[Frontiers in Bioscience 16, 1787-1796, January 1, 2011]

KSRP, many functions for a single protein

Paola Briata[1], Ching-Yi Chen[2], Matteo Giovarelli[3,4], Michela Pasero[3], Michele Trabucchi[5], Andres Ramos[6], Roberto Gherzi[1]

[1]Istituto Nazionale per la Ricerca sul Cancro (IST), 16132 Genova, ITALY, [2]Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, Alabama 35294, USA, [3]RNA Technology Laboratory, Centro di Biotecnologie Avanzate, 16132 Genova, Italy, [4]Universita' degli Studi di Genova, Largo R. Benzi, 10, 16132 Genova, Italy, [5]Howard Hughes Medical Institute, Department and School of Medicine, University of California, San Diego, 9500 Gilman Drive, Room 345, La Jolla, California 92093-0648, USA, [6]Division of Molecular Structure, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

TABLE OF CONTENTS

1. ABSTRACT

KSRP is a single-strand nucleic acids binding protein that affects RNA fate at multiple levels. KSRP modular structure and its complex pattern of post-translational modifications underpin the interaction with a wide spectrum of RNA target sequences, as well as with other RNA-binding proteins and molecular adaptors. These interactions are important to the regulation of different steps of mRNA metabolism and, in turn, modulate several aspects of cellular proliferation and differentiation. In this review we will discuss in detail KSRP ability to i) promote decay of labile mRNAs interacting with some components of the mRNA decay machinery and ii) favor the maturation of a select group of microRNA precursors.

2. INTRODUCTION

2.1. The origins.

In 1996 the Levens laboratory identified novel members of the far upstream element (FUSE)-binding protein family that were named FBP1, FBP2, and FBP3 (1). The FUSE is an AT-rich DNA element 1.7 Kb upstream of the c-myc oncogene promoter. The three members of the FBP family are able to bind to the FUSE and to up-regulate c-myc transcription (1). The inspection of the primary sequences of FBPs revealed the presence of four K homology (KH) motifs (named after their recurrence in the heterogeneous nuclear ribonucleoprotein K, see Figure 1) (2). In 1997 Black and co-workers cloned a cDNA coding for KSRP (KH-type splicing regulatory protein), a 75 KDa

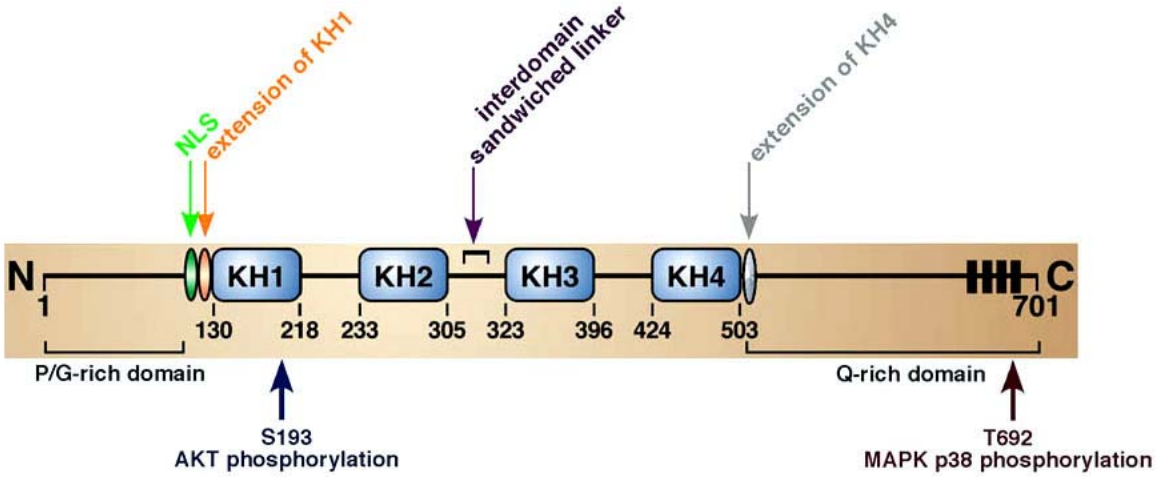

Figure 1. Schematic representation of the primary structure of human KSRP. NLS is for nuclear localization signal. S193 is the AKT1/2 phosphorylation site while T692 is the MAPK p38 phosphorylation site. Y-rich repeats in the C-terminal region are indicated as vertical bars.

component of a multi-protein complex that binds to an intronic splicing enhancer element downstream of the neuron-specific c-src N1 exon (3, 4). FBP2 and KSRP turned out to be the same protein (henceforth indicated as KSRP). The human gene encoding KSRP (called KHSRP) has been mapped to chromosome 19p13.3 (5). As depicted in Figure 1, the amino-terminus of the protein encompasses a low complexity proline-glycine (PG)-rich region, a putative a-helix that is involved in protein-protein recognition, and a nuclear localisation signal. The central region comprises four KH domains, two of which have additional secondary structure elements (see below). The carboxy-terminal part of the protein contains four Y-rich repeats important to up-regulate c-myc transcription (1). Finally, experimentally validated post-translational modification sites have been found in both KH domain 1 (KH1) and in the C-terminal part of the protein and have been shown to regulate regulate its functions (see section 3).

2.2. Discovery of additional functions.

In 2000 Greeve and coworkers purified KSRP as an activating component of the APOBEC-1 cytidine deaminase-containing complex that is responsible for the apoB mRNA editing (6). In 2002, the Kindler laboratory demonstrated that MARTA1, a protein that interacts with the cis-acting dendritic targeting element in the 3' untranslated region (3'UTR) of microtubule-associated protein 2 mRNA, is the rat homologue of KSRP (7, 8). Additional homologues of KSRP in Xenopus Laevis and chicken (VgRBP71 and ZBP2, respectively) were identified as effectors of subcellular localization of specific mRNAs (9, 10). More recently, KSRP was found to interact with the internal ribosomal entry site of the Enterovirus 71 and to negatively regulate viral translation (11).

Altogether, the above findings indicate that KSRP is a versatile RNA-binding protein that is implicated in several biological functions. In the next chapters we will focus on two recently discovered additional functions of KSRP: i) its ability to favor the decay of inherently labile mRNAs which has been by far the most extensively investigated function; ii) its ability to promote miRNA precursor maturation which is an exciting mode of KSRP action to post-transcriptionally control gene expression.

