



LUND UNIVERSITY

Signalling and trafficking of the cysteinyl leukotriene receptors in intestinal epithelial cells

Parhamifar, Ladan

2009

[Link to publication](#)

Citation for published version (APA):

Parhamifar, L. (2009). *Signalling and trafficking of the cysteinyl leukotriene receptors in intestinal epithelial cells*. [Doctoral Thesis (compilation), Cell Pathology, Malmö]. Department of Laboratory Medicine, Lund University.

Total number of authors:

1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

**From the Department of Laboratory Medicine,
Division of Cell Pathology,
Lund University, Malmö, Sweden**

**Signalling and trafficking of the cysteinyl leukotriene receptors
in intestinal epithelial cells**

Ladan Parhamifar



LUND
UNIVERSITY

Academic dissertation


By due permission of the Faculty of Medicine, Lund University, Sweden
To be defended at the main lecture hall, Pathology building, Malmö
University Hospital, Malmö on Friday January 23rd, 2009 at 09.15 for
the degree of Doctor of Philosophy, Faculty of Medicine.

Faculty Opponent: Professor Catherine Godson, College of Life Sciences, Conway
Institute Belfield, Dublin 4, Ireland

Organization LUND UNIVERSITY	Document name DOCTORAL DISSERTATION	
	Date of issue 090123	
	Sponsoring organization	
Author(s) Ladan Parhamifar		
Title and subtitle Signalling and trafficking of the cysteinyl leukotriene receptors in intestinal epithelial cells		
<p>Abstract</p> <p>Inflammation is a response to injury or pathogen invasion. A large proportion of the body's immune system is centred in the gastrointestinal tract (GI). Prolonged inflammatory conditions of the GI have been suggested to increase the risk for developing colon cancer.</p> <p>The cysteinyl leukotrienes (CysLTs), LTC₄, LTD₄ and LTE₄ are inflammatory mediators that can bind to four known receptors, two of which are CysLT₁R and CysLT₂R. Inhibitors of the CysLT₁R are currently used in the clinic as asthma medication. LTD₄ has been shown to induce cell proliferation, migration and survival of non-transformed intestinal epithelial cells (Int407) via the CysLT₁R. These mechanisms are often used by cancer cells to survive and spread. Furthermore, increased expression of the CysLT₁R in colon cancer patient material is correlated with a poorer survival prognosis. Conversely, increased expression of the CysLT₂R correlates with a better survival prognosis. The aim of this thesis was therefore to explore the signalling and trafficking of the CysLT₁R and CysLT₂R. Our results demonstrate that LTD₄ via the CysLT₁R can activate the enzyme, cPLA₂-alpha. This enzyme releases arachidonic acid, the precursor of CysLTs, from the cell membranes upon activation. One major regulatory mechanism of GPCR signalling is their internalization from the cell membrane upon activation. We demonstrate how LTD₄ mainly internalizes the CysLT₁R and accumulates this receptor at the nuclear membrane and that LTC₄ internalizes both the CysLT₁R and CysLT₂R. This information is valuable in developing potential drug targets against CysLT₁ and CysLT₂ in cancer and inflammation.</p>		
Key words: Cysteinyl leukotriene receptors, cytosolic phospholipase A2, trafficking, inflammation, colon cancer		
Classification system and/or index terms (if any):		
Supplementary bibliographical information:		Language English
ISSN and key title: 1652-8220		ISBN 978-91-86059-95-8
Recipient's notes	Number of pages 139	Price
	Security classification	

Distribution by (name and address)

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature 

Date 081215

For my parents

Nothing shocks me. I'm a scientist.
Harrison Ford (1942 -), as Indiana Jones

The highest result of education is tolerance.
Helen Keller (1880 - 1968), 'Optimism,'
1903

TABLE OF CONTENTS

LIST OF PAPERS	5
LIST OF ABBREVIATIONS	6
INTRODUCTION	7
BACKGROUND	8
1. The intestinal epithelium	8
2. Inflammatory Bowel Disease (IBD)	11
3. Colon cancer	13
4. Colon cancer and Inflammatory Bowel Disease	14
Highlighted signalling molecules	17
5. Phospholipase A ₂ (PLA ₂)	17
6. Eicosanoids	22
Cyclooxygenase (COX) and Prostaglandins (PG)	23
Leukotrienes	24
7. Cysteinyl Leukotriene receptors (CysLTRs)	27
8. Mitogen activated protein kinases (MAPKs)	32
9. Nuclear factor kappa B (NFκB)	33
10. Protein Kinase C (PKC)	35
11. G-protein coupled receptors (GPCRs)	36
12. GPCR dimerization and other GPCR interacting proteins	39
13. GPCR desensitization	43
14. Internalisation of GPCRs	44
- Internalisation of GPCRs and caveolin	44
- GPCR internalisation and clathrin	45
- GPCR internalisation and β-arrestin	46
- GPCR internalisation and Rab proteins	48
15. Nuclear GPCR	50
THE PRESENT INVESTIGATION	52
RESULTS AND DISCUSSION Papers I, II and III	53
SUMMARY	59
POPULÄRVETENSKAPLIG SAMMANFATTNING	60
ACKNOWLEDGEMENTS	62
REFERENCES	65

LIST OF PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

- I. **Parhamifar L, Jeppsson B, Sjölander A.**
Activation of cPLA2 is required for leukotriene D4-induced proliferation in CRC cells. *Carcinogenesis*. 2005 Nov; 26(11): 1988-98.
- II. **Parhamifar, L, Yudina, Y, Vilhardt, F and Sjölander, A**
Nuclear trafficking and signalling of the G-protein coupled receptor, CysLT₁ in intestinal epithelial cells (*Manuscript*)
- III. **Parhamifar L, Vilhardt F, Mögerlin M and Sjölander A**
Co-dependent localization and ligand specific trafficking of Cysteinyl Leukotriene receptors (*Manuscript*)

Reprints were made with permission from publisher:
©2005 by the Oxford University Press

LIST OF ABBREVIATIONS

AA	arachidonic acid
ADP	adenosine 5'-diphosphate
APC	adenomatous polyposis coli
ATP	adenosine 5'-triphosphate
BSA	bovine serum albumin
cAMP	Cyclic adenosine mono phosphate
CD	Crohn's Disease
COX	Cyclooxygenase
CRC	Colorectal cancer
CREB	cAMP response element binding
CysLT	Cysteinyl leukotriene
CysLT ₁ R	Cysteinyl Leukotriene 1 receptor
CysLT ₂ R	Cysteinyl Leukotriene 2 receptor
ER	Endoplasmatic reticulum
FAP	Familial adenomatous polyposis
FLAP	Five Lipoxygenase Activating Protein
GDP	Guanosine diphosphate
GI	Gastrointestinal
GPCR	G-protein coupled receptor
HNPCC	Hereditary non-polypus colorectal cancer
IBD	Inflammatory bowel disease
LO	Lipoxygenase
LT	Leukotriene
MAPK	Mitogen-activated protein kinase
NF κ B	Nuclear Factor κ B
NLS	Nuclear localization signal
PBS-T	Phosphate buffer saline – Tween
PG	Prostaglandin
PI	Phosphoinositol
PK	Protein kinase
PL	Phospholipase
PLA ₂	Phospholipase A ₂
PPAR- γ	Peroxisome Proliferator Activated Receptor- γ
PTX	Pertussis toxin
RAMP	Receptor activity modifying protein
RGS	Regulator of G-protein signalling
TCF	T cell factor
TNF- α	Tumour necrosis factor α
TRAF	TNF receptor associated factor
UC	Ulcerative colitis

INTRODUCTION

Inflammatory responses are designated to protect the host from injuries and / or foreign invasion. The colon houses 300-500 species of bacteria, responsible for producing vitamins and other important products from food transversing the digestive system. The bacteria are prevented from entering the rest of the body by a single layer of intestinal epithelial cells, this barrier functions as the first line of defence against foreign invaders. Due to its large number of bacteria the colon maintains a low level of inflammation. An imbalance of this inflammatory state has been suggested as a possible cause of inflammatory bowel disease (IBD). Prolonged inflammatory conditions arise when the inflammatory response fails to resolve the injury, leading to continuous recruitment of inflammatory cells and inflammatory mediators to the site of injury. This continuous inflammatory response leads to tissue damage, such as the disruption of the epithelial barrier. These responses are thought to be the pre disposition to colorectal cancer.

Leukotrienes are lipid mediators produced by inflammatory cells and implicated in various inflammatory responses. High levels of leukotrienes are found in patients suffering from IBD. In intestinal epithelial cells Leukotriene D₄ (LTD₄) can induce cell growth, increase cell survival and induce cell migration by regulating various enzymes and proteins shown to be involved in cancer progression and development. LTD₄ elicits these effects through the Cysteinyl leukotriene receptor 1 (CysLT₁R). However, leukotrienes can bind to four different G-protein coupled receptors (GPCRs), the work in this thesis focuses on two of the best characterized CysLTRs; the CysLT₁R and the CysLT₂R. The balance between the two first receptors has been suggested to play a role in the prognosis of colorectal cancer patients. Increased CysLT₁ expression correlates with a poor survival rate, whereas increased CysLT₂ correlates with a better prognosis.

With this in mind, the aim of the work in this thesis has been to investigate the cysteinyl leukotriene receptors CysLT₁ and ₂ signalling and their regulation in particular the trafficking.

BACKGROUND

1. The intestinal epithelium

The small and large intestine are the last part of the gastrointestinal (GI) tract. In the small intestine food components such as fats, proteins and carbohydrates are broken down. The nutrient components are then transferred to the bloodstream. The large intestine absorbs the remaining water and removes the waste products (1,2).

The intestinal epithelium consists of a single layer of epithelial cells(3). They provide a barrier for the indigestible components and the gut microflora, keeping these within the lumen of the intestine.

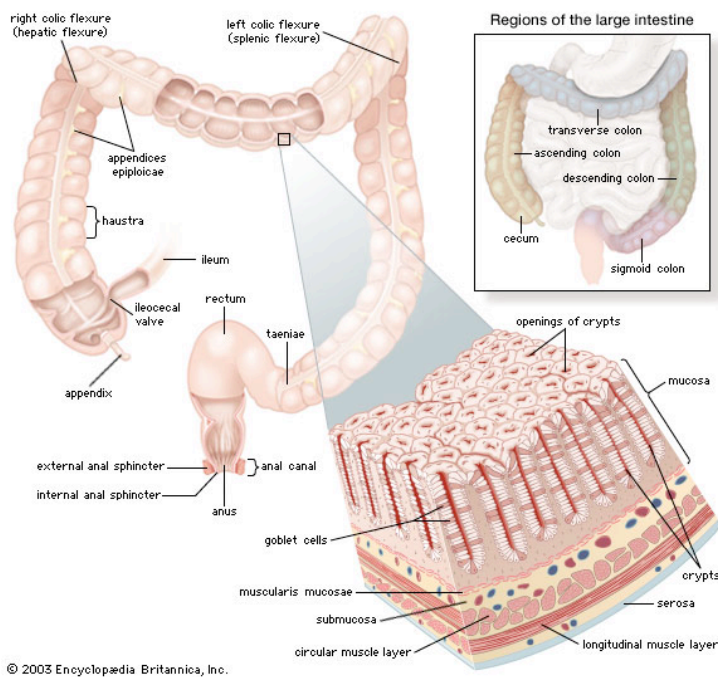


Figure 1. The large intestine.

In the lining of the mammalian intestine different classes of epithelial cells termed goblet, endocrine and paneth cells can be found. Paneth cells are only found in the small intestine and constitute one of the differences between the small and large intestine. Whilst the goblet cells secrete protective mucins, the

endocrine cells secrete various gut hormones and the paneth cells secrete anti-bacterial proteins (4-6). The majority of cells are the absorptive cells are termed enterocytes (small intestine) or colonocytes (large intestine), and are positioned on a basement membrane, where the intra epithelial lymphocytes can be found migrating in and out of the intestinal epithelium.

The small intestine consists of villi and crypts whilst the large intestine consists only of crypts (3,5) (Fig.2). The villi in the small intestine mainly consist of enterocytes. At the bottom of the crypts in the small intestine reside the stem cells and the paneth cells. The paneth cells occupy the three first positions of the crypt whilst the stem cells are positioned fourth. New cells that are made at the bottom of the crypts by the stem cells, migrate up to the villi while dividing and differentiating into specialised intestinal epithelial cells (Fig.2) (4,5).

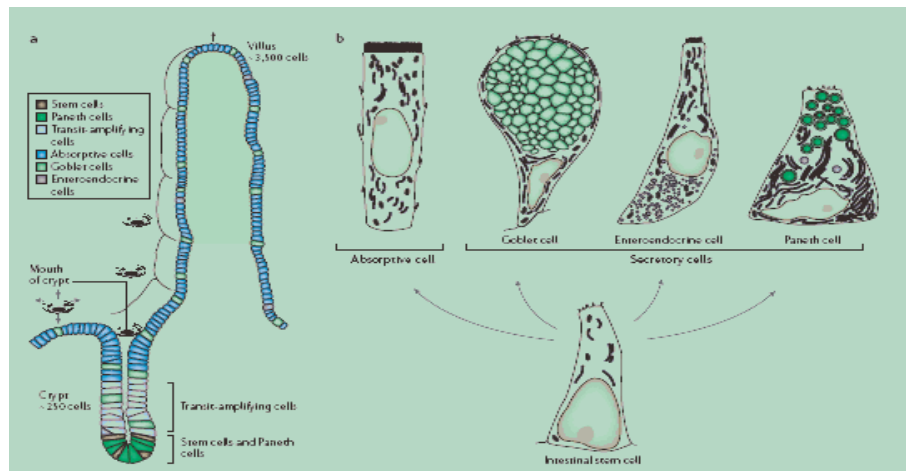


Figure2. Cells of the small intestine (Crosnier, Nature Publishing group, 2006)

Cells in transit divide three to four times before they differentiate into mature cells. Once the cells have reached the top of the villi they undergo apoptosis and slough off into the lumen (6). In the colon, proliferative cells

occupy two thirds of the crypt and the top third consists of the differentiated cells.

Renewal of the epithelium in the small intestine takes place every 4-6 days whilst turnover of the colonic epithelium can take 3-8 days. This high regenerative potential is what makes the intestine so fascinating but it is also its achilles heel as dys-regulation of this system has dangerous outcomes (4,5). Colonisation of the gastrointestinal tract begins after birth and the intestinal tract ends up with between 300-500 different bacterial species. The stomach and the small intestine only have a few species of bacteria but in the colon the microflora outnumber host eukaryotic cells by 10 fold (7). The colonisation of the colon is affected by the environment and the food that the baby first eats. The first bacteria to colonise the colon can decide the gene expression patterns in the epithelial cells and create a more favourable environment for themselves and inhibit the growth of other bacteria. The normal microflora consists of mainly bacteria a few viruses and fungi (8). This normal intestinal flora is essential for the function of the intestine, however some of these bacteria are potentially pathogenic and can cause infections if the epithelial barrier has been disrupted (9). Therefore, due to its commensal bacteria the colon always produces a low level of inflammation (immune surveillance) to be able to distinguish between pathogens and “normal” commensal bacteria. This is accomplished by the existence of intra epithelial immune cells such as macrophages and dendritic cells as well as the Gastrointestinal Associated Lymphoid Tissue (GALT) are lymphoid like sections, important in facilitating immune surveillance along the GI tract, which consist immune cells, such as dendritic cells, T and B lymphocytes. In the small intestine the GALT is known as the Peyer's patches. M cells are specialised epithelial cells that are located on top of the Peyer's Patches. The M cells have loose intercellular binding, allowing the intestinal microflora easy access to the underlying lymphoid tissue. Dendritic cells present in the Payers Patches process the microbes they come into contact with and present

them to the T lymphocytes. In other GALT along the GI tract, the dendritic cells can sample antigens directly from the intestinal lumen, or receive microbes which have transversed the intestinal epithelial cells (10,11).

Specialised membrane and intracellular receptors, the Toll-like receptors (TLRs) and the NOD or NOD-like receptors (NLRs), respectively, are responsible for recognition of the microbial antigens also known as pathogen associated molecular patterns (PAMPS). These PAMPS induce tolerance towards the normal commensal bacteria through stimulating signalling pathways through the TLRs, NODs and NLRs. However dysregulation of the tolerogenic signalling pathways, for example through receptor mutation can lead to the development of prolonged or chronic inflammation (12-14).

Inflammation is a response to injury or pathogen invasion. Under normal conditions the initiation of inflammation also includes signals for the termination and resolution of inflammation (15-18). Prolonged inflammatory conditions arise when the stimulus for the acute state of inflammation is not terminated (8,19) leading to the development of inflammatory bowel disease (IBD) (20).

2. Inflammatory bowel disease

IBD is an umbrella term for several conditions (collagenous colitis, Lymphocytic colitis, Ischemic colitis, Behcet's colitis, infective colitis, intermediate colitis, diversion colitis) however there are two major conditions, ulcerative colitis (UC) and Crohn's disease (CD) (21). Even though it is difficult to compare studies geographically due to methodological differences it is suggested that the incidence of IBD is higher in developed countries(22). Patients suffering from IBD often more vulnerable to other chronic inflammatory conditions. Currently three dispositions are hypothesised to be required for the development of IBD: 1) genetic factors, for example mutations to proteins involved in immune surveillance; 2) luminal factors such

as the microflora and food antigens, for example a low fibre diet is linked to the development of IBD; and 3) environmental factors for example smoking increases the risk for the development of CD (21).

The two major conditions of IBD have similarities but also major differences. CD can affect any part of the GI tract, displaying typical discontinuous transmural inflammation and is associated with a T helper cell -1 response, over-production of IL-12/IL-23 and IFN- γ . Research has also identified mutations in the NOD2 protein as being a cause for CD in a sub-population of patients (23,24). UC on the other hand primarily affects the colon and extending rectum with the inflammation continuous and confined to the mucosa and sub mucosa, is mainly a T helper cell -2 response, mediated by an over-production of IL-13. Interestingly smoking increases the risk for CD whilst providing a protective factor against UC (7,14).

Despite the differences in the two different conditions, UC and CD also share many common features. In both cases inflammation causes mucosal destruction, which leads to loss of mucosal barrier and absorptive function. These two events have similar symptoms, such as abdominal pain and bloody diarrhoea. Furthermore both UC and CD have been associated with alterations in STAT-3 and NKX2-3. Various mouse models demonstrate an important role for the commensal bacteria in IBD (9,14,23,25).

Current non-invasive treatments for IBD mainly centre around the inhibition of the dysregulated inflammatory process. Commonly 5-aminosalicylates are used to dampen the inflammation (26) either alone or in combination with corticosteroids, which are more immunosuppressive drugs (27). However, tolerance can develop, therefore in the case of Crohn's disease several chimeric antibodies have been developed which bind to tumour necrosis factor- α (TNF- α), a major cytokine and driving factor behind IBD. Patients receiving this treatment can also unfortunately relapse (28). Thus,

ultimately, the only cure to date, (which is only possible in ulcerative colitis) is surgical resection (29).

