ELSEVIER

Mechanisms of Development 99 (2000) 159–162

www.elsevier.com/locate/modo

Gene expression pattern

Hexokinase I is a Gli2-responsive gene expressed in the embryonic CNS

R. Brewster[a,b,1], A. Ruiz i Altaba[a,b,*]

[a]*The Skirball Institute, Developmental Genetics Program, NYU School of Medicine, 540 First Avenue, New York, NY 10016, USA*
[b]*Department of Cell Biology, NYU School of Medicine, 540 First Avenue, New York, NY 10016, USA*

Received 20 July 2000; received in revised form 6 September 2000; accepted 6 September 2000

Abstract

Little is known about the downstream genes regulated by Gli zinc finger transcription factors, which are targets and mediators of Hedgehog signaling. Specifically, the identity and regulation of genes which mediate Gli2 function in neurogenesis are unclear. We describe here the cloning of frog *Hexokinase I* (*HKI*) as a Gli2-responsive gene. We show that *HKI* expression is induced by Gli2 and that it is detected in defined neuronal populations. Since the primary energy source of the brain is derived from glucose metabolism and hexokinase catalyses the first and rate limiting step in this process, the conversion of glucose into glucose-6-phosphate, these findings suggest a link between the regulation of neuronal induction and differentiation.

Keywords: Hexokinase; Brain; Nervous system; *Xenopus laevis*; Glucose metabolism; Glycolysis; Primary neurons; Nervous system; Neural plate; Gli2; Neurogenin; Neurogenesis; Neuronal differentiation

1. Results

The Gli family of zinc finger transcription factors is known to play a critical role in early development (Lee et al., 1997; Hynes et al., 1997; Marine et al., 1997; Brewster et al., 1998; Ding et al., 1998; Ruiz i Altaba, 1998; Matise, 1998). Gli2 in particular, has a potent neurogenic function. In an effort to identify downstream Gli targets, we carried out a differential screen for genes whose expression can be induced by Gli2 (Brewster et al., 2000). One clone isolated in this screen is a partial cDNA encoding HK. There are four isoforms of HK in mammals, the amino acid sequences of which are remarkably conserved between one another. Our clone has highest homology to HKI (Fig. 1; gene accession # AF28841), also known as brain HK (Clifton et al.; gene accession # AW871847; Nishi et al., 1988; Schwab and Wilson, 1988 and 1989; Griffin et al., 1989), due to its prominent expression in this tissue.

The identification of *HK1* as a Gli2-responsive gene prompted us to determine if its expression is detected in the neural plate, thus overlapping that of *Gli2* (Lee et al., 1997), since its early endogenous expression is unknown. Using in situ hybridization on whole-mount frog embryos, we determined that by midgastrula (stage ~12.5), *HKI* is localized in the notochord and in scattered cells in the epidermis, possibly neuromasts (Fig. 2A). At mid-neurula (stage 15), *HKI* is expressed in trigeminal ganglia and in three longitudinal stripes on either side of the neural plate, that correspond to the sites where primary neurons form (Fig. 2B,C). A comparison with the expression pattern of *N-Tubulin*, a marker for trigeminal ganglia and primary neurons, confirmed the identity of the *HKI*-positive cells (Fig. 2E,F). At mid-neurula, *HKI* is also expressed at low levels in the medial aspect of somites but has disappeared from the notochord (Fig. 2C). Expression, however, persists in neuromasts (Fig. 2B). In tadpoles (stage ~28), *HKI* is observed in somites and in the heart. *HKI*-positive cells in the nervous system include distinct groups of cells in the neural tube, the eye and several cranial ganglia (Fig. 2G). Examination of cross-sections at different levels along the anterior-posterior axis of tadpoles reveal that *HKI* is anteriorly restricted to ventro-lateral cells in the neural tube and to a subset of cranial nuclei (Fig. 2H). In posterior regions *HKI* is observed in the ventro-lateral neural tube, close to the position of spinal motor neurons, and in the somites (Fig. 2I). *HK1* is thus expressed in a variety of cell groups, although its expression is more restricted than that of *Gli2*, which is detected throughout the neural plate with exception of the midline (Lee et al., 1997). Nevertheless, *HKI* expression in the CNS includes ventral neurons the differentiation of which is known to be responsive to Sonic hedgehog signaling and Gli function (Ruiz i Altaba, 1998; Brewster et al., 1998).

