Nuclear translocation controlled by alternatively spliced isoforms inactivates the QUAKING apoptotic inducer

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The quaking viable mice have myelination defects and develop a characteristic tremor 10 d after birth. The quaking gene encodes at least five alternatively spliced QUAKING (QKI) isoforms that differ in their C-terminal 8–30-amino-acid sequence. The reason for the existence of the different QKI isoforms and their function are unknown. Here we show that only one QKI isoform, QKI-7, can induce apoptosis in fibroblasts and primary rat oligodendrocytes. Heterodimerization of the QKI isoforms results in the nuclear translocation of QKI-7 and the suppression of apoptosis. The unique C-terminal 14 amino acids of QKI-7 confers the ability to induce apoptosis to heterologous proteins such as the green fluorescent protein and a QKI-related protein, Caenorhabditis elegans GLD-1. Thus, the unique C-terminal sequences of QKI-7 may function as a life-or-death ‘sensor’ that monitors the balance between the alternatively spliced QKI isoforms. Moreover, our findings suggest that nuclear translocation is a novel mechanism of inactivating apoptotic inducers.

[Key Words: quaking; QKI; nuclear translocation; apoptosis; alternative splicing]

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Apoptosis, or programmed cell death, is a physiological process characterized by a cascade of events culminating in the destruction of the cell. There are numerous protein families that have been shown to induce apoptosis including the TNF/Fas ligands (Tartaglia and Goeddel 1992, Ashkenazi and Dixit 1998), oncogenes (Evan and Littlewood 1998), the Bcl-2 family (Adams and Cory 1998; Reed 1998; Gross et al. 1999; Porter 1999), and p53 (Livingstone et al. 1992, Ko and Prives 1996). Heterogeneous nuclear ribonucleoprotein particle K (KH) homology domain containing RNA-binding proteins (Gibson et al. 1993, Siomi et al. 1993) are an emerging class of apoptotic inducers. We have shown that mouse QKI-7 and Drosophila KEP1 and Sam50 are potent inducers of apoptosis (Chen and Richard 1998; Di Fruscio et al. 1998). Two other KH domain-containing proteins, Drosophila FMRF and MCG10, have also been shown to induce apoptosis (Wan et al. 2000; Zhu and Chen 2000). The mechanism by which this family of RNA-binding proteins induce cell death is unknown.

The quaking viable (qkv) mice have been studied for more than thirty years and represent an animal model for dysmyelination (Hogan and Greenfield 1984). Ten days after birth these animals develop a rapid tremor that is especially pronounced in the hind limbs (Hogan and Greenfield 1984). The gene responsible for the defect was cloned and termed the quaking (qk) gene (Ebersole et al. 1996). The mouse qk gene expresses at least five alternatively spliced mRNA including QKI-5, QKI-6, QKI-7, and QKI-G that differ in their C-terminal 30 amino acids (Ebersole et al. 1996, Cox et al. 1999, Kondo et al. 1999). The KH domain of the QKI proteins is embedded in a larger conserved domain of ~200 amino acids called the GSG (GRP33, Sam68, GLD-1) domain (Jones and Schedl 1995; Di Fruscio et al. 1998) or the STAR (signal transduction activator of RNA metabolism; Vernet and Artzt 1997) domain. The GSG domain of the QKI proteins is required for RNA binding and dimerization (Chen et al. 1997; Zorn and Krieg 1997; Di Fruscio et al. 1998) or the STAR (signal transduction activator of RNA metabolism; Vernet and Artzt 1997) domain. The GSG domain of the QKI proteins is required for RNA binding and dimerization (Chen et al. 1997; Zorn and Krieg 1997; Chen and Richard 1998; Wu et al. 1999).

