

## ADVANCED REVIEW

# The Quaking RNA-binding proteins as regulators of cell differentiation

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## Abstract

The RNA-binding protein Quaking (QKI) has emerged as a potent regulator of cellular differentiation in developmental and pathological processes. The *QKI* gene is itself alternatively spliced to produce three major isoforms, QKI-5, QKI-6, and QKI-7, that possess very distinct functions. Here, we highlight roles of the different QKI isoforms in neuronal, vascular, muscle, and monocyte cell differentiation, and during epithelial–mesenchymal transition in cancer progression. QKI isoforms control cell differentiation through regulating alternative splicing, mRNA stability and translation, with activities in gene transcription now also becoming evident. These diverse functions of the QKI isoforms contribute to their broad influences on RNA metabolism and cellular differentiation.

This article is categorized under:

RNA Interactions with Proteins and Other Molecules > Protein-RNA Interactions: Functional Implications

RNA Processing > Splicing Regulation/Alternative Splicing

RNA in Disease and Development > RNA in Development

## KEYWORDS

RNA, RNA-binding protein

## 1 | INTRODUCTION

The Quaking RNA-binding proteins (RBPs) are composed of three major isoforms (QKI-5, QKI-6, and QKI-7) that play diverse functions in RNA metabolism with widespread impacts on cellular differentiation. The Quaking gene (Ebersole et al., 1996) derives its name from a “Quaking” phenotype observed in the Quaking viable (*Qk<sup>v</sup>*) mutant mouse (Ebersole et al., 1996; Sidman et al., 1964), resulting from severe demyelination of the central and peripheral nervous system. Close examination of the *Qk<sup>v</sup>* mouse revealed a mosaic pattern of QKI isoform expression, where QKI-5 is retained in less affected regions of the brain while loss of all three major isoforms correlates with severe dysmyelination (Hardy et al., 1996). In addition to neural tissues, QKI proteins are prominently expressed in developing heart and vascular tissues, with *Qki* mutant mice displaying cardiovascular defects (review in Justice & Hirschi, 2010). These pioneering studies pointed to important roles of QKI in cellular differentiation and homeostasis.

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More recently, QKI proteins have been found to contribute to neural stem cell (NSC; Hayakawa-Yano et al., 2017), vascular (van der Veer et al., 2013), muscle (Chen et al., 2021), and monocyte (De Bruin, Shiue, et al., 2016) cell differentiation through an array of mechanisms including regulating alternative splicing, RNA stability and gene transcription. In addition, QKI also plays diverse roles during cancer progression, influencing epithelial–mesenchymal transition (EMT) and associated alternative splicing patterns (Pillman et al., 2018). This review provides a background on the discovery of QKI, function of its major isoforms and focuses on recent discoveries of QKIs functions in cellular differentiation.

## 2 | IDENTIFICATION OF THE QUAKING GENE

The Quaking protein (QKI) was named after the phenotype observed in the *Qk<sup>v</sup>* mouse from which the *QkI* gene was discovered (Sidman et al., 1964). These mice possess a spontaneous recessive mutation which at 10 days after birth causes severe demyelination of the central nervous system leading to the characteristic “Quaking” (meaning shaking or shuddering) phenotype (Sidman et al., 1964). Homozygous male mice were also found to be infertile due to a defect in spermatid differentiation (Bennett et al., 1971).

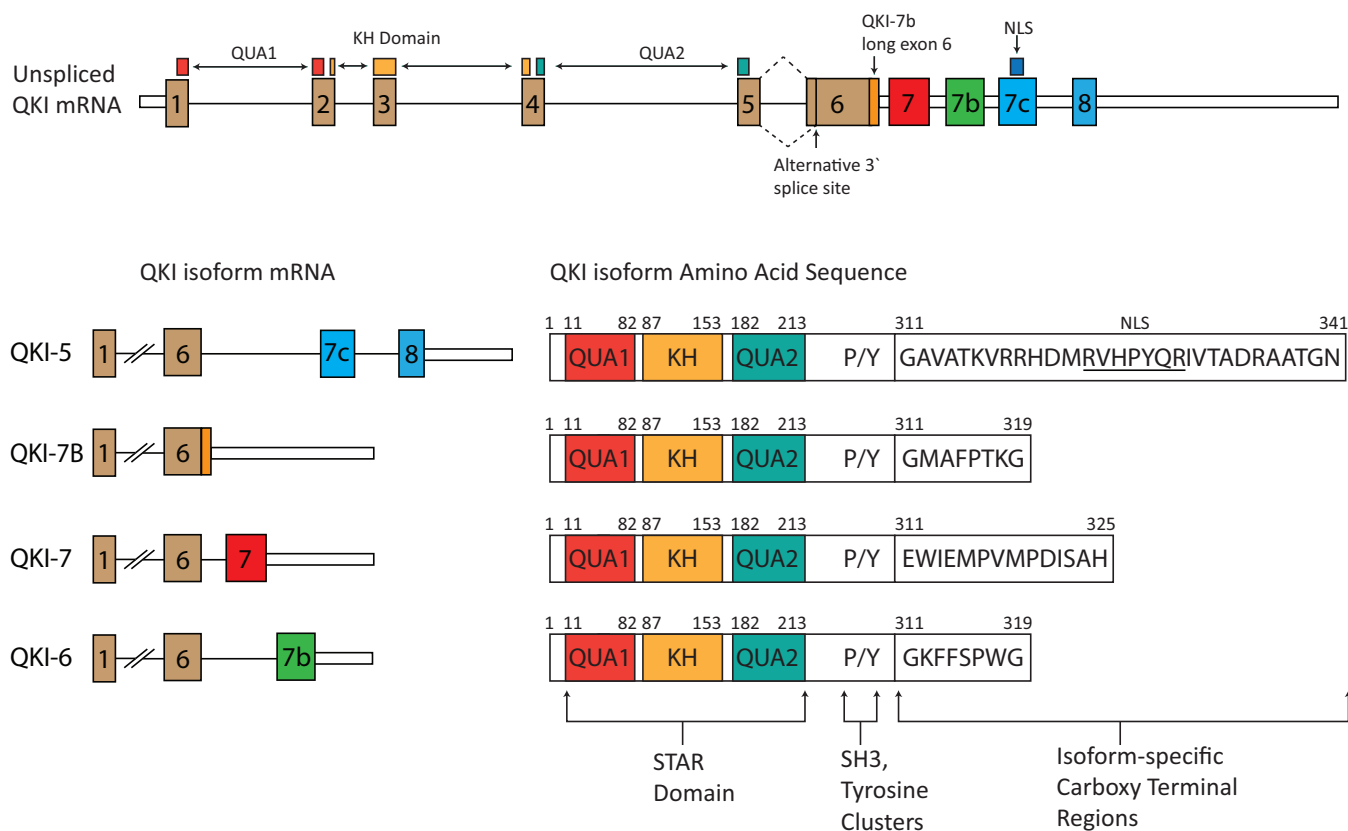
Characterization of the mutation responsible for the Quaking phenotype revealed a 1 Mb deletion in the mouse Chromosome 17 (Ebersole et al., 1992; Ebersole et al., 1996). This deletion was found to impact four distinct genes, *parkin*, Parkin co-regulated gene (*pacrg*) and two previously uncharacterized genes initially named *QkI* and *QkII* (Ebersole et al., 1996). The genes *parkin* and *pacrg* are both associated with juvenile familial Parkinson's disease and their disruption has no influence on the Quaking phenotype, except for *pacrg* which was found to be responsible for the homozygous male infertility (Itier et al., 2003; Lorenzetti et al., 2004). The *QkII* gene transcribed a small single exon gene, which has since been found to code for a long noncoding RNA, now identified as *CAHM* (Pedersen et al., 2014). Mapping of the *Qk<sup>v</sup>* mice deletion break point was localized to 913 bp upstream of the transcription initiation site for *QkI*, leaving the coding region intact, but likely disrupting important promoter and enhancer sequences and consequently reducing transcription of the coded protein (Hardy et al., 1996; Kondo et al., 1999).

