Identification of Lassa virus glycoprotein signal peptide as a *trans*-acting maturation factor

Robert Eichler, Oliver Lenz[†], Thomas Strecker, Markus Eickmann, Hans-Dieter Klenk & Wolfgang Garten^{*}

Institut für Virologie der Philipps-Universität Marburg, Marburg, Germany

Lassa virus glycoprotein is translated as a precursor (pre-GP-C) into the lumen of the endoplasmic reticulum and is cotranslationally cleaved into the signal peptide and GP-C, before GP-C is proteolytically processed into its subunits GP1 and GP2. The signal peptide of pre-GP-C comprises 58 amino acids. The substitution of Lassa virus pre-GP-C signal peptide with another signal peptide still mediates translocation and the release of signal peptide but abolishes the proteolytic cleavage of GP-C into GP1 and GP2. Remarkably, cleavage of GP-C from these hybrid pre-GP-C substrates was restored on coexpression of the wild-type pre-GP-C signal peptide, indicating that the signal peptide functions as a *trans*-acting factor to promote Lassa virus GP-C processing. To our knowledge, this is the first report on a signal peptide that is essential for proteolytic processing of a secretory pathway protein.

EMBO reports 4, 1084–1088 (2003)

doi:10.1038/sj.embor.7400002

INTRODUCTION

Almost half of the proteins of an average cell are translocated across membranes. Proteins directed into the secretory pathway use amino-terminal signal sequences to interact with the translocon machinery (Johnson & van Waes, 1999; Schatz & Dobberstein, 1996). These signal peptides have an important role in the translocation and membrane insertion of secretory and membrane-resident proteins. Signal peptides are cleaved from precursor proteins by an endoplasmic reticulum (ER)-resident signal peptidase or they remain uncleaved and function as a membrane anchor. Signal peptides generally consist of three parts: an N-terminal region of differing length, which usually comprises positively charged amino acids; a hydrophobic domain; and a short carboxy-terminal peptide segment. Owing to their universal structure most signal peptides can switch

between different proteins without loss of translocation function (Izard & Kendall, 1994). During recent years, a more advanced view of signal peptides has evolved, showing that the functions of certain signal peptides are much more versatile than previously anticipated. For example, a conserved segment of the signal peptide of major histocompatibility complex (MHC) class I human leukocyte antigen (HLA) was presented on the cell surface by the HLA-E molecule of nucleated human cells to report the biosynthesis of MHC class I molecules to the immune system (Braud *et al.*, 1998); a signal peptide fragment of the HIV-1 envelope protein was released into the cytosol and found to bind to calmodulin (Martoglio *et al.*, 1997); and a foamy virus glycoprotein signal peptide was shown to have a crucial role in viral assembly (Lindemann *et al.*, 2001).

Lassa virus is a member of the Arenaviridae, a family that includes highly pathogenic members that cause haemorrhagic fever, such as the Guanarito, Junin or Machupo viruses, as well as the less pathogenic lymphocytic choriomeningitis (LCMV). Lassa virus is endemic in West Africa, causing approximately 100,000–500,000 infections annually, of which around one-third results in illness ranging from flu-like symptoms to fulminant haemorrhagic fever with mortality up to 30% (McCormick *et al.*, 1987). So far, no vaccine and only an insufficient ribavirin therapy are available. In recent years, Lassa virus increasingly seems to be distributed from endemic regions to other parts of the world (ter Meulen *et al.*, 2001).

Lassa virions consist of a nucleocapsid surrounded by a lipid envelope in which viral glycoprotein spikes are embedded. The glycoprotein is synthesized as a 76-kDa precursor (pre-GP-C). The N-terminal portion of Lassa virus pre-GP-C contains a signal peptide of highly extended length comprising 58 residues (Eichler *et al.*, 2003; Fig. 1). After cotranslational cleavage of the signal peptide, GP-C of arenaviruses is post-translationally processed downstream from non-basic residues into the distal N-terminal subunit GP1 and the C-terminal membrane-anchored GP2 by subtilase SKI1/S1P (subtilisin-kexin isoenzyme 1/site 1 protease; Lenz *et al.*, 2000, 2001; Beyer *et al.*, 2003). SKI1/S1P belongs to the pyrolysins group of subtilases. It has also been shown to cleave the sterol regulatory element binding protein (SREBP; DeBose-Boyd *et al.*, 1999), the activating transcription factor 6 (ATF6; Ye *et al.*, 2000) and the brain-derived neurotrophic factor (BDNF; Seidah *et al.*, 1999).