The cellular localization of the QKI isoforms differ in oligodendrocytes (OLs), the myelinating cells of the central nervous system. QKI-5 is predominantly nuclear; QKI-6 and QKI-7 are localized in the perikaryal cytoplasm with lower levels in the nucleus (Hardy et al. 1996). In the qkv− mice, part of the quaking enhancer/promoter is deleted (Ebersole et al. 1996) and, as a result, QKI-6 and QKI-7 isoforms are not expressed in OLs (Hardy et al. 1996). Several missense mutations have been generated in the qk gene by using ethynitrosourea, and these mutations are known to be embryonic lethal;

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QKI homologs have been identified in many species including Xenopus [Zorn et al. 1997], chicken [Mezquita et al. 1998], zebrafish (Tanaka et al. 1997), and Drosophila (Bachrecke 1997, Zaffran et al. 1997). Caenorhabditis elegans does not have a QKI homolog but has a closely related protein called GLD-1 (germ-line defective, Francis et al. 1995) that has a high degree of sequence identity with the QKI GLD domain [Jones and Schedl 1995; Vernet and Artzt 1997]. GLD-1 is required for germ cell differentiation and has been shown to function as a tumor suppressor [Jones and Schedl 1995] and as a translational repressor [Jan et al. 1999]. The relatedness between the GSG domains of QKI and GLD-1 suggest that these proteins may recognize similar RNA targets as dimers. We have shown that C. elegans GLD-1 is able to homodimerize and that QKI and GLD-1 associate with one another when ectopically expressed in HeLa cells [Chen et al. 1997]. It was also shown that QKI-6 can replace GLD-1 in repressing the translation of a GLD-1-specific RNA target, tra-2 [Saccomanno et al. 1999]. These observations demonstrate the functional similarity between the QKI and GLD-1 GSG domains. The overexpression of GLD-1 in NIH 3T3 cells did not induce apoptosis as was observed with QKI-7 [Chen and Richard 1998]. These findings suggested that the QKI-7 GSG domain may not be required for the induction of apoptosis.

In this study, we identified a short region of 14 amino acids in QKI-7 that confers the ability to induce apoptosis to heterologous proteins. The other QKI isoforms were unable to induce apoptosis, but induced cell survival when expressed with QKI-7. Here we describe the possible mechanism by which the QKI-7 apoptotic inducer is suppressed by the QKI isoforms. Our data suggest that a balance between the alternatively spliced QKI isoforms is required for cell survival.

**Results**

**Characterization of the apoptosis induced by QKI-7**

The expression of QKI-7 in NIH 3T3 cells induces cell death [Chen and Richard 1998]. To further assess the apoptosis and determine whether early signs of apoptosis were observed, we stained QKI-7-expressing cells with annexin V conjugated with fluorescein isothiocyanate (FITC) and propidium iodide. Abundant staining with annexin V–FITC was observed in NIH 3T3 cells transfected with QKI-7 compared with the control cells transfected with QKI-5 [Fig. 1A, top panels]. Some of the QKI-7 transfected cells that stained with annexin V also stained with propidium iodide [Fig. 1A, bottom panels], implying that they represent later stages of cell death. To confirm that the transfected cells were staining with annexin V–FITC, we performed double staining by using annexin V–FITC and indirect immunofluorescence using anti-myc antibodies followed by a rhodamine-conjugated secondary antibody. Most of the myc epitope-tagged QKI-7 transfected cells that stained with annexin V–FITC also stained with anti-myc antibodies [Fig. 1B, right panel], demonstrating that the QKI-7 transfected cells were dying. In comparison, the myc-QKI-5 transfected cells that stained with annexin V–FITC did not stain with anti-myc antibodies [Fig. 1B, left panel], demonstrating that the cell death observed in these cells corresponds to background cell death and not from the presence of QKI-5.

Two additional experiments were performed to assess whether classical apoptotic/survival pathways were being utilized. We examined the effect of overexpressing a known survival protein such as Bcl-2 [Adams and Cory 1998; Gross et al. 1999]. NIH 3T3 cells were cotransfected with expression vectors encoding GFP alone and Bcl-2, GFP–QKI-7 with pCEP4 empty vector, and GFP–QKI-7 and Bcl-2. The transfected green cells were counted and scored as apoptotic if they displayed irregular, condensed, or fragmented nuclei with the nuclear stain DAPI. The expression of GFP–QKI-7 with pCEP4 empty vector induced >50% apoptosis at 36 h [Fig. 1C], consistent with our previous observations [Chen and Richard 1998]. The expression of GFP alone and Bcl-2 had background levels of cell death (~15%) at 36 h [Fig. 1C]. The cotransfection of Bcl-2 suppressed the apoptosis mediated by GFP–QKI-7 in a dose-dependent manner [Fig. 1C, left panel]. The percentage of green cells or the transfection efficiency ranged from 15%–20% 12 h after transfection and decreased by 36 h, in some cases to ~5%, indicating that some green cells had died and did not remain attached to the dish [Fig. 1C, right panel]. To examine whether the apoptosis was caspase dependent, GFP–QKI-7 transfected cells were treated with 50 µM Z-DEVD.fmk, a known caspase 3 inhibitor [Nicholson et al. 1995], or dimethylsulfoxide as a control. The addition of Z-DEVD.fmk suppressed the apoptosis induced by QKI-7 to near background levels [Fig. 1D, left panel]. In addition, the number of green cells was maintained at 15%, 36 h post-transfection in the presence of Z-DEVD.fmk, consistent with cell survival. Taken together, our results suggest that QKI-7-expressing NIH 3T3 cells exhibit the hallmarks of apoptosis. The QKI-7 transfected cells stain by using the TUNEL assay [Chen and Richard 1998] and annexin V–FITC. The apoptotic process is caspase dependent and suppressed by the overexpression of the survival protein Bcl-2.

