MORPHOLOGICAL CHANGES IN THE NUCLEUS OF THE YEAST Saccharomyces cerevisiae INDUCED BY THE EXPRESSION OF GENE pol OF HIV-1

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ABSTRACT

The present paper reports and discusses the effect of the expression of the gene pol of the human immunodeficiency virus (HIV) on the nucleus of the *Saccharomyces cerevisiae* yeast. This unexpected result is characterized by a loss of the nuclear membrane limits as well as of the characteristic homogeneity of the chromatin. This group of changes gives to the nucleus of the yeast a worm-eaten aspect after the induction of the expression of the pol gene. The possible relation of these changes with apoptosis and the possibility that *S. cerevisiae* may constitute a model for their study are discussed.

Key words: **HIV-1, protease, pol, endonuclease, Apoptosis, *Saccharomyces cerevisiae***

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RESUMEN

En el presente trabajo se reporta y se discute el efecto de la expresión del gen pol del virus de la inmunodeficiencia humana (VIH) sobre el núcleo de la levadura *Saccharomyces cerevisiae*. Este resultado inesperado, se caracteriza por una pérdida de los límites de la membrana nuclear asi como de la homogeneidad característica de la cromatina. Estos cambios en su conjunto le confieren al núcleo de la levadura un aspecto carcomido luego de la inducción de la expresión del gen pol. La posible relación de estos cambios con la apoptosis y la posibilidad que *S. cerevisiae* pudiera constituir un modelo para el estudio de este fenómeno son discutidos.

Palabras claves: **VIH-1, proteasa, pol, endonucleasa, apoptosis, *Saccharomyces cerevisiae***

Introduction

The yeast *Saccharomyces cerevisiae* has been used to express many viral proteins, including the HIV-1 like gag/pol product (1), or reverse transcriptase (2, 3), but cytopathic effects have not been reported. Particularly, the cytopathic effects of the human immunodeficiency virus (HIV) have been related to cell fusion and syncitia formation, involving viral envelope antigens and host CD4 receptor interaction, the accumulation of unintegrated viral DNA and other mechanisms (4). Other less documented factors include the toxic effect on the cell of the pol gene product, particularly the proteinase, (5). In order to obtain reverse transcriptase the gene pol of HIV-1 was expressed in *Saccharomyces cerevisiae* and morphological changes were found in the nucleus of the yeast.

Materials and Methods

The plasmid pBSDC that contains the HIV pol gene under the control of the yeast GAPD promoter was constructed according to standard techniques, using the replicative vector pBS-4 (6). Yeast cells (*Saccharomyces cerevisiae SEY 2201*) were transformed with this plasmid according to (7). Transformants were initially grown for 24 hours in a minimal medium (8) supplemented with 2 % glycerol and were induced daily for the next 72 hours with 2 % glucose and 3 % casein hydrolysate. Expression of the pol gene in yeast cells transformed with plasmid pBSDC was assessed by western blot, reverse transcriptase activity and immunogold staining of thin sections using a human HIV-reactive serum (6), which were visualized at the electron microscope essentially as described (9). The experiment, under the same parameters was reproduced three times. As a control both untransformed yeast and yeast transformed with a 2 mm based replicative vector pBS-4 were used. Also, another aspartic protein, chymosin, cloned under the same promoter, using the same plasmid (pBS-4) and expressed in the same strain of *Saccharomyces cerevisiae* (10) was used as a control.

Results and Discussion

Electron microscope viewing of the cells, before immunogold staining, revealed unexpected morphological alterations in the nucleus specific for the pol expressing cells. These nuclear morphological changes are characterized by both a loss of the limits of the nucleus membrane as well as a detriment of the homogeneous appearance of the chromatin giving the nucleus a worm-eaten aspect. The changes were neither detected in control untransformed yeast, yeast transformed with a 2 mm based replicative vector pBS4 nor in the pol expressing cells before the induction of the GAPD promoter. However, after 96 hours of induction the nuclear morphological changes are clearly visible in yeast expressing the

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1. Kramer RA, Schaber MD, Skalka AM, Gangoly K, Wong-Staal F and Reddy EP. HTLV-III gag protein is processed in yeast cells by the virus pol-protease. Science 1985;231:1580-1584.

