Thesis for doctoral degree (Ph.D.)
2008

A study on the E3 ligase Trim21/Ro52

Alexander Espinosa

Thesis for doctoral degree (Ph.D.) 2008

A study on the E3 ligase TRIM21/Ro52

Alexander Espinosa

Karolinska Institutet

Karolinska Institutet

From the Department of Medicine,
Karolinska Institutet, Stockholm, Sweden

A study on the E3 ligase Trim21/Ro52

Alexander Espinosa

Published by Karolinska Institutet.
Printed by Larserics Digital Print AB, Box 20082, SE-161 02 Bromma, Sweden.

ISBN 978-91-7357-480-8

ABSTRACT

Patients with the systemic autoimmune diseases Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE), often have antibodies against the intracellular protein TRIM21/Ro52. Although the presence of anti-TRIM21/Ro52 autoantibodies is used as a diagnostic tool, the biological function of TRIM21/Ro52 is still unknown. The aim of this thesis is to provide a better understanding of the function of TRIM21/Ro52, especially regarding its role in autoimmunity. To achieve this, TRIM21/Ro52 was studied at a molecular and cellular level in vitro and in vivo. By using circular dichroism, limited proteolysis, mass spectrometry, ultra-centrifugation, and two-hybrid experiments, it was shown that TRIM21/Ro52 is a Zn^{2+} binding protein that forms weak dimers. These results also confirmed the presence of the predicted secondary structure domains of TRIM21/Ro52. A RING domain in the N-terminus of TRIM21/Ro52 suggests that TRIM21/Ro52 is a RING dependent E3 ligase. Through ubiquitination assays, it was shown that TRIM/Ro52 is indeed an E3 ligase and that the E3 ligase activity of TRIM21/Ro52 was dependent on the E2 enzymes UbcH5a-c or UbcH6. Furthermore, anti-RING autoantibodies from patients with SS and SLE blocked the E3 activity of TRIM21/Ro52 in vitro. Since the expression of many TRIM proteins is induced by interferons, the effect of interferon alpha (IFNα) and virus on TRIM21/Ro52 expression was investigated. After exposing the cell lines HeLa, Daudi and Raji to IFNα, TRIM21/Ro52 expression was increased both at the mRNA and protein level. In addition, TRIM21/Ro52 expression was increased in human peripheral blood mononuclear cells (PBMC) exposed to inactivated herpes simplex virus. TRIM21/Ro52 was also expressed at a higher level in PBMCs from patients with SS and SLE than in PBMCs from healthy individuals, which could be interpreted as an effect of the interferon signature typical of SS and SLE patients. To investigate the effect of overexpressing TRIM21/Ro52 in B cells, stable transfected cell lines were made and characterized. B cells overexpressing TRIM21/Ro52 proliferated slower and were more sensitive to induced cell death; which was in contrast to B cells expressing a TRIM21/Ro52 mutant lacking E3 ligase activity. Thus, TRIM21/Ro52 is involved in regulating proliferation, and activation status, of B lymphocytes. The intracellular localization of TRIM21/Ro52 was determined by transfecting HeLa cells with GFP-Ro52 and GFP-Ro52 mutants. Two stretches of amino acids (aa) were found to be important for the cytoplasmic localization and nuclear import of TRIM21/Ro52. When the aa 203-248 were deleted, TRIM21/Ro52 entered the nucleus; but the deletion of aa 381-470 prevented the nuclear import of TRIM21/Ro52. In addition, exposure to IFNα and nitric oxide induced translocation of TRIM21/Ro52 from the cytoplasm to the nucleus. In conclusion, TRIM21/Ro52 is a cytoplasmic E3 ligase that is overexpressed in PBMCs from patients with SS and SLE. TRIM21/Ro52 is induced by IFNα and herpes simplex virus, and inhibits cell proliferation.

LIST OF PUBLICATIONS

I. Ottosson L, Hennig J, Espinosa A, Brauner S, Wahren-Herlenius M, Sunnerhagen M. **Structural, functional and immunologic characterization of folded subdomains in the Ro52 protein targeted in Sjögren's syndrome**. Mol Immunol. 2006 Feb;43(6):588-98.

II. Espinosa A, Zhou W*, Ek M*, Hedlund M, Brauner S, Popovic K, Horvath L, Wallerskog T, Oukka M, Nyberg F, Kuchroo VK, Wahren-Herlenius M. **The Sjögren's syndrome-associated autoantigen Ro52 is an E3 ligase that regulates proliferation and cell death**. J Immunol. 2006 May 15;176(10):6277-85.

III. Espinosa A*, Hennig J*, Ambrosi A, Elfving Å, Sandberg M, Sheng Y, Nyberg F, Arrowsmith C, Sunnerhagen M, Wahren-Herlenius M. **Antibodies from patients with Sjögren's syndrome penetrate living cells and inhibit the E3 ligase of Ro52.** Submitted.

IV. Espinosa A, Oke V, Elfving Å, Covacu R, Wahren-Herlenius M. **The auto-antigen Ro52 is a cytoplasmic E3 ligase that enters the nucleus after nitric oxide exposure.** Submitted.

V. Strandberg L*, Ambrosi A*, Espinosa A, Ottosson L, Eloranta ME, Zhou W, Elfving Å, Greenfield E, Kuchroo VK and Wahren-Herlenius M. **Interferon-alpha induces nuclear translocation of the Ro52 autoantigen as detected by a panel of novel Ro52-specific monoclonal antibodies.** J Clin Immunol (in press).

VI. Popovic K, Ek M, Espinosa A, Padyukov L, Harris HE, Wahren-Herlenius M, Nyberg F. **Increased expression of the novel proinflammatory cytokine high mobility group box chromosomal protein 1 in skin lesions of patients with lupus erythematosus.** Arthritis Rheum. 2005 Nov;52(11):3639-45.

* These authors contributed equally

LIST OF ABBREVIATIONS

aa	**amino acid**
CD	**cluster of differentiation**
cDNA	**complementary deoxyribonucleic acid**
DNA	**deoxyribonucleic acid**
DUB	**deubiquitinating enzyme**
E1	**ubiquitin activating enzyme**
E2	**ubiquitin conjugating enzyme**
E3	**ubiquitin ligase**
ELISA	**enzyme linked immunosorbent assay**
GFP	**green fluorescent protein**
HMGB-1	**High mobility group box 1**
HSV	**herpes simplex virus**
IFN	**interferon**
Ig	**immunoglobulin**
IRES	**internal ribosome entry site**
IRF	**interferon regulatory factor**
ISRE	**interferon stimulated response element**
kb	**kilobases**
kDa	**kilo Dalton**
LMB	**leptomycin B**
MHC	**major histocompatibility complex**
MS	**mass spectrometry**
mRNA	**messenger ribonucleic acid**
NES	**nuclear export signal**
NLS	**nuclear localization signal**
NO	**nitric oxide**
PBMC	**peripheral blood mononuclear cell**
Q-RT-PCR	**quantitative reverse-transcriptase polymerase chain reaction**
RFP	**red fluorescent protein**
RING	**really interesting new gene**
SDS-PAGE	**sodium dodecyl sulfate polyacrylamide gel electrophoresis**
SLE	**systemic lupus erythematosus**
SS	**Sjögren's syndrome**
Tg	**transgenic**
TLR	**toll-like receptor**
TRIM	**tripartite motif**

CONTENTS

INTRODUCTION

The main subject of this thesis is the E3 ligase Ro52, and in particular its role in immunity. Ro52 was first described as an autoantigen in systemic autoimmune rheumatic diseases, although, lately its role as an ubiquitin ligase has gained increased attention. With this in mind, this introduction begins with a description of the ubiquitin system and how the post-translational modification with ubiquitin is important for the degradation, activation, and sorting of intracellular proteins. This is followed by an outline of the immune system, autoimmunity and autoimmune disease, and the role of the ubiquitin system in the regulation of immune responses. Finally, the main protagonist of this thesis, Ro52, will be presented in detail and the eventual role of Ro52 in immunity and autoimmune disease will be discussed.

The ubiquitin system

The intracellular concentration, localization, and activity of proteins must be carefully controlled in order for cells to function normally. This control is achieved by regulating several biological processes, e.g. the translation of messenger ribonucleic acid (mRNA), protein transport, protein modification, and protein turnover. One important regulatory tool in eukaryotic cells is the post-translational covalent modification of proteins with *ubiquitin* (Goldstein et al., 1975), a polypeptide of 76 amino acid (aa) residues (Schlesinger et al., 1975, Schlesinger and Goldstein, 1975, Wilkinson and Audhya, 1981). The post-translational modification with ubiquitin, and the handling of ubiquitinated proteins, is carried out by the ubiquitin system, reviewed in (Weissman, 2001). This system is highly conserved among eukaryotes (Sharp and Li, 1987, Tanaka et al., 1992) and can be divided into two interconnected modules: one, modifying proteins with ubiquitin; and another, recognizing ubiquitinated proteins and connecting them to effector molecules through ubiquitin binding adaptor proteins.

Ubiquitin was discovered in 1975 by Goldstein and coworkers (Goldstein et al., 1975) when searching for novel thymic hormones. They purified ubiquitin from bovine thymus and described it as a differentiation factor for thymocytes and pre-B

cells. However, this described role of ubiquitin in lymphocyte differentiation has failed to be replicated. Instead, the breakthrough in the ubiquitin field came in 1978 when Ciechanover et al. (Ciehanover et al., 1978) demonstrated that ubiquitin was involved in intracellular non-lysosomal proteolysis. Later publications by the same investigators (Hershko et al., 1979, Ciechanover et al., 1980, Hershko et al., 1980, Hershko et al., 1981, Ciechanover et al., 1981) proved that the observed proteolysis was dependent on adenosine triphosphate (ATP) and $MgCl_2$, and required at least three novel protein factors called E1, E2 and E3 (Hershko et al., 1983). This work was later rewarded with the Nobel Prize in Chemistry in 2004.

Ubiquitin and ubiquitin like proteins. The human and mouse genomes have numerous ubiquitin genes. Although most of them are pseudogenes, four functional ubiquitin genes have been identified: UbB, UbC, and two UbA genes (**Table 1**).

Table 1. Ubiquitin genes

Gene		Locus	Genbank ID	Reference
Uba52	(*M. musculus*)	Chr8, 35.0 cM	22186	(Sun and Wuthrich, 1999)
UBA52	(*H. sapiens*)	19p13.1-p12	7311	(Webb et al., 1994)
Rps27A	(*M. musculus*)	Chr11, 11 A3.3	78294	(Gerhard et al., 2004)
RPS27A	(*H. sapiens*)	2p16	6233	(Kenmochi et al., 1998)
Ubc	(*M. musculus*)	Chr5, 64 cM	22190	(Klingenspor et al., 1997)
UBC	(*H. sapiens*)	12q24.3	7316	(Board et al., 1992)
Ubb	(*M. musculus*)	Chr11, 35.0 cM	22187	(Klingenspor et al., 1997)
UBB	(*H. sapiens*)	17p12-p11.2	7314	(Webb et al., 1990)

Ubiquitin is not expressed as a free monomer, but as an inactive precursor; either as a polymer (polyubiquitin) or as fusions to ribosomal subunits (Ozkaynak et al., 1984, Wiborg et al., 1985, Lund et al., 1985, Ozkaynak et al., 1987, Finley et al., 1989, Baker and Board, 1991). In man and mouse, the UbA genes (UbA52 and UbA80/Rps27a) encode one ubiquitin molecule fused to the N-terminus of a ribosomal subunit. After transcription and translation, an active ubiquitin molecule with two C-terminal glycine residues is released from the ribosomal subunit fusion partner through endopeptidase cleavage (Agell et al., 1991). The UbA52 gene encodes ubiquitin fused to the L40 ribosomal subunit (Stoffler et al., 1974), while UbA80/Rps27a encodes ubiquitin fused to the S27a ribosomal subunit (Collatz et

al., 1976). In contrast to the UbA genes, UbB and UbC are polyubiquitin genes encoding head-to-tail tandem repeats of ubiquitin. After translation, active ubiquitin moieties are released by endopeptidase cleavage. To ensure that the ubiquitin precursors are inactive, the last ubiquitin repeat in polyubiquitin genes encodes an incomplete ubiquitin moiety lacking the C-terminal diglycine motif that is necessary for activity.

Table 2. Ubiquitin like proteins

Ubl	Function	Reference
ISG15	Antiviral response; cytokine	(Farrell et al., 1979, Reich et al., 1987, Haas et al., 1987)
Nedd8	Transciption**;** proteolysis	(Kumar et al., 1992)
SUMO-1	Localization; transcription	(Shen et al., 1996, Howe et al., 1998)
FAT10	Apoptosis; proteolysis	(Fan et al., 1995, Bates et al., 1997)

Since the discovery of ubiquitin, several ubiquitin like proteins (Ubls) have been described (**Table 2**). Ubls work in an analogous fashion to ubiquitin, although their roles in the physiology of the cell differ (Yeh et al., 2000, Kirkin and Dikic, 2007). Examples of Ubls are small ubiquitin-related modifier (SUMO), neural precursor cell expressed, developmentally down-regulated 8 (Nedd8), HLA-F adjacent transcript 10 (FAT10) and interferon stimulated gene 15 (ISG15).

The primary structures of ubiquitin and Ubls are not very similar; nevertheless, the adopted folds of ubiquitin and Ubls are almost identical (Bayer et al., 1998) (**Fig 1**). Ubiquitin has a compact folded structure (Vijay-Kumar et al., 1985, Vijay-Kumar et al., 1987b, Vijay-Kumar et al., 1987a) and an extraordinary resilience to extreme temperatures, pH and salt concentrations (Lenkinski et al., 1977).

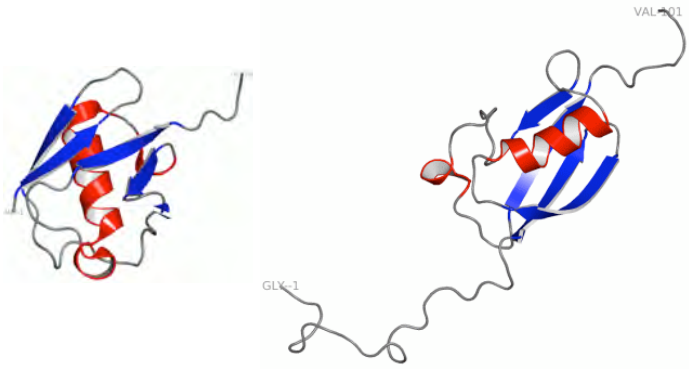

Figure 1. Ubiquitin and SUMO-1. Ubiquitin **(left)** and SUMO-1 **(right)** have a relatively low sequence similarity; even so, the two proteins adopt a similar fold.

Post-translational modification with ubiquitin. In contrast to other post-translational modifications, such as phosphorylation, every single modification with ubiquitin is dependent on several types of enzymes. Before being covalently attached to a target protein, ubiquitin first has to be bound and activated by a ubiquitin activating enzyme (E1) (Ciechanover et al., 1981, Handley et al., 1991, Disteche et al., 1992). This occurs through an ATP dependent mechanism were the C-terminal glycine residue (G76) of ubiquitin forms a thioester bond with a catalytic cysteine residue in the active site of a ubiquitin-activating enzyme (E1) (Hatfield and Vierstra, 1992). The ubiquitin molecule is then transferred from the active site on E1 to a cysteine residue on a ubiquitin-conjugating enzyme (E2) (Jentsch et al., 1987, Goebl et al., 1988, Scheffner et al., 1994). The final step involves a ubiquitin ligase (E3) (Hershko et al., 1986, Bartel et al., 1990) that mediates the transfer of ubiquitin from an E2 to an ε-amino group in a lysine residue of a target protein (Chau et al., 1989). Alternatively, ubiquitin can be attached to the N-termini of proteins (Bachmair et al., 1986, Breitschopf et al., 1998). Due to the simultaneous binding of E2 and E3 to a substrate, the combination of E2 and E3 enzymes determines the specificity of the ubiquitination process (Zheng et al., 2000).

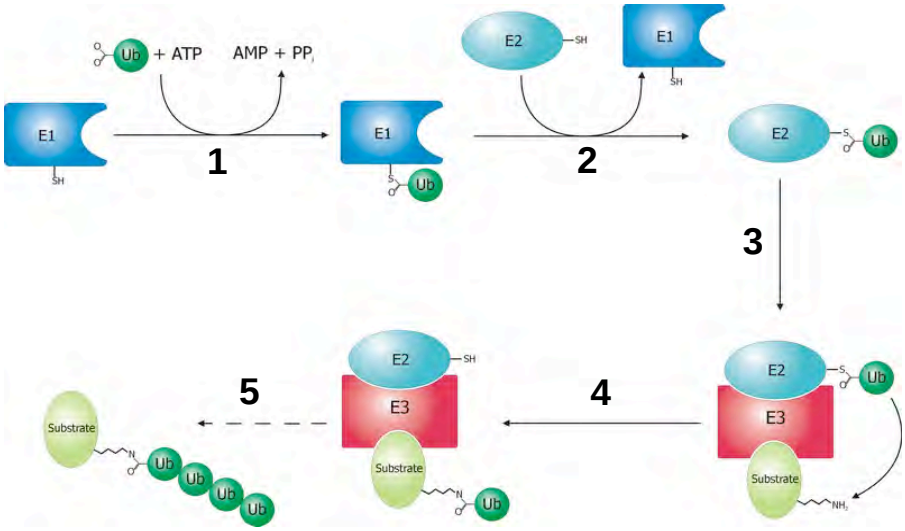

Figure 2. Post-translational modification with ubiquitin. (1) The C-terminal glycine (G76) of a ubiquitin molecule is activated and bound by the E1 enzyme in an ATP dependent manner. **(2)** The ubiquitin molecule is transferred to an E2 enzyme and attached to a protein substrate with the help of an E3 ligase **(3-4)**. Several ubiquitin molecules can be sequentially attached to form a polyubiquitin chain by repeating step 4 **(5)**.

RING E3 ligases. The most studied types of E3 ligases are those dependent on the really interesting new gene (RING) domain (Freemont et al., 1991, Lovering et al., 1993) or the homologous to E6AP carboxyl terminus (HECT) domain (Huibregtse et al., 1995). While RING E3 ligases function as scaffold proteins bringing the E2 and target protein together, HECT E3 ligases act as bona fide enzymes by binding ubiquitin covalently before transferring it to the target protein.

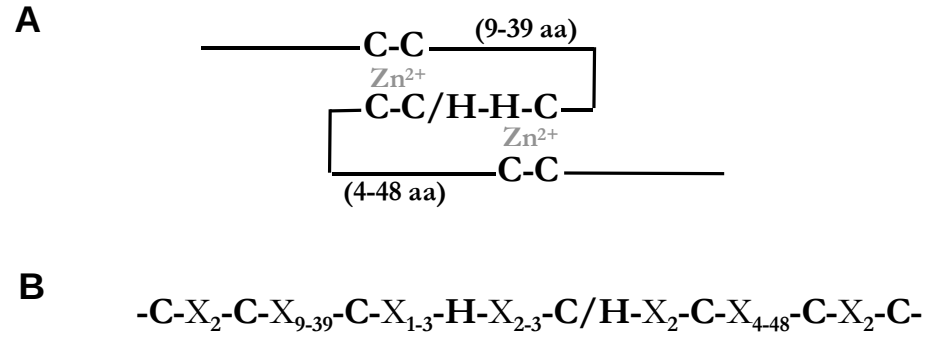

Figure 3. The RING fold and the RING consensus sequence. (A) The RING domain folds around two Zn^{2+} ions in a cross-bracelet like structure. **(B)** The consensus sequence of RING domains; note that the first two, and last two, cysteine residues are always separated by two amino acid residues. The variation in size of the two RING loops is large, from 9 to 39 aa residues and 4 to 48 aa residues respectively.

When first identified through sequence database mining (Freemont et al., 1991), the RING domain, due to its high similarity with the classic zinc finger motif (Berg, 1990), was believed to be a DNA binding domain. Later, however, the RING domain was shown to mediate protein-protein interactions, especially the binding of E3 ligases to E2s (Reiss et al., 1989, Zheng et al., 2000). When the first draft of the humane genome was published, the RING domain was reported to be the 15th most common domain in the human genome (Lander et al., 2001). RING domains are 30-110 aa residues large and fold around two Zn^{2+} ions in a cross-bracelet like structure (**Fig 3A**); this folding is dependent on eight conserved cysteine and histidine residues (**Fig 3B**) that coordinate the Zn^{2+} ions. The first atom level structure of a RING domain was published in 1994 (Barlow et al., 1994).

Ubiquitination is reversible. Analogous to phosphorylation, ubiquitination is a reversible process. This deubiquitination is mediated by a large group of deubiquitinating enzymes (DUBs) (Agell et al., 1991, Amerik and Hochstrasser, 2004), that cleave ubiquitin after the last glycine residue (G76) thereby releasing intact ubiquitin molecules from ubiquitin chains or ubiquitin conjugates.

DUBs are important in the production of active ubiquitin monomers through the processing of inactive ubiquitin precursors. In addition, DUBs edit ubiquitin conjugates on target proteins ('proofreading') and recycles ubiquitin monomers from ubiquitin-modified proteins that are degraded in the proteasome. Consequently, DUBs maintain and regulate the intracellular pool of free ubiquitin (Haas and Bright, 1987).

Ubiquitin topology. Importantly, a protein can be modified by ubiquitin in several ways. Three types of ubiquitination can be identified (**Fig 4**): monoubiquitination, multiubiquitination and polyubiquitination reviewed in (Roos-Mattjus and Sistonen, 2004). However, it cannot be ruled out that combinations of these might occur.

Monoubiquitination is the modification of a target protein with one single ubiquitin moiety, while multiubiquitination is the modification of a target protein with several single ubiquitin moieties, each on a separate lysine residue. In contrast, polyubiquitination is the result of adding a chain of ubiquitin moieties. These polyubiquitin chains are probably built through the sequential attachment of ubiquitin moieties through their lysine residues (Chen and Pickart, 1990), although it has been suggested that polyubiquitination also might occur through the addition of a preformed ubiquitin chain (Li et al., 2007a).

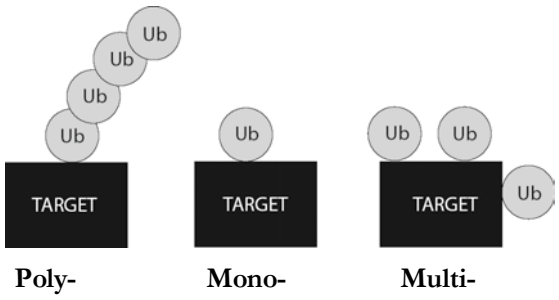

Figure 4. Modification with ubiquitin. A protein can be modified by polyubiquitin, monoubiquitin and multiubiquitin. K48 polyubiquitin chains of >4 ubiquitin moieties are targeted to the proteasome.

Polyubiquitin chains can be formed through different lysine residues in ubiquitin (Kirkpatrick et al., 2005). Since one ubiquitin moiety has seven lysine residues (K6, K11, K27, K29, K33, K48 and K63) at least seven different types of polyubiquitin chains are possible; and even more can be expected since polyubiquitin chains can divide and form branches (Peng et al., 2003, Kim et al., 2007). E.g., the sequential attachment of ubiquitin moieties can occur through lysine 48 (K48) or lysine 63 (K63). Proteins modified with K48 chains are degraded in the 26S proteasome (Hershko and Heller, 1985, Chau et al., 1989), while K63 chains (Spence et al., 1995) may protect proteins from proteasome mediated degradation and is necessary for DNA repair. The topology of K48 and K63 polyubiquitin chains are distinct: whereas K48 chains have a closed formation driven by hydrophobic interactions (Cook et al., 1992, Cook et al., 1994, Beal et al., 1998, Phillips et al., 2001, Varadan et al., 2002) K63 chains adopt a more extended conformation (Varadan et al., 2004). The conformation of K63 chains hypothetically allows for an easier access for binding of ubiquitin binding domain (UBD) containing proteins, while the more compact K48 conformation might be harder to access for UBDs.

Although ubiquitin topology is a fascinating subject, one word of caution is in order. All the studies on ubiquitin topology have been made in cell free reconstituted in vitro systems. It is unclear how these results translate into the situation in vivo. The development of new experimental tools is required to study the importance of different ubiquitin modifications in vivo. E.g., antibodies specific for different polyubiquitin topologies and refinement of existing techniques such as mass spectrometry (MS) (Kirkpatrick et al., 2005), fluorescence resonance energy transfer (FRET) or similar techniques (Boisclair et al., 2000, Hong et al., 2003, Perroy et al., 2004, Hoeller et al., 2006, Fang and Kerppola, 2004),

The different fates of ubiquitin modified proteins. The classical view, that ubiquitination mainly is important for protein degradation in the non-lysosomal compartment, is still dominating ubiquitin research. However, it is now appreciated that ubiquitination is a process that is involved in many facets of cell biology. It was early shown that plasma membrane proteins could be modified by ubiquitin (Yarden et al., 1986, Leung et al., 1987) especially after binding to an extracellular ligand (Mori et al., 1993). That the internalization and degradation of plasma membrane proteins is mediated by ubiquitination was first discovered in yeast (Kolling and Hollenberg, 1994, Hicke and Riezman, 1996) and later in mammalian cells (Strous et al., 1996). In addition, ubiquitination is also necessary for the activation of several transcription factors either in a proteasome dependent manner (Palombella et al., 1994) or independently of the proteasome (Kaiser et al., 2000).

Transcriptional activity is also modified by the ubiquitination of histones (Levinger and Varshavsky, 1982) as reviewed in (Muratani and Tansey, 2003).

Protein degradation in the proteasome. The 26S proteasome (DeMartino and Goldberg, 1979, Rose et al., 1979, Wilk and Orlowski, 1980, Orlowski and Wilk, 1981) is a 2.4 MDa large dynamic protein complex dedicated to degrade proteins modified with K48 polyubiquitin chains, containing at least four ubiquitin moieties (Thrower et al., 2000), into oligopeptides. It consists of two multisubunit complexes: one 20S proteolytic core complex and one 19S regulatory complex (Arrigo et al., 1988) (**Fig 5A**).

The 20S core proteasome is barrel shaped and built out of four stacked heptameric rings (Tanaka et al., 1988b, Tanaka et al., 1988a, Unno et al., 2002) (**Fig 5B)**. The two outer rings are composed of seven α subunits (α1-α7) while the two middle rings our composed of seven β subunits (β1-β7) resulting in a $\alpha_7\beta_7\beta_7\alpha_7$ arrangement. The proteolytic chamber is accessible through the 13Å wide openings at the ends of the cylinder. Entry into the core proteasome is regulated by the α-subunits in the outer rings, while the proteolytic activity of the proteasome is mediated by the β_1, β_2 and β_5 subunits on the two middle rings (Hilt et al., 1993, Zwickl et al., 1994, Lowe et al., 1995, Fenteany et al., 1995).