The C terminus of QKI-7 is necessary and sufficient to signal apoptosis

A structure analysis of QKI-7 was performed to determine the regions or domains required for the induction of apoptosis. Truncations and chimeric proteins were engineered as GFP fusion proteins, transfected in NIH 3T3 cells, and analyzed for their ability to induce apoptosis. The QKI GSG domain is a tripartite protein module with
a central KH domain flanked by the N terminus of KH region [NK] and the C terminus of KH region [CK]. The NK and CK regions are also referred to as the QUA1 and the QUA2 regions [Vernet and Artzt 1997]. To investigate the role of the NK region in QKI, known to mediate dimerization [Chen and Richard 1998], or the entire GSG domain in QKI-7-mediated apoptosis, deletion analyses were performed. The truncated proteins deleted for the NK region [QKI-7:81–325] or entire GSG domain [QKI-7:205–325] induced apoptosis in NIH 3T3 cells to the same extent as QKI-7 [Fig. 2A]. These findings suggest that RNA binding and the GSG domain are not required for the induction of cell death.

The QKI proteins are known to be alternatively spliced at their C termini resulting in at least four different isoforms [Fig. 2B]. The most common isoforms as detected by Northern blot analysis are QKI-5, QKI-6, and QKI-7 [Ebersole et al. 1996]. The deletion of the C-terminal 14 amino acids, unique to isoform QKI-7, was sufficient to prevent cell death [Fig. 2A; QKI:1–311]. Larger deletions as in proteins QKI:1–205 and QKI:1–180 were also unable to induce apoptosis [Fig. 2A]. These data suggest

Figure 1. QKI-7 is a potent apoptotic inducer. (A) Detection of early apoptosis by annexin V. Myc–QKI-5 or myc–QKI-7 were transfected into NIH 3T3 cells and stained with annexin V–FITC and propidium iodide [PI]. The cells were visualized by fluorescence microscopy. The arrowheads align the cells in top and bottom panels. [B] Myc–QKI-5 or myc–QKI-7 were transfected into NIH 3T3 cells stabled live with annexin V–FITC and fixed, and the myc-tagged QKI-7 was visualized by indirect immunofluorescence with anti-myc antibody followed by a rhodamine-conjugated secondary antibody. [C] Suppression of apoptosis by Bcl-2 overexpression. GFP alone or GFP–QKI-7 were cotransfected into NIH 3T3 cells with increasing amounts of a Bcl-2 expression vector [pCEP4–Bcl-2] or empty plasmid [pCEP4]. After 12 h [solid bars] and 36 h [open bars] the cells were fixed and stained with DAPI to visualize the apoptotic nuclei. [D] The caspase inhibitor Z-DEVD.fmk suppresses QKI-7-induced apoptosis. GFP or GFP–QKI-7 were transfected into NIH 3T3 cells and treated with either DMSO [control] or 50 µM Z-DEVD.fmk as indicated. After 12 h [solid bars] and 36 h [open bars] the cells were fixed and stained with DAPI to visualize the apoptotic nuclei. (C,D) The presence of apoptotic nuclei was scored as cells undergoing apoptosis and expressed as % green cells. Each bar represents the mean ± S.E. of 3 experiments [n > 450, n = number of cells counted]. Statistical evaluation was calculated by paired Student’s t-test. (*) Values that differ significantly from GFP at 36 h [P < 0.01].
that the induction of apoptosis may be isoform specific and that the QKI-7 C-terminal 14 amino acids are required. To investigate whether the C-terminal 14 amino acids of QKI-7 were able to confer to heterologous proteins the ability to induce apoptosis, we fused the QKI-7 C-terminal 14 amino acids to nonapoptotic proteins such as GFP and C. elegans GLD-1 (Chen and Richard 1998). The expression of either GFP or GLD-1 with the C-terminal 14 amino acids of QKI-7 (GFP:14, GLD-1:14) was sufficient to induce apoptotic cell death to similar levels as QKI-7 (Fig. 2A). These findings demonstrate that the addition of amino acids EWIEMPVMPDISAH at the C terminus of cytoplasmic proteins is sufficient to induce apoptotic cell death. We next targeted QKI-7 to the nucleus with a strong nuclear localization signal (NLS) and asked whether nuclear QKI-7 could induce apoptosis in NIH 3T3 cells. The GFP fusion protein containing QKI-7:NLS was localized in the nucleus but was unable to induce cell death (Fig. 2A), suggesting that the C-terminal 14 amino acids of QKI-7 signal apoptotic cell death in the cytoplasm.

