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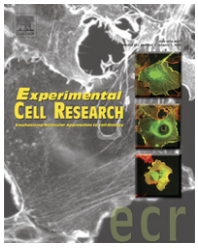

Research Article

Distinct nuclear arrangement of active and inactive *c-myc* genes in control and differentiated colon carcinoma cells

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ABSTRACT

Using sequential RNA–DNA fluorescence *in situ* hybridization, the nuclear arrangement of both the active and inactive *c-myc* gene as well as its transcription was investigated in colon cancer HT-29 cells induced to differentiate into enterocytes. Cytogenetic studies revealed the presence of two chromosomes 8 in HT-29 cells, of which the one containing *c-myc* gene amplicons was substantially larger and easily distinguished from the normal chromosome. This observation enabled detection of both activity and nuclear localization of *c-myc* genes in single cells and in individual chromosome territories. Similar transcriptional activity of the *c-myc* gene was observed in both the normal and derivative chromosome 8 territories showing no influence of the amplification on the *c-myc* gene expression. Our experiments demonstrate strikingly specific nuclear and territorial arrangements of active genes as compared with inactive ones: on the periphery of their territories facing to the very central region of the cell nucleus. Nuclear arrangement of *c-myc* genes and transcripts was conserved during cell differentiation and, therefore, independent of the level of differentiation-specific *c-myc* gene expression. However, after the induction of differentiation, a more internal territorial location was found for the single copy *c-myc* gene of normal chromosome 8, while amplicons conserved their territorial topography.

Introduction

It has become evident that both chromatin modifications (e.g. DNA methylation, histone methylation and acetylation) and the spatiotemporal arrangement of chromatin inside cell nuclei and chromosome territories may play important roles in the regulation of nuclear processes [1–4]. Chromosomes are composed basically of two different types of chromatin, euchromatin and heterochromatin, which occupy discrete compartments in the cell nucleus [5–7]. Transcriptionally active euchromatic genomic regions are preferentially located in the inner parts of cell nuclei, while heterochromatic regions are mostly observed near the nuclear periphery. The nuclear localization of the whole human chromosome 18 and 19 territories provides a striking example: the gene-rich chromosome 19 is positioned in the central part of the cell nucleus while the similarly sized, but gene-poor, chromosome 18 is found at the nuclear periphery [8]. A similar example was found for the nuclear location of the *Mash1* gene. When transcriptionally repressed in embryonic stem cell, this gene

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was located on the nuclear periphery. In contrast, after induction of neural differentiation, the up-regulated *Mash1* gene was repositioned to the nuclear interior [9]. The same conclusions were reached by Zink et al. [10] for selected adjacent loci on human chromosome 7q31, which additionally suggested that transcriptional activity affects nuclear positioning, and not vice versa.

Transcriptional activity of genetic loci might also be related to their territorial arrangement. There have been several attempts to elucidate this relationship. Kurz et al. [11] showed that a non-expressed, anonymous fragment was positioned more interiorly within the chromosome territory while genes tended to be located at the periphery of chromosome territories, irrespective of their activity. It seems that AT-rich DNA is frequently observed more interiorly within the chromosome territories, while GC-rich sequences display a higher variability in their intra-territorial position [12]. The relationship between spatial location of genes within chromosome territories and their transcriptional activity has also been documented in several differentiation systems of embryonic stem cells [13–15]. In addition, transcriptionally active loci were looping out from their related territories in some experiments [13,14,16,17]. Extraterritorial transcriptional activity has also been observed for genes of the epidermal differentiation complex by Williams et al. [18]; for the human β-globin locus by Ragoczy et al. [19]; and for the β-like globin gene cluster in differentiated cells by Galiová et al. [20].

The data described above imply that cell differentiation can be considered as an interesting model for studying correlations between gene nuclear topography and expression. During differentiation, changes in chromatin structure and nuclear organization establish a heritable pattern of gene expression [21] that can be influenced by many proteins (*trans*-acting factors) or DNA sequences (*cis*-acting elements) [22–24]. An example is a differentiation-related gene silencing that was ascribed to heterochromatin protein 1 (HP1) and Ikaros protein that both associate with centromeric regions [25–27]. Inactivation of some differentiation-specific genes has been linked to their distance from centromeres [22,28,29]. Additionally, the association of centromeric clusters with HP1 proteins and transcription intermediary factor TIF1β has been found to be required for induction of several differentiation pathways of mouse embryonic cells [26,27].

