Biometals (2009) 22:557–564
DOI 10.1007/s10534-009-9207-6

Siderocalins: siderophore-binding proteins of the innate immune system

Matthew C. Clifton · Colin Correnti · Roland K. Strong

Received: 30 October 2008 / Accepted: 12 January 2009 / Published online: 30 January 2009

Abstract Recent studies have revealed that the mammalian immune system directly interferes with siderophore-mediated iron acquisition through siderophore-binding proteins and that the association of certain siderophores, or siderophore modifications, with virulence is a direct response of pathogens to evade these defenses.

Keywords Siderophores · Siderophore-binding proteins · Bacterial virulence · Bacterial iron acquisition · Innate immunity

Introduction

Siderocalin [Scn; also referred to as lipocalin 2, neutrophil gelatinase-associated lipocalin (NGAL), 24p3 and uterocalin] was first identified as a component of human neutrophil granules, found there in several forms: a monomer, a disulfide-linked homodimer and a disulfide-linked heterodimer with matrix metalloproteinase 9 (MMP-9; also gelatinase-B) (Triebel et al. 1992; Kjeldsen et al. 1994, 2000; Goetz et al. 2002). Siderocalin is a member of the functionally diverse lipocalin family of proteins, with representatives distributed across many phyla. While also diverse in sequence (beyond characteristic motifs), lipocalins display a highly conserved structural fold shared by Scn: an eight-stranded anti-parallel β-barrel, which encloses a cup-shaped binding site, or calyx, with accessory α- and 3_{10}-helices. Lipocalins are prototypically binding proteins and the function of a particular lipocalin is tied to the ligand bound, with the shape and the chemical character of the lining of the calyx defining ligand specificity. The Scn calyx is uncharacteristically lined with polar and positively-charged residues, where most lipocalin calyces have distinctly hydrophobic linings. The Scn calyx is highly sculpted, with three prominent pockets (1, 2 and 3) defined by the positions of the side-chains of three positively-charged residues, namely Arg81, Lys125 and Lys134 (Fig. 1; Goetz et al. 2000).

Scn ligands

Building on the observation that Scn co-purifies with a dark red chromophore when expressed in bacteria, Goetz and coworkers identified ferric enterochelin (FeEnt; also enterobactin), the primary siderophore of many enteric bacteria, as a candidate ligand. Siderophores are low molecular weight, virtually ferric-specific chelators involved in microbial receptor-mediated iron acquisition. Iron is an essential, but

M. C. Clifton · C. Correnti · R. K. Strong (✉)
Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA
e-mail: rstrong@fhcrc.org

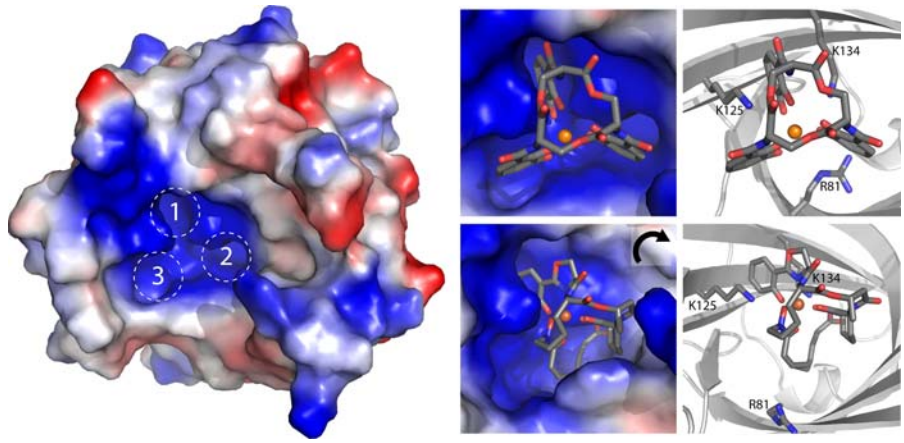

Fig. 1 Crystal structures of Scn. On the *left* is the overall surface energy representation highlighting the prominent features of the calyx and its three sculpted binding pockets. On the *right* are views of the calyx bound to FeEnt (*top*) and CMB (*bottom*)

