatin. On the other hand, TIP60 complex contains two eukaryotic homologs of bacterial RuvB helicase/ATPse, involved in recombination and repair. Accordingly, expression of a dominant negative mutant of TIP60 in living cells interferes with their ability to repair DNA damage, which points out, for the first time, a role for a histone acetyltransferase in a process other than transcription. We also have evidence implicating TIP60 in the apoptotic response to DNA damage.

Key words: chromatin, acetylation, proteome, transcription, apoptosis

Resumen ***Complejos histona acetiltransferasa en mamíferos.*** Durante la década pasada se han hecho grandes progresos para dilucidar cómo opera el genoma en el contexto de la cromatina. Esta publicación describe nuestro trabajo sobre dos acetiltransferasas humanas, PCAF y TIP60 y sus parejas de interacción. Este estudio provee nuevos indicios sobre la función de estas enzimas. En sorprendente paralelo con el factor de transcripción TFIID, el complejo PCAF contiene proteínas con dominios tipo histona. Nosotros especulamos que estas subunidades pueden presumiblemente formar una estructura tipo nucleosoma en el ADN, la cual podría permitir al PCAF contribuir al mantenimiento de la cromatina en estado activo. Por otra parte, los complejos TIP60 contienen dos homólogos eucariotas de la helicasa/ATPasa bacteriana RuvB involucrada en recombinación y reparación. Coincidentemente la expresión de un mutante dominante negativo de TIP60 en células vivas interfiere con su habilidad para reparar el daño en el ADN lo cual señala por primera vez el rol de una histona acetiltransferasa en un proceso distinto al de la transcripción. Nosotros tenemos, además, evidencia de la implicancia del TIP60 en la respuesta apoptótica al daño del ADN.

Given that human genome operates in the context of chromatin, study of the machinery and activities that modify chromatin can greatly help in understanding how the human genome works. Eventually, it can lead to the development of new ways to prevent and treat human health disorders, such as cancer, for example. First part of this paper reviews an approach used in our work to study a subset of the chromatin modifying activities, namely, histone acetyltransferases. it is based on identification of interaction partners of the proteins under study, which can greatly help in figuring out what is the function of a particular protein. Our work on PCAF complex and the model of its function, based on the features of the PCAF complex components, will be described. The second part of the paper reviews new results obtained for TIP60 complex. These results implicate TIP60 acetyltransferase in DNA damage repair and, unexpectedly, in the mechanisms of apoptotic response to DNA damage.

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Histone acetylation

Eukaryotic genome is tightly packed into chromatin, a complex between DNA, histones and nonhistone proteins. For more comprehensive description of chromatin structure and modifications the reader is referred to reviews[1]. What follows is a brief introduction into this subject. The most basal level of chromatin organization is a nucleosome: DNA wrapped twice around the histone octamer. The histone octamer is a complex between four different core histones repeated twice. Each core histone consists of the c-terminal globular domain and the N-terminal tail. The N-terminal tails have been a subject of intensive research for last 5 years. The main reason for this interest is that a postranslational modification of these tails, namely, acetylation of the lysine residues, has been established to play an important role in regula-

tion of gene activity[2]. There is usually a strong correlation between activity of a particular gene and acetylation of chromatin[3]. The enzymes that promote histone acetylational are called histone acetyltransferases and many of them have been identified as transcription activators. Conversely, many histone deacetylases, which promote the reverse reaction, turned out to be generally repressors of transcription[4]. This establishes a mechanistic link between histone acetylation and transcription regulation. In one of the first papers on this subject, we have demonstrated that the transcriptional coactivator p300/CBP is a histone acetyltransferase. P300/CBP is involved in many different signalling pathways in the cell and we proposed that it contributes to activation of transcription by converting inactive chromatin into active chromatin via acetylation of histone tails[5].

