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Calnexin Controls the STAT3-Mediated Transcriptional Response to EGF

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SUMMARY

Calnexin is a well-characterized transmembrane chaperone involved in the folding of newly synthesized glycoproteins in the lumen of the endoplasmic reticulum (ER). Here, we reveal a previously unrecognized function of calnexin in regulating the transcriptional response downstream of epidermal growth factor receptor (EGF), the product of a well-known human oncogene. We find that cell stimulation with EGF leads to the caspase-8-dependent cleavage of the calnexin cytoplasmic domain, preferentially at ER-mitochondria interaction sites. The released fragment translocates into the nucleus, binds to PIAS3—a natural inhibitor of activated STAT3—and, thus, acts as an enhancer of the STAT3-mediated transcriptional response to EGF. Also, we reveal the unsuspected capacity of calnexin to sense ER stress and, in response, prevent the EGF-induced processing of its cytosolic domain. Thus, cells integrate the health status of the ER to determine the amplitude of their response to EGF.

INTRODUCTION

The endoplasmic reticulum (ER) is a large, membrane-bound, architecturally complex organelle that is responsible for diverse key cellular functions, such as calcium homeostasis, the synthesis of major structural lipids, and the folding of membrane and secreted proteins. Protein folding efficiency is enhanced by a large panel of chaperones and folding enzymes, and folding quality is checked by a highly robust control system that ensures that misfolded proteins are rapidly evacuated through the ER-associated degradation (ERAD) pathway (Brodsky, 2012). The accumulation of misfolded proteins leads to ER stress and the subsequent activation of the unfolded protein response (UPR), which orchestrates ER recovery by various means, including arrest in global protein synthesis and the upregulation of chaperones (Hetz, 2012). Failure to restore ER function or persistent ER stress lead to apoptosis (Wang and Kaufman, 2012).

Communication between ER lumen and the cytosol, in both directions, relies mostly on transmembrane proteins. For example, the accumulation of unfolded proteins in the ER lumen leads to the activation of the ER stress sensor Ire1α (Gardner and Walter, 2011), which, in turn, triggers a transcriptional response for enhancing the ER folding potential. In the reverse direction, calnexin, a transmembrane ER chaperone, links ribosome docking on the cytosolic side of the ER to the folding of glycoproteins on the lumenal side (Chevet et al., 1999; Lakkaraju et al., 2012). Recently, we found that, in order to capture newly synthesized glycoproteins as they emerge from the translocation pore, calnexin must be bound to the ribosome-translocon complex through its cytosolic tail, thereby allowing it to capture nascent proteins through its lumenal domain (Lakkaraju et al., 2012).

Calnexin is highly abundant in the ER as well as the outer nuclear membrane (Dreger et al., 2001). It has also been found at ER-mitochondria contact sites (Myhill et al., 2008). Given that these sites are devoid of ribosomes, as observed by electron microscopy (Cosson et al., 2012), calnexin might serve functions there that are unrelated to the folding of glycoproteins. ER-mitochondria contact sites play a crucial role in calcium homeostasis, lipid synthesis, and ER-stress-induced apoptosis (Kornmann, 2013; Michel and Kornmann, 2012). Prolonged ER stress leads to the cleavage of the transmembrane protein Bap31 at ER-mitochondria contact sites, leading to the generation of p20Bap31, a truncated transmembrane proapoptotic form of Bap31, which triggers Ca^{2+} release from the ER followed by the activation of the mitochondrial permeability pore and finally apoptosis (Breckenridge et al., 2003). Bap31 cleavage is mediated by caspase-8 (Breckenridge et al., 2002), the recruitment of which was found to be dependent on the cytosolic tail of calnexin (Delom et al., 2007). Consistently, cells lacking calnexin are more resistant to ER-stress-induced apoptosis and do not undergo Bap31 cleavage (Zuppini et al., 2002).

Here, we investigated whether calnexin has other, nonchaperone functions. Guided by a yeast two-hybrid screen, we found that epidermal growth factor (EGF) triggers caspase-8-mediated cleavage of calnexin in its cytosolic domain. We show that the released fragment interacts with PIAS3, a natural protein inhibitor of signal transducer and activator of transcription 3 (STAT3) (Chung et al., 1997), which is robustly activated by EGF (Quesnelle et al., 2007). As a consequence, calnexin cleavage strongly promotes STAT3-dependent transcription in response to EGF. However, interestingly, calnexin cleavage does not occur under conditions of ER stress. We show that calnexin can sense ER stress through its lumenal domain and become insensitive to caspase-8. Our work reveals an unexpected role of calnexin in promoting gene expression in a manner that is ER-stress-dependent through the oncogenic transcription factor STAT3.