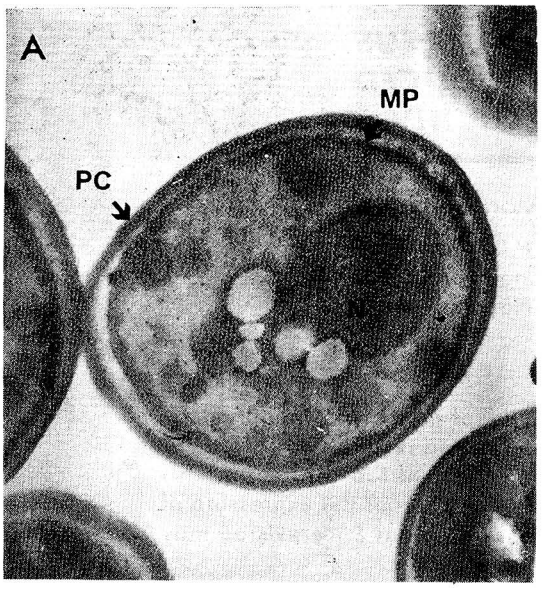
2. Barr PJ, Power MD , Lee-ng C. Ting, Gibson HL and Luciw PA. Expression of active human immunodeficiency virus reverse transcriptase in *Saccharomyces cerevisiae*. Bio/technology 1987;5: 486-489.

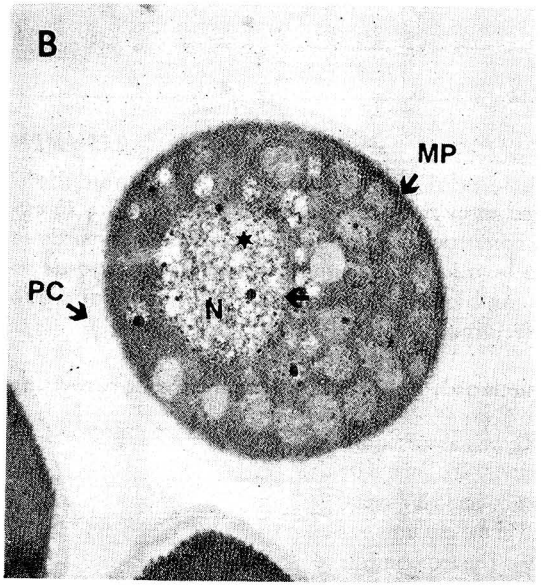
3. Bathurst IC, Moen LK, Lujan MA, Gibson HL, Feucht P H, Pichuantes S, Craik C S, Santi DV and Barr PJ. Characterization of the human immunodeficiency virus type-1 reverse transcriptase enzyme produced in yeast. Bioch. and Bioph. Research. Commun. 1990;171:589-595.

4. Fauci AS, Schnittman SM, Poli G, Koening S and Pantaleo G. Immunopathogenic mechanisms in human immunodeficiency virus (HIV) infection. Ann. Int. Med. 1991;114:678-693.

5. Peng C, Chang NT and Wen Chang T. Identification and characterization of human immunodeficiency virus type 1 gag/pol fusion protein in transfected mammalian cells. J. Virol.1991;65:2751-2756.

6. Sariol CA, Ferbeyre G, Ruiball I, Aguiar J, Palmeiro E and Morales-Grillo J. Processing of gen pol from HIV-1 in S. cerevisiae. Biotecnología Aplicada 1993;10:184-190.





