



## Expression of the human *TIMM23* and *TIMM23B* genes is regulated by the GABP transcription factor

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### ABSTRACT

The TIM23 protein is a key component of the mitochondrial import machinery in yeast and mammals. TIM23 is the channel-forming subunit of the translocase of the inner mitochondrial membrane (TIM23) complex, which mediates preprotein translocation across the mitochondrial inner membrane. In this paper, we aimed to characterize the promoter region of the highly similar human *TIM23* orthologs: *TIMM23* and *TIMM23B*. Bioinformatic analysis revealed putative sites for the GA-binding protein (GABP) and the recombination signal binding protein for immunoglobulin kappa J (RBPJ) transcription factors in both promoters. Luciferase reporter assays, electrophoretic mobility shift assays, and chromatin immunoprecipitation experiments showed three functional sites for GABP and one functional site for RBPJ in both promoters. Moreover, silencing of *GABPA*, the gene encoding the DNA-binding subunit of the GABP transcription factor, resulted in reduced expression of *TIMM23* and *TIMM23B*. Our results show an essential role of GABP in activating *TIMM23* expression. More broadly, they suggest that physiological signals involved in activating mitochondrial biogenesis and oxidative function also enhance the transcription but not the protein level of *TIMM23*, which is essential for maintaining mitochondrial function and homeostasis.

### 1. Introduction

The vast majority of mitochondrial proteins are encoded by nuclear genes, synthesized in the cytosol and imported into the organelle by a multi-protein import complex. The major mitochondrial import pathway transports preproteins with cleavable N-terminal presequences [1–4]. The preproteins are recognized by the receptors TOMM20 and TOMM22 of the translocase of the outer mitochondrial membrane (TOM complex) [5–7] and handed over from the TOM complex [8,9] to the presequence translocase of the inner mitochondrial membrane (TIM23 complex) [10,11] via a two-membrane-spanning super-complex intermediate. In yeast, the TOM complex is phosphorylated and regulated by several cytosolic protein kinases. Among these are casein kinase 2 (CK2), which governs the assembly of TOM complexes, and protein kinase A (PKA), which controls the translocase function [12].

The TIM23 protein is a key component of the mitochondrial import machinery in yeast and mammals [13]. TIM23 is essential in

*Saccharomyces cerevisiae* and *Neurospora crassa* [14], and its own importation to the inner mitochondrial membrane is dependent on the DDP1/TIM13 complex [15]. The importance of Timm23 in mammals was highlighted in experiments carried out using *Timm23* knockout mice. Homozygous *Timm23*<sup>-/-</sup> mice were not viable, whereas heterozygous F1 mutants showed a 50% reduction of Timm23 protein, a neurological phenotype and a markedly reduced life span [16]. Moreover, in humans, alterations in the TIM23 complex have been associated with several diseases [17].

Interestingly, humans have two *TIM23* orthologs (Fig. 1A). *TIMM23* codes for a single protein, TIMM23, which functions as part of the TIM23 complex [18,19]. *TIMM23B* was identified by massive sequencing [20] and codes for two proteins, TIMM23B1 and TIMM23B2, which are highly similar to TIMM23 (Fig. 1B). To our knowledge, the functions of the TIMM23B proteins are unknown.

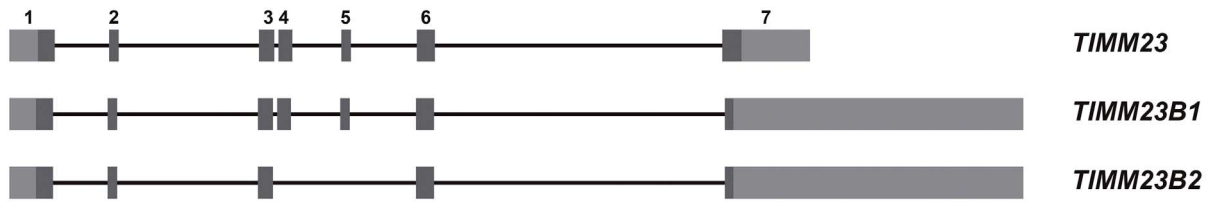
The present work is part of a broader study aimed at better understanding the factors involved in coordinating the nuclear and