limiting resource in the environment for many microorganisms (due to the insolubility of $Fe(OH)_3$), and many bacteria and fungi secrete siderophores in a competition to scavenge this scarce resource (Neilands 1981, 1995; Ratledge and Dover 2000; Winkelmann 2002). Siderophores are highly diverse as well, but can be divided into three broad classes depending on the chemistry of chelation: hydroxamates, phenolates/catecholates or α-hydroxycarboxylates—though other liganding chemistries can be used. Siderophore affinities for iron III can exceed 10^{50} M^{-1}, making them the strongest known iron chelators. Scn was subsequently shown to not only tightly bind FeEnt ($K_D = 0.4 \pm 0.1$ nM) but also a range of related catecholate-type siderophores including parabactin, bacillibactin, 2,3-dihydroxybenzoic acid (DHBA) and, most surprisingly, the quite distinct, mixed catecholate/hydroxamate, soluble siderophores of *Mycobacterium*: carboxymycobactins (CMBs; $K_D = 0.6 \pm 0.04$ μM) (Goetz et al. 2002; Holmes et al. 2005). Scn binds FeEnt and related siderophores by intercalating the side-chains of the three positively-charged calyx residues (Arg81, Lys125 & Lys134) between the three catecholate rings of FeEnt (Fig. 1), generating a novel hybrid of ionic and cation-π interactions (Ent is uncharged, but FeEnt carries a net −3 charge) (Raymond et al. 1984; Goetz et al. 2002). Electrostatic and cation-π bonds both contribute to binding, with retention of significant affinity even with net uncharged ferric complexes of Ent-analogs. Scn binds CMB using similar principles, recognizing the only common element between these two distinct classes of compounds, a highly-polarized phenyl group, while accommodating distinct features, such as the fatty-acid tail of CMB, in sub-pockets unfilled by Ent-like siderophores in an otherwise essentially rigid calyx. Therefore, Scn does not employ induced-fit mechanisms to enable this recognition degeneracy.

Unfortunately, there is extremely limited information about how Ent and Ent-like siderophores are recognized by bacterial siderophore binding proteins. The *Escherichia coli* receptor FepA structure has been determined in the presence of FeEnt, but the ligand and the surrounding protein residues were disordered in the structure (Buchanan et al. 1999). The only other directly relevant structure, CeuE (the *Campylobacter jejuni* FeEnt periplasmic binding protein), was determined in complex with a synthetic Ent analog (MECAM) (Muller et al. 2006). While this complex structure shows some similarities to the Scn/Ent recognition mechanism, with cation-π interactions mediating binding to the catechol rings of MECAM, the FeMECAM ligand shows an unusual bridging interaction, forming an unnatural M_2L_2 complex with iron that dimerizes CeuE, distorts the geometry of the siderophore and likely prevents or limits the closure of the protein over the ligand typical of other periplasmic binding proteins.

Scn, iron and disease

Within the body, the majority of iron is bound up in hemoglobin, though other proteins bind iron directly,

including transferrin, lactoferrin and ferritin. As part of the innate immune response to infection and cancer, available iron in the body, normally tightly controlled, is further reduced to slow or stop the growth of pathogens and tumors through depletion of this necessary resource (Weinberg 1984; Jurado 1997). Lactoferrin is understood to be a bacteriostatic agent, also released from neutrophil granules at sites of inflammation, directly inhibiting the growth of infecting pathogens by sequestering iron (Ellison 1994). The ligand specificity of Scn therefore highlights the complementary role this protein plays in innate immune responses: a neutrophil granule protein, secreted in response to infection or inflammation, that sequesters iron as ferric siderophore complexes rather than free iron, away from microbial pathogens, thus limiting their growth and virulence. Scn complements the activity of lactoferrin by binding ferric siderophore complexes rather than iron directly, therefore targeting iron already earmarked for bacterial use (Goetz et al. 2002). During inflammation, concentrations of Scn can approach 30 nM in the serum, adequate to presumably bind all iron in ferric siderophore complexes (Xu and Venge 2000). Confirming this hypothesis, Scn is a potent bacteriostatic agent in vitro against *E. coli* cultured in iron-limiting conditions; this effect is solely through its affinity for FeEnt and not other bacteriostatic mechanisms (Goetz et al. 2002). Mice are dramatically susceptible to infections by virulent strains of *E. coli* when the Scn gene is knocked-out (Flo et al. 2004; Berger et al. 2006).

