FEBS Letters 354 (1994) 7–11

FEBS 14691

Minireview

The lipocalin protein family: a role in cell regulation

Darren R. Flower*

Department of Physical Chemistry, Fisons Plc, Pharmaceuticals Division, R&D Laboratories, Bakewell Rd., Loughborough, Leicestershire, LE11 0RH, UK

Received 7 July 1994; revised version received 19 September 1994

Abstract The lipocalins, a large, diverse, but relatively poorly understood family of small extracellular proteins, are characterized by the ability to bind small hydrophobic molecules, such as retinol, and by their binding to specific cell surface receptors. These general properties suggest such proteins as appropriate transporters transferring biologically hazardous molecules in a safe and controlled manner between cells. Moreover, many lipocalins have been implicated in the regulation of cell homeostasis: apolipoprotein D, quiescence specific protein, purpurin, α-1-microglobulin, and NGAL. This combination of direct and indirect evidence suggests that the lipocalin protein family may be involved, in a quite general way, in the mediation of cell regulation and that many presently functionless family members might act in this way.

Key words: Lipocalin; Cell regulation; Ligand binding; Cell binding; Apolipoprotein D

1. Introduction

The lipocalin protein family is a large and diverse group of small extracellular proteins [1–3]. A sequence alignment for a sample of the family is shown in Fig. 1 and various representations of the lipocalin fold in Fig. 2. Available data indicates that their action, if not their sole function, is the binding of small, principally hydrophobic molecules [4]. A summary of the properties of lipocalins mentioned in the text is given in Table 1. By analogy with well understood family members such as retinol binding protein (RBP), the lipocalins have been classified as transport proteins [4,5]. It has also become increasingly clear that many lipocalins bind to specific cell surface receptors [3,6,7]. Nevertheless, in general, the family remains poorly understood; functional data, where available, is often either contentious or rudimentary. Much remains to be discovered about this intriguing family of proteins.

However, one recent hypothesis to emerge in the literature speculates that a role in cell regulation may be a common function of the lipocalin family. The following reviews both direct and indirect evidence linking the ability of lipocalins to both transport lipophilic molecules and bind to cellular receptors with the observation that many family members are involved in mediating cell homeostasis.

2. Ligand binding by the lipocalin family

It is well known that many lipocalins bind retinoids: RBP, purpurin, β-lactoglobulin, α-1-microglobulin, and C8γ all bind retinol with high affinity [7]. Rat epididymal secretory protein has been shown to bind retinoic acid, a known regulator of gene expression and morphogen, with high affinity and great specificity [8]. This protein is believed to be important in the process of sperm maturation, able to bind to the plasma membrane of spermatozoa; it may be that this function is related to their ability to bind retinoic acid. Although the role of retinal in vision is well known, it is only relatively recently that the influence of both natural and synthetic retinoids on the regulation of cell growth, development, and survival has become clear [9,10]. Though the mechanisms by which retinoids exert their effects are not yet fully understood, they are generally considered to act, like steroid hormones, via specific nuclear receptors, such as the now well characterized retinoic acid receptors or RARs [11].

Molecules such as retinoids and steroids, with their many and potentially pernicious physical and biological properties, might prove unduly hazardous if permitted to diffuse freely; protein transporters, such as RBP, afford the means to transfer such molecules safely between cells and in a way which may allow their effects to be modulated or controlled [11].

3. Many lipocalins act in cell regulation

It might be reasoned that extracellular proteins, such as certain lipocalins, capable of transporting insoluble retinoid or steroid molecules might fulfil a role in mediating their effects in vivo. Such a conjecture is itself enough to suggest a role for lipocalins, or similar proteins, in cell regulation. However, an examination of the literature reveals that many lipocalins can already be shown to function in this way.

