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Review

IRES trans-acting factors, key actors of the stress response

Anne-Claire Godet, Florian David, Fransky Hantelys, Florence Tatin, Eric Lacazette, Barbara Garmy-Susini, and Anne-Catherine Prats *

UMR 1048-I2MC, Inserm, Université de Toulouse, UT3, Toulouse, France; e-mail@e-mail.com
* Correspondence: Anne-Catherine.Prats@inserm.fr; Tel.: +xx-xxx-xxx-xxxx

Abstract: The cellular stress response corresponds to the molecular changes that cell undergoes in response to various environmental stimuli. It induces drastic changes in the regulation of gene expression, at transcriptional and post-transcriptional levels. Actually, translation is strongly affected with a blockade of the classical cap-dependent mechanism, whereas alternative mechanisms are activated to support translation of specific mRNAs. One of the major mechanisms involved in stress-activated translation is the internal ribosome entry site (IRES)-driven initiation. IRESs, first discovered in viral mRNAs, are present in cellular mRNAs coding for master regulators of cell responses, whose expression must be tightly controlled. IRESs allow translation of these mRNAs in response to different stresses, including DNA damage, amino-acid starvation, hypoxia or endoplasmic reticulum stress, as well as to physiological stimuli such as cell differentiation or synapse network formation. Importantly, cellular mRNA IRESs are regulated by IRES trans-acting factor (ITAFs), exerting their action by at least nine different mechanisms. This review presents an update of the reported ITAFs regulating cellular mRNA translation and of the different mechanisms allowing them to control translation initiation in specific conditions. The impact of ITAFs on coordinated expression of mRNA families and consequences in cell physiology and diseases are also highlighted.

Keywords: gene regulation; translation; mRNA; IRES; ITAF; hnRNP; chaperone; stress; nucleocytoplasmic translocation; ribosome; lncRNA; translation initiation factor; P-bodies

1. Introduction

IRESs are translation regulatory elements of cellular mRNAs involved in multiple processes of cell physiology. Until the late 1980s, it was thought that eukaryote mRNAs could not be translated by internal ribosome entry, and that the only mechanism was the cap-dependent process involving the recruitment of the small ribosome subunit at the mRNA 5′ end, followed by ribosome scanning [1,2]. This dogma has proven incorrect with the discovery, in 1988, of RNA structural elements present in the mRNA 5′ untranslated regions (5′UTR) of two picornaviruses, poliovirus and encephalomyocarditis virus, able to mediate cap-independent translation through internal ribosome entry [3,4]. Picornavirus mRNAs are uncapped, with start codons located several hundreds nucleotides downstream from mRNA 5′ end, rendering improbable translation initiation at these AUG codons by the classical cap-dependent scanning mechanism. These ribosome internal entry elements were given the name of "ribosome landing pad" or internal ribosome entry site (IRES) and were shown later to exist in all *Picornaviridae*, as well as in other viruses such as *Retroviridae* [5–8].

Soon after the finding of the two first IRESs in picornaviruses, two host *trans*-acting factors, La autoantigen and pyrimidine tract binding protein (PTB) were identified as IRES-binding factors required for internal initiation of translation [9,10]. This suggested that the internal initiation process might also concern cellular mRNAs and allow their translation when the cap-dependent process is blocked, which was known to occur during mitosis (G2-M phase) and in stress conditions. Actually,

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the first IRES mediated by the 5′ leader of a cellular mRNA was described in 1991 [11]. Interestingly this messenger codes for the immunoglobulin heavy-chain binding protein (BiP), a chaperone involved in the unfolded protein response occurring during endoplasmic reticulum (ER) stress. Although this first cellular mRNA IRES was indicative of a major role of IRES-dependent translation in the stress response, the physiological relevance of IRESs in the translation of cellular mRNAs was questioned during many years, because these mRNAs are capped, in contrast to the picornavirus mRNAs. Nevertheless, it quickly became clear that the BiP mRNA was not a unique case: IRESs were found in a series of other cellular mRNAs, including transcription factors such as the homeobox (Hox) gene *antennapedia* and the proto-oncogene c-*myc*, angiogenic growth factors such as fibroblast growth factors (FGFs) and vascular endothelial growth factors (VEGFs), as well as many genes coding for master regulators of cell responses [12–19].

The physiological relevance of IRESs clearly appeared with the discovery of the X-linked inhibitor of apoptosis (XIAP) mRNA IRES [20]. This IRES was shown to be induced in apoptotic conditions. This observation was also made for other IRESs of mRNAs coding for factors involved in apoptosis, including the apoptotic peptidase activating factor 1 (APAF1), c-*myc* and p53 [21–24]. These findings provided the first evidence of a crucial role of IRES-dependent translation for cellular mRNAs. Actually, during apoptosis, the cap-dependent translation process is blocked as it is after picornavirus infection, due to the cleavage of a component of the cap-binding complex, the initiation factor 4G (eIF4G) [25]. XIAP and APAF1 have opposite functions during apoptosis, and their relative level due to the differential activation of their IRESs is determinant in the life/death decision of the cell in the progression of the apoptosis pathway [25].

Thereafter, IRES physiological function was evidenced in several reports. First, an important tissue specificity of cellular IRES activities was observed, in contrast to picornavirus IRES activity. This was revealed with the FGF2 IRES in transgenic mice: the IRES was inactive in almost all adult organs, except for brain and testis, where the activity was very strong, much stronger than in cultured cells [26]. Further investigation demonstrated that the FGF2 IRES is a key of FGF2 translational induction during spermatogenesis and during formation of synaptic network between neurons [27,28]. In contrast, the activity of the FGF1 IRES, another member of the FGF family, is strong in skeletal muscle and involved in the control of FGF1 expression during myoblast differentiation and muscle regeneration [29].

