Immunity, Vol. 21, 81–93, July, 2004, Copyright ©2004 by Cell Press

XBP1, Downstream of Blimp-1, Expands the Secretory Apparatus and Other Organelles, and Increases Protein Synthesis in Plasma Cell Differentiation

A.L. Shaffer,[1] Miriam Shapiro-Shelef,[2] Neal N. Iwakoshi,[3] Ann-Hwee Lee,[3] Shu-Bing Qian,[4] Hong Zhao,[1] Xin Yu,[1] Liming Yang,[1] Bruce K. Tan,[1] Andreas Rosenwald,[1] Elaine M. Hurt,[1] Emmanuel Petroulakis,[5] Nahum Sonenberg,[5] Jonathan W. Yewdell,[4] Kathryn Calame,[2] Laurie H. Glimcher,[3] and Louis M. Staudt[1,*]

[1]Metabolism Branch
Center for Cancer Research
National Cancer Institute
National Institutes of Health
9000 Rockville Pike
Bethesda, Maryland 20892

[2]Department of Microbiology and Integrated Program in Biophysical, Cellular and Molecular Studies
Columbia University College of Physicians and Surgeons
New York, New York 10032

[3]Department of Immunology and Infectious Diseases
Harvard School of Public Health
Boston, Massachusetts 02115

[4]Laboratory of Viral Diseases
National Institute of Allergy and Infectious Diseases
Bethesda, Maryland 20892

[5]McGill University
Department of Biochemistry
Montreal, Quebec, H3G 1Y6
Canada

Summary

The differentiation of B cells into immunoglobulin-secreting plasma cells is controlled by two transcription factors, Blimp-1 and XBP1. By gene expression profiling, we defined a set of genes whose induction during mouse plasmacytic differentiation is dependent on Blimp-1 and/or XBP1. Blimp-1-deficient B cells failed to upregulate most plasma cell-specific genes, including *xbp1*. Differentiating *xbp1*-deficient B cells induced Blimp-1 normally but failed to upregulate genes encoding many secretory pathway components. Conversely, ectopic expression of XBP1 induced a wide spectrum of secretory pathway genes and physically expanded the endoplasmic reticulum. In addition, XBP1 increased cell size, lysosome content, mitochondrial mass and function, ribosome numbers, and total protein synthesis. Thus, XBP1 coordinates diverse changes in cellular structure and function resulting in the characteristic phenotype of professional secretory cells.

Introduction

When B cells become plasma cells, they lose expression of most B cell characteristics and undergo a radical restructuring that allows them to secrete large quantities of immunoglobulin (Ig) (Calame et al., 2003; Wiest et al., 1990). Two transcriptional regulators are essential for plasma cell differentiation: Blimp-1, a transcriptional repressor encoded by the *prdm1* gene, and XBP1, a b-ZIP family transcriptional activator (see below; Lin et al., 2003).

In the B cell lineage, Blimp-1 is predominantly expressed at the plasma cell stage where it directly represses genes encoding other transcription factors, such as *c-myc*, *cIIta*, *pax5*, *spiB*, and *id3* (Lin et al., 1997, 2002; Piskurich et al., 2000; Reljic et al., 2000; Shaffer et al., 2002). Global gene expression analysis identified over 250 genes whose expression was extinguished by Blimp-1, including genes mediating BCR expression and signaling, B cell activation and homing, and class switching (Shaffer et al., 2002). Blimp-1 also decreased proliferation and increased Ig synthesis, hallmarks of plasma cell differentiation (Lin et al., 1997; Shaffer et al., 2002). Recent work has shown that *prdm1*-deficient B cells proliferate rapidly upon LPS stimulation in vitro but do not secrete immunoglobulin and fail to produce the secreted form of Ig heavy chain mRNA (Shapiro-Shelef et al., 2003). In vivo, B cell development is grossly normal in *prdm1*-deficient mice, but these B cells fail to develop into Ig-secreting plasma cells upon immunization with T-dependent and -independent antigens.

XBP1 is a positively-acting transcription factor in the CREB/ATF family that is expressed at a high levels in plasma cells (Iwakoshi et al., 2003a; Reimold et al., 1996). Since disruption of *xbp1* in the mouse germ line results in embryonic lethality (Reimold et al., 2000), $xbp1^{-/-}$, $rag2^{-/-}$ chimeric mice were used to assess XBP1 function within the B cell lineage (Reimold et al., 2001). These mice possessed B cells that proliferated and formed germinal centers normally but were dramatically impaired in their ability to secrete immunoglobulin in vitro and in vivo in response to T-dependent or -independent antigens. Most importantly, these *xbp1*-deficient mice were devoid of plasma cells, demonstrating the requirement for XBP1 in plasmacytic differentiation.

XBP1 is also associated with the unfolded protein response (UPR; Calfon et al., 2002; Fewell et al., 2001; Harding et al., 2000; Mori, 2000; Shen et al., 2001; Yoshida et al., 2001), a coordinated change in gene expression that is triggered by perturbations in the function of the endoplasmic reticulum (ER). Experimentally, the UPR can be induced by DTT, which disrupts protein folding in the ER; tunicamycin, which disrupts glycosylation and folding in the ER; and thapsigargin, which depletes ER calcium stores (Fewell et al., 2001; Harding et al., 2001). Overexpression or misfolding of ER proteins can also elicit the UPR (Kozutsumi et al., 1988).

The UPR was first characterized in yeast, where a single signaling pathway governs the response to ER stress (Cox et al., 1993; Patil and Walter, 2001). In this pathway, the ER stress signal is transduced by a type I transmembrane ER protein, IRE1. The ER lumenal portion of IRE1 encodes a kinase for which IRE1 itself ap-

*Corresponding: lstaudt@mail.nih.gov

pears to be the only substrate. Interaction of IRE1 with the ER chaperone Kar2p/BiP prevents IRE1 multimerization and autophosphorylation. Current models propose that during an UPR, BiP is preferentially bound to misfolded client proteins, thereby releasing IRE1 to multimerize and autophosphorylate.

IRE1 phosphorylation is accompanied by activation of an endoribonuclease activity in its cytoplasmic domain, which mediates the posttranscriptional processing of the mRNA encoding HAC1, the yeast ortholog of XBP1. IRE1 removes a 252 nucleotide internal sequence from HAC1 mRNA, and tRNA ligase rejoins the two fragments of HAC1 mRNA. This new version of HAC1 mRNA is translated more efficiently than the unprocessed form and encodes a more stable protein with greater transcriptional activation potential (Chapman and Walter, 1997; Cox and Walter, 1996; Kawahara et al., 1998; Mori et al., 2000; Sidrauski and Walter, 1997). This linear UPR pathway is solely responsible for the upregulation of yeast UPR genes, which encode proteins involved in nearly every aspect of the secretory pathway, including protein entry into the ER, folding, glycosylation, ER-associated degradation (ERAD), and vesicular trafficking (Fewell et al., 2001; Travers et al., 2000).

In higher eukaryotes, ER stress stimulates three distinct but overlapping signaling pathways by activating IRE1, PERK, and ATF6 (Harding et al., 2002; Ma and Hendershot, 2003). The IRE1 UPR pathway has been maintained in higher eukaryotes with some modification. Activated IRE1 functions in mammals by removing 26 nucleotides from the XBP1 mRNA. This new mRNA encodes a protein with increased transcriptional activation potential (Calfon et al., 2002; Shen et al., 2001; Yoshida et al., 2001). XBP1 induces several UPR response genes, similar to those induced by HAC1 in yeast (Lee et al., 2003; Yoshida et al., 2003). However, mouse embryo fibroblasts lacking IRE1 still upregulate several UPR genes in response to ER stress, including *BiP* and *chop*, indicating that IRE1 is not the sole mediator of the UPR in higher eukaryotes (Harding et al., 2002).

Some UPR functions are mediated by PERK, a transmembrane ER kinase (Harding et al., 1999, 2001). Like IRE1, PERK interacts with BiP via its ER lumenal domain, keeping PERK's cytoplasmic kinase domain inactive. BiP is dissociated from PERK during an UPR, which activates PERK to phosphorylate the key translation initiation factor eIF2α. eIF2α phosphorylation acutely inhibits protein translation globally, thereby lessening the load of proteins entering the ER. However, phosphorylated eIF2α allows for the alternative translation initiation of ATF4, a transcription factor that in turn transactivates UPR genes, such as *chop* and *BiP*, and is the only known gene to be regulated in this fashion by PERK (Harding et al., 2003).

A third UPR pathway is initiated by ATF6, a type II ER transmembrane protein (Haze et al., 1999; Yoshida et al., 2000). Two forms of ATF6, α and β, exist in mammalian cells and both are localized to the ER in unstressed cells by association with BiP. Upon UPR induction, ATF6 is released by BiP and exported to the Golgi, where it is cleaved by intramembrane proteases, releasing its cytoplasmic domain. This portion of ATF6 acts as a transcriptional activator that can increase expression of ER chaperones and XBP1 (Okada et al., 2002; Shen et al., 2001; Yoshida et al., 2000, 2003). The exact role of ATF6 is not clear at present since recent studies showed that the activation of several UPR genes (*BiP*, *chop*, *grp94*, and *xbp1*) was unaffected in cells in which ATF6 levels were diminished by RNA interference (Lee et al., 2003).

In the present study, we used gene expression profiling to study the terminal differentiation of B cells derived from Blimp-1-deficient and XBP1-deficient mice in order to elucidate the roles of these transcription factors in plasma cell development. We observed that XBP1 acted downstream of Blimp-1 to regulate a broad complement of genes encoding ER-associated proteins, many of which are involved in protein secretion. XBP1 induced a dramatic physical expansion of the ER but also, unexpectedly, increased cell size, organelle biogenesis, and total protein synthesis, thus demonstrating that XBP1 plays a central role in defining the secretory cell phenotype.

