BiP Binds Type I Procollagen Proα Chains with Mutations in the Carboxyterminal Propeptide Synthesized by Cells from Patients with Osteogenesis Imperfecta*

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Of 20 fibroblast cell strains from patients with osteogenesis imperfecta (OI), a disease caused by mutations in the genes encoding type I procollagen, three had increased synthesis of BiP (GRP78), an hsp70-related, endoplasmic reticulum-resident protein. All three strains carry unique mutations in proc1(I) chains that impair type I procollagen chain association. Immunoprecipitation and pulse-chase experiments show that BiP (immunoglobulin heavy chain-binding protein) stably binds procα1(I) chains in these three cell strains after a brief lag. Ascorbate, which increases procollagen synthesis, increases BiP synthesis and content in these three strains and not in the others. In one of these three strains, BiP content is constitutively elevated prior to ascorbate treatment, and BiP is less inducible. This strain also has relatively high levels of synthesis and content of GRP94, another endoplasmic reticulum-resident stress protein. Pretreating each of the three cell strains to increase their BiP content reduces subsequent ascorbate-mediated BiP induction. BiP synthesis in the 17 other OI strains examined, which had a variety of type I procollagen mutations, was normal. These results suggest that BiP is induced by and binds procollagen with specific types of mutations: ones in the carboxy-terminal propeptide that interfere with chain association. The recognition by BiP of such procollagen in OI cell strains shows that BiP plays a role in the physiological response to the production of some disease-producing abnormal proteins.

Mutations in the genes (COL1A1 and COL2A1) that encode the chains of type I procollagen result in the production of abnormal molecules that fold improperly and cause osteogenesis imperfecta (OI, brittle bone disease)1 (1, 2). Type I procollagen is a trimeric, secretory protein that is made in the endoplasmic reticulum; PAGE, polyacrylamide gel electrophoresis; BiP, immunoglobulin heavy chain-binding protein.

Our results suggest that BiP is only induced by and binds procollagen molecules with mutations that interfere with, but do not prevent, proα chain association. Cells in which BiP has been induced by abnormal type I procollagen vary their BiP content according to the level of synthesis of that protein and do not necessarily have constitutively increased levels of BiP synthesis.

EXPERIMENTAL PROCEDURES

Cell Culture and Strains—Human dermal fibroblast cultures were established and maintained as described previously (13). When used, ascorbate, 50 μg/ml (final concentration), was added to the culture medium from a fresh stock solution made up in serum-free medium or water and was replenished every 24 h during extended treatments.

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1 The abbreviations used are: OI, osteogenesis imperfecta; ER, endoplasmic reticulum; PAGE, polyacrylamide gel electrophoresis; BiP, immunoglobulin heavy chain-binding protein.
Tunicamycin (Sigma) and \(\alpha,\alpha'\)-dipyridyl (Sigma) were used at final concentrations of 5 \(\mu\)g/ml and 300 \(\mu\)M, respectively. In pretreatment experiments, tunicamycin (1 \(\mu\)g/ml) or A23187 (7 \(\mu\)M; Sigma) were added to the media and removed 7–8 h later by rinsing repeatedly with medium containing 10% fetal calf serum (untreated controls were also rinsed).

The OI fibroblast strains used and their causative mutations are listed in Table I. References describing the characterization of most of the mutations can be found in two reviews (1, 2).

**Metabolic Labeling and Pulse-Chase Experiments**—For labeling, 250,000 fibroblasts that had undergone no more than 15 passages during tissue culture were plated into 35-mm dishes and allowed to attach and spread overnight. Cells were preincubated for up to 2 h in methionine-free medium and then labeled for 2–4 h in methionine-free medium supplemented with 100 \(\mu\)Ci/ml \[^{35}S\]methionine (>1000 Ci/mmol; Amersham Corp.). For pulse-chase experiments, the labeling medium was aspirated after 30 min and cells were rinsed with medium supplemented with 10% fetal calf serum and 10 \(\mu\)M methionine and then chased in the same medium. For experiments calling for continuous treatment with ascorbate, tunicamycin, or \(\alpha,\alpha'\)-dipyridyl, these were added to the preincubation, pulse, and rinse/chase media at the same concentrations as used in the culture medium.

