Coordinate Regulation of Phospholipid Biosynthesis and Secretory Pathway Gene Expression in XBP-1(S)-induced Endoplasmic Reticulum Biogenesis*§

Received for publication, October 10, 2006, and in revised form, January 8, 2007 Published, JBC Papers in Press, January 8, 2007, DOI 10.1074/jbc.M609490200

Rungtawan Sriburi†1, Hemamalini Bommasamy†, Gerald L. Buldak§, Gregory R. Robbins§, Matthew Frank§, Suzanne Jackowski§, and Joseph W. Brewer‡1

From the †Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, Maywood, Illinois 60153 and the ‡Department of Infectious Diseases, St. Jude Children’s Research Hospital, Memphis, Tennessee 38105

Development of the expansive endoplasmic reticulum (ER) present in specialized secretory cell types requires X-box-binding protein-1 (Xbp-1). Enforced expression of XBP-1(S), a transcriptional activator generated by unfolded protein response-initiated site-specific cleavage of Xbp-1 mRNA, is sufficient to induce proliferation of rough ER. We previously showed that XBP-1(S)-induced ER biogenesis in fibroblasts correlates with increased production of phosphatidylcholine (PtdCho), the primary phospholipid of the ER membrane, and enhanced activities of the choline cytidylyltransferase (CCT) and cholinephosphotransferase enzymes in the cytidine diphosphocholine (CDP-choline) pathway of PtdCho biosynthesis. Here, we report that the level and synthesis of CCT, the rate-limiting enzyme in the CDP-choline pathway, is elevated in fibroblasts overexpressing XBP-1(S). Furthermore, overexpression experiments demonstrated that raising the activity of CCT, but not cholinephosphotransferase, is sufficient to augment PtdCho biosynthesis in fibroblasts, indicating that XBP-1(S) increases the output of the CDP-choline pathway primarily via its effects on CCT. Finally, fibroblasts overexpressing CCT up-regulated PtdCho synthesis to a level similar to that in XBP-1(S)-transduced cells but exhibited only a small increase in rough ER and no induction of secretory pathway genes. The more robust XBP-1(S)-induced ER expansion was accompanied by induction of a wide array of genes encoding proteins that function either in the ER or at other steps in the secretory pathway. We propose that XBP-1(S) regulates ER abundance by coordinately increasing the supply of membrane phospholipids and ER proteins, the key ingredients for ER biogenesis.

The endoplasmic reticulum (ER) is a multifunctional organelle responsible for the folding and assembly of all proteins targeted to the secretory pathway (1). As such, the ER can adapt to accommodate an increased load of nascent polypeptides. For example, when B-lymphocytes differentiate into antibody-secreting plasma cells, an elaborate network of rough ER develops to facilitate immunoglobulin production (2–4). Likewise, the rough ER is highly developed in other specialized secretory cell types such as pancreatic acinar cells that secrete copious amounts of digestive enzymes (5). In contrast, the ER is sparse in non-secretory cells, such as reticulocytes (6). ER abundance, therefore, is regulated according to the demands on the secretory pathway. However, the mechanisms that regulate ER biogenesis are incompletely defined (7).

A key regulator of ER homeostasis is the unfolded protein response (UPR) pathway, a complex signaling system emanating from the ER membrane (8). When the protein folding capacity of the ER is challenged, the UPR relieves the resulting stress by repressing translation, increasing expression of ER chaperones and folding enzymes, and enhancing ER-associated degradation (8). In addition, recent studies have uncovered a connection between the UPR and ER abundance (9, 10). The UPR-regulated transcription factor X-box-binding protein-1 (XBP-1) is required for proper development and function of plasma cells (11, 12), pancreatic acinar cells, and salivary gland cells (13), all of which normally contain large quantities of rough ER necessary for high level synthesis of their respective secretory cargos. In the absence of Xbp-1 expression, these cell types exhibit poorly developed ER, reduced expression of many ER proteins, and severely compromised secretory activity (11, 13). Xbp-1 mRNA is modified by a novel splicing mechanism initiated by IRE1 (first identified in yeast, inositol requiring mutant), an ER transmembrane kinase/endoribonuclease that serves as a proximal transducer of the UPR (14, 15). IRE1 executes site-specific cleavage of Xhp-1 mRNA and the resulting fragments are ligated to yield a transcript encoding a basic

* This work was supported by National Institutes of Health Grants GM 61970 (to J. W. B.), T32 AI007508 (to H. B. and G. L. B.), and GM 45737 (to S. J.), Cancer Center (CORE) Support Grant CA21765 (to St. Jude Children’s Research Hospital), and by the American Lebanese Syrian Associated Charities. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ The on-line version of this article (available at http://www.jbc.org) contains supplemental Tables S1–S5.

1 Present address: Dept. of Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.
2 To whom correspondence should be addressed: Dept. of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, 2160 South First Ave., Maywood, IL 60153. Tel.: 708-216-5816; Fax: 708-216-9574; E-mail: jbrewer@lumc.edu.

