

Gene expression pattern

Expression of brain subtype *creatine kinase* in the zebrafish embryo

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Abstract

Creatine kinases (CK) play crucial roles in intracellular energy transfer. We have isolated a cDNA from zebrafish embryos, which encodes a CK highly related to the mammalian brain subtype creatine kinase (BCK). The *bck* mRNA is expressed maternally in the zebrafish embryo and transcripts are distributed uniformly in blastula and gastrula stages. Expression becomes restricted to the prechordal plate and the nervous system during subsequent somitogenesis stages. *bck* transcripts are abundant in primary neurons in the developing central nervous system of the 1-day-old embryo. While some *bck* expression persists in the hindbrain, expression vanishes in the spinal cord of the 2-day-old embryo. In summary, the expression pattern of *bck* is highly dynamic and suggests a role for *bck* during gastrulation and neuronal differentiation. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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Creatine kinases (CK) constitute a family of related proteins which serve as phosphate shuttles in cells with high metabolic rate and compartmentalised energy utilisation (Wallimann and Hemmer, 1994; Wyss and Kaddurah-Daouk, 2000). Energy from oxidative metabolism in mitochondria is transferred via creatine phosphate to cellular adenine triphosphatases (ATPases). CKs catalyse the reversible transfer of the γ -phosphate group of ATP to creatine. For example in myocytes, CK supplies energy to myosine ATPases by regenerating ATP from adenine diphosphate (ADP) and creatine phosphate. This is accomplished by a muscle-specific subtype of CK, which is located to the M-line of the muscle fibril. In addition to functions in intracellular energy transport, CKs have been implicated in ATP and Pi-buffering in cells with high and variable rates of energy turnover (Wallimann and Hemmer, 1994; Wyss and Kaddurah-Daouk, 2000).

High levels of CK activity are also present in neural tissues (Wallimann and Hemmer, 1994). Immunocytochemical analysis revealed that expression of the brain (BCK) and mitochondrial (mtCK) isoforms of CK are highly restricted to specific regions of the adult rat brain (Friedman

and Roberts, 1994; Wallimann and Hemmer, 1994). In contrast to the adult brain both brain subtype creatine kinase (BCK) and mtCK are more widely expressed in the developing foetal rat brain (Chen et al., 1995). We report here the cloning and expression of zebrafish *bck*. The gene is initially expressed ubiquitously and subsequently in a highly restricted pattern in the prechordal plate and developing primary neurons of the central nervous system.

1. Results and discussion

We have isolated a cDNA encoding a CK from gastrula stage zebrafish embryos in a screen for nodal responsive genes (to be published elsewhere). This gene is highly related to the mammalian brain subtype of CK with 86% overall amino acid identity to the human protein (Fig. 1A,B). The gene maps to 58.3 cM on linkage group 20 in the zebrafish radiation hybrid panel. The sequence homology suggests that this gene is the zebrafish orthologue of BCK.

The identification of the *bck* cDNA from gastrula stage embryos suggests that the gene is expressed very early during zebrafish development. To investigate embryonic expression of *bck* in further detail, an expression study based on in situ hybridisation of whole mount embryos was carried out. *bck* mRNA was detectable at the 16-cell stage (Fig. 2A) indicat-

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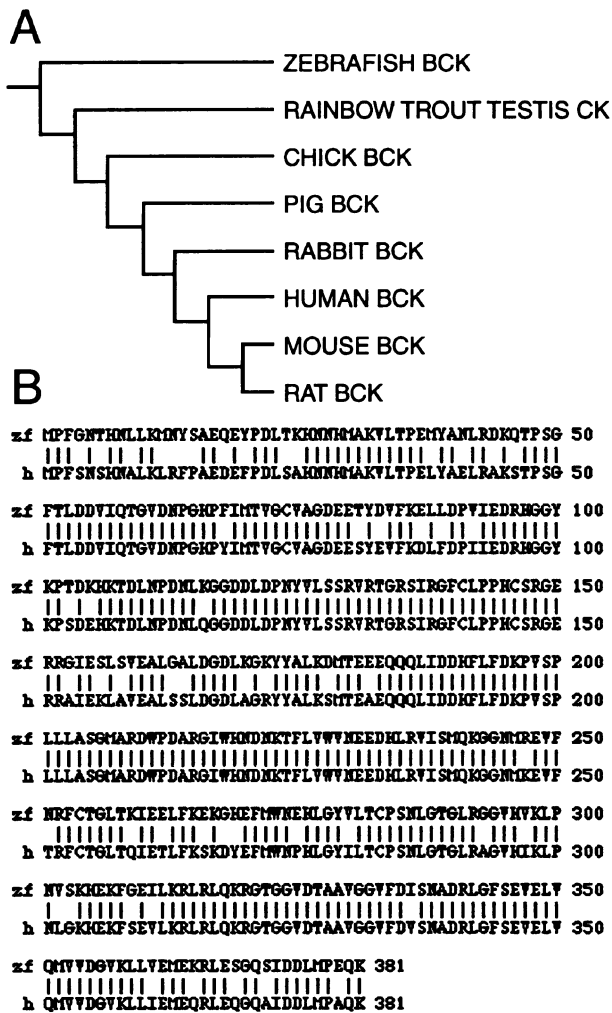