Scn binding properties also contribute to the explanation of the long-known but previously mysterious association of certain siderophores with bacterial virulence. The pathogenesis islands of many bacteria including species of *Yersinia*, *Shigella*, *Klebsiella*, *Salmonella* and *Neisseria*, encode proteins either associated with siderophore synthesis or uptake (Moss et al. 1999; Vokes et al. 1999; Zhou et al. 1999; Klee et al. 2000; Carniel 2001). Biosynthesis of the siderophores aerobactin (Warner et al. 1981) or yersiniabactin (Carniel 2001), for instance, contributes to virulence for many bacteria. Research has shown that Scn has no appreciable affinity for aerobactin (Goetz et al. 2002) or pyochelin (Holmes et al. 2005) and due to the similarity to pyochelin, Scn very likely has limited affinity for yersiniabactin as well. These siderophores thus confer virulence by allowing these bacteria to evade Scn-mediated iron sequestration. This hypothesis explains the apparent conundrum (Crosa 1989; Ratledge and Dover 2000) of why production of a second, seemingly less efficient siderophore aerobactin, one with a considerably lower affinity for iron (III) than Ent, would contribute to virulence. In support of this explanation, the relative susceptibility of Scn knock-out versus wild-type mice to *E. coli* infection is only apparent with bacterial strains unable to synthesize aerobactin; strains that can secrete aerobactin do equally well in culture and in in vivo infections in the presence or absence of added Scn (Flo et al. 2004; Berger et al. 2006). There is also no difference in outcome when wild-type or knock-out mice are infected with *Staphylococcus aureus*; comparable mortality occurs in both contexts. This is consistent with the utilization of siderophores by *S. aureus* (including staphyloferrin) that are predicted to evade Scn binding, a prediction made on the basis of structural homology to siderophores, such as rhizoferrin, that have been directly shown to not bind Scn (Holmes et al. 2005).

Salmonella and uropathogenic strains of *E. coli* are also able to modify Ent by glucosylation (Fischbach et al. 2004) yielding the salmochelins, siderophores that do not bind Scn because of significant steric clashes in the calyx (Abergel et al. 2006a, b; Fischbach et al. 2006). Intriguingly, the CeuE/MECAM complex structure shows pockets large enough to accommodate at least one glucose adduct, potentially showing one mechanism by which a virulence-associated siderophore can evade the Scn defense while still supporting bacterial iron acquisition. Scn-mediated anti-mycobacterial responses may also be limited by the high selectivity of Scn for particular CMB isoforms, with varying fatty acid tail lengths, shown by the structural analysis of Scn/CMB complexes (Holmes et al. 2005; the preferred isoform is show in Fig. 1). Scn may be able to tolerate binding of CMB variants plus or minus one or perhaps two methylene groups in the fatty acid moieties from the optimum, though likely with concurrent reductions in affinity, but it seems unlikely that Scn could accommodate the extremes of the reported CMB spectrum, at least while retaining the overall ligand orientation seen in the co-crystal structures. Therefore, CMB variation may also reflect mycobacterial responses to Scn-mediated defenses, evidenced by the obvious success of mycobacteria as human pathogens.

Pleiotropic functions of Scn

Scn has also been implicated in diverse cellular processes seemingly unrelated to anti-bacterial activities, such as apoptosis (Devireddy et al. 2001; Kamezaki et al. 2003) and kidney cell differentiation (Yang et al. 2002, 2003). Supporting alternate activities is the evidence for mammalian cell-surface Scn receptors reported in at least two different systems (Devireddy et al. 2001; Yang et al. 2002) with two candidate receptor proteins identified: Megalin (Hvidberg et al. 2005) and 24p3R (Devireddy et al. 2005). Megalin, a member of the low-density lipoprotein family and an extracellular matrix component (Saito et al. 1994), also interacts with lactoferrin (Willnow et al. 1992; Meilinger et al. 1995) and other lipocalins: retinol binding protein, α_1-microglobulin/HC, mouse major urinary protein and odorant binding protein (Flower 2000). Megalin binds Scn in the presence or absence of bound ferric siderophores and can mediate its cellular uptake (Hvidberg et al. 2005), though the physiological role of this interaction remains speculative. Megalin also seems to bind lipocalins indiscriminately. Devireddy and coworkers have also reported the cloning of a highly-conserved, multi-pass integral membrane receptor for Scn completely unrelated to Megalin, 24p3R, which can mediate Scn endocytosis (Devireddy et al. 2005).