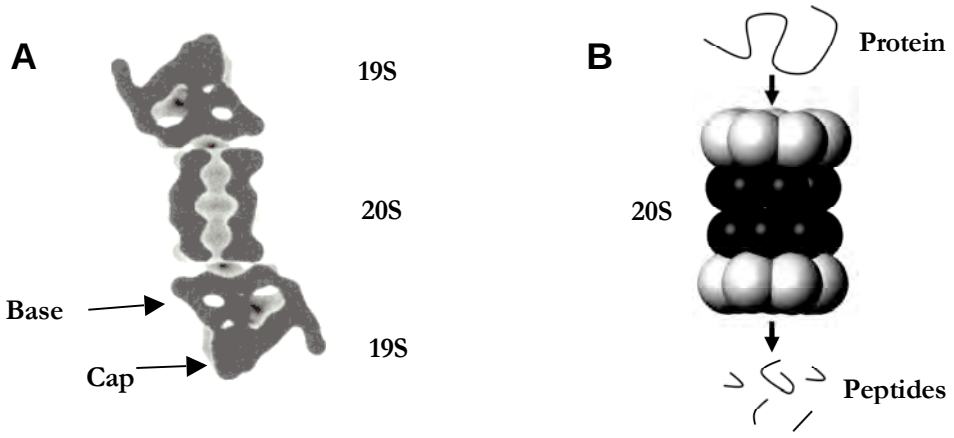

Figure 5. The proteasome. (A) A longitudinal section of the 26S proteasome showing the barrel shaped core proteolytic 20S proteasome, and two regulatory 19S proteasomes. **(B)** The 20S proteasome is composed of four rings each consisting of seven subunits; the proteolytic activity is confined to subunits of the two middle rings.

In contrast to the 20S proteasome, the 19S proteasome does not have proteolytic activity, but rather regulates the entry of ubiquitinated protein substrates into the 20S proteasome. The 19S proteasome 'base' is composed of six subunits (Rpt1-Rpt6) with ATPase activity arranged in a ring like structure and placed axially to the 20S proteasome (Dubiel et al., 1992, Lucero et al., 1995, Glickman et al., 1998, Baumeister et al., 1998). Protruding from this ATPase base is a 'cap' (**Fig 5A**); this 19S cap structure recognizes polyubiquitin chains on proteins to be degraded. In addition, the 19S proteasome unfolds protein substrates, removes ubiquitin chains and funnels proteins into the proteolytic core of the 20S proteasome, reviewed in (Pickart, 1997, Wolf and Hilt, 2004).

The composition of the 20S proteasome is modified in response to e.g. viral infections. Infections with viruses, or intracellular bacteria, induce the production of interferon γ (IFNγ) which in turn leads to the expression of new proteasomal subunits creating a modified proteasome, the immunoproteasome, which produces peptides with enhanced binding to the major histocompatibility complex I (MHC I) cleft. (Yang et al., 1992, Gaczynska et al., 1993).

Proteasome-independent roles of ubiquitination. Ubiquitination of proteins does not necessarily lead to their degradation in the proteasome. The modification of a target protein with ubiquitin may e.g. lead to the sorting into the lysosome (Strous et al., 1996) or modification of transcriptional activity (Ostendorff et al., 2002).

The termination of receptor signaling in response to excessive amounts of extracellular ligands, i.e. receptor 'downregulation', is an important negative feed back mechanism, for reviews see (Fiorini et al., 2001, Dikic and Giordano, 2003). One major such negative feedback mechanism is the endocytosis of transmembrane proteins, after which they are either degraded in the lysosomes, or recycled back to the plasma membrane. The decision between recycling or degradation is regulated by the ubiquitin system (Hicke, 1997, Hicke, 2001), for review see (Mukhopadhyay and Riezman, 2007). It is commonly observed, that mammalian cells surface receptors, such as receptor tyrosine kinases (RTKs) and G-protein coupled receptors (GPCRs), are internalized into the endosomal compartment. E.g., the RTK endothelial growth factor receptor (EGFR) is ubiquitinated and internalized after exposure to high concentration of ligand (Stang et al., 2000, de Melker et al., 2001, Sigismund et al., 2005). In the article by Stang et al., polyubiquitination of the EGFR was reported, indicating a possible role for the proteasome. However, due to technical difficulties of differentiating between multiubiquitination and polyubiquitination (both modifications might be detected as smears after immunoblotting) the issue of polyubiquitination versus

monoubiquitination is not altogether clear. Haglund et al. (Haglund et al., 2003) used molecular biology techniques and antibodies with different affinities for mono- and polyubiquitin, to show that the internalization of EGFR is indeed dependent on multiubiquitination and not polyubiquitination.

Recycling of internalized cargos to the cell surface occurs by default (Mayor et al., 1993); in contrast, cargos to be sorted into the lysosomes need to be labeled with ubiquitin through the coordinated action of E1, E2, and E3 proteins. The exact mechanism by which ubiquitin mediates the sorting of internalized cargos to the lysosomes is still being investigated. In general, the recognition of ubiquitinated cargos is mediated by UBD containing proteins, e.g. Eps15, Hrs, and Tsg10, that function as sorting receptors (Benmerah et al., 1995, Babst et al., 2000, Polo et al., 2002, Lu et al., 2003). Internalized cargos to be degraded in the lysosome are transported through the early endosomal compartment to the late endosomal compartment. Invagination of the limiting membrane of late endosomes leads to the inward budding of intralumenal vesicles (ILVs); forming ILV-containing late endosomes called multivesicular bodies (MVBs). After fusion of MVBs with lysosomes, ILVs and their content (including membrane proteins) are degraded by lysosomal enzymes (Piper and Katzmann, 2007).

In all, ubiquitinated proteins from the cell surface or the late Golgi complex are directed to MVBs, preferentially incorporated into ILVs and finally directed to the lysosomes. Non-ubiquitinated proteins are recycled from the endosomes or MVBs. In contrast to membrane proteins or proteins in the late Golgi network, proteins in the early secretory compartments are degraded by the proteasome through endoplasmic reticulum-associated degradation (ERAD) (Meusser et al., 2005, Kostova et al., 2007).

Ubiquitin binding domains. The recognition of ubiquitin-modified proteins is achieved through ubiquitin binding proteins. Recognition of ubiquitinated protein is mediated through UBD domains reviewed in (Hicke et al., 2005), and the broad recognition specificity is achieved through the use of a multitude of different UBD domains (**Table 3**). UBDs have a low to moderate affinity for ubiquitin and this raises the question whether they bind preferentially to monoubiquitin or polyubiquitin (Hicke et al., 2005). Intuitively, ubiquitin receptors that bind polyubiquitinated proteins and deliver them to the proteasome have a high affinity for polyubiquitin.

The hierarchical organization of the ubiquitin system. Every post-translational modification of a protein presents a problem of specificity. How can a specific protein be ubiquitinated while leaving bystander proteins unmodified?

Moreover, how can the ubiquitin system specifically recognize a certain ubiquitinated protein? The ubiquitin system solves the specificity problem of ubiquitination by using multiple proteins to modify target proteins, and multiple proteins to recognize ubiquitinated proteins. Thus, a large number of E3 ligases (>400) are needed in order to achieve the high specificity; in contrast, fewer E2s (~50) and only one E1 are required.

Table 3. Ubiquitin binding domains

Ubiquitin binding domain		Reference
UBA	(ubiquitin-associated domain)	(Hofmann and Bucher, 1996)
UEV	(ubiquitin-conjugating E2 enzyme variant)	(Sancho et al., 1998)
UIM	(ubiquitin-interacting motif)	(Hofmann and Falquet, 2001)
NZF	(Npl4 zinc finger)	(Meyer et al., 2002)
PAZ	(polyubiquitin-associated zinc finger)	(Hook et al., 2002)
CUE	(coupling of ubiquitin conjugation to ERAD)	(Donaldson et al., 2003, Shih et al., 2003)
VHS	(Vps27, HRS, STAM)	(Mizuno et al., 2003)
GAT	(Gga and Tom1)	(Shiba et al., 2004)
GLUE	(GRAM-like ubiquitin-binding in Eap45)	(Slagsvold et al., 2005)

The deletion of E1 is lethal in yeast and metazoans (McGrath et al., 1991, Zacksenhaus and Sheinin, 1990), whereas the deletion of E2s or E3s may be lethal but often lead to varying less severe phenotypes (**Table 4**). Interestingly, mice lacking some E2s and E3s manifest phenotypes with a large immunologic component. E.g., gene targeted mice deficient in the E2 enzyme Ubc13 ($Ubc13^{-/-}$) die during early embryogenesis, but survive if Ubc13 is genetically ablated only in B lymphocytes (Yamamoto et al., 2006). Interestingly, these $Ubc13^{-/-}$ B lymphocytes are defective in several signaling pathways e.g. toll-like receptor (TLR) signaling. Furthermore, a Drosophila mutant (*bendless*) with a missense mutation in the active site region of the $Ubc13^{-/-}$ ortholog has a defective development of the central nervous system (Muralidhar and Thomas, 1993). Mice lacking either one of the RING E3 ligases Casitas B-lineage lymphoma b or c ($Cbl\text{-}b^{-/-}$ or $Cbl\text{-}c^{-/-}$) have dysregulated T cell signaling, and mice deficient in the E3 ligase tripartite-motif protein 25 ($TRIM25^{-/-}$) have an attenuated antiviral response due to defective retinoic-acid inducible gene 1 (RIG-1) signaling (Murphy et al., 1998, Bachmaier et al., 2000, Gack et al., 2007a).

Table 4. Phenotypes of mice with mutant E2s and E3s

Mutant	Phenotype	Reference
c-Cbl$^{-/-}$	Lymphoid hyperplasia	(Murphy et al., 1998)
Itchy	Autoimmune disease	(Perry et al., 1998)
TRIM25$^{-/-}$	Developmental., antiviral response	(Orimo et al., 1999, Gack et al., 2007a)
Cbl-b$^{-/-}$	Hyperproliferating lymphocytes	(Bachmaier et al., 2000)
TRIM28$^{-/-}$	Embryonic lethal	(Cammas et al., 2000)
Ubc13$^{-/-}$	Embryonic lethal; impaired B cell response.	(Yamamoto et al., 2006)

E3 ligases and immune cell responsiveness. E3 ligases are involved in all fundamental biological processes, e.g. the cell cycle, apoptosis, neurogenesis and the immune response. Interestingly, many E3 ligases seem to regulate the activation threshold of immune cells by ubiquitinating different substrates, ranging from cell surface receptors to transcription factors. E.g., the E3 ligase Itch mediates the ubiquitination and degradation of the activator protein 1 (AP-1) subunit c-Jun (Gao et al., 2004), thus down regulating the expression of many AP-1 dependent cytokine genes. The Cbl E3 ligase family control many signaling events through the ubiquitination of cell surface receptors and signaling molecules, and SMAD ubiquitination regulatory factor (Smurf) 1 and 2 regulate signaling induced by transforming growth factor β (TGF-β) (Zhu et al., 1999, Kavsak et al., 2000, Lin et al., 2000, Zhang et al., 2001).

★

Immunity and the ubiquitin system

The post-translational modification of proteins with ubiquitin and Ubls plays an important role in most biological processes in eukaryotes. As a result, organs and multicellular systems of metazoans are dependent on the ubiquitin system to function normally. One good example is the immune system in which intracellular signaling pathways are regulated through the ubiquitination of several signaling proteins. E.g., the activation of the nuclear factor of kappa light chain gene enhancer in B-cells (NFκB) transcription factors (Sen and Baltimore, 1986b, Sen and Baltimore, 1986a) require the activation of the inhibitor of NFκB kinase (IKK)

and the degradation of the inhibitor of NFκB (IκB). The activation of IKK, and the degradation of IκB, occurs through ubiquitination: IKK activation through K63 polyubiquitination (Deng et al., 2000), and IκB degradation through K48 polyubiquitination (Chen et al., 1995, Scherer et al., 1995, Alkalay et al., 1995, DiDonato et al., 1995, DiDonato et al., 1996). The activation of some NFκB subunits is necessary for an immune response, as evident in immunodeficient $p50^{-/-}$ mice (Sha et al., 1995), while other NFκB subunits are important in modulating immune responses as seen in $RelB^{-/-}$ mice (Weih et al., 1995).

Immunity. The immune system is an intricate system of cells and molecules that gives the host the benefit of *immunity*; a state in were the host is protected, albeit incompletely, against infections with e.g. pathogenic microorganisms and opportunistic parasites.

The immune system is often classified into two branches: the innate, and the acquired immune system. The innate immune system is dependent on germ line encoded pathogen recognition receptors, and is therefore present from birth. In contrast, the acquired immune system requires the creation and selection of new, unique, antigen receptors through the process of somatic recombination (Hozumi and Tonegawa, 1976). Consequently, the acquired immune system needs time to develop and mature before being functional, reviewed in (Landreth, 2002).

Immune cells. Immune cells are produced exclusively in the bone marrow and are found through out the body of both rodents and humans; furthermore, immune cells have the characteristic property of being highly able to migrate. E.g., dendritic cells are stationary at the outer and inner linings of the body until they are activated. They then migrate to secondary lymphatic organs where they present antigen to naïve T cells. In contrast, monocytes and neutrophils circulate in the blood until they eventually migrate into the tissues. By being able to migrate the immune cells can be assembled, and coordinated, to thwart infections.

It was early predicted that each T and B lymphocyte clone would express a single type of antigen receptor with a unique specificity (Burnet, 1959). Although this prediction later was shown to be valid, important exceptions have been discovered (Padovan et al., 1993, Zal et al., 1996).

Both T and B cells originate from the bone marrow but differ in their site of maturation. While the maturation process of B cells occurs in the bone marrow, the T cell maturation take place in the thymus, reviewed in (Hardy and Hayakawa, 2001, Ciofani and Zuniga-Pflucker, 2007, Ye and Graf, 2007). Furthermore, B and T cells differ in how their antigen receptors recognize antigen. The T cell antigen receptor (TCR) only recognizes peptide antigens presented on MHC I or MHC II

molecules (Rosenthal and Shevach, 1973, Zinkernagel and Doherty, 1974), while the B cell antigen receptor (BCR) recognize surfaces of several classes of molecules directly, e.g. proteins, carbohydrates and lipids. Peptides for MHC I are produced by the proteasome (Michalek et al., 1993) while peptides for MHC II are produced in the lysosomes after endocytosis (Waldron et al., 1974, Buus and Werdelin, 1986).

Another type of lymphocyte is the natural killer (NK) cells (Kiessling et al., 1975b, Kiessling et al., 1975a), which are involved in the recognition and killing of virus infected cells and tumor cells. This recognition is not dependent on antigen receptors, but rather on a large number of activating (Anegon et al., 1988, Cassatella et al., 1989, Frey et al., 1991) and inhibitory receptors (Karlhofer et al., 1992, Moretta et al., 1993). While interacting with target cells, NK cells receive signals through activating and inhibitory receptors, and depending on the type of signal that dominates, NK cells might destroy the target cell. Target cells that cannot deliver inhibitory signals, e.g. through MHC I (Karre et al., 1986), are likely to be killed by NK cells. As suggested by the 'missing-self' hypothesis these inhibitory signals are delivered exclusively by 'self cells', i.e. syngeneic, healthy, untransformed and non-infected cells (Kärre, 1981).

Positive and negative selection of T and B cells. During T and B cell ontogeny, their antigen receptor genes are assembled through somatic recombination. This is followed by an imperfect selection process were cells expressing antigen receptors with strong affinity for self-molecules are deleted. Negative selection deletes T cells with TCRs of high affinity for MHC/self-peptides in the thymus (Kappler et al., 1987, McDuffie et al., 1988). Similarly, B cells with BCRs of high affinity for self-antigens in the bone marrow are deleted (Goodnow et al., 1989). Positive selection ensures the survival only of T cells with TCRs of low affinity for MHC/self-peptide (Kisielow et al., 1988, von Boehmer et al., 1988, von Boehmer et al., 1989), and of BCRs with low affinity for self-antigens (Lam et al., 1997, Hayakawa et al., 1999). T cells whose TCR receives a positive selection signal via MHC I develop into cluster of differentiation 8 ($CD8^+$) T cells, while those who receive a positive selection signal via MHC II develop into $CD4^+$ T cells (Teh et al., 1988). Consequently, $CD4^+$ T cells are activated by antigen presenting cells (APCs) presenting antigen on MHCII, while $CD8^+$ cells recognize peptides presented on MHC I.

Positive and negative selection of T and B cells ensures that cells with high affinity for self-antigens die, while cells with low/moderate affinity for self-antigens survive. In this way, only useful cells, with a potential for recognizing foreign antigen, survive while useless and dangerous cells are deleted.

Activation of lymphocytes. The activation of T cells is mediated through the presentation of antigenic peptides by MHC molecules on APCs (Rosenthal and Shevach, 1973, Zinkernagel and Doherty, 1974). Mature DCs can activate naïve T cells, while macrophages and B cells only can reactivate previously activated T cells (Inaba and Steinman, 1984, Inaba and Steinman, 1985, Ronchese and Hausmann, 1993). Activated T cells start to proliferate and may in turn activate B cells and macrophages. Depending on the cytokine environment in which naïve T cells differentiate into their effector phenotype, they can develop into at least four distinct lineages: Th1 and Th2 (Tada et al., 1978, Mosmann et al., 1986), Th17 (Harrington et al., 2005, Park et al., 2005) or regulatory T cells (Tregs) (Sakaguchi et al., 1995).

B cells activated by T cells in secondary lymphoid organs start to proliferate, undergo affinity maturation (Eisen and Siskind, 1964), class switching (Wang et al., 1970) and finally differentiate into memory B cells or antibody producing plasma cells. Affinity maturation requires that dividing activated B cells undergo somatic hyper mutation (Kim et al., 1981). B cells that have, through mutation, produced BCRs with higher affinities for the inducing antigen will be expanded on the expense of the B cells that produced BCRs with lower affinities.

Tolerance. The cells of the immune system are tuned to be activated by infectious or noxious antigens, but not by self-antigens; this state is called *tolerance*. In a classic article, Owen gave the first description of immunological tolerance (Owen, 1945) where he described how bovine dizygotic twins do not reject each other's blood cells despite a common circulation (placental anastomosis). Later, by using skin grafting experiments on mice, it was shown that tolerance develops actively during early fetal life (Billingham et al., 1953), a study that was rewarded with the Nobel Prize in Physiology or Medicine 1960. This work demonstrated that all antigens 'seen' by the immune system during early life are perceived as self.

Tolerance is achieved through central tolerance, where most autoimmune T and B cells are deleted or inactivated through negative selection, and through peripheral tolerance where T and B cells that recognize self-antigens are kept inactive through anergy or ignorance (Nossal and Pike, 1980, Nossal, 1987, Kappler et al., 1987, Goodnow et al., 1989). The molecular pathways of tolerance are still unclear, but ubiquitin E3 ligases have been implicated: e.g., the gene related to anergy in lymphocytes (GRAIL) (Anandasabapathy et al., 2003, Seroogy et al., 2004) and Cbl-b (Bachmaier et al., 2000). In addition, Tregs modulate $CD4^+$ and $CD8^+$ T cell responses and are critical for self-tolerance (Sakaguchi et al., 1995, Hori et al., 2003, Fontenot et al., 2003, Fontenot et al., 2005). Despite all this, tolerance is never complete and therefore autoimmune cells are always present. Thus, under certain

conditions autoimmune cells can be activated and initiate an escalating autoimmune response leading to autoimmune disease.

★

Autoimmunity and autoimmune disease

Several chronic inflammatory diseases, such as rheumatoid arthritis (RA) and multiple sclerosis, are classified as autoimmune diseases; this based on clinical findings, e.g. inflammatory cell infiltrates in the affected organs and high levels of autoantibodies in patient sera. However, it is unknown to which extent these diseases are *bona fide* autoimmune diseases. A true autoimmune disease can be defined by criteria analogous to the famous Henle-Koch postulates (Koch, 1884, Inglis, 2007). These criteria must be fulfilled before affirming that a disease is *directly caused* by autoantibodies or autoimmune cells. **(1)** All patients with the disease must have autoantibodies (autoreactive lymphocytes) directed against at least one autoantigen in the affected tissue, and these autoantibodies (autoreactive lymphocytes) must be present in the affected tissue. **(2)** It must be possible to purify these autoantibodies (autoreactive lymphocytes) from patients. **(3)** These purified autoantibodies (autoreactive cells) must induce the same disease if transferred to a healthy subject. **(4)** Removal of the same autoantibodies (autoreactive cells) from patients must eliminate (or at least ameliorate) the disease progression, and finally **(5)** immunization with the corresponding autoantigen must induce a similar disease.

In animal models, the minimal genetic and environmental variation makes the fulfillment of these criteria possible (Kouskoff et al., 1996, Matsumoto et al., 1999, Korganow et al., 1999, Matsumoto et al., 2002, Schubert et al., 2004). In contrast, the criteria are difficult to fulfill in human subjects. This is due to the large variation in genetic background and environmental exposure between individuals, resulting in a high variation in the susceptibility to autoimmune disease. Consequently, the transfer of autoimmune disease from a susceptible individual to a resistant individual is unlikely. However, circumstantial evidence corroborates the idea that a certain disease is indeed autoimmune in origin. E.g., allogeneic bone marrow transplantations have been reported to transfer disease in a few cases (Wyatt et al., 1990, Haslam et al., 1993, Marazuela and Steegman, 2000). Moreover, immunosuppression is generally beneficial for patients and targeted therapies

against inflammatory mediators (e.g. tumor necrosis factor) or immune cells (e.g. $CD20^+$ B cells) do in many cases improve the disease.

From autoimmunity to autoimmune disease. After tissue trauma, or following an immune response against a non-self antigen, an autoimmune episode might be observed. These transient autoimmune responses may arise after infection and can cause further pathogenicity, e.g. rheumatic heart fever or reactive arthritis. Reportedly, transient autoimmune reactions might be necessary for healing and recovery after tissue trauma as reviewed in (Kipnis and Schwartz, 2005).

In contrast to transient autoimmune episodes, a chronic autoimmune disease does not resolve. What causes the chronicity of the autoimmune reaction? Possibilities range from a defective Treg population unable to modulate an ensuing autoimmune response, to chronic cryptic infections that lead to persistent stimulation of the innate immune system, feeding the autoimmune response.

A chronic inflammatory disease with typical autoimmune features is myasthenia gravis, in which autoimmune plasma cells produce autoantibodies that bind and block the acetylcholine α-receptor at post-synaptic neuromuscular junctions. Consequently, the disease is characterized by muscle weakness and muscle fatigue. Other autoimmune diseases are associated with massive infiltration of autoreactive T cells into the affected tissue. E.g., in insulin dependent diabetes mellitus (type I diabetes) the islet of Langerhans are infiltrated by autoimmune T cells followed by the destruction of the insulin producing β cells.

Per definition autoimmune cells recognize self-antigens, i.e. autoantigens, through the BCR on autoimmune B cells or through the presentation of self-peptides on MHC I and MHC II to the TCRs of autoimmune T cells. Although necessary, the presence of autoimmune cells is not sufficient to initate an autoimmune response. A requirement for the induction of an autoimmune response is that autoimmune T cells are activated by professional APCs presenting peptides from the corresponding autoantigen. An activated autoimmune (autoreactive) T cell clone can then in turn activate autoimmune B cells presenting the peptides from the same autoantigen. Activated autoimmune B cells undergo class switch, affinity maturation and differentiate into autoimmune plasma cells producing antibodies against self-antigens, i.e. autoantibodies.

Autoreactive effector T cells can activate other cells that in turn can mediate direct tissue damage. E.g., Th1 cells secrete IFNγ (Mosmann et al., 1986) that activate macrophages, who in turn secrete proteases and reactive oxygen species (Schultz and Kleinschmidt, 1983), similarly Th17 cells secrete IL-17 species that activates a cell mediated response, reviewed in (Kolls and Linden, 2004). In

contrast, Th2 cells stimulate a humoral immune response through the release of e.g. IL-4 and IL-10 (Mosmann et al., 1986).

Autoantigens and autoantibodies. Intracellular proteins are not normally presented on MHC II in conjunction with costimulatory signals. This since the digestion of apoptotic material by macrophages is a tolerogenic process (Fadok et al., 1998), i.e. no induction of costimulatory signals on the macrophages occurs. Therefore the activation of naïve $CD4^+$ T cells by dendritic cells, or the reactivation of primed $CD4^+$ T cells, mainly occur after tissue damage or infection. Despite this, patients with systemic lupus erythematosus (SLE) produce large amounts of autoantibodies against double stranded deoxyribonucleic acid (dsDNA). Many other intracellular autoantigens have also been described (**Table 5**), e.g. Ro/SSA, La/SSB, phospholipids, and Jo-1. One hypothesis is that due to excessive apoptosis, or defective clearance of apoptotic cells, exposed intracellular autoantigens can be bound by autoimmune B cells, engulfed by endocytosis and then presented to autoimmune T cells via MHC II. The T cells, in turn, provide costimulation for B cells leading to the production of memory B cells and autoantibody secreting plasma cells. These events could initiate an autoimmune response. However, it is not clear how an autoimmune response is perpetuated and escalates into autoimmune disease. One possibility is that a viral infection induces excessive cell death and the exposure of intracellular proteins. This, together with activation of immune cells by the virus, will provide both intracellular proteins and the induction of costimulation (Rosen et al., 1995). The autoimmune reaction could then be driven by the continuous release of endogenous danger signals from the damaged tissue. Recurrent viral infections might feed the ongoing response and lead to a progressive disease. A multitude of scenarios can explain how self-antigens can activate T or B cells. The insulting epitope might not have been present in the thymus or the bone marrow (cryptic epitope) preventing negative selection against it. Alternatively, neo-epitopes might be formed through post-translational modification or epitopes in viruses might induce a response against cross reactive self epitope (molecular mimicry).

Are autoantibodies pathogenic? There is no direct evidence that autoantibodies (**Table 5**) are indeed pathogenic except for in a few autoimmune diseases, e.g. myasthenia gravis, Grave's disease, Goodpasture's syndrome, SLE, and autoimmune congenital heart block. These diseases can be transferred by autoantibodies, either naturally i.e. from mother to fetus, or artificially e.g. during blood transfusions or bone marrow transplantations.

Table 5. Autoantigens in autoimmune rheumatic diseases (Routsias et al., 2006)

Disease	Autoantigen	Frequency
Systemic lupus erythematosus	dsDNA	65-75%
	Ro/SSA (Ro60 and Ro52)	30-40%
	La/SSB (La48)	10-15%
	Phospholipid	30-50%
Sjögren's syndrome	Ro/SSA	70%
	La/SSB	70%
	IgG	85%
Idiopathic inflammatory myopathies	Jo-1	25-30%

Other chronic autoimmune diseases such as RA, idiopathic inflammatory myopathies multiple sclerosis and insulin dependent diabetes mellitus are driven mainly by autoimmune Th1 or/and Th17 cells.