Institut für Virologie der Philipps-Universität Marburg, Robert-Koch-Straße 17, D-35037 Marburg, Germany

[†]Present address: EMBL Grenoble Outstation, 6 rue Jules Horowitz, 38000 Grenoble, France

^{*}Corresponding author. Tel: +49 6421 286 5145; Fax: +49 6421 286 8962; E-mail: garten@mail.uni-marburg.de

Received 18 July 2003; revised 21 August 2003; accepted 3 September 2003
Published online 10 October 2003

Fig. 1 | Overview of Lassa virus glycoprotein. The primary translation product of Lassa virus glycoprotein (pre-GP-C; amino acids (aa) 1–491), the signal peptide (SP; aa 1–58), the precursor glycoprotein (GP-C; aa 59–491), the distal subunits GP1 (aa 59–259) and GP2 (aa 260–491) containing the membrane anchor (aa 427–450, stripes) are shown. Antisera binding sites, Rb- α -SP (aa 2–18) and Rb- α -GP2-N (aa 259–277), the signal peptidase (SPase) cleavage site between threonine residues 58 and 59 (arrow), the SKI1/S1P (subtilisin-kexin isoenzyme 1/site 1 protease) cleavage site carboxy-terminal of leucine 259 (arrow) and putative N-glycosylation sites (Y symbols) are indicated. The total signal peptide sequence is shown in one-letter code at the bottom of the figure.

In this study, we show that the released signal peptide interacts directly with GP-C and promotes the SKI1/S1P cleavage of GP-C, thereby mediating as a *trans*-acting maturation factor.

RESULTS

Substitution of Lassa virus GP-C signal peptide

The Lassa virus GP-C signal peptide was replaced by ordinary signal peptides of other proteins. Although the peptide is unusually long, there is no known reason for its length, and therefore such a substitution was considered appropriate. Influenza virus haemagglutinin and CD8 α -chain have been used frequently as reporter molecules for chimeric proteins (Jackson *et al.*, 1993; Meyer & Radsak, 2000; Schäfer *et al.*, 1995). Therefore, two substitution mutants of pre-GP-C were constructed using the signal peptide of cellular plasma membrane protein CD8 (mutant CD8-SP/GP-C) and that of influenza virus haemagglutinin (mutant HA-SP/GP-C; Fig. 2A, upper panel). The electrophoretic mobility of these mutants compared with a signal peptide cleavage-defective mutant (T58R; Eichler *et al.*, 2003) and the wild-type glycoprotein (WT pre-GP-C) confirm that the signal peptide is cleaved regularly from these mutants (Fig. 2A, upper panel, lanes 3–5). No other signal peptide cleavage site has been predicted using the SignalP prediction program (Nielsen *et al.*, 1997). Regardless of the origin of the substituted signal peptide, Lassa GP-C hybrid molecules were translocated into the ER as judged by the position of the GP-C band, which shows the same electrophoretic mobility as N-glycosylated wild-type GP-C. Surprisingly, GP-C derived from both chimeric constructs was not further proteolytically processed by SKI1/S1P into GP1 and GP2, as is the case with wild-type GP-C (Fig. 2A, lower panel, lanes 2, 3 and 4). Like Lassa virus pre-GP-C, the signal peptide of LCMV glycoprotein is also predicted to consist of 58 residues (Burns & Buchmeier, 1993). Because it shows high sequence similarity to Lassa GP-C signal peptide, we generated the mutant LCMV-SP/GP-C to find out whether these peptides are interchangeable. Lassa virus glycoprotein containing the LCMV signal peptide is cleaved by SKI1/S1P, yielding the subunits GP1 and GP2 (only GP2 is detectable by the antiserum used; Fig. 2A, lower panel, lane 5). This hybrid mutant is thus functionally indistinguishable from wild-type Lassa virus glycoprotein. In conclusion, replacing the signal peptide with unrelated signal sequences still allows ER translocation of GP-C, but proteolytic processing into GP1 and

GP2 by SKI1/S1P depends on the presence of the native or a closely related signal peptide.

