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Brain Research Reviews 49 (2005) 158–166

BRAIN RESEARCH REVIEWS

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Review

Experimental study of MAP kinase phosphatase-3 (*Mkp3*) expression in the chick neural tube in relation to Fgf8 activity

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Accepted 8 December 2004
Available online 7 April 2005

Abstract

Mitogen-activated protein kinase (MAPK) pathways are well known to be involved in signal transduction from extracellular to intracellular compartments in all eukaryotes. The activation of this cascade will have an effect on cell proliferation, differentiation, and apoptosis. In this study, we describe the cloning of the chick *Mkp3* gene that is highly homologous to the mammalian gene and are both expressed in several embryo regions with demonstrated morphogenetic activity. In early developmental stages, *Mkp3* and *Fgf8* have similar expression patterns. Differences in the activation of *Mkp3* transcription in the isthmus and the repression with FGF receptor inhibition suggest that Fgf8 protein controls *Mkp3* transcription. Ectopically, expression of Fgf8 protein induces *Mkp3* in a short period of time in the diencephalon, indicating a positive regulation of *Mkp3* by *Fgf8*. Moreover, we show a distinct tissue competence to express *Mkp3* rostrally and caudally to the zona limitans intrathalamica (ZLI).

Theme: Development and regeneration
Topic: Pattern formation, compartments, and boundaries

Keywords: Mid–hindbrain boundary; Isthmic organizer; Zona limitans intrathalamica; Mkp3; Fgf8; MAPK

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Abbreviations: ANR, anterior neural ridge; ba, branchial arches; cb, cerebellum; cp, commissural plate; cx, cortex; di, diencephalon; dt, dorsal thalamus; e, eye; egl, external granule cell layer; es, ectostriatum; fl, forelimb bud; hf, head fold; hl, hindlimb bud; hsd, dorsal hyperstriatum; hsv, ventral hyperstriatum; hy, hypothalamus; igl, inner granule cell layer; is, isthmus; mes, mesencephalon; ml, molecular layer; nr, neural retina; ns, neostriatum; nsc, neostriatum caudale; ob, olfactory bulb; oc, optic chiasma; os, optic stalk; otv, otic vesicle; pa, pallidum; pcl, Purkinje cell layer; pe, pigmentary epithelium; pi, pineal gland anlage; pit, pituitary gland; rh, rombencephalon; rp, Rathke's pouch; st, striatum; tb, tail bud; tel, telencephalon; vt, ventral thalamus; wm, white matter; zli, zona limitans intrathalamica

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doi:10.1016/j.brainresrev.2004.12.038

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1. Introduction

The mitogen-activated protein (MAP) kinase cascade is highly conserved in all species. Its function is to relay, amplify, and integrate signals activated by a range of extracellular stimuli mediating a number of physiological and pathological changes in cell function. MAP kinase signaling cascades are involved in the control of cell proliferation, differentiation, inflammatory response, and apoptosis. MAP kinase activation requires phosphorylation on threonine and tyrosine residues, which can be mediated by transmembrane receptors of the tyrosine kinase (RTK) type, like the fibroblast growth factor receptors (FGFR). This activation results in the nuclear translocation of the extracellular-signal-regulated MAP kinase (ERK) ending in transcriptional changes. Dual specificity phosphatases dephosphorylate critical tyrosine and threonine residues reversing in this way the activation of MAP kinases. MAP kinase phosphatase-3 (MKP-3), a predominantly cytosolic, dual-specificity MAPK phosphatase dephosphorylates the ERK family preventing its translocation to the nucleus [22]. The changes in cell function mediated by the MAP kinase pathway depend ultimately on the competing influences of MAP kinase activators and inhibitors.

In the present work, we describe the cloning and expression pattern analysis of chick *Mkp3*. In early developmental stages, *Mkp3* has a similar spatio-temporal expression with *Fgf8* in neural and non-neural tissues. Although some differences have been detected in the isthmus, the dorsal thalamus, and in the limb bud that can be related to functional consequences of Fgf8-specific activity at these domains.

Fgf8 is one of the principal molecules responsible for the morphogenetic activity of the isthmic organizer ([4], reviewed in [7]). Fgf8 protein is by itself capable of transforming diencephalic tissue into ectopic midbrain and cerebellar tissue. This event is due to the induction of *Fgf8* mediated by *Otx2* and *Gbx2* interaction [4,10,19]. Taking advantage of the experiments with the Fgf8 protein and our observations that *Mkp3* and *Fgf8* have a very similar expression pattern, we demonstrate that Fgf8 protein induces *Mkp3* transcription in the diencephalon. The ectopic induction of *Mkp3* is possible rostrally and caudally to the zona limitans intrathalamica, but with different levels of intensity. Moreover, inhibition of FGF signaling in the isthmus generates an important reduction of *Fgf8* and *Mkp3* expressions. These results therefore suggest that *Mkp3* transcription is physiologically activated and maintained by Fgf8. The negative regulation of Mkp3 over Fgf8 intracellular functional cascade [8,14], together with the present results, suggests a mechanism of negative regulation mediated by Fgf8 activity at the isthmic organizer.

2. Materials and methods

2.1. Cloning of a chick homologue of Mkp3

We cloned *Mkp3* using RT-PCR from HH12 chick embryos mRNA. The degenerate oligonucleotide primers were designed to the conserved amino acid motifs YILNVTPN and PNFNFMGQ of the MAP kinase phosphatase protein family. The primer sequences used in this study are: 5′-TA(C/T)AT(A/C/T)CT(N/A)AA(T/C)GT(N/A)AC(N/G)CC(N/C)AA-3′ as the forward primer and 5′-CC(N/C)AA(T/C)TT(T/C)AA(T/C)TT(T/C)ATGGG(N/C)GA-3′ as the reverse primer.

A PCR product of 311 bp was obtained, subcloned into pGEM-T Easy, and sequenced. Comparing to the previously published chick *Mkp3* [14], our DNA fragment corresponds to nucleotides 852–1162.

2.2. Experimental embryology

Fertilized chick (*Gallus gallus*) eggs were incubated at 37 °C in a forced air incubator. Embryos were staged according to Hamburger and Hamilton [12].

Implantation of FGF8 beads into the neural tube of chick embryos was performed as previously described [4]. Heparin acrylic beads (Sigma, St. Louis, MO, USA) were rinsed in PBS and then soaked in a solution of 1 μg/μl of FGF8 protein (R&D Systems, Minneapolis, MN, USA) for 1 h at 4 °C. Then, the beads were rinsed in PBS several times and then implanted in the neural tube of the embryos. For the control experiments, the beads were soaked in PBS (phosphate-buffered saline) in the same manner.

PI3K inhibitor (LY294002, Cell Signalling Technology) was diluted in DMSO to a final concentration of 20 μM and then soaked in Affigel blue beads (Bio-Rad, Hercules, CA) for 1 h at 4 °C. FGF receptor inhibitor (SU5402, Calbiochem) was diluted in DMSO at a final concentration of 40 μM and soaked in ion exchange beads (AG1-X2, Bio-