Systemic lupus erythematosus and Sjögren's syndrome. Rheumatic diseases belong to a heterogeneous group of diseases that may be either autoimmune or non-autoimmune. Autoimmune rheumatic diseases such as RA, SLE and Sjögren's syndrome (SS), all have two common features: they are more prevalent among females than males and they are associated with certain human leukocyte antigen (HLA) haplotypes.

SLE and SS are considered to be overlapping diseases but still they are different in many ways. Clinically, SLE is a more severe disease and may lead to multiorgan failure and premature death, while SS most commonly engage exocrine organs. Both patients with SS or SLE have an increased risk of developing B cell lymphomas (Talal and Bunim, 1964, Voulgarelis and Moutsopoulos, 2003), though the diseases also have distinct immunological features. E.g., SLE patients often have an increased $CD27^+$ population in the circulation, consisting of memory B cells and plasma cells, in contrast SS patients have a diminished $CD27^+$ population both in absolute and relative numbers compared both to healthy subjects and SLE patients. In addition, patients with SS are also usually not B cell lymphopenic, which is a very common observation amongst SLE patients (Odendahl et al., 2000, Hansen et al., 2002, Dorner and Lipsky, 2004, Hansen et al., 2004).

★

Tripartite-motif proteins and immunity

Members of the TRIM protein family are E3 ligases (Reymond et al., 2001, Meroni and Diez-Roux, 2005) characterized by an N-terminal TRIM motif, i.e. the co-occurrence of one RING domain, one or two B-box domains (Borden et al., 1993) and one coiled-coil region. The difference in the C-terminal domain between TRIM proteins suggests that the TRIM motif is a highly conserved effector motif, whilst the non-conserved C-terminal domain mediates binding specificity to different targets. The potential role of TRIM proteins in innate antiviral responses has been highlighted (Nisole et al., 2005), emphasizing the importance of E3 ligases in immunity. Many TRIM proteins have been shown to be restriction factors for viruses; e.g. TRIM1 (Yap et al., 2004), TRIM5 (Stremlau et al., 2004), TRIM19 (Chelbi-Alix et al., 1998), TRIM22 (Tissot and Mechti, 1995), TRIM25 (Gack et al., 2007b) and TRIM28 (Wolf and Goff, 2007). Furthermore, the notion of antiviral TRIMs is strengthened by the observation that many TRIMs are upregulated by interferons (Tissot and Mechti, 1995, Rhodes et al., 2002, Asaoka et al., 2005, Zou and Zhang, 2006). That some TRIMs have been reported to be inefficient at restricting viruses (Li et al., 2007b) could be interpreted as if that not all TRIMs are involved in antiviral immunity. However, it could be argued that these TRIMs were only ineffective against the tested viruses; they might have a strong effect against yet non-tested viruses. Thus, it is not unlikely that the TRIM family is a large group of antiviral restriction factors and that more TRIMs will be identified as antiviral proteins

TRIM21/Ro52. The history of the intracellular protein Ro52 (a.k.a. TRIM21, RNF81) is deeply rooted in the field of rheumatology. That patients with systemic rheumatic diseases, in particular SLE and SS, have antibodies to an autoantigen called Ro/SSA has been known since the late 60's (Clark et al., 1969, Mattioli and Reichlin, 1974, Alspaugh and Tan, 1975). These anti-Ro/SSA autoantibodies have been used as a diagnostic tool for decades although the molecular identity of the Ro/SSA autoantigen was unknown. Ro/SSA was consequently found to consist of at least two proteins; Wolin et al. first identified Ro60 (Wolin and Steitz, 1984) subsequently Ben-Chetrit et al. (Ben-Chetrit et al., 1989) and Wang et al. (Wang et al., 1996) cloned the complementary DNA (cDNA) of Ro60. Ben-Chetrit and colleagues gave the first description of Ro52 by discovering that a 52 kD protein (Ro52) was a part of the Ro/SSA autoantigen (Ben-Chetrit et al., 1988). Three years later the cDNA of human Ro52 was cloned independently by two groups (Chan et al., 1990, Chan et al., 1991, Itoh et al., 1991); subsequently the human

Ro52 gene was mapped to chromosome 11 (Frank et al., 1993) and the exon-intron structure was elucidated (Chan et al., 1995).

The nomenclature surrounding Ro52 is confusing. Ro/SSA can mean either Ro52 or Ro60, or both; therefore, Ro52 is often described as the 52 kDa component of Ro/SSA, i.e. Ro/SSA 52 kDa or SSA/Ro 52kD. However, the two most common symbols are Ro52 and TRIM21 (Reymond et al., 2001). TRIM21 is the official gene symbol approved by the HUGO Gene Nomenclature Committee, therefore, the recommendation is to use the symbol TRIM21 to denote both the protein and the gene.

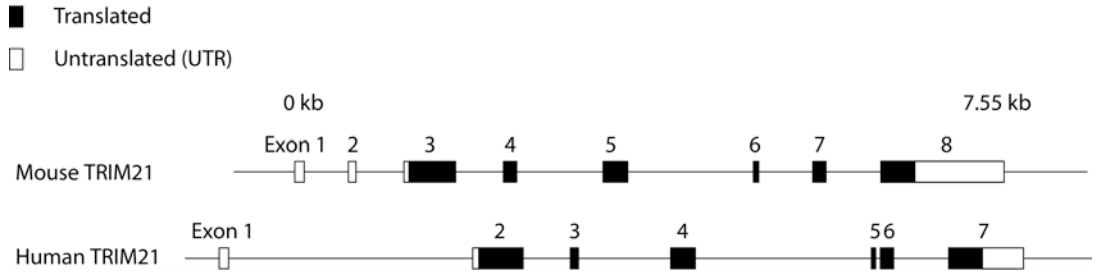

Figure 6. The gene structure of TRIM21/Ro52. The exon-intron structure of the TRIM21 gene is similar in mouse and human. The mouse gene is located on chromosome 7 and the human gene on chromosome 11 (11p15.5).

The TRIM/Ro52 gene is approximately 8 kb in size and consists of eight (mouse) or seven (human) exons and has a conserved exon-intron structure (**Fig 6**). The RING and B-box domains are encoded by exons 3 and the B30.2 domain is encoded by the last exon (**Fig 6 and 7**). In the human and mouse genomes there are two large paralogous TRIM gene clusters. TRIM10, 15, 26, 27, and 31 are located on chromosome 6 in man, and 17 in mouse. TRIM5, 6, 21, 22, and 34 are located on chromosome 11 in man, and 7 in mouse.

The B-box domain (Borden et al., 1993) is unique for the TRIM family and is like the RING domain a Zn^{2+} binding domain although the B-box consensus sequence distinguishes it from the RING domain. Two coiled-coil (CC) domains, CC-1 and CC-2, are predicted by using a coiled-coil prediction algorithm (Lupas et al., 1991). CC-1 has a lower prediction score, especially in mouse, while CC-2 has a high prediction score. In addition, CC-2 contains a putative leucine zipper motif

making CC-2 a good candidate to be a homo- or hetero interaction domain. A B30.2 domain (Vernet et al., 1993) is located C-terminal of the TRIM motif. The B30.2 contain the subdomains splA and RyR (SPRY) and associated with SPRY (PRY) (Ponting et al., 1997), together referred to as the PRYSPRY domain (Rhodes et al., 2005). The B30.2 domain of Ro52 has been implicated in binding to the constant part of the IgG heavy chain (Yang et al., 1999, Yang et al., 2000, Rhodes and Trowsdale, 2006, James et al., 2007) and to the interferon responsive factor (IRF) 8 (Kong et al., 2007).

TRIM21/Ro52 is reportedly a ubiquitously expressed protein as determined by in situ hybridization (Reymond et al., 2001) (www.tigem.it/TRIM); although, ubiquitous expression at the protein level has not been corroborated by experimental data. However, no failed attempt to detect Ro52 at the mRNA and protein level from different tissues has been reported.

MOUSE (aa 1-470)

Domain:	Sequence (aa):	Exon:
RING	(20-58)	3
B-box	(96-127)	3
Coiled-coil 1	(128-166)	3-4
Coiled-coil 2	(186-245)	5
B30.2	(252-470)	7-8

HUMAN (aa 1-475)

Domain:	Sequence (aa):	Exon:
RING	(16-54)	2
B-box	(92-123)	2
Coiled-coil 1	(125-166)	2-3
Coiled-coil 2	(179-235)	4
B30.2	(268-465)	6-7

Figure 7. The domain structure of the protein TRIM21/Ro52. The RING, B-box and coiled-coil domains together constitute the TRIM motif. Two predicted coiled-coil domains and one B30.2 domain are located C-terminal to the TRIM motif. Coiled-coil 2 contains a putative leucine zipper motif.

Anti-Ro/SSA positive sera affinity purified against Ro52 is commonly used to detect Ro52. This is an approach suffering from non-specific staining; instead, the preferred method of detecting Ro52 is by using anti-Ro52 monoclonal antibodies. A powerful approach to detect Ro52 expressing cells in vivo is by using a genetic reporter system wherein a reporter protein, e.g. green fluorescent protein (GFP), is expressed from the endogenous Ro52 promoter. By knowing which cell types express Ro52, it is possible to gain information regarding the function of Ro52.

E.g., if Ro52 expression occur only in differentiated cells of a lymphoid cell lineage, Ro52 is likely to have a role in the differentiation or effector function of these cells.

The function of TRIM21/Ro52. The biological function of Ro52 is not known, however, experimental data accumulating during the last decade have given important clues to its roles in e.g. IgG binding, IL-2 and IL-12 production and in the cell cycle (Yang et al., 1999, Yang et al., 2000, Ishii et al., 2003, Rhodes and Trowsdale, 2006, Sabile et al., 2006, James et al., 2007, Kong et al., 2007).

That Ro52 is an E3 ligase was first demonstrated by (Wada and Kamitani, 2006a) and later corroborated and expanded by the same and other authors (Espinosa et al., 2006, Sabile et al., 2006, Wada and Kamitani, 2006b, Wada et al., 2006, Kong et al., 2007). Although it is clear that Ro52 is an E3 ligase, its substrate is unknown. By searching for interaction partners of Ro52, several research groups have found putative substrates; e.g., IgG heavy chain (Yang et al., 2000, Yang et al., 1999, Takahata et al., 2007), p27 (Sabile et al., 2006) and IRF-8 (Kong et al., 2007).

The best-characterized interaction partner for human Ro52 is human IgG1 and IgG4 heavy chain. This interaction, occuring between the B30.2 (PRYSPRY) domain of Ro52 and the constant part of IgG heavy chain (Yang et al., 1999), was already noted by Buyon et al. (Buyon et al., 1994). Human Ro52 does not, however, seem to interact with mouse IgG (Rhodes and Trowsdale, 2006). The B30.2 domain of TRIM21/Ro52 (and other TRIMs) has a β-sandwich like core domain with six extended variable loops. Analogous to the immunoglobulin fold in antibodies, the variable loops seem to have diverged to different specificities while the core domain is more conserved. The PRY and SPRY subdomains of B30.2 form binding pockets for the CH2 and CH3 domains of IgG heavy chain respectively, the same domains of IgG that are also bound by protein-A and G. The affinity of the TRIM21/Ro52 B30.2 domain for the IgG heavy chain has been estimated to $K_D=3.7x10^{-9}$ M (James et al., 2007), a very high affinity when comparing to the $K_D=8x10^{-7}$ M for the protein-G:IgG interaction (Sjobring et al., 1991), and the TRIM21/Ro52:B30.2 stoichometry to 2:1.. The physiological consequence of this TRIM21/Ro52-IgG interaction is presently unknown, but it cannot be excluded that it plays a role in the pathogenesis. TRIM21/Ro52 released, after e.g. apoptosis, might hinder the clearing of immune complexes by binding the IgG Fc part; blocking the binding of immune complexes by FcγR expressing cells. Although the B30.2 domain does not bind the same part of IgG as FcγR, the binding of IgG by FcγR might still be outcompeted. One possibility is also that TRIM21/Ro52 is involved in controlling IgG levels, or IgG trafficking, by ubiquitination.

In addition to IgG, TRIM21/Ro52 also binds and ubiquitinates IRF-8 in a B30.2 dependent manner (Kong et al., 2007). IRF-8 is ubiquitinated and stabilized by TRIM21/Ro52, thereby increasing the transcription of IRF-8 induced genes, e.g. the IL-12 subunit p40. Furhermore, ectopic overexpression of TRIM21/Ro52 increased the production of IL-12 in macrophages. This enhancement of IRF-8 transcriptional activity through ubiquitination is an example of how ubiquitin acts independently of the proteasome.

In all, data is accumulating demonstrating that TRIM21/Ro52 is an E3 ligase ubiquitinating multiple proteins involved in immunity. However, the cell biological function of TRIM21/Ro52 is still not well studied; and the functional role of TRIM21/Ro52 in regulating immune responses in vivo is completely unknown.

★

AIMS

The protein TRIM21/Ro52 was first described as an autoantigen in rheumatic diseases, consequently, most of the research performed concerning TRIM21/Ro52 has been in relation to its quality as an autoantigen. Much effort has been spent on trying to explain why and how the intracellular TRIM21/Ro52 becomes an autoimmune target, and if this has any importance in the etiology of Sjögren's syndrome and congenital heart block. This thesis has a different focus; its main purpose is to understand the biological function of TRIM21/Ro52. The discovery of the RING domain, and the growing evidence implicating TRIM proteins in innate antiviral responses, has renewed the interest in TRIM21/Ro52. So far, TRIM21/Ro52 has been implicated in the regulation of cytokine expression, binding of IgG and the regulation of the cell cycle.

The aim of this thesis is to contribute to a better understanding of the structure and function of TRIM21/Ro52. The domains of TRIM21/Ro52 have only been predicted; therefore, the integrity and folding of these domains need to be determined experimentally. Furthermore, the putative role of TRIM21/Ro52 in ubiquitination also needs experimental support and the regulation of the intracellular localization of TRIM21/Ro52 is poorly understood. In addition to this in vitro characterization of TRIM21/Ro52, an in vivo characterization of TRIM21/ Ro52 is critical for a more complete understanding of its biology. For relevant in vivo studies, one mouse strain with a disrupted TRIM21/Ro52 gene ($Ro52^{-/-}$), and two TRIM21/Ro52 transgenic (Ro52-Tg) mouse strains were generated.

★

RESULTS

TRIM21/Ro52 forms weak dimers and binds Zn^{2+}

(Paper I)

The RING, B-box, coiled-coil and B30.2 domains of Ro52 are predicted based on sequence homology with other proteins. Although sequence based predictions are reliable, it has never been shown that the predicted domains of Ro52 adopt the corresponding secondary structures. To investigate the secondary structure of the predicted domains, biochemical, biophysical, and molecular biology techniques were used.

By using limited proteolysis, it is possible to find stable secondary structure domains of a protein. Secondary structures are less prone to proteolytic digestion than a random coil, therefore they can be detected as non-degraded fragments after limited proteolysis. Full-length Ro52 was expressed and purified as a maltose-binding protein (MaBP) fusion protein. For limited proteolysis, MaBP-Ro52 was digested with trypsin or chymotrypsin. At different time-points, the proteolysis was stopped, and the reactions were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Two stable Ro52 fragments were identified: the RING-B-box (aa 6-121) and coiled-coil (aa 121-260) domains. The B30.2 domain was not detected as a stable fragment, which could be interpreted as if B30.2 is not a structurally stable domain, or that the lack of a stable B30.2 fragment is due to the expression system used (see discussion). For further analyzes, the identified stable fragments were expressed and purified as 6xHis (His_6) fusions proteins from the strain BL21-Gold (DE3) pLysS.

To determine if the RING-B-box domain was stabilized by Zn^{2+} binding, a limited proteolysis with trypsin was preformed in the presence or absence of Zn^{2+}. As detected by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), the RING-B-box was significantly stabilized by Zn^{2+} but not by DNA, suggesting that RING-B-box folding is dependent on and stabilized by Zn^{2+}. Thus, as predicted by sequence homology, the RING-B-box region of Ro52 is a Zn^{2+} binding region.

Circular dichroism (CD) was used to reveal the secondary structures of the different Ro52 domains. The coiled-coil region had a dominating alpha-helical

contribution, while the RING-B-box and RBCC structures had a large contribution of beta strand structure (**Fig 8**).

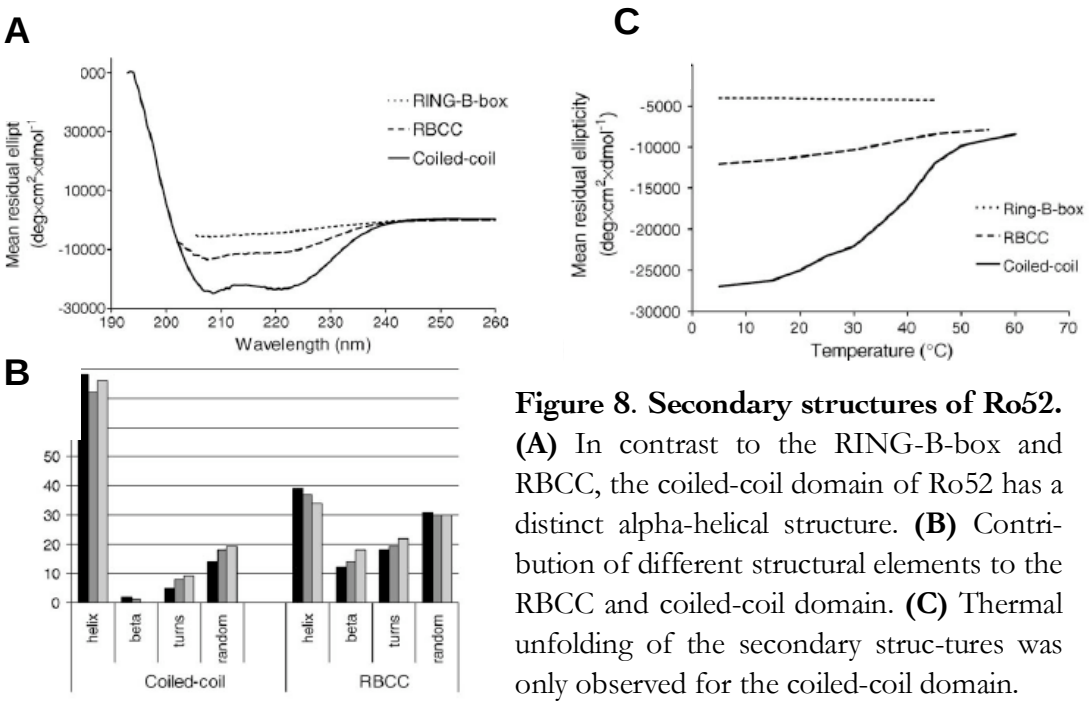

Figure 8. Secondary structures of Ro52. **(A)** In contrast to the RING-B-box and RBCC, the coiled-coil domain of Ro52 has a distinct alpha-helical structure. **(B)** Contribution of different structural elements to the RBCC and coiled-coil domain. **(C)** Thermal unfolding of the secondary struc-tures was only observed for the coiled-coil domain.

Coiled-coil domains are often involved in oligomer formation, and to investigate wether the coiled-coil of Ro52 contribute to oligomer formation, analytical ultracentrifugation and a mammalian two-hybrid assay were used. A weak homo dimerization of the coiled-coil domain was detected (K_D=25 μM) by ultracentrifugation; also, a weak oligomer formation was detected by the two-hybrid assay.

In conclusion, the N-terminal domains of Ro52 adopt stable secondary structures as predicted, and the RING-B-box domain is dependent on Zn^{2+} ions for proper folding. Furthermore, Ro52 may form weak dimers through the coiled-coil domain.

★

TRIM21/Ro52 is an E3 ligase

(Paper II&III)

When the work on paper II began, it was uncertain if Ro52 was indeed an E3 ligase as suggested by the presence of the RING domain. However, it was known that Ro52 was ubiquitinated (Fukuda-Kamitani and Kamitani, 2002) and shortly before the publishing of paper II it was also shown that Ro52 was an E3 ligase (Wada and Kamitani, 2006a). In paper II the finding that Ro52 is an E3 ligase is expanded, and in paper III, it is shown that the E3 ligase activity of Ro52 is inhibited by anti-RING autoantibodies from patients with SS and SLE.

To investigate if Ro52 was an E3 ligase, autoubiquitination assays were used. Autoubiquitination is a phenomenon in where E3 ligases modify themselves with polyubiquitin chains; since this can occur in the absence of a substrate it is unclear whether autoubiquitination is only an experimental artifact or if it also is of physiological relevance. In any case, autoubiquitination assays are useful for demonstrating E3 ligase activity.

A transfection based ubiquitination assay (Treier et al., 1994) was first used to show that ubiquitination of Ro52 is RING dependent, indicative of RING dependent autoubiquitination (paper II). FLAG tagged Ro52 (FLAG-Ro52) and 6xHis tagged ubiquitin (His_6-Ub) were coexpressed in 293T cells by transfection with the corresponding plasmids. After 48 hours, cells were lysed in a denaturing lysis buffer, and proteins covalently modified by His_6-Ub were purified using a Ni^{2+}-nitrilotriacetic acid (Ni-NTA) resin. His_6-Ub modified proteins were separated by SDS-PAGE followed by immunoblotting with an anti-FLAG antibody. FLAG-Ro52 was detected as a high molecular weight smear, showing that it had been modified by polyubiquitin. In contrast, FLAG-Ro52ΔRING was not modified by polyubiquitin, demonstrating that that the ubiquitination of Ro52 was RING dependent.

To ensure that Ro52 was autoubiquitinated, and to exclude that the observed ubiquitination was dependent on another E3 ligase ubiquitinating Ro52 only in the RING domain, an in vitro ubiquitination assay was used (Lorick et al., 1999). Purified His_6-Ro52 was mixed with E1, ubiquitin and different E2s in a reducing Tris-buffer containing ATP and $MgCl_2$ (paper II). After incubating at 25°C for one hour, the reactions were terminated by the addition of Laemmli sample buffer and boiling. The reactions were separated by SDS-PAGE followed by immunoblotting against ubiquitin. Autoubiquitinated Ro52 was observed with several E2s.

To investigate if the RING domain was critical for the E3 ligase activity of Ro52, in vitro autoubiquitination assays were made using Ro52 mutants either lacking the RING domain or with critical cysteine residues in the RING domain mutated to alanine residues (C20A, C32A or C55A, C58A) (paper III). All these Ro52 mutants lacked E3 ligase activity. Also, Ro52 could mediate the formation of K48 and K63 chains as shown by using ubiquitin mutants with only one lysine residue, K48 only and K63 only mutants.

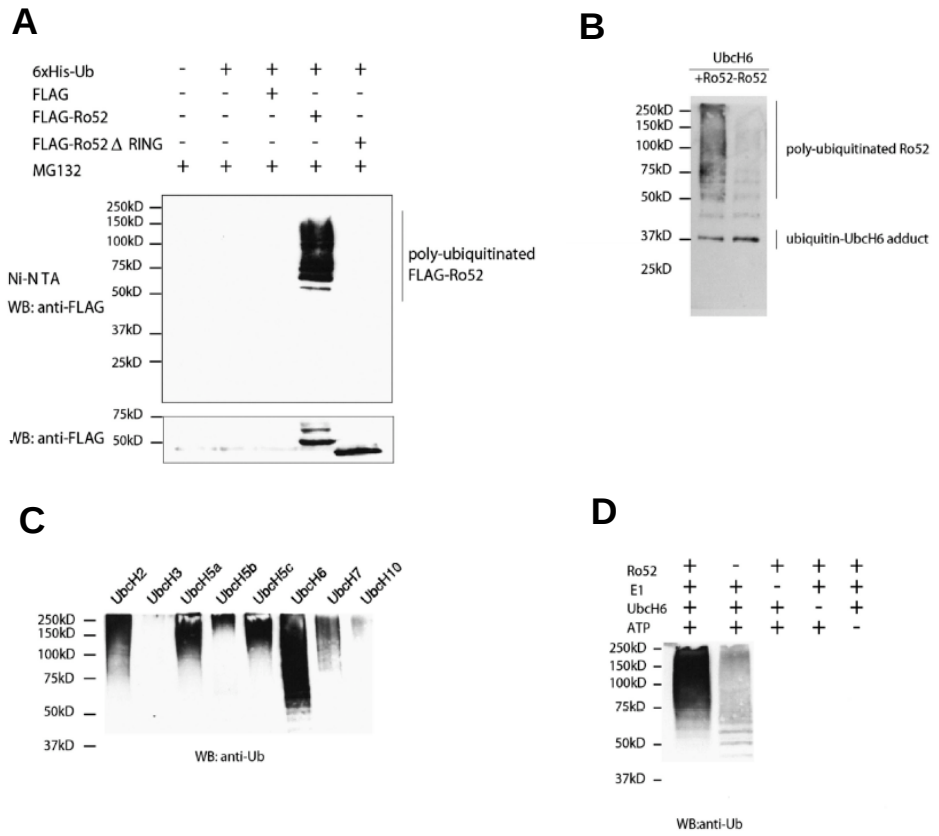

Figure 9. Ro52 is an E3 ligase. RING dependent ubiquitination of Ro52 was detected in cell transfection experiments **(A)**, and autoubiquitination occurs in in vitro autoubiquitination experiments **(B-F)**.

Sera from patients with SLE and, even more so SS, frequently have high titers of anti-Ro52 autoantibodies. These antibodies are mainly directed against dominant epitopes in the coiled-coil region, but antibodies to the RING domain are also detectable. To explore the possibility that anti-RING antibodies could inhibit the E3 ligase activity of Ro52, in vitro ubiquitin assays were performed, into which

purified IgG fractions from different patients were added (paper III). Affinity purified anti-RING antibodies from an anti-RING positive patient were used as a positive control. IgG fractions from anti-RING positive patients inhibited the E3 ligase activity of Ro52, while IgG fractions from anti-RING negative patients did not.

In all, TRIM21/Ro52 is a RING dependent E3 ligase that can use several E2 ubiquitin-conjugating enzymes and mediate the creation of both K48 and K63 polyubiquitin chains. Moreover, anti-RING autoantibodies from patients with SS and SLE can inhibit the E3 ligase activity in vitro by binding to the RING domain.

★

TRIM21/Ro52 expression is induced by IFNα and herpes simplex virus

(Paper II&V)

IFNα is a pleiotropic cytokine expressed mainly by hematopoeitic cells, and whose expression is strongly induced in vivo during viral infections and in cell cultures challenged with live or inactivated viruses. In contrast, uninfected cells produce no, or very low amounts, of IFNα. IFNα has many antiviral effects including the modulation of the cell cycle, induction of pro-apoptotic genes and of MHC I expression, reviewed in (Sen, 2001).