**Harass Conditions and Immunoprecipitations**—Cells were harvested on ice and all solutions were kept cold. When no ATP depletion system was employed, cells were rinsed twice with harvest phosphate-buffered saline (phosphate-buffered saline with 25 mM EDTA) and lysed in 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, single detergent lysis buffer as described (14). Iodoacetamide (10 mM) and \(N\)-ethylmaleimide (10 mM) were, when needed, added to the lysis buffer to prevent new disulfide bond formation. To deplete ATP in some experiments, the cells were rinsed with phosphate-buffered saline and harvested in single detergent lysis buffer that contained hexokinase (8 units/ml; Sigma), dextrose (10 mM), apyrase (25 units/ml; Sigma), and iodoacetamide (20 mM). Lysates were spun in a microcentrifuge to remove nuclei and insoluble material.

Immunoprecipitations were carried out at 4 °C using protein G-Sepharose (Pharmacia LKB Biotechnology Inc.) to bind and precipitate antibody-target protein complexes as described (14) in experiments where ATP was not depleted; cell extracts from equal numbers of cells (85,000 to 125,000) were diluted to 500 \(\mu\)l using NET buffer

**Table I**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Strain</th>
<th>Mutation*</th>
<th>Notes</th>
<th>BIP studies*</th>
</tr>
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<tr>
<td>OI type I (mildest form)</td>
<td>81-002</td>
<td>Unknown</td>
<td>Decreased intracellular ratio, proα1(proα2) mutant proα1 chains rapidly degraded</td>
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<td></td>
<td>82-110</td>
<td>5-bp deletion, proα1(COOH-terminal domain)</td>
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<td>85-132</td>
<td>COL1A1 exon 17 skip</td>
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<td>Gly to Cys at residue 988, proα1</td>
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<td>Residues 586-765 deleted, proα2</td>
<td>Nearly complete retention of abnormal trimer</td>
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<td>Secreted proα1 trimers</td>
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<td>OI types III and IV</td>
<td>82-057</td>
<td>4-bp deletion in proα2(I) COOH-terminal propeptide</td>
<td>Homozygous; secretes proα1(I) homotrimer</td>
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<td>Normal</td>
<td>Newborn</td>
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</table>

* Mutations heterozygous unless noted. Residues are numbered from the first glycine of the triple helix (residue 1) except for carboxy-terminal propeptide residues (bold), which are numbered with the initiating methionine as position 1.

* Studies: 1) metabolic labeling with \[^{35}S\]Met and SDS-PAGE to detect increased BiP synthesis after ascorbate treatment, 2) immunoprecipitation with antibodies to type I procollagen and/or to BiP to detect BiP-type I procollagen binding, 3) Northern or slot blot analysis to examine BiP message levels, 4) Western blot analysis to examine BiP content. The three strains with increased BiP content/synthesis after ascorbate treatment and BiP-procollagen binding (see text) are in bold.
(50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Nonidet P-40, 1 mM EDTA, 0.02% sodium azide) with bovine serum albumin (0.25%). The primary antibody was added, and the immunoprecipitations were incubated for 1 h. Protein G-Sepharose was added, and the precipitations were incubated, with end-over-end rotation, for several hours more. The beads were washed for 30 min in 1 ml of NET buffer 3 times. In experiments where ATP was depleted, cell extracts were diluted with the same buffer used for cell lysis and the immunoprecipitations were washed 3 times with NET buffer. Rat anti-BiP monoclonal antibody (15) was a gift from Dr. David Boile (University of Michigan School of Medicine). Dr. Burton Goldberg (University of Wisconsin School of Medicine) provided a polyclonal antibody, AFP 5, that recognizes the carboxyl-terminal propeptide of human type I procollagen. Another polyclonal antibody to the same region, M98, and one to the amino-terminal propeptide of proα1(I), M58, were gifts from Dr. Yvette Holmes (Washington University). A rat monoclonal antibody to GRP94 was purchased from StressGen (Victoria, British Columbia).

Proteins were separated by SDS-PAGE (16) detected by autoradiography or autoradiofluorography using the fluor ENHANCE (Du Pont-New England Nuclear Research Products), and in some experiments were quantitated using a Phosphor imager (Molecular Dynamics Corp.). When high resolution of collagenous proteins was necessary, 2 M urea was added to the gel and buffer solutions (13). Molecular weight markers were 14C-methylated proteins (Amersham Corp.) and, to accurately determine the positions of BiP and (unglycosylated) GRP94, aliquots of extracts from cells treated overnight with tunicamycin and labeled as above.