Apart from its inhibitory effects on the immune system, α-1-microglobulin, a retinol-binding lipocalin, is also known to exhibit mitogenic properties. Studies on mouse lymphocytes have shown both an increase in thymidine uptake by B and T cells and the proliferation of B cells caused by α-1-microglobulin [12]. The protein has also been reported to induce cell division in both human and mouse lymphocytes; processes which are themselves under complex regulation by other components of the immune system [13].

Purpurin, a lipocalin which binds retinol and is localized in neural cells of the retina, is also believed to function in the

*Corresponding author. Fax: (44) (509) 266 738;
E-mail: FLOWER_DR@FISONSPHARM.CO.UK

Abbreviations: NGAL, neutrophil gelatinase associated lipocalin; QSP, quiescence specific protein; RAR, retinoic acid receptors; RBP, retinol binding protein.

SSDI 0014-5793(94)01078-1

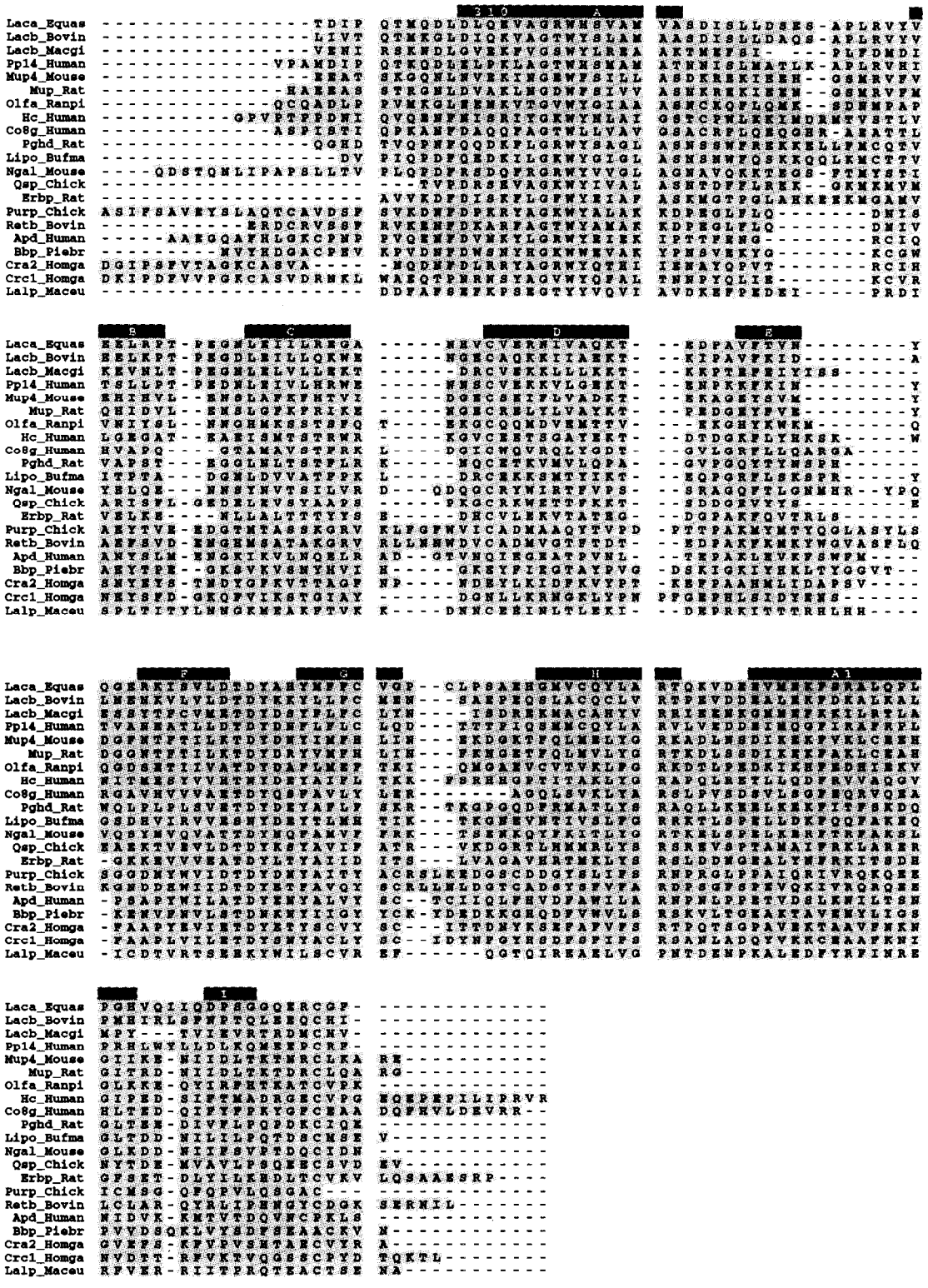

Fig. 1. Lipocalin multiple alignment. A sequence alignment of 21 representative members of the lipocalin protein family based on careful comparison of known crystallographic structures [3,8]. All sequences are drawn from version 23.0 of the OWL composite sequence database [35]. Concensus secondary structure elements are shown at the top of the alignment blocks: β-strands are shown in dark grey (labelled A–I) and helices in lighter grey (labelled 310 and A1), respectively.

control of cell differentiation, adhesion, and survival. Experiments on cultured nerve cells from chick retina have shown the protein to facilitate both cell–substratum and cell–cell adhesion in the absence of other factors, and to promote the survival of cell populations [14,15].