Results

Regulation of Plasma Cell Gene Expression by Blimp-1 and XBP1

Lipopolysaccharide (LPS) treatment of mouse mature splenic B cells in vitro is an established experimental system for studying plasma cell differentiation (Lafrenz et al., 1982; Schliephake and Schimpl, 1996). After 4 days in culture, approximately 30% of cells achieve a plasma cell phenotype (surface expression of syndecan-1 and immunoglobulin secretion; M.S.-S. and N.N.I., unpublished data). To identify genes induced during differentiation and to determine their dependence on Blimp-1 and XBP1, we compared gene expression profiles from LPS-treated wild-type (wt), *prdm1*-deficient, and *xbp1*-deficient B cells (Reimold et al., 2001; Shapiro-Shelef et al., 2003). For these studies, we constructed the mouse Lymphochip, a specialized DNA microarray analogous to the human Lymphochip (Alizadeh et al., 1999), which is enriched for genes expressed in normal lymphocytes and for genes that play important roles in the immune system. Since only a fraction of the B cells in these cultures differentiate to plasma cells, it was not possible to monitor decreases in the expression of genes in the plasma cells, but the induction of plasma cell genes was readily measured. After applying confidence criteria for each element, we focused our analysis on those genes that required *prdm1* or *xbp1* for maximal induction and were also more highly expressed in plasma cells as compared to B cells (Figure 1; Supplemental Figure S1 at http://www.immunity.com/cgi/content/full/20/2/81/DC1 and http://lymphochip.nih.gov/ShafferPCfactors/). To capture the most reliable data, our analyses included only those genes that consistently failed to be induced in factor-deficient B cells as compared to wt B cells by at least 1.8-fold, which represents a difference in induction of approximately 5-fold when the number of differentiating cells in each culture (approximately 30%) is taken into account.

Comparison of wt and *prdm1*-deficient B cells identified 54 genes that required Blimp-1 for induction (Figure 1A). Comparison of wt and *xbp1*-deficient B cells revealed 36 genes as downstream targets of XBP1 (Figure 1B). Notably, *xbp1* mRNA expression was induced in wt but not *prdm1*-deficient B cells. On the other hand,

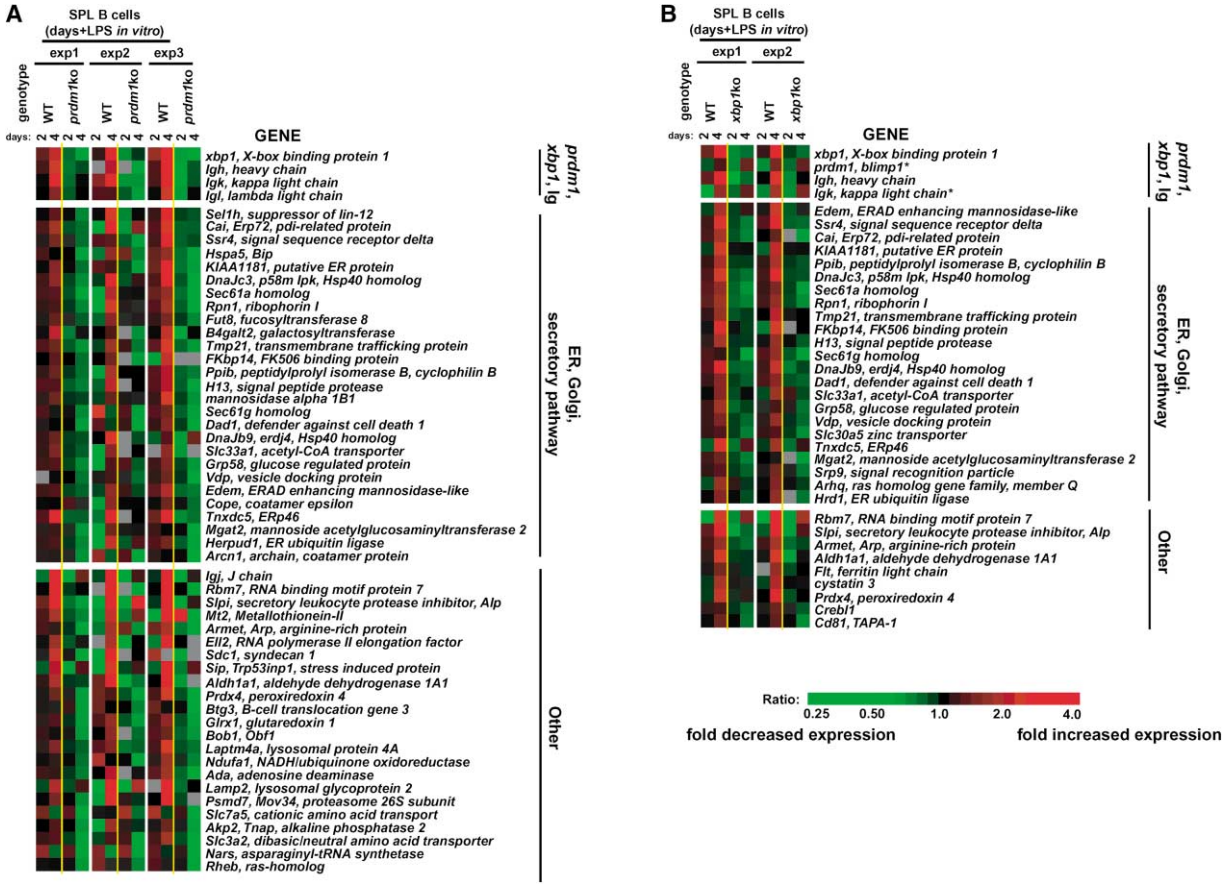

Figure 1. The Roles of *prdm1* and *xbp1* in Promoting Gene Expression during Plasma Cell Differentiation

RNA extracted from purified, LPS-treated wt, *prdm1*-, or *xbp1*-deficient splenic B cells was converted to labeled cDNA (Cy5, red) and cohybridized on mouse lymphochips with cDNA generated from a common reference pool of mouse cell line RNA (Cy3, green). Hybridization was measured by laser scanning (Axon Genepix 4000) and converted to a gene expression ratio (Cy5 experimental/Cy3 control), permitting the direct comparison of all samples by hierarchical clustering. Data were normalized to unstimulated (time zero) controls for each time course. When genes were represented by multiple array elements, data for a single representative feature is displayed. A color bar depicts the magnitude of gene expression differences. (A) Genes whose induction was diminished in *prdm1*-deficient B cells by at least 1.8-fold versus wt at days 2 or 4 in 2 of 3 experiments. (B) Genes whose induction was diminished in *xbp1*-deficient B cells versus wt by at least 1.8-fold in both experiments at days 2 or 4 of culture. *, *prdm1* and Ig_K light chain expression is affected less than 1.8-fold by the absence of *xbp1* (see Results).

prdm1 mRNA expression was induced equally in wt and *xbp1*-deficient B cells (Figure 1B). These observations confirm that Blimp-1 is upstream of XBP1 in the regulatory cascade of terminal B cell differentiation, consistent with previous observations (Shaffer et al., 2002; Shapiro-Shelef et al., 2003).

To better assess the contribution of Blimp-1 and XBP1 to the induction of differentiation-associated genes, we calculated the difference in expression of each gene from Figure 1 between wt B cells and *prdm1*- or *xbp1*-deficient B cells at day 4 following LPS stimulation. Figure 2 depicts the difference in expression for each gene between wt and *prdm1*-deficient B cells (black bars) and wt and *xbp1*-deficient B cells (gray bars). Most differentiation genes (46 of 63) required both *prdm1* and *xbp1*, and these are most likely downstream targets of XBP1, since *xbp1* itself was not induced in the absence of *prdm1*. Secretory pathway genes represented the largest functional category of *prdm1*- and *xbp1*-dependent genes (28), emphasizing the importance of these regulatory factors in preparing cells for high-level immunoglobulin secretion (Figures 1 and 2). Some of these genes have previously been identified as XBP1 targets and are induced during the UPR (*Edem*, *DnaJc3*, *Armet*, *Cai*, *Hspa5*; Lee et al., 2003; Yoshida et al., 2003). However, many of these genes have not been formally associated with the response to ER stress and encode a variety of secretory pathway proteins that play roles in targeting proteins to the ER (*srp9*), translocation of newly synthesized proteins into the ER (*sec61a*, *sec61g*, *ssr4*), folding of ER proteins (*fkbp14*, *ppib*, *grp58*, *txndc5*, *dnaJb9*), ER protein degradation by the ERAD pathway (*edem*, *sel1h*, *hrd1*), protein glycosylation (*dad1*, *fut8*, *slc33a1*, *man1b1*, *b4galt2*, *mgat2*), and vesicle trafficking (*arhq*, *cope*, *vdp*, *arcn1*). The expression of most (28 of 31) of the secretory pathway genes depended on both *prdm1* and *xbp1*, suggesting that XBP1, acting downstream of Blimp-1, plays a critical role in coordinating secretory function.

Several (16 of 63) plasma cell-associated genes (e.g., *syndecan-1*, *sdc1*) required only *prdm1* for their induction, suggesting that Blimp-1 controls part of the plasma

