Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimie 88 (2006) 905–911
BIOCHIMIE
www.elsevier.com/locate/biochi

Involvement of the SH3 domain in Ca^{2+}-mediated regulation of Src family kinases

A.N.A. Monteiro *

Risk Assessment, Detection and Intervention Program, The H. Lee Moffitt Cancer Center and Research Institute, MRC 3 West, 12902 Magnolia Drive, Tampa, FL 33612, USA

Received 1 November 2005; accepted 20 January 2006
Available online 10 March 2006

Abstract

When cells are treated with Ca^{2+} and Ca^{2+}-ionophore, c-Src kinase activity increases, whereas c-Yes kinase activity decreases. This opposite modulation can be reproduced in an in vitro reconstitution assay and is dependent on Ca^{2+} and on soluble factors present in cell lysates. Since c-Src and c-Yes share a high degree of homology, with the exception of their N-terminal "unique" domains, their activity was thought to be coordinately regulated. To assess the mechanism of regulation we generated stable cell lines expressing eight different constructs containing wild type c-Src and c-Yes, as well as swaps of the unique domain alone, unique and Src homology 3 (SH3) domains together and the SH3 domain alone. Swapping of the unique domains was not sufficient to reverse the regulation of the chimeric molecules. On the other hand, chimeras containing swaps of the unique plus the SH3 domains displayed reverse regulation, implicating both domains in the regulation of kinase activity by Ca^{2+}. To rule out the participation of the unique domain, we used chimeric molecules with swapped SH3 domains only and found that the SH3 domain is necessary and sufficient to confer Ca^{2+}-mediated regulation of Src and Yes tyrosine kinases.

Keywords: c-Src; c-Yes; Tyrosine kinases; Calcium; MDCK cells; SH_3 domain

1. Introduction

Protein tyrosine kinases regulate several aspects of cell physiology such as cell growth and transformation. *c-Src* and *c-Yes* encode non-receptor tyrosine kinases that participate in cellular growth signaling pathways, since specific mutations that activate their intrinsic kinase activity lead to altered cell growth and neoplasia [1]. It has been shown that elevation of intracellular calcium levels causes c-Src activation and c-Yes inactivation [2,3]. Results obtained in other systems have supported the view of a cross talk between the tyrosine kinases signaling pathway and intracellular calcium levels [4–6].

The $p62^{c\text{-}yes}$ (c-Yes) protein is highly homologous to $p60^{c\text{-}src}$ (c-Src): their C-terminal region share approximately 90% amino acid similarity and their N-terminal SH2 and SH3 domains share 75–80% amino acid identity [7,8] (Fig. 1A). With a few exceptions [9], it was generally believed that c-Src and c-Yes were coordinately regulated by action of cellular phosphatases and phosphorylation of their C-terminal negative regulatory tyrosine. During Ca^{2+}-induced keratinocyte differentiation, c-Src is activated and c-Yes is inactivated. This phenomenon is also reproducible in several different cell types including fibroblasts, indicating that opposite regulation of c-Src and c-Yes activities is a general phenomenon [2,3]. Moreover, this modulation can be replicated in an in vitro reconstitution assay (IVRA) which demonstrated that it is dependent on factors present in the cell lysates. In the absence of cytosolic factors addition of Ca^{2+} to immunoprecipitates is not sufficient to cause an increase in c-Src activity nor decrease in c-Yes activity [2,3]. The opposite regulation found in c-Src and c-Yes raised the question of how differential regulation is achieved and which domains are responsible for regulation. Here, using

Abbreviations: EDTA, ethylene dinitrilo-tetraacetic acid; GST, glutathione-S-transferase; GT, glutathione; IVKA, in vitro kinase assays; IVRA, in vitro reconstitution assays; MDCK, Madin–Darby canine cells; PMSF, phenylmethyl sulfonyl fluoride; PVDF, polyvinylidene difluoride; RIPA, radioimmunoprecipitation; SDS, sodium dodecyl sulfate; SH_2, Src homology 2 domain; SH_3, Src homology 3 domain.