3. KSRP AND mRNA DECAY CONTROL

mRNA stability varies considerably from one mRNA species to another and plays an important role in determining levels of gene expression (12, for a recent review). Differential mRNA decay rates are determined by specific cis-acting elements within the mRNA molecule. The AU-rich element (ARE) is the most common cis element responsible for rapid mRNA decay in mammalian cells and can be found in the 3' UTRs of short-lived transcripts encoding cytokines, chemokines, transcription factors, proto-oncogenes, and cell-cycle regulators (13, 14). It is now clear that AREs may account for degradation of most unstable mRNAs and that the regulation of mRNA half-life plays a crucial role in the control of gene expression (15). Numerous proteins have been described to bind AREs (ARE-binding protein (ARE-BP), (16)). Some are mRNA decay-promoting factors while others are stabilizing factors. In addition, the function of some of these proteins in destabilizing or stabilizing mRNAs is dependent on the cellular context and/or the expressed protein isoforms (16, 17).

KSRP was shown to be an ARE-BP that specifically and directly binds to ARE-containing mRNAs and recruits the exosome (a multi-subunit protein complex with 3'-5' endonucleolytic activity) and other enzymes necessary to mRNA degradation, to AREs-containing mRNAs (18-20). Subsequently, many laboratories further demonstrated that KSRP is required for rapid decay of several ARE-containing mRNAs both *in vitro* and *in vivo* (19, 21-29) with the third and fourth KH domains (KH3 and KH4, Figure 1) mediating high-affinity binding to the AREs (19).

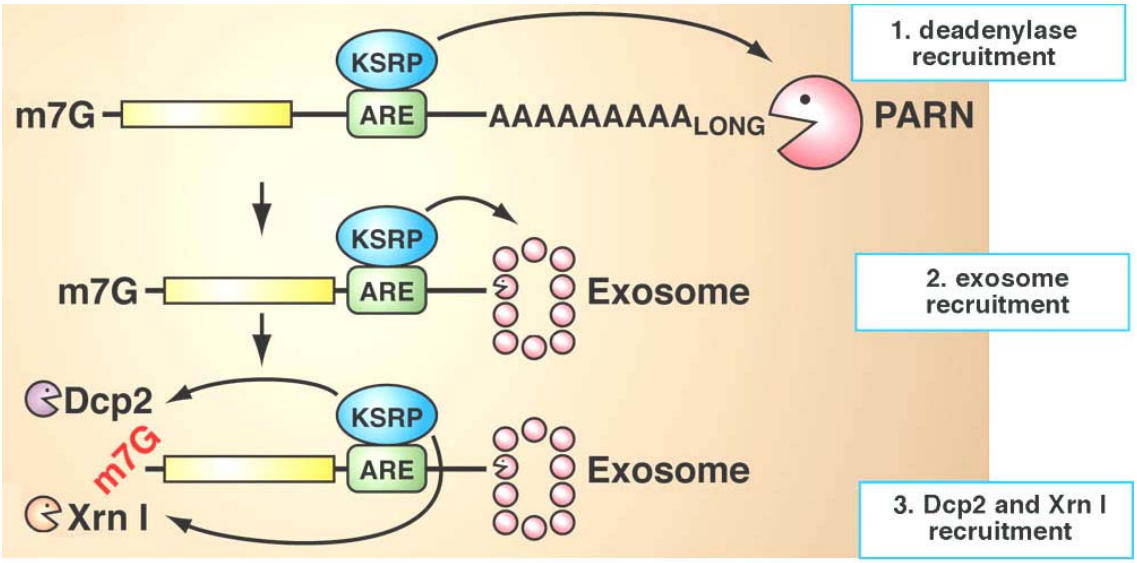

Figure 2. KSRP recruits to the ARE-containing mRNAs the enzymatic machines responsible for the decay of inherently unstable transcripts.

KSRP was found to form a complex with mRNA decay enzymes, including the poly (A) RNase PARN, the exosome component EXOSC2 (also known as RRP4), and the decapping enzyme DCP2 (Figure 2) (18-20). Importantly, it has been shown that two separate regions of KSRP are able to elicit mRNA decay. Both the KH domains and sequences residing in KSRP C-terminal region are required for efficiently triggering decay of mRNA (20). Indeed, immunoprecipitation experiments revealed that KSRP deletion mutants containing KH1–3 or the C terminus associate with mRNA decay enzymes. While experimental evidence suggests that KSRP and the exosome associate via protein-protein interactions (in a RNA-independent way (18)), it is still unknown which exosome subunit (s) contact KSRP. Furthermore, it is not yet clear which region (s) of KSRP interacts with the decay-promoting enzymes and whether all the interactions are mediated by direct contacts or takes place in the context of a larger protein complex.

In vitro studies have indicated that the isolated KH domains of KSRP bind to AU-rich sequences with low affinity (Kd > 100 microM) and that simultaneous binding of at least two KH domains is necessary to obtain Kd values in the nanomolar-to-micromolar range (19, 30). Indeed, although isolated KH3 moderately elicits mRNA decay in transfected cells, efficient degradation requires additional KH domains (either KH2 or KH4) together with KH3 (20).

Adding a further layer of complexity, structural analysis of the four KH domains of KSRP by NMR suggested that KH1 and KH4 have beta-strand additions to the canonical KH fold. These elements, that are outside the KH domain nucleic acid binding groove, are potentially available for protein-protein interactions (30, 31). Further, the arrangement of the KH2 and KH3 domains creates a negatively charged groove that could be involved in protein-protein contacts (32). Interestingly, phosphorylation of KH1 leads to domain unfolding and creates a site for 14-3-3 adaptor protein (31). These data suggest that the KH domains not only mediate ribonucleic acid interactions but also are capable of interacting with other proteins and that KSRP function in ARE-mediated mRNA decay (AMD) may be modulated by a number of associated factors.

4. EXTRACELLULAR SIGNALS CONTROL mRNA DECAY RATES TARGETING KSRP

RNA-binding proteins are often subjected to post-translational modifications - including phosphorylation - that link signals from the extracellular environment to various gene regulation pathways (33-39). At least two signaling pathways, the MAPK p38 and the Akt/PKB, have been shown to target decay-promoting ARE-BPs including TTP, BRF1, and KSRP that modulate AMD (24-26, 35, 40, 41).