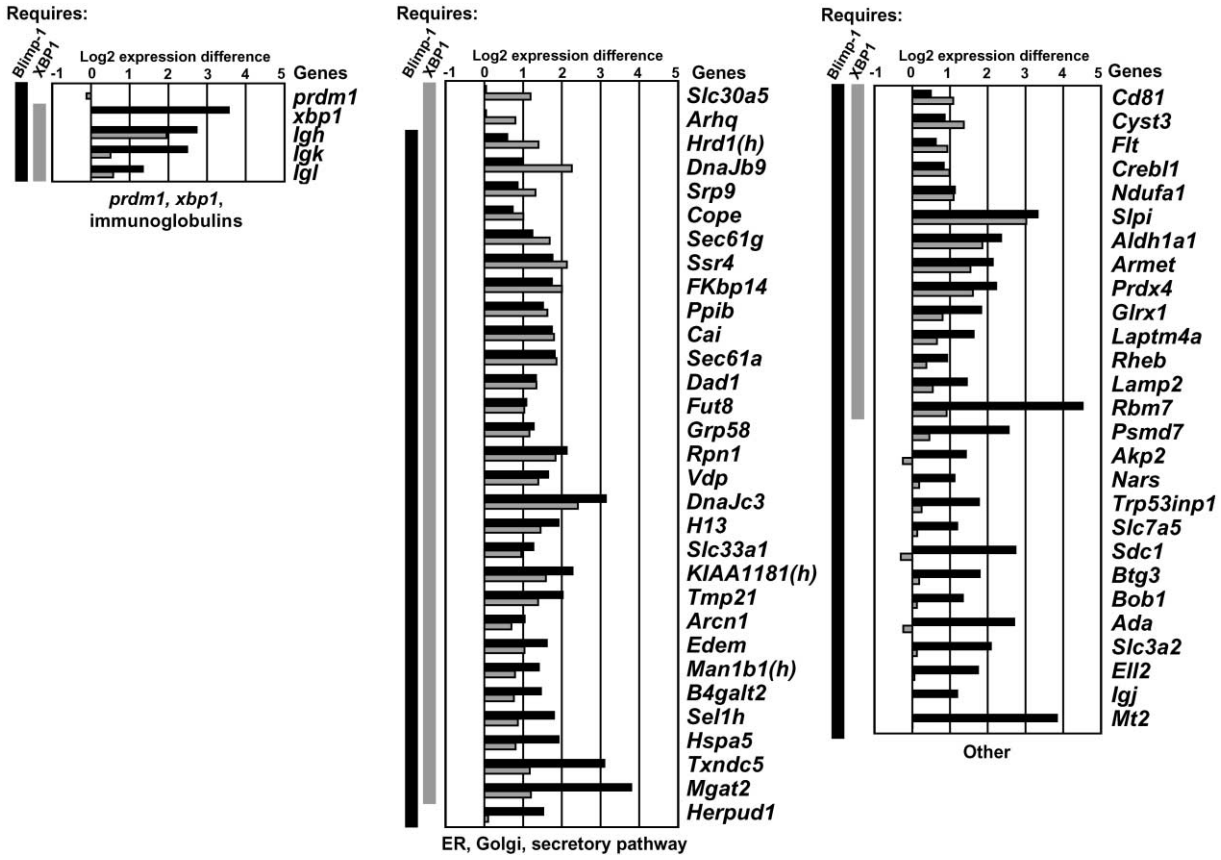

Figure 2. Plasma Cell Gene Induction: Dependence on *prdm1* and/or *xbp1*

The average gene expression ratio at day 4 of LPS culture was calculated for each element identified in Figure 1. The difference in expression of each gene between wt B cells and *prdm1*- (black) or *xbp1*-deficient (gray) B cells is plotted as a bar graph. Bars to next to each graph show the dependence of gene expression on *prdm1* and/or *xbp1*. An (h) indicates the likely mouse ortholog of the human gene.

cell gene expression program in an XBP1-independent fashion (Figure 2). Finally, two genes appeared to depend on XBP1 but not Blimp-1 and are perhaps sensitive to low levels of Blimp-1-independent XBP1 activation.

XBP1 Promotes ER Expansion

To explore further the role of XBP1 in regulating gene expression, we used retroviral transduction to express human XBP1 in cell lines that lack expression of the endogenous *xbp1* gene. Retroviruses bearing a puromycin selectable marker were engineered to coexpress the transcriptionally active, processed form of human *xbp1* mRNA (XBP1-s) or a form of *xbp1* mRNA that cannot be processed by IRE1 (XBP1-u) (Iwakoshi et al., 2003b), which encodes an unstable and transcriptionally inactive factor. Retroviruses expressing only the puromycin resistance gene were used for control transductions.

The human mature B cell line Raji, which expresses little active XBP1-s (A.-H.L., unpublished data), was infected with control, XBP1-s-, or XBP1-u-expressing retroviruses, and gene expression changes were monitored using human Lymphochip cDNA microarrays (Alizadeh et al., 1999; Figure 3). Multiple independent experiments were performed using the XBP1-s (8) and XBP1-u retroviruses (4), allowing the identification of genes whose expression was altered by XBP1-s but not XBP1-u in a statistically significant fashion (paired Student's t test, $p < 0.025$) and were also more highly expressed in primary human plasma cells as compared to other mature B cell populations (Wright et al., 2003; Supplemental Figure S2).

Figure 3A shows genes upregulated by XBP1-s but not XBP1-u in Raji cells. XBP1-s induced many of the same genes that were found to be *xbp1*-dependent in the mouse (indicated by an "*"). Genes found to be *prdm1*-dependent but *xbp1*-independent in the mouse (e.g., *syndecan-1*) were not induced by XBP1-s expression in Raji cells, supporting the notion that Blimp-1 alone regulates these genes. Secretory pathway genes again dominate the list of XBP1-s-induced genes (Figure 3A). These encode proteins involved in translocation of proteins across the ER membrane (*srp54*, *srpr*, *ssr3*, *ssr4*, *rpn1*, *tram1*, *spc22/23*), ER protein folding (*erp70*, *ppib*, *grp58*, *fkbp11*, *dnaJb9*, *hspa5*), protein glycosylation (*gcs1*, *ddost*, *dad1*), and vesicle trafficking (*sec23B*, *sec24C*, *os-9*, *golgB1*, *mcfd2*). XBP1-s also upregulated ER/secretory pathway genes when introduced into the human kidney cell line 293 and the mouse B cell line WEHI-231 (Figures 3B and 3C), suggesting that the program of secretory gene expression is inherent to XBP1-s irrespective of cell type. A number of genes with diverse functions are also upregulated by XBP1-s expression.

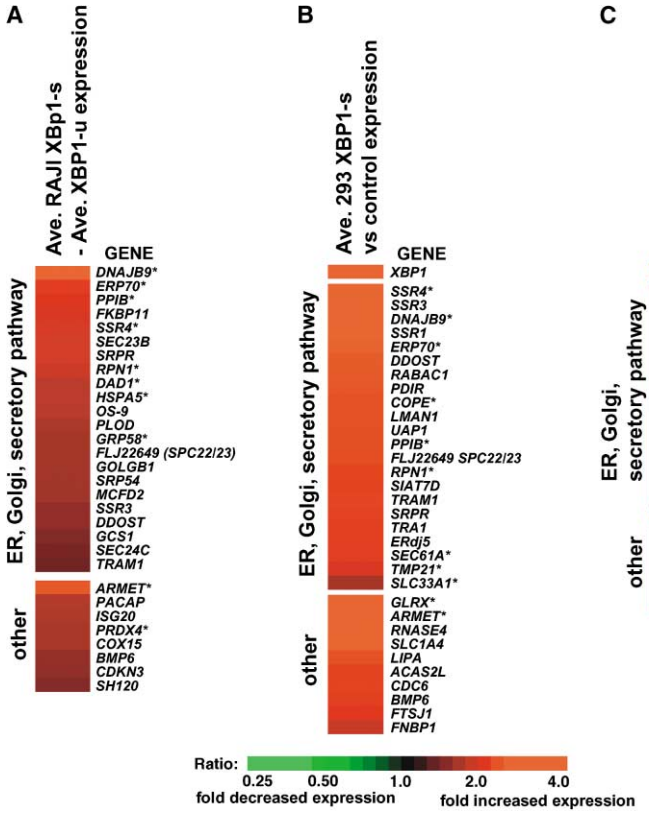

Figure 3. XBP1-s Target Genes

(A) RNA from RAJI cells transduced with retrovirus encoding the active form (XBP1-s) or an unsplicable, unstable form (XBP1-u) of human XBP1 was converted to labeled cDNA (Cy5, red), and cohybridized with cDNA generated from cells transduced with control retrovirus (Cy3, green). Eight independent XBP1-s transductions and four independent XBP1-u transductions were analyzed on human lymphochips (Alizadeh et al., 1999) against control transductions. Average expression ratios for each gene were calculated (XBP1-s/control and XBP1-u/control), and the difference between groups (Ave. XBP1-s − Ave. XBP1-u) is shown, with a color bar indicating its magnitude.

(B) The average expression of genes induced at least 1.8-fold in two independent transductions comparing 293 cells transduced with control (Cy3) or XBP1-s (Cy5) retroviruses.

(C) The average expression of genes induced at least 1.8-fold in two independent transductions comparing WEHI-231 cells transduced with control (Cy3) or XBP1-s (Cy5) retroviruses, measured on mouse lymphochips. *, indicates XBP1 target genes from Figures 1 and 2.

Some (*armet*, *prdx4*, *glrx1*) were also identified in the mouse differentiation system as *xbp1* dependent. The consistent appearance of these genes in two independent systems suggests conserved functions for XBP1-s beyond induction of secretory pathway genes.

We further confirmed several of these genes as XBP1-s targets by independently measuring their induction using quantitative RT-PCR (Supplemental Figure S3). The dependence of these genes on XBP1 expression was also confirmed by RNA interference. Expression of an shRNA directed against XBP1 in the XBP1-expressing human multiple myeloma line H929 decreased XBP1 expression by nearly 3-fold and shows a concomitant drop in expression of ER-related and other XBP1-s-induced genes (Supplemental Figure S3).

Given the profound change in secretory pathway gene expression mediated by XBP1 in both mouse and human cells, it seemed likely that it may also mediate an expansion of the ER and Golgi compartments. We observed changes in the light scattering properties of cells expressing XBP1-s consistent with an increase in organelle content, while interference with XBP1 expression in H929 myeloma cells resulted in a decrease in side scatter (Figure 4A). To specifically assess the expansion of the secretory apparatus, we stained cells with fluorescent brefeldin A (BFA-BODIPY; Deng et al., 1995), which stains both the ER and Golgi. Expression of XBP1-s increased fluorescence by 2- to 3-fold in each cell type tested (Figure 4B), consistent with increased ER mass associated with plasma cell differentiation (Wiest et al., 1990).

To directly visualize this apparent ER expansion, cells were stained with DiOC6, a marker of ER and mitochondria (Figure 4C; Terasaki et al., 1984). The stained area was greatly expanded in cells expressing XBP1-s (white arrow). To further confirm the XBP1-s-driven ER expansion, we transiently expressed a green fluorescent protein (GFP) with an added signal sequence and KDEL ER-retention motif in Raji control- and XBP1-s-expressing cells (Figure 4D). Consistent with the preceding results, the ER-retained GFP painted a small area in control cells that expanded substantially when XBP1-s was expressed.

During our analysis of XBP1-induced ER expansion, we also noted that cell size increased dramatically, as quantitated by measuring cell diameter (Figures 5A and 5B). The XBP1-induced increase in cell size was observed in both B cells (RAJI, WEHI231) and non-B cells (WS1). Furthermore, knockdown of XBP1 expression in a myeloma cell line by RNA interference decreased cell size, demonstrating that in cells that constitutively express XBP1-s, XBP1 maintains cell size. XBP1-s expression also increased nuclear size and nucleolar prominence (yellow arrows, Figure 5B). The increase in nuclear size was not due to increased DNA content or an arrest of cells in the G2/M phase of the cell cycle as determined by cell cycle analysis (data not shown).

