Minireview

Diverse molecular interactions of the hnRNP K protein

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Abstract The hnRNP K protein is a versatile molecule that interacts with RNA, DNA, the proto-oncoprotein VaV, Src-like tyrosine and inducible serine/threonine kinases, the transcription factor TBP and a number of zinc-finger transcriptional repressors. The interaction of K protein with some of its protein partners is modulated by nucleic acids and K protein can alter the in vivo and in vitro rate of transcription. K protein can simultaneously engage several proteins and may facilitate molecular cross-talk. Taken together these diverse interactions suggest that K protein may act as a nucleic acid-regulated docking platform.

Key words: K protein; Docking platform; KH domain

1. K protein primary structure

K protein was first discovered as a component of the heterogenous nuclear ribonucleoprotein (hnRNP) particle [1] from where it derives its name. K protein is encoded by a gene mapped in humans to chromosome 9 [2]. It produces at least five proteins that are products of alternatively spliced transcripts [2]. The deduced molecular weight of the isoforms is in the range of 50–51 kDa but in SDS-PAGE, the K protein has an apparent molecular weight of 65 kDa. The D isoform of K protein has been cloned from human and mouse cDNA libraries [2,3]. On the amino acid level, this isoform in the two species differs by only one conservative substitution: residue 350 in the mouse is Glu and Asp in the human protein. K protein contains the evolutionarily conserved KH repeats (Fig. 1) that are almost completely conserved between *Xenopus laevis* and mammals [4]. KH-like domains are also found in RNA-binding proteins in species as diverse as *Escherichia coli* and *Saccharomyces cerevisiae* [5]. Mutation in the KH domain of the human FMR1 protein is responsible for the fragile X syndrome, the most common hereditary mental retardation disorder [6,7]. The human and murine K protein sequences contain four GRGG repeats, two of which are conserved in *X. laevis*. Human, mouse and *X. laevis* sequences contain RGRP repeats and a cluster of three proline-rich SH3-binding domains [4,8,9] (Fig. 1).

In addition to these highly evolutionarily conserved features, in the mammalian K protein, the carboxyl end of the SH3-binding cluster is adjacent to a domain that recruits an interleukin-1 (IL-1)-responsive K protein kinase [9]. The N-terminus of the K protein is highly acidic and has been reported to contain transcriptional activity [10]. The K protein region that encompasses amino acids 206–327 and contains the GRGG box and the SH3-binding domain, binds the transcriptional repressor, Zik1 [11] (Fig. 1).

2. Interaction of K protein with nucleic acids

The function of K protein in the hnRNP particle has not yet been defined, but it is easily recovered from both cytoplasmic and nuclear extracts indicating that it has a wide intracellular distribution [2,8] and that a large fraction of K protein is not associated with the hnRNP particle. K protein also binds in a sequence-selective fashion to RNA [1,12], and to a single- [8,13–15] and double-stranded [8,16] DNA. Poly(C) RNA is strongly bound by K protein but other RNA homopolymers are bound poorly or not at all [1,12]. The binding of K protein to RNA might involve the KH domains [6], and binding affinity to RNA is diminished when K protein is phosphorylated [2].

A number of specific interactions of K protein with distinct motifs in DNA have also been observed. For example, K protein binds the homopurine/homopyrimidine (CCCC/GGGG) tract present in the CT motif nested within the c-*myc* promoter P1 [16] and the κB motif [8]. Like the CT motif, the κB motif also contains a homopyrimidine/homopurine domain but in the opposite orientation. K protein also binds tenaciously to oligo(dC), which might account for its binding to the late-coding simian virus 40 DNA strand [17].

3. Interaction of K protein with factors involved in signal transduction and gene expression

K protein binds and is phosphorylated in vivo and in vitro by an inducible serine/threonine kinase, K protein kinase, KPK [9,12,18]. The identity of KPK remains to be defined. Phosphorylation of K protein by KPK is modulated by its binding to cognate DNA or RNA motifs. In addition, K protein exists in a complex with a number of important signal transduction molecules. For example, K protein has been shown to bind selectively in vitro to the SH3 domains from Src, Fyn and Lyn and it can be co-immunoprecipitated with c-Src from cell extracts, suggesting that K protein-tyrosine kinase complexes may exist in vivo [9]. Even more interestingly, in the context of K protein, c-Src can reactivate the KPK in vitro, suggesting that K protein can facilitate cross-talk between protein tyrosine and serine/threonine kinases. K protein has also been demonstrated to bind in vivo and in vitro the proto-oncoprotein Vav via SH3 interactions [9,19].

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Vav is a key element of the apparatus for signaling through both the T- and B-cell antigen receptors [20,21], demonstrating involvement in yet another signaling pathway. Finally, K protein and KPK co-immunoprecipitate from cell extracts with either c-Src or Vav, suggesting that K protein permits multienzyme complex formation in vivo [9].