The analysis of KSRP primary structure revealed the presence of several potential phosphorylation sites. At least two of them (Thr 692 and Ser 193) have been proved to be involved in regulation of KSRP function in AMD (24, 25, 32). We showed that the p38 MAPK phosphorylates KSRP at Thr 692 upon serum withdrawal-induced activation at early phases of myoblast differentiation (24). This phosphorylation impairs the ability of KSRP to interact with select ARE-containing “myogenic“ transcripts encoding myogenin and p21 (24). This event results in a 2 to 3-fold enhanced mRNA stability and, in turn, enhanced steady-state expression of these two important regulators of myotube formation. As a consequence, the differentiation of myoblasts is facilitated (24). Interestingly, KSRP is not the only ARE-BP implicated in myoblast differentiation as a role for HuR, another ARE-BP, has also been demonstrated in this process (42-44). Using a different

cellular system, Holtmann and coworkers demonstrated that IL-1 impairs the interaction of KSRP with the IL-8 AREs in a manner dependent on p38 MAPK (26).

As mentioned in section 2, phosphorylation by Akt at Ser 193 promotes the unfolding of the structurally atypical and unstable KH1 of KSRP, creating a site for 14-3-3zeta binding (31). Upon KH1-14-3-3zeta interaction, KSRP accumulates in the nucleus and this prevents its cytoplasmic functions in mRNA decay. Furthermore, phosphorylation of KSRP by Akt decreases its ability to associate with the exosome, whereby the decay of beta-catenin mRNA is inhibited by approximately 3 fold (25). Thus, KSRP phosphorylation creates a link between PI3K-Akt signaling and the control of cellular levels of beta-catenin whose overexpression has been related to the occurrence of several types of cancer (25, 45). Malbon and coworkers confirmed the importance of KSRP in the regulation of beta-catenin mRNA and protein levels demonstrating the relevance in this process of KSRP interaction with the signaling molecule Dishevelled (46). Nechama and coworkers recently reported that the peptidyl-prolyl isomerase Pin1 induces dephosphorylation of KSRP at S181. This event leads to activation of KSRP-dependent PTH mRNA decay (47) that has been previously proposed by the same group (28, 48).

Altogether, these findings strongly suggest that post-translational modifications of KSRP by phosphorylation play an important role in the control of its function in AMD that may be relevant to pathological processes. It would be interesting to investigate whether, in certain conditions, KSRP can be simultaneously phosphorylated by multiple kinases.

5. KSRP ACTS AS A POSITIVE CO-REGULATOR IN THE MATURATION OF A COHORT OF miRNA PRECURSORS

The evolutionarily-conserved mechanism by which primary miRNAs (pri-miRNAs) are processed first to precursor miRNAs (pre-miRNAs) and then to mature miRNAs involves two ordered endonucleolytic cleavages operated by multiprotein complexes including the RNase III enzymes Drosha and Dicer, respectively (49). Drosha cotranscriptionally processes the pri-miRNA into a ~70 nucleotides hairpin pre-miRNA (49). Through the interaction with exportin-5 and Ran-GTP, the pre-miRNA is transported into the cytoplasm, where it undergoes a second round of processing catalyzed by Dicer (49). This cleavage event gives rise to mature miRNAs that are loaded into the RNA Induced Silencing Complex (RISC) (49, 50). In the context of the RISC, miRNAs post-transcriptionally regulate the expression of target genes (49, 50).

Pioneer studies from some laboratories have shown that, at least for some miRNAs, maturation is a finely regulated process that involves co-regulators and that responds to the changing cellular conditions (51-55). We recently demonstrated that KSRP is able to modulate miRNA precursors processing (56). KSRP binds to the terminal loop (TL) of a cohort of miRNA precursors and interacts with both Drosha and Dicer, thus promoting the maturation of select miRNAs (56, 57). Interestingly, hnRNPA1, a molecular partner of KSRP (27) also binds to the TL of a group of pri-miRNAs which partly overlaps those interacting with KSRP (58). However, important differences exist in the function of the two proteins. Contrarily to hnRNPA1, KSRP functions not only in the nuclear maturation of pri-miRNAs into pre-miRNAs but also in the cytoplasmic maturation of pre-miRNAs into miRNAs thus representing a link between nuclear and cytoplasmic events. Indeed, we also obtained evidence that KSRP interacts with exportin-5 in a RNase A-sensitive way (56). Although further details on KSRP-exportin-5 interaction are missing, this observation suggests that KSRP association with the TL of target miRNA precursors is maintained during nucleo-cytoplasmic transit. This is consistent with the published structure of an exportin 5—pre-miRNA complex, the pre-miRNA terminal loop is exposed and free to make contact with additional protein partners (59). Recently, Caceres and coworkers proposed an antagonistic role of KSRP and hnRNPA1 in the regulation of pri-let-7a maturation into pre-let-7a (60).

Contrarily to KSRP, Lin-28 represses the maturation of let-7 family members and this effect is mediated by its direct interaction with the TL of let-7 precursors (61, 62). Some evidence exists to suggest a functional interplay between positive and negative regulators of pri-miRNA maturation. Although we do not have evidence supporting the idea that Lin-28 and KSRP recognize the same nucleotides in the TL of let-7 family precursors (56, 61, 62), our data suggest that when Lin-28 is expressed in undifferentiated P19 embryonic carcinoma cells, KSRP cannot interact with pri-let-7g. When Lin28 is not expressed, as in differentiated P19 cells or in undifferentiated P19 cells upon specific Lin-28 knockdown, KSRP is able to promote let-7g maturation (56).

We propose that the TL is a pivotal element where miRNA processing co-activators (e.g. KSRP) and miRNA processing co-repressors (e.g. Lin-28) function in a coordinated way to convey proliferating and differentiating cues into changes of miRNA expression. In other words, the occurrence of a co-activator and a co-repressor for regulation of miRNA maturation, extends the concept of opposing co-regulators, well established in transcriptional control (Figure 3).

6. KSRP-RNA TARGET SELECTIVITY

The multiple functions of KSRP depend on its specific recognition of the RNA targets that are characterized by significantly diverged sequences. Since they were first described, KH domains, which can accommodate only four nucleic acid bases in their binding clefts, have been found in proteins that perform a wide range of cellular functions (63). An important question in KSRP biology is how does the protein select its different RNA targets in order to perform functions as diverse as mRNA splicing, localization, decay, and miRNA maturation.