Given that XBP1 expression dramatically increased ER content and cell size, we next looked for XBP1-s-related changes in other organelles. The bifluorescent dye JC1 emits green fluorescence as a function of mitochondrial mass (Smiley et al., 1991). By depolarizing mitochondria with the ionophore CCCP and staining with JC1, we observed that XBP1-s expression in-

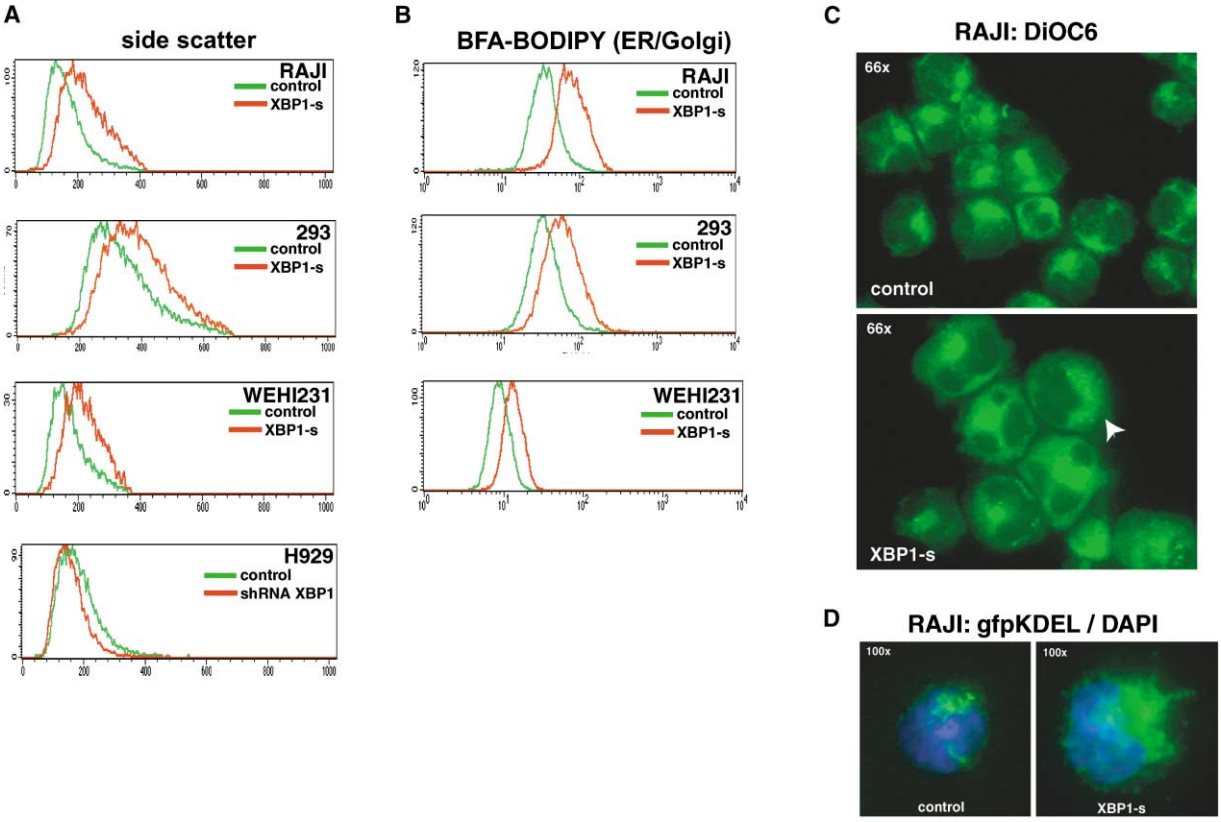

Figure 4. XBP1-s Induces ER Expansion

Phenotypic changes in cells transduced with control, XBP1-s-, or shRNA XBP1-expressing retroviruses.
(A) Side scatter of live cells.
(B) ER/Golgi content measured by staining with BFA-BODIPY.
(C) ER expansion in Raji visualized by microscopy after staining with DiOC6 (white arrow indicates expanded ER).
(D) Control and XBP1-s-expressing Raji cells were transduced with an expression vector for an ER-targeted form of GFP, counterstained with DAPI to identify nuclei (blue), and analyzed by fluorescent microscopy.

creased mitochondrial mass by approximately 40% (Figure 5C). Using another vital dye to assess mitochondrial function (mitotracker red; Poot and Pierce, 1999), we saw a concomitant 2.5-fold increase in mitochondrial respiration (Figure 5D). Cells expressing XBP1-s also had an increase in perinuclear, punctate red staining with acridine orange, a hallmark of lysosomes (Klintworth et al., 1979; data not shown). The XBP1-induced increase in lysosomal content was confirmed by flow cytometry of lysotracker red-stained cells (Figure 5E).

These striking changes in cellular structure prompted us to investigate whether XBP1 expression also altered cellular protein content. Raji cells expressing XBP1-s had a 50% increase in protein content per cell compared to control cells (Figure 6A). We next measured how the expression of XBP1-s affected protein synthesis. Proteins were labeled by incubating cells with ^{3}H-leucine for increasing times. To minimize the confounding effects of protein degradation, radiolabeling was performed in the presence of MG132, a potent inhibitor of proteasomes, lysosomal proteases, and calpains. Raji cells expressing XBP1-s had approximately 50% higher protein synthesis than control cells (Figure 6B), whereas expression of XBP1-u had little effect. Since XBP1-s induces the expression of many ER-targeted gene products, many of which are glycosylated, we attempted to distinguish between the synthesis of glycoproteins and nonglycoproteins. We removed glycoproteins based on their ability to bind to the agarose-coupled lectin concanavalin A (ConA), and the specificity of glycoprotein capture was confirmed by elution of ConA binding proteins with α-methyl mannoside (data not shown). By SDS-PAGE analysis, however, it appeared that some nonglycoproteins (e.g., actin) also bound to ConA, presumably through their interactions with glycoproteins (data not shown). With this in mind, we observed that XBP1 increased the synthesis of non-ConA binding, nonglycosylated proteins to the same extent as observed for all proteins (Figure 6B), leading us to conclude that expression of XBP1-s increases total protein synthesis, not only the synthesis of glycosylated, ER-targeted proteins.

The increase in total protein synthesis mediated by XBP1-s was associated with an increase in the number of assembled ribosomes (80S) per cell compared to control or XBP1-u-expressing cells (Figure 6C). This was not accompanied by an overall induction of ribosomal gene expression (data not shown). It is possible that the increase in assembled ribosomes is related to the condensation and prominence of nucleoli, the sites of

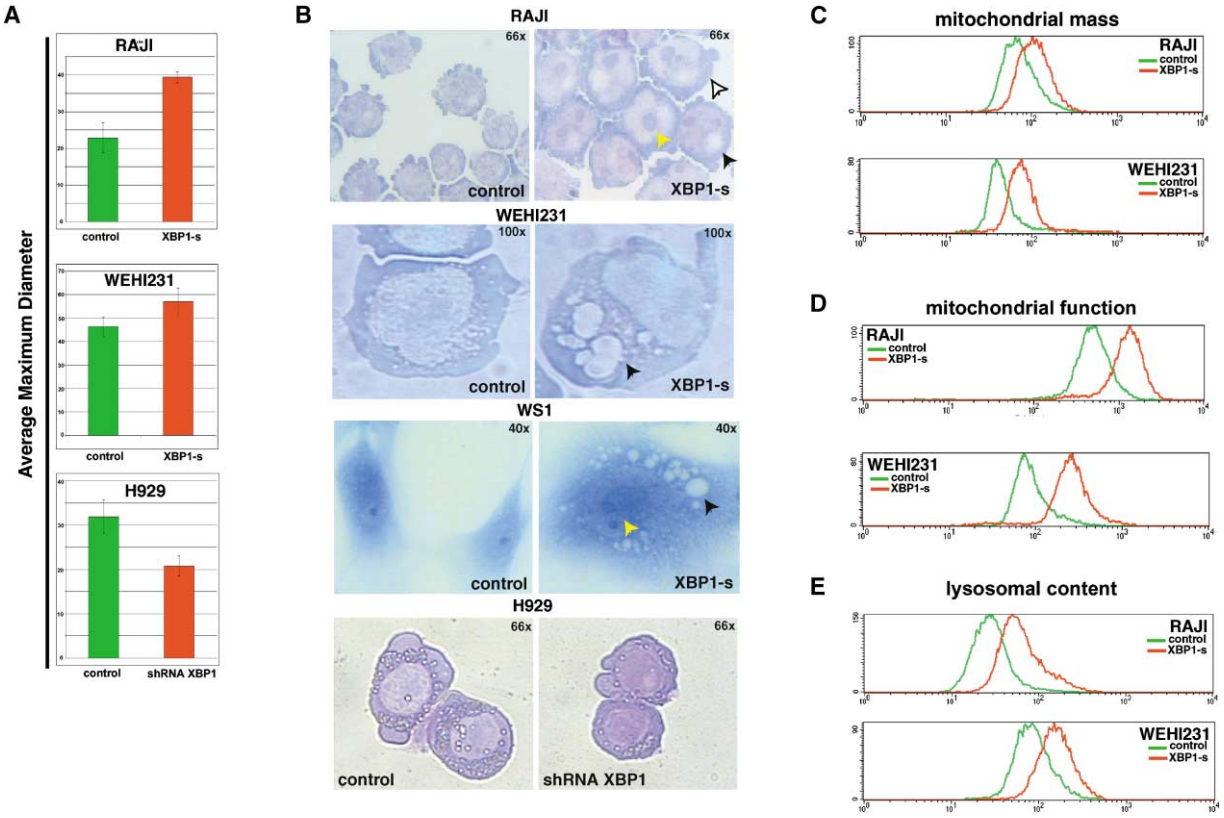

Figure 5. XBP1-s Induces Changes in Cell Size, Structure, and Organelle Content

(A) The average maximum diameter of control and XBP1-s-expressing cells was measured from four to six independent fields of at least five cells each.