Slot blot Analysis of RNA—Cells were plated in 35-mm dishes at the same time that parallel dishes were prepared for protein labeling experiments. Total RNA was extracted in 4 M guanidine isothiocyanate as described (17). Following a standard protocol (14), RNA was slot blotted at two different dilutions onto nitrocellulose using a Minifold II (Schleicher & Schuell). Probes radiolabeled with 32P dCTP (Du Pont-New England Nuclear) were generated using a Minifold I1 (Schleicher manufacturer's instructions, and band intensity was measured using a scanning densitometer (Hoeffer, San Francisco, CA).

RESULTS

Cells from Three Patients with OI Type II Have Increased Synthesis of BiP—To identify proteins that could mediate the retention of abnormal procollagen, we searched for proteins induced in OI cells (skin fibroblasts from patients with OI). The OI cell strains examined all accumulate abnormal type I procollagen intracellularly or have altered intracellular ratios of the proα1(I) and proα2(I) chains of type I procollagen. Control and OI cells were incubated with 35S methionine, and the radiolabeled proteins were examined by SDS-PAGE (Fig. 1; see Table I for list of strains studied and causative mutations). In three of the 20 OI strains examined, 86–251, 86–237, and 86–146, which were derived from patients with OI type II, the perinatal lethal type, there was increased labeling of a protein that comigrated with BiP by SDS-PAGE (Fig. 1) and by two-dimensional nonequilibrium pH gradient electrophoresis/SDS-PAGE (data not shown) and was precipitated with a specific antibody to BiP (see below), confirming its identity. Increased BiP synthesis was most readily apparent in strains 88–251 and 86–237, while, with strain 86–146, it was often more difficult to detect. Labeling of the other major glucose-regulated protein, GRP94 (19), was increased most noticeably in 86–146 cells (not shown). All strains tested increased synthesis of BiP and GRP94 in response to treatment with tunicamycin, a known inducer of the two proteins (20).

The mutations in the three OI strains with increased synthesis of BiP are unique and will be described in detail elsewhere (45). While most infants with OI type II have mutations in the type I collagen triple helical domain that alter the structure of the triple helix (1–3), in these three strains the mutations are in the carboxyl-terminal propeptide of proα1(I), which mediates chain association and interchain disulfide bonding (21, 22). In strain 86–237, there is a substitution of histidine for aspartic acid at residue 1277 of proα1(I), which is bound in the presence of ATP (24, 25). Expression of histidine for aspartic acid at residue 1277 of proα1(I), in strain 86–251, a substitution of arginine for leucine at residue 1338, and in strain 86–146, a deletion of residues 1337 and 1338, a glutamic acid and a tyrosine. These mutations impair, but do not completely prevent, proα chain association, and abnormal type I procollagen molecules are formed that, as in other OI type II strains, have overmodified triple helical domains amino-terminal to the mutation site (45). Overmodification is caused by increased post-translational lysyl hydroxylation and glycosylation of hydroxyllysyl residues and reflects defective helix formation (3).

BiP Binds to Proα1(I) Chains in Cells from the Three Patients—Immunoprecipitation was used to identify proteins that bind to retained abnormal type I procollagen. Fig. 2 shows the results of one such experiment. Proteins were labeled with 35S methionine, harvested, and type I procollagen was immunoprecipitated. As seen in the figure, BiP coprecipitated with intracellular type I procollagen in the three strains with elevated BiP synthesis. In the case of the nine other OI strains and three control strains so investigated (see Table I), no BiP, or, sometimes, trace BiP coprecipitated. Another protein that migrates just above BiP frequently coprecipitated with abnormal type I procollagen. This protein has not been identified, although lysyl hydroxylase (86 kDa) is a candidate (23). BiP has ATPase activity and dissociates from proteins to which it is bound in the presence of ATP (24, 25). Experiments such as the one shown in Fig. 3 (86–237 lanes), in which ATP was added before the cell extract was incubated, confirmed that BiP coprecipitated with the abnormal type I procollagen.
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position of fibronectin procollagen and the resulting samples analyzed by SDS-PAGE under precipitation with protein G-Sepharose. A8496 is the control cell which preferentially binds the normal proα1(1) chains in 88-251, one precipitated with anti-BiP monoclonal antibody on the lanes. The no antibody lanes show the extent of nonspecific binding was also somewhat thermolabile; in the parallel experiment in which no ATP was added, incubation at 22 °C decreased BiP coprecipitation by about 35%. Despite the susceptibility of BiP-procollagen binding to ATP, ATP depletion was not mandatory during harvesting to maintain binding, probably because of the presence of EDTA in the rinse and immunoprecipitation buffers (9) and because the immunoprecipitation reactions were kept cold.

