Expression of ADP-ribosylation factor (ARF)-like protein 6 during mouse embryonic development

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ABSTRACT  ADP-ribosylation factor (ARF)-like protein 6 (ARL6) is a member of the ARF-like protein (ARL) subfamily of small GTPases (Moss, 1995; Chavrier, 1999). ARLs are highly conserved through evolution and most of them possess the consensus sequence required for GTP binding and hydrolysis (Pasquallato, 2002). Among ARLs, ARL6 which was initially isolated from a J2E erythroleukemic cell line is divergent in its consensus sequences and its expression has been shown to be limited to the brain and kidney in adult mouse (Ingley, 1999). Recently, it was reported that mutations of the ARL6 gene cause type 3 Bardet-Biedl syndrome in humans and that ARL6 is involved in ciliary transport in C. elegans (Chiang, 2004; Fan, 2004). Here, we investigated the expression pattern of ARL6 during early mouse development by whole-mount in situ hybridization and found that interestingly, ARL6 mRNA was localized around the node at 7.0-7.5 days post coitum (dpc) embryos, while weak expression was also found in the ectoderm. At the later stage (8.5 dpc) ARL6 was expressed in the neural plate and probably in the somites. Based on these results, a possible role of ARL6 in early development is discussed in relation to the findings in human and C. elegans (Chiang, 2004; Fan, 2004).

KEY WORDS: ADP-ribosylation factor (ARF)-like protein 6 (ARL6), small GTPase, embryonic development, node, organizer

ADP-ribosylation factors (ARFs) have been reported to play an important role in intracellular membrane trafficking (Moss, 1995; Chavrier, 1999). Although ARF-like proteins (ARLs) are very similar to ARFs in amino acid sequences, their biological function remains unclear. ARL1 and ARL3 have recently been shown to be required for localization of GRIP-domain proteins to Golgi membranes (Lowe, 1996; Lu, 2001; Panic, 2003; Setty, 2003). In addition, the expression of ARL4 and ARL5 was found to be developmentally regulated (Lin, 2000; Lin, 2002; Schurmann, 2002). These observations suggest the possibility that the ARL proteins function in membrane traffic which might mediate some developmental processes. ARL6 was first identified in a J2E erythroleukemic cell line. The ARL6 transcript is up-regulated during erythropoietin-induced differentiation of erythroid cells and down-regulated during interleukin-6-induced macrophage differentiation, suggesting a possible role in hemopoietic development (Ingley, 1999). In adult mice, ARL6 shows a tissue-specific expression pattern with the highest expression observed in the brain and kidney. In addition, yeast two-hybrid screening and co-immunoprecipitation reportedly show that ARL6 interacts with the protein-conducting channel subunit SEC61β (Ingley, 1999; Pettersson, 2000). However, its biological functions remain unclear. Recently, ARL6 was identified as the gene that causes Bardet-Biedl syndrome type 3 (BBS3) (Chiang, 2004; Fan, 2004). Four different homozygous substitutions in the regions including the GTP binding domain in ARL6 were found to be involved in BBS3. BBS3 is a multisystemic disorder characterized by obesity, blindness, polydactyly, renal abnormalities and cognitive impairment. Similar to other BBS’s, BBS3 is also thought to result from ciliary dysfunction because loss-of-function mutations of ARL6 in C. elegans impair cilia structure and function (Blacque, 2004). The observations that ARL6 is specifically expressed in ciliated cells including sensory neurons and involved in intraflagellar transport in C. elegans (Chiang, 2004), are good agreement with its involvement in BBS3.

We isolated ARL6 cDNA during a screen for genes which show localized expression patterns in the early mouse embryo and found by RT-PCR that ARL6 mRNA is expressed in the brain of 11.5 dpc embryos. To elucidate the developmental aspect of

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ARL6 function further, we herein report the expression pattern of ARL6 mRNA during early mouse embryonic development.

There are three domains conserved among ARL family members. These domains are thought to be involved in guanine nucleotide binding and hydrolysis (Moss, 1995). Comparison with other ARL members showed that ARL6 lacks some consensus amino acids in these domains; for instance, tryptophan and glycine in domain II and glutamine in domain III (Fig. 1A). These domains were substituted to phenylalanine, serine and methionine, respectively. We also cloned Xenopus ARL6 from cDNA library and found that Xenopus ARL6 shows 88% identity with mouse ARL6 in deduced amino acid sequence. Importantly, the amino acids of these domains were completely conserved among the mouse, human and Xenopus (Fig. 1B). Therefore it is possible that these substitutions may relate to a specific function of ARL6 with regard to the GTP binding and hydrolysis. Furthermore, it is noted that mutations resulting in a nonconservative amino acid change in threonine 31 of domain I and other mutations in invariable glycine 169 and leucine 170 (Fig. 1B), have been found in families affected with BBS3, indicating the importance of these residues for its normal functions.