In addition to their role in specific adult organs, IRESs are important in the control of gene expression during development. The FGF2 and c-*myc* IRESs were as active as the strong EMCV (encephalomyocarditis virus) IRES in E11 mice embryos in contrast to what was observed in adult [26,30]. The early discovery of an IRES in the mRNA of the homeobox Hox gene *Antennapedia* in Drosophila also argued such an hypothesis, but the proof of concept which definitely demonstrated of the key role of IRESs in development was provided 23 years later by Maria Barna and her collaborators who identified IRESs in four HoxA mRNAs [16,31]. These authors showed that such IRESs are conserved in evolution and demonstrated that these IRESs are essential for mouse development by generating the first targeted mouse knockout of a cellular IRES [31]. Moreover the presence of IRESs in cellular mRNAs was investigated in a high throughput study, which uncovered thousands of sequences allowing cap-independent translation and showed that 10% of the mRNAs harbour cap-independent sequences [32]. In this report, two functional classes of IRESs have been defined: local IRESs that can act with a short sequence motif (18S rRNA or ITAF binding site) and global IRESs whose activity depends on a secondary or tertiary structure.

Dysfunction of IRES-dependent translation has also been related with various pathologies. A single mutation in the c-*myc* IRES is responsible for c-myc overexpression in multiple myeloma [33]. Also, single mutations in the connexin 32 and VEGFA IRESs have revealed the essential role of IRESs in two severe neurodegenerative diseases, Charcot-Marie-Tooth disease and amyotrophic lateral sclerosis, respectively [34,35]. More recently, an aberrant increase of IRES-dependent translation of key cancer gene mRNAs has been reported in cancer cells, including the major angiogenic factors FGF1, FGF2 and VEGFA, as well as c-*myc* and insulin growth factor-like receptor (IGF1R) [36]. This study deciphered the mechanism of IRES activation resulting from p53 tumor suppressor

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inactivation, by showing that p53 represses expression of the rRNA methyl-transferase fibrillarin, which modifies the rRNA methylation pattern and generates "cancer ribosomes" that will be preferentially recruited by IRES-containing mRNAs.

These different studies of IRES pathophysiological functions demonstrate the key-role of IRES-dependent translation, revealing the coexistence of cap-dependent and independent translation for capped mRNAs containing IRESs.

2. IRES-dependent translation, a pivotal mechanism in the stress response.

Physiological and environmental stresses induce drastic changes in the regulation of gene expression, which permits cell adaptation and survival, or in contrast triggers programmed cell death. It was long believed that such changes mostly occurred at the transcriptional level. For example, hypoxia, which is one of the major physiological stresses during development, generates the stabilization of the hypoxia-induced factor (HIF) which induces the transcription of a series of target genes. However this strong transcriptional response is not the only way to modify gene expression by stress. Several post-transcriptional mechanisms were shown to participate in the hypoxic response, among which translational control plays a key role.

Above all, global translation is blocked during stress to save energy, as translation is estimated to consume up to 50% of cellular energy [25]. This translation blockade is observed in most stress conditions including hypoxia, nutrient limitation, temperature changes, ultraviolet irradiation, endoplasmic reticulum stress, oxidative stress, viral infection... The two main locks of this blockade are the translation initiation factors eIF4E and eIF2α [37]. The first way of inhibiting translation results from mechanistic target of rapamycin (mTOR) kinase inactivation, which induces hypophosphorylation of 4E-binding proteins (4E-BPs). When dephosphorylated, 4E-BP sequesters the cap-binding protein eIF4E, generating a blockade of cap-dependent translation. The second way of translation inhibition by stress is due to eIF2α phosphorylation, which blocks the exchange of GDP to GTP in the eIF2 complex and prevents the assembly of the ternary complex eIF2-GTP-tRNA$_i^{Met}$ required for binding of the initiator Met-tRNA$_i^{Met}$ to the 40S ribosomal subunit. There are four known stress-responsive eIF2α kinases able to impact global translation: haem-regulated inhibitor kinase (HRI), protein kinase RNA (PKR), PKR-like endoplasmic reticulum kinase (PERK) and general control non-derepressible-2 (GCN2). These kinases are activated by different stresses that induce a common pathway of translation blockade [25,37]. The subtlety of the eIF2 pathway is that it also induces the selective translation of transcripts, mostly coding for master regulators of the cell responses, including transcription factors, growth factors… Such a selective translation occurs by two main initiation mechanisms: small upstream open reading frame (uORF)-regulated initiation and IRES-driven initiation. The best documented example of translation initiation regulated by uORFs is probably the yeast transcriptional activator GCN4 [38]. The GCN4 mRNA contains four uORFs upstream from the GCN4 ORF. When the ternary complex eIF2-GTP-tRNA$_i^{Met}$ is abundant, uORFs are translated, which prevents translation of the GCN4 ORF. In contrast, if the level of ternary complex is low, under amino-acid starvation, scanning ribosomes fail initiation at uORFs and translate the GCN4 ORF. This mechanism has also been described for the mammalian transcription factor ATF4 [37].

The other main mechanism of selective translation upon eIF2α phosphorylation, IRES-dependent translation, is the focus of the present review. Although eIF2α is in principle required for both cap-dependent and independent translation, IRES-dependent translation is selectively increased in condition of phosphorylated eIF2α. This was first observed for the IRES of the Arg/Lys transporter cat-1, as well as for several viral IRESs [39]. Interestingly, the activation of cat-1 IRES observed in response to amino-acid starvation, ER stress and double stranded RNA, requires eIF2α phosphorylation by GCN2, PERK and PKR, respectively. This suggests that the cat-1 IRES can function efficiently when the level of ternary complex eIF2-GTP-tRNA$_i^{Met}$ is low. This was also observed for BiP, XIAP and other stress-responsive transcript IRESs [40,41]. Two models have been proposed for this intriguing observation: i) ribosome recruitment and formation of the initiation complex utilizes initiation factor 5B (eIF5B) that delivers the tRNA directly into the P site of the

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ribosome to form a translation-competent initiation complex [41,42], or ii) IRES-dependent translation is increased following the transcriptional induction of 4E-BP by GCN2 and its downstream transcription factor, activating transcription factor 4 (ATF4): in conditions of limiting ternary complex eIF2-GTP-tRNA$_i^{Met}$, a stronger blockade of cap-dependent translation by 4E-BP results in increased IRES-dependent translation [40].