(B) Cells transduced with control or XBP1-s retroviruses were cytospun onto glass slides, fixed, and stained with DIFFQUIK reagents for microscopy: white arrow, increased cytoplasmic volume; black arrows, perinuclear vacuoles; and yellow arrows, expanded nucleus with prominent nucleoli.

(C) Mitochondrial mass measured by flow cytometry after depolarization using CCCP followed by staining with JC1.

(D) Mitochondrial function assessed by flow cytometry after incubating cells with mitotracker red.

(E) Lysosomal content measured by flow cytometry after staining cells with lysotracker red.

ribosomal biogenesis, in XBP1-s-expressing cells (Figure 5B, yellow arrows). However, it is not clear at present whether this increase in ribosomal content can account for the increased protein synthesis, since the overall loading of mRNAs onto polysomes in these cells was extremely low and therefore difficult to accurately quantitate by ultracentrifugation (data not shown).

We next examined the effects of XBP1-s on protein degradation in Raji cells by radiolabeling cells for 5 min with ^{35}S-methionine and chasing with unlabeled methionine for 30 min. Protein degradation was monitored by measuring the loss of cell-associated and secreted TCA-insoluble radioactivity in the presence and absence of the proteosome inhibitor MG132 over time. In control cells, over 30% of labeled proteins were degraded during the 30 min chase period (Figure 6D), which reflects the rapid proteasome-mediated destruction of newly synthesized proteins (Schubert et al., 2000). Expression of XBP1-s reduced the fraction of rapidly degraded newly synthesized proteins by approximately 40%, from 31% in control cells to 19% with XBP1-s expression, whereas expression of XBP1-u had little effect on the rate of degradation. Using ConA to discriminate between glycosylated and nonglycosylated proteins, we found that XBP1-s expression had little effect on nonglycoprotein degradation, which remained near 9% across all samples (Figure 6D). This suggests that the XBP1-s-associated decrease in protein degradation is the result of a specific decrease in glycoprotein degradation.

Discussion

We have used gene expression profiling to understand how two transcription factors, Blimp-1 and XBP1, control plasma cell differentiation. Most plasma cell genes were under the control of Blimp-1. Many of these were also targets of XBP1 and promote the entry, processing, and movement of proteins through the secretory pathway. Furthermore, XBP1 expression was sufficient to induce many phenotypic changes that characterize plasmacytic differentiation: increased cell size, expanded organelle mass and function, and increased protein synthesis. These findings suggest that the role of XBP1 in higher eukaryotes has been extended beyond its role in the ER stress response and that XBP1 may

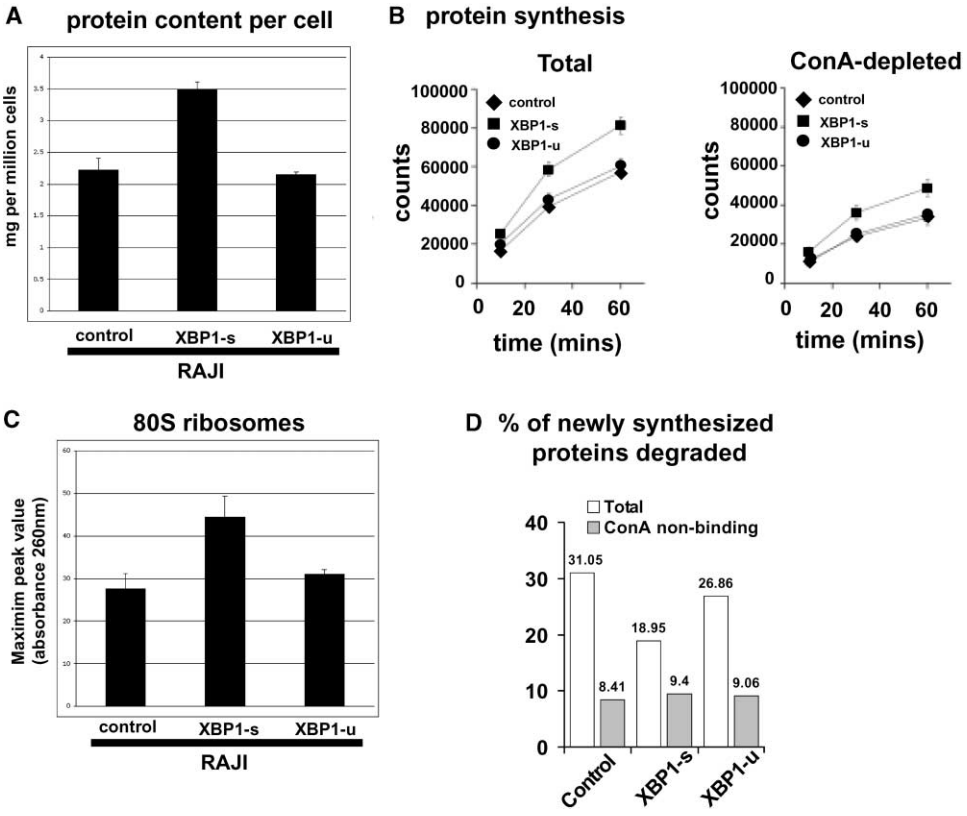

Figure 6. XBP1-s Effects on Protein Synthesis and Degradation

Raji cells transduced with control, XBP1-s-, or XBP1-u-expressing retroviruses were selected and used for the analysis of protein synthesis and degradation.

(A) Cells were lysed by sonication and soluble protein content per cell was measured by absorbance using the Bradford assay.

(B) The rate of protein synthesis was measured by incubating cells with ^{3}H-leucine in the presence of proteosome inhibitor and tracking radioactive amino acid incorporation over time. Proteins were also separated into two fractions based on the binding to ConA (glycosylated and nonglycosylated), and the rate of nonglycosylated protein synthesis is shown.

(C) Assembled (80S) ribosomal content measured per cell by absorbance (260 nm) after arresting translation and separating cellular components by centrifugation through a continuous sucrose gradient. Maximum peak absorbance of the 80S fraction from three independent experiments is shown.

(D) Total protein degradation measured in cells pulsed with S^{35}-methionine and chased with unlabeled amino acids in the presence and absence of proteosome inhibitor. Proteins were separated into two fractions based on binding to ConA. The percentage of total and Con-A nonbinding (nonglycosylated) proteins degraded is shown for a single representative experiment.

be better viewed as a key regulator of the professional secretory cell phenotype (Figure 7).

Blimp-1 Acts Upstream of XBP1 to Regulate Plasma Cell Gene Expression

Blimp-1, a transcriptional repressor, was required for the induction of most plasma cell genes during plasmacytic differentiation. This seemingly paradoxical result is most readily explained by the ability of Blimp-1 to repress genes that encode repressors of plasma cell genes. This "derepression" model of Blimp-1 action has been most clearly elucidated for two plasma cell genes, *J chain* and *xbp1*, both of which are repressed by PAX5 (Reimold et al., 1996; Rinkenberger et al., 1996). Since *pax5* is repressed by Blimp-1, induction of *J chain* and *xbp1* depends on Blimp-1 expression in plasma cells (Lin et al., 2002; Shaffer et al., 2002).

A number of plasma cell genes of diverse function required Blimp-1 but not XBP1 for their expression, including *sdc1*, which encodes the standard plasma cell marker Syndecan-1. By contrast, virtually all of the plasma cell genes that required XBP1 for their expression also required Blimp-1. Further, *xbp1* was not induced in *prdm1*$^{-/-}$ cells, but *prdm1* was induced in *xbp1*$^{-/-}$ cells. Together, these findings suggest a regulatory hierarchy during plasmacytic differentiation in which XBP1 acts downstream of Blimp-1. Consistent with this model, ectopic expression of XBP1 does not restore a plasma cell phenotype in *prdm1*-deficient B cells, though ectopic Blimp-1 does (Shapiro-Shelef et al., 2003).

Blimp-1 was required for maximal induction of Ig genes (Figures 1 and 2), which may be partially explained by the repression of *pax5*, since Pax5 represses expression from these Ig loci (Shaffer et al., 1997; Singh and Birshtein, 1996). Another potential mechanism could involve the Blimp-1-dependent induction of *bob1*, a transcriptional coactivator that regulates a 3′ *Igh* enhancer (Andersson et al., 2000) and a subset of *Ig*κ promoters (Casellas et al., 2002) (Figures 1 and 2). Blimp-1 but not

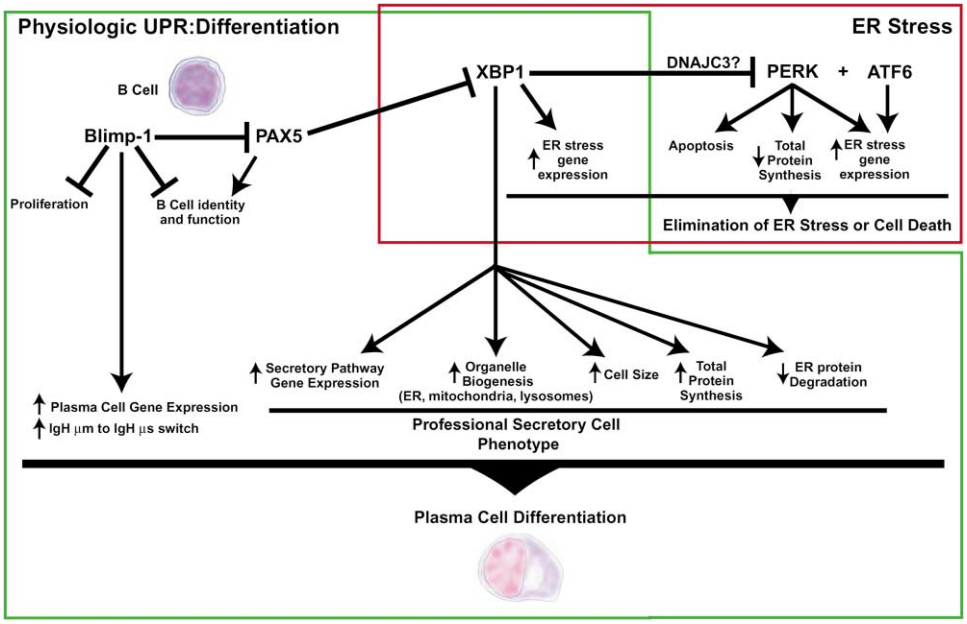

Figure 7. A Model for the Regulatory Biology of Plasma Cell Differentiation

Blimp-1 acts upstream of XBP1 to initiate plasma cell differentiation. ER stress in a nonsecretory cell induces an "ER-stress" UPR in which XBP1, PERK, and ATF6 are activated, leading to decreased ER stress or apoptosis (red box). Alternatively, XBP1 activation in a differentiating secretory cell initiates a "physiological" UPR that antagonizes the effects of PERK on translation, perhaps by inducing DNAJC3. XBP1 also coordinately alters the cell structure and function to create a professional secretory cell. Together, the activities of Blimp-1 and XBP1 drive plasma cell differentiation (green box).