3. Colon cancer

Colorectal cancer (CRC) is the third most common form of cancer in the western world (World health organization 2007). most common malignancy of the GI, with the overall survival rate lower than 50% (30). CRC is the second leading cause of cancer related deaths in the United States. In Sweden 5000 new cases of CRC are diagnosed every year (Swedish National board of Health and Disease, 1998). Most, if not all, malignant colorectal tumours (carcinomas) arise from pre-existing benign tumours (adenomas) (Sugarbaker et al., 1985). The rate of deaths caused by CRC can be and has been reduced by early detection with colonoscopy and testing of stool samples (31). Various factors have been suggested to increase the risk for developing CRC such as; cancer elsewhere in the body, colorectal polyps, CD, family history of CRC and UC (32). Low fibre, high fat diets and smoking have also been suggested to be risk factors for developing CRC. Genetic predisposition increases the chance of developing CRC, however the majority of cases occur sporadically (33). The loss of tumour suppressor genes which drive the sporadic adenoma carcinoma have been proposed to be a contributing factor (33). The three major carcinogenic pathways that lead to sporadic CRC and colitis associated CRC are; chromosomal instability, microsatellite instability and hyper methylation. Hyper methylation of promoter regions of cell cycle(32), DNA repair and cell adhesion genes, leads to silencing of these genes and thereby promotes CRC. In sporadic CRC the dysplastic precursor is a discrete focus of neoplasia, the adenomatous polyp that can be removed. The majority of spontaneous colorectal cancers (about 85%) are the results of chromosomal instability, the rest are due to microsatellite instability (MSI) or failure of the mismatch repair system (34,35). Both of these cases lead to the accumulation of mutations which

eventually could become carcinogenic. Chromosomal instability leads to abnormal chromosomal segregation and abnormal DNA content and eventually loss of chromosomal material and loss of function of tumour suppressor genes such as adenomatous polyposis coli (APC) and p53 (a key regulator of the cell cycle)(36). This results in activation of proto-oncogenes, such as c-myc and cyclin D1. In microsatellite instability, the loss of DNA mismatch repair genes leads to loss of colonic homeostasis and neoplastic growth. Mutations in the RAS/RAF pathway, the p53 pathway, and several other genes/pathways drive tumour progression towards malignancy and metastasis (32).

Genetic predisposition to colorectal cancer depends on the presence or absence of polyps. Patients with hereditary non-polyposis colorectal cancer have an inherited mutation in an allele of a DNA mismatch repair gene(37). Patients with familial adenomatous polyposis (FAP) have a mutation in the APC gene. This pathway however is altered in approximately 95% of CRCs. FAP patients are characterised by the early onset of adenomatous colorectal polyps in the large intestine (38).

4. Colon cancer and Inflammatory Bowel Disease

The link between cancer and inflammation, has long been suggested and dates back to Virchow in 1863. IBD is one of three high risk factors for developing CRC, as patients with UC develop colorectal cancer 10 years earlier than patients with sporadic colorectal cancer (39). The other two high risk factors are having a genetic predisposition namely familial adenomatous polyposis (FAP) and hereditary non-polypus colorectal cancer (HNPCC) (32,39,40).

Dysplasia in IBD can be flat, multifocal, localised or diffuse, with the duration and surface affected by colitis increasing the risk for developing CRC (32). In sporadic CRC the cancer progresses from adenoma to carcinoma, whilst in IBD induced CRC, the progression is from inflammation to dysplasia

and then carcinoma (41). Colitis associated CRC affects individuals at a young age and has a more proximal distribution in the colon.

There is a lot of evidence to support the link between IBD and CRC. Tumours have been observed to behave very similarly to wounds that fail to heal, since they surround themselves with a milieu similar to that of a chronic inflammatory state, which for example gives rise to the production of matrix metalloproteinases, tissue degradation, leukocyte infiltration and angiogenesis. Many proteins have been observed to be highly up-regulated in both IBD tissue and CRC. Possibly one of the most important proteins to be highlighted are the cyclo-oxygenase (COX) enzymes, in particular COX-2. This inducible enzyme is over expressed in 90% of CRC (42). The induction of expression is seen early in acute inflammation, and high expression levels have been shown to be present in UC and CD. COX-2 produces a prostaglandin (PG) precursor (a member of the eicosanoid family of lipid mediators), with the main PG in the intestine being PGE₂. Accordingly, the membrane receptor for PGE₂ along with this PG are also over-expressed in IBD and CRC (43).

A further example linking CRC and IBD include, the observation that p53 mutations which are very common in CRC have been found in the inflamed mucosa from UC patients who did not have cancer. Also, methylation is a way of controlling gene transcription, with many proto-oncogenes being methylated in CRC. Several genes that precede dysplasia have been found methylated in UC (32,41).

Reactive oxygen species (ROS) and nitrogen oxygen species (NOS) are highly reactive molecules that are increased in the inflamed mucosa and remain elevated in colonic neoplasms (Fig. 3). ROS and NOS have also been

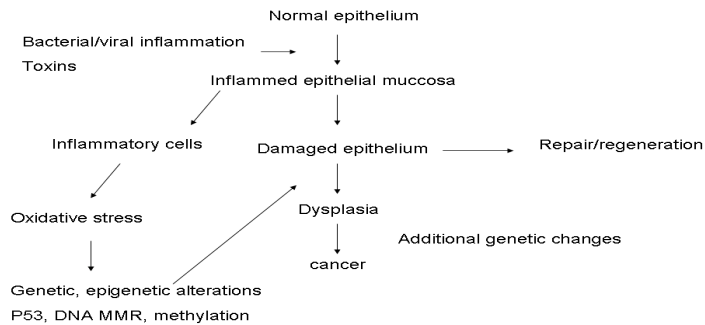


Figure 3. Cascade of colitis associated CRC

implicated in the activation of Nuclear Factor κ B (NF κ B) (44). NF κ B is a significant player in inflammation. This family of transcription factors are known to be highly activated in many chronic inflammatory diseases and cancers, and have been proposed as one of the transforming factors between IBD and CRC (45). NF κ B is a key regulator in inflammation and is also present in many solid tumours. It contributes to tumour formation by providing anti-apoptotic and survival signals to the epithelial cell. One way of triggering NF κ B induced survival pathway in the GI tract is through the commensal bacteria (41). The commensal bacteria activate the appropriate TLR which in turn can activate NF κ B, which can then affect the genes involved in survival. Under normal conditions this pathway is protective against colitis induced damage by blocking inflammation against commensal bacteria. However if the epithelial barrier is damaged these inflammatory signals reach the underlying inflammatory cells that can not distinguish between commensal bacteria and pathogens. This would then lead to a continuous inflammatory response to the commensal bacteria and an over stimulation of the survival signals leading to transformation (46). Another way of colitis induced transformation via this pathway might be when an insult or injury leads to mutate epithelial cells. These cells would then multiply due to the survival signals and lead to neoplasia. NF κ B has been shown to stimulate NO and COX-2 to generate pro-inflammatory prostanooids that in turn can give

eicosanoids (discussed below) are a family of potent mediators involved in many different conditions, such as inflammation and cancer (47,51,52). The lysophospholipids can in turn be converted into lysophosphatidic acid platelet activating factor (PAF) which also is a potent signalling molecule implicated in for example inflammation (53,54). There are currently 15 known groups and subgroups of Phospholipase A₂s (PLA₂) (55-57). These groups are separated by characteristics of the PLA₂s such as their; structure, requirement for calcium and size. This divides the PLA₂s in to five major groups; the sPLA₂s (secreted), the cPLA₂s (cytosolic), the iPLA₂s (calcium independent), the PAF acetyl hydrolases and the lysosomal PLA₂s. The sPLA₂ group have a low molecular weight (14-19kDa) and are calcium dependent (55,56). The different members of the sPLA₂ family have been implicated in the digestion of phospholipids in the stomach (58,59), rheumatoid arthritis (60), hydrolyzing membranes of gram negative bacteria (anti-microbial agents) (61,62), atherosclerosis and IBD (63-65). It has also been demonstrated to decrease the size and development of tumours in mice. Increased expression in mice, has been correlated with a better survival, however this data contradicts the results detected in humans, where the enzyme is up-regulated and may be contributing to the progression of CRC (66). The exact role of sPLA₂ in eicosanoid production remains unexplained, but might depend on cell type and stimuli.

The iPLA₂ group have molecular weights ranging from 28-146 kDa. Whilst they do not have fatty acid chain specificity, they are thought of as house keeping genes but also been shown to be involved in apoptosis by delaying or inhibiting cell death induced by death receptors (55).

The PAF acetylhydrolase group (also shown to be anti-inflammatory) do not require calcium for their activity, they range from 26-45 kDa, and some are secreted and some are intracellular but they all hydrolyse the acetyl group from the sn-2 position of PAF (67-69). The PAF acetylhydrolase IIA is secreted and can also hydrolyse short fatty acids from the sn-2 position, it can

bind to both the high density lipoprotein and low density lipoprotein cholesterol molecules (69,70) and not surprisingly has been implicated in cardiovascular disease (71). Lysosomal PLA₂ is the most recently discovered being found in bovine brain and does not require calcium for its activity (72).

The cPLA₂s are serine esterases, they have a molecular weight of 61-114 kDa, and all but cPLA₂- γ /IVC require calcium. The different cPLA₂s have different specificities for fatty acids in the sn-2 position with cPLA₂- α /IVA being specific for AA containing phospholipids whilst cPLA₂- β and γ have very little specificity for AA (73,74). A major role of cPLA- α is in the production of eicosanoids. cPLA₂- α is an 85kDa enzyme, isolated from neutrophils and platelets (75,76). It is composed of a C2 calcium binding domain, that is required for its membrane translocation, α and β -hydrolase domains with the catalytic site, and the serine 727 and 505 that are phosphorylated by the Mitogen Activated Protein Kinases (MAPK) and MAPK activated proteins. The phosphorylation of the cPLA₂s is important for the lipid enzyme interaction. (55,77). Even though cPLA₂- α requires calcium for its translocation and membrane binding, phosphoinositol diphosphate (PIP₂) has been shown to activate the protein in a calcium independent manner. The PIP₂ binding domain is in the catalytic site but has been shown to require the C2 domain (78). The importance of this protein in inflammation has been demonstrated in knock-out mice, which have post-ischemic injury, acute lung injury and significantly decreased allergic responses. Furthermore, the first study in this thesis demonstrates that the pro-inflammatory mediator leukotriene D₄ (LTD₄) can activate and increase expression of cPLA₂- α (79).

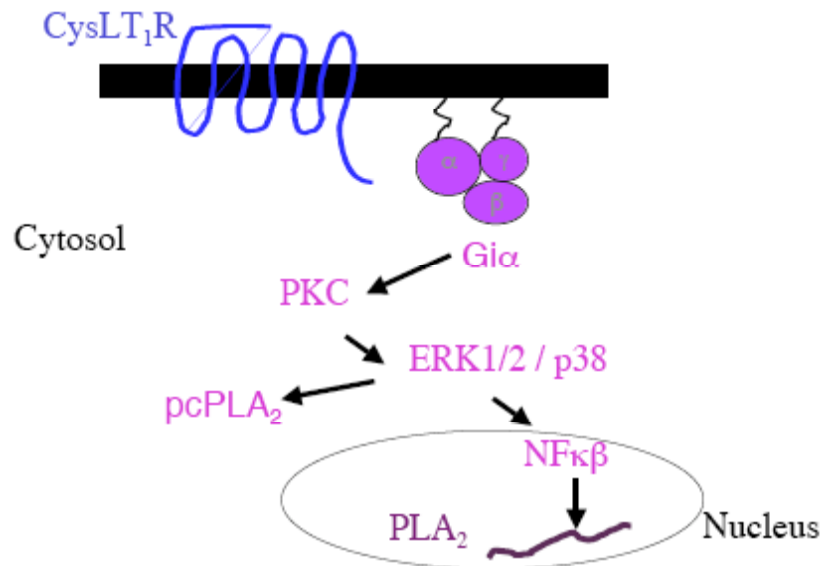


Figure 5. LTD₄ mediated cPLA₂α activation and transcription.

A variety of mediators have been shown to activate cPLA₂ such as; cytokines, chemokines, growth factors, histamine and thrombin. The promoter region of cPLA₂ has been shown to contain potential binding sites for amongst others NFκB, AP1 and AP-2 (73,80). Over-expression of cPLA₂-α has been demonstrated in a variety of cancers such as cholangiocarcinoma and non small lung squamous carcinoma (81-83). The expression and activity of cPLA₂-α in CRC however is a more complicated affair. Early studies of the expression and activation of cPLA₂ in colon tumours demonstrated elevated expression and function compared to non-transformed material (84). Another group demonstrated similar results using primary CRCs. In the study in this thesis, cPLA₂ levels were increased in colon cancer cells compared to non-transformed cells from the small intestine. Tumour samples were also examined, and elevated levels of cPLA₂-α were detected as compared to non-transformed tissue. Another group has also reported that cPLA₂-α and COX-2 were elevated in colorectal adenocarcinomas (85). Here over-expression of cPLA₂-α was found in superficial stromal cells and correlated with an

increased COX-2 expression and high micro vessel density (85), suggesting a regulatory role for cPLA₂- α in COX-2 induced angiogenesis. This group analysed another set of CRC specimens and found elevated levels of cPLA₂- α , but could not correlate them to any specific stage, histological pattern or microsatellite instability (86). They could however, yet again show a correlation between cPLA₂- α and COX-2 in half of the samples (86). A mouse model using APC min mice produced conflicting results concerning cPLA₂- α in colon cancer (87). This group found a decrease in the expression of cPLA₂- α in five human colorectal cancers. The same study also reported that despite low levels of cPLA₂- α , COX-2 expression was elevated. These studies were further explored (88) and 13/27 samples showed elevation of COX-2 and in 11 of these 13 samples cPLA₂- α was reported absent. The authors suggest that the low levels of cPLA₂- α and high levels of COX-2, probably disrupts the levels of AA (high levels of AA induces apoptosis) leading to escape from apoptosis. In another study by the same group, AOM induced colon cancer, was investigated in cPLA₂- α knockout mice, which showed increased tumour size and number in the colon (89). The authors also observed enhanced tumorigenesis despite low levels of COX-2 in their model (89). Further APC min mouse studies have shown that in the small intestine, cPLA₂ is the dominant source of AA for COX-2 (90) and that deletion of cPLA₂ reduces tumour size and/or number even in the colon (90-92). The conflicting results can also be seen in the regulation of cPLA₂- α by AA metabolites. In a study using prostate cancer cells (PC-3), AA was added directly to the cells. This led to a transcriptional activation of cPLA₂- α and COX-2 in a dose dependent manner, and an increase of PGE₂ production. Interestingly the authors show that a COX-2 inhibitor blocked the up-regulation of cPLA₂- α and COX-2, suggesting that PGE₂ can up-regulate cPLA₂- α (100). Again there is a contradictory study performed in mouse lung fibroblasts, where the addition of PGs could only up-regulate COX-2 but not cPLA₂- α (101). These data suggest that the regulation of cPLA₂- α to some extent might be tissue specific.

Surprisingly, a very recent study reported the findings of cPLA₂- α deficiency in a 45 year old white American male of Italian decent. The study showed that this man suffered from multiple ulcers in the small intestine and concluded that cPLA₂- α plays an important role in intestinal homeostasis and integrity. Furthermore he had platelet dysfunction and globally decreased eicosanoid production. The findings suggest that the production of eicosanoids in platelet and leukocytes is almost entirely dependent on cPLA₂- α . However, the study also showed that the outcome of several colonoscopies performed, were normal despite the absence of cPLA₂- α . Moreover, the authors reported that their results indicate that cPLA₂- α provides AA for virtually all biosynthesis of eicosanoids by platelets, leukocytes and the cells from which the CysLTs are derived (93).

6. Eicosanoid

The eicosanoids are biologically active fatty acid metabolites with a twenty carbon chain backbone (94). Their name originates from the Greek word eicosa, which means twenty. Eicosanoid is the umbrella term for metabolites of AA. AA is found in membrane phospholipid bilayers. Once released by PLA₂, AA can be further metabolized by the COXs, LOs, cytochrome p450 or non-enzymatically. When oxygenated by COX, AA gives rise to PGs, prostacyclin or thromboxanes. Oxygenation by LOs gives rise to LTs, lipoxins, hepoxillins or monohydroxy fatty acids (49,95-98). Cytochrome p450 metabolism of AA forms epoxy fatty acids or dihydroxy fatty acids. Non-enzymatic metabolism of AA produces isoprostanes or isoleukotrienes(99-101).

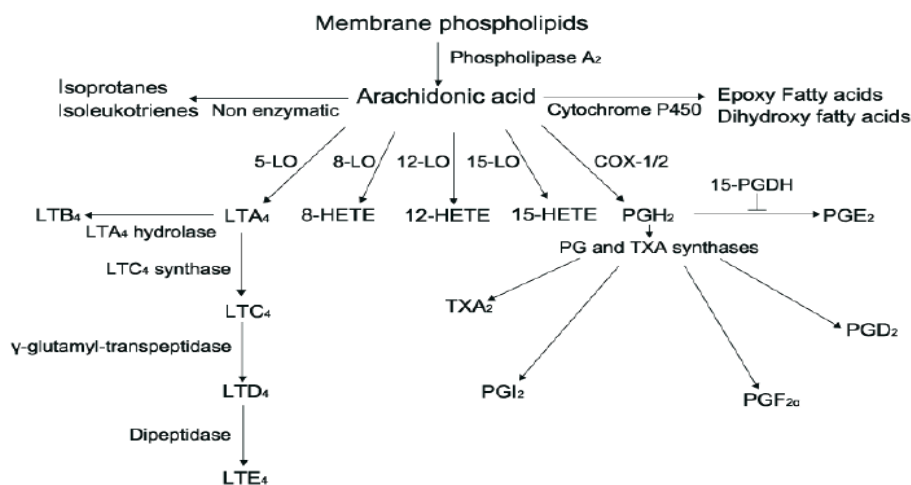


Figure6. Eicosanoid production.

Cyclooxygenases and Prostaglandins

The COX enzymes initiate the synthesis of PGs, and can be divided into two established isoforms; COX-1 and COX-2. The enzymes share a 60% homology (95,102,103) and consist of three domains; an EGF domain, a membrane binding domain and a catalytic domain that controls both its COX and peroxidase activity (104). COX-1 is a house keeping enzyme and produces basal levels of PGs. COX-1 is ubiquitously expressed and needed for homeostasis, for example in maintaining the epithelial barrier (105,106). It should however be mentioned that COX-1 has been shown to play a role in ovarian cancer (107). COX-2 is an inducible enzyme and its expression can be mediated by many inflammatory mediators (108). It has also been shown to be over-expressed in IBD, CRC (90%) and many cancers. Inhibitors of the COXs have been used for over a 100 years (109-111). Non-steroidal anti-inflammatory drugs (NSAIDs; aspirin, indometacin, and ibuprofen) block both COX-1 and COX-2. Specific COX-2 inhibitors have been shown to reduce tumour size and colonic polyps (42). These positive effects are however

clouded by the cardiovascular side effects which were seen with a particular COX-2 specific inhibitor in a subset of patients being treated for Rheumatoid arthritis, leading to it being pulled from the market (105). However, other COX-2 specific inhibitors are still used in treating inflammatory conditions although with greater caution (112,113). With this in mind, there is also data demonstrating that COX-2 also is involved in anti-inflammatory and pro-resolution pathways, and perhaps COX-2 inhibitors are not optimal in this regard either (104,114). AA is metabolized by COXs to the PG precursor PGH₂. PGH₂ is then further metabolized to PGI₂, PGE₂, PGD₂ or PGF₂α via specific PG synthases (42). The PGs were discovered in 1935 and have since then been implicated in various signalling pathways (115). Similarly to COX-2, PGE₂ has been implicated in IBD and CRC. PGE₂ has been shown to increase cell survival, proliferation and migration and be directly involved in angiogenesis (116-118). The effects of PGs are exerted through G-protein coupled receptors termed EP1-4. These receptors have been shown to be expressed at the plasma membrane as well as the nuclear membrane (118-120).

Leukotrienes

The leukotrienes (LTs) are named after their structure and the cells that they were discovered in. Leuko means from white blood cells and trienes stands for the three double bonds in their structure (121). They are inflammatory mediators derived from AA through the action of 5-LO (121). AA is a 20-carbon polyunsaturated fatty acid derived from food or from the conversion of the essential fatty acid linoleic acid (94). It is usually found esterified in the membrane phospholipids and is released by PLA₂ (94). Glucocorticosteroids, common treatments for inflammatory conditions, act by inhibiting the transcription of PLA₂ (122). 5-LO which belongs to a family of LOs comprising of 5-, 8-, 12- and 15-LO (123,124) is responsible for initial step in the production of the LTs converting AA to LTA₄.