* Corresponding author. Tel.: +1 813 745 6321; fax: +1 813 745 1720.
E-mail address: monteian@moffitt.usf.edu (A.N.A. Monteiro).

0300-9084/$ - see front matter © 2006 Elsevier SAS. All rights reserved.
doi:10.1016/j.biochi.2006.01.013

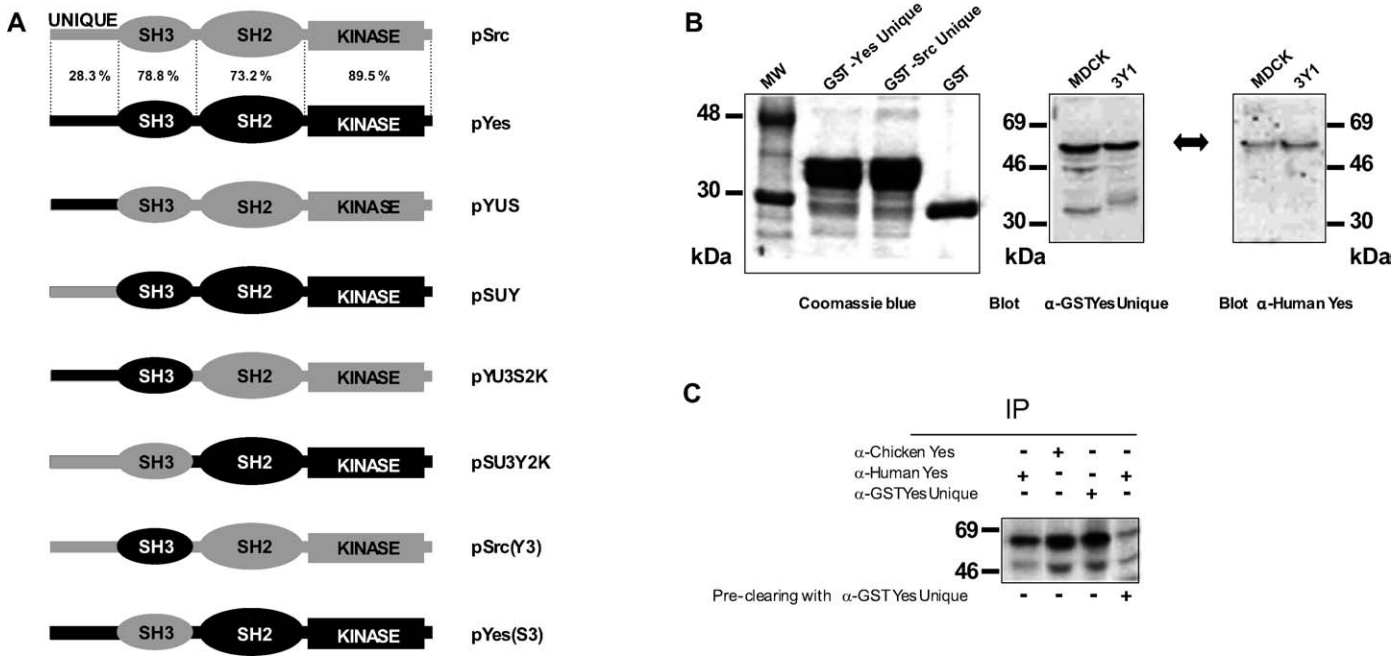

Fig. 1. Generation of wild type and chimeric constructs of Src and Yes kinases, and α-Yes unique antibody. *A.* Constructs used in this study. The percent identity between the different domains of Src and Yes is shown. *B.* Antibody against the unique domain of c-Yes. Coomassie staining of GST-fusion of the unique domain of c-Yes used to raise a rabbit polyclonal antibody (*left panel*). MDCK and 3Y1 cells were harvested, lysed in RIPA buffer, separated on a 10% SDS-PAGE, transferred to a PVDF membrane and blotted against the α-GST c-Yes unique raised in this study (*center panel*) or with a α-human c-Yes (*right panel*). The double-headed arrow indicates the band corresponding to c-Yes. *C.* IVKA of MDCK cells overexpressing c-Yes using different antibodies against c-Yes (lanes 1–4). Lane 4 shows lysates precleared with α-GST c-Yes unique and then subjected to IVKA using α-human c-Yes.