The ability of QKI-7 and GFP:14 to induce apoptosis in a cell line that expresses endogenous QKI proteins was investigated. We have shown previously that C6 glioma cells express three QKI isoforms (Chen and Richard 1998). C6 glioma cells were transfected with GFP–QKI-7 or GFP:14 and analyzed for apoptotic cell death. The transfection of GFP–QKI-7 induced apoptosis in a dose-dependent manner with minimal apoptosis observed with 1 µg of DNA (25%) and a maximal response with 3 µg of DNA (70%, Fig. 2C). In contrast, the expression of GFP:14 induced near maximal levels of apoptosis at 1 µg of DNA (60%) and increased minimally with 3 µg of DNA (70%). These data suggest that elevated concentrations of QKI-7 are required to induce apoptosis in cells expressing endogenous QKI isoforms. The fact that GFP:14 was more potent than QKI-7 at inducing apoptosis in C6 glioma cells suggests that C6 glioma cells, unlike NIH 3T3 cells where QKI-7 and GFP:14 were equipotent, are able to neutralize the ability of QKI-7 to induce apoptosis.

The QKI isoforms

To evaluate the ability of the different QKI isoforms to induce apoptosis, we generated cDNAs for isoforms QKI-5, QKI-6, and QKI-G. The integrity of the QKI isoforms was verified by immunoblotting HeLa cell lysates transfected with myc epitope-tagged QKI proteins by using anti-myc, anti-‘pan’ QKI antibodies, anti-QKI-5-, anti-
QKI-6-, or anti-QKI-7-specific antibodies. The anti-‘pan’ QKI antibody recognizes the KH domain of the QKI isoforms (Chen and Richard 1998) and the anti-QKI-5, -QKI-6, and -QKI-7 antibodies recognize the C-terminal specific sequence of each isoform (Hardy et al. 1996). The QKI isoforms migrated on SDS–polyacrylamide gels with molecular masses ranging from 40 to 47 kD and were all recognized with anti-myc and anti-‘pan’ QKI antibodies (Fig. 3A). The proteins encoded by the QKI-5, QKI-6, and QKI-7 cDNAs were also each recognized with anti-QKI-5, -QKI-6, and -QKI-7 antibodies, respectively (Fig. 3B). The QKI proteins were fused to GFP, transfected in NIH 3T3 cells, and analyzed by immunoblotting with the indicated anti-myc-QKI as in A and analyzed by immunoblotting with anti-QKI-5 [lanes 1–5], anti-QKI-6 [lanes 5–8], and anti-QKI-7 [lanes 9–12] antibodies. The migration of QKI-5, QKI-6, and QKI-7 is indicated. (C) NIH 3T3 cells were transfected with expression plasmids expressing the indicated GFP fusion protein. The cells were lysed and analyzed by immunoblotting with anti-pan QKI antibodies.

In summary, these immunoblotting experiments demonstrate that the cDNAs encode the proper QKI isoforms.

