Available online at www.sciencedirect.com

SCIENCE DIRECT

Gene 347 (2005) 83–97

GENE
SECTION
FUNCTIONAL GENOMICS

www.elsevier.com/locate/gene

GCN5: a supervisor in all-inclusive control of vertebrate cell cycle progression through transcription regulation of various cell cycle-related genes

Hidehiko Kikuchi[a], Yasunari Takami[b,c], Tatsuo Nakayama[a,b,c,*]

[a]*Department of Life Science, Frontier Science Research Center, Miyazaki Medical College, University of Miyazaki, 5200, Kihara, Kiyotake, Miyazaki 889-1692, Japan*
[b]*Department of Biochemistry, Miyazaki Medical College, University of Miyazaki, 5200, Kihara, Kiyotake, Miyazaki 889-1692, Japan*
[c]*CREST, JST (Japan Science and Technology), Kazusa, Kisarazu, Chiba 292-0818, Japan*

Received 4 August 2004; received in revised form 17 November 2004; accepted 6 December 2004

Received by A.J. van Wijnen

Abstract

Histone acetyltransferases (HATs) are involved in the acetylation of core histones, which is an important event for transcription regulation through alterations in the chromatin structure in eukaryotes. To clarify participatory in vivo roles of two such enzymes known as GCN5 and PCAF, we generated homozygous DT40 mutants, ΔGCN5 and ΔPCAF, devoid of two alleles of each of the GCN5 and PCAF genes, respectively, with the help of gene targeting technique. While the PCAF-deficiency exhibited no effect on growth rate, the GCN5-deficiency caused delayed growth rate of DT40 cells. FACS analyses revealed not only that the number of cells in S phase decreased, but also that the cell cycle progression was suppressed at G1/S phase transition for ΔGCN5. RT-PCR analyses revealed that the GCN5-deficiency exhibited opposite influences on transcriptions of G1/S phase transition-related genes, i.e. repressions for E2F-1, E2F-3, E2F-4, E2F-6, DP-2, cyclin A, cyclin D3, PCNA, cdc25B and p107; and activations for p27, c-*myc*, cyclin D2 and cyclin G1. Similarly, the deficiency influenced oppositely transcriptions of apoptosis-related genes, i.e. decreased expression of bcl-xL and increased expression of bcl-2. Immunoblotting analyses using a number of anti-acetylated histone antisera revealed that the GCN5-deficiency led to decreased acetylation levels of K16/H2B and K9/H3, and increased those of K7/H2A, K18/H3, K23/H3, K27/H3, K8/H4 and K12/H4. These results indicate that GCN5 preferentially acts as a supervisor in the normal cell cycle progression having comprehensive control over expressions of these cell cycle-related genes, as well as apoptosis-related genes, probably via alterations in the chromatin structure, mimicked by changing acetylation status of core histones, surrounding these widely distributed genes.

Keywords: DT40; Gene targeting; Histone acetylation; Histone acetyltransferase

1. Introduction

In eukaryotes, the orderly cell cycle events are governed by number of cell cycle-related factors (proteins), such as E2Fs, cyclins, Rb-related proteins, cdcs, cdks, etc. The molecular basis for functions of many of these factors has been clarified in kingdom starting from yeast to mammalian. The orderly appearance and disappearance of the multiple factors to program the sequence of molecular events during normal cell cycle progression are manifested by positive and negative regulations of the cell cycle-related genes. On the

Abbreviations: BrdUrd, 5-bromodeoxyuridine; FACS, fluorescence-activated cell sorting; FITC, fluorescein isothiocyanate-conjugated tyamide; GAPDH, glyceraldehydephosphate dehydrogenase; HAT, histone acetyltransferase; HDAC, histone deacetylase; PCR, polymerase chain reaction; PI, propidium iodide; PVDF, polyvinyliden difluoride; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription-polymerase chain reaction; SDS, sodium dodecyl salfate; TCA, trichloroacetic acid.

* Corresponding author. Department of Biochemistry, Miyazaki Medical, College, University of Miyazaki, 5200, Kihara, Kiyotake, Miyazaki 889-1692, Japan. Tel.: +81 985 85 3127; fax: +81 985 85 6503.
E-mail address: tnakayam@med.miyazaki-u.ac.jp (T. Nakayama).

0378-1119/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2004.12.007

other hand, knowledge concerning the involvement of acetylation of core histones in the regulation of cell functions has rapidly been accumulated (Pazin and Kadonaga, 1997; Carrozza et al., 2003). This chemical modification of core histones has been known to be of fundamental importance as to conformational changes of the chromatin (Cheung et al., 2000), and should be precisely regulated through the sequential and alternative catalytic activities of histone acetyltransferase(s) (HATs) and deacetylase(s) (HDACs). The HAT and HDAC members play much diverse and broader roles in cell functions, such as transcription activation, gene silencing, cell cycle progression and/or arrest, cell differentiation and DNA repair in eukaryotes (Georgakopoulos and Thireos, 1992; Brownell et al., 1996; Pennisi, 1997; Brown et al., 2000; Cheung et al., 2000; Carrozza et al., 2003).

