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Chromosomal localization of genes encoding small heat shock proteins (*HSPB*) in cattle and sheep

Chromosomowa lokalizacja genów kodujących małe białka szoku cieplnego (*HSPB*) u bydła i owiec

Summary. The small heat shock protein gene products (*HSPB*) reveal chaperone and neuroprotective activity contributing to the stabilization of cell homeostasis and cytoskeleton of neurons in conditions of thermal or oxidative stress. The aim of this study was chromosomal localization of five small heat shock protein genes (*HspB1*, *HspB2*, *CRYAB* – alternative name *HspB5*, *HspB6* and *HspB8*) in cattle and sheep, with an application of FISH technique with the probes obtained from BAC clones (derived from the CHORI-240 Bovine BAC Library) containing sequences of these genes. Prior to in situ hybridization, carried out on metaphase chromosomes stained by means of DAPI bands technique, the presence of the studied genes in the selected clones was confirmed by PCR method with the use of the gene-specific primers. As a result of the experiments, FISH signals in the following cattle (BTA) and sheep (OAR) genome regions were obtained: BTA25q22/OAR24q22 (*HSPB1*), BTA15q14-21/OAR15q14-21 (*HSPB2* and *CRYAB*), BTA18q24/OAR14q24 (*HSPB6*), BTA17q24-25/OAR17q24-25 (*HSPB8*). The studies enabled to designate physical localization of the studied genes on the genome maps of these species and confirmed the high level of autosome conservation in Bovidae. The results obtained may provide useful information concerning the genetic background of neurodegenerative diseases in breeding animals.

Key words: cattle and sheep chromosomes, FISH technique, cytogenetic mapping, small heat shock proteins – *HSPB*, neurodegenerative disorders

INTRODUCTION

Small heat shock proteins (*HSPB*) comprise conserved family of molecular chaperones displaying different activities in suppressing protein aggregation. HSPB are found in most organisms where they are induced upon stress and are involved in protecting cells from various unfavorable conditions. The *HSPB* family includes ten members (*HSPB1 – HSPB10*) diverse in size, in sequence and in the N- and C-terminal regions but sharing similar structural and functional characteristics [Acunzo *et al.* 2012]. Their ability of interacting with multitude of proteins account for involvement in as various processes as cell protection from oxidative stress, apoptosis and proteolysis, proliferation, cell motility and muscle contraction [Wettstein *et al.* 2012, Arrigo 2013]. Moreover, mutations of *HSPB* are often associated with severe neurodegenerative disorders (including transmissible spongiform encephalopathies – TSEs), myopathies or motor neuropathies, while their tissue-specific overexpression is closely linked to cardiovascular diseases and cancer [Arrigo 2012, Boncoraglio *et al.* 2012].

Recently, some *loci* modulating resistance/susceptibility to prion diseases (other than *PRNP – prion protein locus*) have been identified in cattle and sheep genomes. Concretely, several QTL influencing BSE and scrapie incubation period have been reported on bovine (BTA5, BTA6, BTA10, BTA13, BTA17, BTA19, BTA20, BTAX) and ovine (OAR6, OAR18) chromosomes [Hernandez-Sanches *et al.* 2002, Zhang *et al.* 2004, Moreno *et al.* 2008, 2010]. Therefore, FISH-based chromosomal assignment of the *HSP* genes in these species is assumed to be a good tool to identify new QTL associated with resistance/susceptibility for TSEs in domestic bovids and verify their genome assemblies [Lewin *et al.* 2009, De Lorenzi *et al.* 2010, Hu *et al.* 2013].

The aim of the presented study was chromosomal assignment of the *HSPB1*, *HSPB2*, *CRYAB (HSPB5)*, *HSPB6* and *HSPB8* small heat shock protein genes, due to their putative involvement in prion diseases development in cattle and sheep.