Mouse 24p3 protein is the product of a single gene, 24p3, and

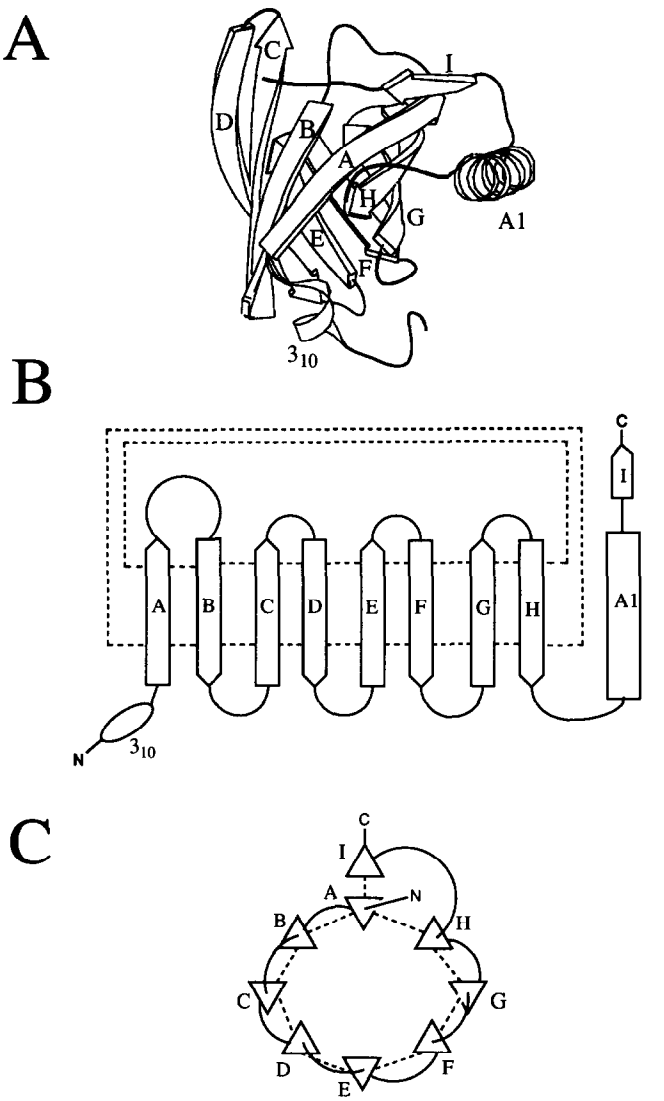

Fig. 2. Representations of the lipocalin fold. (a) Ribbon drawing of a representative lipocalin. The structure of a representative member of the lipoclain family, mouse major urinary protein [31], is shown as a ribbon drawing [36]. β-strands are labelled A–I. The 3_{10} helix before strand A and the α-helix (A1) beyond strand H are also labbelled. (b) Schematic of the lipocalin fold. The nine β-strands of the antiparallel closed sheet of mouse major urinary protein [31] are shown as arrows and labelled A–I. The N-terminal 3_{10} helix and C-terminal α-helix (A1) are also labelled. The hydrogen bonded connection of two strands is indicated by a pair of dotted lines between them. Connecting loops are shown as solid lines. (c) The lipocalin barrel. β-Strands are shown as triangles, after the diagrammatic style of Sternberg and Thornton [37], with triangles pointing downwards indicating a strand direction into the plane of the paper and those pointing upwards indicating a strand direction out of the plane of the paper. The hydrogen-bonded connectedness of strands is represented by a connecting dotted line. Labelling is as before. Connecting loops are shown as solid lines. For simplicity, helices are not shown.

is notable because of the 7- to 10-fold increase in its expression in cultured mouse kidney cells infected by SV40 or other viruses [16]. Its membership of the lipocalin family has been determined [17], as has its near identity to α-2-microglobulin-related-protein. More recently it has been shown that the human homologue of 24p3 is covalently associated with gelatinase (type IV collagenase), thus the protein was named NGAL (neutrophil gelatinase associated lipocalin), although most of the protein is secreted in an uncomplexed form [18]. These authors propose for NGAL a regulatory role on the action of gelatinase, however in another study [19], 24p3 was identified as a major secretory product of lipopolysaccharide stimulated macrophages and it was suggested that the protein might function in the defense against infection. Thus the function of the protein remains unclear; however, Hraba-Renevey et al. considered