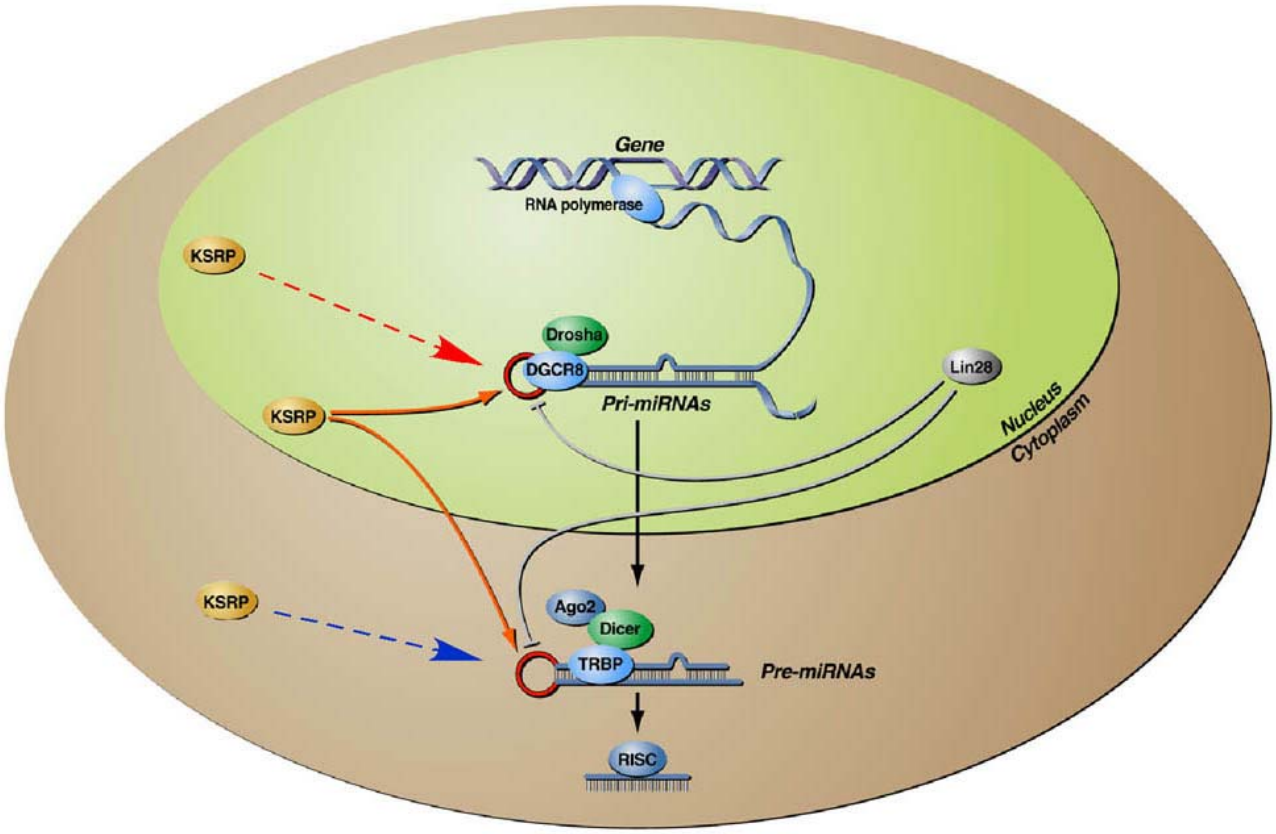

Figure 3. KSRP interacts with the TL of a class of miRNA precursors and participates in both nuclear Drosha and cytoplasmic Dicer complexes. As a consequence, the maturation of select miRNAs is favored. Lin-28 interacts with a partly overlapping group of miRNA precursors and inhibits their maturation. Open questions remain: 1. Does KSRP cotranscriptionally associate with the TL of target pri-miRNAs (red dashed arrow in the nucleus)? 2. Does KSRP participate in the functional organization of the RISC complex (blue dashed arrow in the cytoplasm)?

KSRP-RNA recognition depends both on the availability of single stranded RNA sequences and on the sequence selectivity of the KH domains of the protein (30, 64). Availability of single stranded sequences for KSRP binding is limited by the structure surrounding those sequences: enough space must be available within the RNA 3D structure for several covalently linked protein domains to bind simultaneously. Sequence specificity is different for different domains, although all four domains show a negative specificity for poly-C sequences (64). KH3 shows a strong selectivity towards G-containing sequences, with the affinity increasing proportionally to the number of Gs present within the domain binding site (64). The remaining three domains (KH1, KH2, and KH4) show only a moderate selectivity towards specific sequences (different for each different domain). The contribution of each domain to the binding is likely to depend on the structural context in which the single stranded target is embedded. If a G stretch is available (e.g. in the let-7a precursors), KH3 will dock on this motif, defining a binding frame for the other domains of the protein. However, if the RNA target does not include a run of Gs (e.g. in the miR-21 precursors), the four KH domains of KSRP will explore the available sequences in order to optimize their global binding affinity.

These binding modes also apply to KSRP recognition of the ARE sequences and could explain how the protein can recognize very different mRNA targets (27). Also in the case of ARE, the *in vivo* targeting of a subset of AU-rich elements by KSRP is probably a combination of the sequence preference of the domains (e.g. KH2 shows preference for AU sequences (64)), the structural context the targeted AU-rich sequence sequences are embedded in, their general propensity to be in a single stranded conformation, and the negative selectivity of the KH domains of KSRP for other repetitive sequences that share a single stranded propensity and could act as local competitors (e.g. polyC sequences).

