PARTIAL SEQUENCE OF A TRANSFERRIN HOMOLOG FROM *DANIO RERIO* (ZEBRAFISH)

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Transferrin (Tf) is a vertebrate plasma protein that binds two Fe^{3+} ions in a bicarbonate dependent fashion. Tf functions in the transport and delivery of iron to cells via transferrin receptor-mediated endocytosis. As iron is required for the growth of many bacteria and free Fe^{3+}/Fe^{2+} is involved in the generation of chemically damaging hydroxyl free radicals, Tf also functions to limit bacterial infections and protect against iron toxicity. Lactoferrin (Lf) is an Fe^{3+}-binding homolog of Tf present in milk and secretions that bathe mucosal surfaces. Lf is also secreted by neutrophils and is believed to play a role in modulation of immune and inflammatory responses.

In recent years, novel members of the Tf family have come to light. One such homolog is saxiphilin (Sax), a 91 kDa protein found in bullfrog plasma (Morabito, M. & Moczydlowski, E., *Proc. Nat. Acad. Sci. USA* 91: 2478-2482, 1994). Sax does not bind Fe^{3+}. However, it does specifically bind one molecule of the sodium channel neurotoxin, saxitoxin, with high affinity (K_D = 0.2 nM). Saxitoxin, is the causative agent of the human food poisoning syndrome called paralytic shellfish poisoning. Such poisoning can result from unwitting ingestion of mussels or clams contaminated with saxitoxin accumulated from toxic species of marine dinoflagellates. Another interesting homolog of transferrin is pICA (a 79 kDa inhibitor of carbonic anhydrase II from pig plasma (Wuebbens, et al., *Biochemistry* 36: 4327-4336, 1997). Although these latter two Tf homologs do not bind Fe^{3+}, Sax exhibits 44% identity to human Tf and pICA exhibits 63% identity to pig Tf. The examples of Sax and pICA imply that transferrin-related genes comprise a superfamily of proteins involved in a variety of diverse biological functions including iron metabolism, metal ion toxicity, toxin defense mechanisms, immune modulation, and control of certain enzymatic activities.

To enhance understanding of the physiology and developmental regulation of the Tf gene family, we have begun a project to identify such genes from the zebrafish, *Danio rerio*. The zebrafish is becoming increasingly popular as a vertebrate model system in biology because of favorable features such as rapid reproduction, transparent embryos, and ease of genetic manipulation. This small freshwater fish may also provide a valuable system for studies of iron metabolism and aquatic toxicology. Biochemical assays indicate that zebrafish contain saxiphilin-like binding activity for saxitoxin (Llewellyn et al., *Proc. R. Soc. Lond. B* 264: 891-902, 1997). Although transferrin has been cloned from various fish species such as cod and salmon, it is not known whether the teleost fish genome contains Lf or other Tf-related genes.

Our initial attempt to identify and clone Tf-related genes in zebrafish made use of a known partial sequence in the zebrafish database and a cDNA library prepared from mRNA from

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hum Lf  qssdpdpncv drpvegYlAV AVVRrsdtsL TWnsVkGKKS CHTavdRtAG
hum Tf  d......nce dtpeagYfAV AVVkKsaSdL TWdNLkGKKS CHTavGRtAG
sal Tf  lcsapg.... ..easSYYAV AVakKG.SGL TWktLkGKrS CHTGLGRtAG
cod Tf  lcssag.... .tpqatYfAV AVVkKG.SGv TWdNLrGKrS CHTGLGRtAG
zeb Tf               nsplSYYAV AVVRKG.SGL TWsNLeGKKS CHTGLGRsAG

hum Lf  WnIPmgllfn qtgsCkfDey fSqsCAPGsD PrSNlCaLCi Gdeqg...En
hum Tf  WnIPmgllyn kinhCrfDeF fSEGCAPGsk kdSslCKLCm G..sg...ln
sal Tf  WnIPmglIhq EtndCdftKy fSkGCAPGse vgSpfCaqCK GSGKAVGDEy
cod Tf  WnIPmglvhs ihgsCdfggF fpsGCAPGse PsStfCrqCa GSGsgVeDgS
zeb Tf  WkIPqsaIcg EkdkCtlDKF sSEGCAPGaD PtSNmCKLCK GSGKAVGDES

