The Cyclin-Dependent Kinase Inhibitor Sic1 of *Saccharomyces cerevisiae* Is a Functional and Structural Homologous to the Mammalian $p27^{Kip1}$

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Introduction

In budding yeast Sic1, an inhibitor of cyclin-dependent kinase (Cki), blocks the activity of Cdk1-Clb5/6 (S-Cdk1) kinase required for the initiation of DNA replication that takes place only when Sic1 is removed [1]. Deletion of Sic1 causes premature DNA replication from fewer origins, extension of the S-phase and inefficient separation of sister chromatids during anaphase, whereas delaying S-Cdk1 activation rescues both S and M phase defects [2]. Despite the well documented relevance of Sic1 inhibition on S-Cdk1 for cell cycle control [3] and genome instability, the mechanism by which Sic1 inhibits S-Cdk1 activity remains obscure.
Sic1 has been proposed to be a functional homologous of mammalian Cki $p21^{Cip1}$ [4], that is characterized by a significant sequence similarity with Cki $p27^{Kip1}$, inhibitor of the Cdk2/Cyclin A kinase activity during S-phase.

Results

In this paper we report that the inhibitory domain of Sic1 is structurally related to mammalian $p27^{Kip1}$ of the Kip/Cip family, and that Sic1 and $p27^{Kip1}$ are functional homologues. Despite the low primary sequence homology between Sic1 and mammalian inhibitors, a model of the inhibitory domain of Sic1 [5] bound to the Cdk2/cyclin A complex was builded on the X-ray structure of $p27^{Kip1}$ [6]. Despite the low primary sequence homology between the two inhibitors, a combination of secondary structure prediction methods and hydrophobic pattern analysis is used for to search similarity patterns. Secondary structure prediction for the inhibitory domain of Sic1, as well as for the corresponding domain of $p21^{Cip1}$, have been computed and compared to the secondary structure deduced from the X-ray structure of $p27^{Kip1}$. On this basis, the Sic1 inhibitory domain was modeled using the corresponding $p27^{Kip1}$ domain as a template (Fig. 1), showing that the inhibitory mechanism to the Cdk/cyclin seems to be conserved during the evolutionary process.
Analysis of Sic1 binding to human cyclin A, Cdk2 and their binary complex by real-time biomolecular interaction analysis (BIAcore) confirm this hypothesis, and the kinetic parameters measured indicate that interaction to, and mode of interaction with, the Cdk/cyclin complex has been conserved during evolution for Sic1 and $p27^{Kip1}$ inhibitors. In support to these evidences, Sic1 inhibits *in vitro* the Cdk2/cyclin A kinase activity.
The goal of this study was to establish whether mammalian inhibitor $p27^{Kip1}$ is able to rescue the phenotypes of yeast cells deleted for the *SIC1* gene. The expression of *KIP1* gene in *Saccharomyces cerevisiae* rescues all the phenotypes of cells lacking the *SIC1* gene and, in particular, high levels of *KIP1* restore a phenotype very similar to that of the *SIC1* overexpression.
Taken together these findings strongly support the idea that yeast Sic1 and mammalian $p27^{Kip1}$ inhibitors are functional and structural homologues.

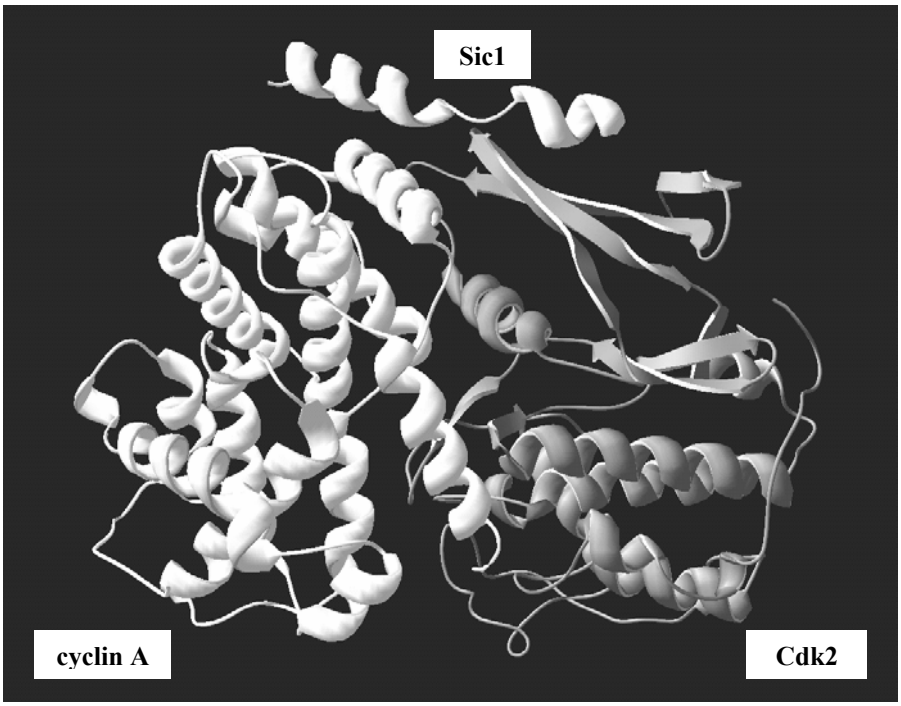

Fig. 1 3D model of Sic1 inhibitory domain bound to Cdk2/cyclin A

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