MATERIAL AND METHODS

Cytogenetic preparation and chromosome identification

Cattle and sheep lymphocytes were cultured and treated with BrdU (10 μ g/ml) and H33258 (20 μ g/ml) (Sigma) 6 h before harvesting to obtain, counterstained by DAPI, late-replicating banded chromosome preparations for FISH detection (according to the protocol reported by Iannuzzi and Di Berardino) [Iannuzzi and Di Berardino 2008]. Chromosome identification followed the standard cattle and sheep karyotypes and ideograms, according to the international chromosome nomenclature for domestic bovids ISCANDB 2000 [Di Berardino *et al.* 2001].

Probe preparation and fluorescence in situ hybridization (FISH)

The bovine BAC clones overlapping *HSPB1*, *HSPB2*, *CRYAB* (*HSPB5*), *HSPB6* and *HSPB8* genes, as indicated in Table 1, were screened by database searching (http://www.ncbi.nlm.nih.gov/clone; http://www.chori.org/bacpac/bovine240.htm) and obtained from CHORI-240 Bovine BAC Library (BACPAC Resources)

(http://bacpac.chori.org/libraries.php). It was not possible to select separate BAC clones for the HspB2 and CRYAB (HSPB5) genes (http://www.ncbi.nlm.nih.gov/gene/508671; http://www.ncbi.nlm.nih.gov/gene/281719), due to their adjacent location in the bovine genome, thus the clone containing sequences of both the genes (CH240-134C10) was used. Each selected clone was verified to contain the studied gene with PCR amplification using specific primers, which are displayed in Table 1. The BAC DNA, extracted according to the alkaline lysis miniprep protocol (Qiagen), was labelled with biotin-16dUTP by standard nick translation kit (Roche) and applied as the probes in the FISH experiments on cattle and sheep chromosomes. Labelled probes with an excess of bovine competitor DNA were denatured for 5 min at 75°C, preannealed for 15 min at 37°C, and applied onto chromosome preparations, denatured previously in formamide for 1 min at 70°C. Hybridizations were carried out overnight (up to three days in cross-species experiments) at 37°C. After detection step with the use of FITC-avidin (fluorescein isothiocyanate-avidin) (Vector Laboratories) and anti-avidin antibodies (Sigma), slides were counterstained with DAPI (4,6-diamidino-2-phenylindole) solution (0.24 µg/ml) in Antifade (Vector Laboratories) to obtain DAPI-banded chromosomes (with patterns corresponding to the O bands). Slides were analyzed in Axio Imager.D2 (Zeiss) fluorescence microscope equipped with Axio Vision computer-assisted image analysis system.

Table 1. PCR protocol verifying presence of the *HSPB* genes in BAC clones Tabela 1. Protokół reakcji PCR weryfikującej obecność genów *HSPB* w klonach BAC

Gene Gen	BAC clone Klon BAC	GenBank Accession numer Numer akcesyjny GenBank	PCR			
			primers (5'-3' sequences) startery (sekwencje 5'-3')	Ta (°c)	product size (bp) dł. produktu (pz)	gene fragment fragment genu
HSPB1	CH240- 362H14	CR850362	ggctacatttcccgttgctt ggtctttacttgtttccggct	57	229	exon 2–3
HSPB2	CH240- 134C10	CR455443	attgtgttggggcacctcg ggtaaagtggctcacgtcca	58	294	exon 2
CRYAB	CH240- 134C10	BI680522	cgccccacactcacctaac gcgctcttcatgtttgccat	58	344	exon 1–3
HSPB6	CH240- 422N21	CF614494	ctgggatgtgccttgaacct agggtagggtcagaaaaggag	57	501	exon 2–3
HSPB8	CH240- 279N2	BT020640	cttcgtggctggagtgtctt ccaagaggcagtcaagtcgt	56	336	exon 1

RESULTS

The FISH experiments allowed for the precise assignment of all BAC clones overlapping sequences o five small heat shock protein genes – *HSPB1*, *HSPB2*, *CRYAB* (*HSPB5*), *HSPB6* and *HSPB8* to the homologous cattle (BTA) and sheep (OAR) chromosomes and chromosome regions, as shown on Figure 1, and displayed in Table 2. FITC signals, which were observed generally as double spots on both metaphase chromosomes and chromatids, had been occurred with frequency of 68–77% in cattle and 55–62% in sheep.



