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F-box motif encoding genes as targets for the development of stress-tolerant traits in *Saccharomyces cerevisiae*

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Stress tolerance is a useful trait actively sought by the bioprocess industry for biofactories dealing with bioconversion of varied raw materials or carbon sources. Fungal or yeast species are useful in bioconversion and are sustainable bioresources for biochemicals and biofuel production. Genetic manipulation strategies are in practice to enhance the tolerance against stress agents for the improved bioconversion process. In this review, we highlight the importance of the F-box motif encoding genes and their interactions in imparting the stress tolerance phenotype to the yeast species. The F-box motif proteins constitute a part of the SCF-E3 ligase complex and are involved in the recruitment, and ubiquitination, followed by degradation of the substrate proteins by the 26S proteasome. It highlights the current scenario on the F-box motif encoding genes and their interaction partners as targets for the stress tolerance phenotype in the yeast and plant species and their utility in the bioconversion processes.

Keywords: Abiotic stress, Bioconversion, Brewer's Yeast, Gene-gene interactions, Stress tolerance, Ubiquitin-proteasome system (UPS)

The bioprocess industries are multibillion-dollar growing enterprises. The current and future sustainability goals also favor bio-based industries for the production of industrial biopharmaceuticals, green chemicals, and biofuels. Fungal strains, including *Saccharomyces cerevisiae* (Brewer's Yeast), have been very useful in the bioprocess industry. The fungal strains used in the industry face many challenges, such as byproduct toxicity, metabolic stresses, and changes in pH and oxygen levels in the surrounding of the biofactory, therefore, impacting the product yields and efficiency[1].

The bioprocess industry requires cost-effective technologies. The biological strains are the backbones of the bioprocess industry that convert substrates into products. To minimize the cost industries are in constant need of biological strains which exhibit stress-tolerant or resistance traits to toxic byproducts for the optimization of product generation for the industry. The industrially important *S. cerevisiae* yeast species[2] requires the development of the stress tolerance phenotypes which are the most sought traits for the bioprocess industry[3].

There are varieties of methods in use for the generation of the stress-tolerant strains, such as isolation from the extreme environment[4], experimental evolution[5], one-step mutagenesis[6,7], and cross-kingdom competition[7,8]. The yeast strains isolated from the extreme environment are unsuitable for direct use in the industrial bioprocess as the industrial environment is quite complex compared to the environment. The utility of such strain in the complex industrial environment requires genetic modification or synthetic biology tools. However, such tools have their limitation and host limitations. The stress tolerance phenotype is considered as polygenic trait and unearthing of genetic determinants needed multiple approaches[1,3,5,9-11].

Here, in this review, we provide evidence and propose that the F-box motif encoding genes in the combination of other genetic determinants could be attractive targets for the development of stress-tolerant traits in the yeast, fungi, and plant species.

F-box motif and molecular function

The F-box protein motif is observed in the proteins of the eukaryotic origin from yeast to humans, that is approximately 50 amino acid long conserved stretch at the N-terminus region[12]. The F-box motif was first observed in the cyclin F protein of the *S. cerevisiae*[13]

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which forms the part of the SCF E3 ligase complex of the ubiquitin-proteasome system (UPS)[12]. The SCF E3 ubiquitin ligases are a tetrameric complex (Fig. 1) which includes, Skp1 (S-phase kinase-associated protein 1), Cul1 (Cullin 1) adaptor protein, RING proteins as a scaffold protein, and F-box substrate recognizing protein, where F-box motif interacts with the Skp1[14]. The number of genes encoding the F-box motif is enormous in the eukaryotic kingdom and their numbers vary according to the species[15]. The genome of *Arabidopsis thaliana* encodes almost 700 F-box motif proteins, *C.elegans*, *500* F-box motif proteins, *Drosophila melanogaster* 33 F-box motif proteins, *Homo sapiens* 69 F-box motif protiens and *S.cerevisiae*, 21 F-box proteins (Table 1)[16]. These F-box motif proteins regulate several biological processes through catabolic action (Fig. 2), such as glucose metabolism, DNA replication, cell cycle process, protein degradation, circadian clock regulation, genome stability regulation, and stress response[17].