The *QkI* gene was found to produce three mRNA transcripts of five, six, and seven kilobases in length, which were named *QkI-5*, *QkI-6*, and *QkI-7*, respectively. All three isoforms share a coding sequence up until a carboxy-terminal region that is unique for each of them (Ebersole et al., 1996) (Figure 1). Three major domains were identified in the coding sequence of *QkI*, based on their conservation in other species (Ebersole et al., 1996). The hnRNP K homology (KH) domain, an RNA-binding domain, is flanked by the upstream QUA1 domain and downstream QUA2 (Ebersole et al., 1996). The QUA1 domain shares strong homology with a sequence in the *Xenopus* version of Quaking, pXqua, which was found to be responsible for homodimerization and is necessary for RNA binding (Chen et al., 1997; Chen & Richard, 1998; Ebersole et al., 1996; Zorn & Krieg, 1997). Studies of the QUA2 domain in the *QkI* homologs, GLD-1 and Splicing factor 1 (SF1), found it to support the KH domain in RNA binding (Daubner et al., 2014; Liu et al., 2001). These three domains form a larger tripartite domain known as the signal transduction and activation of RNA (STAR) domain (Figure 1) and proteins which contain this domain are classified as members of the STAR family (Vernet & Artzt, 1997).

## 3 | STRUCTURE OF QKI PROTEINS AND FUNCTIONS IN RNA REGULATION

### 3.1 | The structure of the QKI pre-mRNA leads to the generation of alternative isoforms

The three major protein isoforms produced from the human QKI locus, QKI-5, QKI-6, and QKI-7, as well as the less studied isoform known as QKI-7B, are generated through complex patterns of alternative splicing (Kondo et al., 1999). Although Exons 1–6 are included in each transcript, the remaining exons are isoform specific with QKI-7 containing Exon 7, QKI-6 containing Exon 7b and QKI-5 containing Exons 7c and 8 (Figure 1). QKI-7B ends at Exon 6, where the 5' splice is not utilized, and a downstream stop codon defines the end of the coding region. This mechanism of splicing is known as alternative last exons (ALE), and is caused by different definitions of the terminal exon through alternative poly(A) site (PAS) selection (Dutertre et al., 2014). Definition of an internal exon requires the recognition of a downstream 5' splice site before the splicing machinery commits to intron excision (Robberson et al., 1990). The definition of



**FIGURE 1** The human *QKI* gene, major isoforms and protein products. The QUA1 dimerization domain, KH RNA binding domain and QUA2 domains are present in all isoforms and are called the STAR domain. Alternative splicing and alternative polyadenylation events generate the major splice isoforms of *QKI*, while an N-terminal truncation in Exon 6 has also been detected. Post-translational phosphorylation can occur in SH3 and tyrosine clusters. *QKI-5* has a distinct 3'UTR and has a NLS in its C-terminal region restricting its activity to the nucleus. NLS, nuclear localization signal; *QKI*, RNA-binding protein Quaking; SRC, SRC homology 3 domain (SH3); STAR, signal transduction and activation of RNA

the terminal exon, and excision of the terminal intron, relies on the presence of a 5' splice site and 3' splice site in the intron and a downstream PAS (Niwa et al., 1990; Niwa & Berget, 1991; Rigo & Martinson, 2008). This phenomenon is likely exploited to produce the *QKI* isoforms where competing PAS sites determine the end of the transcript and a switch between production of *QKI-5* or *QKI-6* and *QKI-7*.

A less studied variation on *QKI* alternative splicing is the presence of an alternative 3' splice site in Exon 6, 24 nucleotides downstream from the major 3' splice site. When used, this splice site causes the loss of eight amino acids encoded within Exon 6. Recently, Polypyrimidine tract-binding protein 1 (PTBP1) was found to regulate the utilization of this splicing site (Mironov et al., 2021); however, further studies are required to determine the consequences of these eight amino acids for *QKI* activity.

As alternative splicing between the *QKI* isoforms is predominantly limited to the 3' end of the transcript, the protein products all contain the STAR domain, and only differ in their carboxy-terminal (C-terminal) tails, which are distinct for each isoform (Ebersole et al., 1996). To date, little is known about the functions of these regions, except for *QKI-5*, which contains a nuclear localization signal (NLS) that directs its localization to the nucleus (Wu et al., 1999). This highlights the importance of performing studies that focus on identifying the functions of these regions.

A striking feature of the *QKI* isoform mRNAs is the lengths of their 3' untranslated regions (UTRs), spanning 4173 bp for *QKI-6*, 5434 bp for *QKI-7*, and 6399 bp for *QKI-7B*, while the *QKI-5* 3' UTR is completely distinct from the other isoforms and predominantly ends 3086 bp downstream from its stop codon (Pillman et al., 2018). Another interesting observation is that these 3' UTRs are highly conserved between vertebrates (Artzt & Wu, 2010). The lengths of these 3' UTRs, their conservation, and the fact that *QKI-5* contains a distinct 3' UTR from *QKI-6*, *QKI-7*, and *QKI-7B* allows for isoform specific regulation by miRNAs and by the *QKI* isoforms themselves (Fagg et al., 2017; Pillman et al., 2018).

### 3.2 | The QUA1 domain mediates the formation of QKI isoform heterodimers

The QUA1 domain is required for the dimerization of the QKI isoforms, which allows them to recognize and bind a bipartite consensus sequence (Chen & Richard, 1998; Galarneau & Richard, 2005). As most KH domain-containing proteins bind using multiple KH domains (Valverde et al., 2008), the ability of QKI and other STAR-domain proteins to dimerize likely overcomes the limitation of having a single KH domain. Interestingly, the QKI homolog, Src-associated substrate in mitosis of 68 kDa (SAM68), requires RNA-binding activity to form a stable homodimer, whereas QKI was found to dimerize in the presence and the absence of RNA (Chen et al., 1997; Chen & Richard, 1998). Another STAR protein, SF1, lacks a QUA1 domain while still retaining the ability to bind RNA as a splicing factor. However, as SF1 recognizes the branchpoint sequence to facilitate intron splicing, it is likely that cooperative binding with the splicing machinery enhances its binding strength and specificity (Berglund et al., 1998).

As all QKI isoforms contain the same QUA1 domain, heterodimerization between different isoforms can occur (Pilotte et al., 2001). This allows the cytoplasmic isoform QKI-7 to trans-localize to the nucleus by shuttling with QKI-5 (Pilotte et al., 2001). Furthermore, hetero-dimerization has been suggested as a mechanism to allow QKI-6 and QKI-7 access to the nucleus to promote the export of nuclear retained mRNAs (Larocque et al., 2002). Some small proteins (<60 kDa) can diffuse into the nucleus freely without a NLS, whereas larger proteins diffuse at a reduced rate (Timney et al., 2016; Wang & Brattain, 2007). Monomeric QKI isoforms are approximately 37 kDa, so it is possible that QKI-6 and QKI-7 can access the nucleus freely as a monomer. However, they may have a reduced ability to enter as dimer, unless heterodimerized with QKI-5. To gain a better understanding of how the QKI isoforms cooperate, more work is required to determine if nuclear localization of QKI-6 and QKI-7 requires heterodimerization with QKI-5.