3 The abbreviations used are: ER, endoplasmic reticulum; UPR, unfolded protein response; XBP-1, X-box-binding protein-1; XBP-1(S), spliced form of X-box-binding protein-1; IRE1, inositol requiring 1; PtdCho, phosphatidylcholine; CDP-choline, cytidine diphosphocholine; CCT, choline cytidylyltransferase; ChK, choline kinase; DAG, diacylglycerol; CPT1, cholinephosphotransferase; CEPT1, choline/ethanolaminephosphotransferase; PtdEtn, phosphatidylethanolamine; BIP, immunoglobulin-binding protein; glucose-regulated protein 78; GRP94, glucose-regulated protein 94; TRAPα, translocon-associated protein α; GFP, green fluorescent protein.
leucine zipper protein, termed XBP-1(S), that bears a strong transactivating domain (16–18). Enforced expression of XBP-1(S) is sufficient to drive expansion of the rough ER (10), increase expression of a large number of ER proteins (9, 19), and augment protein biosynthesis (9). Therefore, XBP-1(S) is necessary and sufficient for the biogenesis of functional ER, yet the mechanisms by which this transcription factor mediates these effects have not been fully delineated.

Synthesis of phosphatidylcholine (PtdCho), the most abundant cellular phospholipid and a major component of ER membranes, is up-regulated in fibroblasts overexpressing XBP-1(S) (10). PtdCho is primarily produced by the cytidine diphosphocholine (CDP-choline), also known as the Kennedy pathway (20). In the rate-limiting step of the pathway, choline cytidylyltransferase (CCT) converts phosphocholine to CDP-choline in the presence of CTP (21). The phosphocholine moiety of CDP-choline is then transferred to diacylglycerol (DAG), yielding PtdCho (20). This final step is catalyzed by either cholinephosphotransferase (CPT1) (22) or choline/ethanolaminephosphotransferase (CEPT1), a bifunctional enzyme that can synthesize both PtdCho and phosphatidylethanolamine (PtdEtn) (23). We previously showed that fibroblasts overexpressing XBP-1(S) exhibit enhanced activities of CCT and CPT1/CEPT1 (10), but the relative role of these alterations in XBP-1(S)-induced PtdCho biosynthesis and ER expansion has not been clarified. Neither is it clear whether an increased supply of PtdCho is sufficient for ER biogenesis.

Here, we report that the level and synthesis of CCT is up-regulated in fibroblasts overexpressing XBP-1(S). Furthermore, our data indicate that enhanced CCT activity in the CDP-choline pathway is the primary means by which XBP-1(S) up-regulates PtdCho production. We also demonstrate that increased synthesis of PtdCho alone in CCT-transduced cells is sufficient for only a meager expansion of rough ER. In contrast, XBP-1(S)-transduced fibroblasts exhibit increased PtdCho synthesis, elevated expression of many ER proteins, and robust ER expansion. Thus, we propose that XBP-1(S) orchestrates ER biogenesis by coordinately regulating phospholipid biosynthesis and expression of ER proteins.

**EXPERIMENTAL PROCEDURES**

*Plasmids—pBMN-I-GFP* (Dr. G. Nolan, Stanford University, Palo Alto, CA) encodes a bicistronic mRNA with a GFP cassette 3’ of the internal ribosomal entry site (I). pBMN-hXBP-1(S)-I-GFP encodes full-length human XBP-1(54) generated by UPR-mediated splicing (10), pBMN-mCEPT1-I-GFP encodes full-length mouse CEPT1 (nucleotides 1–2050; GenBank™ accession number BC023783) and was generated by subcloning a NotI-StuI insert from pCMV-Sport6-mCEPT1 (American Type Culture Collection #9271995) into pBMN-I-GFP. pBMN-mCCTα-I-GFP encodes full-length mouse CCTα (nucleotides 47–1150) and was generated by subcloning a XhoI-HindIII insert from pPJ37-CT into pBMN-I-GFP.

*Cell Culture and Retroviral Transduction—NIH-3T3 fibroblasts and Phoenix-Eco cells (Dr. G. Nolan) were maintained in Dulbecco’s modified Eagle’s medium (24). Ecotropic retroviruses, produced using pBMN plasmids and Phoenix-Eco packaging cells (25), were used for retroviral transduction of NIH-3T3 fibroblasts (26) with ≥95% efficiency, as measured by GFP fluorescence using a FACSCalibur flow cytometer (BD Biosciences) (10).*

*Immunoblotting—Cell lysates of pelleted cells were prepared using a 1% Nonidet P-40 lysis buffer (24). Microsomes were prepared from pelleted cells as described (23). Total protein concentrations of clarified Nonidet P-40 lysates and microsomal preparations were determined using the Bio-Rad protein assay. Proteins were resolved by SDS-PAGE and analyzed by chemiluminescent immunoblotting as previously described (24). The rabbit anti-CCTα antibody (27) was raised against full-length recombinant rodent CCTα (28). The rabbit anti-XBP-1 antibody was raised with the assistance of Rockland Immunociences against the N-terminal 81 amino acids of XBP-1 fused in-frame to glutathione S-transferase. Polyclonal rabbit antisera against BiP/GRP78, GRP94, ERdj3, and calnexin were generously provided by Dr. Linda Hendershot (St. Jude Children’s Research Hospital, Memphis, TN). Rabbit anti-TRAPα was a gift from Dr. Chris Nicchitta (Duke University, Durham, NC). The rabbit anti-protein disulfide isomerase polyclonal antibody (Stressgen Bioreagents), mouse anti-β-actin monoclonal antibody, clone AC-15 (Sigma), peroxidase-conjugated donkey anti-mouse IgG, and peroxidase-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories) were purchased.