In conclusion, we propose a general model for KSRP–RNA interactions based on the differential use of multiple domains that explains the adaptability of the protein to a broad range of single-strand RNA sequences (Figure 4). This concept is underscored by our recent demonstration that the inter-domain linker orients KH2 and

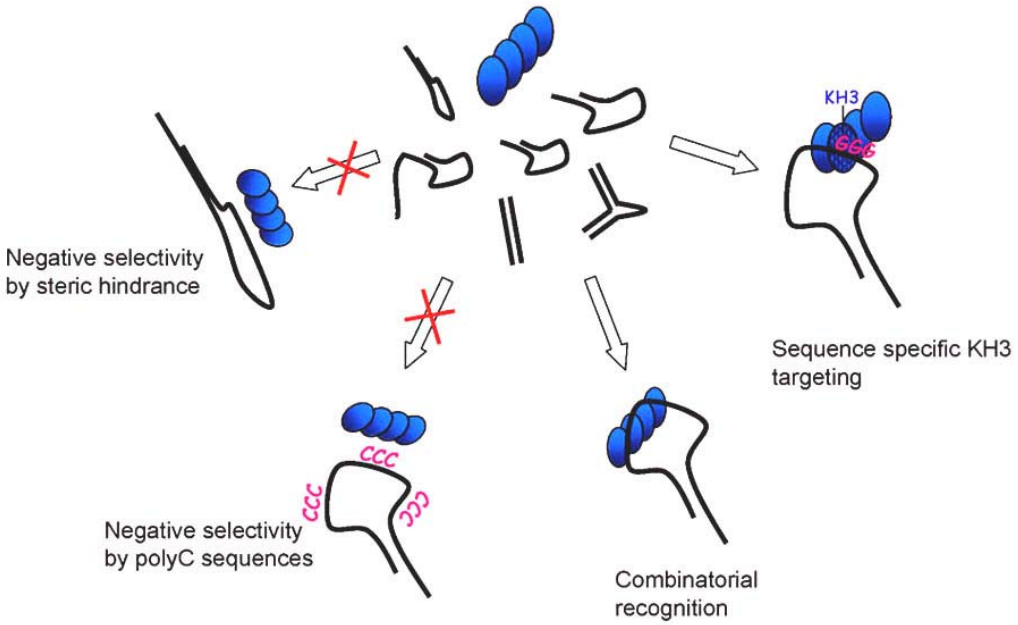

Figure 4. A model for KSRP binding to its diverse targets. The four KH domains of KSRP play different roles in RNA binding depending on the sequence of the targets (64). If a G-stretch is present, KH3 will dock onto it, defining the binding frame of the protein (sequence specific KH3 targeting). However, if the target does not contains such a stretch, the orientation of KH1, KH2/KH3 and KH4 can adapt to the structure of the target optimising the binding of multiple domains to single stranded regions (combinatorial recognition). KSRP can also select against RNA targets where the tertiary structure prevents a suitable arrangement of the multiple KH domains (negative selectivity by steric hindrance) as well as repetitive single stranded sequences that would otherwise be open to protein binding (negative selectivity by polyC sequences). The model shown here specifically refers to a hypothetical miRNA precursor terminal loop.

KH3 providing an arrangement that is important to the interaction between KSRP and its RNA targets (32).

7. PERSPECTIVES

Data reported in this review suggest a strong but still elusive implication of KSRP in fundamental cellular processes such as proliferation and differentiation. Others and we have demonstrated the role of KSRP in the signaling pathways that affects β-catenin expression controlling its mRNA decay (25, 46). Further, miR-155 is induced by LPS in macrophages in a KSRP-dependent way (57). Since LPS activates multiple signaling pathways, an important task of our future work will be to elucidate whether post-translational modification of KSRP regulate its ability to control maturation of specific miRNA precursors. Even though post-translational modification of TRBP, a component of the human miRNA-generating complex, has been recently reported (65), we do not know if KSRP-regulated miRNA maturation is affected by post-translational modifications. An important future challenge will be to systematically dissect pathways that modulate the function of KSRP (and possibly other RNA-binding proteins) in the regulation of miRNA biogenesis.

A role of miRNA biogenesis control in cell transformation and cancer has been suggested by a seminal study from Thomson and co-workers (66). Indeed, the importance of miRNA processing regulation for tumorigenesis has been experimentally proved by the notable global reduction of mature miRNAs observed in cancers (67) and by the consequences of Drosha, Dgcr8 or Dicer knockdown (68) and of a TRBP2 mutation (69). Some miRNAs whose maturation is controlled by KSRP have been implicated in cellular transformation. Consistent with its role in regulating cell proliferation and differentiation during development in different species, the deregulation of members of the let-7 family of miRNAs has been shown to be a feature of many types of cancer (70). Our data showed that KSRP knockdown limits cell proliferation in a let-7a-dependent way by influencing the expression of let-7a targets such as MYC and NRAS (56).

The multiple functions of KSRP and the complexity of its interactions with different proteins and divergent RNA sequences, as discussed above, make a compelling case for a study of the structure and the function of the different complexes in which the protein is involved. Major challenges will be to define the components of the different KSRP-containing ribonucleoprotein complexes, to identify RNA targets specific for each complex in which KSRP is implicated, and to pinpoint the defects associated with dysfunction (s) of KSRP and its protein partners. Therefore, advances in the biochemical isolation of protein complexes and the creation of animal models to investigate KSRP gain and loss of function will shed light on the different roles of KSRP in post-transcriptional regulation of gene expression and its implications in human disorders.

8. ACKNOWLEDGEMENTS

We apologize to colleagues whose work is not cited due to space limitations. The work in the laboratories of P.B. and R.G. has been supported by grants from Italian I.S.S. (# 527B/2B/6), AIRC, CIPE 2007, and Limonte 2010 (from the Regione Liguria and CBA) to R.G. The work on KSRP regulation in the laboratory of A.R. is partially supported by Wellcome Trust Grant WT082088MA and Medical Research Council Grant U117574558.