the over-expression of 24p3 of great importance in virus-induced mitogenesis [16]. It has been suggested that mouse 24p3 protein, and by analogy rat α-2-microglobulin-related-protein and human NGAL, may function in the control of cell regulation, in normal and/or transformed cells, through the transport of retinol, retinoic acid, or other lipophilic molecules [17].

Another lipocalin, quiescence specific protein (QSP), is also involved in cell regulation: dividing, sub-confluent, or transformed cells do not synthesize this protein, whilst confluent cells, including those stimulated by hormones or growth factors, do express the protein, often in a density dependent manner [20,21]. Experiments show that expression of QSP is a specific consequence of the dense confluent state rather than of growth arrest in general, such as might result from cell starvation. These properties seem consistent with a molecule which may function to stabilize or maintain populations of mature cells. The more recent results of Nakano and Graf seem to contradict this: they show that rapidly growing v-myb transformed promyelocytes highly express QSP [22]. However, v-Myb induces an abnormal pattern of gene expression which may abrogate a suppression mechanism present in other growing cells.

Apart from its well documented role in cholesterol metabolism [23], recent studies have shown apolipoprotein D to function in cell regulation. Spreyer et al. [24] and Boyles et al. [25] both report increased apolipoprotein D secretion in regenerating nerve tissue. Spreyer et al. report a 40-fold increase in apolipoprotein D levels in the extracellular space of regenerating crushed rat neurons. Expression of the protein was limited to endoneurial fibroblasts. Boyles et al. report a 500-fold increase in apolipoprotein D expression in the regenerating peripheral nerve of rat, rabbits, and marmoset monkeys. In the central nervous system they find apolipoprotein D produced by astrocytes and oligodendrocytes, while in the peripheral nervous system it is synthesized by neurolemmal cells or fibroblasts. This correlation between apolipoprotein D expression and nerve regeneration is consistent with the protein having an important role to play in the repair process.

