The Xenopus arx gene is expressed in the developing rostral forebrain

Received: 14 August 2002 / Accepted: 3 October 2002 / Published online: 5 November 2002
© Springer-Verlag 2002

Abstract The human aristaless-related homeobox (ARX) gene is mutated in several patients with X-linked mental retardation and/or other neurologic pathologies. We report the isolation and expression pattern of a Xenopus arx gene. Similar to other vertebrate arx genes, Xenopus arx is expressed in the developing telencephalon, diencephalon, and floor plate.

Keywords aristaless · arx · Forebrain · Telencephalon · Homeobox gene

Aristaless (al) was first isolated from Drosophila (Schneitz et al. 1993). A growing number of aristaless-like genes have been isolated from vertebrates (Beverdam and Meijlink 2001; Bienvenu et al. 2002; Galliot et al. 1999; Meijlink et al. 1999; Miura et al. 1997; Stromme et al. 2002). Aristaless-like genes are members of the paired subfamily of homeobox genes (Meijlink et al. 1999; Schneitz et al. 1993). A member of this subfamily, the aristaless-related homeobox gene (arx), is expressed in the central nervous system of zebrafish and mouse embryos (Bienvenu et al. 2002; Miura et al. 1997; Stromme et al. 2002). While the role of arx in development is not yet clear, it has been suggested that arx genes may function as transcriptional repressors (Bienvenu et al. 2002; Stromme et al. 2002).

To facilitate developmental and functional studies of arx, we set out to isolate an ortholog from Xenopus laevis. Xenopus offers many advantages for investigating brain development, including the accessibility of neurula- and tailbud-stage embryos. Additionally, the Xenopus brain is less complex than the mammalian brain, simplifying analysis of expression patterns and developmental events. Finally, comparison of Xenopus arx with arx genes from other species should facilitate recognition of conserved protein domains of potential functional significance. A cDNA encoding the Xenopus arx (XArx) gene was isolated in a degenerate PCR screen for paired-type homeobox genes expressed during early neural development (GenBank accession number AY130460). The XArx coding region is highly related to other vertebrate arx genes (Fig. 1). XArx is more closely related to other vertebrate arx genes (59–64% amino acid identity, 66–72% amino acid similarity) than it is to other aristaless-like homeobox genes, such as alx 3 and 4 (18–21% amino acid identity, 26–30% amino acid similarity; see Fig. 1B for phylogenetic tree). There is a particularly high degree of shared sequence similarity in the stereotypical arx domains, namely the homeodomain, octapeptide, and the OAR domain as well as additional regions throughout the predicted protein (Bienvenu et al. 2002; Miura et al. 1997; Schneitz et al. 1993; Stromme et al. 2002). Notably, a region N-terminal to the homeodomain (aa 252–262 of the Xenopus sequence) is conserved between Xenopus and mammalian genes but is not found in the zebrafish gene. Additionally, a region of mostly acidic amino acids (aa 197–215 of the Xenopus sequence) is significantly larger in Xenopus and mammalian genes than in the zebrafish gene. The Xenopus and zebrafish arx genes each encode one polyalanine tract, while the mammalian arx genes encode four (Fig. 1).

Mutations in the aristaless-related homeobox gene (ARX) have been found in families with some forms of X-linked mental retardation (XLMR; OMIM 300382; Bienvenu et al. 2002; Stromme et al. 2002). Specifically, ARX mutations have been found in several patients with West Syndrome (characterized by mental retardation, infantile spasms, and hypsarrhythmic chaotic electroencephalogram), Partington Syndrome (characterized by mental retardation and dystonic movements of the hands), and...
mental retardation associated with myoclonic epilepsy and spasticity. The N-terminal two polyalanine tracts are mutated in some XLMR patients (Bienvenu et al. 2002; Stromme et al. 2002). The Xenopus gene contains two alanine residues in a similar position as the second mammalian polyalanine tract. There are several other mutations found in human XLMR patients (Bienvenu et al. 2002; Stromme et al. 2002). Three mutations (L33P, Q163R, and P353L of the human protein) involve changes in amino acids that are identical in mammalian and Xenopus arx genes. Another mutation involves a frame-shift in the sequence encoding a conserved amino acid (R483) resulting in miscoding of the fifth (C-terminal) exon of the human ARX gene. This exon encodes the highly conserved C-terminal OAR domain.

The spatio-temporal pattern of ARX expression was examined by in situ hybridization on Xenopus embryos using a digoxigenin-labelled antisense ARX riboprobe (Figs. 2, 3). ARX expression is initially detected in a discrete stripe in the anterior neural plate in early neural