*Metabolic Labeling and Analysis of CCTα Synthesis—Cells were washed twice with warm phosphate-buffered saline and then cultured in warm media lacking methionine and cysteine (Invitrogen) for 20 min. Cells were then labeled for various intervals with 35S-methionine and [35S]cysteine using 100 μCi/ml of Tran35S-label (MB Biomedicals). Labeled cells were washed twice with cold phosphate-buffered saline and then solubilized in the dish on ice in cold lysing buffer (0.5% Nonidet P-40, 0.5% sodium deoxycholate, 150 mM NaCl, 50 mM Tris-HCL, pH 7.5, 20 μg/ml leupeptin, 40 μg/ml aprotonin, 100 μg/ml phenylmethylsulfonyl fluoride). Post-nuclear supernatants were prepared, protein concentrations of clarified Nonidet P-40 lysates and microsomal preparations were determined using the Bio-Rad protein assay. Pre-cleared with protein A-Sepharose beads (Sigma) pre-coated with normal rabbit sera. Pre-cleared lysates were then incubated with protein A-Sepharose beads pre-coated with rabbit anti-CCTα antibodies. In each case, the beads were then washed 4 times with cold washing buffer (0.5% Nonidet P-40, 0.5% sodium deoxycholate, 400 mM NaCl, 50 mM Tris-HCL, pH 7.5), resuspended in reducing SDS-PAGE sample buffer, and boiled for 5 min. Samples were resolved by SDS-PAGE using 10% polyacrylamide gels. Gels were processed, and signals were visualized and quantified using a Typhoon PhosphorImager and ImageQuant software (Amersham Biosciences) as described (24). To measure the incorporation of radiolabeled methionine and cysteine into proteins, lysates from metabolically labeled cells were normalized to cell number, and equivalent amounts were spotted on Whatman filter paper in tripli-
Phospholipid Biosynthesis in XBP-1(S)-transduced Fibroblasts—We previously demonstrated that enforced expression of the transcription factor XBP-1(S) is sufficient to up-regulate PtdCho biosynthesis in NIH-3T3 fibroblasts (10). Furthermore, analysis of the CDP-choline pathway for PtdCho biosynthesis in XBP-1(S)-transduced cells revealed basal activity for choline kinase (CK), enhanced activity for CCT, and markedly elevated activity for CPT (10). To further investigate the status of the CDP-choline pathway in XBP-1(S)-transduced cells, we metabolically labeled cells with \([^{3}H]choline\) and monitored the output of each step in the pathway. Chromatographic analysis of soluble cellular material allowed for analysis of both phosphocholine and CDP-choline, the products of the CK- and CCT-catalyzed steps, respectively. The level of phosphocholine synthesis was similar in empty vector- and XBP-1(S)-transduced cells (Fig. 1A), in keeping with the similar CK activity present in both populations (10). In contrast, the level of CDP-choline synthesis was increased ~2-fold in XBP-1(S)-transduced cells (Fig. 1B), and as expected, this was accompanied by elevated production of PtdCho (Fig. 1C). These data indicate that the enhanced CCT activity present in XBP-1(S)-transduced cells increases the supply of CDP-choline for CPT-catalyzed production of PtdCho.

Similar to PtdCho, the level of PtdEtn, the second most abundant phospholipid in cellular membranes, elevates in XBP-1(S)-transduced fibroblasts (10). To further explore this observation,
we performed metabolic labeling studies with $[^3H]$ethanolamine and found that the synthesis of PtdEtn was increased 2-fold in XBP-1(S)-transduced cells (Fig. 1D). Therefore, enforced expression of XBP-1(S) augments cellular capacity for de novo synthesis of PtdCho and PtdEtn, two key phospholipid components of the ER membrane.

Effect of XBP-1(S) on the Level and Synthesis of CCT in Fibroblasts—PtdCho is the most abundant phospholipid in cellular membranes; thus, we focused our studies on delineating the connection between XBP-1(S) and the regulation of PtdCho biosynthesis. Given that CCT catalyzes the rate-limiting step in the CDP-choline pathway, we investigated the mechanism by which its activity is augmented in XBP-1(S)-transduced cells. We reasoned that the elevation of CCT activity might involve an increase in the level of the CCT enzyme. In testing this hypothesis, we focused on the steady-state level of CCT, the predominant CCT isoform expressed in NIH-3T3 cells (10). Immunoblot analysis of Nonidet P-40 soluble material revealed a nearly 50% increase in CCT in XBP-1(S)-transduced cells (Fig. 2). Thus, the enhanced CCT activity in XBP-1(S)-transduced fibroblasts correlates with an increased pool of CCT enzyme.