Fig. 1. Cytogenetic localization of the *HSPB* genes on chromosomes of cattle (BTA) (A–D) and sheep (OAR) (E–H)

Rys. 1. Cytogenetyczna lokalizacja genów *HSPB* na chromosomach bydła (BTA) (A–D) i owiec (OAR) (E–H)

Gene symbol Symbol genu	Gene name Nazwa genu	Protein function Funkcia białka	Cytogenetic localization Cytogenetyczna lokalizacja	
~) 8	8	j	BTA	OAR
HSPB1	heat shock 27 kDa protein 1	stress resistance, actin organization	25q22	24q22
HSPB2	heat shock 27kDa protein 2	stress response, somatic muscle development	15q14-21	15q14-21
CRYAB	crystallin, alpha B	anti-apoptosis, muscle organ development, response to heat, negative regulation of intracellular transport, camera-type eye development, structural constituent of eye lens, protein homooligomerization activity, unfolded protein binding	15q14-21	15q14-21
HSPB6	heat shock protein beta-6	stress response, protein homodimerization activity, structural constituent of eye lens	18q24	14q24
HSPB8	heat shock 22kDa protein 8	stress response, chaperone activity, identical protein binding	17q24-25	17q24-25

Table 2. Cytogenetic localization of the studied *HSPB* genes in the genomes of cattle (BTA) and sheep (OAR) and functions of encoded proteins Tabela 2. Cytogenetyczna lokalizacja badanych genów *HSPB* w genomach bydła (BTA) i owiec (OAR) oraz funkcje kodowanych białek

DISCUSSION

In this study we present FISH-based chromosomal assignment of five *HSPB* genes (*HSPB1*, *HSPB2*, *CRYAB* (*HSPB5*), *HSPB6*, *HSPB8*) in cattle 25q22, 15q14-21, 18q24, 17q24-25 and sheep 24q22, 15q14-21, 14q24, 17q24-25 genome regions. The *HSPB2* and *CRYAB* (*HSPB5*) *loci* (located at the distance of 1.035 kb in cattle and 0.482 kb in sheep) were mapped to the homologous bovine and ovine chromosomes and chromosome bands, extending the cytogenetic maps of the 15 autosome of the studied species. Likewise in humans, these two neighboring genes (composed of the two and three exons, respectively) are localized in the same genome region and arranged in a head-to-head manner with an inter-genic sequence of less than 1 kb, raising a possibility of shared regulatory elements for their expression [Iwaki *et al.* 1997].

It is noteworthy, that presented in this study physical positions of HSPB loci are in agreement with the corresponding human locations (HGNC) (http://www.genenames.org), taking into account the comparative painting, radiation hybrid or marker mapping data between bovids and humans (BOVMAP (http://dga.jouy.inra.fr/cgi-bin/lgbc/main.pl?BASE=) [Chowdhary *et al.* 1996, Everts-van der Wind *et al.* 2004, Itoch *et al.* 2005, Darlymple *et al.* 2007, Goldammer *et al.* 2009, Schibler *et al.* 2009]. In general, all these five HSPB genes are located in homologous chromosomes and chromosome bands, confirming the significant degree of autosome homologies among cattle and sheep which are very closely related to each other from the

evolutionary point of view [Iannuzzi *et al.* 2009]. Furthermore, the chromosomal assignments of the studied genes are in accordance with results of our earlier provisional comparative mapping of the *HSPB loci* in domestic bovids [Danielak-Czech *et al.* 2014a, 2014b].