The unstable LTA₄ produced by 5-LO is rapidly converted to LTB₄ or the CysLTs; LTC₄, LTD₄ and LTE₄ (52,125,126). All LTs exert their effects through G-protein Coupled Receptors (GPCRs). The CysLT receptors are termed CysLT₁, CysLT₂, CysLTE₄ and GPR17 (127,128) and have been shown to be involved in various inflammatory conditions such as asthma, IBD, cancer, and atherosclerosis (129). The CysLTs are produced upon immune and inflammatory stimuli (50,130).

LTD₄ has been shown to induce cell proliferation, survival (131,132) and cell migration (133), as well as up-regulating several anti-apoptotic and survival proteins such as BCL-2, β -catenin(134) and COX-2, in intestinal epithelial cells.(135). LTD₄ induces proliferation in epithelial cells via two distinct pathways. One pathway is mediated by Protein Kinase C (PKC) - ϵ , MEK1/2 and ERK1/2 leading to p90RSK activation; the other pathway is mediated through PKC- α and CREB (131,132). Furthermore LTD₄ in this thesis has been shown to induce cell proliferation through cPLA₂- α (79). The same study also shows that the activation of cPLA₂- α is mediated through CysLT₁R, a pertussis toxin (PTX) sensitive G-protein, PKC, p38, ERK1/2 and calcium. Correspondingly, in renal mesangial cells, LTD₄ induced proliferation requires activation of ERK1/2, p38, phosphatidylinositol 3-kinase (PI3-K) and PKC (136) and in mast cells, LTD₄ also induces proliferation (137). In addition to cPLA₂- α activation, LTD₄ is shown to induce expression of cPLA₂- α in a similar fashion to the proliferation, which suggested a role for NF κ B. In another study LTD₄ induced CysLT₁R signalling in HEK293 cells stably expressing CysLT₁ lead to IL-8 expression in a NF κ B and AP1 dependent manner (138). Recent data from our group also confirmed the short term activation of NF κ B by LTD₄ (unpublished data), although long term stimulation with LTD₄ does not seem to activate NF κ B (139). Interestingly, in contrast to LTD₄ induction of proliferation, LTC₄ has been demonstrated to promote differentiation in CRC cells (140) and have a chemotactic effect (141).

In addition to the cellular effects named above, the LTs also affect several other cells for example, LTB₄ is a chemoattractant as well as being involved in promoting adhesion and rolling of leukocytes. The actions of LTB₄ are distinct from those of the CysLTs. LTB₄ recruits mainly leukocytes to the site of inflammation and subsequently triggers their degranulation (142,143). The CysLTs are also chemoattractants but mainly for eosinophils and induce leakage and mucus secretion (144,145). They are also known to be potent inducers of smooth muscle contraction that is involved in bronchi constriction and have been shown to be 10,000 times more potent than histamine (146). Due to this, several drugs directed against the effect of the CysLTs have been developed (144,145).

The LTs are predominantly produced in blood cells as suggested by the name, but can be synthesized by other cells, lacking the whole biosynthetic machinery required, through transcellular biosynthesis (147). An example of this is the interaction of endothelial cells and neutrophils. Neutrophils produce LTA₄ and secrete it. Endothelial cells which lack sufficient amounts of 5-LO but express LTC₄ synthase then take up the LTA₄ and convert it to LTC₄ (147).

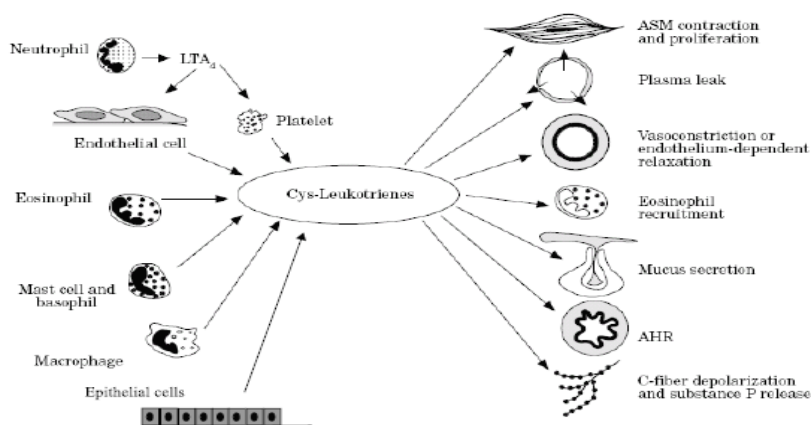


Figure7. Cells producing leukotrienes and the effects of leukotrienes. Modified from Nicosia et al. ,2001

In epithelial cells, LTD₄ can induce the production of CysLTs in an autocrine fashion (148) as well as inducing production of PGs via COX-2 induction (149). The cell death induced by COX-2 inhibitors in intestinal epithelial cells could be escaped by the cells through LTD₄ stimulation (150). LTD₄ has also been shown to induce a rapid calcium response in intestinal epithelial cells as well as CRC cells (151,152). This calcium response has been demonstrated to be mediated through a PTX sensitive G-protein, leading to cAMP release and PKA activation (153,154). In resting cells 5-LO is located in the cytoplasm but when activated it translocates to the nuclear membrane, and with the help of Five Lipoxygenase Activating Protein (FLAP) converts the released AA to LTA₄ (155).

7. Cysteinyl leukotriene receptors

The LT receptors are GPCRs that were cloned and characterised late in the 1990's (156-160). The first receptor to be cloned was the first LTB₄ receptor, BLT₁R (161) and only three years later a second LTB₄ receptor; the BLT₂R was cloned (162). BLT₁ is the high affinity receptor for LTB₄ and BLT₂ is the low affinity receptor. The CysLTs have so far been shown to exert their effects mainly through two receptors; the CysLT₁R and CysLT₂R (156-160). However, recent discoveries reveal the existence of two new receptors; the orphan receptor GPR17 (163) which has higher affinity for LTC₄ than LTD₄ and a potential CysLTE₄R (127) with a high affinity for LTE₄. GPR17 has also been shown to bind uracil nucleotides (124). The CysLTs bind CysLT₁R with the following different affinities; LTD₄<LTC₄<LTE₄. LTD₄ binds CysLT₁R with the highest affinity (EC₅₀ = 0.4nM) whilst LTC₄ binds CysLT₁R with a 350 times lower affinity (EC₅₀ = 21nM). LTE₄ is a less potent agonist and has been shown to sometimes act as a partial agonist (130,159,160,164). LTD₄ and LTC₄ have equal affinity for CysLT₂R and LTE₄ has an even lower affinity (158).



Figure 8. The Cysteinyl leukotriene receptor 1.

The CysLT₁R is encoded on the X chromosomes while the CysLT₂R is encoded by chromosome 13 and the two receptors share a mere 38% homology. Both receptors were cloned almost at the same time in 1999. CysLT₁R has a molecular mass of 38 kDa in its monomeric form (159,160) yet is often detected oligomerized (165). The CysLT₁R has four potential N-glycosylation sites and many potential PKC and PKA phosphorylation sites in its third intracellular loop and C-terminus (160). It is known to be involved in various signalling pathways and cellular functions such as; MAPK activation, calcium increases, actin reorganization, proliferation, survival, differentiation, and migration (79,166,167). In the last 20 years, an array of various inhibitors targeting the CysLT₁R, have been developed. Montelukast, Zafirlukast, Pranlukast (IC₅₀ = 1.8–4.9nM) are some of the examples of inhibitors for the CysLT₁R (159).

The CysLT₂R is a protein of 346 amino acids and migrates at 49-58 kDa (168). It also contains 4 N-glycosylation sites mainly in its N-terminus and PKC and PKA phosphorylation sites in its third internal loop and C terminal tail (158). Binding studies in Cos-7 cells with this receptor reveal the presence of two binding sites, one low and one high for LTD₄. In another study the affinity for LTC₄ for CysLT₂R was detected in the nano molar range (156-158) whilst all of the CysLT₁R selective antagonists were demonstrated to be

inactive in a competition assay. Bay u9773 which has been suggested as a CysLT₂R antagonist but was later shown to also be a partial agonist of both receptors (156,158). HEK-293 cells transfected with the CysLT₂R also demonstrated that LTC₄ and LTD₄ are equipotent agonists but that LTE₄ is a partial agonist (156-158).

The distribution of CysLT₁R and CysLT₂R receptors is mostly overlapping but high expression of CysLT₂R has been detected in heart, brain and the adrenal glands (128,156-158). Intestinal epithelial and CRC cells express both receptors (140,169,170) and the expression of each receptor in CRC cells varies, although in general CysLT₂R seems to be down regulated in CRC patient material whilst CysLT₁R seems to be up-regulated in comparison to the surrounding non-transformed tissue (140,169,171).

The recently discovered GPR17 has been shown to have a higher affinity for LTC₄ than LTD₄ (163). The idea that additional receptors may exist came from a few unexplainable data such as: LTC₄ or LTE₄ failing to activate CysLT₁R or CysLT₂R and also from the fact that the response from LTC₄ was more potent than that of LTD₄, which does not fit with the affinity studies performed on the CysLT₁R and CysLT₂R (172-175). In 2001 a report described that CysLT₁R and another yet unidentified receptor, although not the CysLT₂R, both responded to uracil nucleotides and CysLTs. Later another study investigated the orphan receptor GPR17 and found that it responds to both CysLTs and extracellular nucleotides (163). Interestingly two CysLT₁R inhibitors (pranlukast and montelukast) could block the LTD₄ induced response of GPR17 and as could two antagonists of the P2YR family (163). GPR17 binds the CysLTs in the nano molar range whilst the potency range for the extracellular nucleotides is in the micro molar range (UDP-galactose = UDP > UDP-glucose). All the responses from GPR17 have been demonstrated to be PTX sensitive and both types of agonists induce a calcium response and inhibition of forskolin induced cAMP formation. GPR17 is highly expressed in tissue undergoing ischemic injury such as heart, brain and kidney

suggesting its involvement in these conditions. Infact knock-down or inhibition of GPR17 by either CysLT₁R or P2YR antagonists protects against brain damage (163). A recent study preformed in CysLT₁R / CysLT₂R knockout mice showed a LTE₄ induced vascular permeability response. This led the authors to hypothesize that yet another CysLT receptor existed with high affinity for LTE₄. The authors continued by showing that in mice lacking CysLT₁R and CysLT₂R, LTC₄ and LTD₄ could induce vascular leak but to a lesser extent than LTE₄. The effect of LTE₄ was 64-fold greater in deficient mice than the wild type mice. The response from all three ligands in double deficient mice could be blocked by pranlukast, a CysLT₁R inhibitor. The authors concluded from this data the existence of a new CysLT receptor, termed CysLTE₄R (127).

CysLT₁R internalisation has been studied in Cos-7 cells transfected with the receptor and stimulated with LTD₄. In this study CysLT₁R was internalised in a PKC dependent manner, where PKC phosphorylated the CysLT₁R in the C-terminus, and that lack of this phosphorylation impaired receptor internalisation. Furthermore, the same study identified that the internalisation of CysLT₁R is β -arrestin independent (176). In contrast to this, a separate study preformed in U937 human macrophage like cells, also transfected with CysLT₁R provided evidence that the LTD₄ induced desensitization of CysLT₁R is not PKC dependent but GRK2 dependent. However, the PKC phosphorylation of CysLT₁R was shown to be important in the extracellular nucleotide induced CysLT₁R desensitisation, although this does not lead to its internalisation, rather it shows a rapid recovery of the receptor (165). Data from the second project in this thesis demonstrates a clathrin, EPS15, β -arrestin-2, Rab5 mediated internalisation of CysLT₁R upon LTD₄ stimulation.

Various signalling pathways have been established for CysLT₁R in a variety of cells and tissues. The signalling of CysLT₂R has been less clarified due to the lack of specific inhibitors. Contrasting reports regarding the expression and signalling of CysLT₁ and CysLT₂ in HUVEC cells have been

reported. Some reports have identified the expression of CysLT₁R in these cells coupled with a calcium response from both LTD₄ and LTC₄ in these cells that can be blocked by Pobilukast. Other groups have stated that these cells mainly express the CysLT₂R and have shown that this expression is highly up regulated by IL-4 and also that CysLT₂R is responsible for the calcium signals exerted by LTD₄, LTC₄ and Bay u9773 (177-179). Additionally, LTD₄ induced CysLT₂R activation in HUVEC cells has been shown to up-regulate 37 early inducible genes, which included for example early growth response (EGR) and COX-2 (180). In a study with mast cells the authors use MK 571, a selective CysLT₁R inhibitor, and show that the IL-8 production by the IL-4 primed and CysLT or UDP stimulated cells was not inhibited, but that Bay u9773 was able to evoke IL-8 production. Interestingly, they show that inhibition of p38 blocked the IL-8 production, suggesting a role for p38 downstream of CysLT₂R (181). Studies in human coronary artery stimulated with LTC₄ show a calcium response which is unable to be blocked by CysLT₁R antagonists (141). In oxygen/glucose deprived PC12 cells, cell death was shown to may be mediated by CysLT₂R since Bay u9773 inhibited this effect and CysLT₁R seemed to reduce cell death (182).

A novel mechanism for receptor regulation was described for CysLT₂R, which has been suggested to negatively regulate the plasma membrane expression and signalling of CysLT₁R in mast cells. Moreover the authors gave evidence for dimerization of CysLT₁R and CysLT₂R (183). Studies performed by J.A Boyce and co-workers recently reported by Rovati et. al, suggest CysLT₁R/ CysLT₂R dimers at the nuclear membrane of mast cells (128).

Our group have published data indicating the existence of both receptors at the nuclear envelope in intestinal epithelial cells and CRC cells (140,169). Furthermore, CysLT₁R can localise to the outer nuclear membrane and translocate from the plasma membrane to the nuclear membrane upon stimulation with LTD₄ (169). The COX-2 gene up-regulation mediated by

LTD₄ requires the internalisation of the receptor and we hypothesise that this signal is mediated through the translocation of CysLT₁R to the nuclear membrane. In contrast to COX-2, Cyclin D1 mRNA up-regulation and the LTD₄ induced ERK1/2 phosphorylation are increased when receptor internalisation is blocked. This leads us to hypothesise that the plasma membrane CysLT₁R can continue to signal and respond to LTD₄ when its internalisation is blocked and can therefore increase the signals mediated from the plasma membrane.

8. Mitogen activated protein kinases

Mitogen activated protein kinases (MAPKs) are serine threonine kinases involved in a variety of signalling cascades mediating cellular responses such as proliferation, apoptosis, differentiation and survival. MAPKs need to be phosphorylated for full activation and the pathways they regulate are sometimes cross linked or dependent on each other (184). In contrast, MAPKs are negatively regulated by de-phosphorylation. There are seven families of MAPKs and they can be divided into the classical MAPKs (ERK1/2, p38, JNK and ERK5) and the atypical MAPKs (ERK3, 4, 7 and NLK) (185,186). Phosphorylation of MAPKs occurs in their catalytic loop and these phosphorylations are regulated by upstream MEKs/MAPKK. MEKs in turn are regulated by MAPKKK, such as Raf. In summary different stimuli activate MAPKKK that in turn activate MAPKK and MAPKs (187). Raf can be activated by a family of small monomeric GTPases such as Ras. Ras has been shown to be mutated in 30% of all cancers and B-Raf is mutated in 60% of all malignant melanomas (188). A classical pathway is that of mitogens or growth factors activating PKC which in turn can lead to the activation of Ras and Raf. Raf then activates MEK1,2 which phosphorylate and activate ERK1/2. ERK1/2 activation leads to the activation of various transcription factors such as NFκB, inducing cell survival and proliferation (186,189-192). The p38 MAPK family consists of four isoforms; α, β, γ and ε (189). They are activated

by stress and inflammatory cytokines. Most stimuli that activate p38 can also activate JNK. MEK6 can activate all p38 isoforms whilst MEK3 activates α and β (193). p38 has been shown to be critical for normal immune and inflammatory responses (194). Mitogens or growth factors can also activate (via MEK1-4 or MEK3, 6) the p38 MAPK which has been shown to be involved in differentiation, apoptosis but also cell growth and survival. Downstream targets of p38 include p53 and NF κ B (45,195,196). Both p38 and ERK1/2 have been implicated in IBD. ERK1/2 has been demonstrated to be over expressed and also overly active in IBD (186). Many cytokines can activate ERK1/2 such as IL-21 and IL-1(186,189). p38 and JNK families have however been the centre of investigation concerning MAPKs and IBD (197,198). Inhibition of p38 and JNK have been shown to reduce cytokine production (199).

9. NF- κ B

The NF- κ B family are transcription factors involved in many inflammatory pathways. They have been suggested to play a major role in IBD and CRC (41,45). Epithelial cells isolated from IBD patients show increased expression of NF κ B. Furthermore, constitutively active NF κ B has been found in human cancer cell lines as well as in tumours from patients suffering from CRC, breast cancer, leukemia and prostate cancer. Suppression of NF κ B in these tumour samples inhibits proliferation, causes cell cycle arrest and leads to apoptosis (200). Many cytokines and growth factors such as TNF- α or EGF mediate their proliferative effects through activation of NF κ B. It has been suggested that NF κ B is one of the major links between inflammation and cancer (200). This family consists of five members, p50, p52, p65 (RelA), c-

Rel and RelB, which share an N-terminal Rel homology domain (RHD) responsible for DNA binding, nuclear targeting and homo and heterodimerisation. NF- κ B dimers bind to various pro-inflammatory, anti-apoptotic and cell cycle regulating genes. It is however only the p65, c-Rel and RelB that are able to directly activate transcription of target genes. P50 and p52 need to dimerize with p65, c-Rel and RelB, for gene activation whilst homo-dimerization of p50 and p52 can suppress transcription. NF κ B was first discovered in inflammatory cells but has since been shown to be expressed in various cell types (45,200,201). When activated NF κ B translocates to the nucleus, whilst in its inactive state NF- κ B dimers are bound to specific inhibitory proteins I κ Bs, in the cytoplasm. The I κ Bs can be divided into I κ B- α , β and γ . They keep NF- κ B in its inactive state by masking its nuclear localization sequence. I κ B- α has also been shown to translocate to the nucleus, inhibit NF κ B from binding to its target genes and export NF κ B out of the nucleus. However upon stimuli, the I κ Bs are phosphorylated, ubiquitinated and sent for degradation. This then release NF κ B to translocate to the nucleus and bind to its target genes (201). The activation of NF κ B can be divided into the classical and the alternative pathways. The classical pathway can be activated by for example pro-inflammatory cytokines such as TNF- α or IL-1, bacterial LPS and viruses. These signals lead to activation of IKK (I κ B kinase complex) which is composed of IKK- α and β as well as the regulatory NEMO. The catalytic subunits (IKK α and β) phosphorylate serine residues in the I κ B proteins. In the classical pathway this phosphorylation is mediated mainly through the IKK- β and results in I κ B being sent for degradation and NF κ B translocating to the nucleus. In the alternative pathway, IKK- α is predominant in the activation process. In this pathway p100, the precursor of p52, is cleaved into p52, which then translocates to the nucleus (202-204). NEMO is not absolutely required for the alternative pathway. Some of the inducers of the classical pathway can also induce the alternative pathway, such as TNF-receptor family member CD40. Other NF κ B activators are the Nod proteins (1

and 2) which are cytoplasmic receptors for microbial ligands, or the TLR that via TRAF6 and MEK1/3 can activate the IKK complex. MEK 1,3 can activate and regulate both ERK1/2 and p38, both of which have been shown to be involved in activation of cPLA2- α and proliferation of intestinal epithelial cells (201).

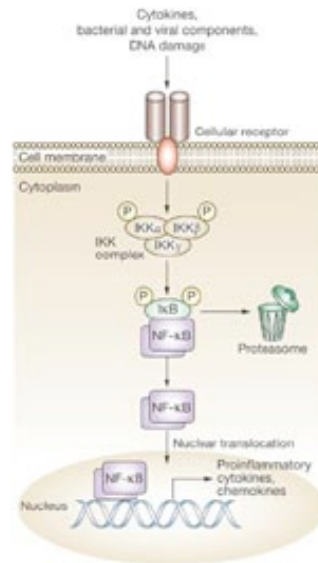


Figure9. Classical and atypical activating pathways of NF κ B.