XBP1 is also required for the switch in the usage of *Igh* mRNA polyadenylation sites, shifting expression from the membrane-bound to the secreted form of Ig heavy chain (Alt et al., 1980; Early et al., 1980; Reimold et al., 2001; Rogers et al., 1980; Shapiro-Shelef et al., 2003). Although the factor(s) mediating this important switch are unknown, an interesting candidate is the RNA polymerase II processivity factor ELL2 (Shilatifard et al., 1997) that we found to be induced during plasmacytic differentiation in a Blimp-1-dependent fashion (Figures 1 and 2). The substantial number of genes, like *ell2*, that depend exclusively on Blimp-1 for induction (Figure 2) point to its unique and essential role in promoting plasma cell differentiation.

XBP1 and Biogenesis of the Secretory Apparatus

Previous work has demonstrated that XBP1, as well as its yeast homolog HAC1p, regulate ER stress-induced genes that promote folding of ER proteins and degradation of misfolded ER proteins through the ERAD pathway (Lee et al., 2003; Mori et al., 1998; Yoshida et al., 2003). Recent studies have also shown that there is overlap in the genes induced during plasmacytic differentiation and those induced by ER stress (Gass et al., 2002; Iwakoshi et al., 2003a; Lee et al., 2003; van Anken et al., 2003).

The present study defines an expanded role for XBP1 in enhancing the secretory capacity of plasma cells. A remarkably consistent set of XBP1 target genes emerged from studies of *xbp1*-deficient mouse B cells and ectopic XBP1 expression, many of which encode proteins that function in the secretory pathway. Our data suggests that XBP1 regulates essentially every stage of the secretory process, including the targeting of proteins to the ER (*srp9*, *srp54*, *rpn1*), translocation of proteins across the ER membrane (*ssr1*, *ssr3*, *ssr4*, *srpr*, *tram1*, *sec61a*, *sec61g*), cleavage of signal peptides (*spc22/23, h13/spp*), folding of ER proteins (*dnaJb9*, *hspa5/BiP*, *erp70*, *grp58*, *pdir*, *txndc5*, *fkbp11, ppib*), degradation of misfolded ER proteins by the ERAD pathway (*edem*, *sel1h*), protein glycosylation (*slc33a1*, *ddost*, *gcs1*, *man1b1*, *siat7d*, *dad1*, *slc33a1*, *b4galt2*, *mgat2*, *fut8*), ER-Golgi vesicular trafficking (*cope*, *os-9*, *sec24C*, *sec23B*, *golgb1*, *mcdf2*, *vdp*, *arcn1*), endosomal trafficking (*rabac*1), and targeting of secretory vesicles to the plasma membrane (*arhq/tc10*). Importantly, XBP1 induces the expression of many components of the multiprotein chaperone complex that binds and processes nascent Ig heavy chains (Erp72, Hsp40 homologs, PDI, PPIB, Bip) (Meunier et al., 2002), which may specifically contribute to the increased efficiency of Ig secretion.

Interestingly, gene expression profiling experiments in yeast cells experiencing ER stress suggested a similarly broad role for HAC1 in the secretory pathway (Travers et al., 2000). During the yeast UPR, HAC1 induces a host of genes involved in protein translocation into the ER, glycosylation, ER protein folding, ER protein degradation in the ERAD pathway, vesicular transport, and phospholipid biosynthesis. Since not every secretory pathway gene was upregulated by the UPR in yeast, HAC1 may be devoted to remodeling the secretory pathway to specifically cope with an excess of unfolded proteins (Travers et al., 2000). Another interesting aspect of this study was the finding that mutations in the UPR pathway and the ERAD pathway were synthetically lethal under

normal growth conditions, revealing a role for HAC1 and the "UPR" in the absence of overt ER stress.

XBP1 has not only retained the broad, UPR-related, transcriptional program of HAC1 but also has the ability to physically expand the ER compartment. In both lymphoid and epithelial cell lines, ectopic XBP1 dramatically augmented the ER, and in myeloma cells, knockdown of XBP1 diminished the ER. Notably, IRE1 and HAC1 are required for the induction of a specialized form of ER known as karmellae that is formed in response to forced overexpression of ER proteins (Cox et al., 1997). In multicellular eukaryotes, unlike yeast, terminal differentiation in some cell lineages results in a "professional" secretory cell that has an expanded ER as a fixed attribute. We propose that the function of HAC1 in inducing ER biogenesis under stress conditions has been adapted in evolution to allow XBP1 to stably increase ER size during secretory cell differentiation. In keeping with this hypothesis, XBP1 is not only highly expressed in plasma cells but also in secretory tissues such as the exocrine pancreas and salivary glands (Clauss et al., 1993).

XBP1: Beyond the Endoplasmic Reticulum

Unexpectedly, ectopic expression of processed XBP1 in diverse cell lines increased overall cell size and, conversely, knockdown of XBP1 expression by RNA interference in a myeloma cell line decreased cell size. In part, these findings could be explained by the ER expansion that is induced by XBP1. However, XBP1 expression also increased nuclear size, mitochondrial mass, and lysosomal content, suggesting that the cell size phenotype is part of a coordinated increase in multiple intracellular structures. These functional capabilities of XBP1 have not been reported for HAC1, suggesting that XBP1 has acquired additional regulatory roles during evolution.

The mechanism by which XBP1 exerts such pleiotropic effects on cellular organelles is not clear at present. Not only did mitochondrial mass increase but so did mitochondrial function, as assessed by the mitochondrial membrane potential. Two XBP1 target genes, *cox15* and *ndufa1*, encode proteins that are required for electron transport in the mitochondrion (Antonicka et al., 2003; Yadava et al., 2002), and the XBP1 target gene *acas2l* encodes a mitochondrial enzyme that synthesizes acetyl-CoA for use in oxidation. However, it is not know whether the increased expression of these mitochondrial proteins would stimulate respiration or whether they play a role in the XBP1-induced increase in mitochondrial mass. Three XBP1 target genes encode lysosomal proteins involved in lipid hydrolysis (*lipa*), fusion of autophagic vesicles with the lysosome (*lamp2*), and possibly in small molecule transport into the lysosome (*laptm4a*). Again, it is not known whether upregulation of these lysosomal proteins can account for the increased lysosomal biogenesis in XBP1-expressing cells.

Another unanticipated observation was that XBP1 increased protein synthesis globally by 30%–50%, which could contribute to the increase in organelle content and cell size. XBP1-s increased the synthesis of both nonglycosylated and glycosylated proteins equivalently. Therefore, the increase in protein synthesis caused by XBP1 cannot be explained by upregulation of mRNAs encoding glycosylated proteins in the secretory pathway but must be due to a more general mechanism. One possible scenario involves the XBP1 target gene DNAJC3 (this study and Lee et al. [2003]), which encodes $p58^{IPK}$, an inhibitor of PERK (van Huizen et al., 2003; Yan et al., 2002). When PERK is activated, it phosphorylates eIF2α, leading to decreased translation initiation of most proteins. Therefore, the upregulation of $p58^{IPK}$ by XBP1 could increase total protein synthesis by antagonizing PERK (Figure 7). XBP1 also increased the abundance of assembled ribosomes per cell, which might contribute to the increase in total protein synthesis, and it is possible that the condensation of nucleoli that we observed in XBP1-expressing cells may play a role in this process. Interestingly, plasma cells are characterized by increased nuclear size and the presence of a single prominent nucleolus (Benjamin et al., 1984), features that may be attributable to XBP1.

Evolution of XBP1 as a Master Regulator of Secretory Cell Differentiation

Whereas yeast have a single pathway that senses unfolded proteins in the ER, multicellular eukaryotes have evolved three parallel pathways: IRE1/XBP1, PERK/ATF4, and ATF6. In evolution, the creation of parallel pathways by genome duplication can set the stage for subsequent functional specialization. It has therefore been proposed that the three mammalian UPR pathways have diverged functionally during evolution (Figure 7; Calfon et al., 2002; Harding et al., 2002), and our data strongly support this hypothesis.

XPB1 has retained important features of its yeast ortholog HAC1, while developing additional functions that promote development of the secretory phenotype. Like HAC1, XBP1 transactivated a host of genes encoding proteins that function throughout the secretory pathway. Further, our experiments revealed that XBP1 is sufficient to expand the ER in a wide variety of mammalian cells, which may be functionally analogous the HAC1-mediated formation of ER-like karmellae.

However, XBP1 has acquired new functional capabilities that have not been reported for HAC1. Most notably, we found that XBP1 increased overall protein synthesis, which clearly would favor high-level protein secretion. In marked contrast, PERK signaling inhibits protein translation, a clearly undesirable attribute for a secretory cell. Indeed, it is possible that XBP1 evolved in order for professional secretory cells to modulate the activity of PERK. In this regard, it is notable that the PERK pathway upregulates *chop* (Harding et al., 2000; Yan et al., 2002), which encodes a mediator of apoptosis during ER stress, whereas the IRE1/XBP1 pathway does not (this study and Gass et al. [2002]; Lee et al. [2003]). Thus, PERK serves as a checkpoint protein during ER stress that can decrease the unfolded protein load in the ER by inhibiting translation or eliminate the cell if the ER stress is severe and prolonged. XBP1, on the other hand, has apparently evolved to promote translation by antagonizing PERK, thereby allowing cells to differentiate into professional secretory cells that can tolerate a constitutively high throughput of ER proteins.