Lassa virus signal peptide restores GP-C cleavage

The previous experiments showed that the signal peptide has an important role in the maturation of GP-C. Because the native or a similar signal peptide is essential for GP-C cleavage we aimed to determine whether the solitary expressed signal peptide of Lassa virus pre-GP-C is able to restore cleavage when GP-C is expressed with an unrelated signal peptide. For this reason, the signal peptide of Lassa virus glycoprotein was coexpressed with the pre-GP-C hybrid mutants CD8-SP/GP-C and HA-SP/GP-C. Interestingly, coexpression of solitary signal peptide allows the proteolytic processing of both GP-C mutants into GP1 and GP2 (Fig. 2B, lanes 3–6). As controls, WT pre-GP-C and a signal peptide deletion mutant cotransfected with the signal peptide, are shown (Fig. 2B, lanes 1 and 2). The non-glycosylated GP-C* of mutant Δ 2-58 is not inserted in the ER membrane, whereas the WT pre-GP-C is inserted and proteolytically processed as expected. Our data show that the native signal peptide is involved in the proteolytic cleavage of GP-C in GP1 and GP2.

How the signal peptide induces the cleavage of GP-C into GP1 and GP2 is not understood. In an attempt to assess whether the Lassa GP-C signal peptide is specifically involved in the folding of the glycoprotein, coprecipitation experiments in a pulse-chase analysis of the glycoprotein with chaperone, calnexin, were performed using an antibody directed against calnexin (Rb- α -calnexin). Figure 2C shows that substituting the wild-type signal peptide with the HA signal peptide results in a time-dependent accumulation of Lassa GP-C with calnexin (middle panels), in contrast to the wild-type glycoprotein (upper panels), suggesting that mutant GP-C is not able to reach its mature conformation without its native signal peptide. The expression level of WT pre-GP-C and HA-SP/GP-C did not differ remarkably during this experiment as confirmed by parallel immunoprecipitation with antiserum directed against GP2 (Rb- α -GP2-N). The calnexin signal is too weak to be seen when GP-C is precipitated using Rb- α -GP2-N (Fig. 2C, lower panels). An interaction between calnexin and the cleaved glycoprotein subunits GP1 and GP2 could not be detected (data not shown). These observations suggest that the Lassa GP-C signal peptide is necessary for correct folding of the glycoprotein.

Fig. 2 | Replacement of Lassa virus glycoprotein (GP-C) signal peptide. Vero cells were mock-transfected (M) and transfected with wild-type (WT) pre-GP-C and pre-GP-C hybrids: mutant haemagglutinin signal peptide (HA-SP)/GP-C, mutant CD8-SP/GP-C and mutant lymphocytic choriomeningitis signal peptide (LCMV-SP)/GP-C. (A) The solitary expressed viral glycoproteins were separated by SDS–polyacrylamide gel electrophoresis (PAGE) on 12% (upper) or 10% (lower) acrylamide gels, immunoblotted and analysed using the rabbit serum Rb- α -GP2-N. (B) Reconstitution of Lassa GP-C cleavage. Mutant HA-SP/GP-C, mutant CD8-SP/GP-C and mutant $\Delta 2-58$ were expressed with or without Lassa GP-C signal peptide. The protein of lysed cells was subjected to SDS–PAGE (10% acrylamide) and immunoblotted using the antiserum Rb- α -GP2-N. Non-glycosylated GP-C is marked by an asterisk. (C) Glycoprotein association with calnexin. Vero cells expressing Lassa pre-GP-C and mutant HA-SP/GP-C, as well as mock-transfected cells (M), were metabolically labelled with 35 S-methionine/-cysteine and chased for various time intervals after 30 min of pulse. Protein was precipitated using anti-calnexin-antiserum (upper and middle panels, left column) or Rb- α -GP2-N antiserum (lower panel, left column) and subjected to SDS–PAGE (10% acrylamide) followed by autoradiography. Precipitated protein was quantified using Tina-Software (Raytest; right column). Phosphor-stimulated luminescence (PSL) mm $^{-2}$ indicates signal intensity.