Murine Scn, in a murine tissue culture system, also acts as an iron delivery protein, acting in concert with transferrin to convert mesenchymal progenitors into tubular epithelium, forming kidney nephrons (Yang et al. 2002). The effect is dependent upon iron bound by Scn as a complex with a chromophore, which is presumed to be an endogenous 'siderophore'. In this system, murine Scn recycles through sub-cellular endosomal compartments distinct from the intracellular trafficking of transferrin. Passage through these low-pH intracellular compartments correlates with release of iron. If mammals do synthesize siderophores that are used to shuttle iron or act as growth factors, Scn may participate in a wide variety of cellular processes by playing a role in regulating their transport, thus potentially explaining Scn's association with tumorigenesis and apoptosis. The existence of an endogenous siderophore confounds proposals to use siderophores and siderophore analogs as therapeutics, either as antibiotics (Roosenberg et al. 2000; Budzikiewicz 2001) or, through the bound iron, as oxygen radical scavengers in various clinical settings, such as the treatment of ischemia associated with congestive heart failure (Horwitz et al. 1998).

Scn and cancer

Scn is also induced in several human cancers and elevated Scn expression levels have been correlated with poor prognosis (Devarajan 2007). The most direct evidence linking Scn with disease progression comes from the Bcr/Abl-induced mouse model of chronic myelogenous leukemia (CML). Suppression of normal hematopoiesis in Bcr/Abl-induced leukemia, leading to mortality, is an active process involving secretion of Scn by mouse leukemia cells, likely through an apoptotic mechanism (Lin et al. 2005). The putative Scn receptor, 24p3R, is also upregulated in Bcr/Abl mice and CML patients (Devireddy et al. 2005) and a shorter splice-variant of 24p3R is expressed in human esophageal cancers (Fang et al. 2007). While the exact molecular mechanisms underlying the role of Scn in tumorigenesis and malignancy are currently unknown, Devireddy and coworkers proposed a model where 24p3R mediates the endocytosis of extracellular Scn/ferric siderophore complexes to donate iron to cells and prevent apoptosis. In contrast, the internalization of apo-Scn by cells is proposed to lead to iron efflux, by binding to and exocytosing intracellular ferric siderophores, resulting in apoptosis and cell death mediated by the pro-apoptotic protein Bim. The effect of apo-Scn is therefore akin to the action of cytotoxic iron chelators, which bind to iron and induce apoptosis (Richardson 2005). This hypothesis requires both functional, endogenous siderophores and an internalizing receptor, like 24p3R. We have evaluated whether bacterial siderophores, like Ent, could serve this role. Scn retains binding to FeEnt at pHs below those expected in endocytic vesicles, consistent with the primary function of Scn to sequester and clear bacterial ferric siderophores in order to halt infections. However, this would be inconsistent with functional endogenous iron transport, leaving the identity of the endogenous siderophore activity unknown (Abergel et al. 2008).

Other 'siderocalins'

While degenerately binding both Ent-like siderophores and CMBs, Scn fails to bind to many bacterial siderophores and essentially all types of fungal siderophores. Therefore, the narrow range of Scn siderophore specificity leaves many holes in this potent innate immune defense, raising the question of whether there are other siderophore-binding proteins or peptides with complementary specificities. Scn is the first non-bacterial siderophore-binding protein characterized. Therefore, our attempts to identify other non-bacterial siderophore-binders have focused on lipocalins related to Scn (Holmes et al. 2005). Typical of the lipocalin family, sequence identities rapidly plummet as the alignments move from Scn itself. However, it is possible to identify candidate, non-orthologous siderophore-binding lipocalins (siderocalins) that display hallmark positively-charged side-chains in the calyx associated with siderophore binding in Scn (Fig. 2; Holmes et al. 2005).

Murine lipocalin 12 (Lcn12) is expressed in the epididymis (Suzuki et al. 2004), but little else is known about its function; its human ortholog has only been identified through analysis of the human genome sequence (Strausberg et al. 2002). Simplistic homology modeling (based on the Scn structure) of Lcn12 reveals a triad of positively charged side-chains in the murine Lcn12 calyx (two in human Lcn12) arranged analogously to Scn (Holmes et al. 2005). The next most-related candidate siderocalins are the highly-homologous proteins chicken Ex-FABP (Descalzi Cancedda et al. 2000) and quail Q83 (Hartl et al. 2003). Ex-FABP is expressed during chicken embryo development in hypertrophic cartilage, muscle fibers and granulocytes and is also a component of egg whites. In chondrocyte and myoblast cultures, Ex-FABP expression is induced by inflammatory agents and inhibited by anti-inflammatory agents. Q83 is a protein strongly induced in v-myc-transformed avian fibroblasts, though no specific candidate ligands or functions have been proposed. The NMR structure of

