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Biochimica et Biophysica Acta 1753 (2005) 257 – 262
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
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Characterization and crystallization of human DPY-30-like protein, an essential component of dosage compensation complex

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Received 18 May 2005; received in revised form 8 August 2005; accepted 8 August 2005
Available online 30 August 2005

Abstract

Human DPY-30-like is a homolog of *C. elegans* DPY-30. DPY-30 is an essential component of dosage compensation machinery and loss of *dpy-30* activity results in XX-specific lethality. In XO animals, DPY-30 is required for developmental processes other than dosage compensation. In yeast, the homolog of DPY-30, Saf19p, functions as a member of histone 3 lysine 4 methylation complex, which is the key part of epigenetic developmental control. In this report, human DPY-30-like protein was overexpressed and purified with the goal of structure determination. It was crystallized at 291 K in hanging drops by the vapour diffusion technique from a precipitant solution consisting of $(NH_4)_2SO_4$ (1.5–2.0 M), Tris–HCl (0.1 M, pH 8.0). The crystal diffracted to 2.7 Å resolution at 100 K in-house and belongs to the space group $P4_12_12$ or $P4_32_12$ with unit–cell parameters of $a=b=74.5$ Å, $c=87.0$ Å, $\alpha=\beta=\gamma=90.0°$. The asymmetric unit contains two molecules with 49% solvent content. We also analyzed its biochemical and biophysical characterizations. Efforts are now under way to determine the molecular structure of the DPY-30-like. These studies will open a new avenue towards the structure–based functional analysis of human DPY-30-like and dosage compensation machinery.

Keywords: DPY-30; Dosage compensation; X chromosome; *C. elegans*

1. Introduction

Dosage compensation is a chromosome-wide regulatory process that controls the expression of numerous genes related solely by their linkage to the same sex chromosome [1]. Dosage compensation mechanisms equalize expression of X-linked genes between the sexes and prevent the sex-specific lethality that would otherwise result from the two-fold difference in X-linked gene dose [2–6]. The mechanisms used to achieve dosage compensation are diverse. In mammals, dosage compensation is accomplished by random inactivation of one of the two female X chromosomes, thereby reducing the effective X chromosome dose to that of males (XY) [7–10]. In *Drosophila*, the dosage compensation machinery acts to transcribe the single X chromosome of males (XY) at twice the rate as each of the two X chromosomes in females (XX) [6,11]. In *C. elegans*, XX hermaphrodites reduce the transcript levels produced by each X chromosome to achieve the same levels produced by the single X of males (XO) [12]. Despite their different mechanisms, the regulators in dosage compensation, such as the *dpy* gene family, are highly conserved throughout evolution (Fig. 1).

In *C. elegans*, four autosomal *dpy* genes, *dpy-21*, *dpy-26*, *dpy-27* and *dpy-28*, were identified by genetic analysis. The products of these *dpy* genes are essential in XX animals for proper dosage compensation, but not for sex determination [13,14]. However, *dpy-30* not only encodes an essential component of the dosage compensation process, but also coordinately regulates sex determination [13]. The *dpy-30* mutant phenotypes superficially resemble those caused by mutations in *dpy-26*, *dpy-27* and *dpy-28*, but detailed phenotypic analysis reveals important differences that distinguish

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1570-9639/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.bbapap.2005.08.011