Interaction of Lassa GP-C with its signal peptide

To investigate a potential direct interaction between GP-C and its released signal peptide, coimmunoprecipitation experiments were performed. When wild-type Lassa GP-C was expressed, the antiserum Rb- α -SP directed against the signal peptide precipitates the signal peptide and GP-C (Fig. 3A, lane 2), and antiserum raised against GP2 precipitates GP-C and the signal peptide (Fig. 3A, lane 3). To address whether the interaction between GP-C and the signal peptide is long-lived we performed long-term pulse-chase experiments. Figure 3B shows that the signal peptide antiserum coprecipitates GP-C even after 12 h of chase (upper panel), and that the GP-C antiserum coprecipitates the signal peptide (lower panel), confirming that the interaction between GP-C and its released signal peptide is stable.

Fig. 3 | Interaction of Lassa virus glycoprotein (GP-C) with its signal peptide. Vero cells were transfected with pre-GP-C. Protein was metabolically labelled with ^{35}S -methionine/-cysteine and precipitated using either Rb- α -SP antiserum (A, lane 2, and B, upper panel), or Rb- α -GP2-N antiserum (A, lane 3, and B, lower panel), or as control with pre-sera of both antisera (M). Immunoprecipitated protein was separated by 10% SDS-polyacrylamide gel electrophoresis (PAGE) (A, upper panel, and B, upper panel) or 16.5% Tricine/PAGE (A, lower panel, and B, lower panel) followed by autoradiography.

DISCUSSION

Signal sequences are excellent substrates for evolution because of their tremendous diversity. Sequence elements that do not disrupt the targeting function but are useful for other purposes could arise and be maintained by selection over time (Hegde, 2002). This might have occurred with Lassa virus glycoprotein during its evolutionary period. A high level of conservation among the glycoprotein signal peptides of arenaviruses, which is unusual among signal peptides of glycoproteins from other virus species, is a strong indication of an additional function besides protein targeting across the ER membrane.

The observation that chimeric Lassa glycoprotein precursor containing non-native signal peptides is not proteolytically processed by SKI1/S1P showed that the native signal peptide indeed has a second function. Interestingly, the second function of the WT pre-GP-C signal peptide, which leads to cleaved GP1/GP2, can be restored by coexpressed signal peptide from Lassa virus pre-GP-C. The possibility that the substitution of the native signal peptide results in incorrect processing of the signal peptide, subsequently leading to the abolition of GP-C cleavage by SKI1/S1P, can be excluded for the following reasons: first, mutant GP-C migrates to the same position as wild-type GP-C in SDS-polyacrylamide gel electrophoresis (PAGE); second, signal sequence cleavage depends only on sequence elements that are N-terminal of the cleavage site; and third, if GP-C was processed incorrectly the coexpression of solitary WT pre-GP-C signal peptide could not restore the maturation cleavage. Notably, the Lassa virus GP-C signal peptide can promote the maturation cleavage of GP-C into GP-1 and GP-2 only when the signal peptide is released from pre-GP-C before, as pre-GP-C with a mutated signal peptide cleavage site was not further processed into GP1 and GP2 (Eichler *et al.*, 2003). The signal peptide might induce GP-C cleavage as follows: the signal peptide is necessary for the transport of GP-C to the cellular compartment where SKI1/S1P cleavage of GP-C occurs. Lassa GP-C, like SREBP, ATF6 and proBDNF, the known cellular substrates of SKI1/S1P, is cleaved early along the secretory pathway before reaching the medial Golgi (Lenz *et al.*, 2001; Chen *et al.*, 2002; Ye *et al.*, 2000; deBose-Boyd *et al.*, 1999). Interestingly, proteolytic processing of SREBP requires SREBP cleavage activating protein (SCAP), which relocates SREBP from the ER to an intermediate or early Golgi compartment. The signal peptide of Lassa GP-C could function similarly in the maturation cleavage of GP-C. However, this possibility seems unlikely given that we could not detect a difference in the cellular localization of wild-type and hybrid GP-C, indicating that the signal peptide is not necessary for the transport of GP-C (data not shown). It may be more tempting to assume that the signal peptide chaperones the N-terminal part of GP-C and keeps it in a conformation that is accessible to the ER resident cleaving enzyme SKI1/S1P. The long persistence of the released signal peptide in the cell (up to 12 h) and the cellular colocalization of the signal peptide with the glycoprotein, as well as the prolonged half-life of the association of GP-C with calnexin in mutants with exchanged signal peptides, support this idea.