One clue for the involvement of K protein in transcription is observation that K protein co-immunoprecipitates with epitope-tagged TBP from nuclear extracts. [^{35}S]TBP translated in vitro binds to recombinant K protein, suggesting that in vivo K protein can interact with TBP directly [22]. K protein also binds in vivo and in vitro a zinc-finger transcriptional repressor, Zik1, an interaction that is likewise direct [11]. The interaction of K protein with Zik1 may reflect a general interaction that exists between Kox1-like zinc-finger transcriptional repressors and K protein because Kid-1 [23] and MZF-1 [24], transcriptional repressors structurally related to Zik1, can also be directly engaged by K protein (Denisenko and Bomsztyk, unpublished results). Importantly, the interaction of K protein with the transcriptional repressors appears to be regulated by cognate nucleic acid motifs. For example, poly(C) RNA, which binds K protein, blocks Zik1-K protein interaction, while poly(A) RNA, which does not bind K protein, has no effect on this interaction. K protein-Zik1 interaction can also be blocked by the κB motif, which like poly(C) RNA also binds K protein [11].

There are also functional studies that implicate K protein in the regulation of transcription. For example, K protein has been shown to increase transcription in vivo [22,25] as well as in vitro [17]. However, the mechanisms by which K protein alters gene transcription are not yet clear because K protein can both activate and repress transcription of reporter gene linked to a heterologous promoter even when that promoter does not contain any obvious K protein-binding sequence-specific DNA motifs [17,22].

4. Perspectives

The most evolutionarily conserved characteristic of K protein is its ability to bind RNA through the KH domains, a motif that occurs in organisms as distant from mammals as bacteria [5]. This extraordinary conservation suggests a very fundamental role of K protein in the processes involving RNA. K protein interacts with a host of protein partners (Table 1) and notably the interaction of K protein with some of these molecular partners is modulated by RNA [8,9,11,12]. Thus, K protein may serve as a RNA linker molecule that would allow RNA to exert effects on protein factors that do not bind nucleic acids directly. As a linker molecule

Table 1
K protein molecular partners

Molecules involved in signal transduction
Tyrosine kinases- Src, Fyn and Lyn
Proto-oncoprotein Vav
Serine/threonine inducible kinases
Molecules involved in gene expression
TFIID TATA-binding protein (TBP)
Transcriptional repressors- Zik-1, Kid-1 and MZF-1
Elongation factor-1α
Sequence-specific single- and double-stranded DNA
Sequence-specific RNA

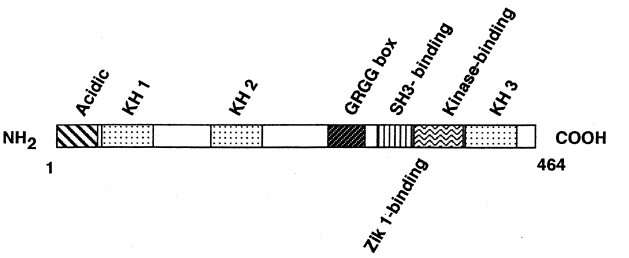

Fig. 1. Diagrammatic illustration of K protein modular domains. Acidic domain is contained in aa 1–40; KH 1 aa 46–98; KH 2 aa 149–197; KH 3 aa 391–439; GRGG box aa 236–273; Src SH3-binding domain aa 289–315; Zik1-binding domain aa 209–337; K protein kinase (KPK)-binding domain aa 337–425.

for nucleic acids, K protein would be expected to interact with tens if not hundreds of different protein factors. Because K protein contains two different dimerization domains (Denisenko, Van Seuningen and Bomsztyk, unpublished observations), it may, in vivo, form oligomers. If so, K protein could assemble multiple factors on RNA and allow multilateral cross-talk that could be regulated by nucleic acids to which K protein binds. Thus K protein may act as an RNA- and DNA-regulated docking platform. As such, K protein would link sequence-specific nucleic acids to protein-protein interactions. Serving as a nucleic acid-linking docking platform, K protein could act in processes such as transcription and translation. Such a role would be consistent with the observation that K protein alters rates of transcription in vivo and in vitro [10,14,25]. The effect of K protein on rates of translation has not been described but it does appear to directly interact with the elongation factor-1α (Denisenko and Bomsztyk, unpublished observations), so it may also have a role in translation. Because K protein also binds single- and double-stranded DNA it could also act in the same linker fashion in processes involving DNA. Depending on the circumstances, K protein could assemble on DNA either transcriptional repressors or activators, accounting for the observations that K protein can either increase or decrease rates of transcription [25].

The ability to engage multiple proteins and nucleic acids is not unique to K protein, since, for example, Sam68 which also contains KH domains, binds RNA and interacts with a number of different protein factors involved in signal transduction [26,27]. Sam68 binds to RNA sequences different from those to which K protein binds, and Sam68 engages some protein factors that K protein is not known to bind. As in the case of K protein [9,11], it has been suggested that Sam68 acts as a docking platform or scaffold to facilitate molecular interactions [28]. The class of proteins that act as nucleic acid-regulated docking platforms is likely to include other factors related to K protein and Sam68, each with unique RNA-, DNA- and protein-binding specificities.

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