Upregulation of IRES-dependent translation by stress has an impact in various pathologies. For instance, hypoxia appears in the center of solid tumors exceeding a volume of 2 mm^3 which are not any more irrigated by blood vessels. As the major angiogenic and lymphangiogenic growth factors of the FGF and VEGF families possess IRESs in their mRNAs, these growth factors are translationally induced as their IRESs are sensitive to hypoxia [13,43–45]. This results in tumoral angiogenesis and lymphangiogenesis, two processes that promote tumor cell invasion and metastasis dissemination. Hypoxic stress also occurs in cardiovascular diseases such as lower limb ischemia and ischemic heart disease. In these pathologies, cells are subjected to hypoxia due to artery occlusion in ischemic leg or in infarcted myocard. In particular, chronic heart failure is a public health issue. IRES-dependent translation plays a major role during ischemia: a very recent mid-scale study show that, unexpectedly, expression of most (lymph)angiogenic factors is not induced at the transcriptome-, but at the translatome level in hypoxic cardiomyocytes (Hantelys F. et al., BioRxiv 2018). The same study indicates that the IRESs of (lymph)angiogenic factors mRNAs, FGF1, FGF2, VEFGA, VEGFC and VEGFD are activated in early hypoxia, while non angiogenic IRESs such as EMCV or c-*myc* IRES are activated in late hypoxia. Furthermore, the FGF1 IRES is also activated in ischemic heart in vivo, in a mouse model of infarcted myocard [46]. IRES-dependent translation in ischemic myocard thus allow a rapid angiogenic response participating in cardiomyocyte survival. These data enlighten the strong pathophysiological impact of IRES-dependent translation to stimulate tumoral and non tumoral (lymph)angiogenesis in response to hypoxia (Figure 1)

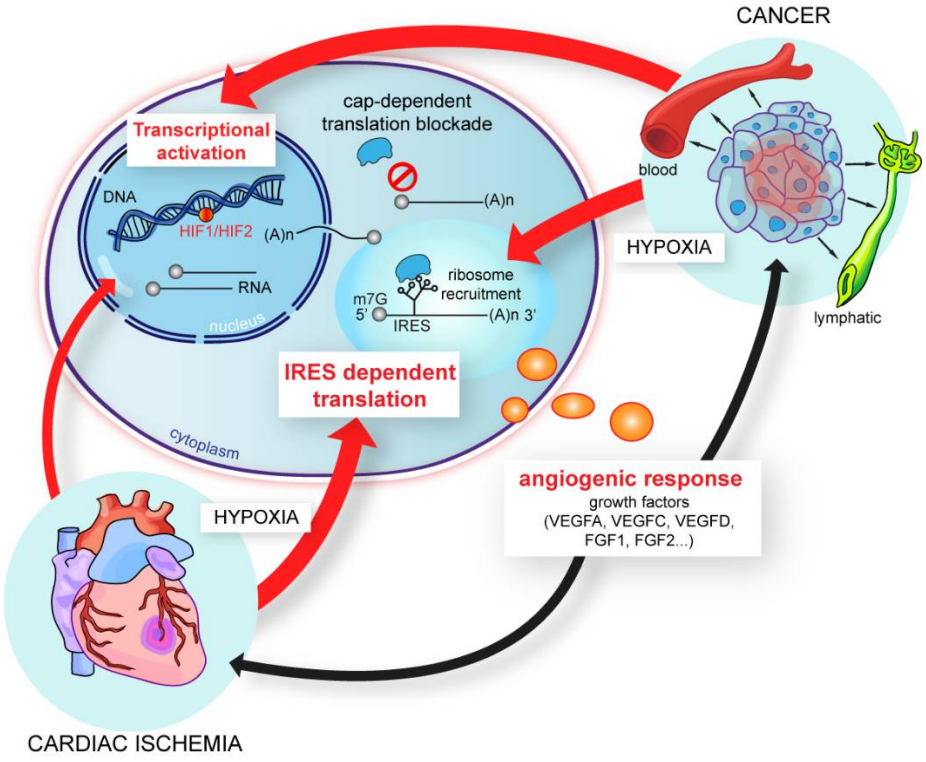

Figure 1. Regulation of (lymph)angiogenic growth factor expression during hypoxia. (Lymph)angiogenic growth factors are regulated at the transcriptional and/or translational levels during hypoxia. In conditions of tumoral hypoxia, regulation is both transcriptional and translational through the IRES-dependent mechanism, whereas during cardiac ischemia, in hypoxic cardiomyocytes, most regulation is translational. IRESs of (lymph)angiogenic growth factor mRNAs are activated during early hypoxia by an HIF1-independent mechanism.

2. IRES trans-acting factors, key regulators of cellular IRESs.

Most IRESs, and in particular cellular IRESs, require IRES trans-acting factors (ITAFs) to function, in addition to several canonical translation initiation factors. Around fifty proteins have been described for their ability to specifically regulate cellular IRESs, while a single long non coding RNA (lncRNA), TP53-regulated modulator of p27 (TRMP), is also able to regulate IRES-dependent translation (Table 1)[47].

A near-exhaustive bibliographic analysis of ITAFs controlling cellular IRESs has been performed here, revealing several classes of ITAFs. The largest class is composed of nuclear proteins able to shuttle from nucleus to cytoplasm to control IRES-dependent translation. This class contains many heterogeneous nuclear ribonucleoproteins (hnRNPs), but also other proteins such as nucleolin, HuR or p54^{nrb}. A second ITAF class is composed of translation machinery associated proteins, with ribosomal and ribosome-associated proteins as well as translation initiation or elongation factors. Other cytoplasmic or membrane-associated proteins have been regrouped in a third class but they share no documented common feature except for being an ITAF. For instance Upstream of N-ras (Unr) is a cytoplasmic cold shock protein which is also associated to the endoplasmic reticulum, hepsin is a plasmic membrane-associated protein able to control the Unr mRNA IRES, while vasohibin 1 (VASH-1) is a mostly cytoplasmic and secreted protein known for its anti-angiogenic and stress resistance features before being identified as an ITAF (Table 1). A fourth ITAF class contains at the moment a single member, the 834 nucleotide lnc RNA TRMP, inhibitor of the p27kip IRES [47]. The discovery of an ITAF function exhibited by a lncRNA is very recent, thus one can expect that TRMP is probably not the only lncRNA to regulate IRES-dependent translation, as many lncRNAs could serve as assembly platforms for regulatory proteins. Interestingly, TRMP is an inhibitor of the p27kip IRES, and is a direct transcriptional target of p53, itself regulated at the IRES-dependent level by sixteen reported ITAFs (Table 1).