Fig. 1. (A) Clustal-X analysis (Thompson et al., 1997) of the isolated zebrafish CK shows the closest relationship to brain subtype CK (bck). (B) Sequence comparison between zebrafish bck (zf) and human BCK (h) protein sequences.

ing that the transcript is expressed maternally. At the sphere stage, a time when zygotic transcription has already commenced, strong ubiquitous expression was noted in whole mounts as well as cross-sections (Fig. 2B and data not shown). Ubiquitous expression faded progressively during gastrulation and, at the bud stage, restricted expression of *bck* mRNA became apparent in the prechordal plate (Fig. 2C,D). At the 10-somite stage, three other domains of expression appeared at the mid/hindbrain level (Fig. 2E–G). Two small groups of cells, which represent the anlagen of the trigeminal ganglia are located on either side of the nascent neural keel. A third group of expressing cells residing within the hindbrain forms a transverse dorsal band in rhombomere 3 as revealed by double-labelling with *Krox20* mRNA (Oxtoby and Jowett, 1993) which is expressed in rhombomeres 3 and 5 (Fig. 2G). In addition, weak expression was variably noted in individual cells in the posterior neural tube of 10-somite stage embryos (Fig. 2E).

At the 18-somite stage, expression of *bck* mRNA was detected in many neurons in the central nervous system (Fig. 3A–C). These include primary Rohon Beard sensory