9. REFERENCES

1. T. Davis-Smyth, R.C. Duncan, T. Zheng, G. Michelotti, and D. Levens: The far upstream element-binding proteins comprise an ancient family of single-strand DNA-binding transactivators. *J Biol Chem*. 271, 31679-31687 (1996)

2. K. Bomsztyk, I. Van Seuningen, H. Suzuki, O. Denisenko, and J. Ostrowski: Diverse molecular interactions of the hnRNP K protein. *FEBS Lett*. 403, 113-115 (1997)

3. H. Min, C.W. Turck, J.M. Nikolic, and D.L. Black: A new regulatory protein, KSRP, mediates exon inclusion through an intronic splicing enhancer. *Genes Dev*. 11, 1023-1036 (1997)

4. M.P. Hall, S. Huang, and D.L. Black: Differentiation-induced colocalization of the KH-type splicing regulatory protein with polypyrimidine tract binding protein and the c-src pre-mRNA. *Mol Biol Cell*. 15, 774-786 (2004)

5. H.Z. Ring, V. Vameghi-Meyers, J.M. Nikolic, H. Min, D.L. Black, and U. Francke: Mapping of the KHSRP gene to a region of conserved synteny on human chromosome 19p13.3 and mouse chromosome 17. *Genomics* 56, 350-352 (1999)

6. H. Lellek, R. Kirsten, I. Diehl, F. Apostel, F. Buck, and J. Greeve: Purification and molecular cloning of a novel essential component of the apolipoprotein B mRNA editing enzyme-complex. *J Biol Chem*. 275, 19848-19856 (2000)

7. M. Rehbein, S. Kindler, S. Horke, and D. Richter: Two trans-acting rat-brain proteins, MARTA1 and MARTA2, interact specifically with the dendritic targeting element in MAP2 mRNAs. *Brain Res Mol Brain Res*. 79, 192-201 (2000)

8. M. Rehbein, K. Wege, F. Buck, M. Schweizer, D. Richter, and S. Kindler: Molecular characterization of MARTA1, a protein interacting with the dendritic targeting element of MAP2 mRNAs. *J Neurochem*. 82, 1039-1046 (2002)

9. T.T. Kroll, W.M. Zhao, C. Jiang, and P.W. Huber: A homolog of FBP2/KSRP binds to localized mRNAs in Xenopus oocytes. *Development* 129, 5609-5619 (2002)

10. W. Gu, F. Pan, H. Zhang, G.J. Bassell, and R.H. Singer RH: A predominantly nuclear protein affecting cytoplasmic localization of beta-actin mRNA in fibroblasts and neurons. *J Cell Biol*. 156, 41-51 (2002)

11. J.Y. Lin, M.L. Li, and S.R. Shih: Far upstream element binding protein 2 interacts with enterovirus 71 internal ribosomal entry site and negatively regulates viral translation. *Nucleic Acids Res*. 37, 47-59 (2009)

12. J. Houseley, and D. Tollervey: The many pathways of RNA degradation. *Cell* 136, 763-776 (2009)

13. K.S. Khabar: The AU-rich transcriptome: more than interferons and cytokines, and its role in disease. *J Interferon Cytokine Res*. 25, 1-10 (2005)

14. W. Eberhardt, A. Doller, el-S. Akool, and J. Pfeilschifter: Modulation of mRNA stability as a novel therapeutic approach. *Pharmacol Ther*. 114, 56-73 (2007)

15. S. Hao, and D. Baltimore: The stability of mRNA influences the temporal order of the induction of genes encoding inflammatory molecules. *Nat Immunol*. 10, 281-288 (2009)

16. N.L. Garneau, J. Wilusz, and C.J. Wilus: The highways and byways of mRNA decay. *Nat Rev Mol Cell Biol*. 8, 113-126 (2007)

17. A. Bevilacqua, M.C. Ceriani, S. Capaccioli, and A. Nicolin: Post-transcriptional regulation of gene expression by degradation of messenger RNAs. *J Cell Physiol*. 195, 356-372 (2003)

18. C.-Y. Chen, R. Gherzi, S.E. Ong, E.L. Chan, R. Raijmakers, G.J. Pruijn, G. Stoecklin, C. Moroni, M. Mann, and M. Karin: AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. *Cell* 107, 451-464 (2001)

19. R. Gherzi, K.Y. Lee, P. Briata, D. Wegmuller, C. Moroni, M. Karin, and C.-Y. Chen: A KH domain RNA binding protein, KSRP, promotes ARE-directed mRNA turnover by recruiting the degradation machinery. *Mol Cell* 14, 571-583 (2004)

20. C.F. Chou, A. Mulky, S. Maitra, W.J. Lin, R. Gherzi, J. Kappes, and C.-Y. Chen: Tethering KSRP, a decay-promoting AU-rich element-binding protein, to mRNAs elicits mRNA decay. *Mol Cell Biol*. 26, 3695-3706 (2006)

21. P. Briata, C. Ilengo, G. Corte, C. Moroni, M.G. Rosenfeld, C.-Y. Chen, and R. Gherzi: The Wnt/beta-catenin-->Pitx2 pathway controls the turnover of Pitx2 and other unstable mRNAs. *Mol Cell* 12, 1201-1211 (2003)

22. E.A. Suswam, L.B. Nabors, Y. Huang, X. Yang, and P.H. King: IL-1beta induces stabilization of IL-8 mRNA in malignant breast cancer cells via the 3' untranslated region: Involvement of divergent RNA-binding factors HuR, KSRP and TIAR. *Int J Cancer* 113, 911-919 (2005)

23. K. Linker, A. Pautz, M. Fechir, T. Hubrich, J. Greeve, and H. Kleinert: Involvement of KSRP in the post-

transcriptional regulation of human iNOS expression-complex interplay of KSRP with TTP and HuR. *Nucleic Acids Res*. 33, 4813-4827 (2005)

24. P. Briata, S.V. Forcales, M. Ponassi, G. Corte, C.-Y. Chen, M. Karin, P.L. Puri, and R. Gherzi: p38-dependent phosphorylation of the mRNA decay-promoting factor KSRP controls the stability of select myogenic transcripts. *Mol Cell* 20, 891-903 (2005)

25. R. Gherzi, M. Trabucchi, M. Ponassi, T. Ruggiero, G. Corte, C. Moroni, C.-Y. Chen, K.S. Khabar, J.S. Andersen, and Briata P: The RNA-binding protein KSRP promotes decay of beta-catenin mRNA and is inactivated by PI3K-AKT signaling. *PLoS Biol*. 5, e5 (2006)

26. R. Winzen, B.K. Thakur, O. Dittrich-Breiholz, M. Shah, N. Redich, S. Dhamija, M. Kracht, and H. Holtmann: Functional analysis of KSRP interaction with the AU-rich element of interleukin-8 and identification of inflammatory mRNA targets. *Mol Cell Biol*. 27, 8388-8400 (2007)

