ISOLATION AND CHARACTERIZATION OF PROTEIN KINASE CK2 CATALYTIC ALPHA SUBUNIT OF THE INSECT *CERATITIS CAPITATA*

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Figure 1: Face to face with the Mediterranean fruit fly *Ceratitis capitata*. Photo: *bugguide.net.*

INTRODUCTION

The Mediterranean fruit fly *Ceratitis capitata* (Diptera: *Tephritidae*) is a widespread fruit pest of great economical importance due to the extensive damages to fruit crops. It is also used as a model insect for the development of biological control programs because it is the best studied fruit pest at the genetic and molecular level.

Protein kinase CK2 is a pleiotropic kinase Ser/Thr composed of two catalytic (α and/or α´) and two regulatory subunits (β) **(Fig.2)**[1].

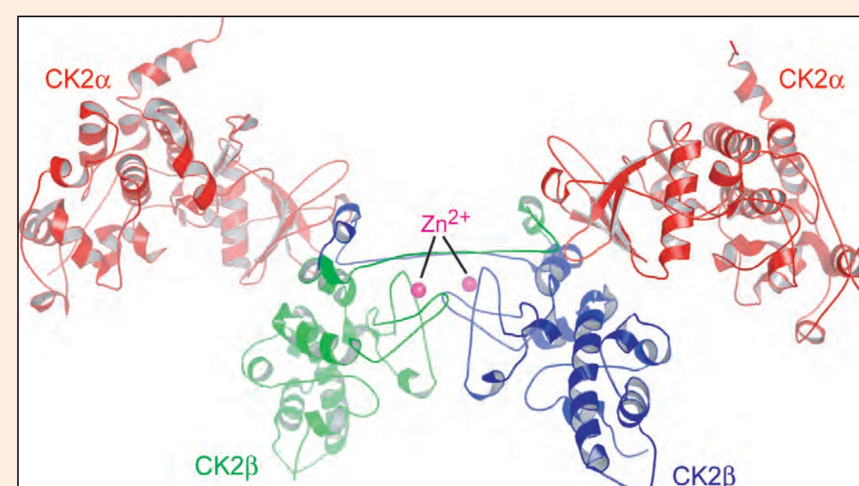

Figure 2: Structure of the human protein kinase CK2 holoenzyme[1]. CK2α subunits are shown in red, CK2β subunits are indicated in green and blue.

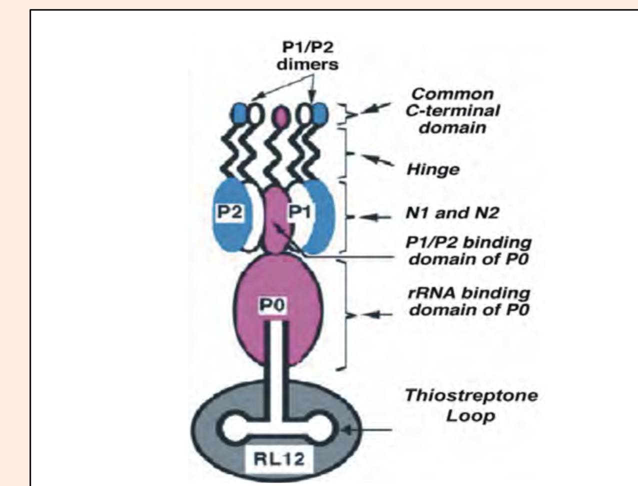

Figure 3: The ribosomal stalk of the large ribosomal subunit consists of a pentamer protein complex[8].

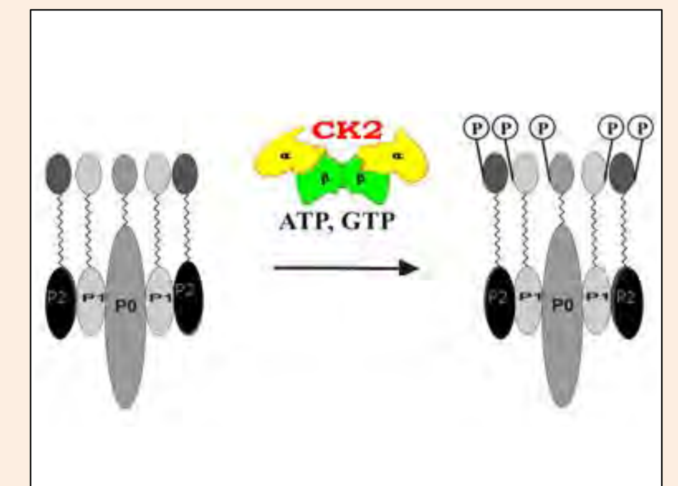

Figure 4: Phosphorylation of the P-proteins of the ribosomal stalk by protein kinase CK2[9].