In Fig. 3, all four cell strains are O1 type II strains and have marked intracellular type I procollagen accumulation. As in Fig. 2, coprecipitation of BiP with procollagen was limited to the three strains with carboxyl-terminal mutations. In the reciprocal experiment using a monoclonal antibody to BiP, coprecipitation of procollagen with BiP was most apparent in the same three strains (Fig. 3, left four lanes). Proc1(I) was the predominant chain that coprecipitated, and, when run adjacent to reduced proα chains on a 2 M urea SDS-PAGE gel, it was evident that primarily the overmodified (abnormal) proc1(I) chains coprecipitated in all three strains (not shown; see Fig. 4b). Coprecipitation of proα2(I) was also evident, especially after prolonged labeling periods (see Fig. 4a, reduced) and with strain 86–237, the strain which exhibited the greatest intracellular levels of unassociated proα2(I) chains (see Fig. 4a, unreduced lanes) (45).

Coprecipitation of BiP with type I procollagen in strain 88–251 was reduced when an antibody to the carboxyl-terminal propeptide that preferentially bound the normal chains, antibody M38, was used (Fig. 3). Coprecipitation of BiP was similar with an antibody (M58) specific for the amino-terminal propeptide of proc1(I) and with one (A5) that recognized both chains. Although coprecipitation was consistent in experiments with the three OI strains and an absence of coprecipitation was consistent with all other strains examined, the calculated extent of coprecipitation was variable. The amount of radiolabeled type I procollagen that coprecipitated with BiP ranged from 3 to 10% of the amount precipitated by an antiprocollagen antibody. The amount of BiP that coprecipitated with type I procollagen ranged from 4 to 16% of total.

Coprecipitation was independent of increased BiP synthesis in the three OI strains, as BiP coprecipitated with procollagen under conditions in which BiP synthesis was normal (prior to ascorbate treatment, data not shown).

To determine whether BiP preferentially bound procollagen molecules or proα chains, we analyzed immunoprecipitated proteins under reducing and nonreducing conditions (Fig. 4a). Following precipitation with the monoclonal antibody to BiP, proc1(I) and proα2(I) chains were detected when samples were analyzed under reducing conditions but under nonreducing conditions, neither proα chains nor procollagen molecules entered the gel. These findings suggested that the proα chains bound by BiP were in disulfide-bonded aggregates. The abnormally large quantities of proα chain monomers that were precipitated from the three OI cell strains by the antibody to type I procollagen (nonreduced lanes) are a manifestation of the impaired proα chain association in those strains.

To determine the time course of BiP-procollagen binding, 88–251 cells were pulsed for 30 min with [35S]methionine and chased in methionine-supplemented medium, and BiP was immunoprecipitated from the resulting lysates (Fig. 4b). While BiP coprecipitated with an antibody to type I procollagen at all time points (not shown), significant amounts of proc1(I) and proα2(I) chains coprecipitated with an antibody to BiP only after 20 min of chase. This suggests that there was a lag before nascent proα chains were bound by BiP, perhaps reflecting a delay between chain synthesis and incorporation into aggregates bound by BiP. BiP-procollagen binding was stable for the remainder of the 3-h chase, and the

2 S. D. Chessler and P. H. Byers, unpublished observation.
BiP induction by abnormal procollagen chains

Defective triple helix folding does not cause BiP induction—Our results suggested that increased BiP synthesis was caused by mutations that hindered the early stages of procollagen assembly. We next asked whether BiP synthesis would be affected by defects in triple helix formation, a later stage of procollagen maturation. The triple helix is propagated from the carboxyl-terminal end of the molecule and stabilized by post-translational modifications, especially prolyl hydroxylation (3). In the absence of these modifications, there is intracellular accumulation of trimers with unfolded triple helical regions. We used two approaches to prevent or inhibit these post-translational modifications: we treated cells with \(\alpha,\alpha'-dipyridyl\), an inhibitor of the hydroxylation enzymes, and we maintained cells in medium deficient in ascorbate, a cofactor for these enzymes (26). Neither ascorbate deficiency nor \(\alpha,\alpha'-dipyridyl\) treatment caused BiP induction (Fig. 5A).