It may therefore be helpful to define a "physiological" UPR (Gass et al., 2002) that is activated constitutively in professional secretory cells through the action of XBP1, and an "ER stress" UPR that is activated by unfolded proteins in nonsecretory cells through the action of PERK (Figure 7). Though activated during in vitro plasma cell differentiation (Gass et al., 2002), the role of ATF6 in the physiological UPR remains unclear and will require the analysis of ATF6-deficient animals to clarify this factor's role in plasma cell differentiation. The physiological UPR that XBP1 coordinates promotes secretion and is characterized by increased protein synthesis and biogenesis of ER, mitochondria, and lysosomes. The increased mitochondrial mass and respiration in the physiological UPR could be used by secretory cells to meet the energy demands of sustained high-level secretion. The benefit to a secretory cell of lysosomal biogenesis is less clear, but it is possible that autophagy, which requires lysosome function, may contribute biosynthetic precursors needed for high-level protein secretion. The present study has expanded our view of XBP1 function from that of a transcription factor that activates a subset of ER stress genes to that of a key regulator responsible for many of the cellular changes that occur during differentiation of secretory cells.

Experimental Procedures

Mice and In Vitro LPS B Cell Differentiation

Please see http://lymphochip.nih.gov/ShafferPCfactors/.

Cell Lines and Retroviral Constructs

Cell lines were grown as described at http://lymphochip.nih.gov/ShafferPCfactors/. Retroviral transduction of human and mouse cell lines was performed as described (Shaffer et al., 2000, 2002) using constructs expressing only the puromycin resistance gene (control) or vectors expressing a form of XBP1 and the puromycin resistance gene as part of an IRES-containing bicistronic vector (see http://lymphochip.nih.gov/ShafferPCfactors/). The pRETROsuper retroviral construct with only a puromycin resistance gene was used as a control vector. An shRNA against human XBP1 was designed using the Dharmacon search engine, converted to a hairpin loop with resistriction enzyme overhanging ends, and cloned via BglII/HindIII sites into pRETROSUPER (see http://lymphochip.nih.gov/ShafferPCfactors/). After infection, pools of cells were selected with puromycin at 1 μg/ml.

RNA Preparation

For mouse lymphochip analysis, the total RNA was prepared using Trizol (Invitrogen), and for human lymphochip analysis, polyA+ RNA was prepared using FastTrack kits (Invitrogen).

Microarray Analysis

The Mouse Lymphochip Microarray

The mouse lymphochip is a spotted cDNA array similar in construction to the human lymphochip (Alizadeh et al., 1999). For murine microarray analysis, experimental sample RNAs were converted to Cy5-labeled cDNA and cohybridized with RNA from a reference pool labeled with Cy3 (http://lymphochip.nih.gov/ShafferPCfactors/).

The Human Lymphochip Microarray

Gene expression analysis using human lymphochips has been previously described (Alizadeh et al., 1999). RNA from control and XBP1-transduced cells were compared directly on arrays with RNAs labeled as described in the figure legends. Microarray data analysis (hierarchical clustering) and presentation was performed using the Cluster and Treeview (Alizadeh et al., 1999). Data files for all experiments can be found at http://lymphochip.nih.gov/ShafferPCfactors/.

Flow Cytometry and Microscopy

Please see http://lymphochip.nih.gov/ShafferPCfactors/. For determination of protein synthesis, degradation, and ribosome content, please see http://lymphochip.nih.gov/ShafferPCfactors/.

Acknowledgments

We would like to thank the members of all contributing labs for helpful discussions and generosity with reagents. We would like to thank all those who have thusfar contributed RNA samples and cDNA clones to the Mouse Lymphochip project (especially H. Morse, M. Potter, S. Janz, Y. Tagaya); Drs. E. Snapp and J. Lippincott-Schwartz for the gift of the ER-targeted gfp vector; and Bob Strausberg and the Cancer Genome Anatomy Project (National Cancer Institute) as well as the Leukemia and Lymphoma Society for funding, in part, the creation of the Mouse Lymphochip Microarray. This work was in part supported by NIH grants AI32412 (LHG), AI50659 (KC) and AI43576 (KC), an Award from the Multiple Myeloma Research Foundation (L.H.G.), and an Irvington Institute Postdoctoral Fellowship Award (N.N.I.).

Received: March 3, 2004
Revised: April 23, 2004
Accepted: May 19, 2004
Published: July 20, 2004

References

Alizadeh, A., Eisen, M., Davis, R.E., Ma, C., Sabet, H., Tran, T., Powell, J., Yang, L., Marti, G., Moore, T., et al. (1999). The lymphochip: a specialized cDNA microarray for the genomic-scale analysis of gene expression in normal and malignant lymphocytes. Cold Spring Harb. Symp. Quant. Biol. *64*, 71–78.

Alt, F.W., Bothwell, A.L., Knapp, M., Siden, E., Mather, E., Koshland, M., and Baltimore, D. (1980). Synthesis of secreted and membrane-bound immunoglobulin mu heavy chains is directed by mRNAs that differ at their 3′ ends. Cell *20*, 293–301.

Andersson, T., Samuelsson, A., Matthias, P., and Pettersson, S. (2000). The lymphoid-specific cofactor OBF-1 is essential for the expression of a V(H) promoter/HS1,2 enhancer-linked transgene in late B cell development. Mol. Immunol. *37*, 889–899.

Antonicka, H., Mattman, A., Carlson, C.G., Glerum, D.M., Hoffbuhr, K.C., Leary, S.C., Kennaway, N.G., and Shoubridge, E.A. (2003). Mutations in COX15 produce a defect in the mitochondrial heme biosynthetic pathway, causing early-onset fatal hypertrophic cardiomyopathy. Am. J. Hum. Genet. *72*, 101–114.

Benjamin, D., Magrath, I.T., Triche, T.J., Schroff, R.W., Jensen, J.P., and Korsmeyer, S.J. (1984). Induction of plasmacytoid differentiation by phorbol ester in B-cell lymphoma cell lines bearing 8;14 translocations. Proc. Natl. Acad. Sci. USA *81*, 3547–3551.

Calame, K.L., Lin, K.I., and Tunyaplin, C. (2003). Regulatory mechanisms that determine the development and function of plasma cells. Annu. Rev. Immunol. *21*, 205–230.

Calfon, M., Zeng, H., Urano, F., Till, J.H., Hubbard, S.R., Harding, H.P., Clark, S.G., and Ron, D. (2002). IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature *415*, 92–96.

Casellas, R., Jankovic, M., Meyer, G., Gazumyan, A., Luo, Y., Roeder, R., and Nussenzweig, M. (2002). OcaB is required for normal transcription and V(D)J recombination of a subset of immunoglobulin kappa genes. Cell *110*, 575–585.

Chapman, R.E., and Walter, P. (1997). Translational attenuation mediated by an mRNA intron. Curr. Biol. *7*, 850–859.

Clauss, I.M., Gravallese, E.M., Darling, J.M., Shapiro, F., Glimcher, M.J., and Glimcher, L.H. (1993). In situ hybridization studies suggest a role for the basic region-leucine zipper protein hXBP-1 in exocrine gland and skeletal development during mouse embryogenesis. Dev. Dyn. *197*, 146–156.

Cox, J.S., and Walter, P. (1996). A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. Cell *87*, 391–404.

Cox, J.S., Shamu, C.E., and Walter, P. (1993). Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. Cell *73*, 1197–1206.

Cox, J.S., Chapman, R.E., and Walter, P. (1997). The unfolded protein response coordinates the production of endoplasmic reticulum protein and endoplasmic reticulum membrane. Mol. Biol. Cell *8*, 1805–1814.

Deng, Y., Bennink, J.R., Kang, H.C., Haugland, R.P., and Yewdell, J.W. (1995). Fluorescent conjugates of brefeldin A selectively stain the endoplasmic reticulum and Golgi complex of living cells. J. Histochem. Cytochem. *43*, 907–915.

Early, P., Rogers, J., Davis, M., Calame, K., Bond, M., Wall, R., and Hood, L. (1980). Two mRNAs can be produced from a single immunoglobulin mu gene by alternative RNA processing pathways. Cell *20*, 313–319.

Fewell, S.W., Travers, K.J., Weissman, J.S., and Brodsky, J.L. (2001). The action of molecular chaperones in the early secretory pathway. Annu. Rev. Genet. *35*, 149–191.

Gass, J.N., Gifford, N.M., and Brewer, J.W. (2002). Activation of an unfolded protein response during differentiation of antibody-secreting B cells. J. Biol. Chem. *277*, 49047–49054.

Harding, H.P., Zhang, Y., and Ron, D. (1999). Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature *397*, 271–274.

Harding, H.P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., Schapira, M., and Ron, D. (2000). Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol. Cell *6*, 1099–1108.

Harding, H.P., Novoa, I., Bertolotti, A., Zeng, H., Zhang, Y., Urano, F., Jousse, C., and Ron, D. (2001). Translational regulation in the cellular response to biosynthetic load on the endoplasmic reticulum. Cold Spring Harb. Symp. Quant. Biol. *66*, 499–508.

Harding, H.P., Calfon, M., Urano, F., Novoa, I., and Ron, D. (2002). Transcriptional and translational control in the Mammalian unfolded protein response. Annu. Rev. Cell Dev. Biol. *18*, 575–599.

Harding, H.P., Zhang, Y., Zeng, H., Novoa, I., Lu, P.D., Calfon, M., Sadri, N., Yun, C., Popko, B., Paules, R., et al. (2003). An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol. Cell *11*, 619–633.

Haze, K., Yoshida, H., Yanagi, H., Yura, T., and Mori, K. (1999). Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. Mol. Biol. Cell *10*, 3787–3799.

Iwakoshi, N.N., Lee, A.H., and Glimcher, L.H. (2003a). The X-box binding protein-1 transcription factor is required for plasma cell differentiation and the unfolded protein response. Immunol. Rev. *194*, 29–38.

Iwakoshi, N.N., Lee, A.H., Vallabhajosyula, P., Otipoby, K.L., Rajewsky, K., and Glimcher, L.H. (2003b). Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. Nat. Immunol. *4*, 321–329.

Kawahara, T., Yanagi, H., Yura, T., and Mori, K. (1998). Unconventional splicing of HAC1/ERN4 mRNA required for the unfolded protein response. Sequence-specific and non-sequential cleavage of the splice sites. J. Biol. Chem. *273*, 1802–1807.

Klintworth, G.K., Hawkins, H.K., and Smith, C.F. (1979). Acridine orange particles in cultured fibroblasts. A comparative study of macular corneal dystrophy, systemic mucopolysaccharidoses types I-H and II, and normal controls. Arch. Pathol. Lab. Med. *103*, 297–299.