CK2 is involved in several cellular responses such as the regulation of cell morphology and mobility, cell cycle control, embryogenesis, cell proliferation, circadian rhythm, apoptosis and tumorigenesis[2-6]. CK2 kinase phosphorylates also the P–proteins of the ribosomal stalk $P0(P1/P2)_2$ of the large ribosomal subunit **(Fig. 3 and 4)**[7].

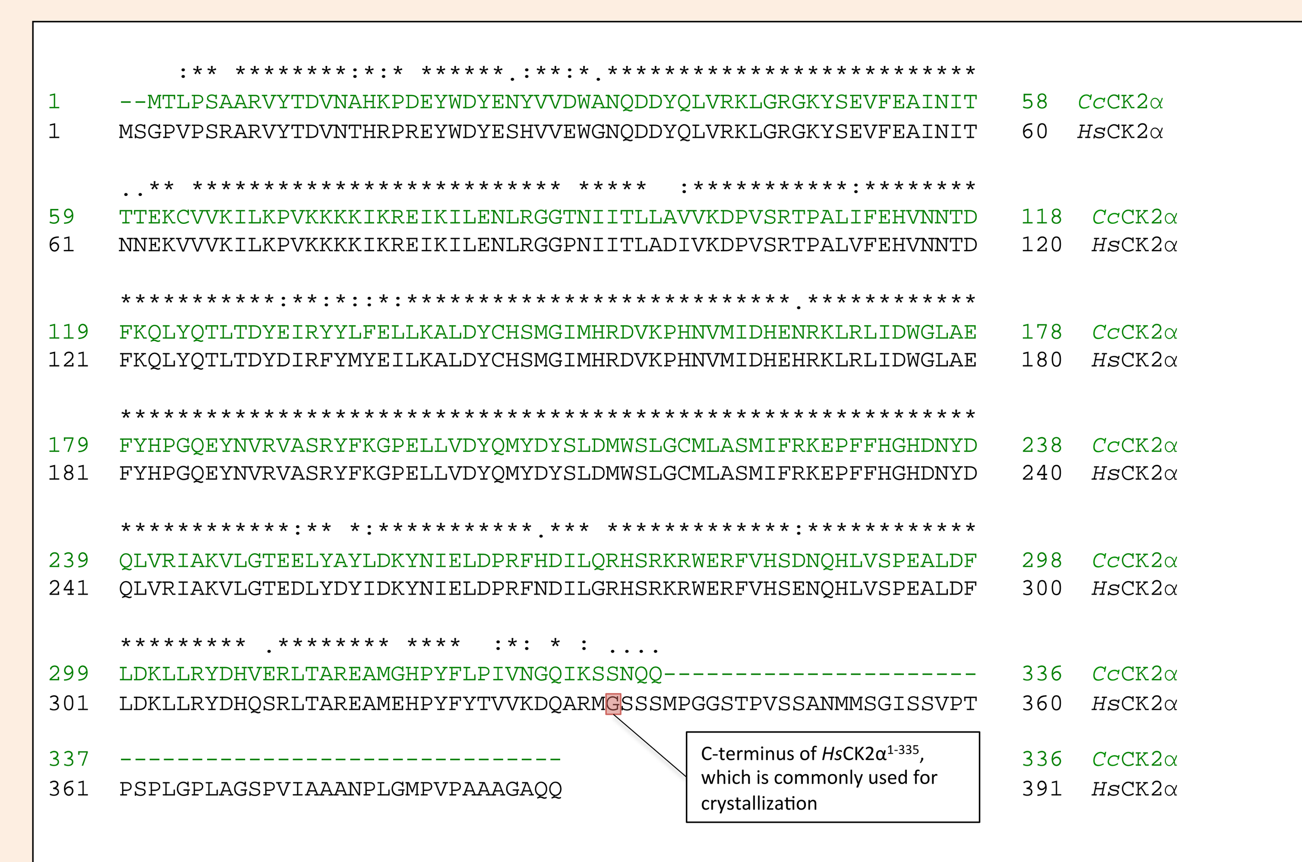

Figure 5: Alignment of the amino acid sequences of *Cc*CK2α and *Hs*CK2α.