Interestingly, many (all?) TRIM genes are induced by IFNα (Lavau et al., 1995, Stadler et al., 1995, Chelbi-Alix et al., 1995, Der et al., 1998, Asaoka et al., 2005). TRIMs have also been shown to be induced by IFNγ, endotoxins and viruses. In particular, Ro52 mRNA levels are induced by IFNα (Der et al., 1998, Wang and Campbell, 2005, Zimmerer et al., 2007, Scherbik et al., 2007), IFNγ (Rhodes et al., 2002, Kota et al., 2006), influenza virus A (Geiss et al., 2002) and lipopolysaccharide (LPS) (Thomas et al., 2006). To verify that Ro52 is indeed induced by IFNα or virus, cell lines and peripheral blood mononuclear cells (PBMCs) were exposed to IFNα and/or herpes simplex virus (HSV) and Ro52 expression was measured at both the mRNA *and* protein levels (**Fig 10**).

Patients with SS and SLE are characterized by a so-called IFN signature, in which interferon stimulated genes (ISGs) are upregulated (Gottenberg et al., 2006, Baechler et al., 2003, Kirou et al., 2004). This, and since IFNα could induce Ro52 expression in vitro, prompted an investigation of the expression of TRIM21/Ro52

in SS and SLE patients. PBMCs were collected from patients, and from healthy individuals, and the mRNA levels of Ro52 were determined by Q-RT-PCR. There was a clear increase of Ro52 expression in patients compared to controls (**Fig 11**).

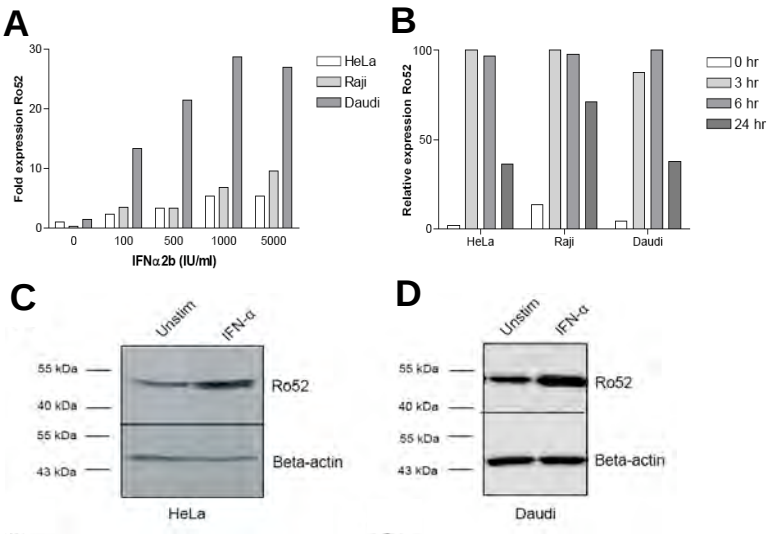

Figure 10. Ro52 expression is increased after exposure to IFNα. Cell lines were exposed to IFNα2b at different concentrations **(A)**; Ro52 was induced up to 30-fold in Daudi and 5-fold in HeLa cells. The expression of Ro52 peaked at 3-6 hours after IFNα2b exposure at 1000 IU/ml **(B)**. Ro52 expression was increased at the protein level both in HeLa cells **(C)** and Daudi cells **(D)** as determined by immunoblotting.

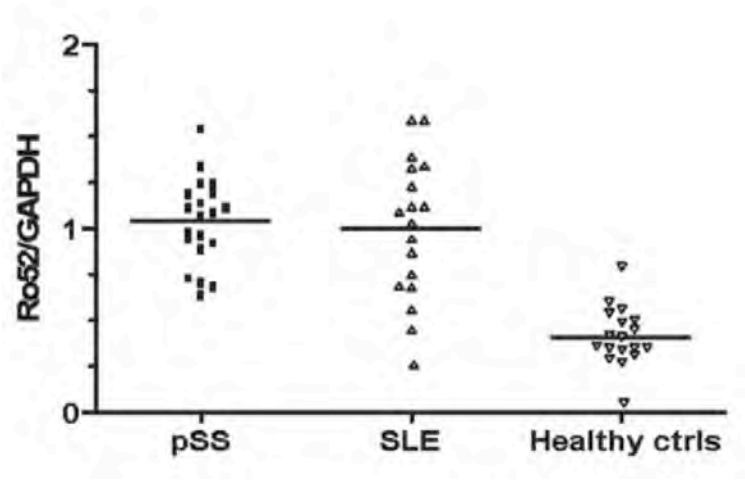

Figure 11. Ro52 expression is elevated in PBMCs from patients with SS or SLE. Ro52 mRNA levels in PBMCs from healthy individuals and patients with SS or SLE were compared. Interestingly, Ro52 mRNA levels were elevated in PBMCs from patients; probably as a consequence of the general increase in expression of IFNα induced genes (i.e. IFNα signature).

★

TRIM21/Ro52 inhibits cell proliferation

(Paper II)

Patients with SS and SLE have elevated Ro52 mRNA levels in PBMCs. Since these patients also have skewed populations of lymphocytes, one possibility is that overexpression of Ro52 affect the proliferation and differentiation of lymphocytes. B cell populations are particularly skewed in SLE and SS as manifested by B cell lymphopenia in SLE and decreased $CD19^{+}CD27^{+}$ frequency in SS.

To investigate the effect of Ro52 overexpression in B cells, B cell lines were stably transfected with Ro52-GFP, Ro52ΔRING-GFP or GFP. B cells over-expressing Ro52 had a significantly lower proliferation than Ro52ΔRING-GFP or GFP expressing cells. Because CD40 signaling induces cell death in the A20 B cell line, the sensitivity to CD40 mediated cell death was tested in the three stably transfected cell lines. Cells overexpressing Ro52 were more sensitive to anti-CD40 induced cell death, than Ro52ΔRING-GFP or GFP expressing cells.

Interestingly, transgenic mice overexpressing Ro52 in B cells have a very low expression level of the transgene; this in contrast to trangenic mice expressing Ro52ΔRING were the expression of the transgene is high (non-submitted data). Since this is observed in independent transgenic lines, it suggests that B cells expressing high levels of Ro52 will proliferate slower leading to a selection and expansion of B cells expressing low levels of Ro52.

★

TRIM21/Ro52 is translocated to the nucleus after exposure to IFNα or nitric oxide

(Paper IV,V&VI)

The intracellular localization of Ro52 has been the focus of surprisingly much attention; probably since it has been reported that Ro52 is located in apoptotic blebs (Miranda-Carus et al., 2000, Lawley et al., 2000, Saegusa et al., 2002, Ohlsson et al., 2002). According to a popular hypothesis, intracellular autoantigens (e.g. Ro52) are modified and exposed after apoptosis leading to break of tolerance, reviewd in (Rosen and Casciola-Rosen, 2004). The purposes of paper IV and V

were not to investigate the exposure of Ro52 after apoptosis, but rather to understand how and when Ro52 can translocate to the nucleus. Although Ro52 is a cytoplasmic protein, low levels of Ro52 is detectable in the nucleus (Pourmand et al., 1998, Ohlsson et al., 2002, Kong et al., 2007). The idea that Ro52 might be working as a transcription factor in an analogous fashion to NFκB, i.e. in a cytoplasma-inactive versus nucleus-active dichotomy, is unlikely since Ro52 contains no DNA binding motifs. However, a nuclear role of Ro52 might be as an E3 ligase modifying a transcription factor (e.g. IRF-8) with K48 or K63 polyubiquitin chains. Furthermore, cytoplasmic Ro52 might have a different target than nuclear Ro52. To investigate this, the intracellular localization of Ro52 and two different E2 enzymes in HeLa cells were determined by staining with antibodies or fusing the proteins to fluorescent reporter proteins.

Ro52 was found to be a cytoplasmic protein, both when staining with anti-Ro52 monoclonal antibodies and when detecting red fluorescent protein (RFP) fused Ro52 (Ro52-RFP) after transfection. Interestingly, UBE2D1 (UbcH5a) was cytoplasmic while UBE2E1 (UbcH6) was strictly nuclear, suggesting that Ro52 might use UbcH5a as an E2 in the cytoplasm and UbcH6 as an E2 in the nucleus.

It has previously been reported that an intact coiled-coil domain is necessary for the cytoplasmic localization of Ro52 (Pourmand et al., 1998). The CC-2 domain contains a putative nuclear export signal (NES) and a leucine zipper. To further investigate what regions of Ro52 are involved in the regulation of Ro52s intracellular localization, GFP-Ro52 and several deletion mutants of GFP-Ro52 were expressed in HeLa cells, and their intracellular localization was determined by fluorescent microscopy. GFP-Ro52 was located in the cytoplasm, but when the leucine zipper of Ro52 was deleted (GFP-Ro52ΔLZ) Ro52 was mainly localized in the nucleus. To investigate if the cytoplasmic localization of Ro52 was due to continuous nuclear export, the Exportin-1 mediated nuclear export inhibitor leptomycin B (LMB) was added to cells transfected with GFP-Ro52. Addition of LMB did not induce nuclear translocation of Ror52. Also, Ro52 with a mutated putative NES (GFP-Ro52mNES) was still located in the cytoplasm. In contrast, cyclin-B1, containing a known NES, accumulate in the nucleus after addition of LMB.

Ro52 is detected in the nucleus of a small proportion of HeLa cells, and the question is what triggers the translocation of Ro52 to the nucleus. Since the expression of Ro52 is induced by IFNα it is plausible that the intracellular localization changes as well. Therefore, HeLa cells were treated with IFNα and the intracellular localization of Ro52 was detected by immunofluorescence using patient sera affinity purified against Ro52 or a monoclonal anti-Ro52 antibody.

After 24 hours, a clear nuclear accumulation of Ro52 was observed, suggesting that Ro52 is involved in IFNα responses.

It has been reported that H_2O_2 induces nuclear accumulation of Ro52 (Nobuhara et al., 2007). Therefore, HeLa cells were transfected with Ro52-GFP or GFP and treated with the nitric oxide (NO) donor diethylenetriamine NONOate (DETA-NONOate) for 6 hours, and the intracellular localization of Ro52-GFP and GFP was determined by fluorescence microscopy. Ro52-GFP translocated from the cytoplasm to the nucleus after treatment with DETA-NONOate.

★

DISCUSSION

Paper I

-Why is the B30.2 domain not stable, and what about Ro52 dimers?

After limited proteolysis of Ro52 the RING/B-box and coiled-coil domains remained as stable fragments, however, the B30.2 domain did not. This can be interpreted as if the B30.2 domain is not a stably folded domain; but this interpretation goes against robust data considering the B30.2 structure (James et al., 2007). A more likely explanation is that the apparent instability of the B30.2 domain is an artifact caused by incomplete mRNA translation. Truncated Ro52 was detected already before limited proteolysis, indicating either degradation or uncompleted translation. Due to the large size of MaBP-Ro52, uncompleted translation is a probable reason for the truncated B30.2 domain. A truncated B30.2 domain would be less stable due to incomplete folding, making it susceptible to even limited proteolysis. The prokaryotic expression system used is the likely cause of the truncated B30.2, this due to the phenomenon of codon bias. Some codons that are used frequently in mammals are rare in prokaryotes; therefore, the t-RNA pool for rare codons in *E. coli* will be depleted when expressing Ro52, leading to stalling of the ribosome and incomplete translation. By using *E. coli* strains genetically supplemented with t-RNAs rarely used in prokaryotes, translation will proceed, leading to a non-truncated protein. The *E. coli* strain TB-1 used for expression of MaBP-Ro52 is not supplemented with rare t-RNAs, explaining the truncated Ro52. In contrast, the production of non-truncated Ro52 is increased when using *E. coli* strains enriched for rare t-RNAs (data not shown).

Together with the two-hybrid data, the ultracentrifugation experiment suggests that Ro52 forms weak dimers through the coiled-coil domain. However, when separating the RING-B-box domain with SDS-PAGE, a weak dimerization product of this domain was observed after Coomassie staining as a band of approximately 30 kDa. In the same experiment, coiled-coil dimers were, however, not observed after Coomassie staining. Rhodes et al. and Li et al. have showed that Ro52 forms trimers by using cross-linking experiments (Rhodes and Trowsdale, 2006, Li et al., 2007b). However, these authors did not explicitly show that the homotypic trimerization occured through the coiled-coil domain; although Rhodes

et al. exclude that it is mediated by the RING, B-box or B30.2 domains. The most thorough investigation of a TRIM protein oligomerization, is by Peng et al. (Peng et al., 2000). By using a wide array of techniques, they demonstrate that TRIM28 form homo- and hetero-oligomers. Specifically, purified TRIM28 exists in a monomer-trimer-hexamer equilibrium.

★

Paper II

-Ro52 expression in PBMCs: High or low?

The mRNA levels of Ro52 differed in PBMCs from healthy subjects compared to PBMCs from patients with SS and SLE. This finding is in line with the interferon signature in SS and SLE patients, and Ro52 being an interferon stimulated gene (paper V). In contrast to the high levels of IFNα detected in SLE patients (Kim et al., 1987), only low levels of IFNα has been detected in sera from patients with SS (Bave et al., 2005). This argues against that the high Ro52 levels in PBMCs from SS patients are due to high IFNα levels. However, measurement of IFNα by ELISA has a low reproducibility and often do not correlate with results from more sensitive bioassays (Jabs et al., 1999, Hua et al., 2006). Moreover, an IFN signature, and infiltrates of plasmacytoid dendritic cells in salivary glands, are present in patients with SS (Gottenberg et al., 2006), indicating that IFNα is indeed overexpressed in patients compare to healthy controls.

Is there a differential expression of Ro52 in PBMCs? To answer this, PBMCs from a healthy individual were purified into T cells ($CD3^+$), monocytes ($CD14^+$), and B cells ($CD19^+$) using antibody coated magnetic beads; and the expression of Ro52 was determined by Q-RT-PCR and immunoblotting. Paradoxically, in $CD19^+$ cells the Ro52 expression was high at the mRNA level but low at the protein level. An interpretation of these data is that B cells are sensitive to high protein levels of Ro52, as shown by lower proliferation of Ro52 overexpressing B cell lines (paper II). The high level of Ro52 mRNA does not necessarily mean that high levels of protein is produced, e.g., due to downregulated translation. Furthermore, in Ro52 knock-out mice, were the deleted exons are replaced with an internal ribosome entry site (IRES) GFP reporter cassette ($Ro52^{-/+GFP}$), the Ro52

gene is expressed at a lower level in B cells than in T cells as determined by the GFP expression (non-submitted data).

-So, Ro52 is an E3 ligase, but what is the substrate?

As shown in paper II, Ro52 is an E3 ligase. However, this result is only based on autoubiquitination assays. To understand the role of Ro52 in vivo it is necessary to know the substrate of Ro52 mediated ubiquitination. Three substrates have been suggested in the literature: p27 (Sabile et al., 2006), IRF-8 (Kong et al., 2007) and IgG heavy chain (Takahata et al., 2007). Although p27 has been suggested as a substrate, Ro52 was not shown to be directly involved. Sabile et al. show that Ro52 is a component of the SCF^{Skp2} multisubunit E3 ligase complex, but do not show that the E3 ligase activity of Ro52 is necessary for the degradation of p27. The strongest candidate for a substrate of Ro52 is IRF-8 as reported by Kong et al. Direct binding of the B30.2 domain to IRF-8 was shown, as well as a Ro52 mediated ubiquitination of IRF-8 in vitro. Interestingly, the ubiquitination of IRF-8 by Ro52 did not lead to degradation of IRF-8 in the proteasome, but instead to an activation of the transcriptional activity by IRF-8. This suggests that Ro52 modifies IRF-8 with K63 polyubiquitin chains, in contrast to Cbl-b that targets IRF-8 for proteasomal degradation through modiciation with K48 polyubiqutin chains (Xiong et al., 2005).

The Fc part of the IgG1 heavy chain is clearly interacting with the B30.2 domain of Ro52, however, if this binding is of physiological relevance is less certain. Takahata et al. suggest that the IgG1 heavy chain is a substrate of Ro52, and that Ro52 plays a role in quality control through ERAD. However, it is not shown explicitly that the heavy chain of IgG1 indeed is a substrate of Ro52 mediated ubiquitination. After expressing FLAG-Ro52 or FLAG-Ro52ΔRING together with the IgG1 heavy chain in 293T cells, Takahata et al. pulled out IgG1 with protein-A beads and did immunoblotting against ubiquitin. A high molecular smear was detected only when IgG1 heavy chain was co-expressed with FLAG-Ro52, but not when co-expressed with FLAG-Ro52ΔRING. Takahata et al. interpret this as a Ro52 mediated ubiquitination of IgG1 that is dependent on the RING domain. However, it cannot be ruled out that the protein-A beads co-purifed Ro52 through a Ro52-IgG1 interaction, and that the detected high molecular weight ubiquitin smear is only a result of autoubiquitination of Ro52.

The identification of a substrate of an E3 ligase is a difficult task. First, an interaction to a substrate needs to be established. By nature, these interactions are often transient, especially if the E3 ligase mediates the degradation of the substrate. Also, it needs to be confirmed that the interaction between the E3 ligase and the

identified substrate leads to ubiquitination of the substrate. This is usually done through in vitro ubiquitination assays that directly show the modification of the substrate with ubiquitin. Furthermore, the in vivo relevance of the ubiquitination of the substrate needs to be demonstrated. E.g., that ubiquitination leads to degradation in the proteasome, internalization from the cell surface, or activation of transcription factors.

Why does TRIM21/Ro52 inhibit cell proliferation?

Interestingly, the human TRIM21/Ro52 gene is located in a locus suspected of harboring a tumor suppressor gene (Kim et al., 2000, Zhao and Bepler, 2001, Schiebe et al., 2001, Zenklusen et al., 1995). To investigate the effect of TRIM21/Ro52 overexpression, B cells stably transfected with Ro52 or an inactive Ro52 mutatnt were made. B cells stably overexpressing Ro52-GFP, but not Ro52ΔRING-GFP or GFP, had a reduced proliferation, implicating Ro52 in the regulation of cell proliferation. Importantly, however, ectopic overexpression of proteins may lead to erroneous conclusions, e.g. due to protein misfolding or mislocalization. Therefore, the use of adequate controls is necessary, in this case an inactive Ro52 mutant and GFP. One problem with the used experimental setup is the commonly observed phenomenon of transgene silencing, which makes it difficult to maintain stably transfected cells. Although the B cells were kept under strong selective pressure with the antibiotic geneticin (G418), Ro52-GFP expressing cell rapidly lost expression; this in contrast to GFP-expressing cells, which expressed high levels of GFP even without selection pressure. Making experiments as early as possible after the verification of stable transfected clones solved this problem. An alternative approach is to make transient transfections, although the interpretation of such experiments might be corrupted by uneven transfection efficiencies. The best approach is to use an inducible expression of Ro52 or Ro52ΔRING in vivo by using Tet-On or Tet-Off mice. Although Tet-Off Ro52-Tg and Ro52ΔRING-Tg mice were made, the expression of the transgenes were unfortunately not detected (data not shown).

The mechanism whereby a high expression of Ro52 inhibited the cellular proliferation is unclear, but might be part of an intracellular anti-proliferation program induced by type I interferons. Signaling through CD40 is known to induce apoptosis in many transformed cells, including some high-grade B cell lymphomas (e.g. A20 cells) (Hess and Engelmann, 1996), but how overexpression of Ro52 enhances this sensitivity is not clear. One mechanism could be through the induction of apoptosis, e.g. by CD95 ligation. Contradicting reports concerning CD40 signaling in A20 cells complicates the interpretation of the results. E.g.,

Hollmann et al. show that anti-CD40 induced apoptosis of A20 cells is not due to increased expression of CD95 but instead they detected activation of caspase-3 (Hollmann et al., 2002). In contrast, Benson et al. demonstrate that anti-CD40 treatment upregulates the expression of CD95 on A20 cells (Benson et al., 2006).

★

Paper III

Blocking the E3 ligase activity of TRIM21/Ro52: are anti-RING autoantibodies really doing it?

In paper III it is demonstrated that autoantibodies against the RING domain of Ro52, but not against the other domains, inhibit the E3 ligase activity of Ro52 in vitro. Furthermore, it is shown that antibodies from sera of patients with SS can penetrate HeLa cells in vitro. Although these data are robust in vitro, it is a controversial issue whether the penetration of cells with antibodies is an in vitro artifact or a finding of physiological relevance. Since the seminal study by Alarcon-Segovia et al (Alarcon-Segovia et al., 1978), several research groups have shown that antibodies can penetrate living cells and even bind intracellular targets and inhibit enzymatic activity (Reichlin et al., 1994, Koren et al., 1995, Zack et al., 1996, Koscec et al., 1997). All these studies are made in vitro, therefore a better way of detecting penetrating antibodies is needed. E.g., the transgenic expression of a GFP-fused antibody light chain in mice, would allow the tracking of antibodies without the need for secondary reagents and fixation/permeabilization of cells. This would drastically decrease the risk of introducing artifacts due to manipulation of cells in vitro.

Originally, the penetration of autoantibodies into human PBMCs was suggested to occur through fragment crystallizable (Fc) receptors (Alarcon-Segovia et al., 1978), resulting in a focus on Fc receptors in subsequent studies. Since HeLa cells were used in paper III, the expression of different Fc receptors was investigated through RT-PCR. Only the MHC I like neonatal Fc receptor (FcRn) (Simister and Mostov, 1989, Burmeister et al., 1994) was expressed (data not shown), suggesting a mechanism whereby antibodies enter the cells by pinocytosis, and bind FcRn in the acidic endosomes, reviewed in (Lencer and Blumberg, 2005). Instead of being directed into the lysosomal compartment for degradation, internalized IgG is

protected by FcRn and is recycled to the cell membrane, or hypothectically released into the cytoplasm. FcRn also binds strongly to albumin (Chaudhury et al., 2003), making it possible to block IgG-FcRn binding by adding albumin. In support of an FcRn binding mechanism, no IgG was detected in HeLa cells after blocking the FcRn with albumin (data not shown).

The transport of IgG via an FcRn dependent mechanism mediates the transfer of antibodies across biological membranes. After internalization of IgG and binding to FcRn, IgG-FcRn containing endosomes fuse with cell membranes and release IgG in a pH dependent manner. Although crossing of membranes is well described, it is unknown if IgG can be released inside the cytoplasm. One explanation for the reported cellular penetration of antibodies, and the binding to intracellular antigen, is that this is an artifact likely caused by the high IgG concentrations used in vitro. High IgG concentrations might saturate the degradation of IgG in lysosomes causing a release of free IgG into the cytoplasm. As mentioned earlier, one way of solving this problems is through the use of transgenic animals expressing a GFP-fused light chain of IgG.

★

Paper IV

What keeps TRIM21/Ro52 in the cytoplasm?

TRIM21/Ro52 is mainly located in the cytoplasm, however, when detecting Ro52 by immunofluorescence or by using fluorescent protein tags, a small amount of nuclear Ro52 is observed. Furthermore, Ro52 is often detected in the nucleus of stressed cells (data not shown). Together, this indicates that Ro52 can be imported to the nucleus. One hypothesis is that Ro52 is located in the cytoplasm due to a continuous export of Ro52 from the nucleus. This would explain the low, but detectable, levels of Ro52 in the nucleus. After either an increased rate of import, or a decreased rate of export, Ro52 would accumulate in the nucleus. Unfortunately, however, there is no classical NLS or NES in Ro52. Therefore, several deletion mutants of Ro52 were analyzed with respect to their intracellular localization in hope of finding new NLS or NES sequences. An intact coiled-coil domain (CC2) was necessary for the cytoplasmic localization of Ro52, and interestingly, a putative leucine rich NES was located in this

region. We hypothesized that the NES was dependent on the nuclear export receptor Exportin-1, which is inhibited by LMB. However, neither addition of LMB, or mutation of the NES, lead to an accumulation of Ro52 in the nucleus. Intriguingly, calreticulin is a nuclear export receptor that has been shown to interact with Ro52 (Holaska et al., 2001, Holaska et al., 2002, Cheng et al., 1996), potentially explaining why LMB and ablation of the putative NES in Ro52 does not affect the nuclear localization of Ro52. Further experimentation is needed to investigate the relationship of Ro52 and calreticulin.

Ro52 translocates to the nucleus of HeLa cells after exposure to IFNα or NO. Since both these molecules are inflammatory mediators, we also investigated the intracellular localization of Ro52 in inflamed tissue. Indeed, in inflamed skin from patients with cutaneous lupus erythematosus, Ro52 was highly expressed in the nucleus. Futhermore, the expression of inducible nitric oxide synthetase (iNOS) was increased, suggesting that the nuclear localization of Ro52 is caused by NO. The nuclear import of Ro52 after exposure to IFNα or NO might be dependent on a post-translational modification, e.g. phosphorylation or nitrosylation.

Both IFNα and NO are inducers of cell cycle arrest, and thus, one possible explanation is that Ro52 is involved in regulating proliferation after its translocation to the nucleus. This hypothesis could be tested in cells from $Ro52^{-/-GFP}$ mice, which hypothetically would have a reduced sensitivity to IFNα and NO induced cell cycle arrest.

★

Paper V

IFN stimulated response elements in the Ro52 promoter?

The TRIM5α promoter contains interferon stimulated response elements (ISRE) (Asaoka et al., 2005), which recruits interferon stimulated gene factor 3 (ISGF3), a transription factor complex consisting of signal transducer and activator of transcription 1 and 2 (STAT1 and STAT2) and IRF-9, for review see (Brierley and Fish, 2005). ISGF3 complexes initiate transcription of TRIM5α after IFNα induced signaling. ISRE elements have not, however, been reported in the proximal promoter region of TRIM21/Ro52. On the other hand, putative ISREs can be found by bioinformatic approaches using algorithms for finding transcription factor binding sites. One experimental approach to find ISREs is to

clone overlapping genomic DNA fragments upstream of TRIM21/Ro52, and using them for reporter assays of promoter activity. By inserting genomic fragments in front of a promoterless luciferase expression cassette, transfecting the reporter constructs into an IFNα responsive cell line, and measuring the luciferase luminescence after addition of IFNα, it is possible to map IFNα stimulated promoter elements. Because TRIM21/Ro52 is induced by IFNα, and possibly also by IFNγ and LPS, more than one type of inducible promoter element is predicted to be present in the TRIM21/Ro52 promoter. E.g., although ISREs are partially activated by IFNγ signaling, it is also possible that the promoter contains gamma activation sites (GAS). Indeed, binding sites for other transcription factors have been predicted; Gilchrist et al. used a computational approach to identify genes regulated by activating transcription factor 3 (ATF3) after TLR4 activation of mactophages; interestingly, TRIM21/Ro52 was among the genes to contain putatice ATF3 and NFκB binding sites (Gilchrist et al., 2006).