In the 88-251 cells, although, ascorbate deficiency, but not \(\alpha,\alpha'-dipyridyl\) treatment, prevented the increased BiP synthesis otherwise observed.

These results indicated that increased BiP synthesis in the 01 strains was dependent on treatment with ascorbate. Furthermore, they suggested that the link between BiP synthesis and ascorbate treatment was unrelated to enzymatic hydroxylation or triple helix formation, but might be related to the effect of ascorbate on procollagen synthesis, a gradual increase in proc chain message levels and translational efficiency (27–30). Fig. 5B shows the effect of the duration of ascorbate treatment on BiP synthesis. With less than 8 h of ascorbate treatment, BiP labeling in 88-251 and 86-237 cells was similar to that in control cells. With longer periods of ascorbate treatment, BiP labeling in 88-251 and 86-237 cells increased, peaked at 20–24 h, and returned to baseline (normal) levels by 48 h. BiP induction was often more difficult to detect in 86-146 cells, but, when apparent, followed roughly the same time course. Increased BiP labeling was due to increased synthesis, not to a change in BiP stability (data not shown).

Patterns of BiP and GRP94 expression differ in the three strains—To more closely examine stress protein induction in the 01 cells, BiP and GRP94 synthesis and BiP message levels were studied in parallel. Cells were treated for 0, 22, or 45 h with ascorbate and labeled, and BiP and GRP94 were immunoprecipitated from the same samples of cell extract. BiP and actin mRNA were quantitated in cells treated in parallel (Fig. 6). In two strains, 88-251 and 86-237, BiP protein synthesis increased approximately 4- (86-237) to 5-fold (88-251) after 22 h of continuous ascorbate treatment and then, after 45 h, decreased to about the same level (88-251) or a level 30% lower (86-237) than before ascorbate treatment (Fig. 6); BiP mRNA followed a similar pattern. Strain 86-146 cells behaved differently. BiP synthesis, although elevated, was less inducible by ascorbate. In addition, and in contrast to the other 01 cell strains and to the control cells, GRP94 synthesis was constitutively high. The ratio of GRP94 synthesis to BiP synthesis in this strain was, on average, 60–70% greater than in the other 3 strains. GRP94 synthesis in 86-146 cells remained about 4-fold greater than in the control strain, with both strains approximately doubling GRP94 synthesis during prolonged ascorbate treatment.

Induction of BiP synthesis by ascorbate was unique to the three cell strains in which BiP binds type I procollagen.

As a result of the ascorbate-induced transient increase in BiP synthesis, intracellular pools of BiP enlarged in cell strains 88-251 and 86-237 (Fig. 7). After 45 h of ascorbate treatment, the BiP pools increased approximately 2-fold. In control and other 01 cell strains (Fig. 7; Table I), ascorbate did not affect BiP content. In cell strain 86-146, there was a different pattern of stress protein expression. First, BiP content was constitutively high and was not increased greatly by prolonged ascorbate treatment. Second, GRP94 content, like GRP94 synthesis (Fig. 6), was even more markedly elevated. Surprisingly, ascorbate treatment increased GRP94 content about 50% in control and most 01 strains tested (most easily seen in 86-237 and A8496 lanes, Fig. 7).

Preinduction of BiP levels inhibits ascorbate-mediated BiP...
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**Fig. 5.** Effect of ascorbate and α,α'-dipyridyl on BiP synthesis. A, strain 88–251 and A8496 (control, CON) cells were incubated overnight with or without ascorbate and labeled with [35S]methionine for 2 h prior to lysis. Lysates were examined by SDS-PAGE. BiP synthesis was induced in 88–251 cells by ascorbate (arrows) but not by a,α'-dipyridyl. Molecular mass markers (kDa) are on the left. B, cells were incubated with ascorbate for the amount of time indicated above the brackets, and BiP synthesis was analyzed by labeling and SDS-PAGE as in A. BiP synthesis after 88–251 and 86–237 cells was maximal about 20 h after the initiation of ascorbate treatment (arrows). BiP induction in strain 86–146, when similarly detectable (not shown), followed the same pattern.