27. T. Ruggiero, M. Trabucchi, M. Ponassi, G. Corte, C.-Y. Chen, L. al-Haj, K.S. Khabar, P. Briata, and R. Gherzi: Identification of a set of KSRP target transcripts upregulated by PI3K-AKT signaling. *BMC Mol Biol*. 8, 28 (2007)

28. M. Nechama, I.Z. Ben-Dov, P. Briata, R. Gherzi, and T. Naveh-Many: The mRNA decay promoting factor K-homology splicing regulator protein post-transcriptionally determines parathyroid hormone mRNA levels. *FASEB J*. 22, 3458-3468 (2008)

29. H. Li, W. Chen, Y. Zhou, P. Abidi, O. Sharpe, W.H. Robinson, F.B. Kraemer, and J. Liu: Identification of mRNA binding proteins that regulate the stability of LDL receptor mRNA through AU-rich elements. *J Lipid Res*. 50, 820-831 (2009)

30. M.F. Garcia-Mayoral, D. Hollingworth, L. Masino, I. Diaz-Moreno, G. Kelly, R. Gherzi, C.F. Chou, C.-Y. Chen, and A. Ramos: The structure of the C-terminal KH domains of KSRP reveals a noncanonical motif important for mRNA degradation. *Structure* 15, 485-498 (2007)

31. I. Diaz-Moreno, D. Hollingworth, T.A. Frenkiel, G. Kelly, S. Martin, S. Howell, M.F. Garcia-Mayoral, R. Gherzi, P. Briata, and A. Ramos. Phosphorylation-mediated unfolding of a KH domain regulates KSRP localization via 14-3-3 binding. *Nat Struct Mol Biol*. 16, 238-246 (2009)

32. I. Diaz-Moreno, D. Hollingworth, G. Kelly, S. Martin, M. Garcia-Mayoral, P.Briata, R. Gherzi, and A. Ramos: Orientation of the central domains of KSRP and its implications for the interaction with the RNA targets. *Nucleic Acids Res*. Apr 12. (Epub ahead of print) PubMed PMID: 20385598 (2010)

33. G.M. Wilson, J. Lu, K. Sutphen, Y. Suarez, S. Sinha, B. Brewer, E.C. Villanueva-Feliciano, R.M. Ysla, S. Charles, and G. Brewer: Phosphorylation of p40AUF1 regulates binding to A + U-rich mRNA-destabilizing elements and protein-induced changes in ribonucleoprotein structure. *J Biol Chem*. 278, 33039-33048 (2003)

34. G. Stoecklin, T. Stubbs, N. Kedersha, S. Wax, W.F. Rigby, T.K. Blackwell, and P. Anderson: MK2-induced tristetraprolin:14-3-3 complexes prevent stress granule association and ARE-mRNA decay. *EMBO J*. 23, 1313-1324 (2004)

35. M. Schmidlin, M. Lu, S.A. Leuenberger, G. Stoecklin, M. Mallaun, B. Gross, R. Gherzi, D. Hess, B.A. Hemmings, and C. Moroni: The ARE-dependent mRNA-destabilizing activity of BRF1 is regulated by protein kinase B. *EMBO J*. 23, 4760-4769 (2004)

36. A. Pascale, M. Amadio, G. Scapagnini, C. Lanni, M. Racchi, A. Provenzani, S. Govoni, D.L. Alkon, and A. Quattrone A: Neuronal ELAV proteins enhance mRNA stability by a PKCalpha-dependent pathway. *Proc Natl Acad Sci U S A* 102, 12065-12070 (2005)

37. M. Brook, C.R. Tchen, T. Santalucia, J. McIlrath, J.S. Arthur, J. Saklatvala, and A.R. Clark: Posttranslational regulation of tristetraprolin subcellular localization and protein stability by p38 mitogen-activated protein kinase and extracellular signal-regulated kinase pathways. *Mol Cell Biol*. 26, 2408-2418 (2006)

38. S. Maitra, C.F. Chou, C.A. Luber, K.Y. Lee, M. Mann, and C.-Y. Chen: The AU-rich element mRNA decay-promoting activity of BRF1 is regulated by mitogen-activated protein kinase-activated protein kinase 2. *RNA* 14, 950-959 (2008)

39. H.H. Kim, K. Abdelmohsen, A. Lal, R. Jr. Pullmann, X. Yang, S. Galban, S. Srikantan, J.L. Martindale, J. Blethrow, K.M. Shokat, and M. Gorospe: Nuclear HuR accumulation through phosphorylation by Cdk1. *Genes Dev*. 22, 1804-1815 (2008)

40. H. Sandler, and G. Stoecklin: Control of mRNA decay by phosphorylation of tristetraprolin. *Biochem Soc Trans*. 36, 491-496 (2008)

41. S. Chiba, M. Tokuhara, E.H. Morita, and S. Abe: TTP at Ser245 phosphorylation by AKT is required for binding to 14-3-3. *J Biochem*. 145, 403-409 (2009)

42. K. van der Giessen, S. Di Marco, E. Clair, and I.E. Gallouzi: RNAi-mediated HuR depletion leads to the inhibition of muscle cell differentiation. *J Biol Chem*. 278, 47119-47128 (2003)

43. A. Figueroa, A. Cuadrado, J. Fan, U. Atasoy, G.E. Muscat, P. Muñoz-Canoves, M. Gorospe, and A. Munoz: Role of HuR in skeletal myogenesis through coordinate regulation of muscle differentiation genes. *Mol Cell Biol*. 23, 4991-5004 (2003)

44. R. Gherzi, M. Trabucchi, M. Ponassi, I.E. Gallouzi, M.G. Rosenfeld, and P. Briata: Akt2-mediated

phosphorylation of Pitx2 controls Ccnd1 mRNA decay during muscle cell differentiation. *Cell Death Differ*. 17, 975-983 (2010)

45. F.M. Watt, and C.A. Collins: Role of beta-catenin in epidermal stem cell expansion, lineage selection, and cancer. *Cold Spring Harb Symp Quant Biol*. 73, 503-512 (2008)

46. R.K. Bikkavilli, and C.C. Malbon: Dishevelled-KSRP complex regulates Wnt signaling through post-transcriptional stabilization of beta-catenin mRNA. *J Cell Sci*. 123, 1352-1362 (2010)

