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Translational Regulation of Estrogen Receptor Alpha Expression

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Estrogen Receptor alpha (ER) is a hormone activated nuclear transcription factor whose presence in breast tumors correlates with favorable prognosis and facilitates antiestrogen therapy. ER is expressed from at least two promoters; the resulting ERs are identical. The Distal promoter is 2 kb upstream of the main translational start of the ER open reading frame (ORF), the proximal (prox) is directly upstream of the ER-ORF. The prox-ER transcript contains a twenty amino-acid ORF (prox_uORF) terminating 50 bp upstream of the ER-ORF. Using flow cytometry and western blot techniques our lab showed that eliminating the prox_uORF increased the expression from ER-Green Fluorescent Protein (GFP) fusion protein constructs of prox-ER transcript upstream sequences and the first 18 ER codons.

Replacing the C-terminal (Phe) codon with a stop-codon further inhibited expression of ER-GFP fusion proteins. This effect seems related to the peptide of the ORF as inhibitory effects were reversed by eliminating the prox_uORF translational start codon. We found similar effects in several ER positive and negative cell lines. Comparison of RNA and protein expression levels suggested that the prox-peptide inhibits translation of ER-GFP at the translational level, rather than at the transcription level. We introduced ATG translational starts within the abolished prox_uORF; the truncated ORFs were also inhibitory on downstream ER-GFP expression, suggesting a role for multiple residues. Further analysis by flow cytometry data on single mutations of codon 13 through 20 to a stop codon showed that the critical region is trp-pro-ala. Tryptophan has a large bulky aromatic side group, proline is very rigid and its presence can create a fixed kink in a protein chain. These features may explain why they are critical for this inhibitory function of the peptide. Our data suggest that the reported lack of the correlation between ER-protein and prox-transcript levels in tumors is due to inhibitory post-transcriptional control by the prox-peptide.