CLONING AND CHARACTERIZATION OF CHINESE HAMSTER HETEROCHROMATIN PROTEIN 1 (HP1) ISOFORMS

Summary of PhD thesis

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Introduction

In the nucleus of eukaryotic cells, the DNA interacts with a large number of proteins, and forms a tightly-packed DNA-protein complex called chromatin. During cell divisions the genetic material is further compacted, which results in the formation of chromosomes. The level of DNA packaging is diverse along the chromosomes, the genome is organized into euchromatic and heterochromatic regions and give rise to distinct functional domains. Heterochromatin is the most condensed region of the genome that is intensively stained throughout the cell cycle. The euchromatin regions contain the majority of the expressed genes; in the heterochromatin, the number of transcriptionally active genes is very low due to the tightly packed structure of the chromatin. There are two functionally distinct forms of heterochromatin. The facultative heterochromatin is condensed and inactive in only certain developmental stages, due to epigenetic modifications. The constitutive heterochromatin remains condensed and inactive throughout the life cycle of the cells on both members of the homolog chromosome pairs. The structural attributes characterizing the facultative or the constitutive heterochromatin are not yet completely known. The main protein component of the constitutive heterochromatin is HP1 (heterochromatin protein 1), which is a highly conserved protein, in the eukaryotes.

There are three isoforms of HP1, in all eukaryotic species studied so far: HP1α, HP1β and HP1γ. The isoforms exhibit different localization patterns along the chromosomes, and presumably have both redundant and specific functions.

There are large constitutive heterochromatic blocks in the chromosomes of most mammals. Chinese hamster is one of the few mammalian species that are characterized by the relatively poor heterochromatin content. The question

arose as to whether or not the absence of these heterochromatic blocks could be attributed to the lack of HP1. It is noteworthy that human satellite DNA-based artificial chromosomes (SATACs) generated in CHO cells are highly heterochromatic. Further, mouse SATACs transferred to CHO cells remained heterochromatic. The questions, whether HP1 in heterochromatic SATACs was substituted by certain heterochromatin-binding hamster proteins or whether they contain genuine HP1 proteins remained open. To answer these questions we attempted to clone hamster HP1 by using human HP1 isoform-specific probes.

Methods

- Recombinant DNA technics
- DNA isolation from cultured cell lines
- DNA sequencing, sequence analysis
- PCR amplification
- Southern blotting
- λ-phage library screen
- Protein electrophoresis on SDS gel
- Western blotting
- Culturing and transfection of cells lines
- *In situ* hybridization
- C- banding
- Immunofuorescent microscopy

Results and Discussion

In our study we identified the hamster HP1 proteins (chHP1α, chHP1β and chHP1γ). All three isoforms known from mouse and human were cloned and characterized. The sequences of the cDNAs were compared with those of the mammalian homologues. The coding regions of the cDNAs demonstrated high-level sequence conservation (>90%). The deduced amino acid sequences were also highly conserved: chHP1α and β differed in only one amino acid from those of the mouse homologues, while the differences between the hamster and human HP1α isoforms were restricted to 5 amino acids. These differences are not in those amino acid positions of the chromo domain which are presumed to be necessary for MetK9 H3 histone binding. The

gamma isoforms were identical in all three species. Similarities were also observed in the 5' and 3'-UTR of the isoforms. These conservative sequences may have regulatory functions but their exact roles remain to be elucidated. The UTRs of the different hamster isoforms were distinct, suggesting that they may be under different translational regulation.

The structure of the hamster HP1α gene proved to be similar to that of the mouse and human orthologues. It contains 5 exons; the translation start point was localized in the second exon. Although the exons of the HP1 isoforms were highly similar in the different mammals, the sizes of the introns and their sequences were different. Some limited homologies present in all three mammals were found in nonrepetitive regions of the introns, implying that these sequences may have regulatory functions.

HP1 was first identified in *Drosophila melanogaster* by its association with heterochromatin. In our study we examined the localisation of the hamster HP1 isoforms. To avoid the possible adverse effects caused by the experimental conditions of immunolocalization, fluorescent protein fusion reporters were used in the localization studies. We used GFP-HP1 and HP1-DsRed fusion reporter constructs in studying the distribution of the hamster HP1 isoforms on native hamster and mouse chromosomes, and on mouse and human satellite DNA-based artificial chromosomes. We demonstrated that the fused fluorescent reporter tags and their positions relative to the HP1 did not influence the localization pattern. The C and N-terminal fusions of the fluorescent proteins gave similar and comparable results on native chromosome preparations. Under completely native conditions all three hamster HP1 isoforms predominantly concentrated in heterochromatic regions. Although in significantly lower amount, all three chHP1 isoforms were observed in the euchromatin too. The similarity in the localization patterns of HP1-DsRed fusion proteins and the

endogenous HP1α (detected by monoclonal antibody) indicates that the DsRed fusion partner does not alter the chromosomal distribution of these proteins.

Despite of the fact that the hamster chromosomes have no large blocks of constitutive heterochromatin, we demonstrated that the three known HP1 isoforms are present in hamster cells, and seem to contribute to the chromatin structure in a similar way as presumed for the mouse and human orthologues. Our results indicate that the indigence of heterochromatin in hamster cells is not a consequence of the absence of HP1 proteins.

In mouse and human cells, the bulk of the satellite DNA components of the heterochromatin consists of tandem reiterations of relatively short repeat units (<1 kb), while in the hamster, apart from the centromeric TTAGGG repeats, the satellite sequences are far more complex and the lengths of the repeat units greatly exceed the sizes of the repeat units of human and mouse satellites. Human and mouse satellite DNA-based artificial chromosomes that build up short tandem repetitive satellite sequences appear as constitutive heterochromatin. This suggests that the limited amount of heterochromatin in hamster cells may be attributed to the special satellite DNA content of the hamster chromosomes.

List of publications used for the present PhD theses:

Szakal B, Cserpan I, Csonka E, Monostori E, Udvardy A, Hadlaczky G. Cloning, characterization and localization of Chinese hamster HP1 isoforms. *Chromosome Res*. 2004;12(5):483-93.

Csonka E, Cserpan I, Fodor K, Hollo G, Katona R, Kereso J, Praznovszky T, **Szakal B**, Telenius A, deJong G, Udvardy A, Hadlaczky G. Novel generation of human satellite DNA-based artificial chromosomes in mammalian cells. *J Cell Sci*. 2000 Sep;113 (Pt 18):3207-16.

List of publications not used for the present PhD theses:

Endre G, Kalo P, Kevei Z, Kiss P, Mihacea S, **Szakal B**, Kereszt A, Kiss GB. Genetic mapping of the non-nodulation phenotype of the mutant MN-1008 in tetraploid alfalfa (Medicago sativa).
Mol Genet Genomics. 2002 Feb;266(6):1012-9. Epub 2002 Jan 23.