Seminars in Cell & Developmental Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

KSRP Controls Pleiotropic Cellular Functions

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ARTICLE INFO

Article history:
Available online xxx

Keywords:
mRNA decay
microRNA maturation
KH domain
Cell differentiation
Immune response
Lipid metabolism

ABSTRACT

The single-strand-RNA binding protein KSRP is able to negatively regulate gene expression operating with at least two distinct and integrated postranscriptional mechanisms: (i) by promoting decay of unstable mRNAs and (ii) by favoring maturation from precursors of select microRNAs (miRNAs) including the prototypical tumor suppressor let-7. Studies performed in primary and cultured cells as well as in mice proved that the ability of KSRP to integrate different levels of gene expression is required for proper immune response, lipid metabolism, cell-fate decisions, tissue regeneration, and DNA damage response.

Contents

1. Introductory remarks

The KH-type splicing regulatory protein (KSRP) is a single-strand RNA-binding protein that contains four hnRNPK-homology (KH) domains and controls gene expression at multiple levels to modulate diverse cellular functions including cell differentiation/proliferation, innate immunity, and lipid metabolism. KSRP is a ubiquitous shuttle protein whose nucleo/cytoplasmic distribution varies in distinct cell types [1,2]. Initially, KSRP was identified as a

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http://dx.doi.org/10.1016/j.semcdb.2014.05.004
1084-9521/

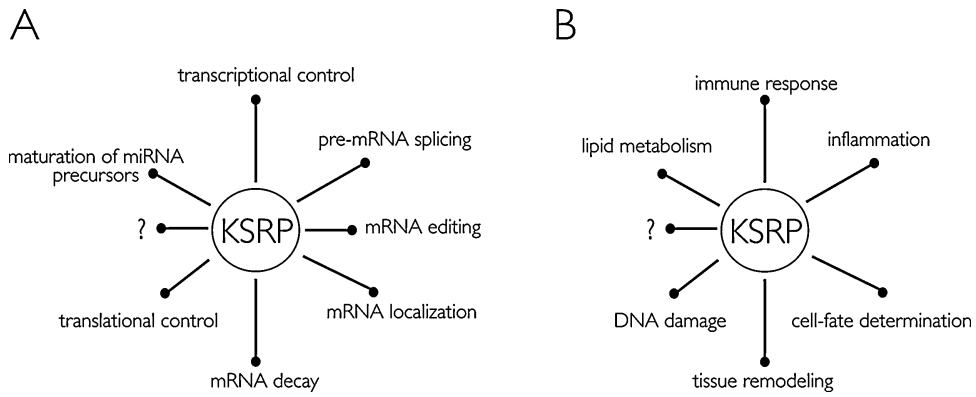

Fig. 1. (A) Schematic representation of KSRP functions in gene expression regulation. (B) Schematic representation of the cellular pathways in which KSRP is implicated.

transcriptional regulator (under the name of FBP2 [3]), as a component of the c-src pre-mRNA splicing complexes [4], and as part of the apolipoprotein B editing complex [5]. However, two distinct functions of KSRP have been investigated in greater detail during the last decade: (i) the ability to favor degradation of labile mRNAs via AU-rich element (ARE)-mediated decay and (ii) the ability to promote maturation from precursors of select microRNAs (miRNAs) [1,2] (Fig. 1A). KSRP function is regulated by a number of post-translational modifications including phosphorylation by AKT (Ser 193), ATM (Ser 132, 274, and 670), and MAPK p38 (p38; Thr 692).

In this review we will not extensively describe the details of the molecular mechanisms underlying KSRP function (se a recent review [1] for additional information) mainly focusing on the distinct cellular functions affected by KSRP in different tissues. However, in order to allow readers to better rationalize KSRP high versatility in terms of target recognition, we will provide in Section 2 biophysical information on the mode of KSRP-RNA interaction.

1.1. KSRP and the rapid decay of labile mRNAs

The ARE is the landmark *cis* element responsible for rapid mRNA decay in mammals and is found in the 3′ untranslated regions (UTRs) of many short-lived transcripts encoding cytokines, cell-cycle regulators, cell type-specific transcription factors, and proto-oncogenes [6]. KSRP is one of the numerous proteins able to bind to AREs (ARE-binding protein (ARE-BP), [6]) and recruits the enzymatic complexes required for mRNA degradation and favors the rapid decay of ARE-containing mRNAs [1].