<http://www.biomedcentral.com/nspprimers/nfkb/full>

10. Protein kinase C

The protein kinase C (PKC) family consists of 10 mammalian isoforms divided into three groups; the classical, novel and the atypical. The classical PKCs include the α , β I, β II, and γ PKCs. These isoforms need calcium, diacylglycerol (DAG) and phosphatidyl serine (PS) for activation. The novel PKCs consist of PKC δ , PKC ϵ , PKC η , PKC μ , PKC θ , and do not require calcium but do require DAG and PS for activation. The atypical PKCs (PKC ζ , PKC ι/λ) only require PS for activation (187,205). PKCs are activated by phosphorylation and binding of PKCs to the cytoplasmic side of the plasma membrane. The membrane targeting domains are the C1 domain which binds DAG and C2 which binds acidic lipids. PKCs are serine threonine kinases and

consist of a regulatory N-terminal that binds calcium, co-factors and lipids (C1 and C2). When co-factors bind these domains they release a pseudo substrate that otherwise blocks the activation site of PKCs. They also have a catalytic C-terminal that is an ATP substrate binding domain (206). PKCs are involved in many different pathways and signalling cascades by phosphorylating many different proteins. Three types of amino acids are known to be phosphorylated; serines, threonines and tyrosines. When PKC phosphorylates other proteins in their serine or threonine site, it changes the state of these downstream proteins and activates or allows them to bind to other proteins. GPCRs, tyrosine kinase receptors, ion channels, and transcription factors and enzymes such as PLA₂s are just some of the types of proteins that are phosphorylated by PKCs (207). In the case of GPCRs PKC has been shown to phosphorylate them once they have been activated by their ligand. This phosphorylation then can desensitize and/or help the GPCR associate with the endocytic machinery (207). Two ways of inhibiting PKCs include the use of calphostin C which competes with DAG/PS and inhibits the membrane translocation of PKC. The other way is used by GFX and Gö inhibitors which inhibit the catalytic activity of PKC by competing with ATP (208,209). We have previously shown several PKC members to be important in LTD₄ mediated signalling. PKC δ was shown to be important for stress fibre production induced by LTD₄ (210), whilst interestingly two PKCs namely PKC α and ϵ , are involved in cell proliferation (132).

11. G-protein coupled receptor structure and signalling

G-protein coupled receptors (GPCRs) are a family of seven transmembrane spanning receptors that sense molecules outside of the cells and activate intracellular signal transduction pathways and cellular responses by coupling to heterotrimeric G-proteins (211).

GPCRs are one of the largest known families in the genome. There are approximately 865 potential GPCRs which equals about 3.5% of the human

genome set (212,213). Approximately 30%-45% of all patented drugs in current clinic use are GPCRs (213,214). GPCRs contain 7 hydrophobic stretches of 22-25 residues which are long enough to cross a membrane when folded (7 transmembrane domains) creating three external and three internal loops with an external N-terminal and a cytoplasmic C-terminus. The transmembrane domains of GPCRs are highly conserved. The bovine rhodopsin GPCR has been crystallized and its structure has served as a template for other GPCRs (215) (216). The GPCRs have a variety of different ligands including; hormones, neurotransmitters, chemokines, calcium ions, sensory receptors for various odorants, tastes and photons of light. There are two broadly defined functional classes of GPCRs; The sensory receptor homologs and the non-sensory receptor homologs. In 2004 the Nobel prize went to Linda Buck and Richard Axel for demonstrating that odour perception results from several different GPCRs on different neurons (217). The GPCRs can further be classified into three families based on their sequence. The first family (Family1/A) has the closest structure to Rhodopsin and is the largest family. They have a short N and C terminus and include most of the olfactory receptors but also 200 of the non-olfactory receptors belong to this group (218). The second family (2/B) only has 25 members and they are characterised by their long N-terminus and the fact that they mainly activate cAMP through the G_s G-protein. This family includes the parathyroid hormone receptor, secretin receptor, adrenomedullin receptor and glucagon receptor (218). The third family (3/C) have long N and C terminals, mostly bind their ligands in the N-terminus and include the $GABA_bR$, calcium sensing receptors, some taste receptors and the orphan receptors, where the natural ligands are unknown (218).

GPCRs have been shown to exist in a conformation equilibrium between inactive and active state in the absence of ligand (219). The binding of ligands shifts the equilibrium either towards the active or inactive state. Three types of ligands exist; agonists which shift the equilibrium to active state, inverse

agonists which shift the state to inactive and also prefer to bind receptors in the inactive equilibrium state. The inverse agonists have been demonstrated to have the opposite effect of agonists. There are also neutral antagonists that bind the GPCR and lock it in an inactive state (220). As mentioned above this activation state can also be induced spontaneously without ligand as well as in receptors containing mutations that lead to constitutive activity which has been demonstrated in various pathological conditions (221). There are four different mutations known in the Rhodopsin receptor that causes it to be constitutively active that can lead to blindness (222). Mutations creating a constitutively α -1- β -adrenergic receptor have been demonstrated to have mitogenic and tumorigenic effects (223).

Malignant cells or their surrounding stromal cells often abuse the functions of GPCRs to be able to proliferate, induce angiogenesis (growth, migration and blood supply) and survive apoptosis. This is often achieved by either expressing constitutively active GPCRs or more commonly by over-expressing particular GPCRs (224). Furthermore over-expression of constitutively active G-proteins have also been implicated in cancer, the most potent ones being G- $\alpha_{12/13}$ (225,226).

Heteromeric G-proteins are composed of the α , β and γ subunits. In their inactive state the α subunit of the G-protein is bound to guanine dinucleotide phosphate (GDP). Upon activation the GDP is exchanged for a guanine trinucleotide phosphate (GTP). This leads to the disassociation of the α subunit from the β and γ subunits. These subunits can stimulate several downstream pathways. The α subunit has an intrinsic ability to hydrolyse the GTP back to GDP. The G-proteins are classified according to their α subunit. The G- α_s stimulates cAMP production by stimulating adenylyl cyclase whilst the G- α_i inhibits cAMP production. The G- $\alpha_{q/11}$ activate PLC which cleaves PIP2 to IP3 and DAG. G- $\alpha_{12/13}$ activate monomeric Rho GTPases. There are several regulating proteins that can catalyze the GDP-GTP and GTP-GDP exchange, but there are also two toxins used that can inhibit these activities. The first one

is Pertussis toxin which is a bacterial toxin that keeps the α subunit of the Gi family of G-proteins in GDP/inactive state by catalysing the ADP-ribosylation thus blocking its interaction with GPCRs. There is also the use of cholera toxin, another bacterial toxin. This toxin inhibits the GTPase activity of the α subunit of the Gs family of G-proteins keeping it in a constitutively active state by ADP ribosylating it (227,228). GPCRs are a family of intrinsic hydrophobic proteins only found in higher eukaryotes, located both at the plasma membrane and nuclear envelope of cells (169).

The β and γ G-protein subunits, are also able to play a signalling role. For example our group has previously shown that LTD₄ stimulation of calcium intracellular release, requires the release of inositoltriphosphate from diacylglycerol. This is achieved through the action of phospholipase C γ , which required “docking” with the β and γ subunits to become functional (229).

12. GPCR dimerization and other GPCR interacting proteins

GPCRs can signal as hetero- and/or homo-dimers or oligomers. Moreover, dimerization has been shown to be needed for proper expression, stronger ligand binding, phosphorylation and internalization.

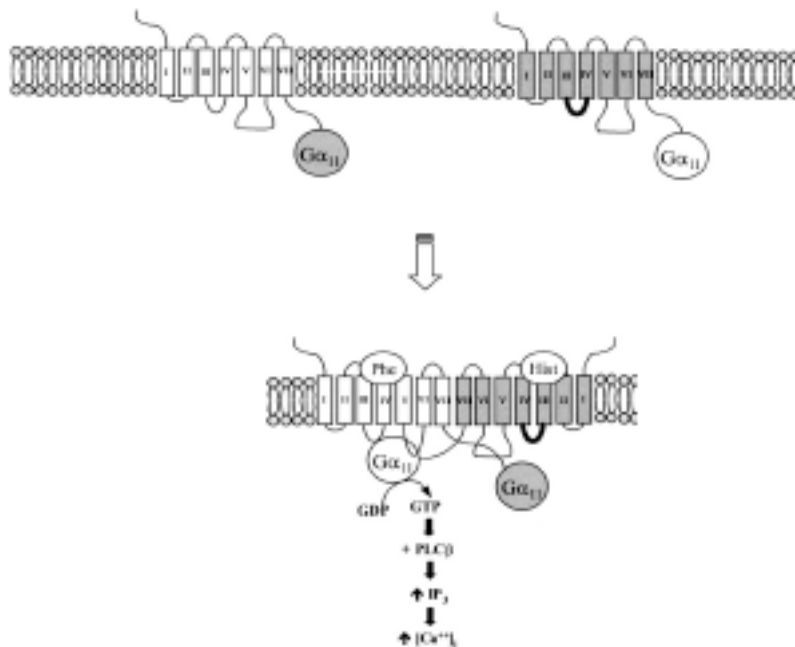


Figure9. GPCR dimerization. Modified from Carrillo, J. J. et al. *J. Biol. Chem.* 2003;278:42578-42587

Dimerized GPCRs have been shown to have signalling properties distinct from those of the monomeric receptors (230,231). The GABA_B receptors are a part of the family C GPCRs and they brought attention to the functional relevance of receptor dimerization. It was demonstrated that when the GABA_BR₁ was expressed alone it did not have the ability to be efficiently trafficked to the cell surface (232). Six years after the cloning of this receptor the GABA_BR₂ was cloned and studies on this receptor showed that it could not bind ligands when expressed alone. However, when the two receptors were expressed together they were efficiently expressed at the plasma membrane and also able to respond to the GABA ligands (233). It has been suggested that GABA_B1 and 2 receptors can dimerize via the transmembrane or N-terminal regions (234). However, the GABA_BR₁ is found in many regions of the brain where expression of the GABA_BR₂ is not detected. (235). Surprisingly it was found that none of the mGluR (closest related family) or 30 other GPCRs could

effectively target the GABA_B1R to the cell surface (236). Several other receptors have been shown to dimerize for different functions. Table 1. demonstrates various receptors, their partners and the effect of the dimerization (237).

Receptor	Heterodimer Partner	Negative Controls	Effect of Heterodimerization
5-HT _{1B}	5-HT _{1B}		n.d.
Adenosine A1	Dopamine D1	Dopamine D2	Signaling
Adenosine A1	P2Y ₁		Pharmacology
Adenosine A1	mGluR1 α	mGluR1 β	Signaling
Adenosine A2A	Dopamine D2	Dopamine D1	Internalization, signaling
Adenosine A2A	mGluR5	mGluR1 β	Signaling
Angiotensin 1A	Angiotensin 2		Signaling
Angiotensin 1A	β_2 AR		Signaling, trafficking
Angiotensin 1A	Bradykinin B2		Internalization, pharmacology, signaling
Calcium sensing receptor	mGluR1 and mGluR5		Trafficking
CCR2	CXCR4 and CCR5		Signaling, reduction of HIV infection
CCR5	δ -, κ -, and μ -OPR		Desensitization
Cholecystokinin A	Cholecystokinin B	β_2 AR	Signaling, internalization
Dopamine D1	Dopamine D2		Signaling
Dopamine D2	SSTR5		Pharmacology, signaling
Dopamine D2	Dopamine D3		Pharmacology
Endothelin A	Endothelin B		Internalization
GABA _B R1	GABA _B R2		Gain of function, pharmacology
Melatonin MT1	Melatonin MT2	β_2 AR	n.d.
Muscarinic M2	Muscarinic M3		Pharmacology
Oxytocin	Vasopressin V1a and V2	GABA _B R2	n.d.
S1P1	S1P2 and S1P3	LPA1	n.d.
SSTR1	SSTR5	SSTR4	Internalization, pharmacology, expression
SSTR2A	SSTR3		Desensitization
SSTR2A	μ -OPR		Desensitization
Substance P (NK1)	μ -OPR		Internalization, desensitization
TRHR1	TRHR2	GnRHR	Internalization
Vasopressin V1a	Vasopressin V2		Internalization
α_{1B} AR	α_{1A} AR	β_2 AR, NK1, CCR5	Internalization
α_{1B} AR	Histamine H ₁		n.d.
α_{1D} AR	α_{1B} AR	α_{1A} AR	Gain of function, internalization
α_{1D} AR	β_2 AR	β_1 AR	Gain of function, internalization
α_{2A} AR	β_1 AR		Internalization, pharmacology
α_{2A} AR	μ -OPR		Signaling
β_1 AR	β_2 AR		Internalization, pharmacology, signaling
β_2 AR	δ -, κ -OPR		Internalization, signaling
β_2 AR	β_2 AR	GABA _B R2	Internalization, signaling
β_2 AR	M71-OR		Gain of function, internalization
κ -OPR	δ -OPR	μ -OPR	Internalization, pharmacology, signaling
μ -OPR	δ -OPR		Pharmacology, signaling

n.d., not determined.

Table1. GPCR heterodimerisation. Modified from Prinster et. al. Pharmacological Reviews.

The importance of homo-dimerization has also been demonstrated as in the case of β_2 ARs where interruption of the homo-dimerization decreases β_2 AR induced cAMP production. (238). Targeting of GPCRs to the correct site has been shown to play an important role in homeostasis as miss localization has been demonstrated in various pathological conditions such as retinitis pigmentosa. This condition arises from intra cellular accumulation of mutant forms of the Rhodopsin receptor. Similar pathological accumulations have been demonstrated for the vasopressin and gonadotropin-releasing hormone receptors (239).

There are various proteins known to interact with GPCRs and deliver them to the cell surface, link them to their downstream effectors and ensure specificity and activation of diverse pathways. Proteins involved in regulating GPCR ER export and GPCR folding include the ER chaperons; calnexins, which help the folding of newly synthesized proteins and send improperly folded proteins to degradation. Accessory proteins cyclophilins are also involved in folding, and RAMPs are involved in ER export as well as proper folding, signalling, internalization, recycling and degradation (240). A-kinase anchoring proteins have been shown to link GPCRs to PKA or G-proteins (241,242). Some AKAPS have been shown to increase receptor phosphorylation and downstream ERK1/2 signalling whilst others have been shown to induce desensitization (242). Other proteins involved in bringing together GPCRs with their downstream effectors for rapid and efficient signalling are the Homer proteins and the InaD proteins (243). The InaD proteins contain PDZ domains, a domain known to interact with the C-termini of proteins including GPCRs (244). Scaffolding/chaperon proteins bind and recognize specific motifs on GPCRs (245). Furthermore, several motifs and domains of GPCRs have been demonstrated to be required for surface expression, nuclear localization and ER-retention (245).

13. GPCR desensitization

There are many regulatory mechanisms involved in GPCR signalling. One of which is receptor desensitization (246). This mechanism involves GPCR phosphorylation which is mediated by PKC, PKA or G-protein receptor kinases (GRKs) (247). The first two mentioned kinases directly uncouple GPCRs from G-proteins. PKC and PKA can mediate both homo and heterologous desensitization. These two terminologies refer to whether the receptor desensitization is initiated by the ligand of the receptor or by another GPCR-ligand interaction. An example of this is the heterologous desensitization of the CysLT₁R by UDP or ATP (165). This desensitization is mediated through PKC and leads to a more rapid receptor recovery than the homologous desensitization (165). In addition, PKA is capable of phosphorylating the GPCR in such a manner that it switches its binding from G_s to G_i. This has been demonstrated for the β_2 adrenergic receptor (β_2 AR) and the prostacyclin receptor. This switch favours downstream activation of MAPKs such as ERK1/2 (248-250). GRKs only phosphorylate ligand occupied/ligand activated GPCRs. It is often associated with the classical β -arrestin dependent desensitization because GRKs are suggested to promote β -arrestin binding to the GPCR which inhibits the GPCR from further binding G-proteins (251). β -arrestins are often involved in receptor internalization (discussed below). There are four arrestins, two of which are expressed in the retina called visual arrestin and cone arrestin. The other two arrestins, β -arrestin-1/arrestin-2 and β -arrestin-2/arrestin-3 are expressed in most tissues. The importance of GRKs and β -arrestin are demonstrated in knock-out mice where various GPCR desensitization, signalling and regulation are impaired (218). Receptor desensitization can be regulated on G-protein level as well by the RGS family. These proteins accelerate the hydrolysis of GTP to GDP speeding up deactivation of the signal induced (252).

14. Internalization of GPCRs

GPCR internalization is a complex yet extensively studied mechanism involving various scaffolding and regulatory proteins. GPCRs can internalize via three known pathways; clathrin coated pits, uncoated vesicles and caveolae (253). Internalization of GPCRs is initiated by ligand binding, which leads to a conformational change initiating receptor signalling. This subsequently leads to phosphorylation of the receptor by PKA, PKC or GRKs (as discussed above). From here the GPCRs are targeted to vesicles transporting them inside the cell to translocate, recycle or be degraded. Some receptors have actually been shown to have the ability of continuing to signal or even initiate new signalling pathways from the endosomes (254). Though internalization is a series of sequentially regulated events, studies have demonstrated that various steps have the ability to determine or play a part in receptor fate. The ligand binding has the ability to determine the fate of the receptor. This has been demonstrated for example for the β -2 adrenergic receptor. Under normal conditions when the receptor is activated by its ligand it predominantly undergoes recycling, however upon prolonged or repeated exposure to its ligand, the receptor is degraded (255). Different ligands have also been implicated in inducing different endocytic sorting and trafficking of the same GPCR (256).

Internalization of GPCRs and caveolin

Caveolae are 50-100nm flasked shaped membrane invaginations that are rich in cholesterol as well as the caveolin proteins (as indicated by the name) (257). There are three caveolins; caveolin1, 2 and 3. Caveolins-1 and 2 are strictly co localised and they have been implicated to require each other for proper function (258-260). It is not fully understood how receptors are targeted or internalized via caveolae or uncoated vesicles. But it is speculated that the transmembrane regions can interact with cholesterol found in caveolae or

uncoated vesicles and it has been shown that cholesterol can modulate the affinity of some GPCRs (261,262). Caveolae are localised both near and on the plasma membranes well as intracellularly. Interestingly many proteins such as the EGFR, heteromeric G-proteins, GPCRs and their interacting proteins are positioned in caveolae (263). This is thought to be a mechanism that makes signalling and trafficking of GPCRs efficient (263). However, disruption of caveolae has been shown to prevent internalization of the endothelin receptor ET_B and VIP receptors (263). Caveolin-1 in vivo and in vitro animal experiments have shown a suppressive effect of caveolin-1 in transformation and breast tumorigenesis (264,265). Contradictory to this, studies on human breast and prostate cancer show a positive correlation of caveolin-1 expression and tumorigenesis. Another study demonstrated elevated levels of caveolin-1 in adenocarcinoma of the colon whilst the adenomas and normal colonic epithelium showed little or no staining and Caveolin-2 absent (266). Conversely in another study CRC cell lines and human tumour epithelial mucosa were shown to express low or undetectable levels of caveolin-1 (267).

GPCR internalization and clathrin

Clathrin mediated endocytosis is a much more extensively studied mechanism. Clathrin coated pits are made up of clathrin heavy and light chain as well as a dozen regulatory proteins (268).

Clathrin mediated endocytosis of GPCRs usually includes the β -arrestin dependent internalization, although β -arrestin independent clathrin mediated internalization will also be addressed in this section.

