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Article

Translation Attenuation through eIF2α Phosphorylation Prevents Oxidative Stress and Maintains the Differentiated State in β Cells

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SUMMARY

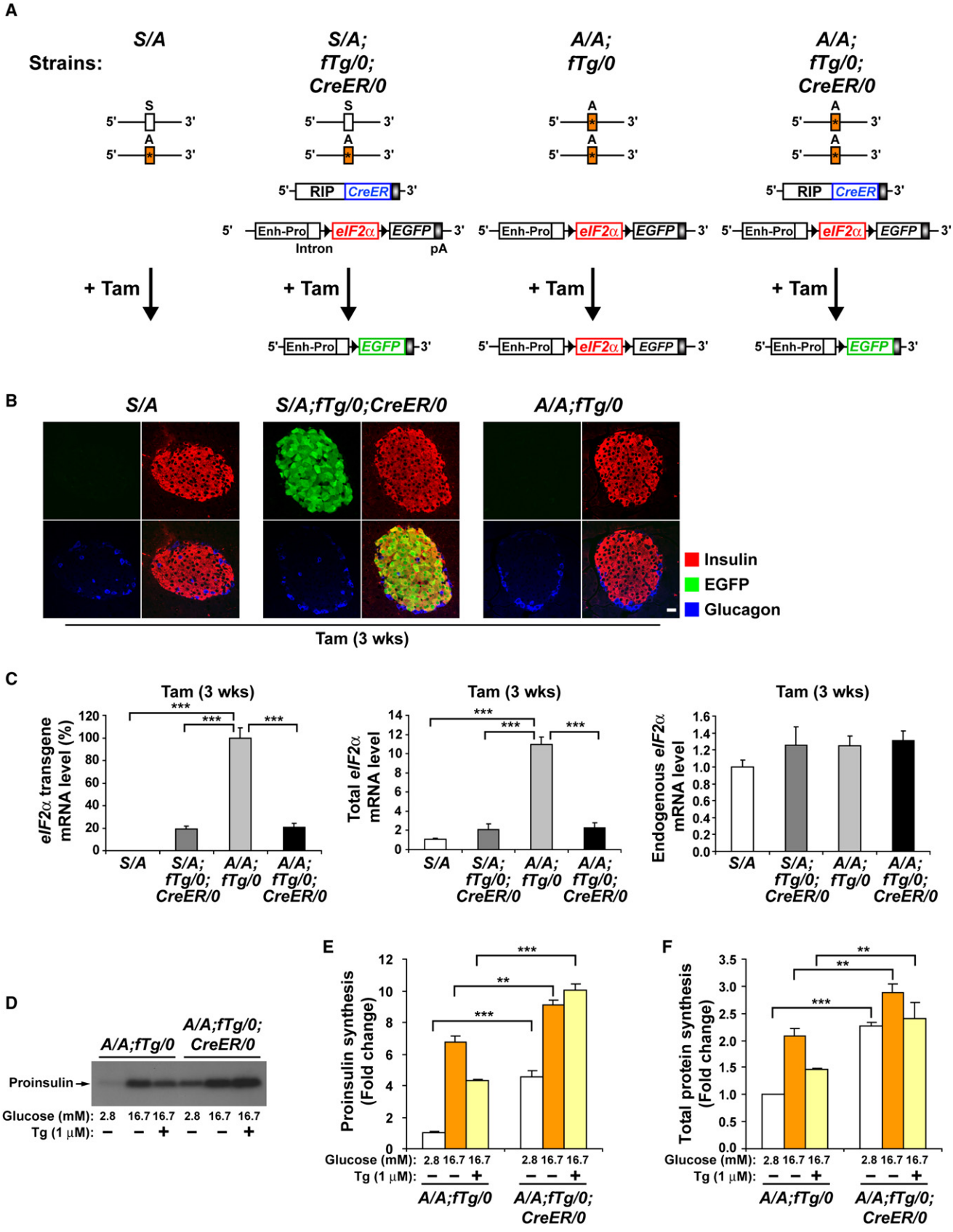
Accumulation of unfolded protein within the endoplasmic reticulum (ER) attenuates mRNA translation through PERK-mediated phosphorylation of eukaryotic initiation factor 2 on Ser51 of the α subunit (eIF2α). To elucidate the role of eIF2α phosphorylation, we engineered mice for conditional expression of homozygous Ser51Ala mutant eIF2α. The absence of eIF2α phosphorylation in β cells caused a severe diabetic phenotype due to heightened and unregulated proinsulin translation; defective intracellular trafficking of ER cargo proteins; increased oxidative damage; reduced expression of stress response and β-cell-specific genes; and apoptosis. However, glucose intolerance and β cell death in these mice were attenuated by a diet containing antioxidant. We conclude that phosphorylation of eIF2α coordinately attenuates mRNA translation, prevents oxidative stress, and optimizes ER protein folding to support insulin production. The finding that increased proinsulin synthesis causes oxidative damage in β cells may reflect events in the β cell failure associated with insulin resistance in type 2 diabetes.