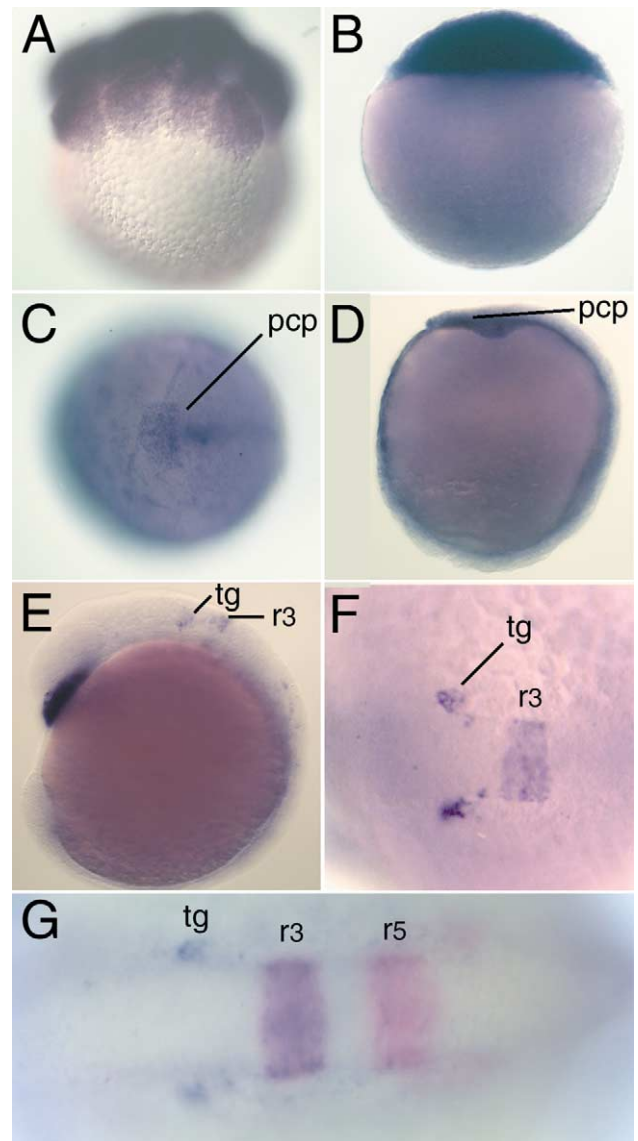


Fig. 2. Ubiquitous *bck* expression becomes restricted to prechordal plate and developing central nervous system during early somitogenesis. (A) Lateral view of 16-cell stage embryo reveals maternally deposited *bck* mRNA. (B) Lateral view of sphere stage embryo shows ubiquitous expression of *bck* mRNA after onset of zygotic transcription. (C, D) *bck* transcription becomes restricted to the prechordal plate (pcp) by the end of gastrulation. (C) Dorsal view of the head region, anterior left, and (D) lateral view of bud stage embryos, dorsal right. (E–G) Start of neural expression at the 10-somite stage. (E) Lateral view, dorsal right, shows expression in prechordal plate, the anlagen of the trigeminal ganglia (tg) and a dorsal stripe in the hindbrain (r3). Neurons of the spinal cord express *bck* weakly. (F) Dorsal view of the hindbrain, anterior left. The bilateral anlagen of the trigeminal ganglia (tg) and the dorsal stripe in the hindbrain (r3) are indicated. (G) Double labelling with *krox20* (red) and *bck* (blue black) reveals that the stripe of *bck* expression in the hindbrain corresponds to the dorsal part of rhombomere 3. Dorsal view, anterior left. Abbreviations: pcp prechordal plate; r3, r5, rhombomeres 3 and 5; tg, trigeminal ganglion.