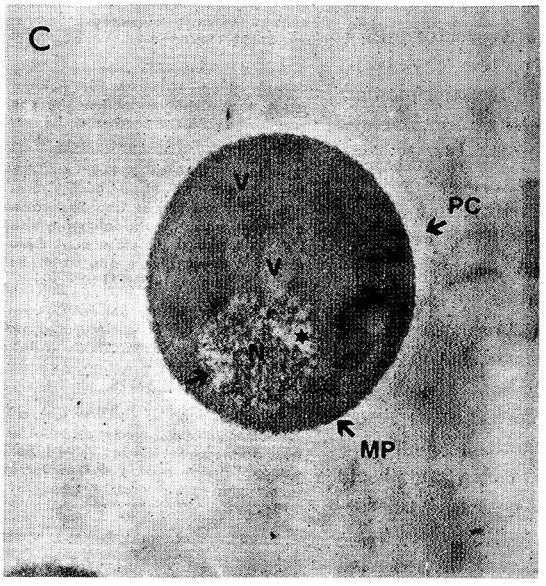
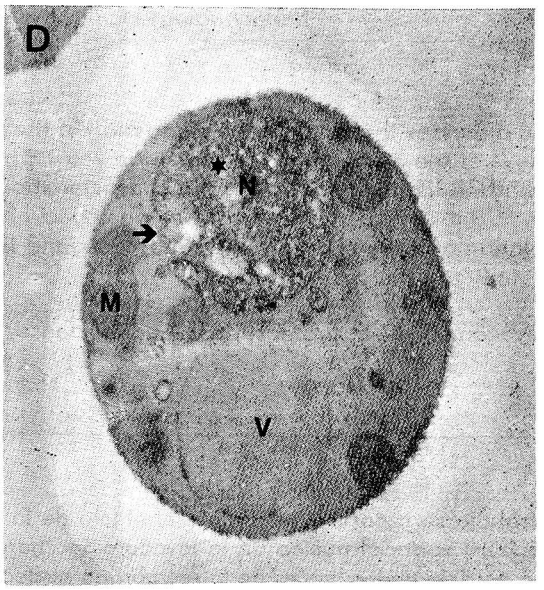
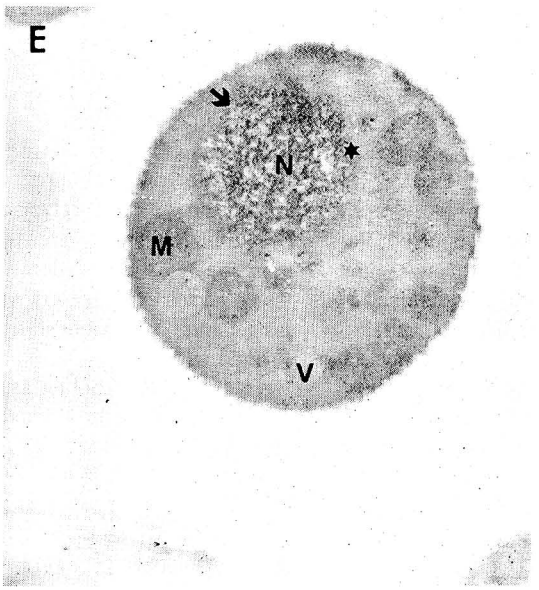


Figure 1. *Electron micrographs of yeast being transformed with plasmid pBSDC before (A), and after 96 hours of induction (B, C, D, E), and the same strain transformed with plasmid pBS-4 before (F) and after induction (G). Nuclei (N) are indicated and nucleal morphological changes as the worm-eaten aspect are clearly visible and marked with a stain. The arrow indicates the loss of the limits of nucleus membrane. PC: Cellular wall, MP: Plasmatic membrane, M: Mitochondria. V: Vacuole. Examination was in a JEOL-JEM-2000 80 KV (X 20K). Bar=500.*