Table 1. An update of reported ITAFs that regulate cellular IRESs. The different reported ITAFs regulating cellular IRESs are indicated. They are dispatched into four classes (see text). For each ITAF are shown the regulated IRESs, the type of regulation (activator or inhibitor), the described stimuli able to trigger their activity, the roles in cell physiology and diseases as well as the corresponding references.

ITAF	Also known as	Regulated IRESs	Regulation	Stimulus	Roles in cell physiology and diseases	References
Class I: ITAFs with nucleocytoplasmic translocation						
Annexin A2		p53	activator	ER stress	cancer	[72]
CUGBP1	CELF1	SHMT-1, p27kip	inhibitor/ activator	UV irradiation	DNA repair, cell proliferation	[85,86]
DAP5	P97, NAT1, eIF4GII	Bcl-2, Bcl-XL, BAX, APAF-1, DAP5, Δ40p53, CDK1, HIAP2, c-*myc*, XIAP	activator	viral infection, apoptosis, ER stress, serum starvation, g-irradiation	cell survival or programmed cell death	[75,77,87–94]
FUS		LEF1	activator		Cancer, amyotrophic lateral sclerosis	[95]
GRSF1		c-*myc*, L-*myc*, N-*myc*	activator		cancer	[96]
H-ferritin		SHMT-1	activator	UV irradiation	DNA repair	[85,97]
HDMX		p53	activator	DNA damage	tumour suppression	[98]

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hnRNPA1		XIAP, FGF2, Nfil3, SREBP1-a, c-*myc*, BCL-XL, cyclin D1, APAF-1, sst2, ER-α HIF1-α	activator/ inhibitor	FGF2, lipid accumulation, ER stress, osmotic shock, UV irradiation	multiple myeloma, circadian oscillation	[55,56,58,61, 62,99–104]
hnRNPC	hnRNP C1/C2	p53, IGF1R, unr, c-*myc*, XIAP	activator	DNA damage, transcription inhibition, growth stimulus, cell cycle	inhibition of apoptosis, cancer	[74,105–108]
hnRNPD	JKTBP1	NRF	activator	UV irradiation	cell survival	[109,110]
hnRNPE	PCBP, alphaCP	c-*myc*, BAG-1	activator	Chemotoxic stress	cell survival, tumorigenesis	[59,111,112]
hnRNPH2		SHMT1	activator	UV irradiation	DNA repair	[85]
hnRNPK		c-myc	activator		myoblast differentiation, proliferation, tumor progression	[111,113]
hnRNPL		Cat-1, p53, LINE-1	activator	Amino-acid deprivation/ DNA damage	transposition inhibition	[114–116]
hnRNPM		FGF1	activator	myoblast differentiation	muscle regeneration	[76]
hnRNPQ	NSAP1	p53, rev-erb-a, Period1, AANAT, Bip, FMRP	activator	apoptosis/ heat shock	circadian oscillation/ cell survival/ axonal growth cone collapse/ Fragile X syndroma, autism	[117–122]
hnRNPR		AANAT	activator		circadian oscillation	[123]
HuR	ELAV1	IGF1R, caspase-2, BcL-XL, XIAP, p27kip, Thrombomodulin	activator/ inhibitor	amino-acid deprivation, IL-1b,	cytoprotection, inhibition of apoptosis, cell proliferation, breast cancer	[74,124–129]
La auto antigen		XIAP, Bip, RRBP1	activator/ inhibitor	serum starvation, paclitaxel, adriamycin	cell survival, malignancy maintenance, hepatocellular carcinoma	[130–132]
Mdm2	HDM2	p53, XIAP	activator	DNA damage, ionizing radiation	resistance to radiation-induced apoptosis	[98,133]
NF45		iIAP1, XIAP, NRF, ELG	activator	ER stress	polyploidy, senescence	[134]
nPTB		IR	activator	cell density, insulin	cell proliferation	[135]

nucleolin		p53, VEGFD, LINE-1	activator/ inhibitor	heat shock, DNA damage	transposition inhibition	[14,115,136, 137]
p54nrb	NONO	c-*myc*, L-*myc*, N-myc, APAF1, FGF1	activator	myoblast differentiation, nucleolar stress, apoptosis	muscle regeneration	[76,96,138]
Pdcd4		P53, INR, IGF1R, BcL-XL, XIAP	activator/ inhibitor	oxidative stress, absence of DNA damage, S6K2 inactivation, FGF2 pathway inhibition	apoptosis, tumour suppression	[64–67]
PSF	SFPQ	p53, c-*myc*, L-*myc*, N-*myc*, BAG-1, LEF1	activator/ inhibitor	nucleolar stress, apoptosis, ER stress	cancer	[72,95, 96,138]
PTB	hnRNPI/ PTBP1	p53, p27kip, PFK1, IR, Cat-1, APAF-1, HIF-1α, IRF2, rev-erb-a, unr, c-*myc*, N-*myc*, BAG-1, Bip, ADAR1	activator/ inhibitor	DNA damage, hypoxia, ER stress, amino-acid deprivation, cell density, insulin	circadian oscillation, cell cycle arrest, apoptosis	[50,70,73,96, 108,112,114, 139–147]
RHA	NDH II	p53	activator	DNA damage	tumour suppression	[148]
SMAR 1		p53	activator/ inhibitor	glucose deprivation	cancer (tumor suppressor)	[149]
YB1	YBX1	c-*myc*, L-*myc*, N-*myc*, p16INK4	activator	hypoxia	multiple myeloma, cancer	[96,140,150]
Class II: Cytoplasmic ITAFs related to translational machinery						
4E-BP1		VEGFA, HIF1a, INR	activator	hypoxia, low nutrients, low insulin	cancer, Parkinson	[44,151]
eeF1A2		utrophin A	activator		muscle regeneration	[152]
eIF4GI		APAF-1, DAP5, Bcl-2, Bip, c-*myc*, L-*myc*, N-*myc*, VEGFA	activator	apoptosis, hypoxia	cancer	[44,92, 146,153]
eL38	Rpl38	Hox	activator		development	[31]
eS19	Rps19	BAG1, CSDE1, LamB1	activator		erythroid differentiation, Diamond-Blackfan anemia	[154]
eS25	Rps25	APAF-1, BAG1, c-*myc*, L-myc, Myb, p53, Set7	activator	ER stress	multiple myeloma	[52,83,103]
Rack1		IGF1R	activator/ inhibitor		Hepatocellular carcinoma	[155]