When a GPCR is activated by its ligand and subsequently phosphorylated, this phosphorylation recruits β -arrestin. Phosphorylation of the GPCR has also been demonstrated to be able to determine the sorting of the receptor. This is exemplified in the case of the CXCR chemokine receptor where mutation of its phosphorylation sites inhibits it from being ubiquitinated and targeted to the lysosomes (269). Ubiquitin is known for its ability to sort

proteins to the proteasome (270), this mechanism includes GPCRs as well, but only in the biosynthetic pathway (271). In endocytic sorting, ubiquitin molecules added on to the C-terminus of the GPCRs targets them for lysosomal degradation (272) or effects the rate of internalization (273,274). The family of ESCRT proteins are highly conserved and have been demonstrated to be involved in the lysosomal sorting of GPCRs. This lysosomal sorting can be through the ubiquitin pathway but GPCRs can be sorted to lysosomes by ESCRT in the absence of ubiquitination as well. (256). This suggests that phosphorylation can be an initial step to the post translational modifications made on a GPCR upon trafficking.

Furthermore, phosphorylated GPCRs recruit β -arrestin that functions as a scaffolding protein and links the GPCR to the heavy chain of clathrin and to β 2 subunit of AP-2 by directly binding to these proteins. AP-2 is a clathrin adaptor protein involved in early events of vesicle formation. AP-2 targets receptors containing tyrosine or di-leucine based motifs and binds them as well as binding clathrin coats and Eps-15 (another clathrin adaptor protein first discovered in the EGFR endocytic pathway). Inhibition of clathrin pit formation is often achieved by using dominant negative Eps-15 constructs. Eps15 has been shown to be both phosphorylated as well as ubiquitinated. The phosphorylation of Eps-15 has been connected to its role in internalization, although the role of the ubiquitination is still poorly understood (275,276). The clathrin coated pit complex recruits the large GTPase Dynamin via β -arrestin. Dynamin is involved in the pinching off the coated pit from the plasma membrane (277).

GPCR internalization and β -arrestin

β -arrestin mediated binding targets the GPCR to clathrin coated pits at the cell surface (268). Furthermore, ubiquitination of β -arrestin has been shown to promote internalization of GPCRs. This is suggested to occur through

enhancing the affinity of β -arrestin to the plasma membrane (278). Furthermore, β -arrestin also plays a role in receptor sorting. Transient binding of GPCRs to arrestin that is terminated shortly after internalization mediates a rapid recycling whilst a more prominent binding mediates a slow recycling (251,253). Once inside the cell, GPCRs can be de-phosphorylated and returned to the plasma membrane, but this requires that β -arrestin is disassociated from the GPCR. ERK 1/2 activation have been implicated in β -arrestin mediated signalling. Dominant negative mutants of arrestins have been shown to attenuate GPCR induced ERK1/2 phosphorylation (279,280). However β -arrestin has also been shown to mediate ERK1/2 signalling in parallel to effecting G-protein mediated signalling. Over-expression of arrestins attenuated G-protein mediated signalling of the angiogenesis 1 receptor (AT₁R) but increased ERK1/2 phosphorylation through the same receptor (281,282). β -arrestin has the ability of effecting the duration and magnitude of ERK1/2 signalling as well as its localization. This has been demonstrated by the fact that weak interactions between β -arrestin and GPCRs mediated ERK1/2 translocation to the nucleus, activation of transcription factors (Elk and c-Myc) and induction of proliferative/mitogenic responses. In a similar manner, stable interactions between β -arrestin and GPCRs can mediate ERK1/2 signalling with the difference that the ERK1/2 signal retained in the cytosol preventing agonist induced cell proliferation (282,283). With all this in mind some GPCR internalization has been suggested to be arrestin-independent. The LTB₄ receptor BLT₁R has been demonstrated to internalize in an arrestin independent but GRK dependent manner. In this study dominant negative constructs of GRK2 could inhibit internalization of the BLT₁R but dominant negative constructs of β -arrestins were ineffective. Furthermore, the authors demonstrated a lack of co-immunoprecipitation and co-localization of β -arrestins with the BLT₁R (284). This does not rule out the possibility that the interaction of β -arrestin and the BLT₁R might be weak and transient and therefore not detected, therefore β -arrestin double (β -arrestin-1 and-2) knock-

out mice models should be used (285,286). This has been preformed in studies with the protease activated receptor 1 where internalization is not effect and suggested to be arrestin-independent (287). Similar studies to both models mentioned above have been preformed for the CysLT₁ receptor, where over-expression of β -arrestin increased CysLT₁ internalization and dominant negative constructs of β -arrestin demonstrate a non-significant decrease of receptor internalization. However, MEF cells from β -arrestin double knockout mice demonstrated a loss of CysLT₁ from the surface similar to that of the wild type mice (176). We preformed β -arrestin-2 siRNA studies in intestinal epithelial and CRC cells and demonstrate an almost abolished internalization of CysLT₁R.

GPCR internalization and Rab proteins

The Rab GTPases are small monomeric G-proteins and constitute the largest family of monomeric GTPases with over 60 different members (288). They are involved in various cellular processes such as trafficking, endocytosis, exocytosis cell growth and differentiation (289). They are post-translationally modified which allows them to associate with various membranes such as the Golgi-, nucleus, mitochondria-, ER-, plasma- membranes, endosomes and lysosomes (290). They act to coordinate the trafficking between these membrane bodies.

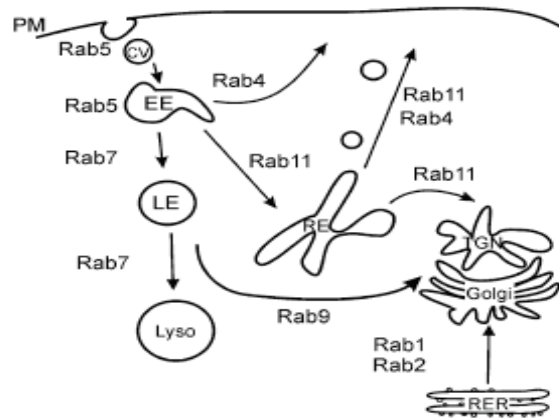


Figure 11. Role of Rab GTPases in GPCR trafficking. From Seachrist et al *Life sciences*, 2003.

Several Rab GTPases have been shown to be involved in the internal trafficking of GPCRs. Rab 5 and Rab 21 are involved in the trafficking of clathrin coated pits and early endosomes (291) whilst Rab 7 is implicated in trafficking to late endosomes and lysosomes(246). Rab 11 and 4 have been shown to be involved in the recycling of GPCRs to the plasma membrane. Rab 4 is involved in the fast recycling whilst Rab 11 is involved in the slow recycling (292). The different Rabs are localised to specific membrane domains and translocate upon activation. Rab 5 for example is localised to the clathrin coated pits and early endosomes (289,290). Even though GPCRs may mainly localize to Rab5 in the early endosomes or clathrin coated pits, they can later be sorted to different types of endosomes controlled by different types of Rabs. This is the case for the endothelin receptors, A and B. The Endothelin A and B receptors are targeted to Rab 5 positive vesicles but Endothelin A is recycled whilst Endothelin B is degraded (293). On the other hand it has also been demonstrated that GPCRs such as the AT1AR can work as Rab protein modulators. AT1AR has been shown to stimulate Rab5 GDP/GTP exchange (290).

15. Nuclear GPCR

Over time a greater number of GPCRs have been found at the nuclear membrane and even inside the nucleus (such as the PAFR) (294). They have either been shown to be there under basal conditions such as the prostaglandin receptors (EP1-4) (295) and be further increased upon stimulation, such as the CysLT₁R (169) or translocate there upon ligand dependent or independent activation (296). The GPCRs have been shown to be inserted into the nuclear membrane and exert signalling pathways or gene transcriptions that are diverse from those of the plasma membrane (297). Interestingly, no difference in the size and sequence has been detected in any of the GPCRs (297). There are several examples of GPCRs signalling at the nucleus. The first examples are GPCRs at the plasma membrane that initiate signalling pathways at the nuclear membrane. The GPCR for LPA (lisophosphatidic acid) activates a G_i dependent pathway that results in activation of phospholipase D1 (PLD1) that in turn leads to conversion of phosphatidylcholine to DAG and phosphatidic acid (PA). The chemokine receptor CXCR4 has been shown to induce ERK1/2 nuclear translocation and induction of transcription and proliferation in a G_i dependent manner. Both of these pathways are mediated through the plasma membrane GPCR eliciting a nuclear response from the plasma membrane (296). As discussed above β-arrestin can retain ERK1/2 in the cytoplasm and this retention can be mediated by GPCRs. The GPCR Neurokinin-1 for example has been shown to induce β-arrestin dependent retention of ERK1/2 in the cytoplasm while the β₂ adrenergic receptor induces β-arrestin dependent ERK1/2 translocation (298-300). An example of different signalling pathways induced by the same receptor at the nuclear membrane compared to the plasma membrane is that of the EP3Rs. The receptors signal for cAMP or IP₃ generation from the plasma membrane whereas the nuclear membrane EP3R induces calcium signals and induces gene-transcription (119,301). Moreover, stimulation of the nuclei with PAF and LPA induces a calcium dependent ERK1/2 pathway and NFκB binding to DNA. The calcium

induction induced by lipid ligands of these three nuclear localised GPCRs lead to specific gene-up regulations (119,297,301,302). PAF or LPA have been demonstrated to induce gene expression of eNOS, COX-2 and iNOS. These signalling cascades were mediated through MAPK and NF κ B (297). Considering the cascade of signalling molecules that reside near or in the nuclear membrane such as; cPLA₂, PKC, ERK1/2, β -arrestin, 5-LO and COX-2 (which give rise to the production of lipid ligands) and calcium channels, it is not so farfetched to believe that signalling pathways can be orchestrated here. Some GPCRs contain nuclear localization signals (NLS) or even ER-retention sequences (PAFR and CysLT₁R) (169) yet deletion or mutation of these sequences do not change the nuclear localization of the GPCRs. In line with this there are GPCRs that do not contain any of these types of sequences, such as the LPA₁R (297). The LPA₁R has been shown in both clathrin and caveolae domains in the plasma membrane but co localizes only with caveolin in nuclear fractions. Considering the role caveolae play in bringing signalling molecules together this is rather interesting. Moreover, not all GPCRs that are found to reside at the nuclear membrane in certain tissue or cells are found to do so in other types of cell or tissue (297).

THE PRESENT INVESTIGATION

AIM

The general aim of this work was to further investigate signalling and trafficking of the cysteinyl leukotriene receptors and their role in CRC.

Specific aims of the present work

1. To explore the role of cPLA₂ in LTD₄ induced cell proliferation
2. To explore the regulatory mechanism of trafficking of the CysLT₁R in non-transformed and CRC cells as increased expression of this receptor has been correlated with a poorer prognosis in CRC.
3. To validate the trafficking of the CysLT₁R and the CysLT₂R in relation to each other as increased expression of the CysLT₂R has been correlated with a good prognosis and the balance and signalling of these receptors may play a role in CRC.

RESULTS AND DISCUSSION

Paper I

One of the characteristics of chronic inflammation is a continuous production of inflammatory mediators (32). LTs are inflammatory mediators derived from AA. AA is released by PLA₂s, particularly cPLA₂- α from the phospholipid bilayer of cellular membranes and can further be metabolized by 5-LO to produce LTs or COX-2 to produce PGs (94). Indeed previous studies from our group have demonstrated that LTD₄ is able to induce COX-2 expression as well as up-regulate LTC₄ synthase and 5-LO in intestinal epithelial cells (303). We have shown that these cells have the ability to produce CysLTs and that this production can mediate proliferation and survival in intestinal epithelial cells (148,303). Additionally, we have published data that LTD₄ can up-regulate 5-LO and LTC₄ synthase in CRC cells (303). In the first paper of this thesis LTD₄ was observed to be able to activate cPLA₂- α after 10 minutes of stimulation, and that this activation consists of phosphorylation of cPLA₂- α and its translocation from the cytosol to an intact nuclear fraction. The same study also revealed that LTD₄ in a similar fashion could induce production of cPLA₂- α after 90 minutes. Both these pathways are mediated through the CysLT₁R, a PTX sensitive G-protein, PKC, ERK1/2, p38 and require a calcium signal. The LTD₄ induced expression of cPLA₂- α is suggested to be mediated through NF κ B as an inhibitor of this protein blocked the cPLA₂- α up-regulation. This is further supported by data from our group that LTD₄ can induce NF κ B expression at both mRNA and protein level at 60 minutes of stimulation (unpublished data). The results from Paper I provide evidence for the theory that LTD₄ can induce its own production in intestinal epithelial and CRC cells. We also demonstrate that LTD₄ can induce cell proliferation, which has been previously shown to go through two separate pathways; one involving a PTX sensitive G-protein and one Ras dependent but PTX independent pathway. This latter pathway activated PKC- ϵ , Raf-1 and ERK1/2 (131). In Paper I the LTD₄ mediated proliferation reveals it to be cPLA₂- α

dependent. Interestingly the cPLA₂- α inhibitors even decreased the basal level of proliferation. Whether this cPLA₂- α proliferation involves a third pathway or a crosstalk with the other pathways was not clear. It is however interesting in the concept of inflammation induced cancer, that LTD₄ induces a rapid activation of cPLA₂- α and that a longer stimulation results in the production of cPLA₂- α and eventually proliferation of the cells. We also observed an increase of cPLA₂- α in CRC cells and tissue compared to non-transformed cells and tissue. This increased expression was similarly seen for COX-2 and correlates well with the theory that cPLA₂- α and COX-2 play a role in different malignancies including CRC, strengthening the idea that cPLA₂- α is the predominant source for eicosanoid production (81-83,85,86,90-92). There are however studies describing decreased cPLA₂- α levels in CRC. The study in this case suggests that cPLA₂- α is involved in apoptosis and the decreased expression of it, leads to cell survival (87,89).

Other studies including our own with colorectal patient material, contradict this data, as increased cPLA₂- α expression was evident. A recent study investigating a patient with cPLA₂- α deficiency indicated an important global role for cPLA₂- α in eicosanoid production and shows no malignancy in the colon (93).

PaperII

A key regulation in GPCR signalling is internalisation. Our group has previously identified two very interesting points about the CysLT₁R; its nuclear localisation and translocation (169) as well as its role in CRC prognosis (171). High levels of the CysLT₁R have been correlated with a poorer survival rate in material from CRC patients. In this context, we wanted to investigate a regulatory mechanism of the receptor in non-transformed intestinal epithelial cells compared to CRC cells. Our results showed a rapid internalisation of the receptor from the plasma membrane already at 5 minutes and recycling of the receptor back to the plasma membrane within 20 minutes after agonist removal in non-transformed cells. However, continuous

stimulation with LTD₄ up to 60 minutes increased of the expression of the receptor in a nuclear fraction in both non-transformed as well as CRC cells. We have previously published that the CysLT₁R is also localised to the outer nuclear membrane (169). This internalisation and increase of the CysLT₁R at the nuclear membrane is a clathrin dependent mechanism, as inhibition of clathrin blocks internalisation and CysLT₁R accumulation at the nuclear membrane. Furthermore, the CysLT₁R internalisation is a β -arrestin-2 dependent process as siRNA against β -arrestin-2 blocks loss of the CysLT₁R from the cells surface. A previous study investigating internalisation of the CysLT₁R demonstrated a PKC dependent but β -arrestin independent internalisation of the receptor (176). Another study showed that the LTD₄ induced internalisation of the CysLT₁R is not PKC dependent but GRK2 dependent (165). These data suggest that the internalisation of the CysLT₁R may vary in different systems. Since our study is preformed on cells endogenously expressing the receptor we believe that the internalisation route characterised in PaperII is representative for intestinal epithelial cells and CRC cells. Moreover, we present data that the internalised CysLT₁R is targeted to Rab5 positive endosomes. The inhibition of clathrin led to blockade of the CysLT₁R internalisation in both non-transformed as well as CRC cells. However, in the CRC cells an increase of the CysLT₁R at the plasma membrane was detected. Indeed CysLT₁R internalisation is delayed compared to non-transformed cells and also difficult to detect endogenously using confocal microscopy (169) and western blot analysis. Intriguingly an accumulation of the CysLT₁R is detected rapidly after LTD₄ stimulation and this accumulation is clathrin sensitive. These results suggest a rapid turnover of the CysLT₁R at the plasma membrane of CRC cells explaining the increase at the plasma membrane detected upon inhibition of clathrin coated pit formation and LTD₄ stimulation. FACS analysis only detects internalised CysLT₁R after 20min to 1h in various CRC cells yet the accumulation of CysLT₁R at the nuclear membrane is detected already 10-15 min of LTD₄

stimulation. This could be explained by a low amount of receptor being internalised from the plasma membrane at earlier time points that are difficult to detect. It does however not exclude the possibility of internal pools of the receptor translocating to both the plasma membrane as well as the nuclear membrane of CRC cells upon LTD₄ stimulation. This would also support the increased amount of CysLT₁R detected at the plasma membrane fo CRC cells. As mentioned above inhibition of clathrin pit formation and stimulation with LTD₄ blocks the internalisation of CysLT₁R from the plasma membrane and its up regulation at the nuclear membrane in non-transformed cells. This study also demonstrates ERK1/2 phsophorylation and cyclinD1 mRNA up regulation induced by LTD₄ in non transformed intestinal epithelial cells. Interestingly both these signalling events further increase upon inhibition of clathrin coated pit formation and LTD₄ stimulation (1h), suggesting that the signalling is mediated through the CysLT₁R at the plasma membrane since this treatment renders the receptor to stay at the plasma membrane and continue signalling. In contrast to this LTD₄ induced COX-2 is inhibited by upon inhibition of clathrin coated pit formation suggesting that this signal is dependent on upon inhibition of CysLT₁R internalisation. Furthermore, we speculate that the COX-2 signal is mediated through the CysLT₁R at the nuclear membrane since CysLT₁R seems to translocate to nuclear membrane upon LTD₄ stimulation. However, we can not exclude the possibility of CysLT₁R signalling from endosomes.

PaperIII

Previous publications from our group have demonstrated different roles for LTD₄ and LTC₄. Whilst LTD₄ has been shown to induce proliferation, migration and cell survival (131-133), LTC₄ has been shown to promote cell differentiation (140). Furthermore, upregulation of the CysLT₁R has been correlated with a poorer survival prognosis whilst increased CysLT₂R correlates with a better prognosis (140,171). In this study we wanted to investigate the trafficking of the two cysteinyl leukotriene receptors; CysLT₁R

and CysLT₂R upon LTD₄ or LTC₄ stimulation. We started our investigation by over-expressing the receptors alone or together in Cos-7 cells to determine the effect of each ligand on each receptor when expressed alone and when expressed together. However, when we tried to over-express the receptors alone, the localization of the receptors to the plasma membrane was disturbed. The majority of the receptors localised to a peri-nuclear region. When co-expressed, the localization of both receptors was observed correctly both at the plasma membrane as well as the nuclear membrane. These results were in direct conflict with studies performed in Cos-7 cells over expressing CysLT₁R. The only difference in these studies and our study was the insertion of a signalling sequence upstream of the tagged receptor in the other studies. This sequence is inserted upstream the receptor to ensure proper expression and localization of the receptor to the plasma membrane(304). It is a cleavable sequence found naturally in certain proteins, but not GPCRs. Our results demonstrate a natural way of achieving proper localization of the CysLT₁R and CysLT₂R. Furthermore, shRNA down regulating either receptor in non-transformed and CRC cells, rendered the majority of both receptors to be restricted to the peri-nuclear region. It is important to mention that not all receptors were miss-localised, especially in the CRC cells. The receptors detected at the plasma membrane are most likely the receptors that were already there before sh-RNA treatment. Another interesting observation was that down regulation of either receptor resulted in poor survival of the cells. This correlates well with previous data from our group demonstrating cell death in normal intestinal epithelial cells upon long-term treatment of CysLT₁R inhibition. The same treatment in CRC cells resulted in decreased proliferation (148). Interestingly despite the restriction of the receptors to the peri-nuclear region of the cells, down regulation of CysLT₁R did not change the expression of the CysLT₂R or the other way around. A recent study demonstrated that CysLT₁R and CysLT₂R can dimerize and that CysLT₂R negatively regulates CysLT₁R plasma membrane expression and

signalling in mast cells (183). These conflicting results may be due to tissue specificity of receptor regulation. Conflicting results by different groups have been demonstrated in the internalisation of the CysLT₁R. Furthermore, we hypothesize that a balance in the expression of CysLT₁R and CysLT₂R is most likely responsible for proper expression of the two receptors as low levels of CysLT₂R has been detected in some cancer cells where the expression of the CysLT₁R at the plasma membrane is not altered. Most likely a low level of CysLT₂R is required for proper localization of the CysLT₁R and vice versa. Furthermore, we investigated the effect of LTC₄ on both the CysLT₁R and the CysLT₂R. Interestingly both receptors were internalised from the plasma membrane upon LTC₄ stimulation. Moreover, a dimerization assay demonstrated that the CysLT₁R and CysLT₂R were dimerized both at basal levels as well as upon LTC₄ stimulation. No dynamics of either were detected at the nuclear membrane upon LTC₄ stimulation. Stimulation with LTD₄ resulted mainly in CysLT₁R internalisation. Furthermore, stimulation with LTD₄ decreases the CysLT₁R and CysLT₂R dimerization. This correlates well with our previous results that the effect of LTD₄ is mediated through the CysLT₁R.