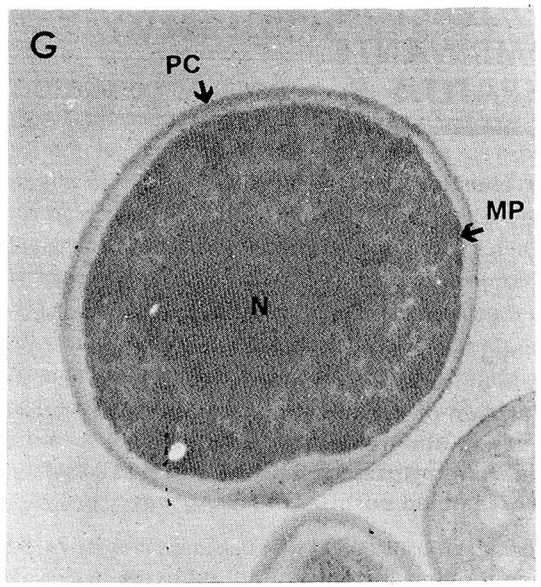


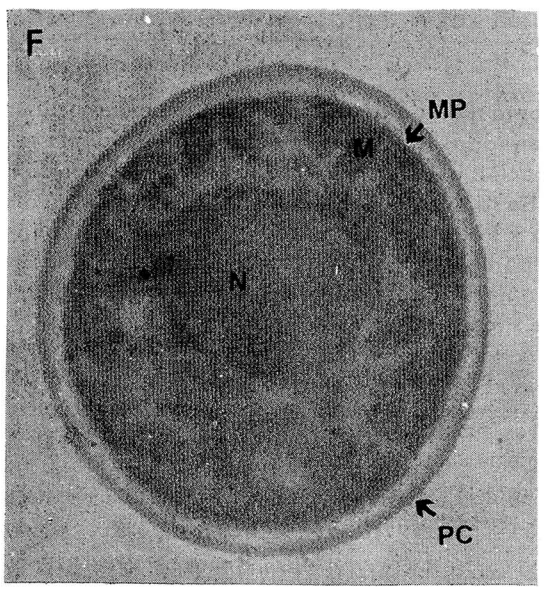
7. Martínez E, García C and Morales-Grillo J. Rapid Transformation of non-Saccharomyces yeast by electroporation. Biotechniques 1993;10:895-896.

8. Galzy P and Soloninski PP. Variation phisiologique de la levadure au cours de la croissance a surl acide lactique comme seule source de carbone. Comptes. Rends. Acad. Sci. (Paris) 1957;245:2423-2433.

9. Bendayan M. Protein-gold electron microscopic immunocytochemistry methods, applications and limitations. J. Electr. Microsc. Tech. 1984;1:243-270.

10. Torrens I, Villegas A, Narciandi R, Santos A, Rodríguez F, Morales-Grillo J, Silva A and Herrera L. La fusion de los genes superóxido dismutasa humana y la proquimosina de ternero se expresa eficientemente en *Saccharomyces cerevisiae*. Biotecnología Aplicada 1989;7:257-264.





HIV-1 pol gene but not in the yeast transformed with plasmid pBS-4 (Figure 1). Expression of another aspartic protease (chymosin) or induction of untransformed yeast, did not show any specific alteration of the nucleus (data not shown).

High proteolytic activity of HIV proteinase in eukaryotic cells has been reported . This activity is directed against several cellular proteins, including cytoskeleton (11) and nuclear chromatin, which is found to condense into a few granules, and nuclear morphology is altered (12).

Recently, apoptosis has been proposed as a mechanism of cell death in HIV pathogenesis (13) which includes oligosomal-size DNA fragments before the onset of cell death.

As was pointed out, after the induction of the pol gene, nuclear morphological changes in *Saccharomyces cerevisiae* are produced, which could be, presumably, performed by HIV proteinase, giving evidence of the toxicity of this product on the yeast cells.

In this case, it is suggested that *Saccharomyces cerevisiae* could be a model for studying apoptosis or specific toxic effects of products from the pol gene on nuclei of eukaryotic cells.

11. Shoeman RL, Honer B, Stoller TJ, Keselmeier C, Miedel MC, Traub P and Graves MC. Human Immunodeficiency virus Type 1 protease cleaves the intermediate filament protein vimentin, desmin and glial fibrillary acidic protein. Proc. Natl. Acad. Sci. USA. 1990;87:6336-6348.

12. Shoeman R L, Honer B, Mothes E and Traub P. Potential role of the viral protease in Human Immunodeficiency virus type 1 associated pathogenesis. Med. Hypoth. 1992;37:137-150.

13. Ameisen JC and Capron A. Cell disfunction and depletion in AIDS: The programmed cell death hypotesis. Immunol Today 1991; 12:102

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