TCP80	NF90, DRBP76	p53	activator	DNA damage	tumour suppression	[148]
uL1	Rpl10A	IGF2, APP, Chmp2A, Bcl-2	activator		Alzheimer, leukemia, mitochodrial dysfunction	[83,84]
uL24	Rpl26	p53	activator	DNA damage	tumour suppression	[136,137]
uL5	Rpl11	BAG1, CSDE1, LamB1	activator		erythroid differentiation, Diamond-Blackfan anemia	[154]
Class III: atypical cytoplasmic ITAFs						
APP (AICD)		Δ40p53	activator		Alzheimer disease	[156]
Hepsin (also in plasmic membrane)		unr	inhibitor		Cell cycle regulation, Prostate cancer	[157]
PINK1 (also mitochondrial)		HIF1	activator	hypoxia	Parkinson	[78]
Unr (also in ER)		APAF-1, unr, c-*myc*, PITSLRE, CDK11P58	activator/ inhibitor		Cell cycle regulation, apoptosis	[70,108,111, 158,159]
VASH1 (also secreted and nuclear)	Vasohibin 1	FGF1	activator	hypoxia	ischemic heart disease	Hantelys BioRxiv 2018
Class IV: ncRNA-constituted ITAFs						
TRMP		p27kip	inhibitor	induced by p53	inhibition of cell proliferation, tumor suppressor	[47]

It has been often reported that viral IRESs harbor specific secondary or tertiary structures with common domains while it is difficult to identify any structural conservation between different cellular IRESs [48,49]. Despite of this difference, most reported ITAFs seem to control IRES-dependent initiation of translation for both cellular and viral IRESs. A well-documented example is PTB (also known as hnRNPI), first described as an ITAF of the EMCV IRES: this protein is able to modulate translation of a dozen reported virus IRESs as well as at least fourteen cellular IRESs (Table 1) [50]. This is not limited to the nucleocytoplasmic ITAF class I. In the cytoplasmic ITAF class II, a good example is provided by the ribosomal protein rps25 (eS25), required for *discitroviridae*, *flaviviridae*, *picornaviridae* and *retroviridae* IRES activities, as well as for at least ten cellular IRESs (Table 1) [51–53]. In class III, Unr is able to regulate viral IRESs such as poliovirus and human rhinovirus IRESs, as well as at least five cellular IRESs [48]. These observations suggest that there is no major mechanistic difference of ITAF mode of action for viral and cellular IRESs. However, it has been reported that cellular IRESs are more tissue-specific than viral IRESs [26]. One can hypothesize that cellular IRESs require specific ITAFs in addition to "general" ITAFs. These specific ITAFs could regulate groups of mRNAs in a coordinated manner, thus defining regulons [31].

While several IRESs can be regulated by the same ITAF, each IRES can be regulated by several ITAFs, which may be positive or negative regulators. As shown in Table 1, we have listed twelve cellular ITAFs able to inhibit IRES dependent translation. Furthermore, nine of them have the double role of IRES activator or inhibitor, depending on the IRES. Among the best-documented IRESs regulated by several ITAFs are the p53 mRNA IRESs [54]. Two p53 IRESs have been described, controlling expression of either the full-length p53 (FL-p53) or of a p53 isoform devoid of N-terminal domain, ΔN-p53. These two IRESs are induced by genetoxic or cytotoxic stress. In basal non-stressed conditions, the IRES activity is inhibited by two negative ITAFs, nucleolin and Programmed cell death protein 4 (Pdcd4), whereas two other ITAFs, translational control protein 80 (TCP80) and RNA helicase A (RHA) are bound to RNA but with an inadequate interaction that cannot activate the IRES. Following stress, the interaction of TCP80 and RHA is increased and several other positive ITAFs including ribosomal protein RPL26 (uL24) and hnRNPQ bind to the IRES, facilitating secondary structure unwinding and enhancing IRES activity. The ΔN-p53 mRNA IRES is activated during stress by several other ITAFs including PTB, death-associated protein 5

(DAP5), PTB-associated splicing factor (PSF) and Annexin A2 [54]. In addition, proteins bound to the 3'UTR of the FLp53 mRNA also influence the IRES activity: the protein Quaking has an inhibitory effect on the IRES activity while HuR binds to the 3'UTR during stress, displaces Quaking and activates translation. It is likely that many IRESs, as well as p53 IRESs, are regulated by a protein complex rather than by a single ITAF. The composition of this complex, called the IRESome, varies among IRESs, and is probably a means to regulate IRES activity specifically. The presence of different partners in the complex may also help us to understand why a given ITAF can be either negative or positive depending on the IRES, as shown for at least nine ITAFs (Table 1).

3. Multifunctional ITAFs: how are they assigned to the translational function?

Strikingly, ITAFs have often other functions in addition to their role in IRES-dependent translation. Most of them have been first discovered for playing roles in alternative splicing (hnRNPs), ribosome biogenesis (nucleolin, TCP80, RHA), mRNA stability (HuR), transcription ($p54^{nrb}$, hnRNPK, -M, RHA, SMAR1)… The question of how they are assigned to their translational function remains to be investigated. However several reports provide some answers. The first one is the intracellular localization. Numerous multifunctional ITAFs are mainly nuclear proteins that can translocate in the cytoplasm. A well-documented example is hnRNPA1 [55–58]: this protein is relocalized to the cytoplasm in stress conditions, resulting in IRES negative or positive regulations. HnRNPA1 activates FGF2 and sterol regulatory element-binding protein 1 (SREBP-1) IRESs while it inhibits APAF1 and XIAP IRESs. Such a relocalization has been reported for other ITAFs, including PTB and poly r(C) binding protein 1 (PCBP1) that act in concert to activate the Bcl-2-associated athanogene 1 (BAG1) IRES in response to chemotoxic stress [59]. Also, nucleolin is translocated from nucleolus to cytoplasm to activate the VEGFD IRES in response to heat shock [14].