The F-box motif-containing proteins through their C terminus protein-protein interaction domain recruit the phosphoproteins for ubiquitination[18,19]. Based on the observed protein-protein interaction domain at the C terminus region, the F-box motif proteins of *S. cerevisiae* are grouped into three categories: FBXL class containing leucine-rich repeats (LRR), FBXW class containing tryptophan-aspartic acid (WD) repeats, and FBXO class containing other protein-protein interaction domains (Fig. 3)[15]. The targets of

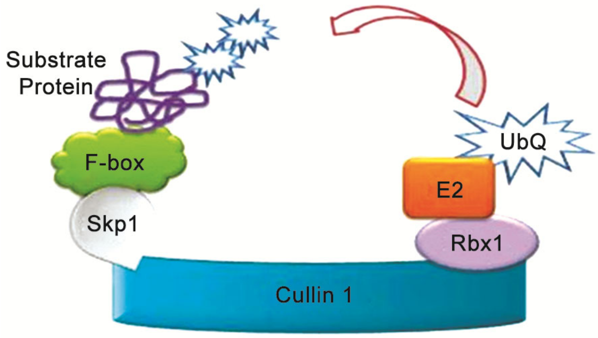


Fig. 1 — Schematics of the tetrameric complex of SCF-E3 ligase. [The F-box motif protein interacts with the Skp1 of the complex through the N-terminal F box motif whereas the C–terminus domain recruits the substrate proteins for ubiquitination]

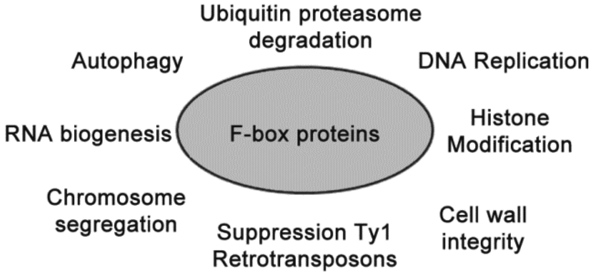
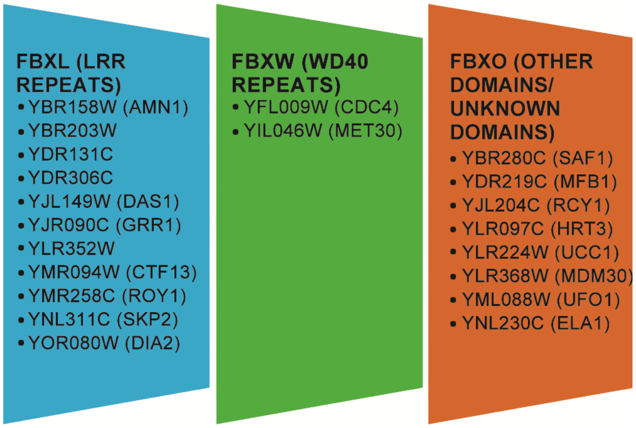


Fig. 2 — Role of F-box motif proteins in the indicated biological processes. [The F-box proteins have been implicated in the biological process which determines the response to the stress thus indirectly involved in the stress response]



FBXL (LRR REPEATS)
- YBR158W (AMN1)
- YBR203W
- YDR131C
- YDR306C
- YJL149W (DAS1)
- YJR090C (GRR1)
- YLR352W
- YMR094W (CTF13)
- YMR258C (ROY1)
- YNL311C (SKP2)
- YOR080W (DIA2)

FBXW (WD40 REPEATS)
- YFL009W (CDC4)
- YIL046W (MET30)

FBXO (OTHER DOMAINS/ UNKNOWN DOMAINS)
- YBR280C (SAF1)
- YDR219C (MFB1)
- YJL204C (RCY1)
- YLR097C (HRT3)
- YLR224W (UCC1)
- YLR368W (MDM30)
- YML088W (UFO1)
- YNL230C (ELA1)

Fig. 3 — The F-box motif proteins of *Saccharomyces cerevisiae* with C terminus protein-protein (Leucine-rich repeat, LRR, WD40 and other domain) interaction domains.

Table 1 — List of F-box motif encoding genes of *Saccharomyces cerevisiae* and associated gene ontology terms.