47. M. Nechama, T. Uchida, I. Mor Yosef-Levi, J. Silver, and T. Naveh-Many: Thepeptidyl-prolyl isomerase Pin1 determines parathyroid hormone mRNA levels and stability in rat models of secondary hyperparathyroidism. *J Clin Invest*. 119, 3102-3114 (2009)

48. M. Nechama, Y. Peng, O. Bell, P. Briata, R. Gherzi, D.R. Schoenberg, and T. Naveh-Many: KSRP-PMR1-exosome association determines parathyroid hormone mRNA levels and stability in transfected cells. *BMC Cell Biol*. 10, 70 (2009)

49. W. Filipowicz, S.N. Bhattacharyya, and N. Sonenberg: Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet*. 9, 102-114 (2008)

50. A.J. Pratt, and I.J. MacRae: The RNA-induced silencing complex: a versatile gene-silencing machine. J Biol Chem. 284, 17897-17901 (2009)

51. G. Obernosterer, P.J. Leuschner, M. Alenius, and J. Martinez: Post-transcriptional regulation of microRNA expression. *RNA* 12, 1161-1167 (2006)

52. S. Guil, and J.F. Caceres: The multifunctional RNA-binding protein hnRNP A1 is required for processing of miR-18a. *Nat Struct Mol Biol*. 14, 591-596 (2007)

53. S.R. Viswanathan SR, G.Q. Daley, and R.I. Gregory: Selective blockade of microRNA processing by Lin28. *Science* 320 97-100 (2008)

54. T. Fukuda, K. Yamagata, S. Fujiyama, T. Matsumoto, I. Koshida, K. Yoshimura, M. Mihara, M. Naitou, H. Endoh, T. Nakamura, C. Akimoto, Y. Yamamoto, T. Katagiri, C. Foulds, S. Takezawa, H. Kitagawa, K. Takeyama, B.W. O'Malley, and S. Kato: DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. *Nat Cell Biol*. 9, 604-611 (2007)

55. B.N. Davis, A.C. Hilyard, P.H. Nguyen, G. Lagna G, and A. Hata: Induction of microRNA-221 byplatelet-derived growth factor signaling is critical for modulation of vascular smooth muscle phenotype. *J Biol Chem*. 284, 3728-3738 (2009)

56. M. Trabucchi, P. Briata, M. Garcia-Mayoral, A.D. Haase, W. Filipowicz, A. Ramos, R. Gherzi, and M.G. Rosenfeld: The RNA-binding protein KSRP promotes the biogenesis of a subset of microRNAs. *Nature* 459, 1010-1014 (2009)

57. T. Ruggiero, M. Trabucchi, F. De Santa, S. Zupo, B.D. Harfe, M.T. McManus, M.G. Rosenfeld, P. Briata, and R. Gherzi R: LPS induces KH-type splicing regulatory protein-dependent processing of microRNA-155 precursors in macrophages. *FASEB J*. 23, 2898-2908 (2009)

58. G. Michlewski, S. Guil, C.A. Semple, and J.F. Caceres: Posttranscriptional regulation of miRNAs harboring conserved terminal loops. *Mol Cell* 32, 383-393 (2008)

59. C. Okada, E. Yamashita, S.J. Lee, S. Shibata, J. Katahira, A. Nakagawa, Y. Yoneda, and T. Tsukihara: A high-resolution structure of the pre-microRNA nuclear export machinery. *Science* 326, 1275-1279 (2009)

60. G. Michlewski, and J.F. Caceres: Antagonistic role of hnRNP A1 and KSRP in the regulation of let-7a biogenesis. *Nat Struct Mol Biol*. 17, 1011-1018 (2010)

61. M. Trabucchi, P. Briata, W. Filipowicz, M.G. Rosenfeld, A. Ramos, and R. Gherzi: How to control miRNA maturation? *RNA Biol*. 6, 536-540 (2009)

62. J. Winter, S. Jung, S. Keller, R.I. Gregory, and S. Diederichs: Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat Cell Biol*. 11, 228-234 (2009)

63. R. Valverde, L. Edwards, and L. Regan: Structure and function of KH domains. *FEBS J*. 275, 2712-2726 (2008)

64. M. Garcia-Mayoral, I. Diaz-Moreno, D. Hollingworth, and A. Ramos: The sequence selectivity of KSRP explains its flexibility in the recognition of the RNA targets. *Nucleic Acids Res*. 36, 5290-5296 (2008)

65. Z. Paroo, X. Ye, S. Chen, and Q. Liu: Phosphorylation of the human microRNA-generating complex mediates MAPK/Erk signaling. *Cell* 139, 112-122 (2009)

66. J.M. Thomson, M. Newman, J.S. Parker, E.M. Morin-Kensicki, T. Wright, and S.M. Hammond: Extensive post-transcriptional regulation of microRNAs and its implications for cancer. *Genes Dev*. 20, 2202-2207 (2006)

67. J. Lu, G. Getz, E.A. Miska, E. Alvarez-Saavedra, J. Lamb, D. Peck, A. Sweet-Cordero, B.L. Ebert, R.H. Mak, A.A. Ferrando, J.R. Downing, T. Jacks, H.R. Horvitz, and T.R. Golub: MicroRNA expression profiles classify human cancers. *Nature* 435, 834-838 (2005)

68. M.S. Kumar, J. Lu, K.L. Mercer, T.R. Golub, and T. Jacks: Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nat Genet*. 39, 673-677 (2007)

69. S.A. Melo, S. Ropero, C. Moutinho, L.A. Aaltonen, H. Yamamoto, G.A. Calin, S. Rossi, A.F. Fernandez, F. Carneiro, C. Oliveira, B. Ferreira, C.G. Liu, A. Villanueva, G. Capella, S. Jr. Schwartz, R. Shiekhattar, and M. Esteller: A TARBP2 mutation in human cancer impairs microRNA processing and DICER1 function. *Nat Genet*. 41, 365-370 (2009)

70. I. Bussing, F.J. Slack, and H. Grosshans: let-7 microRNAs in development, stem cells and cancer. *Trends Mol Med*. 14, 400-409 (2008)

Key Words: mRNA decay, KH-domain, microRNA, AU-rich elements, Review

Send correspondence to: Paola Briata, Istituto Nazionale per la Ricerca sul Cancro (IST), 16132 Genova, Italy, Tel: 390105737402, Fax: 390105737405, E-mail: paola.briata@gmail.com

http://www.bioscience.org/current/vol16.htm