ITAF activity is also regulated by various post-translational modifications. This was first demonstrated for RNA-binding motif protein 4 (RBM4), an ITAF described at the moment only for viral IRESs [60]. Following arsenite exposure, RBM4 is phosphorylated, which accompanies its cytoplasmic relocalization and targeting to stress granules. When phosphorylated, RBM4 both inhibits cap-dependent translation and activates IRES-dependent translation. As regards cellular IRESs, hnRNPA1 constitutes a well-documented example for the role of post-translational modifications: its binding to c-myc and cyclin D IRESs is regulated by Akt phosphorylation [61]. Furthermore, hnRNPA1 dimethylation on its glycine-arginine-rich (GAR) motif by the type II arginine transferase PRMT5 is required for activation of cyclin D1, c-myc, HIF1α and estrogen receptor α (ER-α) IRESs [62]. Another ITAF described more recently, the tumor suppressor Pdcd4, is phosphorylated by protein kinase S6K1 or Akt and subsequently degraded via the ubiquitin ligase β-TCRP [63–66]. Pdcd4, as RBM4, inhibits cap-dependent translation, while it is a negative or positive ITAF depending on the IRES: it is a repressor of p53, BcL-XL and XIAP and an activator INR and IGF1R IRESs [64,66,67]. Multiple post-translational modifications have been described for hnRNPK, a protein overexpressed in many cancers [68]. This multifunctional protein is subjected to

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phosphorylation, methylation, ubiquitination and sumoylation while it interacts with diverse groups of molecular partners involved in transcription, chromatin remodeling, RNA processing, translation and signal transduction [69]. hnRNPK sumoylation on a lysine residue promotes its ITAF function and results in activation of the c-*myc* IRES in Burkitt's lymphoma cells [68].

4. ITAF different mechanisms of action

We have seen above that ITAF activities are regulated by different parameters including nucleocytoplasmic shuttling, post-translational modifications and interaction with diverse partners. The question is now: by which mechanisms are ITAFs able to activate or inhibit IRES-dependent translation? As described below, nine ITAF mechanisms have been documented (Figure 2).

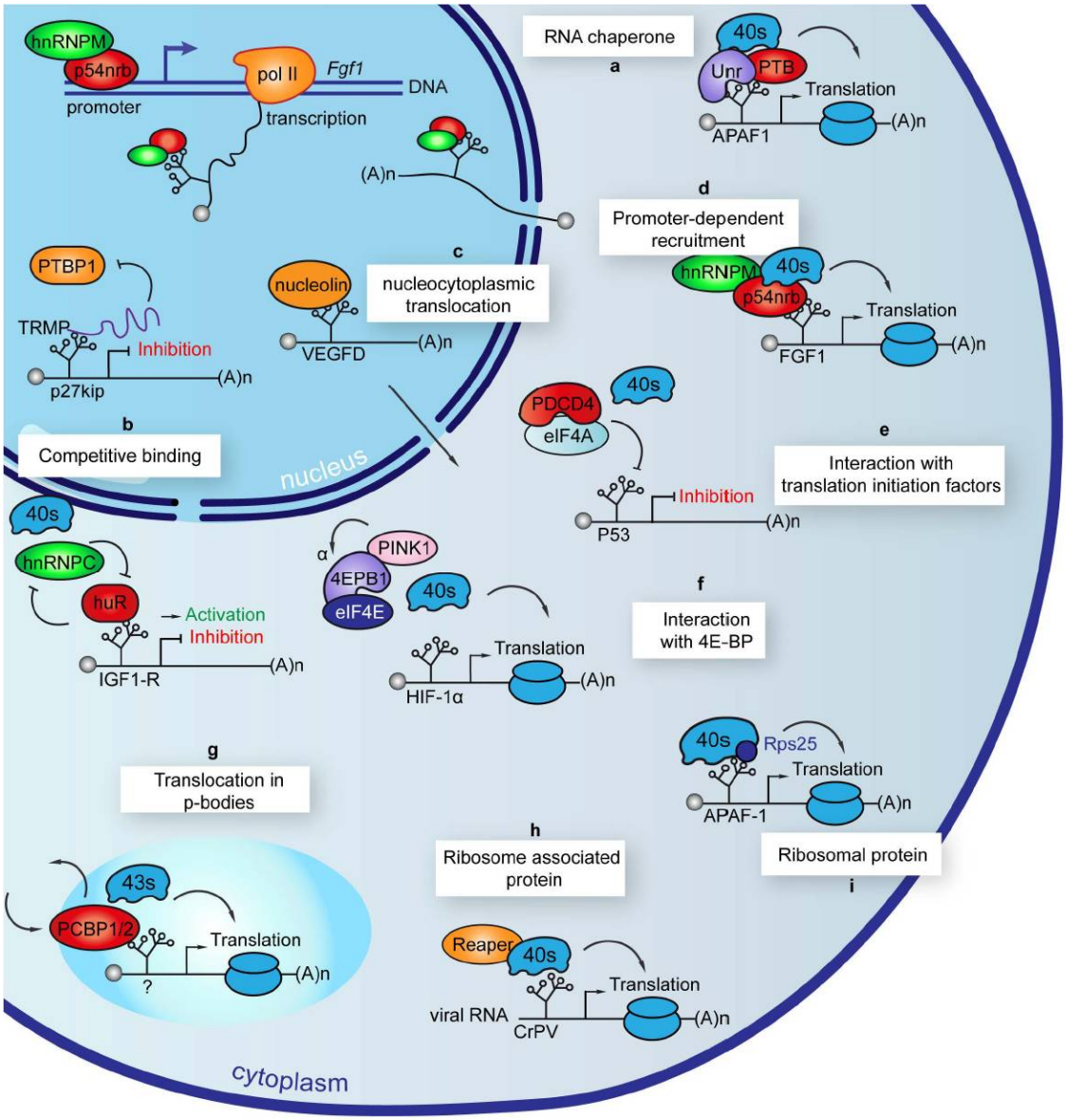

Figure 2. ITAF different mechanisms of action. The different reported mechanisms of ITAFs to regulate IRES activities are schematized. For each mechanism, an example is shown with the names of the ITAF and of the IRES. Each mechanism is detailed in the text.