SIMILARITIES BETWEEN *Cc*CK2α AND *Hs*CK2α

High homology is observed between *Ceratitis capitata* CK2α and human CK2α **(Fig. 5)**. *Hs*CK2α has been successfully crystallized and the construct which is used for crystallization and *in vitro* experiments has a deletion of 56 amino acids at the C-terminus. *Cc*CK2α ends close to the C-terminus of this construct ($HsCK2\alpha^{1-335}$)[10] and the similarity between them is greater than 86%.

RESULTS:

Initially, the cDNA encoding the CK2α subunit of *C. capitata* was cloned into the specific expression vector pRSET[11] and the protein was over expressed in *Escherichia coli* BL21(DE3) cells. After several purification attempts, the procedure was optimized by cloning the *Cc*CK2α cDNA into the pT7-7 expression vector (restriction enzymes: NdeI/HindIII), permitting the expression of the soluble protein without a His-tag **(Fig. 6 and 7)**. The *Cc*CK2α protein was then successfully purified with P11 phosphocellulose and heparin chromatography **(Fig. 8 and 9)**.

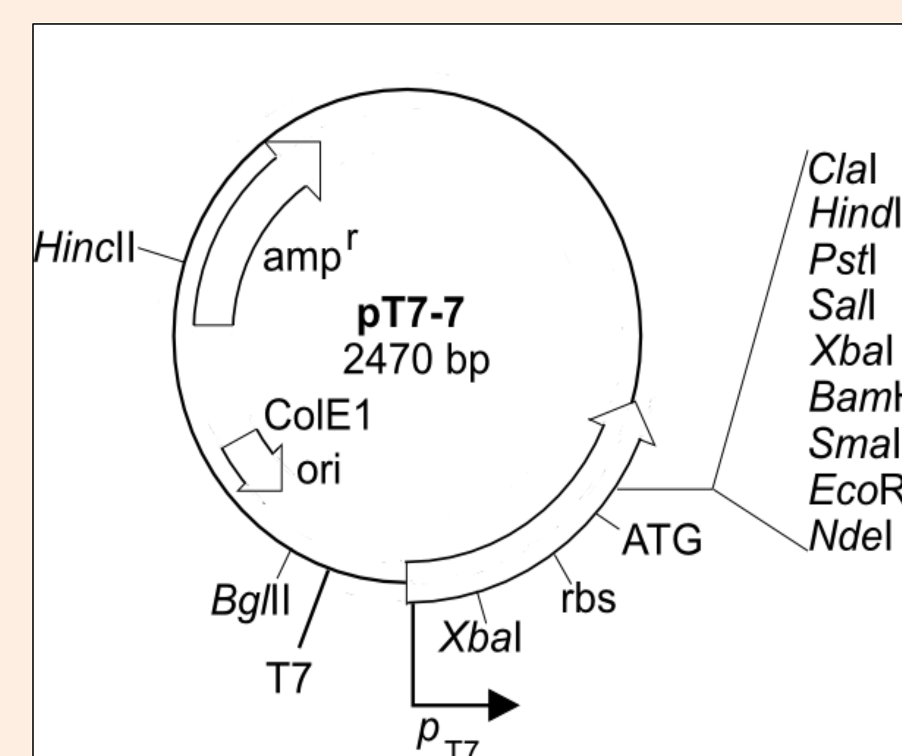

Figure 6: The pT7-7 expression vector.

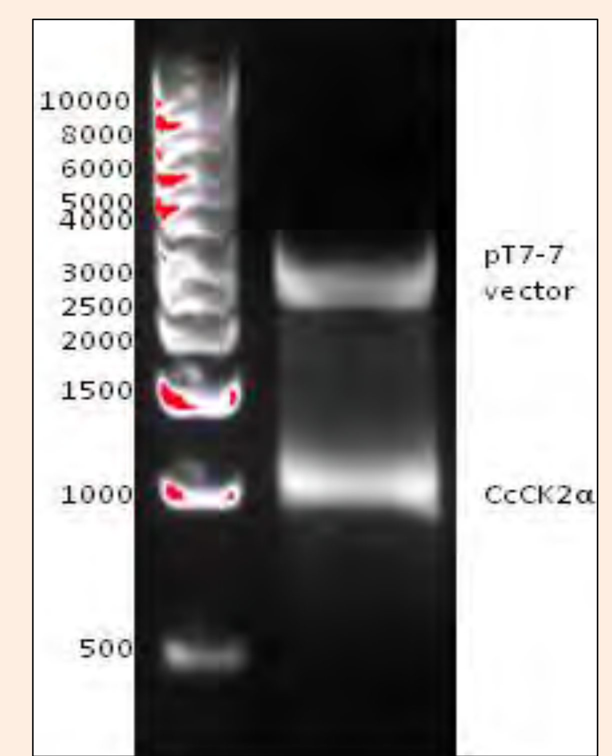

Figure 7: Cloning of the *Cc*CK2α gene (1011bp) into the pT7-7 expression vector.