Ro52, yet another antiviral TRIM?

Mammalian cells contain restriction factors that inhibit infections with retroviruses (Nisole et al., 2005). These restriction factors are dominant, as evident by their titration with purified viral proteins, and they act at an early stage after viral entry. Although many TRIMs have been suggested to be antiviral restriction factors, e.g. TRIM1 (Yap et al., 2004), TRIM5 (Stremlau et al., 2004), TRIM19 (Chelbi-Alix et al., 1998), TRIM22 (Tissot and Mechti, 1995), TRIM25 (Gack et al., 2007b) and TRIM28 (Wolf and Goff, 2007) only the role of TRIM5 has been widely reported. Hypothetically, the TRIM motif is an effector motif shared by many restriction factors, while the C-terminal domain mediates the recognition of viruses. In support of this view, the B30.2 domains of many TRIMs contain variable loops (Song et al., 2005), which might have been positively selected to recognize viruses. E.g., the variable loops of the B30.2 domain of TRIM5 gene show signs of a high degree of positive selection, judged by the high ratio of non-synonymous to synonymous variation (Song et al., 2005).

TRIM21/Ro52 also has variable loops in the B30.2 domain, however, a virus restricting activity for TRIM21/Ro52 has not been reported. Li et al. could not demonstrate any antiviral activity of TRIM21/Ro52 against a number of tested retroviruses, whereas TRIM5 inhibited most of them (Li et al., 2007b). TRIM21/Ro52 could, however, have an antiviral activity against other viruses then the tested ones. E.g., TRIM21/Ro52 in human PBMCs was upregulated by inactivated HSV, indicating a role in antiviral responses. Thus, an antiviral role for TRIM21/Ro52 has not been reported, but certainly cannot be excluded.

★

Paper VI

Could HMGB-1 also do it?

High mobility group box 1 (HMGB-1) is a nuclear DNA binding protein that also has a role as a proinflammatory mediator. HMGB-1 is actively secreted from cells, or released from necrotic cells (Gardella et al., 2002, Scaffidi et al., 2002). As demonstrated in paper VI, extracellular HMGB-1 is detected in skin lesions of patients with cutaneous lupus erythematosus. HMGB-1, like IFNα and NO, is a proinflammatory mediator; and by binding to receptor for advanced glycosylated end products (RAGE), TLR-2 or TLR-4, HMGB-1 can induce the activation of NFκB transcription factors (Kokkola et al., 2005, Park et al., 2006). Thus, addition of HMGB-1 to cells might also lead to the nuclear translocation of Ro52. If true, this might mean that the intracellular localization of Ro52 is regulated by inflammatory signals in general.

★

HYPOTHESIS

To go further, and to design experiments that provide new data regarding the function of TRIM21/Ro52, it is necessary to first design a hypothesis. A hypothesis will be generated by analyzing the data presented in this thesis, together with the data available in the literature.

First, expression analysis can give clues to the function of a protein, this by analyzing the temporal, spatial, and quantitative aspects of gene/protein expression. E.g., a protein that only is detected in the embryonic brain is likely to play an important role in brain development. Similarly, a protein whose expression is restricted to a differentiated cell type is likely to be involved in the effector function of these differentiated cells. Secondly, by analyzing signaling pathways leading to the activation of a protein, it is possible to make predictions of protein function. E.g., a protein upregulated by type I IFNs, could be suspected of playing a role in antiviral defense; either by directly interfering with viral particles, or indirectly, by changing the cellular metabolism to hinder further spreading of a virus. Lastly, by analyzing interaction partners, the effect on cells after overexpression of different mutants, and the biochemical properties of a protein, it is possible to gain functional understanding and to create a new hypothesis.

Expression of TRIM21/Ro52. Using in situ hybridization, Reymond et al. demonstrated that TRIM21/Ro52 is ubiquitously expressed in adult and embryonic mouse tissues (Reymond et al., 2001). In addition, all immune cells in lymph nodes, spleen, and peripheral blood express TRIM21/Ro52 as judged by GFP expression in $Ro52^{-/+GFP}$ reporter mice (non-submitted data). Interestingly, TRIM21/Ro52 is expressed at higher levels in $CD3^+$ cells than in $CD19^+$ and $CD11b^+$ cells.

It could be argued that the ubiquitous expression of TRIM21/Ro52 goes against a cell specific function, e.g. the regulation of IgG metabolism in B cells as described by Takahata et al. (Takahata et al., 2007). Rather, the target of TRIM21/Ro52 mediated ubiquitination could be expected to be a ubiquitously expressed protein. Intriguingly, all TRIMs with reported antiviral effects i.e., TRIM1, TRIM5, TRIM19, TRIM22, TRIM25 and TRIM28, are also ubiquitously expressed (Reymond et al., 2001). Thus, the ubiquitous expression of TRIM21/Ro52 might reflect its role in an ancient innate immune defense.

Induction of TRIM21/Ro52 expression. In paper V, TRIM21/Ro52 is shown to be induced by IFNα and HSV. Furthermore, it has previously been demonstrated that TRIM21/Ro52 is induced by IFNγ and LPS. The IFNα, IFNγ, and LPS signaling pathways (**Fig 12**) are all involved in inflammatory responses against infectious organisms. E.g., HSV induces interferon production by plasmacytoid DCs (Lund et al., 2003) after activating the TLR2 and TLR9 signaling pathways (Sato et al., 2006). Hence, it is plausible that TRIM21/Ro52 expression is upregulated as part of a genetic program designed to fight off invading microorganisms.

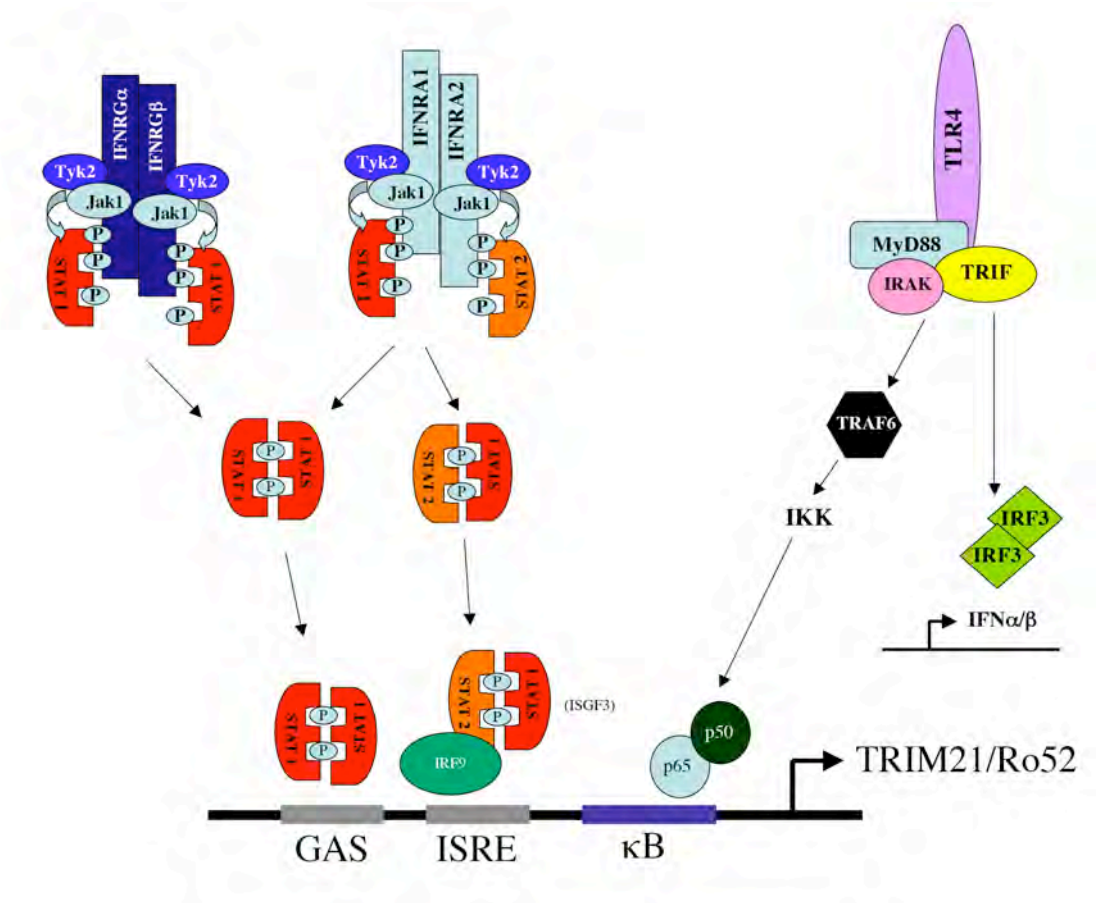

Fig 12. Signaling through type I IFN receptors (IFNRA1, IFNRA2), type II IFN receptor (IFNRGα, IFNRGβ) or through TLR4 leads to an increased expression of TRIM/Ro52; maybe as a part of an antiviral genetic program.

Function! Although the in vitro binding of IgG heavy chain and TRIM21/Ro52 is robust, a physiological consequence of this interaction remains to be convincingly demonstrated. The other proposed substrate of TRIM21/Ro52, p27, is

ubiquitinated by the multisubunit E3 ligase complex SCF^{Skp2}, of which TRIM21/Ro52 has been suggested to be a subunit. In addition, TRIM21/Ro52 has been demonstrated to enhance the ubiquitination of p27 by SCF^{Skp2}, but was not shown to directly ubiquitinate p27. Therefore, TRIM21/Ro52 might be a cofactor of SCF^{Skp2} but not a bona fide E3 ligase for p27. This, and the fact that TRIM21/Ro52 is implicated in IFN signaling pathways, makes IRF-8 the most attractive target since TRIM21/Ro52 is demonstrated to directly bind and ubiquitinate IRF-8.

TRIM21/Ro52 is hypothetically a protein involved in innate immune responses against invading microorganisms. However, like many proteins, TRIM21/Ro52 might play different roles in different cell types, even though it works through the same intracellular mechanisms. E.g., the high expression of TRIM21/Ro52 in T cells could mean that TRIM21/Ro52 is involved in regulating T cell responses to infection, e.g. by regulating IFNγ or IL-2 expression. In contrast, the lower expression in B cells indicates that TRIM21/Ro52 is inducible and might have a different role in B cells. It would be interesting to investigate if the expression of TRIM21/Ro52 is induced (or turned off) by some stimuli, e.g., CD3 and CD28 signaling in T cells or CD40 signaling in B cells. Cells from $Ro52^{-/-GFP}$ mice will be useful in delineating the role of TRIM21/Ro52 in immunity.

In conclusion, it is likely that TRIM21/Ro52 soon will be reported to be necessary for cellular responses against certain types of infections.

★

CONCLUSION

Through the use of biochemical, biophysical, and molecular biological techniques, we demonstrate that the ubiquitously expressed intracellular protein TRIM21/Ro52 is a Zn^{2+} binding protein and a RING dependent E3 ligase. Moreover, TRIM21/Ro52 was found to be overexpressed in PBMCs of patients with SS and SLE, and ectopic overexpression of TRIM21/Ro52 in B cells in vitro inhibited proliferation. We hypothesized that the increased TRIM21/Ro52 expression in patients is a part of the interferon signature seen in SS and SLE. Indeed, expression of TRIM21/Ro52 was induced in cell lines and human PBMCs in vitro after incubation with IFNα. Interestingly, TRIM21/Ro52 expression was also induced in PBMCs after addition of inactivated herpes simplex virus, indicating that TRIM21/Ro52 is an interferon stimulated gene that is involved in a genetic program designed to fight infections. In addition, after incubation with IFNα or nitric oxide, TRIM21/Ro52 was translocated from the cytoplasm to the nucleus.

In all, TRIM21/Ro52 is an E3 ligase that inhibits cell proliferation and whose expresssion is induced by IFNα and herpes simplex.

★

ACKNOWLEDGMENTS

During my time as a PhD student at Karolinska Institutet I have had the pleasure to work and interact with many brilliant people. To some of them I am especially indebted:

My supervisor: Professor *Marie Wahren-Herlenius*, with your energy, enthusiasm, and broad knowledge, it has been a great privilege to have you as a supervisor and mentor. Your scientific intuition, and your ability to give your PhD students the best of opportunities, has been fundamental for the finalization of this thesis.

Head of the Rheumatology Unit: Professor *Lars Klareskog*, thank you for making the lab an excellent place for research.

The MWH group: *Lars*, thank you for being a great lab mate, for our hardcore biochemistry discussions, and for your excellent computer skills. *Stina*, you have always been around to help and to cheer up when necessary; your warm personality has made everything a little more fun. *Linn*, we started the long struggle as PhD students together; with your heart of gold, and will to always help out, it has been a great ride! *Wei*, for your company in the lab; I miss our sessions on molecular biology problem solving, our study of weird bands on Western blot films, and your kindness. *Aurélie*, although you don't like wine, you have given the lab a refreshing french touch; your friendliness, carefulness, and great lab skills are invaluable. *Susanna*, so finally, you have started in the lab; with your immense energy and will to learn, the lab is lucky to have you. *Vilija*, as a member of the 'lupus team' you have enriched the lab discussions with your critical eye and your clinical experience. *Monica*, for our fights with frustrating experiments. *Vijole*, for your delicious pies and cheesecakes! *Åse*, thanks for all your help with experiments. *Karin* and *Filippa*, the other members of the 'lupus team', thank you for your lovely personalities and for providing material and input to the projects. *Robert* for always seeing the details in everybody's work and for your upright personality. *Gabriella*, your soft, yet steady, guidance through my first cell culture adventures was crucial *Evi*, thank you for the introduction to practical lab work; and *Malin* and *Johan* thanks for the help with experiments during your summer projects.

The biophysicists from Linköping: *Janosch*, for your love of protein structures, frank personality, and insight in (german) football tactics; I'm still planning for the crash course in MALDI! *Maria Sunnerhagen*, thank you for the discussions and your input into the projects.

The Boston experience: Professor *Vijay Kuchroo*, your great scientific know-how and generosity made the year at your lab an immensely rewarding experience! *Mohamed*, relaxed molecular biologist extraordinaire, thank you for upgrading my cloning skills, teaching me how to make a good mouse, and for answering all my questions. *Valérie*, aaargh, it is impossible to express my gratitude in just a few sentences. Thanks for everything!! *Maryam*, thanks for friendship and for looking after me when I first came to the lab. *Nasim*, your antibodies served me well, oh, and by the way, your hair looks great! *Amit*, buddy, I hope that you still keep our mañana spirit alive. *Hanspeter*, for your enlightening skepticism. *Shirin* thanks for your friendship and for great times in Boston.

All the co-authors!!!

The past and present members of the Rheumatology and Neuroimmunology labs at CMM, Karolinska Institutet, Solna: Thank you all for great trips to conferences, sharing of reagents, scientific input, and for friendship!

★

The life at a scientific laboratory is nothing without family and friends. In the end, they are the ones that make everything possible.

My friends: guys, thank you for keeping me alive during all these years.

The Espinosa and Covacu families: you have given me so much affection and love. Tack! Gracias! Multumesc!

Ruxandra, you are everything!

REFERENCES

AGELL, N., RYAN, C. & SCHLESINGER, M. J. (1991) Partial purification and substrate specificity of a ubiquitin hydrolase from Saccharomyces cerevisiae. *Biochem J,* 273 (Pt 3)**,** 615-20.

ALARCON-SEGOVIA, D., RUIZ-ARGUELLES, A. & FISHBEIN, E. (1978) Antibody to nuclear ribonucleoprotein penetrates live human mononuclear cells through Fc receptors. *Nature,* 271**,** 67-9.

ALKALAY, I., YARON, A., HATZUBAI, A., ORIAN, A., CIECHANOVER, A. & BEN-NERIAH, Y. (1995) Stimulation-dependent I kappa B alpha phosphorylation marks the NF-kappa B inhibitor for degradation via the ubiquitin-proteasome pathway. *Proc Natl Acad Sci U S A,* 92**,** 10599-603.

ALSPAUGH, M. A. & TAN, E. M. (1975) Antibodies to cellular antigens in Sjogren's syndrome. *J Clin Invest,* 55**,** 1067-73.

AMERIK, A. Y. & HOCHSTRASSER, M. (2004) Mechanism and function of deubiquitinating enzymes. *Biochim Biophys Acta,* 1695**,** 189-207.

ANANDASABAPATHY, N., FORD, G. S., BLOOM, D., HOLNESS, C., PARAGAS, V., SEROOGY, C., SKRENTA, H., HOLLENHORST, M., FATHMAN, C. G. & SOARES, L. (2003) GRAIL: an E3 ubiquitin ligase that inhibits cytokine gene transcription is expressed in anergic CD4+ T cells. *Immunity,* 18**,** 535-47.

ANEGON, I., CUTURI, M. C., TRINCHIERI, G. & PERUSSIA, B. (1988) Interaction of Fc receptor (CD16) ligands induces transcription of interleukin 2 receptor (CD25) and lymphokine genes and expression of their products in human natural killer cells. *J Exp Med,* 167**,** 452-72.

ARRIGO, A. P., TANAKA, K., GOLDBERG, A. L. & WELCH, W. J. (1988) Identity of the 19S 'prosome' particle with the large multifunctional protease complex of mammalian cells (the proteasome). *Nature,* 331**,** 192-4.

ASAOKA, K., IKEDA, K., HISHINUMA, T., HORIE-INOUE, K., TAKEDA, S. & INOUE, S. (2005) A retrovirus restriction factor TRIM5alpha is transcriptionally regulated by interferons. *Biochem Biophys Res Commun,* 338**,** 1950-6.

BABST, M., ODORIZZI, G., ESTEPA, E. J. & EMR, S. D. (2000) Mammalian tumor susceptibility gene 101 (TSG101) and the yeast homologue, Vps23p, both function in late endosomal trafficking. *Traffic,* 1**,** 248-58.

BACHMAIER, K., KRAWCZYK, C., KOZIERADZKI, I., KONG, Y. Y., SASAKI, T., OLIVEIRA-DOS-SANTOS, A., MARIATHASAN, S., BOUCHARD, D., WAKEHAM, A., ITIE, A., LE, J., OHASHI, P. S., SAROSI, I., NISHINA, H., LIPKOWITZ, S. & PENNINGER, J. M.

(2000) Negative regulation of lymphocyte activation and autoimmunity by the molecular adaptor Cbl-b. *Nature,* 403**,** 211-6.
BACHMAIR, A., FINLEY, D. & VARSHAVSKY, A. (1986) In vivo half-life of a protein is a function of its amino-terminal residue. *Science,* 234**,** 179-86.
BAECHLER, E. C., BATLIWALLA, F. M., KARYPIS, G., GAFFNEY, P. M., ORTMANN, W. A., ESPE, K. J., SHARK, K. B., GRANDE, W. J., HUGHES, K. M., KAPUR, V., GREGERSEN, P. K. & BEHRENS, T. W. (2003) Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci U S A,* 100**,** 2610-5.
BAKER, R. T. & BOARD, P. G. (1991) The human ubiquitin-52 amino acid fusion protein gene shares several structural features with mammalian ribosomal protein genes. *Nucleic Acids Res,* 19**,** 1035-40.
BARLOW, P. N., LUISI, B., MILNER, A., ELLIOTT, M. & EVERETT, R. (1994) Structure of the C3HC4 domain by 1H-nuclear magnetic resonance spectroscopy. A new structural class of zinc-finger. *J Mol Biol,* 237**,** 201-11.
BARTEL, B., WUNNING, I. & VARSHAVSKY, A. (1990) The recognition component of the N-end rule pathway. *Embo J,* 9**,** 3179-89.
BATES, E. E., RAVEL, O., DIEU, M. C., HO, S., GURET, C., BRIDON, J. M., AIT-YAHIA, S., BRIERE, F., CAUX, C., BANCHEREAU, J. & LEBECQUE, S. (1997) Identification and analysis of a novel member of the ubiquitin family expressed in dendritic cells and mature B cells. *Eur J Immunol,* 27**,** 2471-7.
BAUMEISTER, W., WALZ, J., ZUHL, F. & SEEMULLER, E. (1998) The proteasome: paradigm of a self-compartmentalizing protease. *Cell,* 92**,** 367-80.
BAVE, U., NORDMARK, G., LOVGREN, T., RONNELID, J., CAJANDER, S., ELORANTA, M. L., ALM, G. V. & RONNBLOM, L. (2005) Activation of the type I interferon system in primary Sjogren's syndrome: a possible etiopathogenic mechanism. *Arthritis Rheum,* 52**,** 1185-95.
BAYER, P., ARNDT, A., METZGER, S., MAHAJAN, R., MELCHIOR, F., JAENICKE, R. & BECKER, J. (1998) Structure determination of the small ubiquitin-related modifier SUMO-1. *J Mol Biol,* 280**,** 275-86.
BEAL, R. E., TOSCANO-CANTAFFA, D., YOUNG, P., RECHSTEINER, M. & PICKART, C. M. (1998) The hydrophobic effect contributes to polyubiquitin chain recognition. *Biochemistry,* 37**,** 2925-34.
BEN-CHETRIT, E., CHAN, E. K., SULLIVAN, K. F. & TAN, E. M. (1988) A 52-kD protein is a novel component of the SS-A/Ro antigenic particle. *J Exp Med,* 167**,** 1560-71.
BEN-CHETRIT, E., GANDY, B. J., TAN, E. M. & SULLIVAN, K. F. (1989) Isolation and characterization of a cDNA clone encoding the 60-kD component of the human SS-A/Ro ribonucleoprotein autoantigen. *J Clin Invest,* 83**,** 1284-92.
BENMERAH, A., GAGNON, J., BEGUE, B., MEGARBANE, B., DAUTRY-VARSAT, A. & CERF-BENSUSSAN, N. (1995) The tyrosine kinase

substrate eps15 is constitutively associated with the plasma membrane adaptor AP-2. *J Cell Biol,* 131**,** 1831-8.
BENSON, R. J., HOSTAGER, B. S. & BISHOP, G. A. (2006) Rapid CD40-mediated rescue from CD95-induced apoptosis requires TNFR-associated factor-6 and PI3K. *Eur J Immunol,* 36**,** 2535-43.
BERG, J. M. (1990) Zinc finger domains: hypotheses and current knowledge. *Annu Rev Biophys Biophys Chem,* 19**,** 405-21.
BILLINGHAM, R. E., BRENT, L. & MEDAWAR, P. B. (1953) Actively acquired tolerance of foreign cells. *Nature,* 172**,** 603-6.
BOARD, P. G., COGGAN, M., BAKER, R. T., VUUST, J. & WEBB, G. C. (1992) Localization of the human UBC polyubiquitin gene to chromosome band 12q24.3. *Genomics,* 12**,** 639-42.
BOISCLAIR, M. D., MCCLURE, C., JOSIAH, S., GLASS, S., BOTTOMLEY, S., KAMERKAR, S. & HEMMILA, I. (2000) Development of a ubiquitin transfer assay for high throughput screening by fluorescence resonance energy transfer. *J Biomol Screen,* 5**,** 319-28.
BORDEN, K. L., MARTIN, S. R., O'REILLY, N. J., LALLY, J. M., REDDY, B. A., ETKIN, L. D. & FREEMONT, P. S. (1993) Characterisation of a novel cysteine/histidine-rich metal binding domain from Xenopus nuclear factor XNF7. *FEBS Lett,* 335**,** 255-60.
BREITSCHOPF, K., BENGAL, E., ZIV, T., ADMON, A. & CIECHANOVER, A. (1998) A novel site for ubiquitination: the N-terminal residue, and not internal lysines of MyoD, is essential for conjugation and degradation of the protein. *Embo J,* 17**,** 5964-73.
BRIERLEY, M. M. & FISH, E. N. (2005) Stats: multifaceted regulators of transcription. *J Interferon Cytokine Res,* 25**,** 733-44.
BURMEISTER, W. P., GASTINEL, L. N., SIMISTER, N. E., BLUM, M. L. & BJORKMAN, P. J. (1994) Crystal structure at 2.2 A resolution of the MHC-related neonatal Fc receptor. *Nature,* 372**,** 336-43.
BURNET, F. M. (1959) *The clonal selection theory of acquired immunity,* Nashville, TN, Vanderbilt University Press.
BUUS, S. & WERDELIN, O. (1986) A group-specific inhibitor of lysosomal cysteine proteinases selectively inhibits both proteolytic degradation and presentation of the antigen dinitrophenyl-poly-L-lysine by guinea pig accessory cells to T cells. *J Immunol,* 136**,** 452-8.
BUYON, J. P., SLADE, S. G., REVEILLE, J. D., HAMEL, J. C. & CHAN, E. K. (1994) Autoantibody responses to the "native" 52-kDa SS-A/Ro protein in neonatal lupus syndromes, systemic lupus erythematosus, and Sjogren's syndrome. *J Immunol,* 152**,** 3675-84.
CAMMAS, F., MARK, M., DOLLE, P., DIERICH, A., CHAMBON, P. & LOSSON, R. (2000) Mice lacking the transcriptional corepressor TIF1beta are defective in early postimplantation development. *Development,* 127**,** 2955-63.
CASSATELLA, M. A., ANEGON, I., CUTURI, M. C., GRISKEY, P., TRINCHIERI, G. & PERUSSIA, B. (1989) Fc gamma R(CD16) interaction with ligand induces Ca2+ mobilization and phosphoinositide

turnover in human natural killer cells. Role of Ca2+ in Fc gamma R(CD16)-induced transcription and expression of lymphokine genes. *J Exp Med,* 169**,** 549-67.

CHAN, E. K., DI DONATO, F., HAMEL, J. C., TSENG, C. E. & BUYON, J. P. (1995) 52-kD SS-A/Ro: genomic structure and identification of an alternatively spliced transcript encoding a novel leucine zipper-minus autoantigen expressed in fetal and adult heart. *J Exp Med,* 182**,** 983-92.

CHAN, E. K., HAMEL, J. C., BUYON, J. P. & TAN, E. M. (1991) Molecular definition and sequence motifs of the 52-kD component of human SS-A/Ro autoantigen. *J Clin Invest,* 87**,** 68-76.

CHAN, E. K., HAMEL, J. C., PEEBLES, C. L., BUYON, J. P. & TAN, E. M. (1990) Molecular characterization and cloning of the 52 kDa SS-A/Ro protein. *Mol Biol Rep,* 14**,** 53.

CHAU, V., TOBIAS, J. W., BACHMAIR, A., MARRIOTT, D., ECKER, D. J., GONDA, D. K. & VARSHAVSKY, A. (1989) A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. *Science,* 243**,** 1576-83.