4.1. Chaperones

The first mechanism to be described is a role of chaperone for PTB (and especially its neuronal form nPTB) and Unr [70]. These two proteins are required for activation of the APAF1 IRES, and act

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by altering the secondary structure of the IRES. According to the report by Mitchell et al., Unr first bind to two stem loops identified in the IRES, generating a conformational change that renders accessible the nPTB or PTB binding sites [70]. Then a second conformational change occurs, providing the correct conformation for 40S ribosome subunit binding. Cooperation of two or more ITAFs in IRES activation through an RNA conformational change has been described for other IRESs: the BAG-1 IRES is also controlled by a couple of ITAFs, PTB and PCBP1 [71]. Again, there is a successive binding of the two ITAFs, with first PCBP1 that opens the RNA, allowing PTB binding and subsequent 40S recruitment. In these study, PTB appears as an essential part of the preinitiation complex.

4.2. Competitive binding

The interplay between different ITAFs can be competitive rather than cooperative: it is the case of Annexin A2, PSF, and PTB [72]. Annexin A2 and PSF would act as chaperones or by stabilizing the preinitiation complex as shown for PTB. These three ITAFs are all activators of the second IRES present in the p53 mRNA, between FL-p53 and ΔN-p53 AUG codons [54]. However they compete for IRES binding as they share overlapping binding sites. Annexin A2 binding is calcium dependent whereas PSF binding is not. The authors propose that the accumulation of more calcium ions in the cytoplasm during ER stress would promote Annexin A2 binding to activate the IRES activity, whereas PSF and PTB would play a role in other stress conditions or physiological stimuli. Actually, it has been proposed that PTB regulates the differential expression of p53 isoforms during the cell cycle, and in response to DNA damage [73]. Competitive binding has also been reported for couples of ITAFs harboring opposite activities. HuR and hnRNPC compete for their binding to the IGF1R IRES, which is silenced by HuR and activated by hnRNPC [74]. The lncRNA TRMP inhibits the $p27^{kip}$ IRES activity by competing with the IRES for PTB binding and thus preventing IRES activation mediated by PTB [47].

4.3. Nucleocytoplasmic translocation

The role of nucleocytoplasmic translocation of many ITAFs in IRES activation (Table 1) does not answer the question of ITAF nuclear or cytoplasmic binding. Actually, the ITAF can be translocated to the cytoplasm upon stress and then bind to the IRES-containing mRNA, or it can bind to its target IRES in the nucleus and then be translocated with the IRES-containing mRNA as a ribonucleoprotein. In such a case the ITAF can also play a role in the nuclear retention of the IRES-containing mRNA in the absence of stress [75]. Clearly, the regulation of APAF1 IRES by successive binding of Unr and PTB suggests that PTB binds to this IRES in the cytoplasm, because Unr is cytoplasmic. In contrast, a set of arguments indicates that certain ITAFs bind to the IRES in the nucleus. First, a nuclear event is required for IRES-dependent translation controlled by certain IRESs: this has been shown for c-*myc* and FGF1 IRESs by demonstrating that these IRESs are not able to drive translation when cells are transfected with a bicistronic in vitro-transcribed mRNA, while the same IRESs are active upon DNA transfection implying mRNA transcription in the nucleus [23,76]. In contrast viral HRV and EMCV IRESs exhibit a similar activity following RNA or DNA transfection, showing that the nuclear event is not required for all IRESs.

4.4. Promoter-dependent recruitment

A second argument favoring the existence of nuclear recruitment of ITAFs onto the IRES is brought by the discovery of a mechanism of coupling between translation and transcription for the FGF1 IRES [29]. The activity of FGF1 IRES is promoter-dependent, a mechanism explained by ITAF recruitment onto the promoter that facilitates the recruitment on the mRNA. These two ITAFs, hnRNPM and p54nrb, are able to enhance both transcription and translation: first they activate the FGF1 promoter, then the FGF1 IRES-dependent translation. The proposed hypothesis is that the two ITAFs might be recruited onto the nascent mRNA in a co-transcriptional manner [76].

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4.5. Interaction with translation initiation factors

Additional ITAF mechanisms of action have been discovered, that strictly occur in the cytoplasm during the translation initiation process. Several ITAFs act by inhibiting translation initiation factors. RBM4 was shown to interact with the initiation factor 4A (eIF4A) in response to arsenite treatment, which promotes the association of eIF4A with the IRES-containing mRNA [60]. By this way, RBM4 simultaneously activates IRES- and inhibits cap-dependent translation. Interestingly, this is concomitant with RBM4 targeting into stress granules. It must be noted that RBM4 has not yet been shown to regulate any cellular IRES. However, an interaction with eIF4A and with eIF2β has been shown for DAP5, an ITAF of the eIF4G family that regulates several cellular IRESs of genes involved in apoptosis as well as its own IRES [77]. Another ITAF acting via eIF4A interaction is the tumor suppressor Pdcd4 [65]. Its interaction with eIF4A was demonstrated by crystal structure and mutation analysis, whereas it also interacts directly with the IRES. Pdcd4 inhibits cap-dependent translation as RBM4. However, in contrast to RBM4, Pdcd4 has been described as a negative ITAF [64]. This reveals that cellular IRESs are also concerned by eIF4A binding mechanism, as Pdcd4 controls at least the five IRESs present in p53, INR, IGF1R, BcL-XL and XIAP mRNAs (Table 1).

4.6. Interaction with 4E–BP

PTEN-induced putative kinase-1 (PINK1), involved in Parkinson's disease, activates the HIF1α mRNA translation during hypoxia by acting on 4E-BP1 [44,78]. It has been shown that PINK1 stimulates the switch of 4E-BP hyperphosphorylated g form (inactive form) to the hypophosphorylated a form (active form) that sequesters eIF4E and inhibits the cap-dependent translation, while it activates IRES-dependent translation by increasing the availability of eIF4G for IRES-dependent translation. PINK1 acts on 4E-BP1 as well as on 4E-BP2, the predominant 4E-BP protein in brain. The activator effect of PINK1 has been shown only for EMCV IRES, however the decrease of HIF1α mRNA translation in $PINK^{-/-}$ mouse strongly suggests that PINK1 is also on activator of the HIF1α IRES [78]. The authors do not rule out that PINK1 could affect the activity of other translation factors such as S6K, eIF4E, eIF4G, eEF2 of eIF2α.

