doi:10.1016/j.jmb.2010.11.023 J. Mol. Biol. (2011) 405, 1154–1169

Contents lists available at www.sciencedirect.com

Journal of Molecular Biology

journal homepage: http://ees.elsevier.com.jmb

Active Mutants of the TCR-Mediated p38α Alternative Activation Site Show Changes in the Phosphorylation Lip and DEF Site Formation

Netanel Tzarum, Ron Diskin, David Engelberg and Oded Livnah*

Department of Biological Chemistry, Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem 91904, Israel
Wolfson Centre for Applied Structural Biology, Hebrew University of Jerusalem, Jerusalem 91904, Israel

Received 7 September 2010; received in revised form 8 November 2010; accepted 10 November 2010
Available online 10 December 2010

Edited by I. Wilson

Keywords:
MAP kinase;
p38α;
T-cells;
signaling;
alternative activation

The p38α mitogen-activated protein kinase is commonly activated by dual (Thr and Tyr) phosphorylation catalyzed by mitogen-activated protein kinase kinases. However, in T-cells, upon stimulation of the T-cell receptor, p38α is activated via an alternative pathway, involving its phosphorylation by zeta-chain-associated protein kinase 70 on Tyr323, distal from the phosphorylation lip. Tyr323-phosphorylated p38α is autoactivated, resulting in monophosphorylation of Thr180. The conformational changes induced by pTyr323 mediating autoactivation are not known. The lack of pTyr323 p38α for structural studies promoted the search for Tyr323 mutations that may functionally emulate its effect when phosphorylated. Via a comprehensive mutagenesis of Tyr323, we identified mutations that rendered the kinase intrinsically active and others that displayed no activity. Crystallographic studies of selected active ($p38\alpha^{Y323Q}$, $p38\alpha^{Y323T}$, and $p38\alpha^{Y323R}$) and inactive ($p38\alpha^{Y323F}$) mutants revealed that substantial changes in interlobe orientation, extended conformation of the activation loop, and formation of substrate docking DEF site (docking site for extracellular signal-regulated kinase FXF) interaction pocket are associated with p38α activation.

Introduction

The p38 kinases are a subgroup of the mitogen-activated protein kinase (MAPK) enzymes[1] that also include extracellular signal-regulated kinases (ERKs), big MAPKs, and c-Jun N-terminal kinases. The p38 subfamily consists of four isoforms, α, β, γ, and δ, which share a high level of sequence similarity[2] but differ in how they are recognized by various MAPK kinases (MKKs)[3] and in their tissue expression pattern.[4] These serine/threonine kinases participate in various cellular processes including inflammatory responses, differentiation, cell death, senescence, and tumor suppression.[5–7] Abnormal activity of p38 is associated with various diseases including chronic inflammatory diseases,[8,9] psoriasis, and cancer,[10–13] making it a viable target for drug design.[10–16]

The p38 enzymes are catalytically activated when cells experience extracellular stimuli, commonly stress signals including osmotic shock and UV radiation and biological signals such as growth

*Corresponding author. Department of Biological Chemistry, Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Safra Campus, Givat Ram, Jerusalem 91904, Israel. E-mail address: oded.livnah@huji.ac.il.

Abbreviations used: MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MKK, MAPK kinase; MKI, MAPK insert; ATF, activating transcription factor; β-OG, *n*-octyl-β-D-glucopyranoside; ESRF, European Synchrotron Radiation Facility; PDB, Protein Data Bank; ZAP-70, zeta-chain-associated protein kinase 70; DEF site, docking site for ERK FXF.

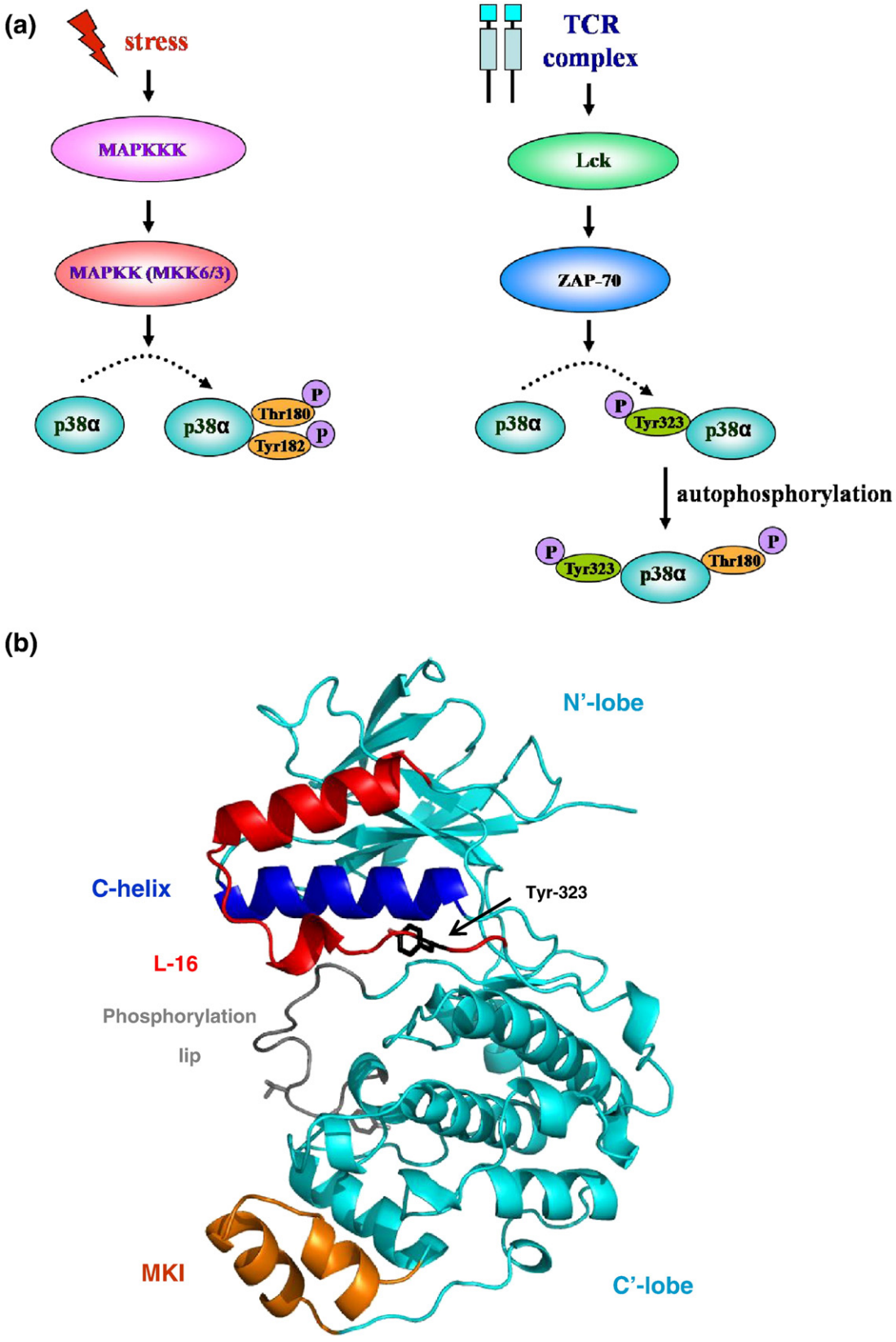

Fig. 1 (*legend on next page*)