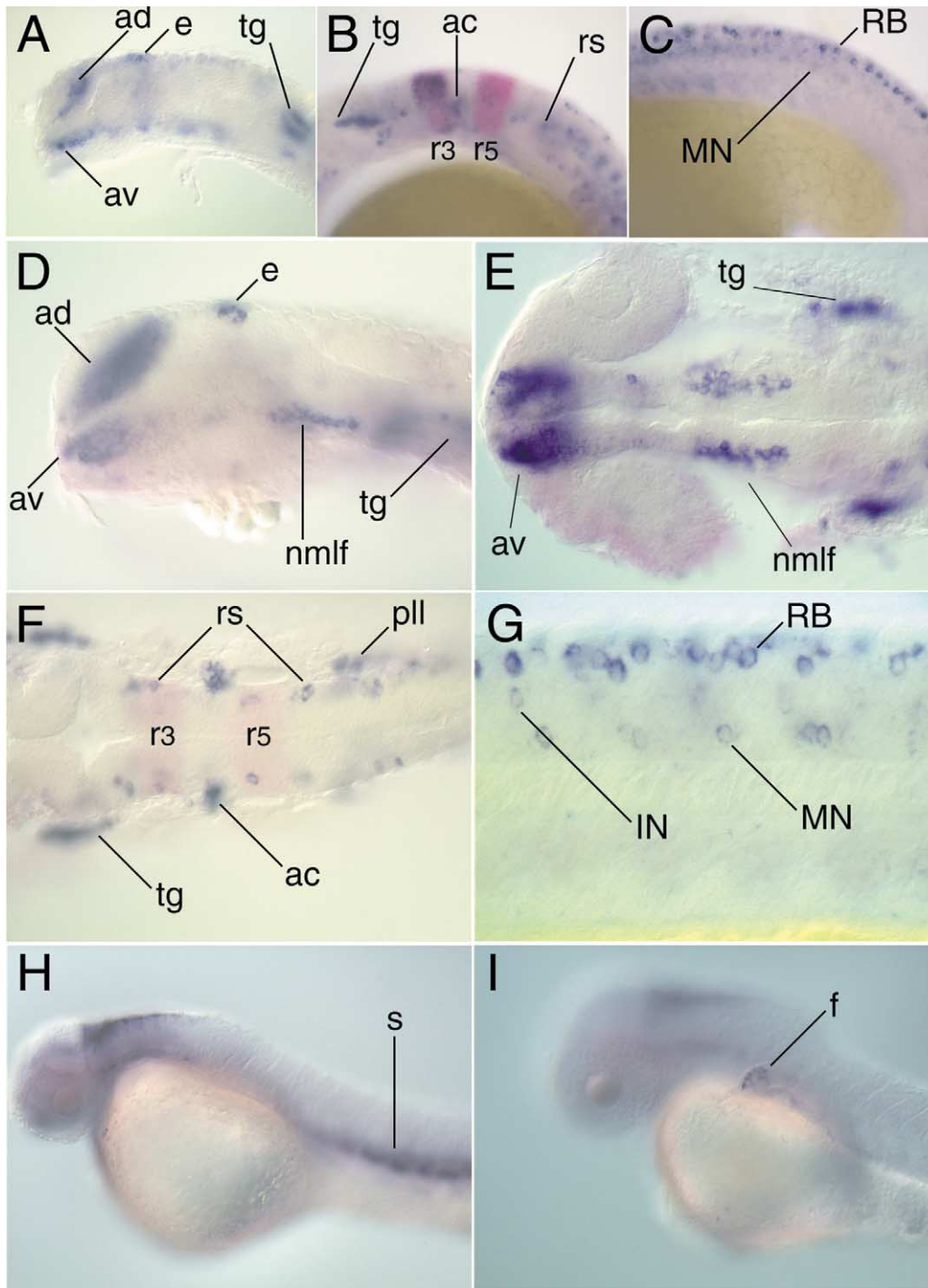


Fig. 3. *bck* is expressed in primary neurons in the central nervous system. (A–C) 18-somite stage embryos in lateral view (dorsal up, anterior left) of the head (A), of the hindbrain (B) and of the trunk (C). Embryos were double-labelled with *bck* (blue black) and *krox20* (red) antisense RNA to label rhombomeres 3 and 5. *bck* and *krox20* are co-expressed at the dorsal aspect of rhombomere 3. (D–G) 24 h embryos stained with *bck* antisense RNA. The embryo in (F) is labelled in addition with the *krox20* probe. Lateral (D) and dorsal (E) view of the forebrain and midbrain, dorsal view of the hindbrain (F) and lateral view of the spinal cord (G) at the level of the hindgut extension are shown. (H, I) 48 h old embryos in lateral views of head and trunk. Abbreviations: ad, anterior dorsal neuronal cluster; av, anterior ventral neuronal cluster; e, epiphysis; f, fin; IN, interneurons; MN, motor neuron; nmlf, nucleus of the medial longitudinal fascicle; pll, posterior lateral line ganglion; r3, r5, rhombomeres 3 and 5; rs, reticulospinal neurons; RB, Rohon Beard sensory neuron; s, somite, tg, trigeminal ganglion. Anterior is to the left.

neurons and motor neurons of the spinal cord (Fig. 3B) and reticulospinal neurons of the hindbrain (Fig. 3C). Neurogenic regions of the fore- and midbrain had also started to express *bck* by the 18-somite stage (Fig. 3A). At the end of somitogenesis, high levels of *bck* transcripts were detected in the anterior dorsal neuronal cluster of the telencephalon, the anterior ventral neuronal cluster of the hypothalamus, the epiphysis and the nucleus of the medial longitudinal fascicle (Fig. 3D,E). The trigeminal, acousticovestibular and posterior lateral line ganglia express *bck* mRNA (Fig. 3F). In the spinal cord of the 24 h old embryo, motor neurons and some interneurons, in addition to the dorsally located Rohon Beard neurons, express *bck* mRNA weakly (Fig. 3G).

High levels of *bck* mRNA persisted in the dorsal hindbrain of 2-day-old embryos (Fig. 3H) while transcripts were no longer detectable in the spinal cord (Fig. 3I). Expression in the ventral somite, already weakly visible in the 24 h embryo, has increased strongly. This somitic expression disappeared again during the next 12 h of development (Fig. 3I) and a new region of strong expression has become apparent in the fin bud by 60 h (Fig. 3I).

The expression of *bck* is highly dynamic, frequently being expressed in specific cells for a very short period only. The ubiquitous maternal expression of *bck* suggests that *bck* may be involved in the transfer of energy deposited by the mother in the egg to power general metabolism during blastula and gastrula stages. In contrast, the restriction to specific tissues at subsequent stages indicates cell-type specific functions. At peak levels of activity, certain regions of the mammalian brain utilise as much energy as contracting striated muscle (Ames, 2000). From estimates of the energy requirement of various neuronal processes, it was proposed that the high energy consumption of neurons is required for maintenance and regeneration of membrane potential, for vesicular transport and neurotransmitter processing (Ames, 2000). However, in a 24 h zebrafish embryo, when very high levels of *bck* expression were noted in neurons, only the very first axonal connections have been formed. Moreover, *bck* expression is absent in the spinal cord of 48 h old embryos when neuronal networks such as the one controlling the startle response have become functional (Kimmel, 1993; Saint-Amant and Drapeau, 1998). This suggests that *bck* may be involved in creatine phosphate metabolism related to differentiation of neurons in the spinal cord rather than to neuronal physiology.

2. Experimental procedures

2.1. Cloning and sequence analysis

The *bck* cDNA was identified in a subtractive screen for Taram-A inducible genes using a macroarray of a shield stage library (RZPD library no. 637 (Clark et al., 1999)). Details of the screen will be published elsewhere (T. Dick-

meis, P. Aanstad, M. Clark, P. Mourrain, H. Lehrach, F. Rosa and U. Strähle, in preparation). Multiple alignments and phylogenetic tree building were carried out using the Clustal X program (Thompson et al., 1997).

2.2. In situ hybridisation

Embryos were staged according to Kimmel et al. (1995). In situ hybridisation was carried out as described previously (Oxtoby and Jowett, 1993; Hauptmann and Gerster, 1994).

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