Two members of HATs, GCN5 and PCAF, show tissue (or cell type) specific expression characteristics, and therefore each is expected to play the distinct role, and may be at a particular time. For instance, the participation of these two HAT enzymes in the cell cycle progression has been identified in yeast (Zhang et al., 1998; Burgess et al., 1999; Krebs et al., 1999; Howe et al., 2001). In late G1 phase of the cell cycle, GCN5 together with the SW1/SNF complex and its transcription coactivators Swi4p/Swi6p is required for expression of the HO gene (Krebs et al., 1999). The combined loss of GCN5 and SAS3 functions results in both an extensive global loss of H3 acetylation and cell cycle arrest in G2/M phase (Howe et al., 2001). GCN5 and Rpd3 play a distinct and opposing role in IME2 transcription during both meiosis and vegetative growth (Burgess et al., 1999). The deficiency of GCN5 in yeast cells leads to the accumulation in G2/M phase, indicating its impact on normal cell cycle progression (Zhang et al., 1998). In addition, Esa1p is found to be required for the cell cycle progression of yeast, potentially through discrete transcriptional regulatory events (Clarke et al., 1999).

Genes associated with cell cycle progression are tightly controlled by E2Fs that are originally identified for their role in G1/S transition. In mammalian cells, GCN5 together with a cofactor TRRAP is required for the transcriptional activation of E2F4 that regulates the temporal activation of genes involved in the cell cycle progression (Lang et al., 2001). E2F family members responsible for the control of the cell cycle progression themselves are acetylated and regulated by p300/CBP (Marzio et al., 2000). Acetylation of E2F1 by PCAF, CBP and p300 has three functional potentialities: increasing its DNA binding ability, its transcriptional activation capacity and its protein half-life (Martinez-Balbas et al., 2000). Human GCN5 and PCAF are reported to be physiologically associated with the ubiquitously distributed transcriptional factor NF-Y, which itself plays a key role in the cell cycle progression through the transcriptional regulation of cyclin A, cdc25 and cdc2 genes (Currie, 1998). This notion was further supported by the fact that following the release of E2Fs/HDACs, a hierarchy of PCAF–NF-Y–p300 interactions and histones H3/H4 acetylations are required for the activation of cell cycle-related promoters as detected by chromatin immunoprecipitation assay (Ceretti et al., 2003). Moreover, other HAT family members have also been reported to be linked with transcriptional regulation of the cell cycle progression related genes. The catalytic HAT activity of CBP was activated by E1A and controlled by the cyclin E-Cdk2 mediated phosphorylation (Ait-Si-Ali et al., 1998). In stable rat cell lines, an important negative regulatory role of p300 has been established for c-Myc expression that is likely to be important in maintaining the cells at G0/G1 phase of the cell cycle (Baluchamy et al., 2003). These studies demonstrate that acetylation represents a novel mechanism for transcriptional activation of several genes essentially required in coordinating cell cycle events.

Although extensive studies with HATs and HDACs have provided valuable clues to their general functions as histone modifying enzymes, their participatory roles in the cell cycle progression have not been explored much. Furthermore, in spite of accumulation of extensive knowledge on functional nature and/or coordinated action of cell cycle-related factors, the way by which amounts of all or most of these factors are maintained throughout the cell cycle progression is still unknown. This could be achieved by three possible ways as follows. Firstly, transcriptions of genes encoding all or most cell cycle-related genes are individually controlled. Secondly, transcriptions of genes for almost all of the factors is collectively and directly regulated by a putative master transcription factor(s). Thirdly, all-inclusive transcriptional regulation for almost all of the genes occurs through alterations in the chromatin structure surrounding them. Among these three and other possibilities, the third is most likely to happen, since alterations in the chromatin structure have been known to be preferentially involved in DNA-utilizing processes, such as gene expression, DNA replication, recombination and repair.

In order to assess the above-mentioned third possibility, we analyzed and characterized GCN5 and PCAF functions from the ΔGCN5 and ΔPCAF mutants. The two genes were found to be dispensable. The deficiency of GCN5 but not PCAF led to reduction in growth rate and suppression at G1 to S phase transition. These phenotypic characteristics were linked with altered transcripts for G1/S transition and apoptosis related genes facilitated by the maintenance of distinct acetylation status of core histone proteins (H2A, H2B, H3 and H4) influencing chromatin structure.

2. Materials and methods

2.1. Cell cultures

DT40 cells and all subclones were cultured essentially as described (Takami et al., 1995). At the indicated times, cells were counted to determine the growth rate.