* Corresponding author. Tel.: +1-212-263-7644; fax: +1-212-263-7760.
E-mail address: ria@saturn.med.nyu.edu (A. Ruiz i Altaba).
[1] Present address: Department of Biology, Haverford College, 370 Lancaster Avenue, Haverford, PA 19041, USA.

0925-4773/00/$ - see front matter
PII: S0925-4773(00)00469-X

We have tested directly whether Gli2 is sufficient to induce *HKI* expression. Ectopic *HKI* was observed in 40% of stage 12 Gli2-injected embryos ($n = 17$ out of 42 injected; Fig. 2D). Cross-sections confirmed the presence of cells expressing ectopic *HKI* in the neural ectoderm (data not shown). In the nervous system, Gli2 is known to function upstream of Neurogenin (Ngnr) (Brewster et al., 1998), a transcription factor that initiates the genetic cascade of inductive events leading to primary neuron differentiation (Ma et al., 1996). We therefore tested whether Ngnr has a similar effect as Gli2 on *HKI* induction. Interestingly, ectopic *HKI* was only observed in a minority of Ngnr-injected embryos ($n = 2$ out of 42 injected; not shown), suggesting that Gli2 may not function through Ngnr to induce *HKI* expression.

2. Discussion

We report here that the expression of *HKI* in the nervous system of frog embryos, like that of HKI and HK3 in rats (Coerver et al., 1998), is surprisingly not observed in all neuronal cell types, given that all mature neurons presumably require glucose metabolism. For example, sensory Rohon-Beard cells in the dorsal neural tube do not express *HKI*. Perhaps neurons that lack *HKI* express other *HK* isoforms, raising the possibility that HKI, like other metabolic enzymes including GSK3 and HMG-CoA reductase (Plyte et al., 1992; Van Doren et al., 1998), could have a role in the development of specific cell types. Nevertheless, the onset of neural expression of *HKI* at mid-gastrula (stage 12) suggests that glucose metabolism in frog embryos coincides with the timing of neuronal differentiation. Early onset of glucose metabolism in frog embryos is further supported by the presence of HKI enzymatic activity after neural induction in toads (Miranda et al., 1982). Thus, the finding that *HKI* is a Gli2-responsive gene, even though *HKI* is likely to be several steps downstream of Gli2, suggests a link between neuronal induction and differentiation. A link between Gli proteins and *HK*-regu-

Fig. 1. Comparison of HKI amino acid sequences. Frog (*Xenopus laevis*) HKI (Genbank # AF288471) has an overall amino acid identity of 85% to human HKI (Genbank #P19367; Nishi et al., 1988), 83% to rat HKI (Genbank # P05708; Schwab and Wilson, 1988, 1989) and 80% to bovine HKI (Genbank # P27595; Griffin et al., 1989). Amino acids that differ from the consensus are boxed. Our partial frog *HKI* cDNA lacks the region encoding the first 269 amino acids and this N-terminal sequence is not shown in this figure.