F-box motif genes	Gene ontology terms	Associated genes	GO ID
UFO1, *MFB1*, *PFU1*, *CDC53CDC34*, *DAS1*, *YDR131C*, *SAF1*, *ROY1*, *HRT3*, *ELA1*, *SKP2*, *MDM30*, *CTF13*, *UCC1*, *RCY1*, *MET30*, *CDC4*,*GRR1*, *DIA2*, *AMN1*	SCF-dependent proteasomal ubiquitin-dependent protein catabolic process	*UCC1*, *AMN1*, *DIA2*, *DAS1*, *CDC34*, *MDM30*, *PFU1*, *MET30*, *HRT3*, *UFO1*, *GRR1*, *SAF1*, *CDC4*, *YDR131C*, *CDC53*, *MFB1*	GO:0031146
	Response to methylmercury	*UCC1*, *HRT3*, *UFO1*, *GRR1*	GO:0051597
	Organonitrogen compound metabolic process	*CDC34*, *DAS1*, *DIA2*, *AMN1*, *UCC1*, *MET30*, *MDM30*, *PFU1*, *ELA1*, *SAF1*, *HRT3*, *UFO1*, *SKP2*, *GRR1*, *YDR131C*, *MFB1*, *CDC53*, *CDC4*	GO:1901564

[Source: SGD]

the many F-box motif proteins of each species remain to be identified for the understanding of their detailed role in the biological process.

F-box proteins and Stress tolerance

The common function of the majority of F-box proteins in the eukaryotic kingdom is to remove the specific target proteins after attaining the function or upon signal transduction. The deletion or over expressions of each of the F-box motif encoding genes have varied phenotypes ranging from alteration of cell morphology to stress tolerance both in yeast and fungi (Fig. 4).

During industrial processes such as fermentation, fungal strains are subjected to many environmental stresses viz. oxidative, osmotic, and nutrient stress. This stress can lead to damage to macromolecules and alteration in the gene transcriptional program to adapt to the environmental changes for survival. For adaptation to the environmental changes, yeast cells apply a variety of stress-tolerant mechanisms such as alteration of transcriptional pattern of the genes involved in the stress response, changes in the concentration of stress protectants, alteration of membrane composition, post-translation modification of proteins, and inhibition of the translational mechanisms[20-22].

Biological processes such as the ubiquitin-mediated protein degradation process have been associated with stress tolerance in plant species[23,24] and yeast[20]. The SCF E3 ligases have been implicated in the regulation of stress response as well[25]. The SCF E3 ligase complex component, F-box proteins are associated with the stress tolerance in the phenotype in the *S. cerevisiae*. However, the deletion of the F-box motif encoding gene and other biological process-related genes together could also contribute to the stress tolerance phenotype albeit their utility in the industrial process needs to be investigated.

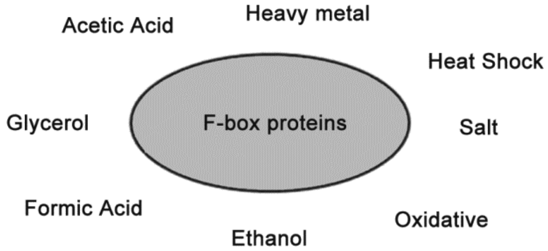


Fig. 4 — F-box proteins and stress agents. [The deletion of the F-box motif encoding gene or overexpression is implicated in the tolerance to the indicated strains]