CHAUDHURY, C., MEHNAZ, S., ROBINSON, J. M., HAYTON, W. L., PEARL, D. K., ROOPENIAN, D. C. & ANDERSON, C. L. (2003) The major histocompatibility complex-related Fc receptor for IgG (FcRn) binds albumin and prolongs its lifespan. *J Exp Med,* 197**,** 315-22.

CHELBI-ALIX, M. K., PELICANO, L., QUIGNON, F., KOKEN, M. H., VENTURINI, L., STADLER, M., PAVLOVIC, J., DEGOS, L. & DE THE, H. (1995) Induction of the PML protein by interferons in normal and APL cells. *Leukemia,* 9**,** 2027-33.

CHELBI-ALIX, M. K., QUIGNON, F., PELICANO, L., KOKEN, M. H. & DE THE, H. (1998) Resistance to virus infection conferred by the interferon-induced promyelocytic leukemia protein. *J Virol,* 72**,** 1043-51.

CHEN, Z., HAGLER, J., PALOMBELLA, V. J., MELANDRI, F., SCHERER, D., BALLARD, D. & MANIATIS, T. (1995) Signal-induced site-specific phosphorylation targets I kappa B alpha to the ubiquitin-proteasome pathway. *Genes Dev,* 9**,** 1586-97.

CHEN, Z. & PICKART, C. M. (1990) A 25-kilodalton ubiquitin carrier protein (E2) catalyzes multi-ubiquitin chain synthesis via lysine 48 of ubiquitin. *J Biol Chem,* 265**,** 21835-42.

CHENG, S. T., NGUYEN, T. Q., YANG, Y. S., CAPRA, J. D. & SONTHEIMER, R. D. (1996) Calreticulin binds hYRNA and the 52-kDa polypeptide component of the Ro/SS-A ribonucleoprotein autoantigen. *J Immunol,* 156**,** 4484-91.

CIECHANOVER, A., ELIAS, S., HELLER, H., FERBER, S. & HERSHKO, A. (1980) Characterization of the heat-stable polypeptide of the ATP-dependent proteolytic system from reticulocytes. *J Biol Chem,* 255**,** 7525-8.

CIECHANOVER, A., HELLER, H., KATZ-ETZION, R. & HERSHKO, A. (1981) Activation of the heat-stable polypeptide of the ATP-dependent proteolytic system. *Proc Natl Acad Sci U S A,* 78**,** 761-5.

CIEHANOVER, A., HOD, Y. & HERSHKO, A. (1978) A heat-stable polypeptide component of an ATP-dependent proteolytic system from reticulocytes. *Biochem Biophys Res Commun,* 81**,** 1100-5.

CIOFANI, M. & ZUNIGA-PFLUCKER, J. C. (2007) The Thymus as an Inductive Site for T Lymphopoiesis. *Annu Rev Cell Dev Biol,* 23**,** 463-493.

CLARK, G., REICHLIN, M. & TOMASI, T. B., JR. (1969) Characterization of a soluble cytoplasmic antigen reactive with sera from patients with systemic lupus erythmatosus. *J Immunol,* 102**,** 117-22.

COLLATZ, E., WOOL, I. G., LIN, A. & STOFFLER, G. (1976) The isolation of eukaryotic ribosomal proteins. The purification and characterization of the 40 S ribosomal subunit proteins S2, S3, S4, S5, S6, S7, S8, S9, S13, S23/S24, S27, and S28. *J Biol Chem,* 251**,** 4666-72.

COOK, W. J., JEFFREY, L. C., CARSON, M., CHEN, Z. & PICKART, C. M. (1992) Structure of a diubiquitin conjugate and a model for interaction with ubiquitin conjugating enzyme (E2). *J Biol Chem,* 267**,** 16467-71.

COOK, W. J., JEFFREY, L. C., KASPEREK, E. & PICKART, C. M. (1994) Structure of tetraubiquitin shows how multiubiquitin chains can be formed. *J Mol Biol,* 236**,** 601-9.

DE MELKER, A. A., VAN DER HORST, G., CALAFAT, J., JANSEN, H. & BORST, J. (2001) c-Cbl ubiquitinates the EGF receptor at the plasma membrane and remains receptor associated throughout the endocytic route. *J Cell Sci,* 114**,** 2167-78.

DEMARTINO, G. N. & GOLDBERG, A. L. (1979) Identification and partial purification of an ATP-stimulated alkaline protease in rat liver. *J Biol Chem,* 254**,** 3712-5.

DENG, L., WANG, C., SPENCER, E., YANG, L., BRAUN, A., YOU, J., SLAUGHTER, C., PICKART, C. & CHEN, Z. J. (2000) Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell,* 103**,** 351-61.

DER, S. D., ZHOU, A., WILLIAMS, B. R. & SILVERMAN, R. H. (1998) Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. *Proc Natl Acad Sci U S A,* 95**,** 15623-8.

DIDONATO, J., MERCURIO, F., ROSETTE, C., WU-LI, J., SUYANG, H., GHOSH, S. & KARIN, M. (1996) Mapping of the inducible IkappaB phosphorylation sites that signal its ubiquitination and degradation. *Mol Cell Biol,* 16**,** 1295-304.

DIDONATO, J. A., MERCURIO, F. & KARIN, M. (1995) Phosphorylation of I kappa B alpha precedes but is not sufficient for its dissociation from NF-kappa B. *Mol Cell Biol,* 15**,** 1302-11.

DIKIC, I. & GIORDANO, S. (2003) Negative receptor signalling. *Curr Opin Cell Biol,* 15**,** 128-35.

DISTECHE, C. M., ZACKSENHAUS, E., ADLER, D. A., BRESSLER, S. L., KEITZ, B. T. & CHAPMAN, V. M. (1992) Mapping and expression of the ubiquitin-activating enzyme E1 (Ube1) gene in the mouse. *Mamm Genome,* 3**,** 156-61.

DONALDSON, K. M., YIN, H., GEKAKIS, N., SUPEK, F. & JOAZEIRO, C. A. (2003) Ubiquitin signals protein trafficking via interaction with a novel ubiquitin binding domain in the membrane fusion regulator, Vps9p. *Curr Biol,* 13**,** 258-62.

DORNER, T. & LIPSKY, P. E. (2004) Correlation of circulating CD27high plasma cells and disease activity in systemic lupus erythematosus. *Lupus,* 13**,** 283-9.

DUBIEL, W., FERRELL, K., PRATT, G. & RECHSTEINER, M. (1992) Subunit 4 of the 26 S protease is a member of a novel eukaryotic ATPase family. *J Biol Chem,* 267**,** 22699-702.

EISEN, H. N. & SISKIND, G. W. (1964) Variations In Affinities Of Antibodies During The Immune Response. *Biochemistry,* 3**,** 996-1008.

ESPINOSA, A., ZHOU, W., EK, M., HEDLUND, M., BRAUNER, S., POPOVIC, K., HORVATH, L., WALLERSKOG, T., OUKKA, M., NYBERG, F., KUCHROO, V. K. & WAHREN-HERLENIUS, M. (2006) The Sjogren's syndrome-associated autoantigen Ro52 is an E3 ligase that regulates proliferation and cell death. *J Immunol,* 176**,** 6277-85.

FADOK, V. A., BRATTON, D. L., KONOWAL, A., FREED, P. W., WESTCOTT, J. Y. & HENSON, P. M. (1998) Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J Clin Invest,* 101**,** 890-8.

FAN, W., LIU, Y. C., PARIMOO, S. & WEISSMAN, S. M. (1995) Olfactory receptor-like genes are located in the human major histocompatibility complex. *Genomics,* 27**,** 119-23.

FANG, D. & KERPPOLA, T. K. (2004) Ubiquitin-mediated fluorescence complementation reveals that Jun ubiquitinated by Itch/AIP4 is localized to lysosomes. *Proc Natl Acad Sci U S A,* 101**,** 14782-7.

FARRELL, P. J., BROEZE, R. J. & LENGYEL, P. (1979) Accumulation of an mRNA and protein in interferon-treated Ehrlich ascites tumour cells. *Nature,* 279**,** 523-5.

FENTEANY, G., STANDAERT, R. F., LANE, W. S., CHOI, S., COREY, E. J. & SCHREIBER, S. L. (1995) Inhibition of proteasome activities and subunit-specific amino-terminal threonine modification by lactacystin. *Science,* 268**,** 726-31.

FINLEY, D., BARTEL, B. & VARSHAVSKY, A. (1989) The tails of ubiquitin precursors are ribosomal proteins whose fusion to ubiquitin facilitates ribosome biogenesis. *Nature,* 338**,** 394-401.

FIORINI, M., ALIMANDI, M., FIORENTINO, L., SALA, G. & SEGATTO, O. (2001) Negative regulation of receptor tyrosine kinase signals. *FEBS Lett,* 490**,** 132-41.

FONTENOT, J. D., GAVIN, M. A. & RUDENSKY, A. Y. (2003) Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol,* 4**,** 330-6.

FONTENOT, J. D., RASMUSSEN, J. P., WILLIAMS, L. M., DOOLEY, J. L., FARR, A. G. & RUDENSKY, A. Y. (2005) Regulatory T cell lineage

specification by the forkhead transcription factor foxp3. *Immunity,* 22**,** 329-41.
FRANK, M. B., ITOH, K., FUJISAKU, A., PONTAROTTI, P., MATTEI, M. G. & NEAS, B. R. (1993) The mapping of the human 52-kD Ro/SSA autoantigen gene to human chromosome 11, and its polymorphisms. *Am J Hum Genet,* 52**,** 183-91.
FREEMONT, P. S., HANSON, I. M. & TROWSDALE, J. (1991) A novel cysteine-rich sequence motif. *Cell,* 64**,** 483-4.
FREY, J. L., BINO, T., KANTOR, R. R., SEGAL, D. M., GIARDINA, S. L., RODER, J., ANDERSON, S. & ORTALDO, J. R. (1991) Mechanism of target cell recognition by natural killer cells: characterization of a novel triggering molecule restricted to CD3- large granular lymphocytes. *J Exp Med,* 174**,** 1527-36.
FUKUDA-KAMITANI, T. & KAMITANI, T. (2002) Ubiquitination of Ro52 autoantigen. *Biochem Biophys Res Commun,* 295**,** 774-8.
GACK, M. U., SHIN, Y. C., JOO, C. H., URANO, T., LIANG, C., SUN, L., OSAMU, T., AKIRA, S., CHEN, Z., INOUE, S. & JUNG, J. U. (2007a) TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. *Nature.*
GACK, M. U., SHIN, Y. C., JOO, C. H., URANO, T., LIANG, C., SUN, L., TAKEUCHI, O., AKIRA, S., CHEN, Z., INOUE, S. & JUNG, J. U. (2007b) TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. *Nature,* 446**,** 916-920.
GACZYNSKA, M., ROCK, K. L. & GOLDBERG, A. L. (1993) Gamma-interferon and expression of MHC genes regulate peptide hydrolysis by proteasomes. *Nature,* 365**,** 264-7.
GAO, M., LABUDA, T., XIA, Y., GALLAGHER, E., FANG, D., LIU, Y. C. & KARIN, M. (2004) Jun turnover is controlled through JNK-dependent phosphorylation of the E3 ligase Itch. *Science,* 306**,** 271-5.
GARDELLA, S., ANDREI, C., FERRERA, D., LOTTI, L. V., TORRISI, M. R., BIANCHI, M. E. & RUBARTELLI, A. (2002) The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-mediated secretory pathway. *EMBO Rep,* 3**,** 995-1001.
GEISS, G. K., SALVATORE, M., TUMPEY, T. M., CARTER, V. S., WANG, X., BASLER, C. F., TAUBENBERGER, J. K., BUMGARNER, R. E., PALESE, P., KATZE, M. G. & GARCIA-SASTRE, A. (2002) Cellular transcriptional profiling in influenza A virus-infected lung epithelial cells: the role of the nonstructural NS1 protein in the evasion of the host innate defense and its potential contribution to pandemic influenza. *Proc Natl Acad Sci U S A,* 99**,** 10736-41.
GERHARD, D. S., WAGNER, L., FEINGOLD, E. A., SHENMEN, C. M., GROUSE, L. H., SCHULER, G., KLEIN, S. L., OLD, S., RASOOLY, R., GOOD, P., GUYER, M., PECK, A. M., DERGE, J. G., LIPMAN, D., COLLINS, F. S., JANG, W., SHERRY, S., FEOLO, M., MISQUITTA, L., LEE, E., ROTMISTROVSKY, K., GREENHUT, S. F., SCHAEFER, C. F., BUETOW, K., BONNER, T. I., HAUSSLER, D., KENT, J.,

KIEKHAUS, M., FUREY, T., BRENT, M., PRANGE, C., SCHREIBER, K., SHAPIRO, N., BHAT, N. K., HOPKINS, R. F., HSIE, F., DRISCOLL, T., SOARES, M. B., CASAVANT, T. L., SCHEETZ, T. E., BROWN-STEIN, M. J., USDIN, T. B., TOSHIYUKI, S., CARNINCI, P., PIAO, Y., DUDEKULA, D. B., KO, M. S., KAWAKAMI, K., SUZUKI, Y., SUGANO, S., GRUBER, C. E., SMITH, M. R., SIMMONS, B., MOORE, T., WATERMAN, R., JOHNSON, S. L., RUAN, Y., WEI, C. L., MATHAVAN, S., GUNARATNE, P. H., WU, J., GARCIA, A. M., HULYK, S. W., FUH, E., YUAN, Y., SNEED, A., KOWIS, C., HODGSON, A., MUZNY, D. M., MCPHERSON, J., GIBBS, R. A., FAHEY, J., HELTON, E., KETTEMAN, M., MADAN, A., RODRIGUES, S., SANCHEZ, A., WHITING, M., MADARI, A., YOUNG, A. C., WETHERBY, K. D., GRANITE, S. J., KWONG, P. N., BRINKLEY, C. P., PEARSON, R. L., BOUFFARD, G. G., BLAKESLY, R. W., GREEN, E. D., DICKSON, M. C., RODRIGUEZ, A. C., GRIMWOOD, J., SCHMUTZ, J., MYERS, R. M., BUTTERFIELD, Y. S., GRIFFITH, M., GRIFFITH, O. L., KRZYWINSKI, M. I., LIAO, N., MORIN, R., PALMQUIST, D., et al. (2004) The status, quality, and expansion of the NIH full-length cDNA project: the Mammalian Gene Collection (MGC). *Genome Res,* 14**,** 2121-7.

GILCHRIST, M., THORSSON, V., LI, B., RUST, A. G., KORB, M., KENNEDY, K., HAI, T., BOLOURI, H. & ADEREM, A. (2006) Systems biology approaches identify ATF3 as a negative regulator of Toll-like receptor 4. *Nature,* 441**,** 173-8.

GLICKMAN, M. H., RUBIN, D. M., FRIED, V. A. & FINLEY, D. (1998) The regulatory particle of the Saccharomyces cerevisiae proteasome. *Mol Cell Biol,* 18**,** 3149-62.

GOEBL, M. G., YOCHEM, J., JENTSCH, S., MCGRATH, J. P., VARSHAVSKY, A. & BYERS, B. (1988) The yeast cell cycle gene CDC34 encodes a ubiquitin-conjugating enzyme. *Science,* 241**,** 1331-5.

GOLDSTEIN, G., SCHEID, M., HAMMERLING, U., SCHLESINGER, D. H., NIALL, H. D. & BOYSE, E. A. (1975) Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells. *Proc Natl Acad Sci U S A,* 72**,** 11-5.

GOODNOW, C. C., CROSBIE, J., JORGENSEN, H., BRINK, R. A. & BASTEN, A. (1989) Induction of self-tolerance in mature peripheral B lymphocytes. *Nature,* 342**,** 385-91.

GOTTENBERG, J. E., CAGNARD, N., LUCCHESI, C., LETOURNEUR, F., MISTOU, S., LAZURE, T., JACQUES, S., BA, N., ITTAH, M., LEPAJOLEC, C., LABETOULLE, M., ARDIZZONE, M., SIBILIA, J., FOURNIER, C., CHIOCCHIA, G. & MARIETTE, X. (2006) Activation of IFN pathways and plasmacytoid dendritic cell recruitment in target organs of primary Sjogren's syndrome. *Proc Natl Acad Sci U S A,* 103**,** 2770-5.

HAAS, A. L., AHRENS, P., BRIGHT, P. M. & ANKEL, H. (1987) Interferon induces a 15-kilodalton protein exhibiting marked homology to ubiquitin. *J Biol Chem,* 262**,** 11315-23.
HAAS, A. L. & BRIGHT, P. M. (1987) The dynamics of ubiquitin pools within cultured human lung fibroblasts. *J Biol Chem,* 262**,** 345-51.
HAGLUND, K., SIGISMUND, S., POLO, S., SZYMKIEWICZ, I., DI FIORE, P. P. & DIKIC, I. (2003) Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. *Nat Cell Biol,* 5**,** 461-6.
HANDLEY, P. M., MUECKLER, M., SIEGEL, N. R., CIECHANOVER, A. & SCHWARTZ, A. L. (1991) Molecular cloning, sequence, and tissue distribution of the human ubiquitin-activating enzyme E1. *Proc Natl Acad Sci U S A,* 88**,** 258-62.
HANSEN, A., GOSEMANN, M., PRUSS, A., REITER, K., RUZICKOVA, S., LIPSKY, P. E. & DORNER, T. (2004) Abnormalities in peripheral B cell memory of patients with primary Sjogren's syndrome. *Arthritis Rheum,* 50**,** 1897-908.
HANSEN, A., ODENDAHL, M., REITER, K., JACOBI, A. M., FEIST, E., SCHOLZE, J., BURMESTER, G. R., LIPSKY, P. E. & DORNER, T. (2002) Diminished peripheral blood memory B cells and accumulation of memory B cells in the salivary glands of patients with Sjogren's syndrome. *Arthritis Rheum,* 46**,** 2160-71.
HARDY, R. R. & HAYAKAWA, K. (2001) B cell development pathways. *Annu Rev Immunol,* 19**,** 595-621.
HARRINGTON, L. E., HATTON, R. D., MANGAN, P. R., TURNER, H., MURPHY, T. L., MURPHY, K. M. & WEAVER, C. T. (2005) Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol,* 6**,** 1123-32.
HASLAM, P. J., PROCTOR, S. J., GOODSHIP, T. H. & ZOUVANI, J. (1993) Immune complex glomerulonephritis, myasthenia gravis and compensated hypothyroidism in a patient following allogeneic bone marrow transplantation. *Nephrol Dial Transplant,* 8**,** 1390-2.
HATFIELD, P. M. & VIERSTRA, R. D. (1992) Multiple forms of ubiquitin-activating enzyme E1 from wheat. Identification of an essential cysteine by in vitro mutagenesis. *J Biol Chem,* 267**,** 14799-803.
HAYAKAWA, K., ASANO, M., SHINTON, S. A., GUI, M., ALLMAN, D., STEWART, C. L., SILVER, J. & HARDY, R. R. (1999) Positive selection of natural autoreactive B cells. *Science,* 285**,** 113-6.
HERSHKO, A., CIECHANOVER, A., HELLER, H., HAAS, A. L. & ROSE, I. A. (1980) Proposed role of ATP in protein breakdown: conjugation of protein with multiple chains of the polypeptide of ATP-dependent proteolysis. *Proc Natl Acad Sci U S A,* 77**,** 1783-6.
HERSHKO, A., CIECHANOVER, A. & ROSE, I. A. (1979) Resolution of the ATP-dependent proteolytic system from reticulocytes: a component that interacts with ATP. *Proc Natl Acad Sci U S A,* 76**,** 3107-10.

HERSHKO, A., CIECHANOVER, A. & ROSE, I. A. (1981) Identification of the active amino acid residue of the polypeptide of ATP-dependent protein breakdown. *J Biol Chem,* 256**,** 1525-8.
HERSHKO, A. & HELLER, H. (1985) Occurrence of a polyubiquitin structure in ubiquitin-protein conjugates. *Biochem Biophys Res Commun,* 128**,** 1079-86.
HERSHKO, A., HELLER, H., ELIAS, S. & CIECHANOVER, A. (1983) Components of ubiquitin-protein ligase system. Resolution, affinity purification, and role in protein breakdown. *J Biol Chem,* 258**,** 8206-14.
HERSHKO, A., HELLER, H., EYTAN, E. & REISS, Y. (1986) The protein substrate binding site of the ubiquitin-protein ligase system. *J Biol Chem,* 261**,** 11992-9.
HESS, S. & ENGELMANN, H. (1996) A novel function of CD40: induction of cell death in transformed cells. *J Exp Med,* 183**,** 159-67.
HICKE, L. (1997) Ubiquitin-dependent internalization and down-regulation of plasma membrane proteins. *Faseb J,* 11**,** 1215-26.
HICKE, L. (2001) Protein regulation by monoubiquitin. *Nat Rev Mol Cell Biol,* 2**,** 195-201.
HICKE, L. & RIEZMAN, H. (1996) Ubiquitination of a yeast plasma membrane receptor signals its ligand-stimulated endocytosis. *Cell,* 84**,** 277-87.
HICKE, L., SCHUBERT, H. L. & HILL, C. P. (2005) Ubiquitin-binding domains. *Nat Rev Mol Cell Biol,* 6**,** 610-21.
HILT, W., HEINEMEYER, W. & WOLF, D. H. (1993) Studies on the yeast proteasome uncover its basic structural features and multiple in vivo functions. *Enzyme Protein,* 47**,** 189-201.
HOELLER, D., CROSETTO, N., BLAGOEV, B., RAIBORG, C., TIKKANEN, R., WAGNER, S., KOWANETZ, K., BREITLING, R., MANN, M., STENMARK, H. & DIKIC, I. (2006) Regulation of ubiquitin-binding proteins by monoubiquitination. *Nat Cell Biol,* 8**,** 163-9.
HOFMANN, K. & BUCHER, P. (1996) The UBA domain: a sequence motif present in multiple enzyme classes of the ubiquitination pathway. *Trends Biochem Sci,* 21**,** 172-3.
HOFMANN, K. & FALQUET, L. (2001) A ubiquitin-interacting motif conserved in components of the proteasomal and lysosomal protein degradation systems. *Trends Biochem Sci,* 26**,** 347-50.
HOLASKA, J. M., BLACK, B. E., LOVE, D. C., HANOVER, J. A., LESZYK, J. & PASCHAL, B. M. (2001) Calreticulin Is a receptor for nuclear export. *J Cell Biol,* 152**,** 127-40.
HOLASKA, J. M., BLACK, B. E., RASTINEJAD, F. & PASCHAL, B. M. (2002) Ca2+-dependent nuclear export mediated by calreticulin. *Mol Cell Biol,* 22**,** 6286-97.
HOLLMANN, A. C., GONG, Q. & OWENS, T. (2002) CD40-mediated apoptosis in murine B-lymphoma lines containing mutated p53. *Exp Cell Res,* 280**,** 201-11.
HONG, C. A., SWEARINGEN, E., MALLARI, R., GAO, X., CAO, Z., NORTH, A., YOUNG, S. W. & HUANG, S. G. (2003) Development of a high throughput time-resolved fluorescence resonance energy transfer

assay for TRAF6 ubiquitin polymerization. *Assay Drug Dev Technol,* 1**,** 175-80.
HOOK, S. S., ORIAN, A., COWLEY, S. M. & EISENMAN, R. N. (2002) Histone deacetylase 6 binds polyubiquitin through its zinc finger (PAZ domain) and copurifies with deubiquitinating enzymes. *Proc Natl Acad Sci U S A,* 99**,** 13425-30.
HORI, S., NOMURA, T. & SAKAGUCHI, S. (2003) Control of regulatory T cell development by the transcription factor Foxp3. *Science,* 299**,** 1057-61.
HOWE, K., WILLIAMSON, J., BODDY, N., SHEER, D., FREEMONT, P. & SOLOMON, E. (1998) The ubiquitin-homology gene PIC1: characterization of mouse (Pic1) and human (UBL1) genes and pseudogenes. *Genomics,* 47**,** 92-100.
HOZUMI, N. & TONEGAWA, S. (1976) Evidence for somatic rearrangement of immunoglobulin genes coding for variable and constant regions. *Proc Natl Acad Sci U S A,* 73**,** 3628-32.
HUA, J., KIROU, K., LEE, C. & CROW, M. K. (2006) Functional assay of type I interferon in systemic lupus erythematosus plasma and association with anti-RNA binding protein autoantibodies. *Arthritis Rheum,* 54**,** 1906-16.
HUIBREGTSE, J. M., SCHEFFNER, M., BEAUDENON, S. & HOWLEY, P. M. (1995) A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. *Proc Natl Acad Sci U S A,* 92**,** 2563-7.
INABA, K. & STEINMAN, R. M. (1984) Resting and sensitized T lymphocytes exhibit distinct stimulatory (antigen-presenting cell) requirements for growth and lymphokine release. *J Exp Med,* 160**,** 1717-35.
INABA, K. & STEINMAN, R. M. (1985) Protein-specific helper T-lymphocyte formation initiated by dendritic cells. *Science,* 229**,** 475-9.
INGLIS, T. J. (2007) Principia aetiologica: taking causality beyond Koch's postulates. *J Med Microbiol,* 56**,** 1419-22.
ISHII, T., OHNUMA, K., MURAKAMI, A., TAKASAWA, N., YAMOCHI, T., IWATA, S., UCHIYAMA, M., DANG, N. H., TANAKA, H. & MORIMOTO, C. (2003) SS-A/Ro52, an autoantigen involved in CD28-mediated IL-2 production. *J Immunol,* 170**,** 3653-61.
ITOH, K., ITOH, Y. & FRANK, M. B. (1991) Protein heterogeneity in the human Ro/SSA ribonucleoproteins. The 52- and 60-kD Ro/SSA autoantigens are encoded by separate genes. *J Clin Invest,* 87**,** 177-86.
JABS, W. J., HENNIG, C., ZAWATZKY, R. & KIRCHNER, H. (1999) Failure to detect antiviral activity in serum and plasma of healthy individuals displaying high activity in ELISA for IFN-alpha and IFN-beta. *J Interferon Cytokine Res,* 19**,** 463-9.
JAMES, L. C., KEEBLE, A. H., KHAN, Z., RHODES, D. A. & TROWSDALE, J. (2007) Structural basis for PRYSPRY-mediated tripartite motif (TRIM) protein function. *Proc Natl Acad Sci U S A,* 104**,** 6200-5.
JENTSCH, S., MCGRATH, J. P. & VARSHAVSKY, A. (1987) The yeast DNA repair gene RAD6 encodes a ubiquitin-conjugating enzyme. *Nature,* 329**,** 131-4.