Enforced expression of XBP-1(S) in fibroblasts does not induce increased levels of CCT transcripts (10); therefore, the rise in CCT enzyme in this system is mediated by a post-transcriptional or post-translational mechanism. A previous study showed that enforced expression of XBP-1(S) in Raji cells, a human B cell line, resulted in an overall increase in translation (9). Similarly, we observed a modest, but measurable, increase in total protein synthesis in XBP-1(S)-transduced NIH-3T3 cells (Fig. 3A). This led us to assess the effect of XBP-1(S) on the level of CCT enzyme translation. First, we verified that radiolabeled CCT could be specifically immunoprecipitated from NIH-3T3 cell lysates (Fig. 3B). We then metabolically labeled empty vector- and XBP-1(S)-transduced cells with $[^35S]$methionine and $[^35S]$cysteine for increasing intervals and assessed the incorporation of radiolabel into newly synthesized CCT (Fig. 3C, upper panel) and into total proteins (Fig. 3C, middle panel). These experiments revealed that CCT synthesis increased...
~60% in XBP-1(S)-transduced cells. Importantly, the effect of XBP-1(S) on translation was not specific for CCTα, as revealed by the increase in total protein synthesis (Fig. 3A) and the profile of labeled proteins (Fig. 3C, middle panel) in XBP-1(S)-transduced cells. Although the relative contributions of transcriptional and translational regulation to the overall increase in total protein synthesis cannot be distinguished from this study, the data indicate that CCTα is one of many proteins produced at a higher rate upon enforced expression of XBP-1(S). In addition, pulse-chase studies indicated that CCTα is a long-lived protein in NIH-3T3 cells (half-life ≥12–14 h) and is not further stabilized by enforced expression of XBP-1(S) (data not shown). These data suggest that elevated translation of CCTα is the primary mechanism by which the pool of CCTα enzyme increases in XBP-1(S)-transduced fibroblasts.

Effect of High Level CCT and CPT Activity on PtdCho Biosynthesis in Fibroblasts—We next determined whether an increase in CCT activity is sufficient to up-regulate PtdCho synthesis in NIH-3T3 fibroblasts. As a comparison, we assessed the effect of elevated CPT activity on PtdCho production. To raise the level of these enzymatic activities, we used retroviral transduction to overexpress CCTα, CPT1, and CEPT1 in NIH-3T3 cells. CCTα-transduced cells exhibited a large increase (~10-fold) in CCT activity, much higher than that observed upon enforced expression of XBP-1(S), whereas neither CPT1 nor CEPT1 overexpression altered CCT activity (Fig. 4A). Total CPT activity was enhanced in both CPT1- and CEPT1-transduced cells to levels comparable with or higher than that present in cells overexpressing XBP-1(S) (Fig. 4B). CCTα overexpression did not modulate the CPT activity (Fig. 4B). These data confirmed that overexpressing CCTα, CPT1, and CEPT1 effectively increased the respective enzymatic activities in NIH-3T3 cells. We then performed metabolic labeling experiments with [3H]choline and found that PtdCho synthesis was induced in cells transduced with CCTα (Fig. 4C). However, neither CPT1 nor CEPT1 overexpression increased PtdCho synthesis in NIH-3T3 cells (Fig. 4C). Therefore, elevated CCT activity is sufficient to increase PtdCho synthesis in fibroblasts, in keeping with its role as the rate-limiting enzyme in the CDP-choline pathway (21). Interestingly, although overexpression of CCTα generated a much larger amount of CCT activity than did enforced expression of XBP-1(S) (Fig. 4A), PtdCho synthesis was up-regulated to a similar level under both conditions (Fig. 4C). These data sug-

---

**FIGURE 3. Synthesis of the CCTα enzyme in XBP-1(S)-transduced NIH-3T3 fibroblasts.** A. NIH-3T3 cells were transduced with either empty vector or XBP-1(S) retroviral vectors. At 48 h post-transduction, cells were metabolically labeled with [35S]methionine and [35S]cysteine for 1 h. Incorporation of radiolabeled amino acids into proteins was assessed by determining the trichloroacetic acid-precipitable activity in labeled cell lysates. Data were normalized to 10^6 cells and plotted as the mean ± S.D. (n = 3; ** denotes p < 0.02 for comparison of XBP-1(S) to empty vector). B, mock and pPJ37-CT-transfected NIH-3T3 cells were metabolically labeled with [35S]methionine and [35S]cysteine for 2 h. Cell lysates were first pre-cleared with protein A-Sepharose beads pre-coated with normal rabbit sera (NRS). CCTα was then immunoprecipitated with rabbit anti-CCTα antibodies and protein A-Sepharose. Immunoprecipitates were resolved by SDS-PAGE under reducing conditions and assessed by phosphorimaging. C, upper panel, NIH-3T3 cells were transduced with either empty vector or XBP-1(S) retroviral vectors. At 48 h post-transduction, cells were metabolically labeled with [35S]methionine and [35S]cysteine for the indicated intervals. CCTα was immunoprecipitated from lysates of equivalent numbers of cells and, as controls, from lysates of metabolically labeled mock and pPJ37-CT transfected NIH-3T3 cells and assessed as in B. Phosphorimaging analysis revealed that the amount of radiolabel incorporated into CCTα in a 2-h interval was 1.64 ± 0.40-fold greater in XBP-1(S)-transduced cells as compared with empty vector controls (n = 3). Middle panel, lysates of equivalent numbers of metabolically labeled cells were resolved by SDS-PAGE under reducing conditions and assessed by phosphorimaging or by (lower panel) immunoblotting for β-actin as a loading control.
suggest that the XBP-1(S)-mediated increase in CCT activity is sufficient for maximal output by the CDP-choline pathway.