Genetic interactions of F-box motif encoding genes and stress response

The ablation of two genes together when results in different phenotypes compared to single-gene deletion indicates a functional relationship between both the genes which is termed genetic interaction[26]. The gene-gene interactions at the global level have been instrumental in assigning the role of genes in the various biological processes[27]. F-box motif proteins impact various biological processes, such as DNA Replication[28,29], metabolism[30], and stress response[31]. The stress adaptation or tolerance requires the rewiring of the genetic networks in the absence of key molecules as the cell has an alternate robust mechanism to deal with the stress condition. The recent studies from our group[29,32,33] and others[34] indicate the F-box motif gene as an attractive target for the stress tolerance phenotype. The study by Sharma *et al.*[32] shows that key transcriptional regulator *RLM1* genetically interacts with the F-box motif encoding gene *SAF1* to regulate the stress response and genome stability. In this study, the authors show that ablation of both the genes (*SAF1* and *RLM1*) together results in a stress tolerance phenotype in the *S. cerevisiae* which could be important for industrial application. In another study by Pandita *et al.*[29], the F-box motif protein Ucc1, characterized as the glyoxylate pathway regulator encoded by *UCC1* genetically interacts with the *SRO9* which encodes a protein product, that is involved in the RNA biogenesis[35]. The genetic interaction between *UCC1* and *SRO9* regulates the response to oxidative, alternate carbon metabolism stress, and growth rate. The study also shows that the absence of both the genes together contributes to the stress resistance. Another study by Shoket *et al.*[33] shows that genetic interactions between F-box motif encoding *YDR131C* and *RTG1* which encodes for a protein involved in the retrograde signaling, regulates the response to oxidative, genotoxic and cell wall stressors. In the study, the authors show that ablation of both the genes together (*YDR131C* and *RTG1*) contributes to the stress tolerance phenotype. The stress tolerance traits in the strains lacking the F-box motif gene and interaction partners are further supported by the induced apoptosis assay in both the studies[29,33] (Table 2).

The F-box motif proteins or genes are emerging as attractive targets for the stress tolerance phenotype as indicated in recent studies (Table 2). Based on recent

Table 2 — Recent literature on the role of F-box proteins in stress response in (A) Yeast; and (B) Plants

Name of Gene and Species	Stress Tolerance	PMID
(A) Yeast		
SAF1/*S. cerevisiae*	Cell wall and Osmo stress	34627408, 34627408
YDR131C/*S. cerevisiae*	Osmo and genotoxic stress	34309121, 34309121
UCC1/*S. cerevisiae*	-do-	33797855
Met30/*S.cerevisiae*	Heavy metal Stress	32817489
Fbp1/*C. neoformans*	SDS Stress	21478432
Dia2/*S.cerevisiae*	Genotoxic stress	23172854, 16751663
(B) Plants		
At5g15710/*Arabidopsis*	Salt and Drought stress	34655724
AT2G16220/*Arabidopsis*	Arsenic stress tolerance	33340971
At1g08710/*Arabidopsis*	Drought stress	33360542
LTSF1 and *LTSF2/Capsicum chinense*	Low-temperature stress tolerance	32933000
SaFbox40, SaFbox51, SaFbox136 and SaFbox170/*Sedum alfredii*	Cadmium stress tolerance	33542339
TaFBA1, *Triticum aestivum*	Heat stress tolerance, Oxidative stress	29740462, 25906259
MdMAX2/*Arabidopsis*	General stress response	27909441

[Source: PUBMED]

emerging literature and the detailed role of F-box motif proteins in the regulation of various biological processes, It is proposed that they could be studied for the development of the stress-tolerant phenotypes in the industrially important fungal species.

Conclusion

The stress tolerance traits development in the industrially important yeast or fungal species is the most desired research endeavor for the bio-based industries. We propose that the F-box motif encoding genes along with their interaction partners can be an attractive target for the development of stress-tolerant traits in the industrially important yeast or fungal species. The approach could be either transitional genetic manipulations or the CRISPER/Cas9 genome editing tools. Further, the strains obtained can be optimized for product generation through the fermentation process or can be utilized for research purposes.

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Conflict of Interest

Authors declare no competing interests.

References

1 Qiu X, Zhang J, Zhou J, Fang Z, Zhu Z, Li J & Du G, Stress tolerance phenotype of industrial yeast: industrial cases, cellular changes, and improvement strategies. *Appl Microbiol Biotechnol*, 103 (2019) 6449.

2 Kong, II, Turner TL, Kim H, Kim SR & Jin YS, Phenotypic evaluation and characterization of 21 industrial *Saccharomyces cerevisiae* yeast strains. *FEMS Yeast Res*, 18 (2018).

3 Deparis Q, Claes A, Foulquié-Moreno MR & Thevelein JM, Engineering tolerance to industrially relevant stress factors in yeast cell factories. *FEMS Yeast Res*, 17 (2017).

4 Mota MJ, Lopes RP, Delgadillo I & Saraiva JA, Microorganisms under high pressure--adaptation, growth and biotechnological potential. *Biotechnol Adv*, 31 (2013) 1426.