More recently, apolipoprotein D has been positively identified as gross cystic disease fluid protein (GCDFP)-24 [26]. This protein was previously shown to be a progesterone/pregnenolone binding protein and to constitute over half the protein component of cyst fluid. Gross cystic breast disease is typified by large, potentially premalignant lesions. Prompted by this observation, Simard et al. [27] studied the effects of steroid hormones on the proliferation of cultured human prostate cancer cells and observed a correspondence between the steroid-induced secretion of apolipoprotein D and the inhibition of cell growth and also note a higher concentration of apolipoprotein D in cultured cells that are well differentiated. In a related study, Provost et al. investigated the secretion of apolipoprotein D in cultured human diploid fibroblasts [28]. They found that little or no apolipoprotein D was expressed by cells in

Table 1
Properties of members of the lipocalin protein family

Protein	M.W.	Number of residues	Oligomeric state	Glycosylation	Receptor	Ligand
Retinol binding protein	21.0	183	M	−	+	Retinol
Purpurin	20.0	175	?	?	?	Retinol
Retinoic acid binding protein	18.5	166	?	?	?	Retinoic acid
α-1 microglobulin	31.0	188	M + C	+	+	Retinol
C8γ	22.0	182	C	−	?	Retinol
NGAL	25.0	179	M + C	+	?	?
Quiescience specific protein	21.0	161	?	?	?	?
Apolipoprotein D	29.0 − 32.0	169	C	+	?	Pregnenolone
β-lactoglobulin	18.0	162	M or D	−	+	Retinol + many
MUP	17.8	161	D	−	+	Pheromones
OBP	37.0 − 40.0	159	D	?	+	Odorants

Certain properties of lipocalins mentioned in the text are summarised as a table. MW is given in kDa. For oligomeric state, M stands for monomeric, D for dimeric and C for for part of a complex. Where a property, such as glycosylation, has been shown to be present experimentally this is indicated by a +, shown to be absent by a −, or is not known by a ?. Data are taken from references cited in the text.

sparse culture, whilst confluent populations of quiescent cells did express the protein. Cells those growth is arrested by serum starvation also express the protein. Provost et al. also note that levels of expression rise as cells reach confluence and hypothesize a role for apolipoprotein D in the control of cell homeostasis.

4. A common function of the lipocalin protein family

The findings presented above suggest that many lipocalins may be involved in mediating cell homeostasis. 24p3 and α-1-microglobulin are mitogenic, acting to enhance cell growth and proliferation. Conversely, apolipoprotein D, QSP, and purpurin act to stabilize or maintain mature cell populations. They may act to inhibit division and maintain or enhance cell competence in mature cell populations, perhaps through the suppression of apoptosis. There is experimental evidence to show that many lipocalins (such as odorant binding protein [6], RBP [29], α-1-microglobulin [30], mouse major urinary protein [31], C8γ [32], and β-lg [33]) have specific cell surface receptors and may be internalized by receptor mediated endocytosis; for example it has been shown that, in the liver, the retinol-RBP complex is taken up by hepatocytes via receptor mediated endocytosis [34]. The internal targeting of a lipocalin protein and/or its retinoid, steroid, or other ligand may allow its delivery to nuclear receptors which, with a concomitant biological response, might provide a possible general mechanism by which the lipocalins elicit these effects. The need to transfer such molecules in a controlled manner between cells is a powerful rationalization of the involvement of such protein carriers.

Although the hypothesis reviewed here is somewhat speculative, will not be valid for all member of the lipocalin family, and is not yet proven, it is nonetheless a compelling conjecture. Although the role of certain family members seems clear, such as the enzyme prostaglandin D synthase [2], many lipocalins, particularly those identified by sequencing, have yet to be ascribed a function; it may be that many of these will also play some part in cell regulation. There is a need to expand and extend the experimental characterization of function and action in the family and, by directing this effort towards their putative roles in cell homeostasis, future work will verify or refute the validity of this hypothesis. The lipocalins may yet demonstrate an involvement in many fundamental biological processes.

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