Because the interaction between the signal peptide and its glycoprotein seems to be highly specific, inhibiting this interaction may be of therapeutic relevance. Inhibitory peptides or peptidomimetics that suppress the interaction between the signal peptide and the glycoprotein would prevent cleavage of GP-C and thus the

production of infectious virus, as only cleaved Lassa virus glycoprotein is incorporated in virus particles (Lenz *et al.*, 2001). However, further studies are needed to clarify the nature of the molecular interaction between signal peptide and glycoprotein.

In this report, we describe a new signal peptide. Besides directing ER translocation, this signal peptide has an additional function. Our data indicate that the signal peptide, presumably acting as a chaperone, is directly responsible for post-translational cleavage of the glycoprotein and, thus, for the formation of infectious virus particles.

MATERIALS AND METHODS

Cell cultures. Vero cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, GIBCO) supplemented with 10% fetal calf serum (FCS), 100 units ml⁻¹ penicillin and 0.1 mg ml⁻¹ streptomycin.

Vectorial expression of Lassa virus glycoprotein and mutagenesis. All constructs were expressed using the β -actin promoter-driven pCAGGS vector (Lenz *et al.*, 2001; Beyer *et al.*, 2003; Eichler *et al.*, 2003). Lassa virus pre-GP-C mutants or mutants thereof were generated by recombinant PCR techniques (Higuchi *et al.*, 1988). Sequences were confirmed by DNA sequencing. Vero cells were transfected with wild-type and mutated recombinant DNA using Lipofectamine 2000 (GIBCO/Invitrogen).

Antibodies. Antiserum Rb- α -GP2-N was raised as described previously for antibodies Rb- α -SP and Rb- α -GP2 (Lenz *et al.*, 2000; Eichler *et al.*, 2003). A rabbit calnexin antibody was purchased from Stressgen Bioreagents.

Acrylamide gel electrophoresis and immunoblotting. Proteins were separated by SDS-PAGE as described by Laemmli (1970) using 10% acrylamide gels and by Schagger & von Jagow (1987) using 16.5% polyacrylamide gel and Tricine buffer (Eichler *et al.*, 2003). Immunoblotting was performed as described previously (Lenz *et al.*, 2000).

Pulse-chase experiments and immunodetection. Plasmid-transfected Vero cells were labelled as described previously (Eichler *et al.*, 2003). The radioactive medium was then replaced by DMEM during a 2-h chase or various chase times as indicated. Labelled cells were lysed in co-immunoprecipitation buffer containing 1% Nonidet P-40, 100 mM sodium chloride, 20 mM Tris and 5 mM EDTA, pH 7.6 and sonicated (40 W, Branson sonifier) for co-immunoprecipitation. Supernatants of the cell lysates were incubated overnight with Protein A-Sepharose coupled to the desired antibodies. Immunoprecipitated proteins were analysed by SDS-PAGE or Tricine-PAGE followed by autoradiography on BioMax films (Kodak; Eichler *et al.*, 2003).

ACKNOWLEDGEMENTS

We thank J. ter Meulen for stimulating discussions. This work was supported by the Deutsche Forschungsgemeinschaft, Sachbeihilfe Ga 282/4-1 and SFB 593 TP B2 and the Graduiertenkolleg *Protein Function at the Atomic Level*. R.E. was supported by the FAZIT-Stiftung, Frankfurt, and performed this work in partial fulfilment of the requirements for a Ph.D. degree from the Philipps-Universität Marburg.