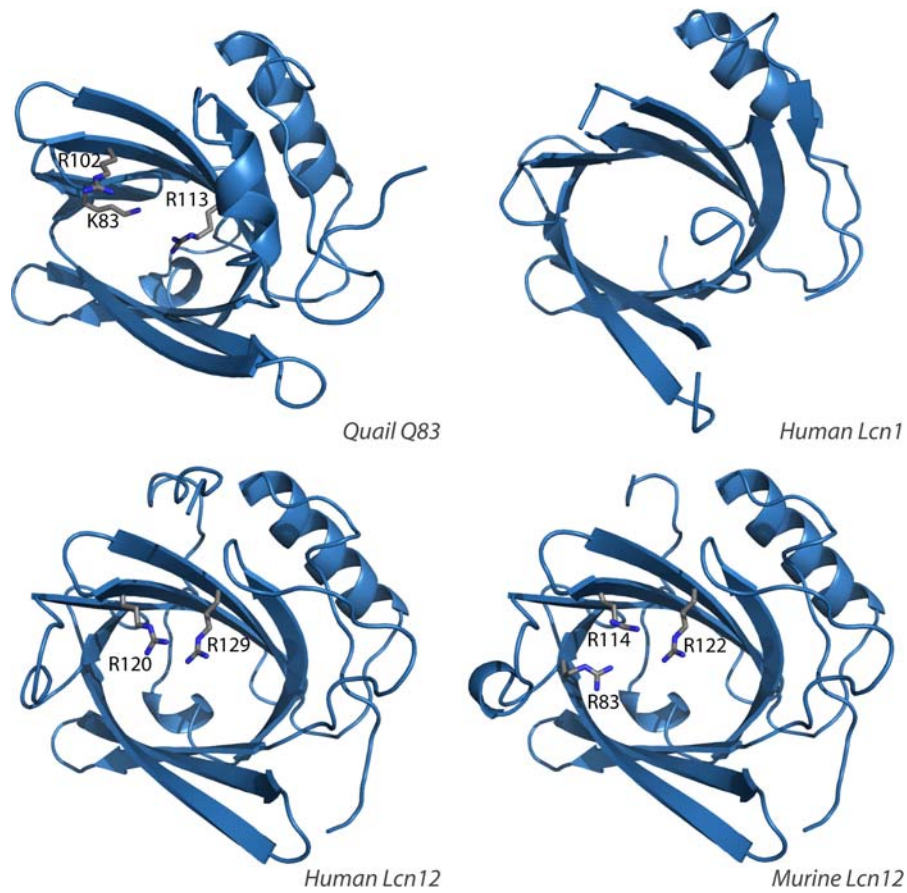

Fig. 2 Structures of other potential siderocalins. Q83 and Lcn1 were solved by NMR and X-ray crystallography, respectively, while human and murine Lcn12 structures are models based on the structure of Scn

Q83 (Hartl et al. 2003) again shows a triad of positively-charged amino acids in the calyx (conserved in Ex-FABP) very reminiscent, in arrangement and character, of the key siderophore-binding residues of Scn. This arrangement is also echoed in the calyx of an even more distantly-related lipocalin, C8γ, a well-studied member of the complement cascade, however, subsequent structural studies showed that C8γ binds peptide ligands and not siderophores (Ortlund et al. 2002; Lovelace et al. 2008).

Yet another lipocalin, Lcn1, also known as tear lipocalin, is even more distantly removed in sequence space but is also reported to function as a siderocalin. Lcn1 broadly inhibits the growth of bacteria and fungi through ferric siderophore sequestration. The nature of the recognition mechanism has yet to be elucidated, but the measured dissociation constants, in the millimolar range, are surprisingly weak (Fluckinger et al. 2004), contrasting the nanomolar dissociation constants for Scn/siderophore interactions. The structure of Lcn1 (Breustedt et al. 2005), though partially disordered, shows no immediately recognizable structural similarity to Scn in the calyx. Lcn1/siderophore complex structures also have yet to be reported. Lastly, it is possible that there are short, but structured peptides that also bind siderophores. Hepcidin, an anti-bacterial and -fungal peptide hormone 20–25 residues in length (McGrath and Rigby 2004), is involved in regulating iron homeostasis through interactions with ferroportin (Nemeth et al. 2004). The structure of Hepcidin (Hunter et al. 2002) also displays a triad of positively-charged side-chains (Arg16, Lys18 & Lys24) on its concave surface almost exactly superimposable on the positively-charged, ligand-interacting calyx residues of Scn. Therefore, the possibility exists that siderophore recognition in eukaryotic systems is much broader, with multiple implications for physiological processes, then heretofore appreciated.