This paper will review our work on two other mammalian histone acetyltransferases: PCAF and TIP60. PCAF was originally cloned in the laboratory of Yoshihiro Nakatani[6], as a human counterpart of the yeast transcriptional coactivator GCN5[7]. TIP60 was originally cloned as a coactivator of the HIV transcriptional activator Tat[8], and later it was also shown to possess HAT activity[9, 10]. We were interested to compare these two proteins because of their distinct and nonoverlapping substrate specificity. All four core histones can be acetylated *in vivo,* however, PCAF acetylates preferentially H3, whereas TIP60 prefers H4 and H2A histones. Comparison of these two enzymes could help us in answering the following open question in the field of histone acetylation: althougth the connection between the histone acetylation and transcription regulation is well established, almost nothing is known about the different roles that acetylation of different histone tails plays in the structure and function of chromatin. These tails belong to different proteins, their primary sequence is different and even the enzymes that acetylate them are often different, therefore it is very likely that the function of the tails can be also different. Thus, we were hoping that study of the enzymes with different substrate specificity, and in particular, identification of their interacting partners, could eventually help us in answering the aforementioned question.

'Proteome walking'

For identification of the interacting partners of PCAF and TIP60, we used a general approach named 'Proteome walking' (Fig. 1). The main objective of the 'Proteome walking' project is to take advantage of the vast volume of sequencing information being accumulated as a result of Human Genome Project. All human genome sequence is going to be determined very soon, and the challenge is going to be to understand the function of all 50-100 000 of the newly identified human genes. One systematic way to assign at least some function to a particular protein is to identify its interaction partners. An approach that was used successfully in our work was a combination of the following steps:

Fig. 1.– 'Proteome Walking' strategy. For description, see the text.

1. Using an efficient retroviral transfer, create cell lines that express an epitope-tagged version of the protein of interest.

2. Using affinity chromatography against the epitope tag, purify the complex of this protein with interacting polypeptides.

3. Using very sensitive mass-speck sequencing technique, identify the interacting polypeptides.

4. Obtain the ORF, corresponding to these newly identified proteins, either by conventional cDNA library screening or directly from an EST database.

5. The new ORF can be epitope tagged again and the described cycle can be repeated again, to identify the interaction partners for the new protein.

By doing the second step of the 'proteome walk', at least two goals are pursued. First, we expect the original protein to be found in the complex with the new polypeptide. This would confirm that the new polypeptide is the *bona fide* interaction partner of the original protein. Second, the new protein often has interaction partners that are not present in the original complex with the first protein. This is because many complexes in the cell have a

combinatorial and dynamic nature and often one and the same protein can be a part of many different complexes. Thus, iteration of this procedure can be a systematic way to walk down the proteome, with every cycle identifying new interactions in the cell. In the case if the new polypeptides are not known, this is a way to assign them at least some meaning via the fact that they interact with a known protein (guilt by association). If the new proteins are already known, this can be a way to discover new and often unexpected connections between previously unrelated processes.

PCAF complex

We used the strategy described above to identify the interacting partners of PCAF acetyltransferase[11]. We found that it interacts most strongly with at least 20 different polypeptides ranging in size from 10 kd to 400 kd (Fig. 2A). Almost all of them have been identified, however, this review will mention only a subset of them that, very unexpectedly, connect the PCAF complex with a general transcription factor TFIID.

TFIID is a complex of TBP (TATA-Binding Protein) and TAFs (TBP Associated Factors)[12]. It has been shown to participate in activation of transcription by providing communication between transcription activators and basal transcriptional machinery, as well as by contributing to the recognition of core promoter sequences. Already some time ago, an intriguing connection between TFIID and chromatin has been found: some of the TAFs (subunits of TFIID) contained domains that looked very similar to histones H3, H4, H2B[13] (lately an H2A-like TAF has been identified as well[14]). Moreover, Yoshihiro Nakatani in collaboration with Bob Roeder and Steve Burley have shown that the histone-like domains of the H3- and H4-like TAFs form a complex that looks, by crystallographic analysis, strikingly similar to the heterotetramer of regular histones H3 and H4[15]. This data, together with some additional evidence, allowed the authors to suggest that there is a substructure in the TFIID complex that looks like the histone octamer, the protein core of the nucleosome[16].

Unexpectedly, the PCAF complex turned out to contain components that are either identical or very similar to the histone-like TAFs. The H3-like TAF31 and H2B-like TAF 20/15 from TFIID are present in the PCAF complex. PAF65β is very similar to the histone H4-like TAF80, and importantly, the similarity extends beyond the histone-like domain. Not only the PAF65β has a domain similar to H4, but this domain interacted stoichiometrically with the H3-like domain of the TAF31, in a manner analogous to the regular H3 and H4 histones[17].