KAISER, P., FLICK, K., WITTENBERG, C. & REED, S. I. (2000) Regulation of transcription by ubiquitination without proteolysis: Cdc34/SCF(Met30)-mediated inactivation of the transcription factor Met4. *Cell,* 102**,** 303-14.
KAPPLER, J. W., ROEHM, N. & MARRACK, P. (1987) T cell tolerance by clonal elimination in the thymus. *Cell,* 49**,** 273-80.
KARLHOFER, F. M., RIBAUDO, R. K. & YOKOYAMA, W. M. (1992) MHC class I alloantigen specificity of Ly-49+ IL-2-activated natural killer cells. *Nature,* 358**,** 66-70.
KARRE, K., LJUNGGREN, H. G., PIONTEK, G. & KIESSLING, R. (1986) Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature,* 319**,** 675-8.
KAVSAK, P., RASMUSSEN, R. K., CAUSING, C. G., BONNI, S., ZHU, H., THOMSEN, G. H. & WRANA, J. L. (2000) Smad7 binds to Smurf2 to form an E3 ubiquitin ligase that targets the TGF beta receptor for degradation. *Mol Cell, 6***,** 1365-75.
KENMOCHI, N., KAWAGUCHI, T., ROZEN, S., DAVIS, E., GOODMAN, N., HUDSON, T. J., TANAKA, T. & PAGE, D. C. (1998) A map of 75 human ribosomal protein genes. *Genome Res,* 8**,** 509-23.
KIESSLING, R., KLEIN, E., PROSS, H. & WIGZELL, H. (1975a) "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. *Eur J Immunol,* 5**,** 117-21.
KIESSLING, R., KLEIN, E. & WIGZELL, H. (1975b) "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur J Immunol,* 5**,** 112-7.
KIM, H. T., KIM, K. P., LLEDIAS, F., KISSELEV, A. F., SCAGLIONE, K. M., SKOWYRA, D., GYGI, S. P. & GOLDBERG, A. L. (2007) Certain pairs of ubiquitin-conjugating enzymes (E2s) and ubiquitin-protein ligases (E3s) synthesize nondegradable forked ubiquitin chains containing all possible isopeptide linkages. *J Biol Chem,* 282**,** 17375-86.
KIM, S., DAVIS, M., SINN, E., PATTEN, P. & HOOD, L. (1981) Antibody diversity: somatic hypermutation of rearranged VH genes. *Cell,* 27**,** 573-81.
KIM, T., KANAYAMA, Y., NEGORO, N., OKAMURA, M., TAKEDA, T. & INOUE, T. (1987) Serum levels of interferons in patients with systemic lupus erythematosus. *Clin Exp Immunol,* 70**,** 562-9.
KIM, Y. C., CAO, Y., PITTERLE, D. M., O'BRIANT, K. C. & BEPLER, G. (2000) SSA/RO52gene and expressed sequence tags in an 85 kb region of chromosome segment 11p15.5. *Int J Cancer,* 87**,** 61-7.
KIPNIS, J. & SCHWARTZ, M. (2005) Controlled autoimmunity in CNS maintenance and repair: naturally occurring CD4+CD25+ regulatory T-Cells at the crossroads of health and disease. *Neuromolecular Med,* 7**,** 197-206.
KIRKIN, V. & DIKIC, I. (2007) Role of ubiquitin- and Ubl-binding proteins in cell signaling. *Curr Opin Cell Biol,* 19**,** 199-205.

KIRKPATRICK, D. S., DENISON, C. & GYGI, S. P. (2005) Weighing in on ubiquitin: the expanding role of mass-spectrometry-based proteomics. *Nat Cell Biol,* 7**,** 750-7.

KIROU, K. A., LEE, C., GEORGE, S., LOUCA, K., PAPAGIANNIS, I. G., PETERSON, M. G., LY, N., WOODWARD, R. N., FRY, K. E., LAU, A. Y., PRENTICE, J. G., WOHLGEMUTH, J. G. & CROW, M. K. (2004) Coordinate overexpression of interferon-alpha-induced genes in systemic lupus erythematosus. *Arthritis Rheum,* 50**,** 3958-67.

KISIELOW, P., TEH, H. S., BLUTHMANN, H. & VON BOEHMER, H. (1988) Positive selection of antigen-specific T cells in thymus by restricting MHC molecules. *Nature,* 335**,** 730-3.

KLINGENSPOR, M., BODNAR, J., WELCH, C., XIA, Y. R., LUSIS, A. J. & REUE, K. (1997) Localization of ubiquitin gene family members to mouse chromosomes 5, 11, and 18. *Mamm Genome,* 8**,** 789-90.

KOCH, R. (1884) Die Aetiologie der Tuberkulose. *Mitt Kaiser Gesundh 2***,** 1-88.

KOKKOLA, R., ANDERSSON, A., MULLINS, G., OSTBERG, T., TREUTIGER, C. J., ARNOLD, B., NAWROTH, P., ANDERSSON, U., HARRIS, R. A. & HARRIS, H. E. (2005) RAGE is the major receptor for the proinflammatory activity of HMGB1 in rodent macrophages. *Scand J Immunol,* 61**,** 1-9.

KOLLING, R. & HOLLENBERG, C. P. (1994) The ABC-transporter Ste6 accumulates in the plasma membrane in a ubiquitinated form in endocytosis mutants. *Embo J,* 13**,** 3261-71.

KOLLS, J. K. & LINDEN, A. (2004) Interleukin-17 family members and inflammation. *Immunity,* 21**,** 467-76.

KONG, H. J., ANDERSON, D. E., LEE, C. H., JANG, M. K., TAMURA, T., TAILOR, P., CHO, H. K., CHEONG, J., XIONG, H., MORSE, H. C., 3RD & OZATO, K. (2007) Cutting edge: autoantigen Ro52 is an interferon inducible E3 ligase that ubiquitinates IRF-8 and enhances cytokine expression in macrophages. *J Immunol,* 179**,** 26-30.

KOREN, E., KOSCEC, M., WOLFSON-REICHLIN, M., EBLING, F. M., TSAO, B., HAHN, B. H. & REICHLIN, M. (1995) Murine and human antibodies to native DNA that cross-react with the A and D SnRNP polypeptides cause direct injury of cultured kidney cells. *J Immunol,* 154**,** 4857-64.

KORGANOW, A. S., JI, H., MANGIALAIO, S., DUCHATELLE, V., PELANDA, R., MARTIN, T., DEGOTT, C., KIKUTANI, H., RAJEWSKY, K., PASQUALI, J. L., BENOIST, C. & MATHIS, D. (1999) From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. *Immunity,* 10**,** 451-61.

KOSCEC, M., KOREN, E., WOLFSON-REICHLIN, M., FUGATE, R. D., TRIEU, E., TARGOFF, I. N. & REICHLIN, M. (1997) Autoantibodies to ribosomal P proteins penetrate into live hepatocytes and cause cellular dysfunction in culture. *J Immunol,* 159**,** 2033-41.

KOSTOVA, Z., TSAI, Y. C. & WEISSMAN, A. M. (2007) Ubiquitin ligases, critical mediators of endoplasmic reticulum-associated degradation. *Semin Cell Dev Biol.*

KOTA, R. S., RUTLEDGE, J. C., GOHIL, K., KUMAR, A., ENELOW, R. I. & RAMANA, C. V. (2006) Regulation of gene expression in RAW 264.7 macrophage cell line by interferon-gamma. *Biochem Biophys Res Commun,* 342**,** 1137-46.

KOUSKOFF, V., KORGANOW, A. S., DUCHATELLE, V., DEGOTT, C., BENOIST, C. & MATHIS, D. (1996) Organ-specific disease provoked by systemic autoimmunity. *Cell,* 87**,** 811-22.

KUMAR, S., TOMOOKA, Y. & NODA, M. (1992) Identification of a set of genes with developmentally down-regulated expression in the mouse brain. *Biochem Biophys Res Commun,* 185**,** 1155-61.

KÄRRE, K. (1981) On the immunobiology of natural killer cells. Stockholm, Karolinska Institutet.

LAM, K. P., KUHN, R. & RAJEWSKY, K. (1997) In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. *Cell,* 90**,** 1073-83.

LANDER, E. S., LINTON, L. M., BIRREN, B., NUSBAUM, C., ZODY, M. C., BALDWIN, J., DEVON, K., DEWAR, K., DOYLE, M., FITZHUGH, W., FUNKE, R., GAGE, D., HARRIS, K., HEAFORD, A., HOWLAND, J., KANN, L., LEHOCZKY, J., LEVINE, R., MCEWAN, P., MCKERNAN, K., MELDRIM, J., MESIROV, J. P., MIRANDA, C., MORRIS, W., NAYLOR, J., RAYMOND, C., ROSETTI, M., SANTOS, R., SHERIDAN, A., SOUGNEZ, C., STANGE-THOMANN, N., STOJANOVIC, N., SUBRAMANIAN, A., WYMAN, D., ROGERS, J., SULSTON, J., AINSCOUGH, R., BECK, S., BENTLEY, D., BURTON, J., CLEE, C., CARTER, N., COULSON, A., DEADMAN, R., DELOUKAS, P., DUNHAM, A., DUNHAM, I., DURBIN, R., FRENCH, L., GRAFHAM, D., GREGORY, S., HUBBARD, T., HUMPHRAY, S., HUNT, A., JONES, M., LLOYD, C., MCMURRAY, A., MATTHEWS, L., MERCER, S., MILNE, S., MULLIKIN, J. C., MUNGALL, A., PLUMB, R., ROSS, M., SHOWNKEEN, R., SIMS, S., WATERSTON, R. H., WILSON, R. K., HILLIER, L. W., MCPHERSON, J. D., MARRA, M. A., MARDIS, E. R., FULTON, L. A., CHINWALLA, A. T., PEPIN, K. H., GISH, W. R., CHISSOE, S. L., WENDL, M. C., DELEHAUNTY, K. D., MINER, T. L., DELEHAUNTY, A., KRAMER, J. B., COOK, L. L., FULTON, R. S., JOHNSON, D. L., MINX, P. J., CLIFTON, S. W., HAWKINS, T., BRANSCOMB, E., PREDKI, P., RICHARDSON, P., WENNING, S., SLEZAK, T., DOGGETT, N., CHENG, J. F., OLSEN, A., LUCAS, S., ELKIN, C., UBERBACHER, E., FRAZIER, M., et al. (2001) Initial sequencing and analysis of the human genome. *Nature,* 409**,** 860-921.

LANDRETH, K. S. (2002) Critical windows in development of the rodent immune system. *Hum Exp Toxicol,* 21**,** 493-8.

LAVAU, C., MARCHIO, A., FAGIOLI, M., JANSEN, J., FALINI, B., LEBON, P., GROSVELD, F., PANDOLFI, P. P., PELICCI, P. G. & DEJEAN, A. (1995) The acute promyelocytic leukaemia-associated PML gene is induced by interferon. *Oncogene,* 11**,** 871-6.

LAWLEY, W., DOHERTY, A., DENNISS, S., CHAUHAN, D., PRUIJN, G., VAN VENROOIJ, W. J., LUNEC, J. & HERBERT, K. (2000) Rapid lupus autoantigen relocalization and reactive oxygen species accumulation following ultraviolet irradiation of human keratinocytes. *Rheumatology (Oxford),* 39**,** 253-61.

LENCER, W. I. & BLUMBERG, R. S. (2005) A passionate kiss, then run: exocytosis and recycling of IgG by FcRn. *Trends Cell Biol,* 15**,** 5-9.

LENKINSKI, R. E., CHEN, D. M., GLICKSON, J. D. & GOLDSTEIN, G. (1977) Nuclear magnetic resonance studies of the denaturation of ubiquitin. *Biochim Biophys Acta,* 494**,** 126-30.

LEUNG, D. W., SPENCER, S. A., CACHIANES, G., HAMMONDS, R. G., COLLINS, C., HENZEL, W. J., BARNARD, R., WATERS, M. J. & WOOD, W. I. (1987) Growth hormone receptor and serum binding protein: purification, cloning and expression. *Nature,* 330**,** 537-43.

LEVINGER, L. & VARSHAVSKY, A. (1982) Selective arrangement of ubiquitinated and D1 protein-containing nucleosomes within the Drosophila genome. *Cell,* 28**,** 375-85.

LI, W., TU, D., BRUNGER, A. T. & YE, Y. (2007a) A ubiquitin ligase transfers preformed polyubiquitin chains from a conjugating enzyme to a substrate. *Nature,* 446**,** 333-7.

LI, X., GOLD, B., O'HUIGIN, C., DIAZ-GRIFFERO, F., SONG, B., SI, Z., LI, Y., YUAN, W., STREMLAU, M., MISCHE, C., JAVANBAKHT, H., SCALLY, M., WINKLER, C., DEAN, M. & SODROSKI, J. (2007b) Unique features of TRIM5alpha among closely related human TRIM family members. *Virology,* 360**,** 419-33.

LIN, X., LIANG, M. & FENG, X. H. (2000) Smurf2 is a ubiquitin E3 ligase mediating proteasome-dependent degradation of Smad2 in transforming growth factor-beta signaling. *J Biol Chem,* 275**,** 36818-22.

LORICK, K. L., JENSEN, J. P., FANG, S., ONG, A. M., HATAKEYAMA, S. & WEISSMAN, A. M. (1999) RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc Natl Acad Sci U S A,* 96**,** 11364-9.

LOVERING, R., HANSON, I. M., BORDEN, K. L., MARTIN, S., O'REILLY, N. J., EVAN, G. I., RAHMAN, D., PAPPIN, D. J., TROWSDALE, J. & FREEMONT, P. S. (1993) Identification and preliminary characterization of a protein motif related to the zinc finger. *Proc Natl Acad Sci U S A,* 90**,** 2112-6.

LOWE, J., STOCK, D., JAP, B., ZWICKL, P., BAUMEISTER, W. & HUBER, R. (1995) Crystal structure of the 20S proteasome from the archaeon T. acidophilum at 3.4 A resolution. *Science,* 268**,** 533-9.

LU, Q., HOPE, L. W., BRASCH, M., REINHARD, C. & COHEN, S. N. (2003) TSG101 interaction with HRS mediates endosomal trafficking and receptor down-regulation. *Proc Natl Acad Sci U S A,* 100**,** 7626-31.
LUCERO, H. A., CHOJNICKI, E. W., MANDIYAN, S., NELSON, H. & NELSON, N. (1995) Cloning and expression of a yeast gene encoding a protein with ATPase activity and high identity to the subunit 4 of the human 26 S protease. *J Biol Chem,* 270**,** 9178-84.
LUND, J., SATO, A., AKIRA, S., MEDZHITOV, R. & IWASAKI, A. (2003) Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. *J Exp Med,* 198**,** 513-20.
LUND, P. K., MOATS-STAATS, B. M., SIMMONS, J. G., HOYT, E., D'ERCOLE, A. J., MARTIN, F. & VAN WYK, J. J. (1985) Nucleotide sequence analysis of a cDNA encoding human ubiquitin reveals that ubiquitin is synthesized as a precursor. *J Biol Chem,* 260**,** 7609-13.
LUPAS, A., VAN DYKE, M. & STOCK, J. (1991) Predicting coiled coils from protein sequences. *Science,* 252**,** 1162-4.
MARAZUELA, M. & STEEGMAN, J. L. (2000) Transfer of autoimmune hypothyroidism following bone marrow transplantation from a donor with Graves' disease. *Bone Marrow Transplant,* 26**,** 1217-20.
MATSUMOTO, I., MACCIONI, M., LEE, D. M., MAURICE, M., SIMMONS, B., BRENNER, M., MATHIS, D. & BENOIST, C. (2002) How antibodies to a ubiquitous cytoplasmic enzyme may provoke joint-specific autoimmune disease. *Nat Immunol,* 3**,** 360-5.
MATSUMOTO, I., STAUB, A., BENOIST, C. & MATHIS, D. (1999) Arthritis provoked by linked T and B cell recognition of a glycolytic enzyme. *Science,* 286**,** 1732-5.
MATTIOLI, M. & REICHLIN, M. (1974) Heterogeneity of RNA protein antigens reactive with sera of patients with systemic lupus erythematosus. Description of a cytoplasmic nonribosomal antigen. *Arthritis Rheum,* 17**,** 421-9.
MAYOR, S., PRESLEY, J. F. & MAXFIELD, F. R. (1993) Sorting of membrane components from endosomes and subsequent recycling to the cell surface occurs by a bulk flow process. *J Cell Biol,* 121**,** 1257-69.
MCDUFFIE, M., ROEHM, N., KAPPLER, J. W. & MARRACK, P. (1988) Involvement of major histocompatibility complex products in tolerance induction in the thymus. *J Immunol,* 141**,** 1840-7.
MCGRATH, J. P., JENTSCH, S. & VARSHAVSKY, A. (1991) UBA 1: an essential yeast gene encoding ubiquitin-activating enzyme. *Embo J,* 10**,** 227-36.
MERONI, G. & DIEZ-ROUX, G. (2005) TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases. *Bioessays,* 27**,** 1147-57.
MEUSSER, B., HIRSCH, C., JAROSCH, E. & SOMMER, T. (2005) ERAD: the long road to destruction. *Nat Cell Biol,* 7**,** 766-72.
MEYER, H. H., WANG, Y. & WARREN, G. (2002) Direct binding of ubiquitin conjugates by the mammalian p97 adaptor complexes, p47 and Ufd1-Npl4. *Embo J,* 21**,** 5645-52.

MICHALEK, M. T., GRANT, E. P., GRAMM, C., GOLDBERG, A. L. & ROCK, K. L. (1993) A role for the ubiquitin-dependent proteolytic pathway in MHC class I-restricted antigen presentation. *Nature,* 363**,** 552-4.
MIRANDA-CARUS, M. E., ASKANASE, A. D., CLANCY, R. M., DI DONATO, F., CHOU, T. M., LIBERA, M. R., CHAN, E. K. & BUYON, J. P. (2000) Anti-SSA/Ro and anti-SSB/La autoantibodies bind the surface of apoptotic fetal cardiocytes and promote secretion of TNF-alpha by macrophages. *J Immunol,* 165**,** 5345-51.
MIZUNO, E., KAWAHATA, K., KATO, M., KITAMURA, N. & KOMADA, M. (2003) STAM proteins bind ubiquitinated proteins on the early endosome via the VHS domain and ubiquitin-interacting motif. *Mol Biol Cell,* 14**,** 3675-89.
MORETTA, A., VITALE, M., BOTTINO, C., ORENGO, A. M., MORELLI, L., AUGUGLIARO, R., BARBARESI, M., CICCONE, E. & MORETTA, L. (1993) P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificities. *J Exp Med,* 178**,** 597-604.
MORI, S., HELDIN, C. H. & CLAESSON-WELSH, L. (1993) Ligand-induced ubiquitination of the platelet-derived growth factor beta-receptor plays a negative regulatory role in its mitogenic signaling. *J Biol Chem,* 268**,** 577-83.
MOSMANN, T. R., CHERWINSKI, H., BOND, M. W., GIEDLIN, M. A. & COFFMAN, R. L. (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol,* 136**,** 2348-57.
MUKHOPADHYAY, D. & RIEZMAN, H. (2007) Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science,* 315**,** 201-5.
MURALIDHAR, M. G. & THOMAS, J. B. (1993) The Drosophila bendless gene encodes a neural protein related to ubiquitin-conjugating enzymes. *Neuron,* 11**,** 253-66.
MURATANI, M. & TANSEY, W. P. (2003) How the ubiquitin-proteasome system controls transcription. *Nat Rev Mol Cell Biol,* 4**,** 192-201.
MURPHY, M. A., SCHNALL, R. G., VENTER, D. J., BARNETT, L., BERTONCELLO, I., THIEN, C. B., LANGDON, W. Y. & BOWTELL, D. D. (1998) Tissue hyperplasia and enhanced T-cell signalling via ZAP-70 in c-Cbl-deficient mice. *Mol Cell Biol,* 18**,** 4872-82.
NISOLE, S., STOYE, J. P. & SAIB, A. (2005) TRIM family proteins: retroviral restriction and antiviral defence. *Nat Rev Microbiol,* 3**,** 799-808.
NOBUHARA, Y., KAWANO, S., KAGEYAMA, G., SUGIYAMA, D., SAEGUSA, J. & KUMAGAI, S. (2007) Is SS-A/Ro52 a hydrogen peroxide-sensitive signaling molecule? *Antioxid Redox Signal,* 9**,** 385-91.
NOSSAL, G. J. (1987) How is tolerance generated? *Ciba Found Symp,* 129**,** 59-72.
NOSSAL, G. J. & PIKE, B. L. (1980) Clonal anergy: persistence in tolerant mice of antigen-binding B lymphocytes incapable of responding to antigen or mitogen. *Proc Natl Acad Sci U S A,* 77**,** 1602-6.

ODENDAHL, M., JACOBI, A., HANSEN, A., FEIST, E., HIEPE, F., BURMESTER, G. R., LIPSKY, P. E., RADBRUCH, A. & DORNER, T. (2000) Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. *J Immunol,* 165**,** 5970-9.

OHLSSON, M., JONSSON, R. & BROKSTAD, K. A. (2002) Subcellular redistribution and surface exposure of the Ro52, Ro60 and La48 autoantigens during apoptosis in human ductal epithelial cells: a possible mechanism in the pathogenesis of Sjogren's syndrome. *Scand J Immunol,* 56**,** 456-69.

ORIMO, A., INOUE, S., MINOWA, O., TOMINAGA, N., TOMIOKA, Y., SATO, M., KUNO, J., HIROI, H., SHIMIZU, Y., SUZUKI, M., NODA, T. & MURAMATSU, M. (1999) Underdeveloped uterus and reduced estrogen responsiveness in mice with disruption of the estrogen-responsive finger protein gene, which is a direct target of estrogen receptor alpha. *Proc Natl Acad Sci U S A,* 96**,** 12027-32.

ORLOWSKI, M. & WILK, S. (1981) A multicatalytic protease complex from pituitary that forms enkephalin and enkephalin containing peptides. *Biochem Biophys Res Commun,* 101**,** 814-22.

OSTENDORFF, H. P., PEIRANO, R. I., PETERS, M. A., SCHLUTER, A., BOSSENZ, M., SCHEFFNER, M. & BACH, I. (2002) Ubiquitination-dependent cofactor exchange on LIM homeodomain transcription factors. *Nature,* 416**,** 99-103.

OWEN, R. D. (1945) Immunogenetic Consequences Of Vascular Anastomoses Between Bovine Twins. *Science,* 102**,** 400-401.

OZKAYNAK, E., FINLEY, D., SOLOMON, M. J. & VARSHAVSKY, A. (1987) The yeast ubiquitin genes: a family of natural gene fusions. *Embo J,* 6**,** 1429-39.

OZKAYNAK, E., FINLEY, D. & VARSHAVSKY, A. (1984) The yeast ubiquitin gene: head-to-tail repeats encoding a polyubiquitin precursor protein. *Nature,* 312**,** 663-6.

PADOVAN, E., CASORATI, G., DELLABONA, P., MEYER, S., BROCKHAUS, M. & LANZAVECCHIA, A. (1993) Expression of two T cell receptor alpha chains: dual receptor T cells. *Science,* 262**,** 422-4.

PALOMBELLA, V. J., RANDO, O. J., GOLDBERG, A. L. & MANIATIS, T. (1994) The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. *Cell,* 78**,** 773-85.

PARK, H., LI, Z., YANG, X. O., CHANG, S. H., NURIEVA, R., WANG, Y. H., WANG, Y., HOOD, L., ZHU, Z., TIAN, Q. & DONG, C. (2005) A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol,* 6**,** 1133-41.

PARK, J. S., GAMBONI-ROBERTSON, F., HE, Q., SVETKAUSKAITE, D., KIM, J. Y., STRASSHEIM, D., SOHN, J. W., YAMADA, S., MARUYAMA, I., BANERJEE, A., ISHIZAKA, A. & ABRAHAM, E. (2006) High mobility group box 1 protein interacts with multiple Toll-like receptors. *Am J Physiol Cell Physiol,* 290**,** C917-24.

PENG, H., BEGG, G. E., SCHULTZ, D. C., FRIEDMAN, J. R., JENSEN, D. E., SPEICHER, D. W. & RAUSCHER, F. J., 3RD (2000) Reconstitution of the KRAB-KAP-1 repressor complex: a model system for defining the molecular anatomy of RING-B box-coiled-coil domain-mediated protein-protein interactions. *J Mol Biol,* 295**,** 1139-62.

PENG, J., SCHWARTZ, D., ELIAS, J. E., THOREEN, C. C., CHENG, D., MARSISCHKY, G., ROELOFS, J., FINLEY, D. & GYGI, S. P. (2003) A proteomics approach to understanding protein ubiquitination. *Nat Biotechnol,* 21**,** 921-6.

PERROY, J., PONTIER, S., CHAREST, P. G., AUBRY, M. & BOUVIER, M. (2004) Real-time monitoring of ubiquitination in living cells by BRET. *Nat Methods,* 1**,** 203-8.

PERRY, W. L., HUSTAD, C. M., SWING, D. A., O'SULLIVAN, T. N., JENKINS, N. A. & COPELAND, N. G. (1998) The itchy locus encodes a novel ubiquitin protein ligase that is disrupted in a18H mice. *Nat Genet,* 18**,** 143-6.

PHILLIPS, C. L., THROWER, J., PICKART, C. M. & HILL, C. P. (2001) Structure of a new crystal form of tetraubiquitin. *Acta Crystallogr D Biol Crystallogr,* 57**,** 341-4.

PICKART, C. M. (1997) Targeting of substrates to the 26S proteasome. *Faseb J,* 11**,** 1055-66.

PIPER, R. C. & KATZMANN, D. J. (2007) Biogenesis and Function of Multivesicular Bodies. *Annu Rev Cell Dev Biol,* 23**,** 519-547.

POLO, S., SIGISMUND, S., FARETTA, M., GUIDI, M., CAPUA, M. R., BOSSI, G., CHEN, H., DE CAMILLI, P. & DI FIORE, P. P. (2002) A single motif responsible for ubiquitin recognition and monoubiquitination in endocytic proteins. *Nature,* 416**,** 451-5.

PONTING, C., SCHULTZ, J. & BORK, P. (1997) SPRY domains in ryanodine receptors (Ca(2+)-release channels). *Trends Biochem Sci,* 22**,** 193-4.

POURMAND, N., BLANGE, I., RINGERTZ, N. & PETTERSSON, I. (1998) Intracellular localisation of the Ro 52kD auto-antigen in HeLa cells visualised with green fluorescent protein chimeras. *Autoimmunity,* 28**,** 225-33.

REICH, N., EVANS, B., LEVY, D., FAHEY, D., KNIGHT, E., JR. & DARNELL, J. E., JR. (1987) Interferon-induced transcription of a gene encoding a 15-kDa protein depends on an upstream enhancer element. *Proc Natl Acad Sci U S A,* 84**,** 6394-8.