4.7. Translocation to P-bodies

Translocation between cytoplasm and processing bodies (P-bodies) has been described for PCBP1 and PCBP2 upon stress conditions [79]. These authors suggest that PCBPs could play a role in shifting rapidly certain untranslated mRNAs into a translationally active state. However the link between PCBP this translocation and IRES-dependent translation has not been elucidated yet.

4.8. Association to ribosome

Another cytoplasmic mechanism of IRES regulation concerns ribosome-associated proteins. Reaper, a potent apoptosis inducer, inhibits cap-dependent translation by direct binding to the 40S ribosome subunit, while it allows IRES-dependent translation to occur via the Cricket paralysis (CrPV) IRES [80]. Although Reaper has not yet been documented for its effect on cellular IRESs, one can hypothesize that certain cellular IRESs may also be regulated by this mechanism.

4.9. Ribosome inherent constituent.

Finally, it appears that ribosomal proteins can be directly involved in the control of IRES-dependent translation. The ribosome has been viewed during the last decades as an apparatus able to translate the genetic code without having intrinsic regulatory capacity. However, several reports have shifted the view of ribosome function by revealing the existence of specialized ribosomes with specific features rendering them able to control gene expression ([81]. The first demonstration of a ribosomal protein that is specifically required for IRES-mediated translation initiation, while not necessary for cap-dependent translation, was provided by Landry et al. [82]. These authors showed that rps25 (eS25) is required for activation of CrPV and hepatitis C virus (HCV) IRESs. Additional studies demonstrated that this protein is globally required for viral IRES as well as for cellular IRES

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activities. Rps25 is an activator of many cellular IRESs including APAF-1, BAG1, c-*myc*, L-*myc*, Myb, p53 and Set7 IRESs (Table 1). Other ribosomal proteins seem to regulate families of messengers, thus defining regulons. It has been documented in a report showing that RPL38 (eL38) is required for ribosome recruitment onto IRESs of the hox gene family, constituted of homeobox genes involved in development [31]. A recent report has definitely demonstrated that heterogeneous ribosomes are able to preferentially translate distinct subpools of mRNAs [83]. This study highlights the role of RPL10A (uL1) in the activation of IGF2, amyloid precursor protein (APP), charged multivesicular body protein 2A (Chmp2A) and Bcl-2 IRESs [83,84]. Such IRES activation would occur by direct interaction of the ribosomal protein with the IRES, resulting in ribosome recruitment.

5. Conclusion

This update highlights the discovery of about fifty ITAFs able to regulate IRES-dependent translation of cellular mRNAs. This indicate that the control of gene expression by the IRES-dependent process is far from marginal. These different ITAFs play a key role in many physiological processes including development, cell differentiation, cell cycle regulation, apoptosis or circadian oscillation. Furthermore, they are pivotal in the cell response to all possible stress conditions (Table 1). Given that ITAFs regulate expression of families of genes involved in these processes, they have a strong impact in different pathologies. ITAFs are important actors in many cancers, but also in cardiovascular diseases such as ischemic heart disease and neurodegenerative diseases including Parkinson's disease, Alzheimer disease or amyotrophic lateral sclerosis. Thus ITAFs provide important perspectives to find new targets to block translation of specific genes or gene networks in a therapeutic objective.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

IRES	Internal ribosome entry site
ITAF	IRES trans-acting factor
UTR	Untranslated region
PTB	Pyrimidine tract binding protein
ER	Endoplasmic reticulum
FGF	Fibroblast growth factor
VEGF	Vascular endothelial growth factor
XIAP	X-linked inhibitor of apoptosis
APAF1	Apoptotic peptidase activating factor 1
HIF	Hypoxia-inducible factor
mTOR	Mechanistic target of rapamycin
4E-BP	4E binding protein
HRI	Haem-regulated inhibitor kinase
PERK	PKR-like endoplasmic reticulum kinase
PKR	Protein kinase RNA
GCN2	General control non-derepressible 2
GCN4	General control non-derepressible 4
uORF	Upstream open reading frame
ATF4	Activating transcription factor 4
eIF	Eukaryotic initiation factor
EMCV	Encephalomyocarditis virus
TRMP	TP53-regulated modulator of p27
hnRNP	Heterogeneous nuclear ribonucleoprotein
Unr	Upstream of N-ras
VASH-1	Vasohibin 1
BAG1	Bcl-2-associated athanogene 1
SREBP-1	sterol regulatory element-binding protein 1

RBM4	RNA-binding motif protein 4
BiP	Immunoglobulin heavy-chain binding protein
Pdcd4	Programmed cell death protein 4
TCP80	translational control protein 80
RHA	RNA helicase A
DAP5	Death-associated protein 5
PSF	PTB-associated splicing factor
PRMT5	Protein arginine methyltransferase
PCBP	poly r(C) binding protein
IGF1R	insulin growth factor-like receptor
PINK1	PTEN-induced putative kinase-1
CrPV	Cricket paralysis virus
APP	amyloid precursor protein
Chmp2A	charged multivesicular body protein 2A
HCV	hepatitis C virus
CUGBP1	CUG triplet repeat RNA-binding protein 1
SHMT-1	serine hydroxymethyltransferase 1
NAT-1	N-acétyltransferase 1
ER	Estrogen receptor
CDK1	Cyclin-dependent kinase 1
HIAP2	Human inhibitor of apoptosis 2
FUS	Fused in sarcoma
LEF1	Lymphoid enhancer binding factor 1
GRSF1	G-rich RNA sequence binding factor 1
AANAT	arylalkylamine N-acyltransferase
FMRP	fragile X mental retardation protein
RRBP1	ribosome binding protein 1
NSAP	nephritis strain-associated protein
ELAV	embryonic lethal abnormal vision
ELAV1	ELAV-like protein 1
NRF	NFKB repressing factor
IR	Insulin receptor
ELG	Elongatus
CSDE1	Cold shock domain containing E 1
ADAR1	Adenosine deaminase RNA specific 1
IRF2	Interferon regulatory factor 2
Hox	Homeobox

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