Abundance of ER in CCTα- Versus XBP-1(S)-transduced Fibroblasts—PtdCho is the most abundant phospholipid in cellular membranes, including ER membranes. Thus, the comparable increase in PtdCho synthesis in CCTα- and XBP-1(S)-transduced fibroblasts prompted us to ask whether an increase in the supply of PtdCho is sufficient for ER expansion. Visual inspection of electron micrographs revealed that overexpression of CCTα, like that of XBP-1(S), resulted in an apparent increase in cell size and in the abundance of intracellular membrane-bound organelles, many of which appeared to be ribosome-studded rough ER (Fig. 5A). Quantitative analysis of cell size confirmed that CCTα-transduced cells were ~20% larger than control cells (empty vector cells, 145.6 ± 8.9 μm²; CCTα cells, 174.2 ± 11.4 μm²), whereas XBP-1(S) induced an ~50% increase in cell size (XBP-1(S) cells, 218.7 ± 13.9 μm²) as previously reported (10). Stereological analysis of high power electron micrographs confirmed that both the surface area and volume of rough ER were enhanced in cells overexpressing CCTα, 1.8- and 1.5-fold, respectively (Fig. 5B). Immunoblot analysis indicated that the modest increase in rough ER abundance in CCTα-transduced cells could not be attributed to induction of XBP-1(S) synthesis (see Fig. 7). In contrast, as expected from our previous studies (10), XBP-1(S)-transduced cells exhibited a nearly 3-fold increase in both the surface area and volume of rough ER (Fig. 5B). These findings demonstrate that the magnitude of XBP-1(S)-mediated ER biogenesis was greater than that attained in cells overexpressing CCTα despite the equivalent level of PtdCho synthesis in both conditions.

Phospholipids and ER Proteins in CCTα- Versus XBP-1(S)-transduced Fibroblasts—The varying degrees of ER expansion in CCTα- and XBP-1(S)-transduced fibroblasts provided an opportunity to assess how cellular lipid content and ER protein levels correspond to overall ER abundance. First, we compared the lipid content of CCTα- and XBP-1(S)-transduced cells and found that the total amount of PtdCho was increased similarly in both cell populations (Fig. 6). However, although the amount of PtdEtn was elevated in the XBP-1(S)-transduced cells, it was diminished in cells overexpressing CCTα (Fig. 6). There was a small increase in the level of cholesterol in CCTα-transduced cells and little or no change in sphingolipid, cholesterol ester, and triglyceride levels upon enforced expression of either XBP-1(S) or CCTα (Fig. 6). Thus, CCTα- and XBP-1(S)-transduced fibroblasts exhibited differences in lipid composition and content, including the ratio of PtdCho to PtdEtn, the two most abundant ER membrane phospholipids. Next, immunoblotting revealed that many ER resident proteins, including soluble components of the ER protein folding machinery (BiP/GRP78, GRP94, Erdj3, and protein disulfide isomerase) and a transmembrane subunit of ER translocons (TRAPα), were up-regulated in XBP-1(S)- but not CCTα-transduced cells (Fig. 7). These data demonstrate that elevated levels of PtdCho, PtdEtn, and resident ER proteins correlated with the greatest increase in rough ER.

Gene Expression Associated with ER Development—Our comparison of CCTα- and XBP-1(S)-transduced cells suggested that an increased supply of PtdCho was not sufficient for maximal ER expansion (Figs. 4–6). Building upon these observations, we used microarray analysis to compare the profile of gene expression in fibroblasts 48 h after XBP-1(S) and CCTα transduction. Gene ontology analysis using the NetAffx Analysis Center (32) correlated the regulated probe sets with the most significantly perturbed cellular components in cells transduced by XBP-1(S). Similar to the findings of previous studies (9, 19), our analysis indicated that XBP-1(S) exerts its greatest effects on genes involved with the ER network and ER to Golgi vesicle-mediated transport (supplemental Tables S1 and S2). Specifically, we found that enforced expression of
XBP-1(S) in NIH-3T3 cells up-regulated transcripts for 122 identified genes (≥2-fold, \( p \leq 0.05 \)) that function either in the ER or at other steps in the secretory pathway (Table 1). The cohort of ER genes up-regulated in the XBP-1(S)-transduced cells included factors involved in targeting and translocation of nascent polypeptides into the ER (such as \( \text{Srp} \) genes for components of the signal recognition particle and \( \text{Sec61} \) genes for translocon subunits), folding and assembly of nascent polypeptides in the ER lumen (such as \( \text{Hspa5} \), which encodes BiP/GRP78, \( \text{Dnaj} \) genes for ER resident DnaJ proteins, and protein disulfide isomerase genes for protein disulfide isomerases), \( N \)-linked glycosylation (such as the \( \text{Ddost} \) and \( \text{Dad1} \) genes for components of the oligosaccharyltransferase), and ER-associated degradation (such as \( \text{Derl1} \) and \( \text{Edem1} \), which encode Derlin1 and Edem). In addition, the XBP-1(S)-transduced cells exhibited increased expression of a large number of genes involved in vesicular trafficking and transport. These included genes implicated in anterograde transport (such as \( \text{Sec} \) genes for components of COPII vesicles), retrograde transport (such as \( \text{Cop} \) genes for components of COPI vesicles), and distal transport through the Golgi and beyond (such as \( \text{Vamp} \) genes for \( \alpha \)-SNARE proteins).

In sharp contrast to the gene expression profile in XBP-1(S)-transduced fibroblasts, microarray analysis revealed that no secretory pathway genes were up-regulated (≥2-fold, \( p \leq 0.05 \)) in cells overexpressing \( \text{CCT} \). This striking difference was consistent with the immunoblot analysis of ER proteins (Fig. 7). In fact, enforced expression of \( \text{CCT} \) induced expression of only 13 genes and reduced expression of only 4 genes (supplemental Table S5).