References

Abergel RJ, Moore EG, Strong RK et al (2006a) Microbial evasion of the immune system: structural modifications of enterobactin impair siderocalin recognition. J Am Chem Soc 128(34):10998–10999. doi:10.1021/ja062476+

Abergel RJ, Wilson MK, Arceneaux JE et al (2006b) Anthrax pathogen evades the mammalian immune system through stealth siderophore production. Proc Natl Acad Sci USA 103(49):18499–18503. doi:10.1073/pnas.0607055103

Abergel RJ, Clifton MC, Pizarro JC et al (2008) The siderocalin/enterobactin interaction: a link between mammalian immunity and bacterial iron transport. J Am Chem Soc 130(34):11524–11534. doi:10.1021/ja803524w

Berger T, Togawa A, Duncan GS et al (2006) Lipocalin 2-deficient mice exhibit increased sensitivity to *Escherichia coli* infection but not to ischemia-reperfusion injury. Proc Natl Acad Sci USA 103(6):1834–1839. doi:10.1073/pnas.0510847103

Breustedt DA, Korndorfer IP, Redl B et al (2005) The 1.8-a crystal structure of human tear lipocalin reveals an extended branched cavity with capacity for multiple ligands. J Biol Chem 280(1):484–493

Buchanan SK, Smith BS, Venkatramani L et al (1999) Crystal structure of the outer membrane active transporter FepA from *Escherichia coli*. Nat Struct Biol 6(1):56–63. doi:10.1038/4931

Budzikiewicz H (2001) Siderophore-antibiotic conjugates used as trojan horses against *Pseudomonas aeruginosa*. Curr Top Med Chem 1(1):73–82. doi:10.2174/1568026013395524

Carniel E (2001) The Yersinia high-pathogenicity island: an iron-uptake island. Microbes Infect 3(7):561–569. doi:10.1016/S1286-4579(01)01412-5

Crosa JH (1989) Genetics and molecular biology of siderophore-mediated iron transport in bacteria. Microbiol Rev 53(4):517–530

Descalzi Cancedda F, Dozin B, Zerega B et al (2000) Ex-FABP: a fatty acid binding lipocalin developmentally regulated in chicken endochondral bone formation and myogenesis. Biochim Biophys Acta 1482(1–2):127–135

Devarajan P (2007) Neutrophil gelatinase-associated lipocalin: new paths for an old shuttle. Cancer Ther 5(B):463–470

Devireddy LR, Teodoro JG, Richard FA et al (2001) Induction of apoptosis by a secreted lipocalin that is transcriptionally regulated by IL-3 deprivation. Science 293(5531):829–834. doi:10.1126/science.1061075

Devireddy LR, Gazin C, Zhu X et al (2005) A cell-surface receptor for lipocalin 24p3 selectively mediates apoptosis and iron uptake. Cell 123(7):1293–1305. doi:10.1016/j.cell.2005.10.027

Ellison RT (1994) The effects of lactoferrin on gram-negative bacteria. Adv Exp Med Biol 357:71–90

Fang WK, Xu LY, Lu XF et al (2007) A novel alternative spliced variant of neutrophil gelatinase-associated lipocalin receptor in oesophageal carcinoma cells. Biochem J 403(2):297–303. doi:10.1042/BJ20060836

Fischbach MA, Lin H, Liu DR et al (2004) In vitro characterization of IroB, a pathogen-associated C-glycosyltransferase. Proc Natl Acad Sci USA 102(3):571–576

Fischbach MA, Lin H, Zhou L et al (2006) The pathogen-associated IroA gene cluster mediates bacterial evasion of lipocalin 2. Proc Natl Acad Sci U S A. 103(44):16502–16507

Flo TH, Smith KD, Sato S et al (2004) Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature 432(7019):917–921. doi:10.1038/nature03104