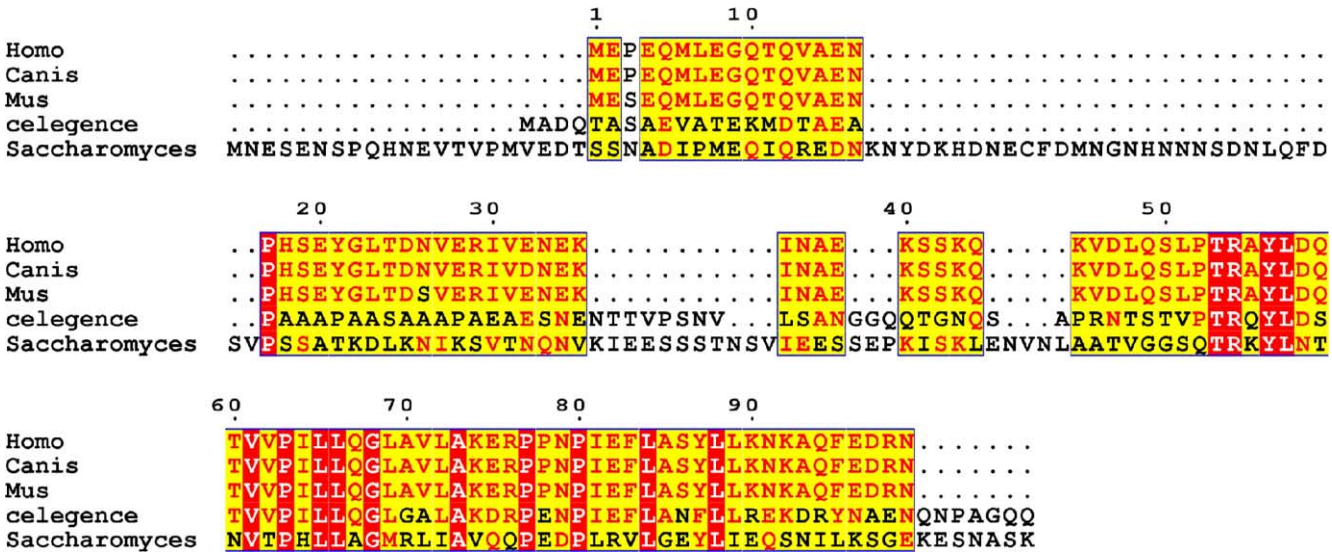

Fig. 1. Alignment of DPY-30 proteins. The protein is highly conserved throughout different species.

dpy-30 from these genes [15]. Analysis of all mutants indicates that DPY-30 is the upstream regulator of other *dpy* genes and is very important in the balance of the sex determination and dosage compensation processes. So, in addition to acting in the dosage compensation process, *dpy-30* may play a more general role in the development of both XX and XO animals [1,15].

The high degree of conservation in the *dpy* gene family raises an important question about how members of the same gene family act in different dosage compensation mechanisms of different species. To address this issue, studies on the characterization, structure and molecular mechanism of DPY-30 are necessary and urgent. As the upstream regulator, detailed studies of DPY-30 will be of great significance.

Recent studies in *Saccharomyces cerevisiae* demonstrated that Saf19p, the homolog of *C. elegans* DPY-30, was identified as an important component of the seven-member complex (Set1 protein complex) and can specifically function as a histone 3 lysine 4 (H3-K4) methyltransferase [16–18]. Epigenetic control was revealed to be the central molecular mechanism of dosage compensation since DPY-30 is the upstream regulator, and H3-K4 methylation is one of the key points of "histone code". The "histone code" is known to be a fundamental regulatory mechanism that has an impact on most, if not all, chromatin-templated processes, with far-reaching consequences for cell fate decisions and both normal and pathological development. [19]. All of these suggest the functional importance of DPY-30.

It is speculated that there is a human complex with similar function to the Set1 protein complex in *Saccharomyces cerevisiae* and DPY-30-like protein, the homolog of *C. elegans* DPY-30, was considered as an essential member. However, no studies have been reported so far. Here, we describe the subcloning, expression, purification and crystallization of the protein and also provide its biochemical and biophysical characterizations. Our results show that the pure recombinant protein forms an oligomer and is suitable for crystallization. These studies are likely to give insight into the precise illustration of the functions of DPY-30-like protein and molecular mechanism of dosage compensation.

2. Materials and methods

2.1. Construction of the expression vector

The human *dpy-30-like* gene was identified from a large scale sequencing of human brain cDNA library. A PCR product containing the coding sequence of *dpy-30-like* was generated from the recombinant pUC plasmid carrying the *dpy-30-like* gene. Two PCR primers, 5′-cgc gga tcc atg gag cca gag cag atg c-3′, 5′-ccg ctc gag tca gtt tcg atc ttc aaa ctg tgc c-3′ were designed. The PCR product was restricted with *Bam*HI and *Xho*I, purified and ligated into *Bam*HI and *Xho*I restricted sites of the pGEX-6P-1 plasmid vector (Amersham Biosciences) with T4 DNA ligase. A further transformation into *E. coli* BL21 was performed and the positive clones with an insert of the expected size were identified by double enzyme digestion with *Bam*HI and *Xho*I. The sequence of the insert was verified by sequencing. The clones harbouring the expected recombinant plasmid were used for protein expression.

2.2. Protein expression, purification, and gel-filtration analysis

The recombinant plasmid was overexpressed in LB medium in a 37 °C incubator. When the culture density (OD600) reached 0.6–0.8, the culture was induced with 0.2 mM IPTG and continuously grown for an additional 10 h at 16 °C before the cells were harvested. Bacterial cells were homogenized by sonication in phosphate-buffered saline (PBS, 10 mM sodium phosphate, pH 7.4; 150 mM NaCl). The lysates were clarified by

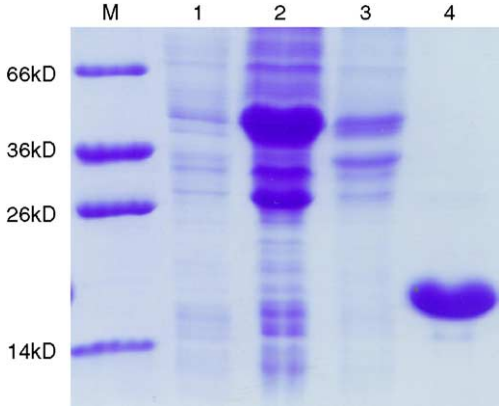

Fig. 2. 15% SDS-PAGE analysis of the expression and purification of DPY-30-like. M: molecular mass standards in kDa; Lane 1: non-induced whole cell lysate; Lane 2: the supernatant; Lane 3: the precipitant; Lane 4: purified DPY-30-like.