In format provided by Leppek, K. *et al.* (doi:10.1038/nrm.2017.103)

Supplementary information S3 | **Experimental probing of cellular IRES RNA structures**

Experimental methods applied to probing cellular IRES structures, sorted chronologically and indicating where their functional relevance has been tested by compensatory mutagenesis. To our knowledge, the RNA structures of the IRESs of vascular endothelial growth factor A (VEGFA), immunoglobulin heavy chain binding protein (BiP) and fibroblast growth factor 9 (FGF9) have not yet been examined.

IRES mRNA*	Biological role of protein	Probing method**	Probe***	Has the RNA structure model been tested?	Compensatory mutagenesis	Refs
c-myc	proliferation, apoptosis	chemical probing	DMS, kethoxal, CMCT	structural deletions and mutagenesis, structure model: two domain structure and pseudoknot	yes	1
IGF2	proliferation, development	chemical probing	DMS, kethoxal, CMCT	structure model	-	2
Apaf1	apoptosis	chemical and enzymatic probing	DMS, kethoxal, CMCT, RNase V1	structure model, DMS probing in presence of ITAFs UNR and nPTB to map binding sites, structure changes upon binding, structural mutants abolish ITAF binding	-	3
CAT1	proliferation, cell survival	chemical and enzymatic probing	RNAse T1, T1/A, V1; DEPC/aniline	structure model in presence and absence of uORF translation, analysis of uORF stop codon mutants in IRES	yes	4,5
FGF2	development	chemical and enzymatic probing	DMS, kethoxal, CMCT, RNase T1, V1	structure model: two stem-loops and RG4; structural mutagenesis: domain deletion and loop mutations; cation-dependent RT termination to probe RG4 structure in IRES	yes	6
Bag1	cell survival, apoptosis	chemical and enzymatic probing	DMS, kethoxal, RNase V1	structure model, DMS, kethoxal, V1 probing in presence of ITAFs PTB and PCBP1 to map binding sites, structure changes upon binding, probing of structural mutants at binding site that abolish ITAF binding	-	7
FGF1A	angiogenesis and metastasis	chemical and enzymatic probing	DMS, CMCT, RNase T1, T2, V1	structure model, probing of human IRES; model for six mammalian species, two stem-loops conserved in mammals; domain and point mutations in human IRES	yes	8
L-myc	proliferation	chemical and enzymatic probing	DMS, kethoxal, CMCT, RNase V1	structure model, predicted pseudoknot	-	9
XIAP	cell survival, apoptosis	chemical and enzymatic probing	NMIA, RNase T1, T2, A, V1	structure model, structural deletions and mutagenesis	-	10
N-myc	proliferation, development	chemical and enzymatic probing	DMS, kethoxal, RNase V1	structure model	-	11
cIAP1	cell survival	enzymatic probing	RNase T1, T2, V1	structure model, DNA oligo bound to IRES structure competes with ITAF NF45 binding, structural mutagenesis to test ITAF binding	yes	12
p53	cell cycle	chemical and enzymatic probing	DMS, RNase T1, V1	structure model, enzymatic probing of WT and mutant structure with cancer-derived silent mutations that change structure, reduced IRES activity of mutants, 5'-3' UTR interaction by base pairing	limited	13,14
VEGF-C	angiogenesis and metastasis	chemical and enzymatic probing, SHAPE	DMS, RNase T1, T2, V1; BzCN (SHAPE)	structure model, SHAPE analysis of mouse and human structure	-	15

Hoxa	development	chemical probing, SHAPE, mutate-and-map, MOHCA-Seq	1M7 (SHAPE)	structure model, SHAPE of Hoxa5 and Hoxa9 IRES, pseudoknot; mutate-and-map, MOHCA-seq, structural mutagenesis of Hoxa9	yes	16,17
VEGFD	angiogenesis and metastasis	chemical and enzymatic probing, SHAPE	RNase T1, T2, V1; BzCN (SHAPE)	structure model, SHAPE of VEGF-D 5' UTR, predicted pseudoknot	-	18

* IRES-containing mRNAs: IGF2, insulin-like growth factor 2; Apaf1, apoptotic protease activating factor 1; CAT1, cationic amino acid transporter 1; FGF, fibroblast growth factor; Bag1, BCL2-associated athanogene 1; XIAP, X-linked inhibitor of apoptosis protein; c-IAP1, inhibitor of apoptosis protein; VEGF, vascular endothelial growth factor; Hoxa; Homeobox a.

** Most RNA structures of cellular IRESs have been probed individually using chemical modification or enzymatic cleavage of RNA, reverse transcription (RT) and analysis of primer extension products *in vitro*. The accessibility of an RNA nucleotide for a probe or an enzyme indicates whether an RNA molecule is base-paired.

*** Reagents and enzymes adapted to modify single-stranded bases in RNA show nucleotide selectivity: DMS, dimethyl sulfate: A, C, G; CMCT, 1-cyclohexyl-(2-morpholinoethyl)carbodiimide metho-p-toluene sulfonate: U; kethoxal: G; lead, Pb^{2+}: non-specific. Single-strand RNA-specific nucleases cleave the following substrates: RNase T1: after unpaired G; RNase T2: non-specific with preference for A; RNase A: after C and U, RNase I: non-specific; RNase S1: non-specific; and double-strand specific RNase V1. SHAPE uses the probe N-methylisatoic anhydride (NMIA), its derivative 1-methyl-7-nitroisatoic anhydride (1M7), or benzoyl cyanide (BzCN) to mark the 2'OH of flexible, single-stranded RNA positions at all four nucleotides[19].

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