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Polypyrimidine Tract Binding Protein Regulates IRES-Mediated Gene Expression during Apoptosis

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Summary

During apoptosis there is a substantial reduction in the rate of protein synthesis, and yet some mRNAs avoid this translational inhibition. To determine the impact that receptor-mediated cell death has on the translational efficiency of a large number of mRNAs, translational profiling was performed on MCF7 cells treated with the apoptosis-inducing ligand TRAIL. Our data indicate that approximately 3% of mRNAs remain associated with the polysomes in apoptotic cells, and genes that are involved in transcription, chromatin modification/remodeling, and the Notch signaling pathway are particularly prevalent among the mRNAs that evade translational inhibition. Internal ribosome entry segments (IRESs) were identified in several of the mRNAs that remained associated with the polysomes during apoptosis, and, importantly, these IRESs functioned efficiently in apoptotic cells. Finally, the data showed that polypyrimidine tract binding protein (PTB, a known IRES *trans*-acting factor or ITAF) is pivotal in regulating the apoptotic process by controlling IRES function.

Introduction

Apoptosis is a physiological process that is essential for the proper regulation of cell growth, tissue architecture, differentiation, and development. In apoptotic cells, there is a substantial reduction in the rate of polypeptide synthesis that occurs soon after the onset of cell death and is not simply due to a loss of cell viability (Clemens et al., 2000). The data suggest that translation initiation is inhibited during apoptosis (Morley et al., 1998), and indeed numerous alterations to the translation initiation factors have been observed during cell death, including cleavage or degradation of eIF4GI, eIF4GII, eIF4B, eIF3j, eIF2α, and 4EBP1; increases in eIF2α phosphorylation; and dephosphorylation of 4EBP1 (Clemens et al., 2000).

However, a number of proteins, in particular those involved in cell death, are still synthesized during apoptosis, including c-*myc*, DAP5, and XIAP (Stoneley et al., 2000; Henis-Korenblit et al., 2000; Holcik et al., 1999). Furthermore, in each case there is evidence that a cap-independent mechanism of internal translation initiation is involved, whereby ribosomes are recruited to the mRNA by a complex RNA structural element known as an internal ribosome entry segment (IRES) (Hellen and Sarnow, 2001; Komar and Hatzoglou, 2005; Stoneley and Willis, 2004). Thus, modification of the translational apparatus during apoptosis affects differential regulation of gene expression, and this is thought to be brought about by specific *trans*-acting factors that are in turn regulated during apoptosis. Relatively few of the proteins that are required for IRES-mediated translation have been identified, but it is apparent that polypyrimidine tract binding protein (PTB) is required by many cellular IRESs (Mitchell et al., 2005).

A genome-wide analysis was performed to identify changes in the association of specific mRNAs with the translational apparatus during receptor-mediated cell death. Our data demonstrate that mRNAs are subject to differential translational regulation during cell death. Most notably, we identified a considerable number of mRNAs that evade translational inhibition during apoptosis, approximately 30% of which encode proteins with roles in chromatin organization and the control of transcription. Many of the mRNAs that remain associated with the polysomes were shown to contain IRESs that are dependent upon PTB for function in vitro and in vivo.

Results

TRAIL Treatment of MCF7 Cells Causes a Reduction in Protein Synthesis

MCF7 cells are sensitive to tumor necrosis factor (TNF) family receptor-mediated cell death (Janicke et al., 1998; Liang et al., 2001), and following treatment with TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) there was an approximately 50% reduction in the rate of protein synthesis 4 hr after the addition of the ligand (Figure 1A). Moreover, the degradation and partial cleavage of poly(ADP-ribose) polymerase (PARP) clearly indicated that cell death was initiated during this time period, and a total cleavage of PARP was observed after 8 hr (Figure 1B). There was also a decrease in the association of eIF4G with eIF4E, a concurrent increase in the association of eIF4E with 4EBP1 (Figure 1C), and dephosphorylation of 4EBP1 during TRAIL-induced cell death (Figure 1C). Thus, TRAIL reduces the abundance of the eIF4F complex through dephosphorylation of the eIF4E inhibitor protein 4EBP1. At this early stage in apoptosis in this cell type, there is little or no degradation of eIF4G. These time points were chosen since we and others have shown previously that later in apoptosis, when cleavage of eIF4G occurs, there is also significant RNA degradation (Bushell et al., 2004; Del Prete et al., 2002; Mondino and Jenkins, 1995). In agreement with these data, Northern blot analysis to compare the steady-state levels of actin and ribosomal protein S6 mRNAs in control cells and TRAIL-treated