The similarity between TFIID and PCAF complex extends beyond the histone-like subunits. For example, the PAF65α is very similar to the WD40 repeat containing TAF100[18], moreover, the similarities between these two proteins extend beyond the WD40 repeat domain.

Fig. 2.– TIP60 and PCAF complexes. Polypeptides specific for TIP60 or PCAF complex and their molecular masses are indicated. Positions of the heavy and light chains of IgG in the PCAF complex preparation are shown by asterisks.

This unexpected parallel between the general transcription factor TFIID and the histone acetyltransferase PCAF complex provides a new dimension in the connections between histone acetylation and transcription activation. We are considering the following model that takes into account the potential for PCAF (and TFIID) complex to form a nucleosome-like structure on DNA (Fig. 3). After acetylation of histones, the conventional nucleosome can be replaced by the histone octamer-like structure in the PCAF (or TFIID) complex and thus contribute to the establishment and maintenance of an active state of chromatin[19]. During mitosis, most of the transcription factors come off DNA[20]. However, regular nucleosomes remain on DNA during mitosis. If PCAF coplex can form the nucleosome-like structure on DNA, it might also survive mitosis and remain on DNA. After the mitosis is completed, PCAF complex

Fig. 3.– Role of octamer-like structure and PCAF bromodomain in establishment and maintenance of an active state of chromatin. A. After acetylation of a nucleosome, PCAF complex can replace it with the octamer-like structure. Given that the histone-like factors in the PCAF complex do not have the n-terminal tails, involved in inter-nucleosome interactions[28, 29], this will create "permanently activated" nucleosome, resistant to deacetylatin. B. During replication, the PCAF complex remains associated with one of the daughter strands. Second PCAF complex can be recruited to the sister strand via itneraction of the PCAF bromodomain with acetylated nucleosome on this strand. This can contribute to replication of active chromatin state. C. During mitosis, most of the transcription factors and other components of transcriptional machinery (TM) are displaced from DNA[20]. The nucleosomes, and presumably, nucleosome-like structure in the PCAF complex remain on the DNA. After completion of mitosis, the PCAF complex can facilitate recruitment of transcriptional machinery back to the site of active transcription. Thus, PCAF complex can provide a molecular bookmart that helps to re-establish transcription after mitosis[30].

can recruit the transcriptional machinery back to the site of transcribed genes. This will contribute to the reestablishment of an active state of chromatin after mitosis and hence, to maintenance of an active state of gene expression during the cell cycle. In addition to the proposed role for the histone octamer-like structure, the model also incorporates the recent finding by Zhou group of specific interaction of the PCAF bromodomain with acetylated lysine[21].

It is worth noting that the ability to maintain a particular pattern of gene expression is an essential aspect of differentiation; moreover, the defects in this ability can often contribute to an oncogenic process. Thus, the investigation of the mechanisms of maintenance of a particular state of gene activity is an important part of gene regulation studies[22].

TIP60 complex

The interacting partners of TIP60 (Fig. 2B) were identified in a manner very similar to that of PCAF[23]. This paper will describe two of these partners, TAP54α and TAP54β. These proteins are the human homologues of the bacterial ATPase/helicase RuvB[24]. RuvB is involved in recombination and recombination-dependent repair. It acts as a motor protein that catalyses migration of the Holliday junction during recombination and repair. It functions in the form of a hexameric ring, usual for other members of AAA protein superfamily[25]. First, we confirmed that TAP54s are the *bona fide* interacting partners of the TIP60 acetyltransferase. For this purpose, we performed the second proteome walking step described above. TAP54β was epitope-tagged, then the cell line expressing this protein was generated and TAP54β was purified from the nuclear extract together with interacting polypeptides. Almost all of the TIP60 complex components are present in the TAP54β prep. This confirms that TAP54 is a TIP60 interacting partner. Intriguingly, the TAP54β prep also contains polypeptides that are not present in the original TIP60 complex. We found, by size fractionation, that this prep contains additional complexes, indicating involvement of TAP54s in other interactions independent of TIP60. Currently, we are working on the identification of these new complexes.