On the whole, the study performed may help to elucidate the role of HSPB genes in the development of neurodegenerative disorders in domestic bovids and other livestock species. Such an approach appears to be justified on the ground that a huge amount of data demonstrates neuroprotective role of endogenous expressed or stress-induced HSPB (HSPB1, CRYAB) in infectious protein deposit diseases (with particular emphasis on prion diseases) in humans, rodents and domestic bovids [Tortosa et al. 2008, Vidal et al. 2009, Brownell et al. 2012, Arrigo 2013]. In addition, some heat shock protein loci (among them HSPB) have been defined as a putative positional or functional candidate genes influencing polygenic response to prion diseases [Serrano et al. 2011, Bae et al. 2012]. The four members of the heat shock protein gene families HSP90, HSP70 and HSPB have been chosen for their functions as chaperones and apoptosis modulators, as well as their possible protective effect against the stress-related infectious protein aggregation and neuronal degeneration in prion diseases [Sawiris et al. 2007, Serrano et al. 2011, Brownell et al. 2012, Brown et al. 2014]. Structural and functional analysis of the inducible form HSP90AA1 gene and distribution of polymorphisms among sheep with different responses to scrapie revealed variability in the HSP90AA1 5' flanking region, associated with scrapie incubation period [Marcos-Carcavilla et al. 2008, 2010]. The subsequent studies displayed negative correlation between prion protein deposition and the expression of HSP90, HSP73 and HSPB1 genes, suggesting that high levels of HSP gene expression are associated to the prevention or degradation of prion protein aggregates and the presence of reactive astrocytosis in natural scrapie [Serrano et al. 2011]. The above mentioned experiments confirmed previous findings, which had been proved that HSPB1 gene expression level contribute to the development of the prion protein deposits and morphological lesions such as spongiosis or gliosis of classical scrapie in sheep [Vidal et al. 2009]. The similar studies showed increased HSPB1 expression as a stress response of the central nervous system in a mouse model of BSE [Tortosa et al. 2008]. Generally, it should be stressed that research on the small heat shock protein genes has been to a large degree limited to prions and tauropathies in humans and there is rather no evidence whether mutations of these genes can affect TSE development in domestic bovids [Brownell et al. 2012].

CONCLUSIONS

The experiments carried out indicated that FISH mapping is still useful to validate the data on physical gene location and improve *Bovidae* genome assemblies.

The reported chromosomal localizations of the small heat shock protein genes may be a basis for identifying new QTL associated with response for TSE in *Bovidae* species.

The presented study adds further information to the cytogenetic maps of bovids and precisely assigns *HSPB loci* related to prion disease development in cattle and sheep, which are the major domestic bovid species of great economic importance.

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Streszczenie. Produkty genów małych białek szoku cieplnego (HSPB) wykazują aktywność chaperonową i neuroprotekcyjną, przyczyniając się do stabilizacji równowagi komórkowej i cytoszkieletu neuronów w warunkach stresu oksydacyjnego lub termicznego. Celem badań była chromosomowa lokalizacja pięciu genów małych białek szoku cieplnego (HspB1, HspB2, CRYAB – alternatywna nazwa HspB5, HspB6 i HspB8) u bydła i owiec, przy zastosowaniu techniki FISH z sondami uzyskanymi z klonów BAC (pochodzących z biblioteki genomowej CHORI-240 Bovine BAC Library) zawierających sekwencje tych genów. Przed hybrydyzacją in situ, przeprowadzoną na chromosomach metafazowych barwionych techniką prążków DAPI, potwierdzono obecność badanych genów w wyselekcjonowanych klonach metodą PCR z wykorzystaniem genowo specyficznych starterów. W wyniku przeprowadzonych eksperymentów uzyskano sygnały FISH w następujących regionach chromosomów bydła (BTA) i owiec (OAR): BTA25q22/OAR24q22 (HSPB1), BTA15q14-21/OAR15q14-21 (HSPB2 and CRYAB), BTA18q24/OAR14q24 (HSPB6), BTA17q24-25/OAR17q24-25 (HSPB8). Badania umożliwiły określenie fizycznej lokalizacji badanych genów na mapach genomowych tych gatunków i potwierdziły wysoki poziom konserwatyzmu autosomów u Bovidae. Uzyskane wyniki mogą dostarczyć przydanych informacji dotyczących genetycznego podłoża chorób neurodegeneracyjnych u zwierząt hodowlanych.

Słowa kluczowe: chromosomy bydła i owiec, technika FISH, mapowanie cytogenetyczne, małe białka szoku cieplnego – *HSPB*, zaburzenia neurodegeneracyjne