Flower DR (2000) Beyond the superfamily: the lipocalin receptors. Biochim Biophys Acta 1482(1–2):327–336

Fluckinger M, Haas H, Merschak P et al (2004) Human tear lipocalin exhibits antimicrobial activity by scavenging microbial siderophores. Antimicrob Agents Chemother 48(9):3367–3372. doi:10.1128/AAC.48.9.3367-3372.2004

Goetz DH, Willie ST, Armen RS et al (2000) Ligand preference inferred from the structure of neutrophil gelatinase associated lipocalin. Biochemistry 39(8):1935–1941. doi:10.1021/bi992215v

Goetz DH, Holmes MA, Borregaard N et al (2002) The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell 10(5):1033–1043. doi:10.1016/S1097-2765(02)00708-6

Hartl M, Matt T, Schuler W et al (2003) Cell transformation by the v-myc oncogene abrogates c-Myc/Max-mediated suppression of a C/EBP beta-dependent lipocalin gene. J Mol Biol 333(1):33–46. doi:10.1016/j.jmb.2003.08.018

Holmes MA, Paulsene W, Jide X et al (2005) Siderocalin (Lcn 2) also binds carboxymycobactins, potentially defending against mycobacterial infections through iron sequestration. Struct Camb 13(1):29–41

Horwitz LD, Sherman NA, Kong Y et al (1998) Lipophilic siderophores of *Mycobacterium tuberculosis* prevent cardiac reperfusion injury. Proc Natl Acad Sci USA 95(9):5263–5268. doi:10.1073/pnas.95.9.5263

Hunter HN, Fulton DB, Ganz T et al (2002) The solution structure of human hepcidin, a peptide hormone with antimicrobial activity that is involved in iron uptake and hereditary hemochromatosis. J Biol Chem 277(40):37597–37603. doi:10.1074/jbc.M205305200

Hvidberg V, Jacobsen C, Strong RK et al (2005) The endocytic receptor megalin binds the iron transporting neutrophil-gelatinase-associated lipocalin with high affinity and mediates its cellular uptake. FEBS Lett 579(3):773–777. doi:10.1016/j.febslet.2004.12.031

Jurado RL (1997) Iron, infections, and anemia of inflammation. Clin Infect Dis 25(4):888–895. doi:10.1086/515549

Kamezaki K, Shimoda K, Numata A et al (2003) The lipocalin 24p3, which is an essential molecule in IL-3 withdrawal-induced apoptosis, is not involved in the G-CSF withdrawal-induced apoptosis. Eur J Haematol 71(6):412–417. doi:10.1046/j.0902-4441.2003.00160.x

Kjeldsen L, Bainton DF, Sengelov H et al (1994) Identification of neutrophil gelatinase-associated lipocalin as a novel matrix protein of specific granules in human neutrophils. Blood 83(3):799–807

Kjeldsen L, Cowland JB, Borregaard N (2000) Human neutrophil gelatinase-associated lipocalin and homologous proteins in rat and mouse. Biochim Biophys Acta 1482(1–2):272–283

Klee SR, Nassif X, Kusecek B et al (2000) Molecular and biological analysis of eight genetic islands that distinguish *Neisseria meningitidis* from the closely related pathogen *Neisseria gonorrhoeae*. Infect Immun 68(4):2082–2095. doi:10.1128/IAI.68.4.2082-2095.2000

Lin H, Monaco G, Sun T et al (2005) Bcr-Abl-mediated suppression of normal hematopoiesis in leukemia. Oncogene 24(20):3246–3256. doi:10.1038/sj.onc.1208500

Lovelace LL, Chiswell B, Slade DJ et al (2008) Crystal structure of complement protein C8gamma in complex with a peptide containing the C8gamma binding site on C8alpha: implications for C8gamma ligand binding. Mol Immunol 45(3):750–756. doi:10.1016/j.molimm.2007.06.359

McGrath H Jr, Rigby PG (2004) Hepcidin: inflammation's iron curtain. Rheumatology (Oxford) 43(11):1323–1325. doi:10.1093/rheumatology/keh345

Meilinger M, Haumer M, Szakmary KA et al (1995) Removal of lactoferrin from plasma is mediated by binding to low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor and transport to endosomes. FEBS Lett 360(1):70–74. doi:10.1016/0014-5793(95)00082-K