a series of chimeric molecules containing swaps of the unique and SH3 domains of Src and Yes, I identify the SH3 domains as the domains responsible for the Ca^{2+}-mediated regulation of Src and Yes.

2. Material and methods

2.1. Constructs

pSrc: c-Src was amplified by PCR (first cycle: denature 94 °C, 5 min; annealing 54 °C, 2 min; extension 72 °C, 3 min; subsequent 30 cycles: denature 94 °C, 1 min; annealing 54 °C, 2 min; extension 72 °C, 3 min; and last cycle: denature 94 °C, 1 min; annealing 54 °C, 2 min; extension 72 °C, 10 min) using primers S1 (5′ ATCGCGGATCCATGGGGAGCAA 3′) and S2 (5′ GGAGGGGTACCAGGCCTATAGGTT 3′) and plasmid p5H as template [10]. pYes: c-Yes was amplified by PCR (same conditions as above) using primers Y1 (5′ GACG GATCCAAGCAACCATGGGGTG 3′) and H (5′ CTGTTGGTACCTAAATTGTCCC 3′) and plasmid p6a as template [11].

The chimeric constructs were obtained using splicing by overlapping extension by PCR [12]. For each construct two initial (first round) separate PCR reactions were performed to generate the 5′ and the 3′ ends of the chimeras. The products of the two separate reactions were combined and used as templates for the second round of PCR. pYUS first round: primers Y1 and Y3 (5′ GCCCCGGCACGCTGCGGTGGCACAGCT GAAAATGA 3′) using p6a as template; primers S2 and S3 (5′ TCATTTTCAGCTGTGCCACCGCAGCGTGCCGGGG CACT 3′) using p5H as template. pYUS second round: primers Y1 and S2 with first round products combined and used as template. pSUY first round: primers S1 and F (5′ AGTACTAG GATATGGACTCGACGTAACGGTGTCAGA 3′) with p5H as template; primers H and G (5′ TCTGACACCGTTACGTC GAGTCCATATCCTAGTACT 3′) with p6a as template. pSUY second round: primers S1 and H with first round products combined and used as template. pYU3S2K first round: primers Y1 and R (5′ CTGGATGGAGTCTGAGGGAGCTA CATAATTGCTTGG 3′) with p6a as template; primers U (5′ CCAAGCAATTATGTAGCTCCCTCAGACTCCATCCAG 3′) and S2 with p5H as template. pYU3S2K second round: primers Y1 and S2 with first round products combined and used as template. pSU3Y2K first round: primers S1 and S (5′ TTGAATGGAGTCTGCAGGCGCGACATAGTTACTGGG 3′) with p5H as template; primers T (5′ CCCAGTAAC TATGTCGCGCCTGCAGACTCCATTCAA 3′) and H with p6a as template. pSU3Y2K second round: primers S1 and H with first round products combined and used as template. pYes (S3) first round: primers Y1 and S with pYUS as template; T and H with p6a as template. pYes(S3) second round: primers Y1 and H with first round products combined and used as template. pSrc(Y3) first round: primers S1 and R with pSUY as template; primers U and S2 with p5H as template. pSrc(Y3) second round: primers S1 and S2 with first round products combined and used as template. The final PCR products were cloned at *Bam*H1 and *Kpn*I sites into pMEXneo and confirmed by sequencing.