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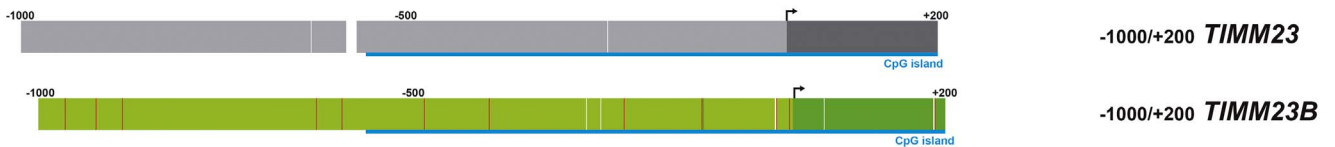
### A *TIMM23* and *TIMM23B* and their protein coding mRNAs



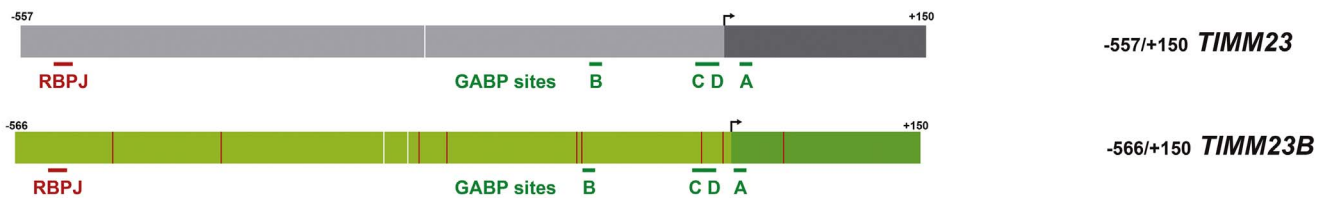
### B *TIMM23* and *TIMM23B* proteins



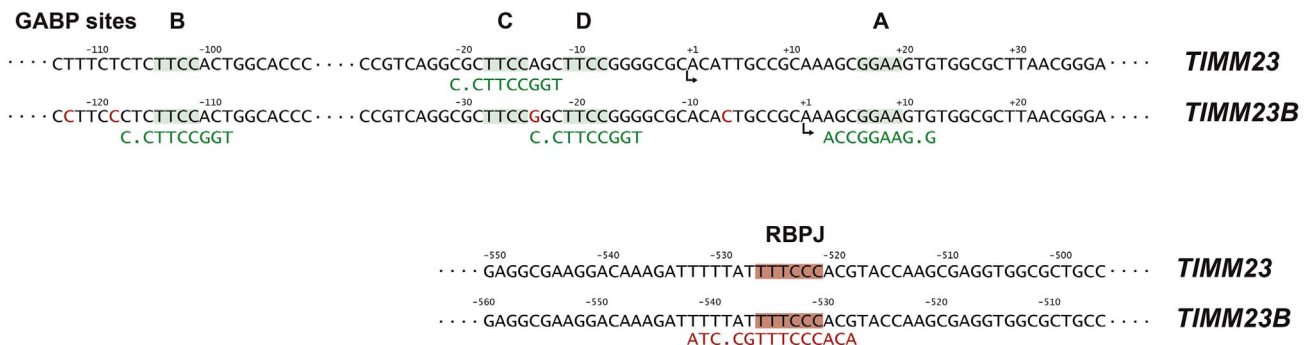
### C *TIMM23* and *TIMM23B* promoter regions



### D *Cis*-elements in the *TIMM23* and *TIMM23B* promoter regions



### E GABP and RBPJ binding sites in *TIMM23* and *TIMM23B* promoters



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