REICHLIN, M., MARTIN, A., TAYLOR-ALBERT, E., TSUZAKA, K., ZHANG, W., REICHLIN, M. W., KOREN, E., EBLING, F. M., TSAO, B. & HAHN, B. H. (1994) Lupus autoantibodies to native DNA cross-react with the A and D SnRNP polypeptides. *J Clin Invest,* 93**,** 443-9.

REISS, Y., HELLER, H. & HERSHKO, A. (1989) Binding sites of ubiquitin-protein ligase. Binding of ubiquitin-protein conjugates and of ubiquitin-carrier protein. *J Biol Chem,* 264**,** 10378-83.

REYMOND, A., MERONI, G., FANTOZZI, A., MERLA, G., CAIRO, S., LUZI, L., RIGANELLI, D., ZANARIA, E., MESSALI, S., CAINARCA,

S., GUFFANTI, A., MINUCCI, S., PELICCI, P. G. & BALLABIO, A. (2001) The tripartite motif family identifies cell compartments. *Embo J,* 20**,** 2140-51.
RHODES, D. A., DE BONO, B. & TROWSDALE, J. (2005) Relationship between SPRY and B30.2 protein domains. Evolution of a component of immune defence? *Immunology,* 116**,** 411-7.
RHODES, D. A., IHRKE, G., REINICKE, A. T., MALCHEREK, G., TOWEY, M., ISENBERG, D. A. & TROWSDALE, J. (2002) The 52 000 MW Ro/SS-A autoantigen in Sjogren's syndrome/systemic lupus erythematosus (Ro52) is an interferon-gamma inducible tripartite motif protein associated with membrane proximal structures. *Immunology,* 106**,** 246-56.
RHODES, D. A. & TROWSDALE, J. (2006) TRIM21 is a trimeric protein that binds IgG Fc via the B30.2 domain. *Mol Immunol.*
RONCHESE, F. & HAUSMANN, B. (1993) B lymphocytes in vivo fail to prime naive T cells but can stimulate antigen-experienced T lymphocytes. *J Exp Med,* 177**,** 679-90.
ROOS-MATTJUS, P. & SISTONEN, L. (2004) The ubiquitin-proteasome pathway. *Ann Med,* 36**,** 285-95.
ROSE, I. A., WARMS, J. V. & HERSHKO, A. (1979) A high molecular weight protease in liver cytosol. *J Biol Chem,* 254**,** 8135-8.
ROSEN, A. & CASCIOLA-ROSEN, L. (2004) Altered autoantigen structure in Sjogren's syndrome: implications for the pathogenesis of autoimmune tissue damage. *Crit Rev Oral Biol Med,* 15**,** 156-64.
ROSEN, A., CASCIOLA-ROSEN, L. & AHEARN, J. (1995) Novel packages of viral and self-antigens are generated during apoptosis. *J Exp Med,* 181**,** 1557-61.
ROSENTHAL, A. S. & SHEVACH, E. M. (1973) Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. *J Exp Med,* 138**,** 1194-212.
ROUTSIAS, J. G., VLACHOYIANNOPOULOS, P. G. & TZIOUFAS, A. G. (2006) Autoantibodies to intracellular autoantigens and their B-cell epitopes: molecular probes to study the autoimmune response. *Crit Rev Clin Lab Sci,* 43**,** 203-48.
SABILE, A., MEYER, A. M., WIRBELAUER, C., HESS, D., KOGEL, U., SCHEFFNER, M. & KREK, W. (2006) Regulation of p27 degradation and S-phase progression by Ro52 RING finger protein. *Mol Cell Biol,* 26**,** 5994-6004.
SAEGUSA, J., KAWANO, S., KOSHIBA, M., HAYASHI, N., KOSAKA, H., FUNASAKA, Y. & KUMAGAI, S. (2002) Oxidative stress mediates cell surface expression of SS-A/Ro antigen on keratinocytes. *Free Radic Biol Med,* 32**,** 1006-16.
SAKAGUCHI, S., SAKAGUCHI, N., ASANO, M., ITOH, M. & TODA, M. (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol,* 155**,** 1151-64.

SANCHO, E., VILA, M. R., SANCHEZ-PULIDO, L., LOZANO, J. J., PACIUCCI, R., NADAL, M., FOX, M., HARVEY, C., BERCOVICH, B., LOUKILI, N., CIECHANOVER, A., LIN, S. L., SANZ, F., ESTIVILL, X., VALENCIA, A. & THOMSON, T. M. (1998) Role of UEV-1, an inactive variant of the E2 ubiquitin-conjugating enzymes, in in vitro differentiation and cell cycle behavior of HT-29-M6 intestinal mucosecretory cells. *Mol Cell Biol,* 18**,** 576-89.

SATO, A., LINEHAN, M. M. & IWASAKI, A. (2006) Dual recognition of herpes simplex viruses by TLR2 and TLR9 in dendritic cells. *Proc Natl Acad Sci U S A,* 103**,** 17343-8.

SCAFFIDI, P., MISTELI, T. & BIANCHI, M. E. (2002) Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature,* 418**,** 191-5.

SCHEFFNER, M., HUIBREGTSE, J. M. & HOWLEY, P. M. (1994) Identification of a human ubiquitin-conjugating enzyme that mediates the E6-AP-dependent ubiquitination of p53. *Proc Natl Acad Sci U S A,* 91**,** 8797-801.

SCHERBIK, S. V., STOCKMAN, B. M. & BRINTON, M. A. (2007) Differential expression of interferon (IFN) regulatory factors and IFN-stimulated genes at early times after West Nile virus infection of mouse embryo fibroblasts. *J Virol,* 81**,** 12005-18.

SCHERER, D. C., BROCKMAN, J. A., CHEN, Z., MANIATIS, T. & BALLARD, D. W. (1995) Signal-induced degradation of I kappa B alpha requires site-specific ubiquitination. *Proc Natl Acad Sci U S A,* 92**,** 11259-63.

SCHIEBE, M., OHNESEIT, P., HOFFMANN, W., MEYERMANN, R., RODEMANN, H. P. & BAMBERG, M. (2001) Loss of heterozygosity at 11p15 and p53 alterations in malignant gliomas. *J Cancer Res Clin Oncol,* 127**,** 325-8.

SCHLESINGER, D. H. & GOLDSTEIN, G. (1975) Molecular conservation of 74 amino acid sequence of ubiquitin between cattle and man. *Nature,* 255**,** 42304.

SCHLESINGER, D. H., GOLDSTEIN, G. & NIALL, H. D. (1975) The complete amino acid sequence of ubiquitin, an adenylate cyclase stimulating polypeptide probably universal in living cells. *Biochemistry,* 14**,** 2214-8.

SCHUBERT, D., MAIER, B., MORAWIETZ, L., KRENN, V. & KAMRADT, T. (2004) Immunization with glucose-6-phosphate isomerase induces T cell-dependent peripheral polyarthritis in genetically unaltered mice. *J Immunol,* 172**,** 4503-9.

SCHULTZ, R. M. & KLEINSCHMIDT, W. J. (1983) Functional identity between murine gamma interferon and macrophage activating factor. *Nature,* 305**,** 239-40.

SEN, G. C. (2001) Viruses and interferons. *Annu Rev Microbiol,* 55**,** 255-81.

SEN, R. & BALTIMORE, D. (1986a) Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism. *Cell,* 47**,** 921-8.

SEN, R. & BALTIMORE, D. (1986b) Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell,* 46**,** 705-16.
SEROOGY, C. M., SOARES, L., RANHEIM, E. A., SU, L., HOLNESS, C., BLOOM, D. & FATHMAN, C. G. (2004) The gene related to anergy in lymphocytes, an E3 ubiquitin ligase, is necessary for anergy induction in CD4 T cells. *J Immunol,* 173**,** 79-85.
SHA, W. C., LIOU, H. C., TUOMANEN, E. I. & BALTIMORE, D. (1995) Targeted disruption of the p50 subunit of NF-kappa B leads to multifocal defects in immune responses. *Cell,* 80**,** 321-30.
SHARP, P. M. & LI, W. H. (1987) Ubiquitin genes as a paradigm of concerted evolution of tandem repeats. *J Mol Evol,* 25**,** 58-64.
SHEN, Z., PARDINGTON-PURTYMUN, P. E., COMEAUX, J. C., MOYZIS, R. K. & CHEN, D. J. (1996) UBL1, a human ubiquitin-like protein associating with human RAD51/RAD52 proteins. *Genomics,* 36**,** 271-9.
SHIBA, Y., KATOH, Y., SHIBA, T., YOSHINO, K., TAKATSU, H., KOBAYASHI, H., SHIN, H. W., WAKATSUKI, S. & NAKAYAMA, K. (2004) GAT (GGA and Tom1) domain responsible for ubiquitin binding and ubiquitination. *J Biol Chem,* 279**,** 7105-11.
SHIH, S. C., PRAG, G., FRANCIS, S. A., SUTANTO, M. A., HURLEY, J. H. & HICKE, L. (2003) A ubiquitin-binding motif required for intramolecular monoubiquitylation, the CUE domain. *Embo J,* 22**,** 1273-81.
SIGISMUND, S., WOELK, T., PURI, C., MASPERO, E., TACCHETTI, C., TRANSIDICO, P., DI FIORE, P. P. & POLO, S. (2005) Clathrin-independent endocytosis of ubiquitinated cargos. *Proc Natl Acad Sci U S A,* 102**,** 2760-5.
SIMISTER, N. E. & MOSTOV, K. E. (1989) An Fc receptor structurally related to MHC class I antigens. *Nature,* 337**,** 184-7.
SJOBRING, U., BJORCK, L. & KASTERN, W. (1991) Streptococcal protein G. Gene structure and protein binding properties. *J Biol Chem,* 266**,** 399-405.
SLAGSVOLD, T., AASLAND, R., HIRANO, S., BACHE, K. G., RAIBORG, C., TRAMBAIOLO, D., WAKATSUKI, S. & STENMARK, H. (2005) Eap45 in mammalian ESCRT-II binds ubiquitin via a phosphoinositide-interacting GLUE domain. *J Biol Chem,* 280**,** 19600-6.
SONG, B., GOLD, B., O'HUIGIN, C., JAVANBAKHT, H., LI, X., STREMLAU, M., WINKLER, C., DEAN, M. & SODROSKI, J. (2005) The B30.2(SPRY) domain of the retroviral restriction factor TRIM5alpha exhibits lineage-specific length and sequence variation in primates. *J Virol,* 79**,** 6111-21.
SPENCE, J., SADIS, S., HAAS, A. L. & FINLEY, D. (1995) A ubiquitin mutant with specific defects in DNA repair and multiubiquitination. *Mol Cell Biol,* 15**,** 1265-73.
STADLER, M., CHELBI-ALIX, M. K., KOKEN, M. H., VENTURINI, L., LEE, C., SAIB, A., QUIGNON, F., PELICANO, L., GUILLEMIN, M. C., SCHINDLER, C. & ET AL. (1995) Transcriptional induction of the PML growth suppressor gene by interferons is mediated through an ISRE and a GAS element. *Oncogene,* 11**,** 2565-73.

STANG, E., JOHANNESSEN, L. E., KNARDAL, S. L. & MADSHUS, I. H. (2000) Polyubiquitination of the epidermal growth factor receptor occurs at the plasma membrane upon ligand-induced activation. *J Biol Chem,* 275**,** 13940-7.

STOFFLER, G., WOOL, I. G., LIN, A. & RAK, K. H. (1974) The identification of the eukaryotic ribosomal proteins homologous with Escherichia coli proteins L7 and L12. *Proc Natl Acad Sci U S A,* 71**,** 4723-6.

STREMLAU, M., OWENS, C. M., PERRON, M. J., KIESSLING, M., AUTISSIER, P. & SODROSKI, J. (2004) The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature,* 427**,** 848-53.

STROUS, G. J., VAN KERKHOF, P., GOVERS, R., CIECHANOVER, A. & SCHWARTZ, A. L. (1996) The ubiquitin conjugation system is required for ligand-induced endocytosis and degradation of the growth hormone receptor. *Embo J,* 15**,** 3806-12.

SUN, L. & WUTHRICH, R. P. (1999) Molecular identification of a murine ubiquitin/60S ribosomal fusion protein and expression study in mouse kidney. *Biochem Genet,* 37**,** 139-47.

TADA, T., TAKEMORI, T., OKUMURA, K., NONAKA, M. & TOKUHISA, T. (1978) Two distinct types of helper T cells involved in the secondary antibody response: independent and synergistic effects of Ia- and Ia+ helper T cells. *J Exp Med,* 147**,** 446-58.

TAKAHATA, M., BOHGAKI, M., TSUKIYAMA, T., KONDO, T., ASAKA, M. & HATAKEYAMA, S. (2007) Ro52 functionally interacts with IgG1 and regulates its quality control via the ERAD system. *Mol Immunol.*

TALAL, N. & BUNIM, J. J. (1964) The Development Of Malignant Lymphoma In The Course Of Sjoegren's Syndrome. *Am J Med,* 36**,** 529-40.

TANAKA, K., TAMURA, T., YOSHIMURA, T. & ICHIHARA, A. (1992) Proteasomes: protein and gene structures. *New Biol,* 4**,** 173-87.

TANAKA, K., YOSHIMURA, T., ICHIHARA, A., IKAI, A., NISHIGAI, M., MORIMOTO, Y., SATO, M., TANAKA, N., KATSUBE, Y., KAMEYAMA, K. & ET AL. (1988a) Molecular organization of a high molecular weight multi-protease complex from rat liver. *J Mol Biol,* 203**,** 985-96.

TANAKA, K., YOSHIMURA, T., KUMATORI, A., ICHIHARA, A., IKAI, A., NISHIGAI, M., KAMEYAMA, K. & TAKAGI, T. (1988b) Proteasomes (multi-protease complexes) as 20 S ring-shaped particles in a variety of eukaryotic cells. *J Biol Chem,* 263**,** 16209-17.

TEH, H. S., KISIELOW, P., SCOTT, B., KISHI, H., UEMATSU, Y., BLUTHMANN, H. & VON BOEHMER, H. (1988) Thymic major histocompatibility complex antigens and the alpha beta T-cell receptor determine the CD4/CD8 phenotype of T cells. *Nature,* 335**,** 229-33.

THOMAS, K. E., GALLIGAN, C. L., NEWMAN, R. D., FISH, E. N. & VOGEL, S. N. (2006) Contribution of interferon-beta to the murine macrophage response to the toll-like receptor 4 agonist, lipopolysaccharide. *J Biol Chem,* 281**,** 31119-30.

THROWER, J. S., HOFFMAN, L., RECHSTEINER, M. & PICKART, C. M. (2000) Recognition of the polyubiquitin proteolytic signal. *Embo J,* 19**,** 94-102.
TISSOT, C. & MECHTI, N. (1995) Molecular cloning of a new interferon-induced factor that represses human immunodeficiency virus type 1 long terminal repeat expression. *J Biol Chem,* 270**,** 14891-8.
TREIER, M., STASZEWSKI, L. M. & BOHMANN, D. (1994) Ubiquitin-dependent c-Jun degradation in vivo is mediated by the delta domain. *Cell,* 78**,** 787-98.
UNNO, M., MIZUSHIMA, T., MORIMOTO, Y., TOMISUGI, Y., TANAKA, K., YASUOKA, N. & TSUKIHARA, T. (2002) The structure of the mammalian 20S proteasome at 2.75 A resolution. *Structure,* 10**,** 609-18.
VARADAN, R., ASSFALG, M., HARIRINIA, A., RAASI, S., PICKART, C. & FUSHMAN, D. (2004) Solution conformation of Lys63-linked di-ubiquitin chain provides clues to functional diversity of polyubiquitin signaling. *J Biol Chem,* 279**,** 7055-63.
VARADAN, R., WALKER, O., PICKART, C. & FUSHMAN, D. (2002) Structural properties of polyubiquitin chains in solution. *J Mol Biol,* 324**,** 637-47.
VERNET, C., RIBOUCHON, M. T., CHIMINI, G., JOUANOLLE, A. M., SIDIBE, I. & PONTAROTTI, P. (1993) A novel coding sequence belonging to a new multicopy gene family mapping within the human MHC class I region. *Immunogenetics,* 38**,** 47-53.
VIJAY-KUMAR, S., BUGG, C. E. & COOK, W. J. (1987a) Structure of ubiquitin refined at 1.8 A resolution. *J Mol Biol,* 194**,** 531-44.
VIJAY-KUMAR, S., BUGG, C. E., WILKINSON, K. D. & COOK, W. J. (1985) Three-dimensional structure of ubiquitin at 2.8 A resolution. *Proc Natl Acad Sci U S A,* 82**,** 3582-5.
VIJAY-KUMAR, S., BUGG, C. E., WILKINSON, K. D., VIERSTRA, R. D., HATFIELD, P. M. & COOK, W. J. (1987b) Comparison of the three-dimensional structures of human, yeast, and oat ubiquitin. *J Biol Chem,* 262**,** 6396-9.
VON BOEHMER, H., KISHI, H., UEMATSU, Y., TEH, H. S., SCOTT, B. & KISIELOW, P. (1988) T cell repertoire selection in T cell receptor transgenic mice. *Princess Takamatsu Symp,* 19**,** 107-13.
VON BOEHMER, H., TEH, H. S. & KISIELOW, P. (1989) The thymus selects the useful, neglects the useless and destroys the harmful. *Immunol Today,* 10**,** 57-61.
VOULGARELIS, M. & MOUTSOPOULOS, H. M. (2003) Lymphoproliferation in autoimmunity and Sjogren's syndrome. *Curr Rheumatol Rep,* 5**,** 317-23.
WADA, K. & KAMITANI, T. (2006a) Autoantigen Ro52 is an E3 ubiquitin ligase. *Biochem Biophys Res Commun,* 339**,** 415-21.
WADA, K. & KAMITANI, T. (2006b) UnpEL/Usp4 is ubiquitinated by Ro52 and deubiquitinated by itself. *Biochem Biophys Res Commun,* 342**,** 253-8.

WADA, K., TANJI, K. & KAMITANI, T. (2006) Oncogenic protein UnpEL/Usp4 deubiquitinates Ro52 by its isopeptidase activity. *Biochem Biophys Res Commun,* 339**,** 731-6.
WALDRON, J. A., JR., HORN, R. G. & ROSENTHAL, A. S. (1974) Antigen-induced proliferation of guinea pig lymphocytes in vitro: functional aspects of antigen handling by macrophages. *J Immunol,* 112**,** 746-55.
WANG, A. C., WILSON, K. S., HOPPER, J. E., FUDENBERG, H. H. & NISONOFF, A. (1970) Evidence for control of synthesis of the varible regions of the heavy chains of immunoglobulins G and M by the same gene. *Proc Natl Acad Sci U S A,* 66**,** 337-43.
WANG, D., BUYON, J. P. & CHAN, E. K. (1996) Cloning and expression of mouse 60 kDa ribonucleoprotein SS-A/Ro. *Mol Biol Rep,* 23**,** 205-10.
WANG, J. & CAMPBELL, I. L. (2005) Innate STAT1-dependent genomic response of neurons to the antiviral cytokine alpha interferon. *J Virol,* 79**,** 8295-302.
WEBB, G. C., BAKER, R. T., COGGAN, M. & BOARD, P. G. (1994) Localization of the human UBA52 ubiquitin fusion gene to chromosome band 19p13.1-p12. *Genomics,* 19**,** 567-9.
WEBB, G. C., BAKER, R. T., FAGAN, K. & BOARD, P. G. (1990) Localization of the human UbB polyubiquitin gene to chromosome band 17p11.1-17p12. *Am J Hum Genet,* 46**,** 308-15.
WEIH, F., CARRASCO, D., DURHAM, S. K., BARTON, D. S., RIZZO, C. A., RYSECK, R. P., LIRA, S. A. & BRAVO, R. (1995) Multiorgan inflammation and hematopoietic abnormalities in mice with a targeted disruption of RelB, a member of the NF-kappa B/Rel family. *Cell,* 80**,** 331-40.
WEISSMAN, A. M. (2001) Themes and variations on ubiquitylation. *Nat Rev Mol Cell Biol,* 2**,** 169-78.
WIBORG, O., PEDERSEN, M. S., WIND, A., BERGLUND, L. E., MARCKER, K. A. & VUUST, J. (1985) The human ubiquitin multigene family: some genes contain multiple directly repeated ubiquitin coding sequences. *Embo J,* 4**,** 755-9.
WILK, S. & ORLOWSKI, M. (1980) Cation-sensitive neutral endopeptidase: isolation and specificity of the bovine pituitary enzyme. *J Neurochem,* 35**,** 1172-82.
WILKINSON, K. D. & AUDHYA, T. K. (1981) Stimulation of ATP-dependent proteolysis requires ubiquitin with the COOH-terminal sequence Arg-Gly-Gly. *J Biol Chem,* 256**,** 9235-41.
WOLF, D. & GOFF, S. P. (2007) TRIM28 mediates primer binding site-targeted silencing of murine leukemia virus in embryonic cells. *Cell,* 131**,** 46-57.
WOLF, D. H. & HILT, W. (2004) The proteasome: a proteolytic nanomachine of cell regulation and waste disposal. *Biochim Biophys Acta,* 1695**,** 19-31.
WOLIN, S. L. & STEITZ, J. A. (1984) The Ro small cytoplasmic ribonucleoproteins: identification of the antigenic protein and its binding site on the Ro RNAs. *Proc Natl Acad Sci U S A,* 81**,** 1996-2000.

WYATT, D. T., LUM, L. G., CASPER, J., HUNTER, J. & CAMITTA, B. (1990) Autoimmune thyroiditis after bone marrow transplantation. *Bone Marrow Transplant,* 5**,** 357-61.

XIONG, H., LI, H., KONG, H. J., CHEN, Y., ZHAO, J., XIONG, S., HUANG, B., GU, H., MAYER, L., OZATO, K. & UNKELESS, J. C. (2005) Ubiquitin-dependent degradation of interferon regulatory factor-8 mediated by Cbl down-regulates interleukin-12 expression. *J Biol Chem,* 280**,** 23531-9.

YAMAMOTO, M., OKAMOTO, T., TAKEDA, K., SATO, S., SANJO, H., UEMATSU, S., SAITOH, T., YAMAMOTO, N., SAKURAI, H., ISHII, K. J., YAMAOKA, S., KAWAI, T., MATSUURA, Y., TAKEUCHI, O. & AKIRA, S. (2006) Key function for the Ubc13 E2 ubiquitin-conjugating enzyme in immune receptor signaling. *Nat Immunol,* 7**,** 962-70.

YANG, Y., EVERSOLE, T., LEE, D. J., SONTHEIMER, R. D. & CAPRA, J. D. (1999) Protein-protein interactions between native Ro52 and immunoglobulin G heavy chain. *Scand J Immunol,* 49**,** 620-8.

YANG, Y., WATERS, J. B., FRUH, K. & PETERSON, P. A. (1992) Proteasomes are regulated by interferon gamma: implications for antigen processing. *Proc Natl Acad Sci U S A,* 89**,** 4928-32.

YANG, Y. S., YANG, M. C., WANG, B. & WEISSLER, J. C. (2000) Autoantigen Ro52 directly interacts with human IgG heavy chain in vivo in mammalian cells. *Mol Immunol,* 37**,** 591-602.

YAP, M. W., NISOLE, S., LYNCH, C. & STOYE, J. P. (2004) Trim5alpha protein restricts both HIV-1 and murine leukemia virus. *Proc Natl Acad Sci U S A,* 101**,** 10786-91.

YARDEN, Y., ESCOBEDO, J. A., KUANG, W. J., YANG-FENG, T. L., DANIEL, T. O., TREMBLE, P. M., CHEN, E. Y., ANDO, M. E., HARKINS, R. N., FRANCKE, U. & ET AL. (1986) Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. *Nature,* 323**,** 226-32.

YE, M. & GRAF, T. (2007) Early decisions in lymphoid development. *Curr Opin Immunol,* 19**,** 123-8.

YEH, E. T., GONG, L. & KAMITANI, T. (2000) Ubiquitin-like proteins: new wines in new bottles. *Gene,* 248**,** 1-14.

ZACK, D. J., STEMPNIAK, M., WONG, A. L., TAYLOR, C. & WEISBART, R. H. (1996) Mechanisms of cellular penetration and nuclear localization of an anti-double strand DNA autoantibody. *J Immunol,* 157**,** 2082-8.

ZACKSENHAUS, E. & SHEININ, R. (1990) Molecular cloning, primary structure and expression of the human X linked A1S9 gene cDNA which complements the ts A1S9 mouse L cell defect in DNA replication. *Embo J,* 9**,** 2923-9.

ZAL, T., WEISS, S., MELLOR, A. & STOCKINGER, B. (1996) Expression of a second receptor rescues self-specific T cells from thymic deletion and allows activation of autoreactive effector function. *Proc Natl Acad Sci U S A,* 93**,** 9102-7.

ZENKLUSEN, J. C., OSHIMURA, M., BARRETT, J. C. & CONTI, C. J. (1995) Human chromosome 11 inhibits tumorigenicity of a murine squamous cell carcinoma cell line. *Genes Chromosomes Cancer,* 13**,** 47-53.

ZHANG, Y., CHANG, C., GEHLING, D. J., HEMMATI-BRIVANLOU, A. & DERYNCK, R. (2001) Regulation of Smad degradation and activity by Smurf2, an E3 ubiquitin ligase. *Proc Natl Acad Sci U S A,* 98**,** 974-9.

ZHAO, B. & BEPLER, G. (2001) Transcript map and complete genomic sequence for the 310 kb region of minimal allele loss on chromosome segment 11p15.5 in non-small-cell lung cancer. *Oncogene,* 20**,** 8154-64.

ZHENG, N., WANG, P., JEFFREY, P. D. & PAVLETICH, N. P. (2000) Structure of a c-Cbl-UbcH7 complex: RING domain function in ubiquitin-protein ligases. *Cell,* 102**,** 533-9.

ZHU, H., KAVSAK, P., ABDOLLAH, S., WRANA, J. L. & THOMSEN, G. H. (1999) A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. *Nature,* 400**,** 687-93.

ZIMMERER, J. M., LESINSKI, G. B., RADMACHER, M. D., RUPPERT, A. & CARSON, W. E., 3RD (2007) STAT1-dependent and STAT1-independent gene expression in murine immune cells following stimulation with interferon-alpha. *Cancer Immunol Immunother,* 56**,** 1845-52.

ZINKERNAGEL, R. M. & DOHERTY, P. C. (1974) Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. *Nature,* 248**,** 701-2.

ZOU, W. & ZHANG, D. E. (2006) The interferon-inducible ubiquitin-protein isopeptide ligase (E3) EFP also functions as an ISG15 E3 ligase. *J Biol Chem,* 281**,** 3989-94.

ZWICKL, P., KLEINZ, J. & BAUMEISTER, W. (1994) Critical elements in proteasome assembly. *Nat Struct Biol,* 1**,** 765-70.