### 3.3 | Posttranslational modification of QKI and its regulation by signal transduction pathways

A characteristic of STAR proteins is their regulation in response to signal transduction pathways (Vernet & Artzt, 1997). Proteins of this classification are found to contain various sites that permit posttranslational modification, including tyrosine residues, proline-rich SH3-binding domains, WW-binding sites, and arginine-glycine methylation sites. STAR proteins have been found to be directed to regulate mRNA metabolism as downstream targets of cell signaling and, in the case of SAM68, as a mediator of cell signals (Lock et al., 1996; Richard et al., 1995). However, little is known about the role that QKI plays in cell signaling.

The QKI amino acid sequence was found to contain RG boxes, tyrosine clusters, and putative SH3-binding sites (Ebersole et al., 1996). Subsequently, QKI has been shown to be phosphorylated by Src-protein tyrosine kinases and arginine methylated (Côté et al., 2003; Zhang et al., 2003). Phosphorylation of QKI by Src was discovered to decrease its ability to bind RNA, repressing its ability to regulate mRNAs (Zhang et al., 2003). During myelination, decreased phosphorylation of QKI coincides with myelin basic protein (MBP) mRNA accumulation (Zhang et al., 2003) and the Src family member, Fyn, was shown to be required for the upregulation of important myelin genes, with loss of Fyn leading to reduction of the Exon 2 containing MBP isoform (Lu et al., 2005). As QKI was shown to promote the expression of this isoform, it is suggested that Fyn might phosphorylate QKI to indirectly repress this isoform (Lu et al., 2005).

### 3.4 | The QKI RNA-binding interface and discovery of the QKI regulatory element

The QKI-binding motif was initially identified using the systematic evolution of ligands by exponential enrichment method, which uncovered a motif, NACUAAY, followed by a half site, UAAAY, within 20 nucleotides away (Galarneau & Richard, 2005). Since then, various cross-linked immunoprecipitation (CLIP)-Seq experiments have been performed that have also confirmed the QKI motif to be ACUAAY (Hafner et al., 2010; Pillman et al., 2018; Teplova et al., 2013; Van Nostrand et al., 2016). The tendency of QKI to bind a full site and half site in combination provides strong evidence that QKI preferentially recognizes two sites as a dimer (Galarneau & Richard, 2005; Teplova et al., 2013).

The QKI isoforms, like other STAR proteins, all have a QUA2 domain adjacent to the KH domain that strengthens their association with RNA. In the case of the QKI homolog SF1, the QUA2 domain was found to specifically recognize the 5' bases of the QKI motif (ACU), while the KH domain was found to recognize the 3' bases (UAAC) (Daubner

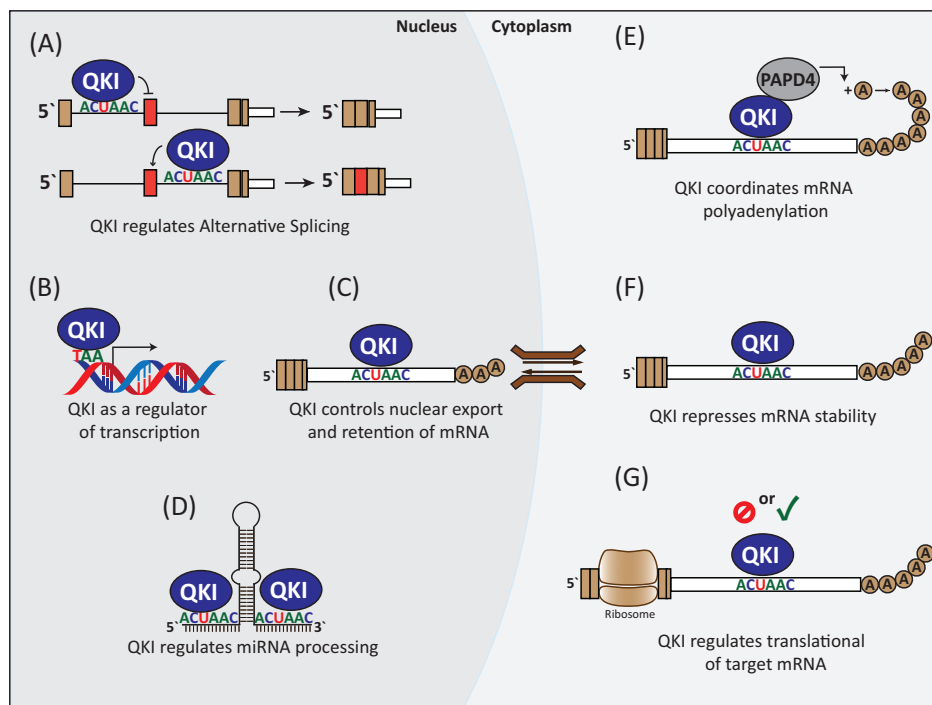
et al., 2014; Liu et al., 2001). The QUA2 domain is required for successful binding of SF1 to the QKI motif and deletion of the QUA2 domain from mouse *Qk* has been shown to severely disrupt RNA binding (Chen & Richard, 1998; Liu et al., 2001).

### 3.5 | Distinct mechanisms of RNA regulation by the QKI isoforms

The QKI isoforms engage in many different mechanisms of mRNA regulation including pre-mRNA splicing, nuclear export, differential localization, translation, and mRNA stability (Figure 2). Since each isoform shares the same RNA-binding interface, it likely that the differences in function of the QKI isoforms are due to their unique C-terminal regions. The only established function of any of the C-terminal regions is for QKI-5, which contains a NLS that permits its nuclear function in splicing (Wu et al., 1999). Most of the differences in function between the nuclear QKI-5 and cytoplasmic QKI-6 and QKI-7 are likely due to localization. However, the C-terminal regions of QKI-6 and QKI-7 are distinct from one another and so it is likely that these isoforms have unique functions.

#### 3.5.1 | The balance of the QKI isoforms determine subcellular localization of target mRNAs

Early work on QKI-6 and QKI-7 in oligodendrocytes found that both isoforms associate with microtubules, while QKI-6 alone interacts with actin filaments (Wu et al., 2001). It was suggested that both isoforms could direct traffic of mRNAs along microtubules away from the cell body while QKI-6 might also direct mRNAs to the cell periphery via the actin cytoskeleton (Wu et al., 2001). More recently, studies of the QKI isoforms in astrocytes found that many mRNAs are locally translated at the peripheral processes and that these mRNAs had higher occurrence of the QKI motif in their 3' UTRs, with QKI-6 binding many of these mRNAs (Sakers et al., 2017; Sakers et al., 2021). It is possible that the ability



**FIGURE 2** QKI coordination of gene expression and RNA metabolism. (a) Nuclear expression of QKI controls alternative splicing via binding upstream (exon inclusion) or downstream (exon exclusion) to specific motifs. (b) QKI can act as a co-factor to influence gene transcription. (c) Binding of QKI to mRNAs can facilitate nuclear export or retention. (d) Processing of miRNAs can be enhanced through QKI interaction with the primary miRNA transcript. (e) In the cytoplasm QKI interacts with PABP4 (TENT2) and coordinates cytoplasmic polyadenylation of specific transcripts. (f) QKI can also repress mRNA stability through unknown mechanisms. (g) QKI can enhance or repress translation of mRNAs, in some cases in specific cellular locations. QKI, RNA-binding protein Quaking



of QKI-6 and QKI-7 to associate with the cytoskeleton allows them to shuttle mRNAs to differentially localize their translation at specific locations in the cell.

The balance of QKI-5 to QKI-6 and QKI-7 has been shown to control the nuclear export of target mRNAs. In oligodendrocytes, the balance in the QKI isoforms is important for MBP mRNA to be exported from the nucleus where it can be translated into protein (Larocque et al., 2002). This phenomenon is discussed in greater detail in Section 4.1.2. It is currently unknown how widespread the QKI-controlled nuclear export of mRNA is and what the consequences are for different physiological and disease states.