1.2. KSRP and the miRNA biogenesis from precursors

miRNAs are short non-coding RNAs that regulate nearly every aspect of cell life interacting with their target mRNAs, inhibiting their translation and favoring their decay ([7] and literature cited therein). Mature miRNAs are generated through a complex and highly regulated series of molecular events. Once transcribed, the long primary miRNAs (pri-miRNAs) are cleaved into stem-loop-structured precursor miRNAs (pre-miRNAs) by the ribonuclease Drosha-containing complex. Pre-miRNAs are actively exported into the cytoplasm where they are cleaved by the ribonuclease Dicer-containing complex into short miRNA duplexes with one strand being incorporated into the effector machinery known as the RNA-induced silencing complex (RISC) that inhibits translation and favors decay of mRNA targets ([7] and literature cited therein). We have demonstrated that KSRP binds to a single strand region (the terminal loop (TL)) of a cohort of miRNA precursors and interacts with both Drosha and Dicer to promote miRNA maturation [8]. Among miRNAs whose maturation is enhanced by KSRP, it is worthy to mention the let-7 family members, whose deregulation in many cancers has been reported, as well as a group of miRNAs whose role in DNA damage response will be reviewed in Section 6.

2. KSRP structure and RNA binding

KSRP interacts with its nucleic acid targets using four KH domains grouped in a central nucleic acid binding region [1] (Fig. 2). This region is flanked on both sides by two flexible regions that contain motifs potentially responsible for protein-protein interactions as well as less conserved low-sequence complexity regions. The four KH domains within the protein central region recognize KSRP RNA targets in a combinatorial fashion, with each domain recognizing a short RNA sequence with low affinity [9,10]. The KH fold encodes a α/β domain that binds to single-stranded (ss) nucleic acid sequences with varying degrees of affinity and specificity [11]. In contrast to RNA recognition motifs (RRM domains), KH domains are not known to recognize RNA structures and isolated KH domains recognize their RNA target sequences with Kds in the micromolar range. With one exception (the KH4 domain of IMP1,), KH domains have been shown to recognize up to four nucleotides in a single-stranded conformation and share the same overall recognition mode. The conserved GxxG loop of the KH domain makes contact with the phosphate backbone of the nucleic acid; this represents the basis for specific recognition within a hydrophobic groove on the protein surface. Specific nucleobase recognition is based on hydrogen bonding between the protein and the Watson–Crick edge of the bases as well as hydrophobic interactions and steric hindrance. Indeed, the width of the hydrophobic groove varies depending on whether the recognized nuclobase is, for example, a G or a C [11,12]. The solution structure of the four KH domains of KSRP, in isolation and in different combinations (KH1 + KH2, KH2 + KH3, KH3 + KH4) has been solved [9,13,14]. Inter-domain dynamics have shown that the two distal domains of KSRP (KH1 and KH4) can re-orient freely in solution with respect to a central core formed by KH2 and KH3. That is, the protein possesses several structural independent ssRNA binding units that can adapt to the structural context of different protein-RNA regulatory particles.

The range of RNA targets to which KSRP binds are diverse, not only functionally, as described below, but also in terms of RNA sequence [15]. Biophysical, structural and cellular studies have lead to a working model where KSRP-RNA recognition of diverse targets is mediated by the combinatorial action of several protein domains where the role of each domain can vary in regards to the recognition of different targets. TNF-α ARE, one of the best-studied targets of KSRP, is repetitive and comprises two classical ARE motifs: AUUUA and AUUA. The core AU-rich sequence is flanked by two short self-complementary sequences and is part of the highly structured TNF-α 3′ UTR. In TNF-α-KSRP recognition, the low affinity (10^{-4} Kd)

Please cite this article in press as: Gherzi R, et al. KSRP Controls Pleiotropic Cellular Functions. Semin Cell Dev Biol (2014), http://dx.doi.org/10.1016/j.semcdb.2014.05.004