Gastrointestinal epithelial cells represent an attractive model for studies of cell differentiation. In our experiments, we have used the human colon adenocarcinoma cell line HT-29 that can be induced *in vitro* to enterocytic differentiation by sodium butyrate (NaBt) [30–32], which has physiological relevance in colonic epithelium function [33]. Colorectal cancers are mostly characterized by mutations in the *APC* gene, involved in the Wnt/β-catenin pathway [34,35]. It is known that the *APC* product is normally localized in the cytoplasm where it sequesters β-catenin, promotes its degradation, and prevents its entry to the cell nucleus. In colon cancer cells, the mutated APC protein is unable to bind β-catenin, which results in β-catenin entry to the cell nucleus and the *c-myc* gene over-express [36–38].

In our experiments, the *c-myc* gene was studied to find inter-relations between its nuclear topography and expression during enterocytic differentiation of adenocarcinoma HT-29 cells. These cells are characterized by mutations in the *APC* gene leading to the formation of a truncated protein product [39]. The HT-29 cell line provides a unique model of two distinguishable territories of chromosome 8: (a) the normal territory (HSA8n) that contains a single copy of the *c-myc* gene, and (b) the derivative territory (HSA8d) that contains a homogeneously staining region (HSR) involving *c-myc* gene amplicons and telomeric sequences of the q arm. We found that HSA8n was positioned more centrally, while the large derivative territory was tightly associated with the nuclear envelope. Distinctions were also observed after anti-RNAP II staining, which revealed reduced RNAP II signals in the region of the HSA8d just beneath the nuclear membrane. On the other hand, the whole HSA8n was densely labeled by RNAP II, which implies a higher transcriptional activity in this region. Changes in the territorial arrangement, involving relocation of the normal *c-myc* gene inside the territory, were associated with differentiation processes. On the other hand, the *c-myc* gene amplicons did not change their territorial topography during enterocytic maturation. Both normal and derivative chromosomes displayed *c-myc* transcriptional activity, determined by RNA-FISH at all differentiation intervals, but because of the number of *c-myc* copies in the derivative chromosome, the activity per copy was substantially lower. The reduced size and intensity of the transcription sites corresponded well with transient *c-myc* down-regulation studied by RT-PCR during the differentiation process. In comparison with non-transcribed coding sequences, the *c-myc* transcripts and the active *c-myc* genes were located more peripherally within the chromosome territory. In addition, the active *c-myc* genes from both territories were positioned in a very central part of the interphase nucleus independently of differentiation and, therefore, independently of the differentiation-specific changes in the level of *c-myc* gene expression. The *c-myc* gene transcriptional activity in HT-29 cells was similar for both the normal and derivative HSA8 territories in control and during differentiation. Consequently, amplification of the *c-myc* gene did not contribute to its increased expression in HT-29 cells.

Materials and methods

Cell cultivation and differentiation

The human adenocarcinoma colon cell line HT-29 (ATCC) was plated at a density of 2×10^5/ml in Dulbecco's modified Eagle's medium (D-MEM PAN, Germany, # P03-0710) supplemented with 10% fetal calf serum and 100 i.u./ml penicillin and 0.1 mg/ml streptomycin. The leukemic HL-60 cells were cultivated according to Bártová et al. [40] and A-549 cells according to Bártová et al. [41]. The human primary colon fibroblasts (CCD-18Co) were a generous gift of W. Schmitt from Martin Luther University in Halle. These cells were grown in D-MEM containing 1 g/l glucose and 2 mM L-glutamine supplemented with 10% fetal calf serum without antibiotics. All cell lines were cultivated under standard conditions at 37°C in a humidified atmosphere containing 5% CO_2.

HT-29 cells under normal culture conditions display an undifferentiated phenotype. In response to various inducers, including the inhibitor of histone deacetylases sodium