### 3.5.2 | The QKI isoforms have complex roles in regulating mRNA stability and translation

QKI-5, QKI-6, and QKI-7 have been shown to stabilize or destabilize many different mRNAs (Azam et al., 2019; Cochrane et al., 2017; Doukhanine et al., 2010; Guo et al., 2014; Larocque et al., 2005; S. Li, Lin, et al., 2018; Thangaraj et al., 2017; Zhang et al., 2016). Similarly, the QKI isoforms have been implicated in both translational repression and activation (De Bruin, Van Der Veer, et al., 2016; Saccomanno et al., 1999; Sakers et al., 2017; Yamagishi et al., 2016; Zhao, Ku, et al., 2006). Recently, QKI-7, but not QKI-5 or QKI-6, was found to directly promote translation of bound mRNAs by recruiting PAP-associated domain containing 4 (PAPD4, also known as Terminal nucleotidyltransferase 2 or TENT2) to increase poly(A) tail length (Yamagishi et al., 2016). The complex and sometimes contradictory effects of QKI isoforms likely arise from recruitment of different partner proteins. However, due to the complication of heterodimerization, nuclear-cytoplasmic shuttling, and autoregulation between the isoforms, it is difficult to determine the precise isoform contribution to mRNA regulation in contexts where all isoforms are expressed. This highlights the importance of finding methods to study the QKI isoforms in isolation, to identify mechanisms of RNA regulation that are specific to each isoform.

### 3.5.3 | Functions for QKI in regulation of microRNAs

The QKI isoforms have also been implicated in the regulation of microRNAs. QKI-5 and QKI-6 have been shown to bind a QKI motif in the primary transcript (pri-miR) encoding miR-7-1 in glial cells where it repressed the formation of mature miR-7-1 (Wang et al., 2013). The pri-miR-7-1 was retained in the nucleus and tightly bound to Drosha in the presence of QKI-5 and QKI-6, but not QKI-7 (Wang et al., 2013). Conversely, QKI-5 has been shown to promote the maturation of pri-miR-124-1 during erythropoiesis by facilitating its interaction with the microprocessor component, DGCR8, through directly binding the QKI-5 KH domain (F. Wang, Song, et al., 2017). More recently, QKI-7 was shown to stabilize the mature miR-122 by promoting adenylation at its 3' end. This occurs through the formation of a ternary structure involving Ago2, which recognizes the QUA2 domain, and PAPD4, which recognizes the C-terminal region of QKI-7 (Hojo et al., 2020). Lastly, the co-localization of QKI-6 with Ago2 within stress granules has been previously reported, providing further evidence of a role for the QKI isoforms in regulating miRNAs (Wang et al., 2010).

### 3.5.4 | QKI-regulated alternative splicing

One of the most established functions of QKI is the regulation of pre-mRNA alternative splicing (Wu et al., 2002). QKI has been found to regulate many alternative splicing events in different contexts including during EMT (Conn et al., 2015; Pillman et al., 2018; Yang et al., 2016), monocyte differentiation (De Bruin, Shiue, et al., 2016), myelination (Darbelli et al., 2016; Darbelli et al., 2017; Mandler et al., 2014), microglia function (Lee et al., 2020; J. Ren, Dai, et al., 2021), muscle differentiation (Hall et al., 2013), and various aspects of cancer progression (Brosseau et al., 2014; de Miguel et al., 2016; Zong et al., 2014). The nuclear isoform, QKI-5, has been found to bind primarily within introns, suggesting that its major function is regulation of splicing (Pillman et al., 2018). Interestingly, QKI-6 has been found to bind the first intron of HDAC7 and regulate its splicing (Caines et al., 2019). This suggests that although QKI-5 is the predominant regulator of alternative splicing, the other isoforms might have a minor role in regulating splicing, possibly through the formation of heterodimers with QKI-5.

Whether QKI-5 promotes inclusion or exclusion of a cassette exon appears dependent on its location of binding, promoting skipping when it binds within or 100 base pairs upstream from a cassette exon or inclusion when it binds in the

downstream intron (Pillman et al., 2018). This has been shown in muscle cell differentiation, where QKI-5 promotes the retention of Exon 9 of *Capzb* by binding its motif in the downstream intron (Hall et al., 2013). Another RBP, PTBP1 was found to repress this exon by binding in the same intron as QKI-5, which suggests that the two proteins could compete to regulate the exon in opposite directions (Hall et al., 2013). Further studies are required to define clear rules for how QKI promotes exon inclusion.

In the opposite case, an explanation for how QKI-5 might promote skipping of a target exon is through direct competition with the U2 snRNP or SF1 for the branchpoint (Zong et al., 2014). The QKI motif bears striking resemblance to the branchpoint sequence and has been shown to compete with SF1 for binding to the branchpoint of the target gene *NUMB* (Zong et al., 2014). It is currently not known how often QKI-5 interacts with branchpoint sites and the extent of this mechanism of regulation.

### 3.6 | The QKI isoforms engage in complex autoregulation

A feature of the QKI isoforms that complicates their study is autoregulation. Expression of QKI-5 is required to allow the formation of QKI-6 and QKI-7, which is suggested to be controlled through alternative splicing (Darbelli et al., 2017; Fagg et al., 2017). This is consistent with the presence of QKI motifs within introns of the QKI pre-mRNA; however, the mechanism behind this phenomenon has not been determined. The 3' end of the QKI locus is organized in an unusual manner, where last exons that are specific for each isoform are alternatively included (see Figure 1). This phenomenon of ALE is thought to have emerged late in evolution (Dutertre et al., 2014) and occurs frequently in neuronal cells, with alternative isoforms bearing distal exons often localizing to neurites (Taliaferro et al., 2016). Some evidence for RBP-mediated regulation of ALE exists, such as ELAV1/HUR promoting widespread inclusion of ALEs through direct exon binding (Dutertre et al., 2014). Furthermore, regulation of alternative polyadenylation through direct binding of polyadenylation sites by RBPs is an established phenomenon (Goering et al., 2021; Tian & Manley, 2017) and is a possible mechanism of regulating ALEs. Further study is required to determine the specific mechanism by which QKI-5 controls splicing of the cytoplasmic isoforms.

Recent work has uncovered more complex interactions between the QKI isoforms. QKI-5 was found to inhibit its own expression by binding a QKI motif in its 3' UTR (Fagg et al., 2017). Interestingly, QKI-6 was found to promote its own translation through binding its 3' UTR and to repress QKI-5 protein production, potentially through binding its coding region (Fagg et al., 2017). The full extent of autoregulation by the QKI isoforms remains to be determined.