SUMMARY

In summary, we have shown that:

1. LTD₄ via the CysLT₁R can produce and activate cPLA2- α , which is involved in LTD₄ mediated intestinal epithelial cell proliferation.
2. CysLT₁R is internalised and increased at the nuclear membrane in a clathrin, β -arrestin, Rab 5 dependent manner, and that the LTD₄ induced COX-2 requires CysLT₁R internalisation.
3. CysLT₁ and CysLT₂ may need each other for proper plasma membrane localization.
4. LTC₄ internalizes both CysLT₁ and CysLT₂ receptors, whilst LTD₄ mainly internalizes the CysLT₁R.
5. CysLT₁ and CysLT₂ receptors can hetero-dimerize, this dimerization is decreased upon LTD₄ stimulation

POPULÄRVETENSKAPLIG

SAMMANFATTNING

Inflammation är en mekanism som kroppen använder för att skydda oss mot patogener och fysiska skador i olika vävnader. Detta karaktäriseras av feber, svullnad och rodnader. Ett nätverk av specialiserade signalvägar och mediatorer initierar, reglerar och avslutar den inflammatoriska processen. Rubbnings i dessa signalvägar kan leda till att inflammationsprocessen inte avslutas eller fungerar som den ska. Detta kan t.ex. hända när kroppen börjar reagera på sina egna antikroppar och detta tillstånd kallas för autoimmun sjukdom. En stor del av vårt immunförsvar sitter i mag-tarmkanalen, främst i tjocktarmen som innehåller ett stort antal bakterier. Dessa bakterier börjar leva i tarmen från det att vi föds och fortsätter föröka sig i arter allt eftersom vi utsätts för miljön och maten omkring oss. Balansen av dessa bakterier är viktig för en välfungerande tarm och ett effektivt immunsystem. Specialiserade celler i tarmen omsluter dessa bakterier och håller de skilda från immunceller och resten av kroppen. Dessa specialiserade celler består till största del av olika epitelceller som kan känna av bakterieprodukter genom speciella receptorer. Dessa celler kan skilja på vilka bakterier som är egna och vilka som inte är egna. Detta medför att en låg nivå av inflammation alltid existerar i tarmen. Om epitelcellerna av olika anledningar skulle ta skada och tarmbakterierna skulle komma i kontakt med de underliggande cellerna uppstår en inflammation som karaktäriseras som akut. Men om kroppen inte kan reparera denna skada övergår den akuta inflammationen till ett tillstånd som kallas kronisk inflammation (Inflammatory bowel disease, IBD). Detta innebär att en konstant nivå av inflammation hela tiden påverkar cellerna i tarmen. Epitelcellerna kan då ta skada av det här genom muteringar i sitt DNA. Dessa muteringar leder oftast till ökad cell delning vilket i sin tur kan leda till fler muterade celler. Om tillräckligt många muteringar och muterade celler ansamlas och fortsätter delar sig kan detta leda till uppkomsten av en tumör som sedan utnyttjar de inflammatoriska cellerna för att sprida sig. Denna

fortskridning från en inflammatorisk respons till cancer är en teori som föreslogs redan 1863 av en forskare vid namn Virchow. Den är också grunden för de studier som presenteras i denna avhandling. Den modell som används är normala epitelceller och koloncancer celler och de inflammatoriska mediatorerna som undersöks är cysteinyl leukotrienerna (LTC_4 , LTD_4 och LTE_4). Dessa inflammatoriska mediatorer bildas från arakidonsyra som frisätts från cellmembran främst av ett enzym kallat $cPLA_2\alpha$. Tidigare studier från vår grupp har visat att LTD_4 kan inducera celldelning, cellöverlevnad samt cellmigration i normala tarmepitelceller, tre mekanismer som används av cancer celler för överlevnad. Dessa LTD_4 inducerade mekanismer har visats vara genom hög affinitets receptorn för LTD_4 , nämligen $CysLT_1R$. LTC_4 har föreslagits ha en roll i celldifferentiering. Leukotrienerna har 4 olika receptorer; $CysLT_1$, $CysLT_2$, GPR17 och $CysLTE_4R$. Ett ökat uttryck av $CysLT_1R$ har påvisats i material från koloncancer patienter. Detta ökade uttryck har visats ge en dålig överlevnadsprognos medan ökat uttryck av $CysLT_2R$ ger en bättre prognos. Den första studien i denna avhandling visar att LTD_4 via $CysLT_1R$ kan aktivera $cPLA_2\alpha$ och att denna aktivering är ett av stegen i en signalväg som leder till LTD_4 inducerad celldelning. Signalvägen som aktiverar $cPLA_2\alpha$ via LTD_4 har också karaktäriserats i större detalj denna studie. Vi visar involveringen av protein kinase C (PKC), MAP kinaserna; ERK1/2 och p38 samt transkriptionsfaktorn NF κ B. I den andra studien har internaliseringen av $CysLT_1R$ undersökts. Internalisering av receptorer är ett nyckelsteg som definierar varaktigheten av en signalväg. Vi visar här att $CysLT_1R$ internaliseras via clathrin vesiklar, Rab5 positiva endosomer och β -arrestin-2. Utöver detta visar vi att $CysLT_1R$ ökar i kärnan och föreslår att den där kan inducera ökad COX-2 mRNA. I den tredje studien visar vi att $CysLT_1R$ och $CysLT_2R$ kan dimerisera. Vi visar även att LTC_4 kan inducera internalisering av båda receptorena, men att LTD_4 främst internaliserar $CysLT_1R$. Utöver detta visar vi att LTD_4 minskar dimeriseringen mellan $CysLT_1$ och $CysLT_2R$.

ACKNOWLEDGEMENTS

The work presented in this thesis has been performed at Cell and Experimental Pathology, Malmö University Hospital. It has been supported by grants from the Royal Physiographic Society in Lund.

First and foremost, I want to express my sincere gratitude to my supervisor **Anita Sjölander**. I have thoroughly enjoyed being a part of your lab, learning from your experience, and being influenced by your never-ending enthusiasm for science. Your criticisms and our long discussions about projects and science have been of great help to me. I learned a lot more about science than just lab-work, grant applications and scientific writing from you. Your honesty and easy going attitude has been of great help during my PhD.

Prof. Tommy Andersson, during most of my PhD time, you were the head of the department and I would like to thank you for your contribution to our working environment.

Maria Juhas, thank you for maintaining the lab so well and for being so patient with us. It has been so much fun working with you and your advice and excellent lab-skills have been invaluable throughout the years. We have had a lot of laughs about food, fashion and all that other stuff that exists in a land far far away from the lab. You are the definition of a true lady. Next time we are in San Francisco together we will find a good sushi bar.

Lena Axelsson, for always lending me things (trolley, keys mm.) from your lab, and for the much appreciated attempt at getting calcium signals from cells in a passage where they just don't want to respond.

Anki, Monica and Ulla; you are like the three little angels from Sleeping beauty. You work your magic and make sure all the paper work, trips and salaries are fixed. Thank you for that. We have shared many laughs. See you in Australia. **Alva**, you were the first one to help me with all these things, I miss your stories.

Frederik Vilhardt, thank you for all the work with the shRNA, the antibodies and the discussions about trafficking and microscope pictures.

Mattias Mögerlin, thank you for all the help with the electron microscope pictures, despite microscope and memory sticks breaking down.

The past members of Cell and Experimental pathology: Christian Kamp-Nielsen and Joan **Campel-Tofte** thank you for the initial help on the receptor

projects. **Yulyana Yudina**, my co-author in the first manuscript of this thesis. Thank you for sharing the frustration of working with GPCR internalization. **Sailaja** for sharing my enthusiasm for Disney and for always being so kind and friendly. Your secret dance for good western blots is safe with me. **Karim**, for making me laugh so much, I think Ireland is the perfect place for you. **Marina**, thank you for being such a great person to share my office with and for all the Ukrainian celebrations, massages, dinners and drinks outside of the lab. **Tarras**, you have a great voice and a wonderful personality, it has been a pleasure getting to know you. **Christian H** when I finally started understanding what you say in your Danish accent, I have found you to be a really funny and kind person. I can even over-look your love for football. **Shamsa** you are the best older sister ever! You are like Anthony Hopkins I could listen to your voice for hours. Thank you for being such a compassionate and kind person. **Karin L**, thank you for all the molecular biology help and your wonderful personality. **Catharina**, for being such a cool person and for the chats and medical advise. **Annette, Simone, Veronika and Maite** I enjoyed working with you in the lab, thank you for making me feel welcome in the “PhD-room” when I first started. **Totte**, you are a breath of fresh air with your insane sport interests and your enthusiasm.

The present member of Cell and Experimental Pathology

I would like to thank all my colleagues in Cell Pathology for a great working environment (**Jian, Yuan, Hala, Astrid, Cecilia**). **Jian** you are so funny, **Yuan** thank you for teaching me how to make dumplings and for discussing weird food customs, **Hala** for speaking to me in French all the time even though I have no idea what you are saying I know you are a lovely person. Thank you for the tea. **Astrid** for introducing me to all the boards on the medical faculty. My two brilliant students **Emily** and **Linda**, I could not have asked for more enthusiastic and smart students. Thank you for your contribution to my work. **Ramins group: Ramin**, it was nice to come to a lab that had such a good impression of Persian people, thank you for that and for hiring Gina. **Gina**, my other room mate, thank you for being so happy all the time. I look forward to laughing and talking to you every day in the office. Thank you for all the wonderful food recipes. You have become a very good friend. **Gang**, you have a great sense of humour (and wonderful hair). **Raji**, Thank you for teaching me about Indian culture.

Exp-pathology: Jill, thank you for so many things; the advice, the help in and out of the lab, the discussions but most of all for being a true friend and being

on my side and standing up for me when I really needed it the most. **Richard**, thank you for being such a good friend and for bringing me Maryan Keyes books, even though you don't like buying them. I think you are the funniest person I know. **Caroline**, thank you for all the fun times and the walks in the mornings. **Elin** thank you for all the chats, laughs and for Endnote. **Vicky**, for the short time I have known you I have really grown to like you a lot. Thank you for taking me home when I had hick-ups. **Anders**, thank you for all the coffee times, I admire your somewhat hidden sarcasm. I would also like to thank **all the other present and past members of cell and Experimental pathology** for creating a creative atmosphere.

Friends and Family: I would like to thank my parents **Parviz and Mitra** to whom this thesis is dedicated and my brother **Amir**. Without your support, endless encouragement and unconditional love I could not have accomplished any of this. Thank you for always guiding me.

I would also like to thank my mother and father in laws **Gillian and Ernest** for the wonderful times in England and for your love and support.

My aunt **Roya** who has been like my second mother in Malmö. Thank you for always listening and understanding, for all the good food and cosy movie nights. My cousins **Aida, Sanaz, Sara and Lilly** for all the good times growing up together and for still being like sisters.

All my family in Stockholm **The Hassanis: Manijeh, Farhad, Yohannes and Abtin**, thank you for being there and being such a wonderful part of my life. **The Welin-Berger family: Katty, Johan, Philip, Nadia and Simon** thank you for all the lovely weeks, ski-trips, shopping and movie nights. My family in Australia; **Mohsen, Mandana, Ranna, Ramtin and Moshtaba** for showing me such a great time in Australia. My closest friends; **Azade and Mariam (and your families)** for showing me the other side of life and for always being there for me. You are the best. **Kaveh** for your sense of humour, your wonderful personality and just so much more. I am lucky to have you as a friend. **Alex & Richard** all the laughs and great time together. **Sussie** for filling me in on all the latest celebrity happenings and for introducing me to painting classes, where we can bitch about science. **Karin** for helping sort out all the mess I make from time to time. I big thank you to all my other friends and family.

Saving the best for last; I would like to thank my wonderful husband **Oliver**. You are my inspiration and the joy in my life. I don't know what I would do without you. There are no words to express how I feel about you.

REFERENCES

1. Llopis, M., Antolin, M., Guarner, F., Salas, A., and Malagelada, J. R. (2005) *Gut* 54, 955-959
2. Flint, H. J., Duncan, S. H., Scott, K. P., and Louis, P. (2007) *Environ Microbiol* 9, 1101-1111
3. Sancho, E., Batlle, E., and Clevers, H. (2004) *Annu Rev Cell Dev Biol* 20, 695-723
4. Crosnier, C., Stamataki, D., and Lewis, J. (2006) *Nature reviews* 7, 349-359
5. Fre, S., Vignjevic, D., Schoumacher, M., Duffy, S. L., Janssen, K. P., Robine, S., and Louvard, D. (2008) *Advances in cancer research* 100, 85-111
6. Radtke, F., and Clevers, H. (2005) *Science* 307, 1904-1909
7. McKay, D. M. (2005) *Memorias do Instituto Oswaldo Cruz* 100 Suppl 1, 205-210
8. Tlaskalova-Hogenova, H., Stepankova, R., Hudcovic, T., Tuckova, L., Cukrowska, B., Lodinova-Zadnikova, R., Kozakova, H., Rossmann, P., Bartova, J., Sokol, D., Funda, D. P., Borovska, D., Rehakova, Z., Sinkora, J., Hofman, J., Drastich, P., and Kokesova, A. (2004) *Immunology letters* 93, 97-108
9. Guarner, F., and Malagelada, J. R. (2003) *Lancet* 361, 512-519
10. Sanders, D. S. (2005) *J Clin Pathol* 58, 568-572
11. Hanaway, P. (2006) *Altern Ther Health Med* 12, 52-60; quiz 61-52
12. Fukata, M., and Abreu, M. T. (2007) *Biochemical Society transactions* 35, 1473-1478
13. Fukata, M., Chen, A., Vamadevan, A. S., Cohen, J., Breglio, K., Krishnareddy, S., Hsu, D., Xu, R., Harpaz, N., Dannenberg, A. J., Subbaramaiah, K., Cooper, H. S., Itzkowitz, S. H., and Abreu, M. T. (2007) *Gastroenterology* 133, 1869-1881
14. Strober, W., Fuss, I., and Mannon, P. (2007) *J Clin Invest* 117, 514-521
15. Serhan, C. N. (2008) *J Periodontol* 79, 1520-1526
16. Serhan, C. N., and Chiang, N. (2008) *Br J Pharmacol* 153 Suppl 1, S200-215

17. Serhan, C. N., Chiang, N., and Van Dyke, T. E. (2008) *Nat Rev Immunol* 8, 349-361
18. Serhan, C. N., Yacoubian, S., and Yang, R. (2008) *Annu Rev Pathol* 3, 279-312
19. Verdu, E. F., and Collins, S. M. (2004) *Best Pract Res Clin Gastroenterol* 18, 315-321
20. Rieder, F., Brenmoehl, J., Leeb, S., Scholmerich, J., and Rogler, G. (2007) *Gut* 56, 130-139
21. McFarland, L. V. (2008) *World J Gastroenterol* 14, 2625-2629
22. Gismera, C. S., and Aladren, B. S. (2008) *World J Gastroenterol* 14, 5491-5498
23. Cho, J. H. (2008) *Nat Rev Immunol* 8, 458-466
24. Shi, D., Das, J., and Das, G. (2006) *Cell Res* 16, 70-74
25. Guarner, F. (2006) *Digestion* 73 Suppl 1, 5-12
26. van Bodegraven, A. A., and Mulder, C. J. (2006) *World J Gastroenterol* 12, 6115-6123
27. Pierik, M., Rutgeerts, P., Vlietinck, R., and Vermeire, S. (2006) *World J Gastroenterol* 12, 3657-3667
28. Shepela, C. (2008) *Minn Med* 91, 42-45
29. Hwang, J. M., and Varma, M. G. (2008) *World J Gastroenterol* 14, 2678-2690
30. Boyle, P., and Leon, M. E. (2002) *Br Med Bull* 64, 1-25
31. Lieberman, D. (2008) *Gastrointest Endosc Clin N Am* 18, 595-605, xi
32. Itzkowitz, S. H., and Yio, X. (2004) *Am J Physiol Gastrointest Liver Physiol* 287, G7-17
33. Fearon, E. R., and Vogelstein, B. (1990) *Cell* 61, 759-767
34. Grady, W. M., and Carethers, J. M. (2008) *Gastroenterology* 135, 1079-1099
35. Alrawi, S. J., Schiff, M., Carroll, R. E., Dayton, M., Gibbs, J. F., Kulavlat, M., Tan, D., Berman, K., Stoler, D. L., and Anderson, G. R. (2006) *Anticancer Res* 26, 107-119
36. Watson, A. J. (2006) *Crit Rev Oncol Hematol* 57, 107-121
37. Su, L. K., Kinzler, K. W., Vogelstein, B., Preisinger, A. C., Moser, A. R., Luongo, C., Gould, K. A., and Dove, W. F. (1992) *Science* 256, 668-670
38. Senda, T., Iizuka-Kogo, A., Onouchi, T., and Shimomura, A. (2007) *Med Mol Morphol* 40, 68-81
39. Balkwill, F., and Mantovani, A. (2001) *Lancet* 357, 539-545
40. Coussens, L. M., and Werb, Z. (2002) *Nature* 420, 860-867

41. Clevers, H. (2004) *Cell* 118, 671-674
42. Wang, D., and Dubois, R. N. (2006) *Gut* 55, 115-122
43. Cha, Y. I., and DuBois, R. N. (2007) *Annu Rev Med* 58, 239-252
44. Zhang, Y., and Chen, F. (2004) *Cancer Res* 64, 1902-1905
45. Atreya, I., Atreya, R., and Neurath, M. F. (2008) *J Intern Med* 263, 591-596
46. Burstein, E., and Fearon, E. R. (2008) *J Clin Invest* 118, 464-467
47. Stenson, W. F. (1990) *Scand J Gastroenterol Suppl.* 172, 13-18
48. Balsinde, J., Balboa, M. A., Li, W. H., Llopis, J., and Dennis, E. A. (2000) *J Immunol* 164, 5398-5402
49. Balsinde, J., Winstead, M. V., and Dennis, E. A. (2002) *FEBS Lett* 531, 2-6
50. Funk, C. D. (2001) *Science* 294, 1871-1875
51. Schumert, R., Towner, J., and Zipser, R. D. (1988) *Dig Dis Sci.* 33, 58S-64S
52. Soberman, R. J., and Christmas, P. (2003) *J Clin Invest.* 111, 1107-1113
53. Moolenaar, W. H., van Meeteren, L. A., and Giepmans, B. N. (2004) *Bioessays* 26, 870-881
54. Prescott, S. M., Zimmerman, G. A., Stafforini, D. M., and McIntyre, T. M. (2000) *Annu Rev Biochem* 69, 419-445
55. Schaloske, R. H., and Dennis, E. A. (2006) *Biochim Biophys Acta* 1761, 1246-1259
56. Six, D. A., and Dennis, E. A. (2000) *Biochim Biophys Acta* 1488, 1-19
57. Dennis, E. A. (1994) *J Biol Chem* 269, 13057-13060
58. Eerola, L. I., Surrel, F., Nevalainen, T. J., Gelb, M. H., Lambeau, G., and Laine, V. J. (2006) *Biochim Biophys Acta* 1761, 745-756
59. Richmond, B. L., Boileau, A. C., Zheng, S., Huggins, K. W., Granholm, N. A., Tso, P., and Hui, D. Y. (2001) *Gastroenterology* 120, 1193-1202
60. Seilhamer, J. J., Pruzanski, W., Vadas, P., Plant, S., Miller, J. A., Kloss, J., and Johnson, L. K. (1989) *J Biol Chem* 264, 5335-5338
61. Buckland, A. G., and Wilton, D. C. (2000) *Biochim Biophys Acta* 1488, 71-82