REFERENCES

- Beyer, W.R., Popplau, D., Garten, W., von Laer, D. & Lenz, O. (2003) Endoproteolytic processing of the lymphocytic choriomeningitis virus glycoprotein by the subtilase SKI-1/S1P. *J. Virol.*, **77**, 2866–2872.
- Braud, V.M. *et al.* (1998) HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature*, **391**, 795–799.
- Burns, J.W. & Buchmeier, M.J. (1993) in *The Arenaviridae* (ed. Salvato, M.S.), 17–33. Plenum, New York, USA.
- Chen, X., Shen, J. & Prywes, R. (2002) The luminal domain of ATF6 senses endoplasmic reticulum (ER) stress and causes translocation of ATF6 from the ER to the Golgi. *J. Biol. Chem.*, **277**, 13045–13052.
- DeBose-Boyd, R.A. *et al.* (1999) Transport-dependent proteolysis of SREBP: relocation of site-1 protease from Golgi to ER obviates the need for SREBP transport to Golgi. *Cell*, **99**, 703–712.
- Eichler, R., Lenz, O., Strecker, T. & Garten, W. (2003) Signal peptide of Lassa virus glycoprotein GP-C exhibits an unusual length. *FEBS Lett.*, **538**, 203–206.
- Hegde, R.S. (2002) Targeting and beyond: new roles for old signal sequences. *Mol. Cell*, **10**, 697–698.
- Higuchi, R., Krummel, B. & Saiki, R.K. (1988) A general method of *in vitro* preparation and specific mutagenesis of DNA fragments: study of protein and DNA interactions. *Nucleic Acids Res.*, **16**, 7351–7367.
- Izard, J.W. & Kendall, D.A. (1994) Signal peptides: exquisitely designed transport promoters. *Mol. Microbiol.*, **13**, 765–773.
- Jackson, M.R., Nilsson, T. & Peterson, P.A. (1993) Retrieval of transmembrane proteins to the endoplasmic reticulum. *J. Cell Biol.*, **121**, 317–333.
- Johnson, A.E. & van Waes, M.A. (1999) The translocon: a dynamic gateway at the ER membrane. *Annu. Rev. Cell Dev. Biol.*, **15**, 799–842.
- Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.
- Lenz, O., ter Meulen, J., Feldmann, H., Klenk, H.D. & Garten, W. (2000) Identification of a novel consensus sequence at the cleavage site of the Lassa virus glycoprotein. *J. Virol.*, **74**, 11418–11421.
- Lenz, O., ter Meulen, J., Klenk, H.D., Seidah, N.G. & Garten, W. (2001) The Lassa virus glycoprotein precursor GP-C is proteolytically processed by subtilase SKI-1/S1P. *Proc. Natl Acad. Sci. USA*, **98**, 12701–12705.
- Lindemann, D. *et al.* (2001) A particle-associated glycoprotein signal peptide essential for virus maturation and infectivity. *J. Virol.*, **75**, 5762–5771.
- Martoglio, B., Graf, R. & Dobberstein, B. (1997) Signal peptide fragments of preprolactin and HIV-1 p-gp160 interact with calmodulin. *EMBO J.*, **16**, 6636–6645.
- McCormick, J.B. *et al.* (1987) A case-control study of the clinical diagnosis and course of Lassa fever. *J. Infect. Dis.*, **155**, 445–455.
- Meyer, G.A. & Radsak, K.D. (2000) Identification of a novel signal sequence that targets transmembrane proteins to the nuclear envelope inner membrane. *J. Biol. Chem.*, **275**, 3857–3866.
- Nielsen, H., Engelbrecht, J., Brunak, S. & von Heijne, G. (1997) Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Prot. Eng.*, **10**, 1–6.
- Schäfer, W. *et al.* (1995) Two independent targeting signals in the cytoplasmic domain determine trans-Golgi network localization and endosomal trafficking of the proprotein convertase furin. *EMBO J.*, **14**, 2424–2435.
- Schagger, H. & von Jagow, G. (1987) Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.*, **166**, 368–379.
- Schatz, G. & Dobberstein, B. (1996) Common principles of protein translocation across membranes. *Science*, **271**, 1519–1526.
- Seidah, N.G. *et al.* (1999) Mammalian subtilisin/kexin isozyme SKI-1: A widely expressed proprotein convertase with a unique cleavage specificity and cellular localization. *Proc. Natl Acad. Sci. USA*, **96**, 1321–1326.
- ter Meulen, J. *et al.* (2001) Short communication: Lassa fever in Sierra Leone: UN peacekeepers are at risk. *Trop. Med. Int. Hlth.*, **6**, 83–84.
- Ye, J. *et al.* (2000) ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol. Cell*, **6**, 1355–1364.