**Induction**—To test whether increasing BiP content prior to ascorbate treatment would prevent subsequent BiP induction, strain 88–251 and 86–237 cells were pretreated with a BiP-inducing agent, either tunicamycin or calcium ionophore A23187 (31). After 8 h, the agent was washed away, and the cells were allowed to fully recover. During recovery, BiP and GRP94 synthesis decreased and the labeling of all other proteins and their glycosylation returned to normal, as determined by examination of SDS-PAGE gels of labeled proteins. Four or 5 days after pretreatment, cells with normal (no pretreatment) or increased BiP content were treated with ascorbate for different times, and BiP synthesis was examined by SDS-PAGE of labeled cell extracts and by immunoprecipitation of BiP (Fig. 8, a and b). Western blot analysis confirmed that, due to BiP's high stability (half-life > 48 h) (32), BiP content remained elevated after the recovery period (Fig. 8c). In the pretreated OI cells, BiP induction by ascorbate was greatly reduced. Inhibition of BiP induction was likely due to the increased levels of BiP in the cells rather than to another effect of tunicamycin or A23187 as both agents caused such inhibition. Basal BiP synthesis was also reduced in the pretreated cells (Fig. 8b, 4-h lanes and data not shown).

**DISCUSSION**

We have shown that BiP content is constitutively increased or that synthesis can be enhanced by ascorbate in cells from a small subset of individuals with OI. In these cells, retained procollagen chains are stably bound by BiP. Three of 20 OI fibroblast strains examined have this phenotype. Each carries a mutation that impairs the assembly of the procollagen chains of type I procollagen into molecules. The three strains harbor mutations within the carboxyl-terminal propeptide of procollagen, the region of the chain that mediates its assembly into trimers (21, 22). The mechanism by which the cells detect production of the abnormal procollagen chains and respond by increased synthesis of BiP is uncertain. The most striking correlation we observed is with long duration ascorbate treatment. Ascorbate has two principal known effects on collagen metabolism. First, it acts as a cofactor for prolyl hydroxylase and lysyl hydroxylase, the former essential for prolyl hydroxylation and stabilization of the collagen triple helix. Increased post-translational hydroxylation after ascorbate treatment is rapid with maximal effects occurring within 60 min. Second, it acts over a much longer period to increase collagen synthesis, apparently through transcriptional and post-transcriptional mechanisms (27–30). The time course of increased BiP synthesis after ascorbate treatment resembles that of this second effect.

Although the folding of the triple helical domains of procollagen molecules synthesized in the absence of ascorbate or the presence of α,α'-dipyridyl is abnormal due to the resulting inhibition of prolyl hydroxylation, neither ascorbate deprivation nor α,α'-dipyridyl treatment induced BiP synthesis in control or OI cells. Furthermore, mutations within the triple helical domain of the procollagen chain did not result in BiP induction in the OI strains that carried such mutations under the same conditions that induction occurred in the three OI strains with carboxyl-terminal propeptide mutations. Thus, our results suggest that BiP synthesis increases in response to the synthesis of procollagen chains with a specific type of defect: one that impairs, but does not totally disable, chain association and that perhaps renders the procollagen chains capable of forming aberrant intermolecular disulfide bonds. Carboxyl-terminal mutations that completely prevent incorporation of the affected chains into trimers (strain 82–057; see Ref. 33) or that result in rapid turnover of the affected chains (strain 82–110) do not result in increased BiP accumulation. We searched for previously characterized OI strains with carboxyl-terminal mutations that impair but do not prohibit chain association and found one such strain (34). As
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**FIG. 6. Induction of ER-resident stress proteins after ascorbate treatment.** a, two sets of 35-mm dishes of each strain were plated at the same time and treated in parallel with ascorbate for the amounts of time indicated above the lanes. One set was labeled for 3 h with [35S]methionine and harvested. Extracts from equal numbers of cells were immunoprecipitated simultaneously with antibodies to BiP and GRP94, and the precipitated proteins examined by SDS-PAGE. The other set was used for RNA extraction. The RNA was slot-blotted onto nitrocellulose at two different dilutions (bands with more RNA shown) and hybridized with a BiP probe and, after stripping the blot, with an actin probe. SDS-PAGE gels of the total cell extracts (not shown) confirmed that the immunoprecipitation results accurately reflected the pattern of stress protein synthesis. b, the immunoprecipitation and slot blot results shown in a, as well as the higher dilution slot blot bands, were quantitated with a Molecular Dynamics PhosphorImager and graphed. Differences in mRNA recovery and loading were adjusted for by standardizing against actin mRNA levels. Synthesis and message levels in strain A8496 were set to 1 with no ascorbate treatment. Levels in the other strains are shown relative to levels in A8496. In another trial of this experiment, BiP mRNA levels closely matched those shown here, except in strain 88–251, in which BiP induction was weaker.