62. Buckland, A. G., Heeley, E. L., and Wilton, D. C. (2000) *Biochim Biophys Acta* 1484, 195-206
63. Nevalainen, T. J., Haapamaki, M. M., and Gronroos, J. M. (2000) *Biochim Biophys Acta* 1488, 83-90
64. Pruzanski, W., Lambeau, L., Lazdunsky, M., Cho, W., Kopilov, J., and Kuksis, A. (2005) *Biochim Biophys Acta* 1736, 38-50
65. Murakami, M., and Kudo, I. (2003) *Curr Opin Lipidol* 14, 431-436
66. Kennedy, B. P., Soravia, C., Moffat, J., Xia, L., Hiruki, T., Collins, S., Gallinger, S., and Bapat, B. (1998) *Cancer Res* 58, 500-503
67. Tjoelker, L. W., Eberhardt, C., Unger, J., Trong, H. L., Zimmerman, G. A., McIntyre, T. M., Stafforini, D. M., Prescott, S. M., and Gray, P. W. (1995) *J Biol Chem* 270, 25481-25487
68. Tjoelker, L. W., Wilder, C., Eberhardt, C., Stafforini, D. M., Dietsch, G., Schimpf, B., Hooper, S., Le Trong, H., Cousens, L. S., Zimmerman, G. A., and et al. (1995) *Nature* 374, 549-553
69. Min, J. H., Jain, M. K., Wilder, C., Paul, L., Apitz-Castro, R., Aspleaf, D. C., and Gelb, M. H. (1999) *Biochemistry* 38, 12935-12942
70. Gardner, A. A., Reichert, E. C., Topham, M. K., and Stafforini, D. M. (2008) *J Biol Chem* 283, 17099-17106
71. Koenig, W., and Khuseyinova, N. (2008) *Cardiovasc Drugs Ther*
72. Hiraoka, M., Abe, A., and Shayman, J. A. (2002) *J Biol Chem* 277, 10090-10099
73. Clark, J. D., Lin, L. L., Kriz, R. W., Ramesha, C. S., Sultzman, L. A., Lin, A. Y., Milona, N., and Knopf, J. L. (1991) *Cell* 65, 1043-1051
74. Ghosh, M., Tucker, D. E., Burchett, S. A., and Leslie, C. C. (2006) *Prog Lipid Res* 45, 487-510
75. Kramer, R. M., Checani, G. C., Deykin, A., Pritzker, C. R., and Deykin, D. (1986) *Biochim Biophys Acta* 878, 394-403
76. Alonso, F., Henson, P. M., and Leslie, C. C. (1986) *Biochim Biophys Acta* 878, 273-280
77. Kita, Y., Ohto, T., Uozumi, N., and Shimizu, T. (2006) *Biochim Biophys Acta* 1761, 1317-1322

78. Six, D. A., and Dennis, E. A. (2003) *J Biol Chem* 278, 23842-23850
79. Parhamifar, L., Jeppsson, B., and Sjolander, A. (2005) *Carcinogenesis* 26, 1988-1998
80. Kramer, R. M., and Sharp, J. D. (1997) *FEBS Lett* 410, 49-53
81. Han, C., Demetris, A. J., Michalopoulos, G., Shelhamer, J. H., and Wu, T. (2002) *Am J Physiol Gastrointest Liver Physiol* 282, G586-597
82. Heasley, L. E., Thaler, S., Nicks, M., Price, B., Skorecki, K., and Nemenoff, R. A. (1997) *J Biol Chem* 272, 14501-14504
83. Van Putten, V., Refaat, Z., Dessev, C., Blaine, S., Wick, M., Butterfield, L., Han, S. Y., Heasley, L. E., and Nemenoff, R. A. (2001) *J Biol Chem* 276, 1226-1232
84. Soydan, A. S., Tavares, I. A., Weech, P. K., Temblay, N. M., and Bennett, A. (1996) *Eur J Cancer* 32A, 1781-1787
85. Wendum, D., Comperat, E., Boelle, P. Y., Parc, R., Masliah, J., Trugnan, G., and Flejou, J. F. (2005) *Mod Pathol* 18, 212-220
86. Panel, V., Boelle, P. Y., Ayala-Sanmartin, J., Jouniaux, A. M., Hamelin, R., Masliah, J., Trugnan, G., Flejou, J. F., and Wendum, D. (2006) *Cancer Lett* 243, 255-263
87. Dong, M., Guda, K., Nambiar, P. R., Rezaie, A., Belinsky, G. S., Lambeau, G., Giardina, C., and Rosenberg, D. W. (2003) *Carcinogenesis* 24, 307-315
88. Dong, M., Johnson, M., Rezaie, A., Ilsley, J. N., Nakanishi, M., Sanders, M. M., Forouhar, F., Levine, J., Montrose, D. C., Giardina, C., and Rosenberg, D. W. (2005) *Clin Cancer Res* 11, 2265-2271
89. Ilsley, J. N., Nakanishi, M., Flynn, C., Belinsky, G. S., De Guise, S., Adib, J. N., Dobrowsky, R. T., Bonventre, J. V., and Rosenberg, D. W. (2005) *Cancer Res* 65, 2636-2643
90. Hong, K. H., Bonventre, J. C., O'Leary, E., Bonventre, J. V., and Lander, E. S. (2001) *Proc Natl Acad Sci U S A* 98, 3935-3939
91. Takaku, K., Sonoshita, M., Sasaki, N., Uozumi, N., Doi, Y., Shimizu, T., and Taketo, M. M. (2000) *J Biol Chem* 275, 34013-34016

92. Oshima, M., Dinchuk, J. E., Kargman, S. L., Oshima, H., Hancock, B., Kwong, E., Trzaskos, J. M., Evans, J. F., and Taketo, M. M. (1996) *Cell* 87, 803-809
93. Adler, D. H., Cogan, J. D., Phillips, J. A., 3rd, Schnetz-Boutaud, N., Milne, G. L., Iverson, T., Stein, J. A., Brenner, D. A., Morrow, J. D., Boutaud, O., and Oates, J. A. (2008) *J Clin Invest* 118, 2121-2131
94. Dennis, E. A. (2000) *Am J Respir Crit Care Med.* 161, S32-35
95. Smith, W. L., DeWitt, D. L., and Garavito, R. M. (2000) *Annu Rev Biochem* 69, 145-182
96. Smith, W. L., Marnett, L. J., and DeWitt, D. L. (1991) *Pharmacol Ther* 49, 153-179
97. Murakami, M., Das, S., Kim, Y. J., Cho, W., and Kudo, I. (2003) *FEBS Lett* 546, 251-256
98. Murakami, M., and Kudo, I. (2002) *J Biochem (Tokyo)* 131, 285-292
99. Harrison, K. A., and Murphy, R. C. (1995) *J Biol Chem.* 270, 17273-17278
100. Morrow, J. D., Awad, J. A., Boss, H. J., Blair, I. A., and Roberts, L. J. (1992) *Proc Natl Acad Sci U S A.* 89, 10721-10725
101. Rzigalinski, B. A., Willoughby, K. A., Hoffman, S. W., Falck, J. R., and Ellis, E. F. (1999) *J Biol Chem.* 274, 175-182
102. Wendum, D., Masliah, J., Trugnan, G., and Flejou, J. F. (2004) *Virchows Arch* 445, 327-333
103. Warner, T. D., and Mitchell, J. A. (2002) *Proc Natl Acad Sci U S A* 99, 13371-13373
104. Marnett, L. J., and DuBois, R. N. (2002) *Annu Rev Pharmacol Toxicol* 42, 55-80
105. Ferrandez, A., Prescott, S., and Burt, R. W. (2003) *Curr Pharm Des* 9, 2229-2251
106. Sakamoto, C. (1998) *J Gastroenterol* 33, 618-624
107. Li, S., Miner, K., Fannin, R., Carl Barrett, J., and Davis, B. J. (2004) *Gynecol Oncol* 92, 622-627
108. Wu, K. K., Liou, J. Y., and Cieslik, K. (2005) *Arteriosclerosis, thrombosis, and vascular biology* 25, 679-685
109. Cao, Y., and Prescott, S. M. (2002) *J Cell Physiol* 190, 279-286

110. Singer, II, Kawka, D. W., Schloemann, S., Tessner, T., Riehl, T., and Stenson, W. F. (1998) *Gastroenterology* 115, 297-306
111. Williams, C. S., Shattuck-Brandt, R. L., and DuBois, R. N. (1999) *Expert Opin Investig Drugs* 8, 1-12
112. Singh, P., and Mittal, A. (2008) *Mini Rev Med Chem* 8, 73-90
113. Sooriakumaran, P. (2006) *Postgrad Med J* 82, 242-245
114. Rossi, A., Kapahi, P., Natoli, G., Takahashi, T., Chen, Y., Karin, M., and Santoro, M. G. (2000) *Nature* 403, 103-108
115. Simmons, D. L., Botting, R. M., and Hla, T. (2004) *Pharmacol Rev* 56, 387-437
116. Wang, M. T., Honn, K. V., and Nie, D. (2007) *Cancer Metastasis Rev* 26, 525-534
117. Stasinopoulos, I., O'Brien, D. R., Wildes, F., Glunde, K., and Bhujwala, Z. M. (2007) *Mol Cancer Res* 5, 435-442
118. Konturek, P. C., Kania, J., Burnat, G., Hahn, E. G., and Konturek, S. J. (2005) *J Physiol Pharmacol* 56 Suppl 5, 57-73
119. Bhattacharya, M., Peri, K., Ribeiro-da-Silva, A., Almazan, G., Shichi, H., Hou, X., Varma, D. R., and Chemtob, S. (1999) *J Biol Chem* 274, 15719-15724
120. Breyer, M. D., and Breyer, R. M. (2000) *Am J Physiol Renal Physiol* 279, F12-23
121. Samuelsson, B. (2000) *Am J Respir Crit Care Med.* 161, S2-6
122. Bailey, J. M. (1991) *Biofactors* 3, 97-102
123. Levick, S. P., Loch, D. C., Taylor, S. M., and Janicki, J. S. (2007) *J Immunol* 178, 641-646
124. Flamand, N., Mancuso, P., Serezani, C. H., and Brock, T. G. (2007) *Cell Mol Life Sci* 64, 2657-2670
125. Murphy, R. C., and Gijon, M. A. (2007) *Biochem J* 405, 379-395
126. Leier, I., Jedlitschky, G., Buchholz, U., and Keppler, D. (1994) *Eur J Biochem* 220, 599-606
127. Maekawa, A., Kanaoka, Y., Xing, W., and Austen, K. F. (2008) *Proc Natl Acad Sci U S A* 105, 16695-16700
128. Rovati, G. E., and Capra, V. (2007) *ScientificWorldJournal* 7, 1375-1392
129. Samuelsson, B. (1983) *Science* 220, 568-575
130. Capra, V., Thompson, M. D., Sala, A., Cole, D. E., Folco, G., and Rovati, G. E. (2007) *Med Res Rev* 27, 469-527

131. Paruchuri, S., Hallberg, B., Juhas, M., Larsson, C., and Sjolander, A. (2002) *J Cell Sci* 115, 1883-1893
132. Paruchuri, S., and Sjolander, A. (2003) *J Biol Chem* 278, 45577-45585
133. Paruchuri, S., Broom, O., Dib, K., and Sjolander, A. (2005) *J Biol Chem* 280, 13538-13544
134. Mezhybovska, M., Wikstrom, K., Ohd, J. F., and Sjolander, A. (2006) *J Biol Chem* 281, 6776-6784
135. Massoumi, R., and Sjolander, A. (2007) *ScientificWorldJournal* 7, 1413-1421
136. McMahan, B., Stenson, C., McPhillips, F., Fanning, A., Brady, H. R., and Godson, C. (2000) *J Biol Chem* 275, 27566-27575
137. Jiang, Y., Kanaoka, Y., Feng, C., Nocka, K., Rao, S., and Boyce, J. A. (2006) *J Immunol* 177, 2755-2759
138. Thompson, C., Cloutier, A., Bosse, Y., Thivierge, M., Gouill, C. L., Larivee, P., McDonald, P. P., Stankova, J., and Rola-Pleszczynski, M. (2006) *Am J Respir Cell Mol Biol* 35, 697-704
139. Bengtsson, A. M., Massoumi, R., and Sjolander, A. (2008) *Prostaglandins Other Lipid Mediat* 85, 100-106
140. Magnusson, C., Ehrnstrom, R., Olsen, J., and Sjolander, A. (2007) *Cancer Res* 67, 9190-9198
141. Kamohara, M., Takasaki, J., Matsumoto, M., Matsumoto, S., Saito, T., Soga, T., Matsushime, H., and Furuichi, K. (2001) *Biochem Biophys Res Commun* 287, 1088-1092
142. Dahlen, S. E., Bjork, J., Hedqvist, P., Arfors, K. E., Hammarstrom, S., Lindgren, J. A., and Samuelsson, B. (1981) *Proc Natl Acad Sci U S A* 78, 3887-3891
143. Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E., and Smith, M. J. (1980) *Nature* 286, 264-265
144. Hasday, J. D., Meltzer, S. S., Moore, W. C., Wisniewski, P., Hebel, J. R., Lanni, C., Dube, L. M., and Blecker, E. R. (2000) *Am J Respir Crit Care Med* 161, 1229-1236
145. Tohda, Y., Fujimura, M., Taniguchi, H., Takagi, K., Igarashi, T., Yasuhara, H., Takahashi, K., and Nakajima, S. (2002) *Clin Exp Allergy* 32, 1180-1186
146. Sala, A., Zarini, S., and Bolla, M. (1998) *Biochemistry* 63, 84-92
147. Folco, G., and Murphy, R. C. (2006) *Pharmacol Rev* 58, 375-388

148. Paruchuri, S., Mezhybovska, M., Juhas, M., and Sjolander, A. (2006) *Oncogene* 25, 6660-6665
149. Mezhybovska, M., Wikstrom, K., Ohd, J. F., and Sjolander, A. (2005) *Biochemical Society transactions* 33, 698-700
150. Öhd, J. F., Wikström, K., and Sjölander, A. (2000) *Gastroenterology* 119, 1007-1018
151. Sjolander, A., Gronroos, E., Hammarstrom, S., and Andersson, T. (1990) *J Biol Chem* 265, 20976-20981
152. Massoumi, R., Nielsen, C. K., Azemovic, D., and Sjölander, A. (2003) *Exp Cell Res* 289, 342-351
153. Grönroos, E., Thodeti, C. K., and Sjölander, A. (1998) *Cell Calcium* 24, 9-16.
154. Sjölander, A., Grönroos, E., Hammarström, S., and Andersson, T. (1990) *J Biol Chem* 265, 20976-20981
155. Peters-Golden, M., and Brock, T. G. (2003) *Prostaglandins Leukot Essent Fatty Acids* 69, 99-109
156. Nothacker, H. P., Wang, Z., Zhu, Y., Reinscheid, R. K., Lin, S. H., and Civelli, O. (2000) *Mol Pharmacol* 58, 1601-1608
157. Takasaki, J., Kamohara, M., Matsumoto, M., Saito, T., Sugimoto, T., Ohishi, T., Ishii, H., Ota, T., Nishikawa, T., Kawai, Y., Masuho, Y., Isogai, T., Suzuki, Y., Sugano, S., and Furuichi, K. (2000) *Biochem Biophys Res Commun.* 274, 316-322
158. Heise, C. E., O'Dowd, B. F., Figueroa, D. J., Sawyer, N., Nguyen, T., Im, D. S., Stocco, R., Bellefeuille, J. N., Abramovitz, M., Cheng, R., Williams, D. L., Jr., Zeng, Z., Liu, Q., Ma, L., Clements, M. K., Coulombe, N., Liu, Y., Austin, C. P., George, S. R., O'Neill, G. P., Metters, K. M., Lynch, K. R., and Evans, J. F. (2000) *J Biol Chem* 275, 30531-30536.
159. Sarau, H. M., Ames, R. S., Chambers, J., Ellis, C., Elshourbagy, N., Foley, J. J., Schmidt, D. B., Muccitelli, R. M., Jenkins, O., Murdock, P. R., and al., e. (1999) *Mol. Pharmacol.* 56, 657-663
160. Lynch, K. R., O'Neill, G. P., Liu, Q., Im, D. S., Sawyer, N., Metters, K. M., Coulombe, N., Abramovitz, M., Figueroa, D. J., Zeng, Z., Connolly, B. M., Bai, C., Austin, C. P., Chateaneuf, A., Stocco, R., Greig, G. M., Kargman, S., Hooks, S. B., Hosfield, E., Williams, D. L., Jr., Ford-

- Hutchinson, A. W., Caskey, C. T., and Evans, J. F. (1999) *Nature* 399, 789-793.
161. Yokomizo, T., Izumi, T., Chang, K., Takuwa, Y., and Shimizu, T. (1997) *Nature* 387, 620-624
 162. Yokomizo, T., Masuda, K., Kato, K., Toda, A., Izumi, T., and Shimizu, T. (2000) *Am J Respir Crit Care Med* 161, S51-55.
 163. Ciana, P., Fumagalli, M., Trincavelli, M. L., Verderio, C., Rosa, P., Lecca, D., Ferrario, S., Parravicini, C., Capra, V., Gelosa, P., Guerrini, U., Belcredito, S., Cimino, M., Sironi, L., Tremoli, E., Rovati, G. E., Martini, C., and Abbracchio, M. P. (2006) *EMBO J* 25, 4615-4627
 164. Bandeira-Melo, C., and Weller, P. F. (2003) *Prostaglandins Leukot Essent Fatty Acids* 69, 135-143
 165. Capra, V., Ravasi, S., Accomazzo, M. R., Citro, S., Grimoldi, M., Abbracchio, M. P., and Rovati, G. E. (2005) *J Cell Sci* 118, 5625-5636
 166. Pollock, K., and Creba, J. (1990) *Cell Signal* 2, 563-568
 167. Winkler, J. D., Sarau, H. M., Foley, J. J., and Crooke, S. T. (1988) *Biochem Biophys Res Commun* 157, 521-529
 168. Gauvreau, G. M., Plitt, J. R., Baatjes, A., and MacGlashan, D. W. (2005) *J Allergy Clin Immunol* 116, 80-87
 169. Nielsen Kamp, C., Campbell, J., Öhd, J. F., Mörgelin, M., Riesbeck, K., Landberg, G., and Sjölander, A. (2005) *Cancer Res.* 65
 170. Nielsen, C. K., Massoumi, R., Sonnerlind, M., and Sjölander, A. (2005) *Exp Cell Res* 302, 31-39
 171. Öhd, J. F., Nielsen, C. K., Campbell, J., Landberg, G., Löfberg, H., and Sjölander, A. (2003) *Gastroenterology* 124, 57-70
 172. Pedersen, K. E., Bochner, B. S., and Udem, B. J. (1997) *The Journal of pharmacology and experimental therapeutics* 281, 655-662
 173. Bandeira-Melo, C., Woods, L. J., Phoofolo, M., and Weller, P. F. (2002) *J Exp Med* 196, 841-850
 174. Back, M., Norel, X., Walch, L., Gascard, J., de Montpreville, V., Dahlen, S., and Brink, C. (2000) *Eur J Pharmacol* 401, 389-395
 175. Walch, L., Norel, X., Back, M., Gascard, J. P., Dahlen, S. E., and Brink, C. (2002) *Br J Pharmacol* 137, 1339-1345