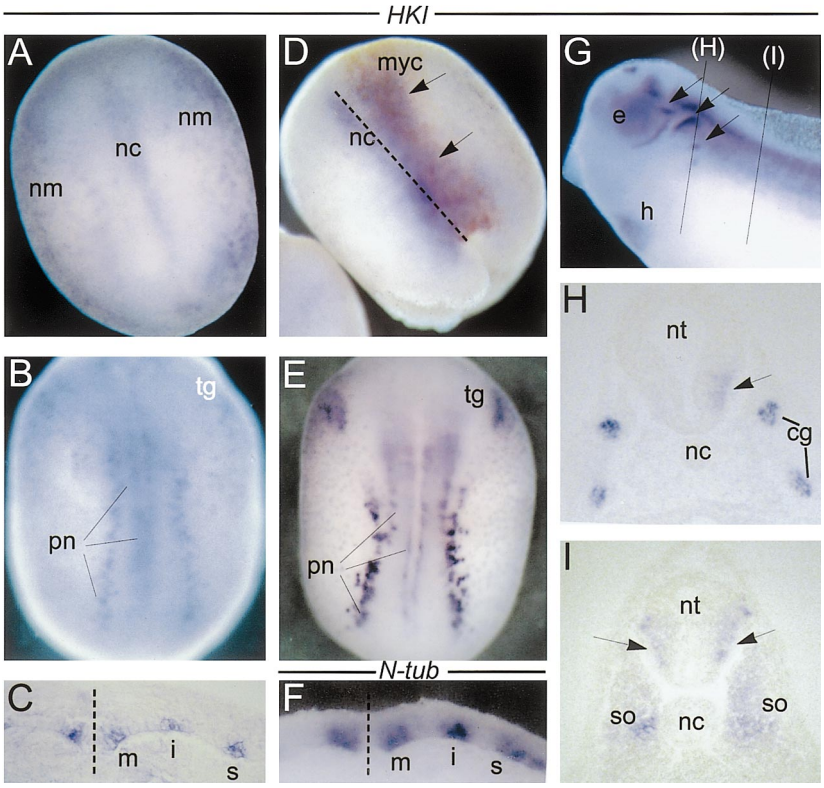

Fig. 2. Normal and Gli2-induced *HKI* expression. In situ hybridizations with an *HKI* probe (A–D,G–I) and a neural-specific *N-Tubulin* (*N-tub*) probe (E,F) were performed on stage ~12.5 (A,D), ~15 (B,C,E,F) and ~28 (G–I) embryos. (A–C,E–I) are uninjected embryos and (D) was injected with *Gli2* RNA into one blastomere at the two-cell stage, resulting in unilateral expression of myc-tagged Gli2 protein (brown staining labeled 'myc'). (A,B,D,E) anterior is to the top or top-left and the dorsal side is facing up. (G) anterior is to the left and the dorsal side is to the top. (C,F,H,I) Cross-sections of embryos; the dorsal side is to the top. Abbreviations: cg, cranial ganglia; e, eye; h, heart; i, interneurons; nc, notochord; nm, cells that may be neuromasts; nt, neural tube; m, motorneurons; s, sensory neurons; so, somites; tg, trigeminal ganglia. Dashed lines indicate the axes of symmetry. Solid black lines and letters in parenthesis next to them in (G) indicate the approximate level corresponding to the sections shown in panels (H,I). Arrows in (D) point to ectopic expression of *HKI* (blue), in (G) arrows point to cranial ganglia, and in (H,I) arrows point to ventral neurons, possibly motorneurons.

lation is also suggested by the presence of abnormally high levels of glycolysis (e.g. Warburg, 1956; Paggi et al., 1990), and thus of HK, in a variety of tumor cells that may express Gli proteins (Dahmane et al., 1997; see Ruiz i Altaba, 1999 for review).

3. Experimental procedures

Albino *Xenopus laevis* embryos, microinjections and *Gli2* RNA were obtained, performed or synthesized as previously described (Lee et al., 1997; Brewster et al., 1998).

Digoxygenin-labeled anti-sense *HKI* RNA for whole-mount in situ hybridization was synthesized using *Bam*HI and T7. Antibody incubations, HRP reactions and histological sections were performed as descried in Ruiz i Altaba (1998); Brewster et al. (1998).

Acknowledgements

We thank Nadia Dahmane and Mark Van Doren for comments on the manuscript. This work was supported by grants from the NCI, NINDS and March of Dimes to A.R.A.

References

Brewster, R., Lee, J., Ruiz i Altaba, A., 1998. Gli/Zic factors pattern the neural plate by defining domains of cell differentiation. Nature 393, 579–583.

Brewster, R., Mullor, J.L., Ruiz i Altaba, A., 2000. Gli functions downstream of FGF signaling during anteroposterior patterning. Development in press.

Coerver, K.A., Gray, S.M., Barnes, J.E., Armstrong, D.L., McCabe, E.R.B., 1998. Developmental expression of hexokinase 1 and 3 in rats. Histochem. Cell Biol. 109, 75–86.