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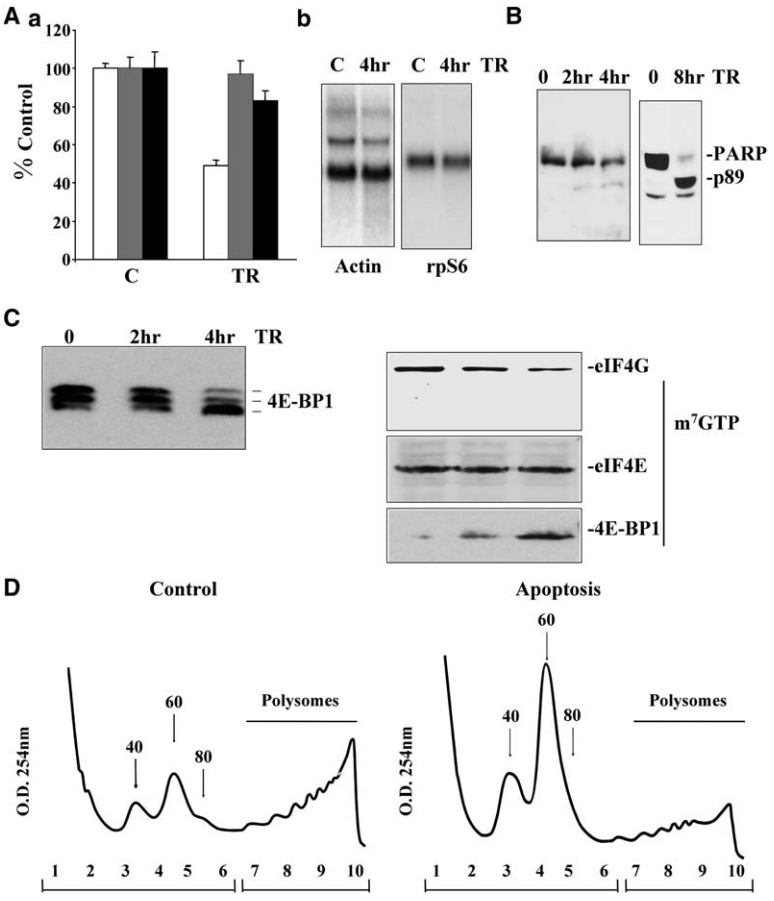

Figure 1. Polypeptide Synthesis in MCF7 Cells Is Inhibited by a Translational Mechanism during TRAIL-Induced Apoptosis without Total mRNA Levels Decreasing

(A) (Aa) MCF7 cells were exposed to 1 μg/ml of TRAIL for 4 hr (TR) or were mock treated (C). The rate of polypeptide synthesis in both control and apoptotic cells was assessed by measuring the incorporation of [^{35}S]-methionine into trichloroacetic acid (TCA) precipitate (white bars). Error bars represent the SD obtained from three independent experiments. (Ab) In addition, total RNA was prepared from control (C) and apoptotic (TR) cells, and samples of RNA were subjected to Northern blot analysis. Radiolabeled gene-specific probes were used to determine the abundance of β-actin (gray bars) and ribosomal protein S6 (rpS6) mRNAs (black bars). Quantification of the mRNA levels was performed using PhosphorImager analysis.

(B) MCF7 cells were exposed to 1 μg/ml of TRAIL for the times indicated, and cytoplasmic cell extract was prepared. Cytoplasmic cell lysate was resolved by SDS gel electrophoresis, and PARP and the cleavage product of PARP (p89) were detected by Western blot analysis.

(C) Cells were treated as above, and cytoplasmic S10 extracts were prepared. The phosphorylation status of 4EBP1 was determined by performing Western blot analysis on a fraction of the lysate. In addition, extracts were subjected to affinity chromatography on m^{7}GTP-Sepharose to purify eIF4E and associated proteins (m^{7}GTP). The recovered proteins were resolved by SDS gel electrophoresis, and the levels of eIF4E, eIF4G, and 4E-BP1 were determined by Western blot analysis.

(D) Lysates from untreated MCF7 cells (control) or MCF7 cells treated for 4 hr with 1 μg/ml TRAIL (apoptosis) were fractionated by sucrose density gradient centrifugation. The fractions from the top to the bottom of the sucrose gradient are displayed from left to right. The absorbance profile at 254 nm is shown, and the position of the 40S, 60S, and 80S ribosomal subunits and polysomes are indicated. Fractions from sucrose density gradient analysis were pooled into subpolysomes (fractions 1–6) and polysomes (7–10).

cells demonstrated that there was no change after 4 hr (Figure 1A).

Cytoplasmic extracts prepared from both untreated MCF7 cells and cells treated with TRAIL were then subjected to sucrose density gradient analysis. Exposure of MCF7 cells to TRAIL resulted in a considerable decrease in the amount of polysomes and a corresponding increase in the abundance of the 40S and 60S complexes (Figure 1D) consistent with inhibition at the initiation stage of protein synthesis.

cDNA Microarray Analysis Shows that a Subset of mRNAs Are Subject to Differential Translational Regulation during Apoptosis

To identify changes in the translational efficiency of individual mRNAs during apoptosis, RNA derived from sucrose gradient fractions 7–10 (polysomes) was compared with RNA from fractions 1–6 (subpolysomes) to obtain a measure of the translational status of mRNAs in both control and apoptotic cells (Figure 1D). While the majority of mRNAs displayed reduced association with the polysomes in apoptotic cells (Figure 1D and see Figures S1 and S2 in the Supplemental Data available with this article online), approximately 6% of mRNAs are severely translationally repressed in early apoptosis. Moreover, approximately 3% of mRNAs are resistant to the translational inhibition and do not relocate from the polysomes into the subpolysomes during apoptosis (Figures S1 and S2). Together, these data imply that certain mRNAs are subject to differential translational regulation during cell death. Distinct categories of mRNAs that displayed either increased or decreased relative translational efficiency during cell death were identified (Tables 1 and 2). Strikingly, within the group of genes whose relative translational efficiency was increased during cell death, approximately 30% encode proteins with roles in transcription and chromatin organization. These include several genes encoding transcription factors, some of which have previously been implicated in the control of cell death and survival, such as c-*myc*, STAT6, and msx2 (Table 1). Also prevalent among the genes in this category are transcriptional corepressors and coactivators, a large proportion of which are involved in histone modification or chromatin remodeling, e.g., HAT1 and TAF1 (Table 1). In addition, several members of the Notch signaling pathway (Haines and Irvine, 2003), such as Notch 2, seven-in-absentia-2, hairy, transducin-like enhancer of split,