Moss JE, Cardozo TJ, Zychlinsky A et al (1999) The selC-associated SHI-2 pathogenicity island of *Shigella flexneri*. Mol Microbiol 33(1):74–83. doi:10.1046/j.1365-2958.1999.01449.x

Muller A, Wilkinson AJ, Wilson KS et al (2006) An [{Fe(mecam)}2]6- bridge in the crystal structure of a ferric enterobactin binding protein. Angew Chem Int Ed Engl 45(31):5132–5136. doi:10.1002/anie.200601198

Neilands JB (1981) Microbial iron compounds. Annu Rev Biochem 50:715–731. doi:10.1146/annurev.bi.50.070181.003435

Neilands JB (1995) Siderophores: structure and function of microbial iron transport compounds. J Biol Chem 270(45):26723–26726

Nemeth E, Tuttle MS, Powelson J et al (2004) Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 306(5704):2090–2093. doi:10.1126/science.1104742

Ortlund E, Parker CL, Schreck SF et al (2002) Crystal structure of human complement protein C8gamma at 1.2 A resolution reveals a lipocalin fold and a distinct ligand binding site. Biochemistry 41(22):7030–7037. doi:10.1021/bi025696i

Ratledge C, Dover LG (2000) Iron metabolism in pathogenic bacteria. Annu Rev Microbiol 54:881–941. doi:10.1146/annurev.micro.54.1.881

Raymond KN, Müller G, Matzanke BF (1984) Complexation of iron by siderophores. a review of their solution and structural chemistry and biological function. In: Boschke FL (ed) Topics in current chemistry, vol 123. Springer, Berlin, pp 50–102

Richardson DR (2005) Molecular mechanisms of iron uptake by cells and the use of iron chelators for the treatment of cancer. Curr Med Chem 12(23):2711–2729. doi:10.2174/092986705774462996

Roosenberg JM II, Lin YM, Lu Y et al (2000) Studies and syntheses of siderophores, microbial iron chelators, and analogs as potential drug delivery agents. Curr Med Chem 7(2):159–197

Saito A, Pietromonaco S, Loo AK et al (1994) Complete cloning and sequencing of rat gp330/"megalin", a distinctive member of the low density lipoprotein receptor gene family. Proc Natl Acad Sci USA 91(21):9725–9729. doi:10.1073/pnas.91.21.9725

Strausberg RL, Feingold EA, Grouse LH et al (2002) Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc Natl Acad Sci USA 99(26):16899–16903. doi:10.1073/pnas.242603899

Suzuki K, Lareyre JJ, Sanchez D et al (2004) Molecular evolution of epididymal lipocalin genes localized on mouse

chromosome 2. Gene 339:49–59. doi:10.1016/j.gene.2004.06.027

Triebel S, Bläser J, Reinke H et al (1992) A 25 kDa a_2-microglobulin-related protein is a component of the 125 kDa form of human gelatinase. FEBS Lett 3:386–388. doi:10.1016/0014-5793(92)81511-J

Vokes SA, Reeves SA, Torres AG et al (1999) The aerobactin iron transport system genes in *Shigella flexneri* are present within a pathogenicity island. Mol Microbiol 33(1):63–73. doi:10.1046/j.1365-2958.1999.01448.x

Warner PJ, Williams PH, Bindereif A et al (1981) ColV plasmid-specific aerobactin synthesis by invasive strains of *Escherichia coli*. Infect Immun 33(2):540–545

Weinberg ED (1984) Iron withholding: a defense against infection and neoplasia. Physiol Rev 64(1):65–102

Willnow TE, Goldstein JL, Orth K et al (1992) Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance. J Biol Chem 267(36):26172–26180

Winkelmann G (2002) Microbial siderophore-mediated transport. Biochem Soc Trans 30(4):691–696. doi:10.1042/BST0300691

Xu S, Venge P (2000) Lipocalins as biochemical markers of disease. Biochim Biophys Acta 1482(1–2):298–307

Yang J, Goetz D, Li JY et al (2002) An iron delivery pathway mediated by a lipocalin. Mol Cell 10(5):1045–1056. doi:10.1016/S1097-2765(02)00710-4

Yang J, Mori K, Li JY et al (2003) Iron, lipocalin, and kidney epithelia. Am J Physiol Renal Physiol 285(1):F9–F18

Zhou D, Hardt WD, Galan JE (1999) *Salmonella typhimurium* encodes a putative iron transport system within the centisome 63 pathogenicity island. Infect Immun 67(4):1974–1981