INTRODUCTION

The rough endoplasmic reticulum (ER) is a network of interconnected tubules, vesicles, and sacs that serves many specialized functions in the cell, including calcium storage and gated release, biosynthesis and folding of membrane and secretory proteins, and production of lipids and sterols. Cells have a unique adaptive capability to adjust and coordinate the ER protein-folding capacity with the protein-folding load. One profound example of physiological fluctuations in the protein-folding load is the unique translational response of pancreatic β cells to variations in blood glucose. Proinsulin synthesis imposes a heavy biosynthetic burden upon the β cell, as ~1 × 10^6 proinsulin molecules are synthesized per minute upon glucose stimulation (Schuit et al., 1988). Conditions that interfere with productive protein folding in the ER lumen result in the accumulation of unfolded or misfolded (herein referred to as misfolded) proteins, a condition known as ER stress. Accumulation of misfolded proteins within the lumen of the ER in β cells occurs as a consequence of increased proinsulin translation, expression of inherently misfolded proinsulin, defects in adaptive response-signaling pathways, and physiological stimuli, including glucose, cytokines, lipids, and nitric oxide (Scheuner and Kaufman, 2008).

The unfolded protein response (UPR) is an adaptive signaling program that evolved to resolve the accumulation of misfolded proteins in the ER (Ron and Walter, 2007; Rutkowski and Kaufman, 2007). The UPR increases ER protein-folding capacity, decreases protein synthesis to reduce the folding demand, and enhances clearance of misfolded proteins. When these mechanisms fail to reestablish ER homeostasis, numerous death-signaling pathways are activated (Kim et al., 2008). Recent studies suggest that there is a close interrelationship between misfolded protein-mediated cell death and oxidative stress (Malhotra and Kaufman, 2007). During disulfide bond formation in the ER, electrons are transferred from cysteine residues through protein disulfide isomerase (PDI) and ER oxidoreductase 1 (ERO1) to reduce molecular oxygen and form hydrogen peroxide (Tu and Weissman, 2004). In addition, glutathione is consumed in the process of reducing mispaired disulfide bonds (Cuozzo and Kaiser, 1999) that may deplete the cellular glutathione pool that is required to neutralize reactive oxygen species (ROS) and prevent oxidation of cytosolic proteins. Therefore, cells with a high biosynthetic load, defective UPR, or inadequate ER-associated protein degradation (ERAD) are vulnerable to oxidative stress (Harding et al., 2003; Malhotra et al., 2008; Merksamer et al., 2008; Song et al., 2008). ER to mitochondria calcium crosstalk may also compromise mitochondrial function and induce oxidative stress in response to protein misfolding (Sharaf El Dein et al., 2009). The studies suggest that the UPR has evolved as a protective mechanism to prevent oxidative stress associated with protein folding in the ER.

In metazoan cells, the UPR is signaled through three ER transmembrane sensors of unfolded protein: inositol-requiring 1α (IRE1α), activating transcription factor 6α (ATF6α), and PKR-like ER kinase (PERK) (Ron and Walter, 2007; Rutkowski and Kaufman, 2007). IRE1α has ER stress-regulated kinase/endoribonuclease activities that initiate unconventional splicing of the


A
Strains:
S/A
S/A; fTg/0; CreER/0
A/A; fTg/0
A/A; fTg/0; CreER/0
RIP
CreER
Enh-Pro
eIF2α
EGFP
Intron
pA
+ Tam
B
S/A
S/A;fTg/0;CreER/0
A/A;fTg/0
Insulin
EGFP
Glucagon
Tam (3 wks)
C
Tam (3 wks)
eIF2α transgene mRNA level (%)
Total eIF2α mRNA level
Endogenous eIF2α mRNA level
D
A/A;fTg/0
A/A;fTg/0; CreER/0
Proinsulin
Glucose (mM): 2.8 16.7 16.7 2.8 16.7 16.7
Tg (1 μM): – – + – – +
E
Proinsulin synthesis (Fold change)
F
Total protein synthesis (Fold change)