XBP-1(S) also altered the expression of a small number of cytoskeletal and lipid metabolic genes in the transduced fibroblasts (supplemental Tables S3 and S4), and these were not up-regulated in the \( \text{CCT} \)-transduced cells. Cytoskeletal genes that were up-regulated in response to XBP-1(S) expression included those encoding myosin VIIa and ankyrin 3. On the other hand, the expression of ankyrin 2, plakophilin 4, paralemmin, and protein band 4.1 was reduced at least 2-fold. Lipid metabolic genes whose expression increased included those involved in fatty acid modification (\( \text{Pecr} \) and \( \text{Cyb5} \)) and \( \text{de novo} \) phosphatidic acid formation (\( \text{Agpat6} \)), whereas the \( \text{Elovl4} \) gene that mediates fatty acid elongation was down-regulated. The largest change in lipid metabolic gene expression involved the Lipin genes \( \text{Lpin3} \) and \( \text{Lpin1} \), which increased more than 8- and 3.4-fold respectively. Lipin, primarily studied in hepatocytes and adipocytes, has been implicated in regulating expression of lipid metabolic genes (34–38). In addition, the yeast homolog of Lipin was recently shown to function as a phosphatidic acid phosphatase that catalyzes for-

**FIGURE 5.** Electron microscopy analysis of the ER in XBP-1(S)- and \( \text{CCT}\alpha \)-transduced NIH-3T3 fibroblasts. A, at 48 h post-transduction with the indicated retroviral vectors, thin sections were prepared from NIH-3T3 cells and examined by transmission EM. Representative micrographs of increasing magnification are shown with scale bar = 1 \( \mu \)m (lower right corner). Top panels, \( \times 3500 \); bottom panels, \( \times 8000 \); the \( \rightarrow \) indicates representative ER; \( \text{N} \), nucleus. B, stereological analysis of rough ER (RER) volume (left panel) and rough ER surface area (right panel) were performed on electron micrographs, and the means ± S.D. are plotted (empty vector, \( n = 22 \); XBP-1(S), \( n = 23 \); \( \text{CCT}\alpha \), \( n = 28 \)). The asterisk denotes \( p < 0.02 \) when compared with empty vector cells; the double asterisk denotes \( p < 0.0001 \) when compared with either empty vector- or \( \text{CCT}\alpha \)-transduced cells.
mation of DAG from phosphatidic acid (39). In contrast to the Lipin genes, expression of Ppap2b (40, 41), which encodes a distinct phosphatidic acid phosphatase, was reduced after XBP-1(S) transduction.

In summary, the microarray analysis revealed that the marked expansion of the rough ER induced by XBP-1(S) correlated with enhanced expression of a wide array of resident ER proteins, a broad group of factors that function at other steps in the secretory pathway, a small group of lipid metabolic genes, and a small set of cytoskeletal genes. This profile of gene expression did not accompany the more modest increase in rough ER that occurred in the CCTα-transduced cells.

DISCUSSION

Our data provide the first mechanistic insight into how the UPR, via XBP-1(S), can modulate the activity of an enzyme that plays a pivotal role in phospholipid production. The level and synthesis of CCTα protein, the rate-limiting enzyme in the CDP-choline pathway of PtdCho biosynthesis, is up-regulated in XBP-1(S)-transduced fibroblasts (Figs. 2 and 3), providing an explanation for the increased CCT activity (10) and elevated production of CDP-choline (Fig. 1B). Given that the CCTα gene is not a target of XBP-1(S) (10), we speculate that XBP-1(S) regulates expression of another gene(s) that influences the translation of CCTα and many other proteins in this experimental system. In the Raji human B cell line, enforced expression of XBP-1(S) led to an increase in assembled (80 S) ribosomes (9). It follows that such a mechanism could enhance total protein synthesis as was observed both in Raji cells (9) and NIH-3T3 fibroblasts overexpressing XBP-1(S) (Fig. 3A). However, there is evidence that XBP-1(S) might mediate events that target specific mRNAs for translation. Specifically, XBP-1(S) is required for optimal synthesis of immunoglobulin heavy chains but not of light chains in activated B cells (42). Delineation of the mechanisms by which XBP-1(S) regulates translation will require further study, and a reasonable next step would be to examine the status of ribosome assembly and the association of CCTα transcripts with polysomes in our experimental system.

Although translational regulation appears to be the primary means by which the level of CCTα increases in XBP-1(S)-transduced fibroblasts, we emphasize that CCTα abundance in different cell types and/or during various developmental processes might be controlled by other mechanisms (43). In this regard, we have found evidence for stabilization of CCTα protein turnover in lipopolysaccharide-stimulated CH12 B cells, a murine B cell lymphoma.4 LPS triggers murine B cells to proliferate and to differentiate into antibody-secreting cells, a process that includes induction of PtdCho biosynthesis and expansion of rough ER (2–4). Thus, multiple mechanisms can influence the level of CCTα, and its activity can also be regulated post-translationally by phosphorylation/dephosphorylation (43) and subcellular localization (44). Whether XBP-1(S)
mediates any post-translational control of CCTα activity and the relative roles of the various CCT regulatory mechanisms in modulating PtdCho biosynthesis under different conditions await further investigation.