### 3.7 | QKI as a transcriptional regulator and chromatin-binding protein

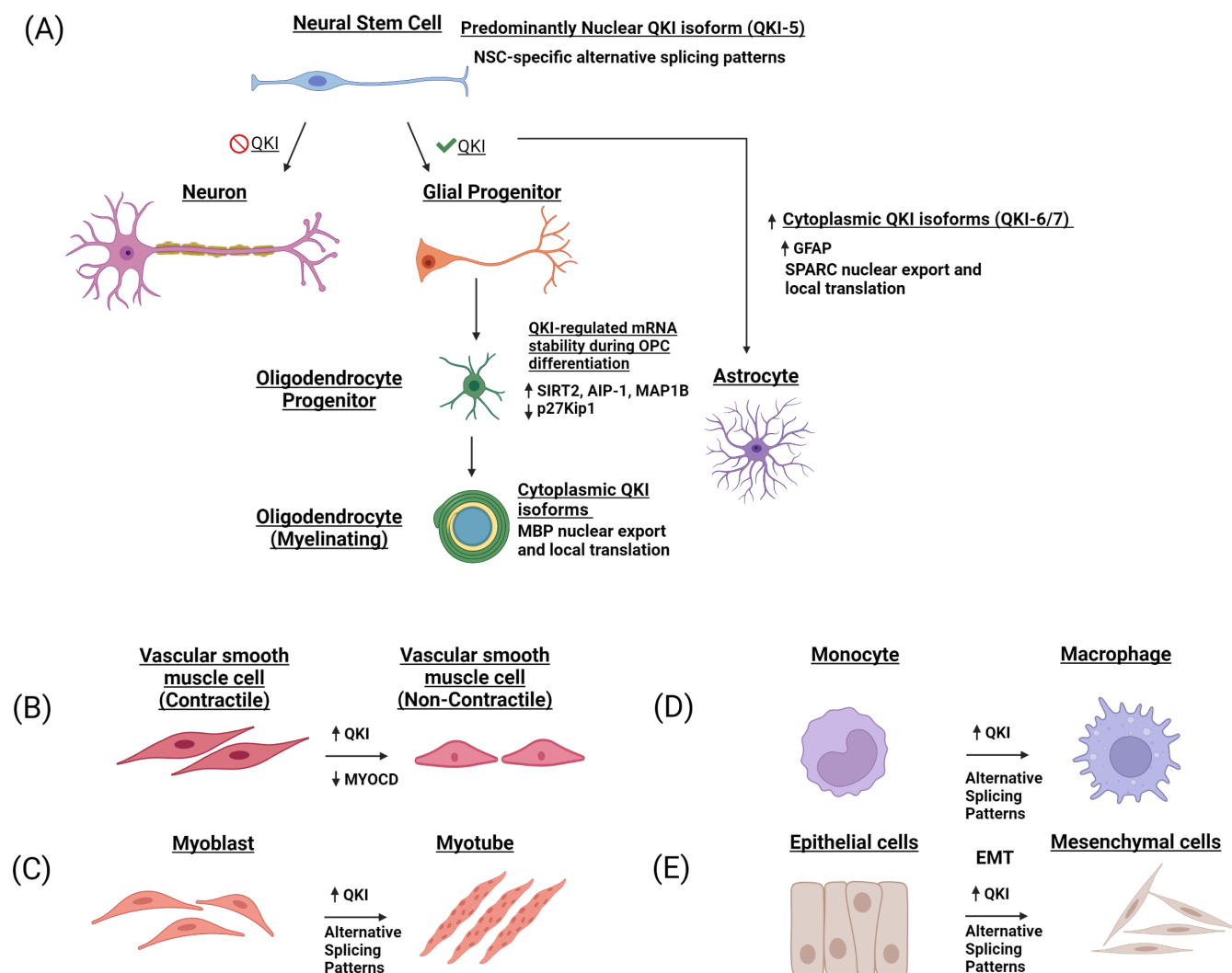
More recently, the realm of QKI functions was expanded to include transcriptional co-activation and single-stranded DNA-binding. QKI was found to regulate the structural lipid components of myelin, through interaction with the peroxisome proliferator-activated receptor  $\beta$ -retinoid X receptor  $\alpha$  complex, leading to the differential regulation of genes associated with lipid metabolism (Zhou et al., 2020). The same group discovered that QKI also regulates cholesterol metabolism in myelinogenesis, again through transcription, in this instance through co-activation with the transcriptional activator, Sterol regulatory element-binding protein 2 (Srebp2) (Zhou et al., 2021). This work was expanded to show that QKI co-activates Srebp2-mediated transcription of cholesterol-metabolism genes in eye lens cells, where mice deficient in QKI have loss of lens transparency (Shin et al., 2021). Strikingly, in this context QKI was shown to function by directly binding single-stranded DNA (ssDNA), which has been suggested for KH domain-containing proteins (Galarneau & Richard, 2009), but is a novel finding for QKI (Shin et al., 2021). Similar to the RNA binding motif, ACUAAC, the central trinucleotide TAA was uncovered as being selective for ssDNA-binding by QKI (Shin et al., 2021). More recently, a large-scale analysis of RBPs with functions in chromatin-binding uncovered QKI-5 as directly binding to promoters of genes associated with hematopoiesis (Y. Ren, Huo, et al., 2021). QKI-5 was able to facilitate differentiation of monocytes by transcriptionally activating *CXCL2*, a cytokine required for monocyte differentiation (Y. Ren, Huo, et al., 2021). However, an alternative possibility is that QKI may also bind nascent transcripts that anchor it to chromatin and facilitate its transcriptional activity (Lee et al., 2015; Pompon & Garcia-Blanco, 2015). The combined functions of RNA and DNA-binding increases the repertoire of QKI functions in gene expression and raises the possibility of QKI regulating a target gene from transcription to translation, and as such it would be worthwhile exploring whether such targets exist.

## 4 | FUNCTIONS OF QKI IN CELL DIFFERENTIATION

### 4.1 | QKI in NSC fate and the maturation of cells of glial lineage

#### 4.1.1 | Role of QKI in neural cells and differentiation of oligodendrocytes

During embryogenesis, NSCs differentiate into neural progenitor cells, which either progress down a neuron-restricted pathway, or a glial restricted pathway. QKI-5 and QKI-6 expression is detected in NSCs and is retained in cells that differentiate down the glial lineage, but is lost in cells that differentiate into neurons (Hardy, 1998; Hayakawa-Yano et al., 2017) (Figure 3). In primary NSCs, QKI was found to contribute to the alternative splicing of a large number of genes, and to maintain a gene expression profile consistent with apical radial glia, which suggests that QKI maintains the NSC state and prevents differentiation toward the neuron lineage (Hayakawa-Yano et al., 2017). Furthermore, Sox10, an important transcription factor for NSC differentiation to glial cells (Pozniak et al., 2010), contains a QKI motif within its 3' UTR and is reduced in expression along with QKI in the brains of schizophrenic patients (Åberg et al., 2006). Expression of QKI reemerges in glial cells and is one of the earliest



**FIGURE 3** Overview of QKI regulation of cellular differentiation. (a) QKI mediates alternative splicing in neural stem cells and is reduced during differentiation to mature neurons. QKI exhibits a range of metabolic functions in glial to oligodendrocyte differentiation and in astrocyte maturation. (b–e) Upregulation of QKI influences alternative splicing patterns that facilitate differentiation of smooth muscle, non-smooth muscle, monocyte to macrophage differentiation and EMT. Created with [BioRender.com](https://www.biorender.com). EMT, epithelial to mesenchymal transition; QKI, RNA-binding protein Quaking



markers of glial cell differentiation, leading to the proposition that QKI contributes to neuron-glial cell fate determination (Hardy, 1998; Takeuchi et al., 2020).

Expression of QKI is also vital for oligodendrocyte progenitor cell (OPC) differentiation into mature oligodendrocytes (Chen et al., 2007). The QKI isoforms are known to affect the stability of several mRNAs during OPC differentiation (Figure 3). Interestingly, while loss of QKI prevents OPC differentiation, QKI-5 and QKI-6 can only partially restore it, suggesting that multiple isoforms are likely required for OPC differentiation (Chen et al., 2007). The QKI target, p27Kip1 is a regulator of the cell cycle and promotes cell cycle exit to decrease proliferation, which is a key step for the differentiation of OPCs into mature oligodendrocytes (Casaccia-Bonnel et al., 1999; Durand et al., 1998; Tikoo et al., 1998). In primary rat OPC cells, QKI-6 and QKI-7 stabilize p27Kip1 mRNA, promoting cell cycle arrest, a key step in OPC differentiation (Larocque et al., 2005). Overexpression of QKI-6 and QKI-7 in mouse embryos is sufficient to induce OPC differentiation (Larocque et al., 2005), however, ectopic expression of p27Kip1 alone cannot induce OPC differentiation (Tang et al., 2000), suggesting that QKI-6- and QKI-7-induced differentiation of OPCs is controlled by multiple QKI targets.