hum Lf  KCvPnsnErY YGYtGAFRCL AEnAGDVAFv KdvtVlqnTD GnnneaWAKD
hum Tf  lCePnnkEgY YGYtGAFRCL vEK.GDVAFv KHqtVpqnTg GKnpDpWAKn
sal Tf  rCKarsEEQY YGYtGAFRCL vEdAGDVAFI KHTiVpesTD GnGpD.WAKD
cod Tf  KCsaSsvEkY YGYaGAFRCL vdgAGDVAFI KHTiVaDnsD GqGpa.WAta
zeb Tf  KCKPSaEEQY YGYdGAFRCL AEKAGDVAFI KHTvVgDyTD GKGrD.WAKD

hum Lf  LKlaDFaLlC ldg..krkpv TearsChLAm aPnHAVvsrm Dkveelkqvl
hum Tf  LnekDyELlC ldg..Trkpv eeyanChLAr aPnHAVvTrk DkeacVhkiL
sal Tf  LKSsDFELlC qdgt.Tq.pv TkFseChLAk VPaHAVITrp etRgdVVSiL
cod Tf  LKSsDyqLIC Pggv.graei sDFasCnLAa VPsHAVvTrq DiRddVVkmL
zeb Tf  LKSeDFELIC PntpdItmky IDFekCnLAq VPvHAVITRe DaRsaVVSfL

hum Lf  lhqQaKfgrn gsdcpdkfcl fqsetKNLLF nDnTeCLarl hGkTtyekyL
hum Tf  rqqQhlfgsn vtdcsgnfcl frsetKdLLF rDdTvCLakl hdrNtyekyL
sal Tf  leLQaKfgss gsdssfrm.f qSsveKNLLF kDsTKCLQEI pkgTkyqDFL
cod Tf  lDqQrKfgid gsdplfri.y eSKDGnNLLF kDsTKCLkEI pslTtaDaFL
zeb Tf  yDiQsKnnd. .......l.f tSKDGKNLLF tDgTKCLQEI kG..svDDFL

hum Lf  gpqYvagItn lkKcS..tsp LleACEflrk
hum Tf  geeYvkavgn lrKcS..tss LleACTfrrp
sal Tf  gKeYmiamqs lrKcSdstsD LeKACTfhsC qqke
cod Tf  gtgYvnaIms lrqcpetase LeKtCisssC stae
zeb Tf  tKkYidmIer tyKtSqnvpD LvKACTlgnC iss
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whole adult zebrafish. We designed sense and antisense oligonucleotide primers for the use in polymerase chain reactions (PCR) according to the 5' and 3' ends of the known partial sequence. Synthetic oligonucleotides were used to amplify a ~ 0.8 kb DNA fragment using the zebrafish library as a template. The amplified cDNA fragment was gel purified and cloned into the TA plasmid vector. The cloned DNA fragment was sequenced in both directions.

The cloned sequence appears to encode 260 residues of a Tf-homolog. The figure shows an alignment of the translated zebrafish sequence (zeb Tf) with that of human Lf (residues 438-711, Acc. No. P02788), human Tf (residues 435-698, Acc. No. P02787), salmon Tf (Acc. No. L20313), and cod Tf (Acc. No. L40370). The alignment approximately corresponds to the C-terminal third of known Tf proteins. The capitalized and underlined residues indicate positions of identity to the zebrafish sequence.

In summary, sequence data confirms that we have isolated a large portion of a zebrafish cDNA that encodes a member of the Tf family. The presence of four conserved residues known to reside in the Fe^{3+}-binding site of Tf and Lf suggests that this homolog binds Fe^{3+}. We are currently analyzing four other potential Tf-related clones obtained by hybridization screening of the cDNA library. This work was partially supported by grants to E. M. from NIH (GM 51172) and a New Investigator Award from MDIBL.