Kozutsumi, Y., Segal, M., Normington, K., Gething, M.J., and Sambrook, J. (1988). The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. Nature *332*, 462–464.

Lafrenz, D., Koretz, S., Stratte, P.T., Ward, R.B., and Strober, S. (1982). LPS-induced differentiation of a murine B cell leukemia (BCL1): changes in surface and secreted IgM. J. Immunol. *129*, 1329–1335.

Lee, A.H., Iwakoshi, N.N., and Glimcher, L.H. (2003). XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol. Cell. Biol. *23*, 7448–7459.

Lin, Y., Wong, K., and Calame, K. (1997). Repression of c-myc transcription by Blimp-1, an inducer of terminal B cell differentiation. Science *276*, 596–599.

Lin, K.-I., Angelin-Duclos, C., Kuo, T.C., and Calame, K. (2002). Blimp-1-dependent repression of Pax-5 is required for differentiation of B cells to IgM secreting plasma cells. Mol. Cell Biol. *22*, 4771–4780.

Lin, K.I., Tunyaplin, C., and Calame, K. (2003). Transcriptional regulatory cascades controlling plasma cell differentiation. Immunol. Rev. *194*, 19–28.

Ma, Y., and Hendershot, L.M. (2003). The stressful road to antibody secretion. Nat. Immunol. *4*, 310–311.

Meunier, L., Usherwood, Y.K., Chung, K.T., and Hendershot, L.M. (2002). A subset of chaperones and folding enzymes form multiprotein complexes in endoplasmic reticulum to bind nascent proteins. Mol. Biol. Cell *13*, 4456–4469.

Mori, K. (2000). Tripartite management of unfolded proteins in the endoplasmic reticulum. Cell *101*, 451–454.

Mori, K., Ogawa, N., Kawahara, T., Yanagi, H., and Yura, T. (1998). Palindrome with spacer of one nucleotide is characteristic of the cis-acting unfolded protein response element in Saccharomyces cerevisiae. J. Biol. Chem. *273*, 9912–9920.

Mori, K., Ogawa, N., Kawahara, T., Yanagi, H., and Yura, T. (2000). mRNA splicing-mediated C-terminal replacement of transcription factor Hac1p is required for efficient activation of the unfolded protein response. Proc. Natl. Acad. Sci. USA *97*, 4660–4665.

Okada, T., Yoshida, H., Akazawa, R., Negishi, M., and Mori, K. (2002). Distinct roles of activating transcription factor 6 (ATF6) and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) in transcription during the mammalian unfolded protein response. Biochem. J. *366*, 585–594.

Patil, C., and Walter, P. (2001). Intracellular signaling from the endoplasmic reticulum to the nucleus: the unfolded protein response in yeast and mammals. Curr. Opin. Cell Biol. *13*, 349–355.

Piskurich, J.F., Lin, K.I., Lin, Y., Wang, Y., Ting, J.P., and Calame, K. (2000). BLIMP-I mediates extinction of major histocompatibility class II transactivator expression in plasma cells. Nat. Immunol. *1*, 526–532.

Poot, M., and Pierce, R.H. (1999). Detection of changes in mitochondrial function during apoptosis by simultaneous staining with multiple fluorescent dyes and correlated multiparameter flow cytometry. Cytometry *35*, 311–317.

Reimold, A.M., Ponath, P.D., Li, Y.S., Hardy, R.R., David, C.S., Strominger, J.L., and Glimcher, L.H. (1996). Transcription factor B cell lineage-specific activator protein regulates the gene for human X-box binding protein 1. J. Exp. Med. *183*, 393–401.

Reimold, A.M., Etkin, A., Clauss, I., Perkins, A., Friend, D.S., Zhang, J., Horton, H.F., Scott, A., Orkin, S.H., Byrne, M.C., et al. (2000). An essential role in liver development for transcription factor XBP-1. Genes Dev. *14*, 152–157.

Reimold, A.M., Iwakoshi, N.N., Manis, J., Vallabhajosyula, P., Szomolanyi-Tsuda, E., Gravallese, E.M., Friend, D., Grusby, M.J., Alt, F., and Glimcher, L.H. (2001). Plasma cell differentiation requires the transcription factor XBP-1. Nature *412*, 300–307.

Reljic, R., Wagner, S.D., Peakman, L.J., and Fearon, D.T. (2000). Suppression of signal transducer and activator of transcription 3-dependent B lymphocyte terminal differentiation by BCL-6. J. Exp. Med. *192*, 1841–1848.

Rinkenberger, J.L., Wallin, J.J., Johnson, K.W., and Koshland, M.E. (1996). An interleukin-2 signal relieves BSAP (Pax5)-mediated repression of the immunoglobulin J chain gene. Immunity *5*, 377–386.

Rogers, J., Early, P., Carter, C., Calame, K., Bond, M., Hood, L., and Wall, R. (1980). Two mRNAs with different 3′ ends encode membrane-bound and secreted forms of immunoglobulin mu chain. Cell *20*, 303–312.

Schliephake, D.E., and Schimpl, A. (1996). Blimp-1 overcomes the

block in IgM secretion in lipopolysaccharide/anti-mu F(ab′) 2-co-stimulated B lymphocytes. Eur. J. Immunol. *26*, 268–271.

Schubert, U., Anton, L.C., Gibbs, J., Norbury, C.C., Yewdell, J.W., and Bennink, J.R. (2000). Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. Nature *404*, 770–774.

Shaffer, A.L., Peng, A., and Schlissel, M.S. (1997). In vivo occupancy of the kappa light chain enhancers in primary pro- and pre-B cells: a model for kappa locus activation. Immunity *6*, 131–143.

Shaffer, A.L., Yu, X., He, Y., Boldrick, J., Chan, E., and Staudt, L. (2000). BCL-6 represses genes that function in lymphocyte differentiation, inflammation and cell cycle control. Immunity *13*, 199–212.

Shaffer, A.L., Lin, K.-I., Kuo, T.C., Yu, X., Hurt, E.M., Rosenwald, A., Giltnane, J.M., Yang, L., Zhao, H., Calame, K., et al. (2002). Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. Immunity *17*, 51–62.

Shapiro-Shelef, M., Lin, K.I., McHeyzer-Williams, L.J., Liao, J., McHeyzer-Williams, M.G., and Calame, K. (2003). Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells. Immunity *19*, 607–620.

Shen, X., Ellis, R.E., Lee, K., Liu, C.Y., Yang, K., Solomon, A., Yoshida, H., Morimoto, R., Kurnit, D.M., Mori, K., et al. (2001). Complementary signaling pathways regulate the unfolded protein response and are required for *C. elegans* development. Cell *107*, 893–903.

Shilatifard, A., Duan, D.R., Haque, D., Florence, C., Schubach, W.H., Conaway, J.W., and Conaway, R.C. (1997). ELL2, a new member of an ELL family of RNA polymerase II elongation factors. Proc. Natl. Acad. Sci. USA *94*, 3639–3643.

Sidrauski, C., and Walter, P. (1997). The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. Cell *90*, 1031–1039.

Singh, M., and Birshtein, B.K. (1996). Concerted repression of an immunoglobulin heavy-chain enhancer, 3′ alpha E(hs1,2). Proc. Natl. Acad. Sci. USA *93*, 4392–4397.

Smiley, S.T., Reers, M., Mottola-Hartshorn, C., Lin, M., Chen, A., Smith, T.W., Steele, G.D., Jr., and Chen, L.B. (1991). Intracellular heterogeneity in mitochondrial membrane potentials revealed by a J-aggregate-forming lipophilic cation JC-1. Proc. Natl. Acad. Sci. USA *88*, 3671–3675.

Terasaki, M., Song, J., Wong, J.R., Weiss, M.J., and Chen, L.B. (1984). Localization of endoplasmic reticulum in living and glutaraldehyde-fixed cells with fluorescent dyes. Cell *38*, 101–108.

Travers, K.J., Patil, C.K., Wodicka, L., Lockhart, D.J., Weissman, J.S., and Walter, P. (2000). Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell *101*, 249–258.

van Anken, E., Romijn, E.P., Maggioni, C., Mezghrani, A., Sitia, R., Braakman, I., and Heck, A.J. (2003). Sequential waves of functionally related proteins are expressed when B cells prepare for antibody secretion. Immunity *18*, 243–253.

van Huizen, R., Martindale, J.L., Gorospe, M., and Holbrook, N.J. (2003). P58IPK, a novel endoplasmic reticulum stress-inducible protein and potential negative regulator of eIF2alpha signaling. J. Biol. Chem. *278*, 15558–15564.

Wiest, D.L., Burkhardt, J.K., Hester, S., Hortsch, M., Meyer, D.I., and Argon, Y. (1990). Membrane biogenesis during B cell differentiation: most endoplasmic reticulum proteins are expressed coordinately. J. Cell Biol. *110*, 1501–1511.

Wright, G., Tan, B., Rosenwald, A., Hurt, E.H., Wiestner, A., and Staudt, L.M. (2003). A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. Proc. Natl. Acad. Sci. USA *100*, 9991–9996.

Yadava, N., Potluri, P., Smith, E.N., Bisevac, A., and Scheffler, I.E. (2002). Species-specific and mutant MWFE proteins. Their effect on the assembly of a functional mammalian mitochondrial complex I. J. Biol. Chem. *277*, 21221–21230.

Yan, W., Frank, C.L., Korth, M.J., Sopher, B.L., Novoa, I., Ron, D., and Katze, M.G. (2002). Control of PERK eIF2alpha kinase activity by the endoplasmic reticulum stress-induced molecular chaperone P58IPK. Proc. Natl. Acad. Sci. USA *99*, 15920–15925.

Yoshida, H., Okada, T., Haze, K., Yanagi, H., Yura, T., Negishi, M., and Mori, K. (2000). ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. Mol. Cell. Biol. *20*, 6755–6767.

Yoshida, H., Matsui, T., Yamamoto, A., Okada, T., and Mori, K. (2001). XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell *107*, 881–891.

Yoshida, H., Matsui, T., Hosokawa, N., Kaufman, R.J., Nagata, K., and Mori, K. (2003). A time-dependent phase shift in the mammalian unfolded protein response. Dev. Cell *4*, 265–271.