Developmental Biology ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

An Elk transcription factor is required for Runx-dependent survival signaling in the sea urchin embryo

Francesca Rizzo [a,1], James A. Coffman [b], Maria Ina Arnone [a,*]

[a] Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Napoli 80121, Italy
[b] MDI Biological Laboratory, Salisbury Cove, ME 04672, USA

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form
23 May 2016
Accepted 23 May 2016

Keywords:
Sea urchin
Embryo
Elk
ERK
Apoptosis
Runx
PKC

ABSTRACT

Elk proteins are Ets family transcription factors that regulate cell proliferation, survival, and differentiation in response to ERK (extracellular-signal regulated kinase)-mediated phosphorylation. Here we report the embryonic expression and function of *Sp-Elk*, the single Elk gene of the sea urchin *Strongylocentrotus purpuratus*. *Sp-Elk* is zygotically expressed throughout the embryo beginning at late cleavage stage, with peak expression occurring at blastula stage. Morpholino antisense-mediated knockdown of *Sp-Elk* causes blastula-stage developmental arrest and embryo disintegration due to apoptosis, a phenotype that is rescued by wild-type Elk mRNA. Development is also rescued by Elk mRNA encoding a serine to aspartic acid substitution (S402D) that mimics ERK-mediated phosphorylation of a conserved site that enhances DNA binding, but not by Elk mRNA encoding an alanine substitution at the same site (S402A). This demonstrates both that the apoptotic phenotype of the morphants is specifically caused by Elk depletion, and that phosphorylation of serine 402 of Sp-Elk is critical for its anti-apoptotic function. Knockdown of *Sp-Elk* results in under-expression of several regulatory genes involved in cell fate specification, cell cycle control, and survival signaling, including the transcriptional regulator *Sp-Runt-1* and its target *Sp-PKC1*, both of which were shown previously to be required for cell survival during embryogenesis. Both *Sp-Runt-1* and *Sp-PKC1* have sequences upstream of their transcription start sites that specifically bind Sp-Elk. These results indicate that Sp-Elk is the signal-dependent activator of a feed-forward gene regulatory circuit, consisting also of *Sp-Runt-1* and *Sp-PKC1*, which actively suppresses apoptosis in the early embryo.

1. Introduction

Apoptosis plays two major roles during development, sculpting tissues during morphogenesis and metamorphosis, and removing damaged or rogue cells (Lockshin and Zakeri, 2002). Apoptosis and proliferation are intimately linked (Alenzi, 2004), and the survival and proliferation of cells both become dependent on genomically-controlled intercellular signaling following the initial cleavage stage of animal development (Coffman, 2003, 2009; Kagoshima et al., 2007; Nimmo and Woollard, 2008; Robertson et al., 2013). Aberrant activities of key players in the complex signal pathways that control cell proliferation and survival can have catastrophic consequences (Pucci et al., 2000; Vermeulen et al., 2003).

The Ets-like (ELK) protein group, also named TCF (ternary complex factor, including Elk-1, SAP-1 and SAP-2/ERP/Net), represents a subfamily of Ets transcription factors (reviewed in Sharrocks (2002), Shaw and Saxton (2003), Treisman (1994)) implicated in regulation of cell proliferation (Sharrocks, 2001; Zhong et al., 2007), apoptosis (Townsend et al., 1999; Vickers et al., 2004), cell migration (Buchwalter et al., 2005), and cell fate determination (Beitel et al., 1995; Vanhoutte et al., 2001). The mammalian and fish ELK proteins contain four domains of high sequence and functional similarity: the ETS DNA-binding domain, the B-box, the D-domain and the C-domain, containing multiple S/TP motifs that act as sites for phosphorylation by MAP kinases and their subsequent regulation (Gille et al., 1995b; Janknecht et al., 1993; Marais et al., 1993). Phosphorylation of ELK proteins results in enhancement of both DNA-binding and transcriptional activation (reviewed in Sharrocks (2002), Shaw and Saxton (2003), Wasylyk et al. (1998)). Except for the extensively studied Elk-1 homologue Lin-1 gene in *Caenorhabditis elegans* (Beitel et al., 1995; Guerry et al., 2007; Jacobs et al., 1998; Leight et al., 2005; Leight et al., 2015; Miley et al., 2004), little is known about the role of Elk

* Corresponding author.
E-mail address: miarnone@szn.it (M.I. Arnone).
[1] Present address: Laboratory of Molecular Medicine and Genomics, Department of Medicine and Surgery, University of Salerno, Baronissi, Italy.

http://dx.doi.org/10.1016/j.ydbio.2016.05.026
0012-1606/