The similarity between the TAP54α, β and the bacterial RuvB motor protein implicates TIP60 complex in repair process. In order to test this hypothesis, we used a dominant negative approach to investigate whether an ability to repair DNA damage is compromised in the cells with inhibited function of TIP60 complex. For this purpose, we introduced two point mutations into the acetylCoA binding site of the HAT domain of TIP60 and then created cell lines that were expressing epitope-tagged version of this TIP60 mutant. According to affinity purification, this mutant was still able to bind at least part of the TIP60 interacting proteins. However, as expected, it was inactive enzymatically as a histone acetyltransferase *in vitro.* We tested whether the ability to repair DNA after ionizing radiation is affected in the cells that express this form of TIP60. Compared to the control cells and to the cells expressing the wild type TIP60, the TIP60 mutant

expressing cells were repairing damaged DNA with much slower kinetics. This implicated TIP60 complex in repair process. The involvement of a TIP60 histone acetyltransferase in repair is the first demonstration of a role of a histone acetylation in a process other than transcription regulation.

Given the fact that all processes with DNA occur in the context of chromatin, the finding that TIP60 complex is involved in repair could be expected. However, the cells with the dominant negative TIP60 behave very unexpectedly with respect to another test. In addition to the ability to repair DNA damage, we also analyzed the ability of ionizing radiation to induce apoptosis. As these cells are compromised in their DNA damage repair capabilities, one might expect that they would be more sensitive to irradiation and would undergo apoptosis more readily than the control cells. However, the opposite turned out to be the case -the cells expressing the TIP60 mutant were much more resistant to irradiation and developed apoptotic response much less efficiently than both control cells and the cells expressing the wild type TIP60. These, quite counterintuitive results, are consistent with the idea that cells have a mechanism that can sense that some DNA is damaged and then direct the cell towards repair or/and apoptotic pathway. We hypothesize that TIP60 complex may be a part of this sensing mechanism. Similar phenomena have been already observed, for example, in the case of mismatch repair mutants[26, 27]. Future studies will show how general is the involvement of TIP60 complex in the different kinds of DNA repair and in the mechanisms that direct a defective cell onto the apoptotic pathway.

In summary, study of histone acetyltransferases and other chromatin modyfing activities has a great potential in helping to understand the way of how human genome operates in the chromatin context. Identification of the interacting partners of histone acetyltransferases is a fruitful approach to understand their function. Due to the involvement of these enzymes in many aspects of DNA metabolism, they might be promising new targets of therapeutic intervention for a variety of health disorders.

Acknowledgements: The author is in a deep debt to his mentor Yoshihiro Nakatani, whose leadership and support made this work possible. The author would also like to acknowledge invaluable contribution of the members of Dr. Y. Nakatani and B. Howard group in this work.

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The developing countries are still losing too many of their best scientists to the world's wealthier research centers. Yet the negative aspects of this movement can be in part counteracted by increased mobility and information sharing, current trends that facilitate intensive training courses, the sharing of big science facilities, and networks linking expatriate researchers to their home country institutions -all strategies supported by UNESCO. In this drive to establish new ways of doing science, the leading industrialized nations clearly have much to offer. The United States, in particular, has the world's strongest science base, a tradition of individual rights, a record of reacting positively to change, and some of the world's largest knowledge-intensive corporations. If the business sector takes note of the potential benefits of a new relationship between science and society, then public and private interests would converge, generating a force for progress powerful enough to meet the challenges of the new century.

Los países en vías de desarrollo siguen perdiendo demasiado de sus mejores científicos hacia los centros de investigación más ricos del mundo. Sin embargo, los aspectos negativos de este movimiento pueden ser en parte contrarrestados al compartir mayor movilidad e información, tendencias facilitadas por cursos intensivos, por compartir facilidades, y tender redes entre expatriados y las instituciones de sus países -todas estrategias auspiciadas por la UNESCO. En esa intención de establecer nuevas formas de hacer investigación, las naciones industrializadas tienen mucho que ofrecer. Los Estados Unidos especialmente, con su fuerte base en ciencia, tiene una tradición en derechos individuales, el record de reaccionar positivamente frente a todo cambio, y algunas de las corporaciones relacionadas al conocimiento más importantes del mundo. Si el sector privado toma nota de los beneficios potenciales de una nueva relación entre la ciencia y la sociedad, entonces podrían converger los intereses públicos y privados, generando una fuerza hacia el progreso suficientemente poderosa como para enfrentar a los desafíos del nuevo siglo.

Federico Mayor

The World Conference on Science. *Science* 1999; 285: 529.