Our studies reveal that although XBP-1(S)-transduced cells exhibit enhanced activities for both CCT and CPT (10), it is the increase in CCT activity that augments production of PtdCho in this system. Elevation of CCT activity by overexpression of CCTα was sufficient to up-regulate PtdCho biosynthesis to a level equivalent to that observed in XBP-1(S)-transduced cells, whereas elevation of CPT activity by overexpression of either CPT1 or CEPT1 had no effect on PtdCho biosynthesis (Fig. 4). These data are in agreement with previous studies of PtdCho biosynthesis has been linked to UPR activation (51), the increased supply of PtdCho in CCTα-transduced NIH-3T3 cells did not trigger the UPR or induction of ER protein expression. These data underscore that PtdCho, the most abundant major lipids, rises along with PtdCho in XBP-1(S)-transduced cells overexpressing CCTα, as compared to empty vector controls, p < 0.05).

Finally, our comparative analyses of XBP-1(S)- and CCTα-transduced fibroblasts provide insight into the basic "ingredients" required to build and equip a larger ER. It was fortuitous that the level of PtdCho increased in the CCTα-transduced NIH-3T3 fibroblasts as cells typically balance enforced induction of PtdCho biosynthesis with PtdCho degradation (27, 45, 47). At least some of the accumulated PtdCho in CCTα-transduced cells was utilized for ER membrane assembly as these cells exhibited a measurable increase in rough ER (Fig. 5B). The increase in rough ER in cells overexpressing CCTα was not accompanied by induction of XBP-1(S) (Fig. 7) or genes encoding ER proteins. Thus, although inhibition of PtdCho biosynthesis has been linked to UPR activation (51), the increased supply of PtdCho in CCTα-transduced NIH-3T3 cells did not trigger the UPR or induction of ER protein expression. These data underscore that PtdCho, the most abundant phospholipid in ER membranes, can be a major determinant of ER abundance and provide further evidence that CCTα plays a key regulatory role in ER biogenesis. On the other hand, it is striking that the level of PtdEtn, but not other major lipids, rises along with PtdCho in XBP-1(S)-transduced fibroblasts (Fig. 6). It would be interesting to investigate the role of PtdEtn in ER biogenesis and the possibility that the PtdEtn supply might influence the UPR and expression of secretory pathway genes.

Only a small number of genes implicated in lipid metabolism were up-regulated in the XBP-1(S)-transduced fibroblasts. The potential roles of these genes in regulating lipid biosynthesis and ER membrane biogenesis, particularly the Lipin genes that have recently been implicated in DAG synthesis (39), certainly warrant further study. However, we note that elevated expres-
XBP-1 and ER Biogenesis

We propose that XBP-1(S) coordinates increased production of the pro-

tion of this set of lipid metabolic genes is apparently not essen-
tial for induction of PtdCho biosynthesis in NIH-3T3 fibro-

sion of the lipid metabolism, and possibly for the increased pro-
The potential role of XBP-1(S)-regulated lipid metabolic genes, such as the Lipin genes, in augmenting lipid biosynthesis remains to be investigated. Although the mechanism remains to be determined, XBP-1(S) has the capacity to enhance translation of CCTα, thereby increasing the supply of the rate-limiting enzyme in the CDP-choline pathway and increasing PtdCho biosynthesis. Thus, we propose that XBP-

FIGURE 8. Model of XBP-1(S)-induced ER biogenesis. We propose that XBP-

REFERENCES
1. Anken, E., Braakman, I., and Craig, E. (2005) Crit. Rev. Biochem. Mol. Biol. 40, 191–228
2. Shohat, M., Janossy, G., and Dourmashkin, R. R. (1973) Eur. J. Immunol. 3, 680–687
3. Wiest, D. L., Burkhardt, J. K., Hester, S., Hortsch, M., Meyer, D. L., and Argon, Y. (1990) J. Cell Biol. 110, 1501–1511
4. Rush, J. S., Sweitzer, T., Kent, C., Decker, G. L., and Waechter, C. J. (1991) Arch. Biochem. Biophys. 284, 63–70
5. Bolender, R. P. (1974) J. Cell Biol. 61, 269–287
6. Grasso, J. A., Sullivan, A. L., and Chan, S. C. (1978) J. Cell Sci. 31, 165–178
7. Federovitch, C. M., Ron, D., and Hampton, R. Y. (2005) Curr. Opin. Cell Biol. 17, 409–414
8. Schroder, M., and Kaufman, R. J. (2005) Annu. Rev. Biochem. 74, 739–789
9. Shaffer, A. L., Shapiro-Shleef, M., Ikawa, N. S., Lee, A. H., Quian, S. B., Zhao, H., Yu, X., Yang, L., Tan, B. K., Rosenwald, A., Hurt, E. M., Petroulakis, E., Sonenberg, N., Yewdell, J. W., Calame, K., Glimcher, L. H., and Staudt, L. M. (2004) Immunity 21, 81–93
10. Sriburi, R., Jackowski, S., Mori, K., and Brewer, J. W. (2004) J. Cell Biol. 167, 35–41
11. Reimold, A. M., Ikawa, N. S., Manis, J., Vallabhajosyula, P., Szomolanyi-Tsuda, E., Gravalisse, E. M., Friend, D., Grusby, M. J., Alt, F., and Glimcher, L. H. (2001) Nature 412, 300–307
12. Ikawa, N. S., Lee, A. H., Vallabhajosyula, P., Otipoby, K. L., Rajewsky, K., and Glimcher, L. H. (2003) Nat. Immunol. 4, 321–329
13. Lee, A. H., Chu, G. C., Ikawa, N. S., and Glimcher, L. H. (2005) EMBO J. 24, 4368–4380
14. Tiraphon, W., Welihinda, A. A., and Kaufman, R. J. (1998) Genes Dev. 12, 1812–1824
15. Wang, X. Z., Harding, H. P., Zhang, Y., Joliceur, E. M., Kuroda, M., and Ron, D. (1998) EMBO J. 17, 5708–5717
16. Calfon, M., Zeng, H., Urano, F., Till, J. H., Hubbard, S. R., Harding, H. P., Clark, S. G., and Ron, D. (2002) Nature 415, 92–96
17. Shen, X., Ellis, R., Lee, K., Liu, C.-Y., Yang, K., Solomon, A., Yoshida, H., Morimoto, R., Kurin, D. M., Mori, K., and Kaufman, R. J. (2001) Cell 107, 893–903
18. Yoshida, H., Matsui, T., Yamamoto, A., Okada, T., and Mori, K. (2001) Cell
XBP-1 and ER Biogenesis