176. Naik, S., Billington, C. K., Pascual, R. M., Deshpande, D. A., Stefano, F. P., Kohout, T. A., Eckman, D. M., Benovic, J. L., and Penn, R. B. (2005) *J Biol Chem* 280, 8722-8732
177. Sjostrom, M., Jakobsson, P. J., Heimburger, M., Palmblad, J., and Haeggstrom, J. Z. (2001) *Eur J Biochem* 268, 2578-2586
178. Heimburger, M., and Palmblad, J. E. (1996) *Clin Exp Immunol* 103, 454-460
179. Sjostrom, M., Johansson, A. S., Schroder, O., Qiu, H., Palmblad, J., and Haeggstrom, J. Z. (2003) *Arteriosclerosis, thrombosis, and vascular biology* 23, e37-41
180. Uzonyi, B., Lotzer, K., Jahn, S., Kramer, C., Hildner, M., Bretschneider, E., Radke, D., Beer, M., Vollandt, R., Evans, J. F., Funk, C. D., and Habenicht, A. J. (2006) *Proc Natl Acad Sci U S A* 103, 6326-6331
181. Mellor, E. A., Frank, N., Soler, D., Hodge, M. R., Lora, J. M., Austen, K. F., and Boyce, J. A. (2003) *Proc Natl Acad Sci U S A* 100, 11589-11593
182. Qian, X. D., Wei, E. Q., Zhang, L., Sheng, W. W., Wang, M. L., Zhang, W. P., and Chen, Z. (2006) *Eur J Pharmacol* 549, 35-40
183. Jiang, Y., Borrelli, L. A., Kanaoka, Y., Bacskai, B. J., and Boyce, J. A. (2007) *Blood* 110, 3263-3270
184. Schindler, J. F., Monahan, J. B., and Smith, W. G. (2007) *J Dent Res* 86, 800-811
185. Coulombe, P., and Meloche, S. (2007) *Biochim Biophys Acta* 1773, 1376-1387
186. Chen, Z., Gibson, T. B., Robinson, F., Silvestro, L., Pearson, G., Xu, B., Wright, A., Vanderbilt, C., and Cobb, M. H. (2001) *Chem Rev* 101, 2449-2476
187. Ron, D., and Kazanietz, M. G. (1999) *FASEB J* 13, 1658-1676
188. Lawrence, M. C., Jivan, A., Shao, C., Duan, L., Goad, D., Zaganjor, E., Osborne, J., McGlynn, K., Stippec, S., Earnest, S., Chen, W., and Cobb, M. H. (2008) *Cell Res* 18, 436-442
189. Roux, P. P., and Blenis, J. (2004) *Microbiol Mol Biol Rev* 68, 320-344
190. Ralph, J. A., and Morand, E. F. (2008) *Expert Opin Ther Targets* 12, 795-808

191. Delaney, J., Chiarello, R., Villar, D., Kandalam, U., Castejon, A. M., and Clark, M. A. (2008) *Neurochem Res* 33, 545-550
192. Guha, M., O'Connell, M. A., Pawlinski, R., Hollis, A., McGovern, P., Yan, S. F., Stern, D., and Mackman, N. (2001) *Blood* 98, 1429-1439
193. Zhang, R., He, X., Liu, W., Lu, M., Hsieh, J. T., and Min, W. (2003) *J Clin Invest* 111, 1933-1943
194. Dong, W., Liu, Y., Peng, J., Chen, L., Zou, T., Xiao, H., Liu, Z., Li, W., Bu, Y., and Qi, Y. (2006) *J Biol Chem* 281, 26029-26040
195. Hollenbach, E., Neumann, M., Vieth, M., Roessner, A., Malfertheiner, P., and Naumann, M. (2004) *FASEB J* 18, 1550-1552
196. Subbaramaiah, K., Marmo, T. P., Dixon, D. A., and Dannenberg, A. J. (2003) *J Biol Chem* 278, 37637-37647
197. Waetzig, G. H., Seegert, D., Rosenstiel, P., Nikolaus, S., and Schreiber, S. (2002) *J Immunol* 168, 5342-5351
198. Dahan, S., Roda, G., Pinn, D., Roth-Walter, F., Kamalu, O., Martin, A. P., and Mayer, L. (2008) *Gastroenterology* 134, 192-203
199. Lee, J. C., Laydon, J. T., McDonnell, P. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Heys, J. R., Landvatter, S. W., and et al. (1994) *Nature* 372, 739-746
200. Aggarwal, B. B. (2004) *Cancer Cell* 6, 203-208
201. Hayden, M. S., and Ghosh, S. (2004) *Genes Dev* 18, 2195-2224
202. Karin, M., and Ben-Neriah, Y. (2000) *Annu Rev Immunol* 18, 621-663
203. Karin, M., and Delhase, M. (2000) *Semin Immunol* 12, 85-98
204. Gilmore, T. D. (2006) *Oncogene* 25, 6680-6684
205. Mellor, H., and Parker, P. J. (1998) *Biochem J.* 332, 281-292
206. Newton, A. C., and Johnson, J. E. (1998) *Biochim Biophys Acta* 1376, 155-172
207. Liu, J. P. (1996) *Mol Cell Endocrinol* 116, 1-29
208. Bradshaw, D., Hill, C. H., Nixon, J. S., and Wilkinson, S. E. (1993) *Agents Actions.* 38, 135-147

209. Davies, S. P., Reddy, H., Caivano, M., and Cohen, P. (2000) *Biochem J.* 351, 95-105
210. Massoumi, R., Larsson, C., and Sjölander, A. (2002) *J Cell Sci* 115, 3509-3515
211. Schoneberg, T., Schulz, A., and Gudermann, T. (2002) *Rev Physiol Biochem Pharmacol* 144, 143-227
212. Fredriksson, R., Lagerstrom, M. C., Lundin, L. G., and Schioth, H. B. (2003) *Mol Pharmacol* 63, 1256-1272
213. Drews, J. (2000) *Science* 287, 1960-1964
214. Hopkins, A. L., and Groom, C. R. (2002) *Nat Rev Drug Discov* 1, 727-730
215. Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Le Trong, I., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M., and Miyano, M. (2000) *Science* 289, 739-745
216. Van Rhee, A. M., Fischer, B., Van Galen, P. J., and Jacobson, K. A. (1995) *Drug Des Discov* 13, 133-154
217. Buck, L., and Axel, R. (1991) *Cell* 65, 175-187
218. Pierce, K. L., Premont, R. T., and Lefkowitz, R. J. (2002) *Nat Rev Mol Cell Biol* 3, 639-650
219. Kenakin, T. (2004) *Trends in pharmacological sciences* 25, 186-192
220. Dupre, D. J., Rola-Pleszczynski, M., and Stankova, J. (2004) *Biochem Cell Biol* 82, 676-680
221. Lefkowitz, R. J., Cotecchia, S., Samama, P., and Costa, T. (1993) *Trends in pharmacological sciences* 14, 303-307
222. Parnot, C., Miserey-Lenkei, S., Bardin, S., Corvol, P., and Clauser, E. (2002) *Trends Endocrinol Metab* 13, 336-343
223. Allen, L. F., Lefkowitz, R. J., Caron, M. G., and Cotecchia, S. (1991) *Proc Natl Acad Sci U S A* 88, 11354-11358
224. Dorsam, R. T., and Gutkind, J. S. (2007) *Nat Rev Cancer* 7, 79-94
225. Dhanasekaran, N., and Dermott, J. M. (1996) *Cell Signal* 8, 235-245
226. Radhika, V., and Dhanasekaran, N. (2001) *Oncogene* 20, 1607-1614
227. Spiegelberg, B. D., and Hamm, H. E. (2007) *Curr Opin Genet Dev* 17, 40-44
228. Medkova, M., Preininger, A. M., Yu, N. J., Hubbell, W. L., and Hamm, H. E. (2002) *Biochemistry* 41, 9962-9972

229. Thodeti, C. K., Adolfsson, J., Juhas, M., and Sjölander, A. (2000) *J. Biol. Chem.* 275, 9849-9853
230. Milligan, G. (2004) *Mol Pharmacol* 66, 1-7
231. Bai, M. (2004) *Cell Signal* 16, 175-186
232. Couve, A., Filippov, A. K., Connolly, C. N., Bettler, B., Brown, D. A., and Moss, S. J. (1998) *J Biol Chem* 273, 26361-26367
233. Marshall, F. H., White, J., Main, M., Green, A., and Wise, A. (1999) *Biochemical Society transactions* 27, 530-535
234. Pagano, A., Rovelli, G., Mosbacher, J., Lohmann, T., Duthey, B., Stauffer, D., Ristig, D., Schuler, V., Meigel, I., Lampert, C., Stein, T., Prezeau, L., Blahos, J., Pin, J., Froestl, W., Kuhn, R., Heid, J., Kaupmann, K., and Bettler, B. (2001) *J Neurosci* 21, 1189-1202
235. Charara, A., Galvan, A., Kuwajima, M., Hall, R. A., and Smith, Y. (2004) *J Comp Neurol* 476, 65-79
236. Balasubramanian, S., Teissere, J. A., Raju, D. V., and Hall, R. A. (2004) *J Biol Chem* 279, 18840-18850
237. Prinster, S. C., Hague, C., and Hall, R. A. (2005) *Pharmacol Rev* 57, 289-298
238. Hebert, T. E., Moffett, S., Morello, J. P., Loisel, T. P., Bichet, D. G., Barret, C., and Bouvier, M. (1996) *J Biol Chem* 271, 16384-16392
239. Tan, C. M., Brady, A. E., Nickols, H. H., Wang, Q., and Limbird, L. E. (2004) *Annu Rev Pharmacol Toxicol* 44, 559-609
240. Brady, A. E., and Limbird, L. E. (2002) *Cell Signal* 14, 297-309
241. Dodge, K., and Scott, J. D. (2000) *FEBS Lett* 476, 58-61
242. Fraser, I. D., Cong, M., Kim, J., Rollins, E. N., Daaka, Y., Lefkowitz, R. J., and Scott, J. D. (2000) *Curr Biol* 10, 409-412
243. Tsunoda, S., Sierralta, J., Sun, Y., Bodner, R., Suzuki, E., Becker, A., Socolich, M., and Zuker, C. S. (1997) *Nature* 388, 243-249
244. Hall, R. A., and Lefkowitz, R. J. (2002) *Circ Res* 91, 672-680
245. Dong, C., Filipeanu, C. M., Duvernay, M. T., and Wu, G. (2007) *Biochim Biophys Acta* 1768, 853-870
246. Ferguson, S. S. (2001) *Pharmacol Rev* 53, 1-24

247. Pitcher, J. A., Freedman, N. J., and Lefkowitz, R. J. (1998) *Annu Rev Biochem* 67, 653-692
248. Daaka, Y., Luttrell, L. M., and Lefkowitz, R. J. (1997) *Nature* 390, 88-91
249. Zamah, A. M., Delahunty, M., Luttrell, L. M., and Lefkowitz, R. J. (2002) *J Biol Chem* 277, 31249-31256
250. Lawler, O. A., Miggin, S. M., and Kinsella, B. T. (2001) *J Biol Chem* 276, 33596-33607
251. Krupnick, J. G., and Benovic, J. L. (1998) *Annu Rev Pharmacol Toxicol* 38, 289-319
252. De Vries, L., Zheng, B., Fischer, T., Elenko, E., and Farquhar, M. G. (2000) *Annu Rev Pharmacol Toxicol* 40, 235-271
253. Claing, A., Laporte, S. A., Caron, M. G., and Lefkowitz, R. J. (2002) *Prog Neurobiol* 66, 61-79
254. Sorkin, A., and Von Zastrow, M. (2002) *Nat Rev Mol Cell Biol* 3, 600-614
255. Tsao, P., Cao, T., and von Zastrow, M. (2001) *Trends in pharmacological sciences* 22, 91-96
256. Hanyaloglu, A. C., and von Zastrow, M. (2008) *Annu Rev Pharmacol Toxicol* 48, 537-568
257. Anderson, R. G. (1998) *Annu Rev Biochem* 67, 199-225
258. Okamoto, T., Schlegel, A., Scherer, P. E., and Lisanti, M. P. (1998) *J Biol Chem* 273, 5419-5422
259. Engelman, J. A., Zhang, X. L., and Lisanti, M. P. (1998) *FEBS Lett* 436, 403-410
260. Scherer, P. E., Lewis, R. Y., Volonte, D., Engelman, J. A., Galbiati, F., Couet, J., Kohtz, D. S., van Donselaar, E., Peters, P., and Lisanti, M. P. (1997) *J Biol Chem* 272, 29337-29346
261. Gimpl, G., Burger, K., Politowska, E., Ciarkowski, J., and Fahrenholz, F. (2000) *Exp Physiol* 85 Spec No, 41S-49S
262. Gimpl, G., and Fahrenholz, F. (2000) *Eur J Biochem* 267, 2483-2497
263. Smart, E. J., Graf, G. A., McNiven, M. A., Sessa, W. C., Engelman, J. A., Scherer, P. E., Okamoto, T., and Lisanti, M. P. (1999) *Molecular and cellular biology* 19, 7289-7304
264. Engelman, J. A., Lee, R. J., Karnezis, A., Bearss, D. J., Webster, M., Siegel, P., Muller, W. J., Windle, J. J., Pestell, R. G., and Lisanti, M. P. (1998) *J Biol Chem* 273, 20448-20455

265. Yang, G., Truong, L. D., Timme, T. L., Ren, C., Wheeler, T. M., Park, S. H., Nasu, Y., Bangma, C. H., Kattan, M. W., Scardino, P. T., and Thompson, T. C. (1998) *Clin Cancer Res* 4, 1873-1880
266. Fine, S. W., Lisanti, M. P., Galbiati, F., and Li, M. (2001) *Am J Clin Pathol* 115, 719-724
267. Bender, F. C., Reymond, M. A., Bron, C., and Quest, A. F. (2000) *Cancer Res* 60, 5870-5878
268. Wolfe, B. L., and Trejo, J. (2007) *Traffic* 8, 462-470
269. Marchese, A., and Benovic, J. L. (2001) *J Biol Chem* 276, 45509-45512
270. Glickman, M. H., and Ciechanover, A. (2002) *Physiol Rev* 82, 373-428
271. Petaja-Repo, U. E., Hogue, M., Bhalla, S., Laperriere, A., Morello, J. P., and Bouvier, M. (2002) *EMBO J* 21, 1628-1637
272. Shenoy, S. K., McDonald, P. H., Kohout, T. A., and Lefkowitz, R. J. (2001) *Science* 294, 1307-1313
273. Wolfe, B. L., Marchese, A., and Trejo, J. (2007) *J Cell Biol* 177, 905-916
274. Hanyaloglu, A. C., McCullagh, E., and von Zastrow, M. (2005) *EMBO J* 24, 2265-2283
275. Benmerah, A., Poupon, V., Cerf-Bensussan, N., and Dautry-Varsat, A. (2000) *J Biol Chem* 275, 3288-3295
276. Klapisz, E., Sorokina, I., Lemeer, S., Pijnenburg, M., Verkleij, A. J., and van Bergen en Henegouwen, P. M. (2002) *J Biol Chem* 277, 30746-30753
277. Zhang, J., Ferguson, S. S., Barak, L. S., Menard, L., and Caron, M. G. (1996) *J Biol Chem* 271, 18302-18305
278. Shenoy, S. K., and Lefkowitz, R. J. (2003) *J Biol Chem* 278, 14498-14506
279. DeFea, K. A., Zalevsky, J., Thoma, M. S., Dery, O., Mullins, R. D., and Bunnnett, N. W. (2000) *J Cell Biol* 148, 1267-1281
280. Charest, P. G., and Bouvier, M. (2003) *J Biol Chem* 278, 41541-41551
281. Wei, H., Ahn, S., Barnes, W. G., and Lefkowitz, R. J. (2004) *J Biol Chem* 279, 48255-48261
282. Tohgo, A., Pierce, K. L., Choy, E. W., Lefkowitz, R. J., and Luttrell, L. M. (2002) *J Biol Chem* 277, 9429-9436

283. Tohgo, A., Choy, E. W., Gesty-Palmer, D., Pierce, K. L., Laporte, S., Oakley, R. H., Caron, M. G., Lefkowitz, R. J., and Luttrell, L. M. (2003) *J Biol Chem* 278, 6258-6267
284. Chen, Z., Gaudreau, R., Le Gouill, C., Rola-Pleszczynski, M., and Stankova, J. (2004) *Mol Pharmacol* 66, 377-386
285. Kohout, T. A., Lin, F. S., Perry, S. J., Conner, D. A., and Lefkowitz, R. J. (2001) *Proc Natl Acad Sci U S A* 98, 1601-1606
286. van Koppen, C. J., and Jakobs, K. H. (2004) *Mol Pharmacol* 66, 365-367
287. Vines, C. M., Revankar, C. M., Maestas, D. C., LaRusch, L. L., Cimino, D. F., Kohout, T. A., Lefkowitz, R. J., and Prossnitz, E. R. (2003) *J Biol Chem* 278, 41581-41584
288. Colicelli, J. (2004) *Sci STKE* 2004, RE13
289. Schwartz, S. L., Cao, C., Pylypenko, O., Rak, A., and Wandinger-Ness, A. (2007) *J Cell Sci* 120, 3905-3910
290. Seachrist, J. L., and Ferguson, S. S. (2003) *Life Sci* 74, 225-235
291. Bucci, C., Parton, R. G., Mather, I. H., Stunnenberg, H., Simons, K., Hoflack, B., and Zerial, M. (1992) *Cell* 70, 715-728
292. Sonnichsen, B., De Renzis, S., Nielsen, E., Rietdorf, J., and Zerial, M. (2000) *J Cell Biol* 149, 901-914
293. Bremnes, T., Paasche, J. D., Mehlum, A., Sandberg, C., Bremnes, B., and Attramadal, H. (2000) *J Biol Chem* 275, 17596-17604
294. Schievella, A. R., Regier, M. K., Smith, W. L., and Lin, L. L. (1995) *J Biol Chem* 270, 30749-30754
295. Gobeil, F., Jr, D. I., M., M. A., Vazquez-Tello, A., Bernier, S. G., Abran, D., Hou, X., Beauchamp, M. H., Quiniou, C., Bouayad, A., Choufani, S., Bhattacharya, M., Molotchnikoff, S., Ribeiro-Da-Silva, A., Varma, D. R., Bkaily, G., and Chemtob, S. (2002) *Circ Res* 90, 682-689
296. Goetzl, E. J. (2007) *FASEB J* 21, 638-642
297. Zhu, T., Gobeil, F., Vazquez-Tello, A., Leduc, M., Rihakova, L., Bossolasco, M., Bkaily, G., Peri, K., Varma, D. R., Orvoine, R., and Chemtob, S. (2006) *Can J Physiol Pharmacol* 84, 377-391
298. Kobayashi, H., Narita, Y., Nishida, M., and Kurose, H. (2005) *Cell Signal* 17, 1248-1253

299. Gripenrog, J. M., and Miettinen, H. M. (2005) *Cell Signal* 17, 1300-1311
300. Jafri, F., El-Shewy, H. M., Lee, M. H., Kelly, M., Luttrell, D. K., and Luttrell, L. M. (2006) *J Biol Chem* 281, 19346-19357
301. Bhattacharya, M., Peri, K. G., Almazan, G., Ribeiro-da-Silva, A., Shichi, H., Durocher, Y., Abramovitz, M., Hou, X., Varma, D. R., and Chemtob, S. (1998) *Proc Natl Acad Sci U S A* 95, 15792-15797
302. Bazan, N. G., Squinto, S. P., Braquet, P., Panetta, T., and Marcheselli, V. L. (1991) *Lipids* 26, 1236-1242
303. Yudina, Y., Parhamifar, L., Bengtsson, A. M., Juhas, M., and Sjolander, A. (2008) *Prostaglandins Leukot Essent Fatty Acids* 79, 223-231
304. Guan, X. M., Kobilka, T. S., and Kobilka, B. K. (1992) *J Biol Chem* 267, 21995-21998