We performed whole mount in situ hybridization to characterize the expression pattern of ARL6 during early mouse embryogenesis. Whereas no obvious expression of ARL6 was detected at early to mid-streak stage (Fig. 2A), at the late streak stage, when the primitive streak reached to its distal end, we observed localized ARL6 expression at the node, which is located at the anterior tip of the primitive streak and functionally corresponds to the gastrula organizer (Fig. 2B). This localized expression was still observed at early bud stage and weak signal was also detected throughout the embryonic portion (Fig. 2C). At this stage, the node consists of two germ layers: a dorsal layer that is continuous with the epiblast or ectoderm and a ventral layer that is continuous with the endoderm. Sagittal sectioning of this embryo revealed that the strong signal was localized to the ventral layer of the organizer and the weak signal was observed throughout the ectoderm (Fig. 2E). The similar expression pattern was maintained at early head fold stage, when the node became morphologically evident (Fig. 2D). At late head fold stage, strong expression of ARL6 was not restricted to the node but was observed throughout embryonic portion (Fig 3 A,B). At later stage (somite stage, 8.5 dpc), ARL6 expression was mainly observed in the neural plate and probably in the somites (Figs. 3 C,D). No obvious signal was observed in the extraembryonic tissues throughout the stages analyzed (6.5-8.5 dpc, Figs. 2 and 3). Because ARL4 is reportedly expressed in the somites and at the junction of forebrain and midbrain at the 10-12 somite stage (8.5 dpc) (Lin, 2000), the localization of transcript in the neural plate is characteristic to ARL6 at this stage.

Our data showed that ARL6 displays a dynamic pattern of expression during early mouse development. Its localized expression at the node that plays a central role in establishing the basic body plan as the gastrula organizer implies possible involvement of this small GTPase in early mouse embryonic development. Interestingly, our whole mount in situ hybridization analysis revealed that the strong signal of ARL6 expression was localized to the ventral layer of the node, which is distinguished by the presence of a single, motile, central cilium, showing good agreement with the observation that ARL6 is expressed specifically in ciliated cells and mediate intraflagellar transport in C. elegans. However, the mutations of ARL6 found in BBS3 did not seem to affect the body axis formation and left-
Mouse embryonic ARL6 893

Experimental Procedures

Mouse ARL6 cDNA was amplified with RNA from 11.5 dpc embryos by RT-PCR using primers, 5'-cctttggattggcgtcaaagatcag-3' and 5'-cactgaggtctccagggactatctc-3' and cloned into pBlue-script KS(+) plasmid. Thirty cycles of PCR were carried out at 94ºC for 30 sec, 55ºC for 1 min and 72ºC for 1 min. Xenopus ARL6 cDNA was also amplified from a cDNA library (stage 17/18) by PCR using primers 5'-cgggatccaccatgggattgtttgacaag-3' and 5'-ccgctcgagttactgcagggtgtcttcatc -3' and cloned into pCS107 plasmid (a gift from R Harland). Inserts were sequenced on both strands. Mouse embryos were collected from ICR (CLEA, Japan). Noon of the day on which a vaginal plug was observed was considered 0.5 dpc. Staging of mouse embryos were performed by their morphology (Downs, 1993). Embryos were fixed overnight at 4ºC in 4 % paraformaldehyde, dehydrated through a graded ethanol series then stored at -20ºC until use. Whole mount in situ hybridization was performed as described (Wilkinson, 1992). The full-length ARL6 cDNA (KS+ARL6) was used to generate digoxygenin-labeled sense or antisense riboprobe by transcribing using T7 or T3 RNA polymerase, respectively and digoxygenin-labeling RNA mix (Roche) according to the manufacturer’s protocol. All probes were run on a 2% agarose/formaldehyde gel before use to verify yield and length. After whole mount staining, some late streak stage embryos were subsequently embedded in paraffin and sectioned at 7 µm to further characterize the expression pattern. Images were captured with a digital camera and imported into Adobe Photoshop for assembly of the final figures.

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References


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