Localization of the QKI isoforms

The cellular localization of GFP–QKI-5, GFP–QKI-6, GFP–QKI-7, and GFP–QKI-G was determined. HeLa cells were transfected with expression vectors encoding the QKI isoforms for 12 h and visualized by fluorescence microscopy. GFP–QKI-5 was predominantly nuclear (Fig. 4B), consistent with the presence of a nuclear localization signal at its C terminus (Wu et al. 1999). GFP–QKI-6- and GFP–QKI-G-expressing cells contained the GFP fusion protein in the nucleus and the cytoplasm (Fig. 4C,E). QKI-7 was predominantly cytoplasmic (Fig. 4D). Some cytoplasmic punctate staining was observed with QKI-6, QKI-7, and QKI-G that may represent focal adhesion structures. In summary, QKI-7 is predominantly cytoplasmic, QKI-5 is predominantly nuclear, and the QKI isoforms QKI-6 and QKI-G are localized in both the cytoplasm and the nucleus.

Figure 3. Expression of the QKI isoforms. (A) Untransfected (control) or myc–QKI-5, myc–QKI-6, myc–QKI-7, and myc–QKI-G were expressed in HeLa cells. The cell lysates were separated by SDS-PAGE and analyzed by immunoblotting with anti-myc [lanes 1–5] or anti-pan QKI antibodies [lanes 6–10]. The molecular mass markers are shown at left in kD. (B) HeLa cells were transfected with the indicated myc–QKI as in A and analyzed by immunoblotting with anti-QKI-5 [lanes 1–4], anti-QKI-6 [lanes 5–8], and anti-QKI-7 [lanes 9–12] antibodies. The migration of QKI-5, QKI-6, and QKI-7 is indicated. (C) NIH 3T3 cells were transfected with expression plasmids expressing the indicated GFP fusion protein. The cells were lysed and analyzed by immunoblotting with anti-pan QKI antibodies.

Figure 4. The localization of the transfected QKI isoforms in HeLa cells. Expression plasmids expressing GFP [A], GFP–QKI-5 [B], GFP–QKI-6 [C], GFP–QKI-7 [D], or GFP–QKI-G [E] were transfected in HeLa cells. After 12 h the cells were fixed and visualized using fluorescence microscopy.
The different QKI isoforms and apoptosis

The ability of the different QKI isoforms to induce apoptosis was examined by transfecting NIH 3T3 cells with expression vectors encoding the GFP–QKI isoforms and analyzing the green cells for the presence of apoptosis by fluorescence microscopy at 12 and 36 h after transfection. GFP–QKI-7 was the only wild-type isoform able to induce apoptosis at 36 h (Fig. 5A). Approximately 60% of the GFP–QKI-7 transfected cells were apoptotic 36 h after DNA transfection. In contrast, background levels of apoptosis (~15%) were observed at 36 h with cells transfected with GFP–QKI-5, GFP–QKI-6, GFP–QKI-G, or GFP alone (Fig. 5A). These findings demonstrate that the QKI isoforms do not have a general toxic effect but that QKI-7 has the unique ability to signal to the cell death machinery.

The ethylnitrosourea-induced mutation qk<sup>kit4</sup> altering glutamic acid 48 to glycine (E48G) (Justice and Bode 1986; Ebersole et al. 1996) was introduced in the different isoforms and the resulting proteins were examined for their ability to induce apoptosis in NIH 3T3 cells. Surprisingly, all QKI isoforms including QKI-5, QKI-6, QKI-7, and QKI-G containing the E48G substitution were now able to induce apoptosis when expressed in NIH 3T3 cells (Fig. 5A; data not shown). The number of green cells remaining 36 h after DNA transfection was also reduced, consistent with cell death. These data suggest that the apoptosis observed with the substitution of QKI glutamic acid 48 to glycine may represent a gain-of-function that is independent of the C-terminal unique sequences.