Two other genes with known roles in OPC differentiation, MAP1B (Zhao, Ku, et al., 2006) and Sirtuin 2 (SIRT2; Thangaraj et al., 2017) also produce mRNAs that are stabilized by QKI. The NAD<sup>+</sup>-dependent tubulin deacetylase (North et al., 2003), SIRT2, is expressed in OPCs prior to myelination (Zhu et al., 2012), and it is known to promote OPC differentiation and to facilitate the growth of extensions (Ji et al., 2011). MAP1B mRNA is also stabilized by QKI-6 in OPCs, and, like SIRT2, its expression precedes differentiation into mature oligodendrocytes (Vouyioukiis & Brophy, 1993). In neurons, MAP1B has functions in axon regeneration and neurite branching (Barnat et al., 2016). It is feasible that MAP1B could participate in OPC differentiation through a similar mechanism involving regulation of microtubules to affect morphology.

Unlike SIRT2 and MAP1B, AIP-1 mRNA stability is decreased by QKI-6 during OPC differentiation into mature oligodendrocytes (Doukhanine et al., 2010). AIP-1 expression is required earlier in OPC cells where it modulates actin disassembly and its premature loss is associated with impaired process outgrowth (Doukhanine et al., 2010). These findings highlight the complex and dynamic regulation of mRNAs by QKI in the context of late OPC differentiation, although further work is required to determine its full repertoire of targets and to elucidate the rules for QKI-mediated alteration to target mRNA stability.

#### 4.1.2 | The *Qk<sup>v</sup>* mouse

Most of what we know about the function of QKI in oligodendrocyte differentiation and myelination is through the study of the *Qk<sup>v</sup>*. Comparative examination of QKI isoforms between wild type and *Qk<sup>v</sup>* mouse brains uncovered cell type-specific alterations in their expression (Hardy et al., 1996). Oligodendrocytes express all three QKI isoforms during myelination, but expression of QKI-6 and QKI-7 is lost in *Qk<sup>v</sup>* oligodendrocytes (Hardy et al., 1996). QKI-5 expression is retained in most cells; however, it is entirely absent in oligodendrocytes derived from areas of the brain that had the most severe dysmyelination phenotype (Hardy et al., 1996). The cell type specific loss of QKI-6 and QKI-7 suggests that the deletion causing the *Qk<sup>v</sup>* phenotype affects an enhancer region that is active in oligodendrocytes alone (Hardy et al., 1996), however, the function of the region deleted in the *Qk<sup>v</sup>* mouse has not yet been determined.

Loss of QKI is now known to cause the *Qk<sup>v</sup>* mouse dysmyelination phenotype by altering posttranscriptional regulation of mRNAs that are required for oligodendrocyte function. The most studied and important of these mRNAs is the transcript for MBP. All three major QKI isoforms bind MBP mRNA via QKI motifs in its 3' UTR, leading to different consequences for the fate of the transcript (Larocque et al., 2002; Li et al., 2000; Zhang & Feng, 2001). Accumulation of MBP mRNA is prevented in the *Qk<sup>v</sup>* mouse brain through destabilization (Li et al., 2000; Zhang & Feng, 2001), and ectopic QKI-6 expression in HEK293T cells increases stability of mRNA from an MBP reporter (Zhang et al., 2003), suggesting that QKI-6 stabilizes MBP mRNA. In oligodendrocytes and Schwann cells, MBP mRNA is locally translated at the myelin sheath, where the MBP protein can incorporate itself in the sheath immediately posttranslation (Ainger et al., 1993; Colman et al., 1982; Trapp et al., 1987). Interestingly, in *Qk<sup>v</sup>* oligodendrocytes, which have lost expression of QKI-6 and QKI-7, the MBP transcript is predominantly localized in the nucleus, preventing translation to protein (Larocque et al., 2002). Similarly, ectopic expression of QKI-5 in wild-type oligodendrocytes forces nuclear retention of MBP mRNAs, preventing its translation (Larocque et al., 2002), suggesting that the disrupted balance between QKI-5 and the cytoplasmic isoforms prevents nuclear export of MBP mRNA in *Qk<sup>v</sup>* oligodendrocytes. This was supported when the effect was replicated by transfecting ectopic MBP and QKI-5 in COS cells, which do not express QKI, while

co-transfection of QKI-5 with QKI-6, QKI-7, or both, rescued export to the cytoplasm and promoted their translation (Larocque et al., 2002). In fact, expression of QKI-6 alone is able to restore expression of MBP and partially rescue the  $Qk^V$  phenotype in oligodendrocytes (Zhao, Tian, et al., 2006). As QKI-6 and QKI-7 localize to peripheral processes of oligodendrocytes (Hardy et al., 1996; Wu et al., 2001), it is possible that controlled shuttling of MBP mRNA by these isoforms is necessary for their correct local translation at the myelin sheath. It is not currently known if other targets are regulated by QKI in the same manner.

Alternative splicing is also affected in the  $Qk^V$  mouse brain, suggesting that QKI might regulate alternative splicing in to facilitate myelination. In the brains harvested from  $Qk^V$  mice, aberrant splicing of MBP, myelin proteolipid protein (PLP) and myelin-associated glycoprotein (MAG) is detected (Wu et al., 2002). QKI-5 expression was found to cause dose-dependent exclusion of MAG Exon 12, dependent on the presence of a 53 nucleotide region in the downstream intron, which QKI-5 interacted with (Wu et al., 2002). However QKI-6 was found to repress translation of another RBP, hnRNPA1, which promotes inclusion of MAG Exon 12 (Zhao et al., 2010), suggesting that QKI regulates MAG splicing both directly and indirectly. Lastly, in an oligodendrocyte-specific conditional QKI knockout mouse, alternative splicing of several genes were detected including MBP, PLP, SIRT2 and neurofascin (Darbelli et al., 2016).

### 4.1.3 | QKI functions in astrocytes

QKI has also been implicated in astrocyte function through several mechanisms. The astrocyte marker, GFAP, appears to have its mRNA stabilized by QKI-7 in primary human astrocytes (Radomska et al., 2013) (Figure 3). Although knock-down of QKI-7 represses GFAP mRNA, loss of total QKI does not, which suggests that affecting the balance of QKI isoforms is important in this context (Radomska et al., 2013). Next-generation sequencing of RNAs captured from the peripheral processes of astrocytes of mice brains has uncovered a significant enrichment of QKI motifs (Sakers et al., 2017). One of these mRNAs, Sparc, which regulates the morphology of astrocytes (Au et al., 2007), was detected after immunoprecipitation of QKI-5 and QKI-6 suggesting that QKI promotes nuclear export of Sparc mRNA to mediate astrocyte functions.

Using QKI-6 QKI CLIP-Seq performed on developing mouse forebrains brains, it was found that many astrocyte-associated mRNAs are bound by QKI-6 through their 3' UTRs, and a large subset of these transcripts are locally translated in the peripheral processes of astrocytes (Sakers et al., 2021). Conditional KO of QKI in mice leads to decreased association of QKI-6 target mRNAs on the ribosomes of GFAP+ astrocytes, affecting mRNAs important for astrocyte maturation, suggesting that QKI affects the stability or translation of these mRNAs. These observations suggest a significant and complex role for QKI in the regulation of RNA in astrocytes.