5 Swamy KBS & Zhou N, Experimental evolution: its principles and applications in developing stress-tolerant yeasts. *Appl Microbiol Biotechnol*, 103 (2019) 2067.

6 Yadav V, Shitiz K, Pandey R & Yadav J, Chlorophenol stress affects aromatic amino acid biosynthesis-a genome-wide study. *Yeast*, 28 (2011) 81.

7 Guaragnella N & Bettiga M, Acetic acid stress in budding yeast: from molecular mechanisms to applications. *Yeast*, 38 (2021) 391.

8 Krogerus K, Magalhães F, Vidgren V & Gibson B, Novel brewing yeast hybrids: creation and application. *Appl Microbiol Biotechnol*, 101 (2017) 65.

9 Shima J &Takagi H, Stress-tolerance of baker's-yeast (*Saccharomyces cerevisiae*) cells: stress-protective molecules and genes involved in stress tolerance. *Biotechnol Appl Biochem*, 53 (2009) 155.

10 Nakagawa Y, Seita J, Komiyama S, Yamamura H, Hayakawa M & Iimura Y, A new simple method for isolating multistress-tolerant semidominant mutants of *Saccharomyces cerevisiae* by one-step selection under lethal hydrogen peroxide stress condition. *Biosci Biotechnol Biochem*, 77 (2013) 224.

11 Treseder KK & Lennon JT, Fungal traits that drive ecosystem dynamics on land. *Microbiol Mol Biol Rev*, 79 (2015) 243.

12 Craig KL & Tyers M, The F-box: a new motif for ubiquitin dependent proteolysis in cell cycle regulation and signal transduction. *Prog Biophys Mol Biol*, 72 (1999) 299

13 Bai C, Richman R & Elledge SJ, Human cyclin F. *EMBO J*, 13 (1994) 6087.

14 Bai C, Sen P, Hofmann K, Ma L, Goebl M, Harper JW & Elledge SJ, Skp1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-Box. *Cell*, 86 (1996) 263.

15 Cenciarelli C, Chiaur DS, Guardavaccaro D, Parks W, Vidal M & Pagano M, Identification of a family of human F-Box proteins. *Curr Biol*, 9 (1999) 1177.

16 Kipreos ET & Pagano M, The F-box protein family. *Genome Biol*, 1 (2000) 3002.

17 Jonkers W & Rep M, Lessons from fungal F-box proteins. *Eukaryot Cell*, 8 (2009) 677.

18 Hermand D, F-box proteins: more than baits for the SCF? *Cell Div*, 1 (2006) 30.

19 Skowyra D, Craig KL, Tyers M, Elledge SJ & Harper JW, F-box proteins are receptors that recruit phosphorylated substrates to the Scf ubiquitin-ligase complex. *Cell*, 91 (1997) 209.

20 Takagi H, Molecular mechanisms and highly functional development for stress tolerance of the yeast *Saccharomyces cerevisiae*. *Biosci Biotechnol Biochem*, 85 (2021) 1017.

21 Brandt BA, Jansen T, Volschenk H, Görgens JF, Van Zyl WH & Den Haan R, Stress modulation as a means to improve yeasts for lignocellulose bioconversion. *Appl Microbiol Biotechnol*, 105 (2021) 4899.

22 Boothby TC, Mechanisms and evolution of resistance to environmental extremes in animals. *Evodevo*, 10 (2019) 30.

23 Kurepa J, Wang S, Li Y&Smalle J, Proteasome regulation, plant growth and stress tolerance. *Plant Signal Behav*, 4 (2009) 924.

24 Xu FQ & Xue HW, The ubiquitin-proteasome system in plant responses to environments. *Plant Cell Environ*, 42 (2019) 2931.

25 Baudouin-Cornu P & Labarre J, Regulation of the cadmium stress response through scf-like ubiquitin ligases: comparison between *Saccharomyces cerevisiae, Schizosaccharomyces pombe* and mammalian cells. *Biochimie*, 88 (2006) 1673.

