CLONING OF TRANSFERRIN AND p53 HOMOLOGS FROM ZEBRAFISH

Nancy Berliner[1], Benjamin Barnes[2], Dukagjin Blakaj[3], Adriana Blakaj[4], Rachel B. Plattus[1], Edward J. Benz, Jr.[5]
[1]Department of Medicine/Hematology, Yale School of Medicine, New Haven, CT [2]Dartmouth College, Hanover, New Hampshire
[3]Wesleyan University, Middletown, CT
[4]Taft School, Watertown, CT
Department of Medicine, Johns Hopkins School of Medicine, Baltimore, MD

Transferrin (Tf) is an iron (Fe)-binding protein that is responsible for the transport and delivery of Fe to cells. It is one of a family of related proteins that includes lactoferrin (LF), a neutrophil granule protein that is secreted upon neutrophil activation. Bacteria require Fe for growth, and it is hypothesized that LF functions to limit bacterial infections by sequestering iron (Lonnerdahl B & Iyer S. Ann Rev Nutr 5:93-110, 1995). It has also been hypothesized that Tf, in addition to mediating Fe transport, may also play a role in limiting bacterial infection and in protecting against iron toxicity. Surprisingly, the lactoferrin-null phenotype is a very early embryonic lethal, suggesting that LF has functions other than that of a protection against bacterial overgrowth. We hypothesize that it may function as a very early Fe transporter necessary for early stages of embryogenesis.

The protein product of the p53 gene has proven to be a critical regulator of entry into the cell cycle and apoptosis. P53 is a transcription factor cofactor, whose expression is induced by a number of stimuli, most of which involve DNA damage either directly or indirectly. High levels of p53 in the nucleus prohibit progression into the cell cycle and can trigger apoptosis. Thus, p53 may lead to the death of cells potentially mutagenized by DNA damaging agents. Indeed, over 60% of the human cancers that have been studied carry mutations that impair or abolish p53 gene expression. We hypothesize that p53 may also play a role in the response to toxins.

In order to delineate further the function of Tf, LF, and p53, we have sought to isolate their cDNA homologues from zebrafish (*Danio rerio*). The zebrafish is an increasingly popular model for genetic studies because of the ease of the manipulation of the organism for transgenic studies (Lever W & Fishman MC. J Clin Invest 98: S41-46, 1996). LF-null and p53-null mice both die in early embryogenesis, making analysis of their function in that model impractical. Study in zebrafish embryos, which are transparent, offers the opportunity to obtain insight into the functional localization of these genes, and may offer clues as to why they are necessary for successful embryonic development.

Last summer, we used PCR-based techniques to isolate a partial cDNA clone of Tf from a zebrafish (ZF) cDNA library (Moczydlowski E, et al. The MDIBL Bulletin 38: 3-4, 1999). Based on the sequence of that clone, we identified an EST that appeared subsequently in the ZF database. We therefore obtained the EST for further study this summer. Restriction enzyme and sequence analysis confirmed that the EST represents a full-length cDNA clone for the ZF Tf. We have nearly completed the full-length sequence of this cDNA. Further planned studies include *in situ* hybridization to ZF embryos using this cDNA clone, and low stringency screening of the cDNA library to obtain related family members.

Last summer we were unsuccessful in obtaining a P53 clone by a PCR-based strategy. During this summer, we again obtained a new EST from the ZF database that corresponds to P53. This clone is quite short, and we initiated screening of the ZF cDNA library to obtain a full-length cDNA. We also plan to use this clone for *in situ* hybridization to ZF embryos. This work was partially supported by an REU grant to BB (*NSF DBI-9820400*)