## 4.2 | QKI in vascular smooth muscle and endothelial cell differentiation

Unlike the  $Qk^V$  mutation, several *N*-ethyl-*N*-nitrosourea-induced mutations were generated that affected the coding sequence of the *QKI* gene, and these are embryonically lethal for homozygous mice before the dysmyelination phenotype has an opportunity to emerge (Justice & Bode, 1988; Shedlovsky et al., 1988). Although the mutations vary in consequence for QKI function, they either impact the ability of QKI to dimerize (Chen & Richard, 1998; Ebersole et al., 1996), bind RNA, ablate all QKI function, or prevent the specific production of QKI-5 (Cox et al., 1999), suggesting that functional QKI-5 is vital for embryogenesis. A study of vascular development in the  $qk^{k2}$  mouse embryos uncovered defective blood vessel formation, which is the proposed mechanism for embryonic lethality (Noveroske et al., 2002). Similarly, mice homozygous for the  $qk^{I-1}$  mutation, which causes loss of QKI-5, also died during midgestation from vascular defects (Bohnsack et al., 2006). The generation of a QKI null mouse reproduced the lethal defective vascular formation, and the cause of death was determined to be disturbed vascular remodeling in angiogenesis, presumably from loss of QKI expression in both vascular smooth muscle cells (VSMCs) and endothelial cells (Li et al., 2003).

QKI also has a role in the regulation of mature VSMCs where it promotes de-differentiation to a non-contractile phenotype (van der Veer et al., 2013). In injured blood vessels, where VSMC cells lack the ability to contract and blood vessels are narrowed, QKI expression is increased and promotes skipping of myocardin (Myocd) Exon 2a, removing the MEF-interacting domain important for its function (van der Veer et al., 2013) (Figure 3). Myocd is known to regulate transcription of genes associated with VSMC differentiation, and its expression is sufficient to induce a contractile

phenotype (Chen et al., 2002; Long et al., 2008). Furthermore, another RBP strongly associated with VSMC differentiation, was found to compete against QKI to promote Myocd Exon 2a inclusion, highlighting the significance of Myocd splicing in VSMC function (Nakagaki-Silva et al., 2019). Treatment of mouse inducible pluripotent stem cells (iPSCs) with platelet-derived growth factor BB to promote differentiation into VSMCs increases QKI-6, but not QKI-5, expression (Caines et al., 2019). Interestingly, QKI-6 was localized in the nucleus in this context and found to regulate splicing of HDAC7 from a cytoplasmic (HDAC7u) to a nuclear (HDAC7s) isoform, which interacts with serum response factor and Myocd to promote VSMC differentiation (Margariti et al., 2009).

In endothelial cells, QKI has been shown to regulate both endothelial barrier function and angiogenesis. Mechanistically, QKI-5 binds the 3' UTRs of beta-catenin and VE-cadherin (CD144) mRNA to promote their translation and to STAT3 mRNA to promote its stability (Cochrane et al., 2017; De Bruin, Van Der Veer, et al., 2016), which impacts these cellular processes. QKI-7 expression was found to be high in diabetic human iPSC-derived endothelial cells, as well as high-glucose treated mouse iPSC-derived endothelial cells, with its overexpression mimicking features of endothelial dysfunction that are seen in diabetic patients (Yang et al., 2020). Expression of QKI-7 was found to be controlled by a balance between the RBPs CUGBP Elav-like family member 1 and heterogeneous nuclear ribonucleoprotein M (Yang et al., 2020). Interestingly, in contrast to QKI-5, QKI-7 expression caused destabilization of VE-cadherin mRNA (as well as NLGN1 and TSG6), suggesting that these isoforms compete for binding of the VE-cadherin 3' UTR, leading to opposing outcomes (Yang et al., 2020). Recently, QKI was found to act as a promoter of angiogenesis in the tumor microenvironment by stabilizing CCND1 mRNA and facilitating endothelial cell cycle progression (Azam et al., 2019). In this case, QKI levels in endothelial cells were modulated by the miR-200 family (Azam et al., 2019; Gregory, 2019), which also regulates QKI activity and alternative splicing in the context of EMT (Pillman et al., 2018).

### 4.3 | QKI in nonsmooth muscle cell differentiation

QKI also has an emerging role in cardiac and skeletal muscle differentiation. During myoblast differentiation into myotubes, QKI-5 and QKI-6 increase in expression (Fagg et al., 2017; Hall et al., 2013) and influence alternative splicing patterns, including Exon 9 of the *Capzb*, which regulates growth of actin filaments in muscle cells (Hall et al., 2013) (Figure 3). In competition with QKI, PTB promotes skipping of Exon 9, and an estimated 39% of alternative exons are controlled by either of these proteins during myogenesis (Hall et al., 2013). RNA-seq and CLIP-seq performed on C2C12 mouse myoblast cells uncovered multiple putative QKI splicing targets, as well as mRNAs that are either upregulated or downregulated in an isoform-dependent manner by QKI-6 and QKI-7 (Fagg et al., 2017).

In addition, QKI has also been implicated in cardiomyocyte development. Human embryonic stem cells with QKI knocked out can still differentiate into early cardiogenic progenitor cells, but fail to differentiate into functional cardiomyocytes (Chen et al., 2021). In this context, QKI-5 was found to be strongly upregulated in differentiated cardiomyocytes, and necessary for alternative splicing of genes associated with contractile physiology (Chen et al., 2021). QKI also controls the formation of muscle myofibrils by binding and stabilizing the muscle-specific tropomyosin 3 transcript, promoting its accumulation (Bonnet et al., 2017). Collectively, these reports highlight important roles for QKI in the splicing and stabilization of mRNAs in nonsmooth muscle cells.

### 4.4 | QKI and differentiation of monocytes

Several studies have implicated functions for QKI in the differentiation and maturation of monocytes into macrophages. In committed macrophage progenitors, QKI represses colony-stimulating factor 1 receptor tyrosine kinase (CSF1R) mRNA (Fu et al., 2012), a key regulator of macrophage differentiation (Bourette & Rohrschneider, 2000; Pixley & Stanley, 2004). Intriguingly, transcriptional activation of QKI and CSF1R during macrophage progenitor maturation is both governed by CCAAT/enhancer-binding protein  $\alpha$  in macrophage progenitor cells (Fu et al., 2012). This keeps CSF1R transcriptionally active while posttranscriptionally repressed by QKI, suggesting that a disruption of QKI-mediated repression of CSF1R is likely required before macrophage maturation can continue (Fu et al., 2012).

During monocyte differentiation into pro-atherosclerotic macrophages, QKI causes changes expression of many transcripts enriched in monocyte-macrophage differentiation pathways, as well as cause changes in splicing of mRNAs including Adducin 3 (ADD3), Fc fragment of IgG receptor IIb (FCGR2B), very low density lipoprotein receptor, Protein bicaudal D homolog 2, and Kinesin family member 13A (De Bruin, Shiue, et al., 2016) (Figure 3). Two transcription

factors associated with macrophage development, PU.1 (Zhang et al., 1994) and Kruppel-like factor 4 (Shankman et al., 2015), were found to bind the QKI promoter region, suggesting a possible mechanism for how QKI is up-regulated during macrophage differentiation (De Bruin, Shiue, et al., 2016; Feinberg et al., 2007; Gertz et al., 2013). Furthermore, QKI was shown to have functions in the macrophage lipid metabolism and the formation of foam cells (De Bruin, Shiue, et al., 2016). An important foam cell marker, Scavenger receptor A, was found to be directly targeted and repressed by QKI during monocyte differentiation (Wang et al., 2015). In addition, functions for QKI have also been implicated in mature macrophages (L. Wang, Zhai, et al., 2017; W. Wang, Zhai, et al., 2021), re-enforcing the notion that QKI participates in the differentiation and maintenance of macrophage phenotypes.