Suppression of apoptosis by coexpressing QKI-5 and QKI-6

The observation that elevated levels of QKI-7 were required to induce apoptosis in C6 glioma cells and the fact that the E48G substitution induces apoptosis implied that dimerization plays a role in the regulation of apoptosis. To determine the role of dimerization in QKI-mediated apoptosis, GFP–QKI-7 was cotransfected with either GFP alone, GFP–QKI-5, or GFP–QKI-6 and assessed for apoptotic cell death by using the nuclear stain DAPI. The expression of GFP–QKI-5 or GFP–QKI-6 with GFP–QKI-7 suppressed the ability of GFP–QKI-7 to induce apoptosis (Fig. 5B). This suppressive effect was not observed when GFP alone was transfected with GFP–QKI-7. We next examined the ability of GFP–QKI-5:E→G or GFP–QKI-6:E→G to suppress the apoptosis mediated by GFP–QKI-7 (Fig. 5B). GFP–QKI-5:E→G and GFP–QKI-6:E→G failed to suppress the apoptosis induced by GFP–QKI-7 demonstrating that dimerization is essential for preventing cell death. To further establish that dimerization is essential for the suppression of apoptosis, we investigated the ability of QKI-5 and QKI-6 to suppress the apoptosis induced by GFP:14, a protein that cannot dimerize. Thirty-six hours after transfection, ~60% of the cells expressing GFP:14 were apoptotic regardless of whether GFP–QKI-5 or GFP–QKI-6 was expressed (Fig. 5B). These data suggest that QKI-5 and QKI-6 suppress apoptosis by forming heterodimers with QKI-7.

Our major concern with the cotransfection studies in Figure 5B was the difficulty in assessing whether the green cells expressed both isoforms. To identify the cells that were transfected with two isoforms GFP alone, GFP–QKI-1–311, or GFP–QKI-5 were cotransfected with
myc–QKI-7 in NIH 3T3. The expression of myc–QKI-7 was visualized by indirect immunofluorescence by using anti-myc antibodies followed by a rhodamine-conjugated secondary antibody. The cells that were both green and red were visualized for apoptotic cell death by using the nuclear stain DAPI. Representative fields are shown [Fig. 6A] and the data were quantitated and expressed as percentage apoptosis [Fig. 6B]. The cells expressing myc–QKI-7 alone, myc–QKI-7/GFP, or myc–QKI-7/GFP–QKI:1–311 exhibited characteristics of apoptosis including cell shrinkage, cytoplasm condensation [Fig. 6A, panels A, D, and G, respectively], and irregular, condensed, and fragmented nuclei [Fig. 6A, panels B, E, and H, respectively]. The myc–QKI-7-expressing cells displayed ∼50% apoptosis and ∼5% of the transfected cells remained attached to the dish 36 h after transfection [Fig. 6B]. The cells that were cotransfected with GFP–QKI-5 and myc–QKI-7 displayed a healthy and normal appearance 36 h post-transfection [Fig. 6A, panels I–K]. These cells had background apoptosis at 36 h (∼10%, Fig. 6B, left) and most of the cells transfected survived or remained on the dish [Fig. 6B, right]. We also noted that the majority of cells that expressed one isoform also expressed the other [Fig. 6A]. These findings suggested that our cotransfection procedure resulted in a high percentage of cells that expressed both isoforms, implying that the majority of the green cells in Figure 5B contained both GFP fusion proteins. The experiments demonstrate the ability of isoforms QKI-5 and QKI-6 [data not shown], but not QKI:1–311, to suppress the apoptosis induced by QKI-7. QKI:1–311 is expressed exclusively in the cytoplasm [Fig. 2A] and was unable to rescue the apoptosis induced by QKI-7 [Fig. 6]. These findings suggest that the expression of a QKI isoform or protein fragment able to heterodimerize with QKI-7 is not sufficient to suppress the apoptosis, but that the heterodimerizing QKI isoform or protein fragment must have access to the nucleus.

### Heterodimerization of the QKI isoforms in mammalian cells

We noticed that QKI-7 entered the nucleus in the presence of QKI-5 [Fig. 6A, panel J]. These findings suggested that heterodimerization was regulating the localization