107, 881–891
19. Lee, A. H., Iwakoshi, N. N., and Glimcher, L. H. (2003) Mol. Cell. Biol. 23, 7448–7459
20. Lykidis, A., and Jackowski, S. (2001) Prog. Nucleic Acid Res. Mol. Biol. 65, 361–393
21. Kent, C. (1997) Biochim. Biophys. Acta 1348, 79–90
22. Henneberry, A. L., Wistow, G., and McMaster, C. R. (2000) J. Biol. Chem. 275, 29808–29815
23. Henneberry, A. L., and McMaster, C. R. (1999) Biochem. J. 339, 291–298
24. Gass, J. N., Gifford, N. M., and Brewer, J. W. (2002) J. Biol. Chem. 277, 49047–49054
25. Gunn, K. E., Gifford, N. M., Mori, K., and Brewer, J. W. (2004) Mol. Immunol. 41, 919–927
26. Brewer, J. W., and Diehl, J. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 12625–12630
27. Jackowski, S. (1994) J. Biol. Chem. 269, 3858–3867
28. Luche, M. M., Rock, C. O., and Jackowski, S. (1993) Arch. Biochem. Biophys. 301, 114–118
29. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917
30. Lykidis, A., Baburina, I., and Jackowski, S. (1999) J. Biol. Chem. 274, 26992–27001
31. Lykidis, A., Wang, J., Karim, M. A., and Jackowski, S. (2001) J. Biol. Chem. 276, 2174–2179
32. Liu, G., Loraine, A. E., Shigeta, R., Cline, M., Cheng, J., Valmeekam, V., Sun, S., Kulp, D., and Siani-Rose, M. A. (2003) Nucleic Acids Res. 31, 82–86
33. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
34. Peterfy, M., Phan, J., Xu, P., and Reue, K. (2001) Nat. Genet. 27, 121–124
35. Phan, J., Peterfy, M., and Reue, K. (2004) J. Biol. Chem. 279, 29558–29564
36. Phan, J., Peterfy, M., and Reue, K. (2005) Drug News Perspect. 18, 5–11
37. Phan, J., and Reue, K. (2005) Cell Metab. 1, 73–83
38. Peterfy, M., Phan, J., and Reue, K. (2005) J. Biol. Chem. 280, 32883–32889
39. Han, G. S., Wu, W. L., and Carman, G. M. (2006) J. Biol. Chem. 281, 9210–9215
40. Kai, M., Wada, I., Imai, S., Sakane, F., and Kanoh, H. (1997) J. Biol. Chem. 272, 24572–24578
41. Roberts, R., Sciorto, V. A., and Morris, A. J. (1998) J. Biol. Chem. 273, 22059–22067
42. Tirosh, B., Iwakoshi, N. N., Glimcher, L. H., and Ploegh, H. L. (2005) J. Exp. Med. 202, 505–516
43. Jackowski, S., and Fagone, P. (2005) J. Biol. Chem. 280, 32883–32889
44. Cornell, R. B., and Northwood, I. C. (2000) Trends Biochem. Sci. 25, 441–447
45. Walker, C. J., Kalmar, G. B., and Cornell, R. B. (1994) J. Biol. Chem. 269, 5742–5749
46. Lykidis, A., Murti, K. G., and Jackowski, S. (1998) J. Biol. Chem. 273, 14022–14029
47. Baburina, I., and Jackowski, S. (1999) J. Biol. Chem. 274, 9400–9408
48. Ridsdale, R., Tseu, I., Roth-Kleiner, M., Wang, J., and Post, M. (2004) J. Biol. Chem. 279, 55946–55957
49. Wright, M. M., Henneberry, A. L., Lagace, T. A., Ridgway, N. D., and McMaster, C. R. (2001) J. Biol. Chem. 276, 25254–25261
50. Araki, W., and Wurtman, R. J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11946–11950
51. van der Sanden, M. H., Houweling, M., van Golde, L. M., and Vaandrager, A. B. (2003) Biochem. J. 369, 643–650
52. Becker, F., Block-Alper, L., Nakamura, G., Harada, J., Wittrup, K. D., and Meyer, D. I. (1999) J. Cell Biol. 146, 273–284
53. Wanker, E. E., Sun, Y., Savitz, A. J., and Meyer, D. I. (1995) J. Cell Biol. 130, 29–39
54. Langley, R., Leung, E., Morris, C., Berg, R., McDonald, M., Weaver, A., Parry, D. A., Ni, J., Su, J., Gentz, R., Spurr, N., and Krissansen, G. W. (1998) DNA Cell Biol. 17, 449–460