Dahmane, N., Lee, J., Robins, P., Heller, P., Ruiz i Altaba, A., 1997. Activation of the transcription factor Gli1 and the Sonic hedgehog signalling pathway in skin tumours. Nature 389, 876–881.

Ding, Q., Motoyama, J., Gasca, S., Mo, R., Sasaki, H., Rossant, J., Hui, C.C., 1998. Diminished Sonic hedgehog signaling and lack of floor plate differentiation in Gli2 mutant mice. Development 125, 2533–2543.

Griffin, L.D., MacGregor, G.R., Muzny, D.M., Harter, J., Cook, R.G., McCabe, E.R., 1989. Synthesis and characterization of a bovine hexokinase 1 cDNA probe by mixed oligonucleotide primed amplification of

cDNA using high complexity primer mixtures. Biochem. Med. Metab. Biol. 41, 125–131.

Hynes, M., Stone, D.M., Dowd, M., Pitts-Meek, S., Goddard, A., Gurney, A., Rosenthal, A., 1997. Control of cell pattern in the neural tube by the zinc finger transcription factor and oncogene Gli-1. Neuron 19, 15–26.

Lee, J., Platt, K.A., Censullo, P., Ruiz i Altaba, A., 1997. Gli1 is a target of Sonic hedgehog that induces ventral neural tube development. Development 124, 2537–2552.

Ma, Q., Kintner, C., Anderson, D.J., 1996. Identification of neurogenin, a vertebrate neuronal determination gene. Cell 87, 43–52.

Marine, J.C., Bellefroid, E.J., Pendeville, H., Martial, J.A., Pieler, T., 1997. A role for Xenopus Gli-type zinc finger proteins in the early embryonic patterning of mesoderm and neuroectoderm. Mech. Dev. 63, 211–225.

Matise, M.P., Epstein, D.J., Park, H.L., Platt, K.A., Joyner, A.L., 1998. Gli2 is required for induction of floor plate and adjacent cells, but not most ventral neurons in the mouse central nervous system. Development 125, 2759–2770.

Miranda, M., Botti, D., Pantani, C., Ragnelli, N.M., Di Cola, M., 1985. Developmental aspects of hexose metabolism in Bufo bufo. J. Exp. Zool. 224, 211–215.

Nishi, S., Seino, S., Bell, G.I., 1988. Human hexokinase: sequences of amino- and carboxyl-terminal halves are homologous. Biochem. Biophys. Res. Commun. 157, 937–943.

Paggi, M.G., Fanciulli, M., Del Carlo, C., Citro, G., Carapella, C.M., Floridi, A., 1990. The membrane-bound hexokinase as a potential marker for malignancy in human gliomas. J. Neurosurg. Sci. 34, 209–213.

Plyte, S.E., Hughes, K., Nikolakaki, E., Pulverer, B.J., Woodgett, J.R., 1992. Glycogen synthase kinase-3: functions in oncogenesis and development. Biochim. Biophys. Acta 1114, 147–162.

Ruiz i Altaba, A., 1998. Combinatorial Gli gene function in floor plate and neuronal inductions by Sonic hedgehog. Development 125, 2203–2212.

Ruiz i Altaba, A., 1999. Gli proteins and Hedgehog signaling: development and cancer. Trends Genet 15, 418–425.

Schwab, D.A., Wilson, J.E., 1988. The complete amino acid sequence of the catalytic domain of rat brain hexokinase, deduced from the cloned cDNA. J. Biol. Chem. 263, 3220–3224.

Schwab, D.A., Wilson, J.E., 1989. Complete amino acid sequence of rat brain hexokinase, deduced from the cloned cDNA, and proposed of a mammalian hexokinase. Proc. Natl. Acad. Sci. USA 86, 2563–2567.

Van Doren, M., Broihier, H.T., Moore, L.A., Lehmann, R., 1998. HMG-CoA reductase guides migrating primordial germ cells. Nature 396, 466–469.

Warburg, O., 1956. On the origin of cancer cells. Science 123, 309–314.