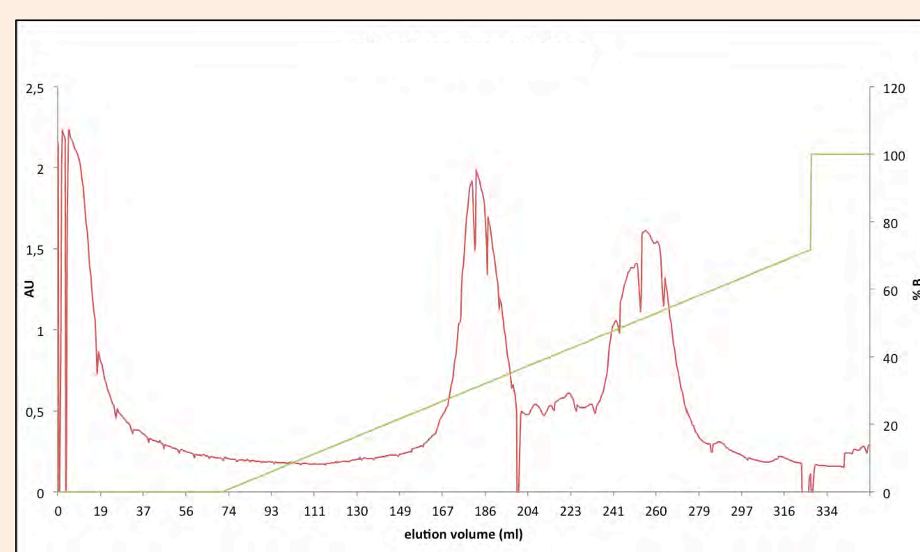

Figure 8: Purification of *Cc*CK2α with P11 phosphocellulose using a NaCl gradient with 25 mM Tris/HCl, 100 mM NaCl, pH 8.5 as low salt buffer and 25 mM Tris/HCl, 1 M NaCl, pH 8.5 as elution buffer.

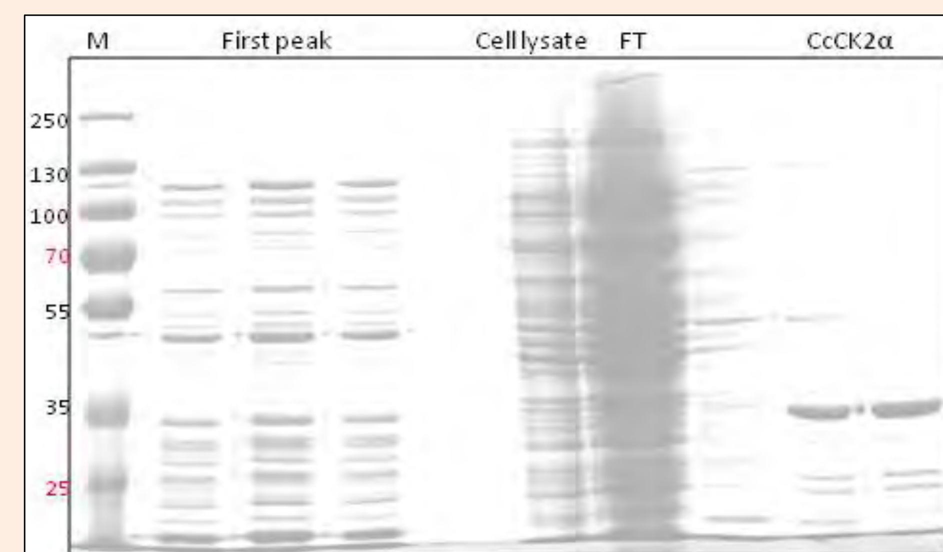

Figure 9: Isolation of the *Cc*CK2α after expression in *E. coli* and purification with P11 phosphocellulose SDS-PAGE 12% - Coomassie Brilliant Blue staining

OUTLOOK

- The purified recombinant protein *Cc*CK2α will be further characterized regarding kinase activity and thermostability and will be used for structural analysis.
- Due to the high homology between *Cc*CK2α and *Hs*CK2α, the characterization of the CcCK2α will allow the comparison with the human CK2α.
- In a second approach we are also working on the expression and purification of the *C. capitata* CK2β subunit in order to study the interaction of the *Cc*CK2α and *Cc*CK2β subunits.

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