in the three strains discussed here, BiP bound abnormal proc(1) chains in this strain and was inducible by ascorbate.3

Gething and Sambrook (35) note that BiP likely recognizes regions that function as subunit interfaces, such as the CH1 domain of immunoglobulin heavy chain, a hypothesis that is consistent with our results. The other OI cell strains we examined have mutations in the triple helical regions of COL1A1 and COL1A2 or synthesize proc(1) and proc(2) chains in altered ratios. Because BiP binds poorly to peptides that contain proline and lysine (36), abundant residues in collagenous triple helices, if increased BiP synthesis depends on recognition of aberrantly folded proteins, then collagens with mutations in their triple helical domains might not actuate BiP synthesis, as was observed here (see also Ref. 6).

The findings presented here demonstrate that BiP plays a role in the cellular response to the production of some disease-producing abnormal proteins. Subtle changes in BiP expression have been reported in cytotologically normal neurons in the brains of patients with Alzheimer’s disease, but the reason for these changes is unclear (37). Increased BiP synthesis has so far not been reported in conjunction with other diseases,

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3 S. D. Chessler, J. F. Bateman, and P. H. Byers, unpublished observation.
Although it has been looked for in the case of α1-antitrypsin deficiency (38, 39), our results do not clarify the function of BiP in the cells in which it binds abnormal procollagen and its synthesis is increased. We did not find evidence that BiP mediates the retention of malformed procollagen. While all the OI type II cell strains we studied exhibited some degree of secretory block, in only three were BiP-procollagen binding or increased deficiency (38, 39). Although it has been looked for in the case of α1-antitrypsin deficiency (38, 39), our results do not clarify the function of BiP in the cells in which it binds abnormal procollagen and its synthesis is increased. We did not find evidence that BiP mediates the retention of malformed procollagen. While all the OI type II cell strains we studied exhibited some degree of secretory block, in only three were BiP-procollagen binding or increased deficiency (38, 39). It has been suggested that BiP facilitates oligomer formation and/or stabilizes monomeric subunits (12, 35). However, the procollagen chains that coprecipitated with BiP entered a polyacrylamide gel only under reducing conditions; BiP did not seem to bind stably to monomeric proα chains.

The transient induction of BiP following ascorbate treatment resembles the pattern of BiP synthesis after transfection of immunoglobulin μ heavy chain cDNA into COS cells (41) or after thymotropin treatment of rat thyroid epithelial cells (42). These examples and others (43, 44) suggest that cells can "sense" whether their BiP content is "adequate" and adjust BiP synthesis accordingly. Accordingly, the induction of endogenous BiP in response to tunicamycin or A23187 is reduced in cells overexpressing BiP from a transfected construct (10). In the OI strains we studied, BiP induction by ascorbate was decreased in cells with pre-enlarged BiP pools, emphasizing the importance of BiP content, as opposed to synthesis levels, in the regulatory mechanism.

More work is needed to clarify the function of BiP in cells from patients with OI and to elucidate the signalling pathway used by those cells to maintain BiP content in the ER sufficient for the load of malformed procollagen accumulating there. The data presented here provide additional support for the hypotheses that the level of BiP synthesis is responsive to BiP content in the ER, that BiP functions to moderate the effects of malformed proteins, especially those with abnormal sites important for subunit assembly, and, finally, that increased BiP expression and the stable binding of BiP to aberrant proteins are not limited to situations in which abnormal proteins are introduced artificially into cells but, instead, constitute a response to certain physiological conditions.

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