#### 4.5 | QKI as a regulator of EMT

EMT is a dynamic cell differentiation process that facilitates embryonic development and occurs during cancer progression, aiding cancer cells to acquire invasive properties (Thiery et al., 2009). QKI was recently shown to be induced during EMT through loss of the epithelial miRNA family, miR-200 (Pillman et al., 2018). The miR-200 family is critical regulators of EMT, operating within a reciprocal feedback loop with the mesenchymal transcription factors Zinc finger E-box-binding homeobox (ZEB) 1 and ZEB2 to control cell plasticity (Burk et al., 2008; Gregory et al., 2008; Gregory et al., 2011). Numerous miR-200 target mRNAs have been uncovered that are relevant to EMT, particularly mRNAs coding for proteins involved in the actin cytoskeleton (Bracken et al., 2014). Using large cancer datasets, QKI-5 was found to be amongst the strongest targets negatively correlated with the miR-200 family and miR-375, both of which are repressed during EMT and allow for increased expression of QKI-5 (Kim et al., 2019; Pillman et al., 2018).

During EMT, QKI-5 causes changes in alternative splicing of many genes by directly binding within introns adjacent to the alternative exon or within the alternative exon itself (Pillman et al., 2018; Yang et al., 2016) (Figure 3). Similar to miR-200 target genes, QKI-5 was predominantly found to regulate genes that control the dynamics of the actin cytoskeleton (Pillman et al., 2018). This allows miR-200 to directly manipulate the actin cytoskeleton by altering gene expression while indirectly affecting splicing through QKI-5. Furthermore, QKI regulation of alternative splicing of the actin-associated protein Filamin B (FLNB) was found to promote an intermediate mesenchymal state and enhance tumorigenicity in breast cancer models (J. Li, Choi, et al., 2018).

Functional studies in mesenchymal breast cancer cell lines show QKI enhances features of the mesenchymal phenotype including promoting an elongated cell morphology with increased cell migration and invasion, and mammosphere forming potential (J. Li, Choi, et al., 2018; Pillman et al., 2018). In other contexts, such as lung and oral squamous cell carcinoma, QKI appears to repress EMT-associated properties, and in some cases these involve splicing of transcripts, such as NUMB and ADD3, that are promoted during EMT (Kim et al., 2019; J. Z. Wang, Fu, et al., 2021; S. Wang, Tong, et al., 2021; Zong et al., 2014). These apparent discrepancies likely reflect contextual differences, including relative levels of QKI isoform, target genes, and associated splicing factors, that are important for QKI's function in cancer cells.

## 5 | CONCLUSION

Upon reviewing the literature, a consistent theme emerges where QKI isoforms promote or affect cell differentiation. Early studies in the mutant QKI mouse models demonstrated improper development of the vasculature and nervous system, with subsequent investigation revealing underlying changes in a range of cell differentiation processes may mediate these effects. The balance of QKI isoforms is an important determinant of QKI function, for example, during myelination cytoplasmic QKI isoforms influence nuclear export and local translation of MBP mRNA (Larocque et al., 2002; Zhao, Tian, et al., 2006). It is possible that similar mechanisms occur in other physiological contexts, where the stages of gene expression during differentiation and maturation are coordinated by QKI isoforms. Transcription factors associated with cell type-specific differentiation, including QKI-5 itself, could increase transcription of genes while QKI-5 affects their alternative splicing and regulates their nuclear export. Following this, cytoplasmic expression of QKI-6 and QKI-7 could control cytoplasmic localization, stability, and translation of these transcripts. This could allow QKI to temporally control posttranscriptional regulation of genes driven by different transcription factors.

QKI is not the only RBP that regulates cell differentiation and altered expression of RBPs is often observed during differentiation. For instance, Muscleblind-like splicing regulators 1/2 (Han et al., 2013), PTBP1/2 (Linares et al., 2015), NOVA (Leggere et al., 2016), fragile X mental retardation protein (Liu et al., 2018), and Pumilio (PUM) 1/2 (Zhang



et al., 2017) have all been reported to have roles in differentiation including alternative splicing and mRNA localization. The reported overlap in function between RBPs during cell differentiation suggests they engage in a complex regulatory network. The full extent of how QKI cooperates and competes with other RBPs to affect cell differentiation is yet to be determined and warrants further exploration.

One important observation is the dynamic regulation of QKI expression through different stages of cell differentiation. During glial (Hardy, 1998; Takeuchi et al., 2020) and monocyte (De Bruin, Shiue, et al., 2016; Fu et al., 2012) differentiation, QKI is expressed highly in progenitor cell populations and is reduced after differentiation but is re-expressed upon terminal differentiation when the cell gains its functional identity. These studies indicate QKI has distinct effects on gene expression in different stages of development and reinforces the role that QKI and other RBPs have in coordinating the complexity involved in cellular differentiation.

More research is likely to identify additional examples of alternate localization and local translation of mRNAs by the QKI isoforms. The complicated nature of autoregulation between the isoforms and the limited number of specialized techniques are available to detect these modes of regulation likely pose challenges. However, as cancer EMT-associated features like migration and cell morphology are controlled through localization and local translation of target mRNAs (Liao et al., 2015; Mardakheh et al., 2015), QKI is a promising candidate regulator of this phenomenon, warranting further exploration.

It is also important to characterize the QKI-specific posttranscriptional regulation across multiple cell types to find a core QKI-specific signature. Although QKI is expressed in many different cell lineages, there is likely a consistent overlap in target transcripts between these contexts. For instance, the splicing event of ADD3 Exon 14 exclusion is regulated by QKI in monocyte to macrophage differentiation (De Bruin, Shiue, et al., 2016), breast and prostate cancer EMT (Pillman et al., 2018) and lung cancer (Wang, Fu, et al., 2021; Zong et al., 2014). It might be that ADD3 Exon 14 splicing is part of a core QKI-directed transcriptomic signal that exists across multiple contexts of tumorigenesis and cellular differentiation alongside cell type-specific regulation of genes like MBP or GFAP. Identifying such a signature and characterizing the functions of its constituent genes could lead to a better understanding of how posttranscriptional regulation and splicing broadly facilitates differentiation.

Uncovering the binding partners and co-regulators that associate with QKI during the regulation of alternative splicing will also be a fruitful area of exploration. Recent work characterizing alternative splicing events during development uncovered distinct patterns for putative QKI targets during development of the brain and heart (Mazin et al., 2021). This highlights the important and complex role that QKI plays in regulation of tissue development and differentiation through alternative splicing. Elucidating the mechanisms governing this process, including identifying RBPs that co-regulate target genes with QKI (e.g., RNA-binding fox-1 homolog 1/2; J. Li, Choi, et al., 2018), or antagonize (e.g., PTB; Hall et al., 2013) will help to generate a mechanistic model of how QKI directly regulates splicing.

QKI is consistently identified as regulating cell fate decisions, playing a role in cellular differentiation in development or in cancer. The balance of the QKI isoforms during oligodendrocyte appears to be tightly and temporally regulated for healthy maturation of myelin producing cells (Larocque et al., 2002; Larocque et al., 2005; Takeuchi et al., 2020; Thangaraj et al., 2017). Studies on cellular differentiation using single-cell transcriptomics have uncovered the stochastic nature of cell fate decisions (Zhang et al., 2019). As most transcriptional analyses rely on bulk methods, which provide a gene expression average that ignores intratissue heterogeneity, it is possible that the majority of QKI's function in differentiation and cancer has not yet been identified. Future studies using single-cell and spatial transcriptomics to uncover cell type and tissue niche-specific expression will help further unravel functions of QKI isoforms.

## AUTHOR CONTRIBUTIONS

**Daniel Neumann:** Conceptualization (equal); writing – original draft (lead); writing – review and editing (equal). **Gregory Goodall:** Writing – review and editing (equal). **Philip Gregory:** Conceptualization (equal); funding acquisition (lead); writing – original draft (supporting); writing – review and editing (equal).

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**CONFLICT OF INTEREST**


The authors declare no potential conflict of interest.

**DATA AVAILABILITY STATEMENT**

Data sharing is not applicable to this article as no new data were created or analyzed in this study

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