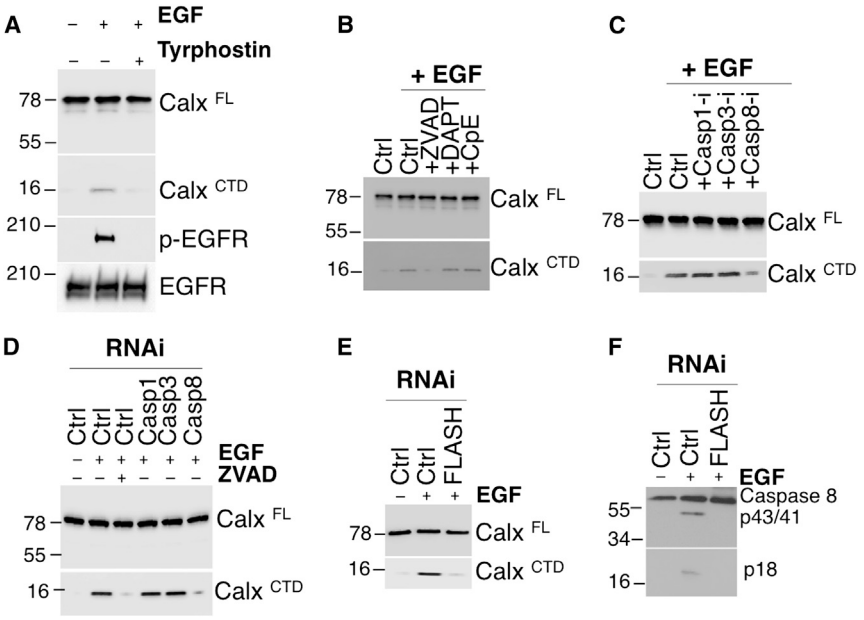

Figure 1. EGF Stimulation Leads to Calnexin Cleavage by Caspase-8

(A) A431 cells were serum starved for 24 hr, pretreated with or without tyrphostin (200 nM) for 30 min, and stimulated with EGF (100 ng/ml) for 10 min. Cells were lysed and analyzed on a 15% SDS-PAGE. Western blotting was performed with antibodies against the C terminus of calnexin, EGFR, and phosphoEGFR. Please note that, for calnexin staining, the high- and low-molecular-weight parts of the SDS gel were transferred separately onto nitrocellulose (see the Experimental Procedures) but were stained with the same antibody against the C terminus of calnexin.

(B) A431 cells were serum starved for 24 hr and pretreated with or without ZVAD-fmk (10 μM) for 2 hr and with γ-secretase inhibitors and CpE (10 μM) and DAPT (10 μM) for 4 hr after being stimulated with EGF (100 ng/ml) for 10 min. Then, cells were incubated in normal medium for an additional 20 min. The cells were lysed, and proteins were migrated on a 15% SDS-PAGE, and western blotting was performed with an antibody against the C terminus of calnexin.

(C) A431 cells were serum starved for 24 hr and pretreated with the inhibitors of caspase-1, -3, and -8 (all used at 10 μM) for 2 hr prior to stimulation with EGF. The cell lysates were migrated on 15% SDS-PAGE, and western blotting was performed with an antibody against the C terminus of calnexin.

(D) A431 cells were treated with either the control RNAi or RNAi against caspase-1, -3, or -8 for 72 hr. The cells were serum starved for the last 24 hr and, before stimulation with EGF (100 ng/ml), were pretreated with or without ZVAD. The cell lysates were migrated on 15% SDS-PAGE, and western blotting was performed with an antibody against the C terminus of calnexin.

(E) A431 cells were treated with control RNAi or RNAi against FLASH for 72 hr. After serum starvation, cells were stimulated with or without EGF, and calnexin cleavage was monitored in the cell lysates with an antibody against the C terminus of calnexin.

(F) A431 cells were treated with either control RNAi or RNAi against FLASH for 72 hr after stimulation with EGF. The cells were lysed, and the cell lysate was migrated on a 15% SDS-PAGE. Western blotting was performed with an antibody against caspase-8.

RESULTS

Yeast Two-Hybrid Screen with the Cytoplasmic Tail of Calnexin

To investigate the potential roles of the cytosolic domain of calnexin, we performed a yeast two-hybrid screen using a placental library to identify interacting proteins. The screen led to two potentially interesting hits: FLASH and PIAS3. FLASH, also called caspase-8-associated protein 2 (CASP8A2), is involved in the activation of caspase-8 in response to Fas-induced apoptosis (Imai et al., 1999). FLASH was also found to be an essential component of cajal bodies in the nucleus, and it plays a role in the transcription of histone genes (Barcaroli et al., 2006a; Barcaroli et al., 2006b) and steroid hormone receptors (Kino et al., 2004). PIAS3 is one of the protein inhibitors of activated STAT (PIAS), which modulates transcription factors of the STAT family (Chung et al., 1997).

EGF Triggers Caspase-8-Mediated Calnexin Cleavage

PIAS3 has been shown to negatively regulate STAT3 (Chung et al., 1997), a transcription factor that is activated in response to EGF (Quesnelle et al., 2007). The identification of both PIAS3 and FLASH led us to investigate whether the stimulation of cells with EGF would lead to the proteolytic processing of calnexin. Cells were serum starved, treated for 10 min with EGF, and treated for 20 min in normal medium. Cell extracts were analyzed by western blotting. Note that transfer onto nitrocellulose membranes was differentially optimized for low- and high-molecular-weight proteins. After EGF treatment, our antibody against the C terminus of calnexin revealed not only the full-length protein (Figure 1A, upper blot) but also a 16 kDa fragment (Figure 1A, lower blot Calx^{CTD}) that was absent in untreated cells. Quantification of the western blots indicated that 22% ± 7% (on four independent experiments) of the total calnexin population underwent processing, keeping in mind the limited linearity of western blotting. Cleavage could also be witnessed with an antibody against the N terminus of calnexin, which could not recognize the Calx^{CTD} but revealed the appearance of a lower-molecular-weight truncated form. Quantification of the western blots with this antibody led to a very similar estimate: 21% ± 10% of the total calnexin population underwent processing (Figures S1A and S1B available online).

Cleavage required the activation of the EGF receptor (EGFR), given that it was inhibited by tyrphostin, a specific inhibitor of EGFR kinase activity (Figure 1A). EGF-induced calnexin cleavage was sensitive to the pan-caspase inhibitor ZVAD, but not to DAPT and CpE, two inhibitors of γ-secretase, an enzyme that is present in the ER (Bot et al., 2011) (Figure 1B). Inhibitors of caspase-8, but not of caspase-1 or -3, also affected EGF-induced calnexin cleavage (Figure 1C). Direct involvement of caspase-8 and its interacting protein FLASH was confirmed by small interfering RNA (siRNA) gene silencing of these proteins