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**Figure 6.** QKI-7-induced apoptosis is suppressed by QKI-5. (A) NIH 3T3 cells were transfected with an expression plasmid that encodes myc–QKI-7 alone (A,B) or with expression vectors for GFP (C–E), GFP–QKI:1–311 (F–H), or GFP–QKI-5 (I–K). After 36 h the cells were fixed and visualized by indirect immunofluorescence by using the anti-myc antibody followed by rhodamine-conjugated secondary antibody. DAPI was used to visualize the nuclei. Each column represents the same field of cells as visualized under the green [top], red [middle], and blue [bottom] filters. The arrows are used to align the panels. (B) Quantitation of the apoptosis suppressed by QKI-5. NIH 3T3 cells were transfected as in A. For the cells expressing both a GFP fusion protein and a myc epitope-tagged protein, the apoptotic cells were expressed as a percentage of the cells that were both green and red. The panel at right indicates the percentage of cells that were transfected from the total number of cells. Each bar represents the mean ± S.E. of 3 experiments (n > 450). * Values that differ significantly from GFP at 36 h (P < 0.01).
of QKI-7. To confirm that the QKI isoforms were forming heterodimers in mammalian cells, we cotransfected the different isoforms in HeLa cells and performed co-immunoprecipitation studies. Hemagglutinin (HA)-tagged QKI-5 was transfected alone or cotransfected with myc–QKI-5, myc–QKI-6, or myc–QKI-7. The cells were lysed, the lysates were divided equally, and the proteins immunoprecipitated with control immunoglobulin G (IgG) and anti-myc antibodies. The proteins were separated by SDS-PAGE and analyzed by immunoblotting with anti-HA antibodies. HA–QKI-5 coimmunoprecipitated with myc–QKI-5, myc–QKI-6, and myc–QKI-7 [Fig. 7A, lanes 1–12], demonstrating that heterodimerization occurs in vivo. Similar findings were obtained with HA–QKI-6 [Fig. 7A, lanes 13–21]. These observations show that heterodimerization occurs between QKI isoforms that display predominant cytoplasmic and nuclear localizations such as QKI-7 and QKI-5. We next examined whether the E48G substitution could prevent heterodimerization. HA-tagged QKI-5:E→G or QKI-6:E→G were cotransfected with myc–QKI-5, myc–QKI-6, or myc–QKI-7 and analyzed as described above. HA–QKI-5:E→G and HA–QKI-6:E→G did not coimmunoprecipitate with any of the wild-type isoforms [Fig. 7B, lanes 1–21]. These results suggest that the heterodimerization domain is similar to the homodimerization domain located in the NK region of the GSG domain.

**Heterodimerization controls QKI-7 localization**

The relocalization of the QKI isoforms was further investigated in HeLa cells, a cell type where it could be easily observed. Myc–QKI-7 was transfected or cotransfected with GFP–QKI-5 or GFP–QKI-6 in HeLa cells and visualized by using fluorescence microscopy 12 h post-transfection. The 12-h time point was chosen because no apoptosis was observed at this time. Myc–QKI-7 localized exclusively to the cytoplasm of HeLa cells [Fig. 8A,B]. However, myc–QKI-7 staining could be observed throughout the cell, with prominent nuclear localization, when coexpressed with GFP–QKI-5 [Fig. 8C–E]. Interestingly, the localization of QKI-5 did not relocalize to the cytoplasm with the coexpression of QKI-7 [Fig. 8, C–E]. The coexpression of GFP–QKI-6 also relocalized myc–QKI-7 to the nucleus [Fig. 8F–H], but less nuclear staining was observed as compared with GFP–QKI-5 [Fig. 8, cf. D and G]. Although significant cytoplasmic staining of QKI-7 was observed with QKI-6 (Fig. 8G), QKI-6 was able to suppress the apoptosis by QKI-7 (Fig. 5B). These findings suggest that in addition to relocalizing...
QKI-7 to the nucleus, QKI-6 may also inactivate cytoplasmic QKI-7 by forming heterodimers. If dimerization is required for relocalization, then the E48G substitution should impair the relocalization. HeLa cells were cotransfected with myc–QKI-7 and GFP–QKI-5:E → G or GFP–QKI-6:E → G and visualized by fluorescence microscopy. Myc–QKI-7 failed to relocalize to the nucleus with the coexpression of GFP–QKI-5:E → G or GFP–QKI-6:E → G (Fig. 8I–N). These results suggest that the nuclear localization of QKI-7 is controlled by heterodimerization.

QKI-7 adenovirus induces apoptosis in primary rat OLs

The dysmyelination phenotype observed in the qk-null mice suggests that the QKI isoforms are involved in the normal physiology of the OLs (Hogan and Greenfield 1984). We wanted to examine whether QKI-7 was a potent apoptotic inducer in a cell type where it is known to have a physiological role. We constructed adenoviruses that express tetracycline-inducible QKI-5, QKI-6, and QKI-7. The QKI adenoviruses also express GFP constitutively and it is a marker of infection. Initially, we performed experiments with HeLa cells to verify that the adenoviruses were expressing the QKI isoforms in an inducible manner. HeLa cells were coinfectected with two adenoviruses: one supplying constitutive levels of the tetracycline-regulated transactivator (tTA) and the other expressing the desired QKI isoform under a tetracycline-inducible promoter. Induced and noninduced infected HeLa cells were lysed, and the proteins were separated by SDS-PAGE and immunoblotted with anti-myc antibodies. QKI-5, QKI-6, and QKI-7 were expressed in a tetracycline-inducible manner (Fig. 9A). The expression of the QKI isoforms was tightly regulated, as little or no expression was observed in the noninduced cultures.

