EXPRESSION OF THE DTK RECEPTOR TYROSINE KINASE DURING ZEBRAFISH DEVELOPMENT

Marta JANSA PEREZ, Jennifer A. WALSHE, Kathryn E. CROSIER and Philip S. CROSIER
Department of Molecular Medicine, School of Medicine, University of Auckland, Private Bag 92019, Auckland, New Zealand

The activation of receptor tyrosine kinases (RTKs) following ligand binding plays a key role in many developmental processes (Schlessinger and Ullrich, 1992). Dtk (Crosier *et al.,* 1994) belongs to a subfamily of receptor tyrosine kinases which includes Axl (O'Bryan *et al.,* 1991) and Mer (Graham *et al.,* 1994). Members of this subfamily are structurally characterized by the presence of two immunoglobulin-like domains juxtaposed with two fibronectin type III domains in their extracellular regions. Both Dtk and Mer are highly expressed in the adult brain. In the rat, Dtk expression increases during late embryonic neuronal development and remains at high levels postnatally (Lai and Lemke, 1991). Other adult mouse tissues which express significant levels of Dtk include testis, ovary, lung, bladder and portions of the gastrointestinal tract. In contrast to its restricted expression in adults, the Dtk gene is widely expressed during embryonic development (Crosier *et al.,* 1994). Dtk ligands have been identified as the protein encoded by the growth-arrest-specific gene (Gas6) and the anticoagulation factor protein S (Stitt *et al.,* 1995; Mark *et al.,* 1996), both belonging to the vitamin K-dependent protein family.

The zebrafish is an excellent system for investigating the function of genes involved in embryonic development, especially in the central nervous system (CNS) (Kimmel, 1993). A cDNA that encodes the zebrafish homologue for the Dtk receptor tyrosine kinase has been isolated and sequenced, showing overall 48% amino acid identity with the human counterpart and 72% identity in the region of the kinase domain (Walshe *et al.,* submitted).

We have undertaken the study of the expression pattern of Dtk during zebrafish embryonic development using different approaches. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis using specific oligonucleotides for Dtk transcripts was carried out on total zebrafish RNA and the specificity of the amplified products was confirmed by hybridization to probes containing sequences internal to the primers. Dtk expression could be detected from the beginning of gastrulation through the segmentation period, as well as in whole adult.

Poly $(A)^+$ RNAs from these different stages were analysed by Northern blot using a cDNA probe that contained sequences encoding part of the extracellular domain of Dtk. Using this technique, the full length transcript was only detected in adult, suggesting that the level of expression is higher in this period.

To gain insight into the pattern of expression of this receptor during the embryonic development of zebrafish, whole-mount *in situ* hybridization using Digoxigenin-labelled RNA probes corresponding to different regions of Dtk have been performed. Zebrafish embryos were grown in Phenyl thiourea (PTU) treated water to inhibit the development of pigment. The *in situ* hybridization method was based on that of Harland (1991) for *Xenopus* with several modifications. As observed by RT-PCR analysis, Dtk expression was first detected during early gastrulation in the zebrafish embryo. By the end of gastrulation Dtk transcripts were detected throughout the embryo. During the early segmentation period low levels of Dtk expression were detected in the brain and by 48 hours post fertilization Dtk expression appeared to be localized to posterior borders of the telencephalon, anterior regions of the midbrain, and discrete cells within the hindbrain.

In order to locate more accurately the sites of expression, we have undertaken comparative whole-mount *in situ* hybridizations with genes which are expressed in discrete domains of the zebrafish brain during the pharyngula period (24-48 h). These include Distal-Less 2, expressed near the ventricular surface of the telencephalon (Akimenko *et al.,* 1994) and hlx-1, which is expressed in reticulospinal interneurons defining rhombomere boundaries (Fjose *et al.,* 1994). These results suggested a correlation with the areas of expression of Dtk, although two-color whole-mount RNA *in situ* hybridization is necessary to precisely determine the cells containing the receptor.

Our results provide insights into the dynamic expression patterns which exist for Dtk during zebrafish development and extend knowledge of the regions in the brain which express Dtk. Further studies including perturbation of Dtk expression in zebrafish embryos in conjunction with identification of receptor ligand(s) for this species will contribute to the understanding of the role of Dtk in brain development.

References

Akimenko, M-A., Ekker, M., Wegner, J., Lin, W. and Westerfield, M. (1994). Combinatorial expression of three zebrafish genes related to Distal-Less: part of a homeobox gene code for the head. J. Neurosci. 14: 3475-3486.
Crosier, P.S., Lewis, P.M., Hall, L.R., Vitas, M.R., Morris, C.M., Beler, D.R., Wood, C.R. and Crosier K.E. (1994). Isolation of a receptor tyrosine kinase (Dtk) from embryonic stem cells: structure, genetic mapping and analysis of expression. Growth Factors 11: 125-136.
Fjose, A., Izpisua-Belmonte, J.C., Fromental-Ramain, C. and Duboule, D. (1994). Expression of the zebrafish gene hlx-1 in the precordal plate and during CNS development. Development 120: 71-81.
Graham, D.K., Dawson, T.L., Mullaney, D.L., Snodgrass, H.R. and Earp, H.S. (1994). Cloning and mRNA expression of a novel human protooncogene, c-mer. Cell Growth Differ. 5: 647-657.
Harland, R.M. (1991). *In situ* hybridization: an improved whole-mount method for *Xenopus* embryos. Methods Cell. Biol. 36: 685-695.
Kimmel, C.B. (1993). Patterning the brain of the zebrafish embryo. Annu. Rev. Neurosci. 16: 707-732.
Lai, C. and Lemke, G. (1991). An extended family of protein-tyrosine kinase genes differentially expressed in the vertebrate nervous system. Neuron 6: 691-704.
O'Bryan, J.P., Frye, R.A., Cogswell, P.C., Neubauer, A., Kitch, B., Prokop, C., Espinosa, R., Le Beau, M., Earp, H. and Liu, E. (1991). Axl, a transforming gene isolated from primary human myeloid leukaemia cells, encodes a novel receptor tyrosine kinase. Mol. Cell. Biol. 11: 5016-5031.

Mark, M.R., Chen, J., Hammond, R.G., Sadlek, M. and Godowski, P.J. (1996). Characterisation of gas6, a member of the superfamily of G domain-containing proteins, as a ligand for Rse and Axl. J. Biol. Chem. 271: 9785-9789.
Schlessinger, J. and Ullrich, A. (1992). Growth factor signalling by receptor tyrosine kinases. Neuron 9: 383-391.
Stitt, T.N., Conn, G., Gore, M., Lai, C., Bruno, J., Radziejewski, C., Mattson, K., Fisher, J., Gies, D.R., Jones, P.F., Masiakowski, P., Ryen, T.E., Tobkes, N.J., Chen, D.H., Di Stefano, P.S., Long, G.L., Basilico, C., Goldfarb, M.P., Lemke, G., Glass, D.J. and Yancopoulos, G.D. (1995). The anticoagulation factor protein S and its relative, Gas6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases. Cell 80: 661-670.
Walshe, J.A., Jansa Perez, M., Crosier, K.E., Evans, C.W. and Crosier, P. (1996). Expression of the Dtk/Tyro3 receptor tyrosine kinase during zebrafish brain development. (Submitted).