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Full paper / Mémoire

Cloning and heterologous expression in *Escherichia coli* of the fission yeast *vip1* gene, showing differential expression after aldosterone treatment

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Abstract

The steroid hormone aldosterone was found to be associated with fibrosis of the heart. Therefore, we are investigating the effects of the mineralocorticoids aldosterone and 11-deoxycorticosterone (DOC), its precursor, on the proteome, focussing on the receptor-independent, non-genomic actions in the model organism *Schizosaccharomyces pombe*. One of the proteins that was found to be significantly regulated by both DOC and aldosterone, vip1, seems to be involved in the regulation of the cytoskeleton and in non-genomic steroid hormone actions, but its functionality remains unclear. Further investigations of vip1 require the availability of larger amounts of the protein. Therefore, we heterologously expressed vip1 in *Escherichia coli* and performed a bioinformatic analysis of the protein as well as an *in vitro* characterization. ***To cite this article: Bernhard Josef Lauer et al., C. R. Chimie 12 2009.***

keywords: Aldosterone; 11-Deoxycorticosterone; Differential protein patterns; Non-genomic effect; *Schizosaccharomyces pombe*; Steroid hormones; vip1

1. Introduction

Steroid hormones are of extraordinary physiological importance. Sexual hormones (androgens, estrogens) regulate the development of secondary sexual characteristics, glucocorticoids such as cortisol are involved in the stress response of mammals and mineralocorticoids such as aldosterone regulate the blood pressure due to their role in modulating the water and salt housekeeping of the body [1–6]. Generally, their effects are mediated through specific nuclear receptors. Besides this classic way of affecting the cellular gene expression, steroid hormones also exert part of their action via non-genomic mechanisms [7–11].

In the biosynthesis of steroid hormones, several cytochromes P450 are involved. Since many pathological conditions are associated with increased or decreased levels of specific steroid hormones, these enzymes are promising therapeutic targets, and their selective regulation is highly desirable in order to influence the amounts of steroid hormones produced [12–17].

Aldosterone, the most important mineralocorticoid, has been associated with hypertension, fibrosis of the

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heart, and severe heart failure [18–21]. Although the mechanisms of these damaging effects are, as yet, poorly understood they may at least partly be mediated through non-genomic pathways [22–26]. To focus on the investigation of these non-genomic actions of aldosterone, a model system has been developed in our group to study the effects of aldosterone and other steroid hormones without interference of steroid hormone nuclear receptors. We chose the fission yeast *Schizosaccharomyces pombe* (*S. pombe*), which does not contain any nuclear steroid receptors, but many genes and regulatory mechanisms that are close to those of mammals [27,28].

The differential protein patterns in *S. pombe* upon mineralocorticoid administration were studied using 2D-gel electrophoresis and mass spectrometry. Eleven proteins that were differentially regulated by aldosterone [29] and 19 proteins that were specifically affected by the aldosterone-precursor 11-deoxycorticosterone (DOC) [30] have been identified. Four of these proteins showed altered levels in both cases [31] and therefore may be specifically associated with non-genomic actions, which makes them potential targets for the development of new drugs (Table 1).

Among the latter, the protein vip1 seems to be of special interest. This protein first had been identified using a polyclonal p53-antiserum while searching for a potential homologue of the human tumour suppressor protein p53 in *S. pombe* [32], which does not possess any known p53-homologues itself [28]. p53 is of crucial importance for the conservation of the genomic integrity in higher eukaryotes [33–36]. Loss of p53-activity in a cell is frequently the first step in the development of tumours that may become a threat to the existence of the entire organism.

In the C-terminal area covering 34 amino acids, vip1 displays a 50% sequence homology to a part of the so-called prolin-rich domain of human p53 [32] (Fig. 1A). Further examinations in order to characterize the vip1 protein, however, showed that vip1 is most probably not a p53-homologue, but rather seems to be involved in the organization of the cytoskeleton [37]. Bioinformatic analysis of the vip1 nucleotide sequence revealed three potential domains in its protein structure: an N-terminal RNA-recognition motif (RRM) [38], a central area, and a C-terminal region containing two PEST-motifs as potential binding sites for ubiquitin [37] (Fig. 1A). Besides the similarities in the central domain between vip1 and myosin, it could be shown that tubulin and vip1 are capable of binding to each other *in vitro*. In addition, co-localization of green fluorescence protein (GFP)-labelled vip1 to the microtubules during cell division *in vivo* could be demonstrated. On the basis of findings in cold-shock experiments using GFP-vip1, the possible participation of this protein in reconstitution of a functional microtubule framework was corroborated [37]. However, little is known about the functionality of this 27.5 kDa protein so far.

As aldosterone is known to induce fibrosis in various tissues [18,19,39] and since fibrosis is always associated with an increase in connective tissue [40,41], it is interesting that aldosterone has been shown to reduce the amount of vip1 [29,31], which seems to be associated with the cytoskeleton and thus with scleroproteins that are known to be involved in the function of connective tissues. The mechanism underlying the aldosterone-mediated action of vip1 are completely unknown at present. To get a deeper insight

Table 1
Differentially regulated proteins by both aldosterone (Aldo) and by DOC. The average intensities (absolute and relative values) of the spots containing the indicated proteins on the respective 2D-gels are listed. NAD-dependent malic enzyme and glyceraldehyde-3-phosphate dehydrogenase 1 were present in two different spots each. The spot intensity data are taken from Böhmer et al. (2006) [29] (Aldo) and estimated according to Hwang (2007) [30] (DOC), respectively. Upregulation (increased intensity) is indicated by ↑ arrows, downregulation (decreased intensity) is indicated by ↓ arrows. n.d., not detectable.

Protein name	*S. pombe* gene	Average spot intensities								Regulation by	
		DOC control		DOC sample		Aldosterone control		Aldosterone sample		DOC	Aldo
		Absolute [ppm]	Relative (%)	Absolute [ppm]	Relative (%)	Absolute [ppm]	Relative (%)	Absolute [ppm]	Relative (%)		
Enolase 1-1	eno101	26,000	100%	53,000	203.9%	1102.9	100%	314.8	28.5%	↑	↓
NAD-dependent malic enzyme	mae2	42,000 23,000	100% 100%	7,000 6,000	16.7% 26.1%	2666.0	100%	1114.7	41.8%	↓	↓
Glyceraldehyde-3-phosphate dehydrogenase 1	tdh1	88,000 48,000	100% 100%	13,000 22,500	14.8% 46.9%	207.4	100%	n.d.	n.d.	↓	↓
Protein vip1	vip1	49,000	100%	23,000	46.9%	1796.4	100%	259.5	14.5%	↓	↓

(Data taken from Hwang (2007) [30], Böhmer et al. (2006) [29], and Zöllner et al. (2008) [31]).