26 Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Lucau-Danila A, Anderson K, Andre B, Arkin AP, Astromoff A, El-Bakkoury M, Bangham R, Benito R, Brachat S, Campanaro S, Curtiss M, Davis K, Deutschbauer A, Entian KD, Flaherty P, Foury F, Garfinkel DJ, Gerstein M, Gotte D, Guldener U, Hegemann JH, Hempel S, Herman Z, Jaramillo DF, Kelly DE, Kelly SL, Kotter P, LaBonte D, Lamb DC, Lan N, Liang H, Liao H, Liu L, Luo C, Lussier M, Mao R, Menard P, Ooi SL, Revuelta JL, Roberts CJ, Rose M, Ross-Macdonald P, Scherens B, Schimmack G, Shafer B, Shoemaker DD, Sookhai-Mahadeo S, Storms RK, Strathern JN, Valle G, Voet M, Volckaert G, Wang CY, Ward TR, Wilhelmy J, Winzeler EA, Yang Y, Yen G, Youngman E, Yu K, Bussey H, Boeke JD, Snyder M, Philippsen P, Davis RW & Johnston M, Functional profiling of the *Saccharomyces cerevisiae* Genome. *Nature*, 418 (2002) 387.

27 Tong AH, Lesage G, Bader GD, Ding H, Xu H, Xin X, Young J, Berriz GF, Brost RL, Chang M, Chen Y, Cheng X, Chua G, Friesen H, Goldberg DS, Haynes J, Humphries C, He G, Hussein S, Ke L, Krogan N, Li Z, Levinson JN, Lu H, Menard P, Munyana C, Parsons AB, Ryan O, Tonikian R, Roberts T, Sdicu AM, Shapiro J, Sheikh B, Suter B, Wong SL, Zhang LV, Zhu H, Burd CG, Munro S, Sander C, Rine J, Greenblatt J, Peter M, Bretscher A, Bell G, Roth FP, Brown GW, Andrews B, Bussey H & Boone C, Global mapping of the yeast genetic interaction network. *Science*, 303 (2004) 808.

28 Mimura S, Komata M, Kishi T, Shirahige K & Kamura T, Scf(Dia2) regulates DNA replication forks during S-phase in budding yeast. *Embo j*, 28 (2009) 3693.

29 Pandita M, Shoket H, Rakewal A, Wazir S, Kumar P, Kumar R & Bairwa NK, Genetic interaction between glyoxylate pathway regulator Ucc1 and La-motif-encoding Sro9 regulates stress response and growth rate improvement in *Saccharomyces cerevisiae. J Biochem Mol Toxicol*, 35 (2021) e22781

30 Nakatsukasa K, Nishimura T, Byrne SD, Okamoto M, Takahashi-Nakaguchi A, Chibana H, Okumura F & Kamura T, The ubiquitin ligase Scf(Ucc1) acts as a metabolic switch for the glyoxylate cycle. *Mol Cell*, 59 (2015) 22.

31 Chen R, Guo W, Yin Y & Gong ZH, A Novel F-Box protein Caf-Box is involved in responses to plant hormones and abiotic stress in pepper (*Capsicum Annuum L.*). *Int J Mol Sci*, 15 (2014) 2413.

32 Sharma M, Verma V& Bairwa NK, Genetic interaction between Rlm1 and F-box motif encoding gene Saf1 contributes to stress response in *Saccharomyces cerevisiae. Genes Environ*, 43 (2021) 45.

33 Shoket H, Pandita M, Sharma M, Kumar R, Rakwal A, Wazir S, Verma V, Salunke DB & Bairwa NK, Genetic interaction between F-box motif encoding Ydr131c and retrograde signaling-related Rtg1 regulates the stress response and apoptosis in *Saccharomyces cerevisiae. J Biochem Mol Toxicol*, 35 (2021) e22864.

34 Zhang YW, Brognard J, Coughlin C, You Z, Dolled-Filhart M, Aslanian A, Manning G, Abraham RT & Hunter T, The F box protein Fbx6 regulates Chk1 stability and cellular sensitivity to replication stress. *Mol Cell*, 35 (2009) 442.

35 Rother S, Burkert C, Brunger KM, Mayer A, Kieser A & Strasser K, Nucleocytoplasmic shuttling of the La motif-containing protein Sro9 might link its nuclear and cytoplasmic functions. *RNA*, 16 (2010) 1393.