Primary rat OLs were isolated from newborn rats as described (Lubetzki et al. 1991). After 2 d of maturation in the presence of growth factors, the cells displayed an OL appearance with little or no contaminating astrocytes (Fig. 9B, left). Approximately 43% of the cells stained with anti-galactocerebroside antibody conjugated to FITC, a well-known marker for mature OLs (Fig. 9B, right). Thus, our cultures contained ~40% mature OLs and ~60% immature OLs. These cells were coinfected as described above with a tTA-expressing adenovirus and the QKI-inducible adenoviruses and could be visualized by indirect immunofluorescence by using the anti-myc antibody. The myc epitope-tagged QKI iso-

Figure 8. Nuclear translocation of QKI-7 with QKI-5 or QKI-6 but not with QKI-5:E → G or QKI-6:E → G. Myc–QKI-7 was either transfected alone (A, B) or cotransfected with GFP–QKI-5 (C–E), GFP–QKI-6 (F–H), GFP–QKI-5:E → G (I–K), or GFP–QKI-6:E → G (L–N) into HeLa cells. After 12 h the cells were fixed, immunostained with an anti-myc antibody followed by a rhodamine-conjugated secondary antibody, and mounted onto a glass slide in the presence of the nuclear stain DAPI. The cells were visualized by fluorescence microscopy. Each column represent the same field of cells as visualized under the green [top], red [middle], and blue [bottom] filters. The arrows are used to align the panels.
forms were expressed in their respective compartments in the OLs. QKI-5 was nuclear, QKI-6 localized throughout the cell, and QKI-7 was cytoplasmic (Fig. 9C). We next examined whether the overexpression of the QKI isoforms resulted in cell death. The induced and noninduced infected cells were stained live with annexin V–phycoerythrin to detect early signs of apoptosis. The stained cells were analyzed by flow cytometry for the expression of GFP and annexin V–phycoerythrin. Approximately ∼65% of the cells infected with the QKI-7 adenovirus in the ON state stained positive for annexin V–phycoerythrin (Fig. 9D, bottom right). The infection with QKI-5 or QKI-6 induced and noninduced had no significant increase in staining with annexin V (Fig. 9D, top right). All OLs cultures were infected at equivalent MOIs as observed with the expression of the GFP marker (Fig. 9D, left). These data suggest that QKI-7 is an apoptotic inducer in mature and immature primary rat OLs.

Discussion

In this study, we show that a balance of the QKI isoforms regulates the activity of the QKI-7 apoptotic inducer. The expression of the different QKI isoforms including QKI-5, QKI-6, and QKI-G in fibroblasts or primary rat OLs did not induce apoptosis. The coexpression of either QKI-5 or QKI-6 with QKI-7 caused the nuclear translocation of QKI-7 and suppressed the apoptosis normally observed with the expression of QKI-7. These data suggest that heterodimerization causes the nuclear translocation of QKI-7 and may be the major mechanism responsible for its inactivation.

The region necessary for the induction of apoptosis was mapped to the unique C-terminal 14 amino acids of QKI-7. This represents a new functional domain in the QKI proteins. Other functional domains characterized previously in QKI include the GSG domain required for dimerization and RNA binding (Chen and Richard 1998) and an NLS in the unique sequences of QKI-5 (Wu et al. 1999). The finding that the C-terminal sequences of QKI-7 are sufficient to confer the ability to induce apoptosis to GFP and GLD-1 suggests that the 14 amino acids of QKI-7 signal to the apoptotic machinery independent of other functional domains. Thus, the RNA-binding activity of QKI-7 is not required for induction of cell death. Mutations or deletions within the QKI-7 GSG domain.
did not suppress the apoptosis [data not shown]. Because
the 14 amino acids of QKI-7 confer the ability to induce
apoptosis to heterologous proteins, we propose the name
‘killer sequence’ for this sequence. Database searches
using the 14-amino-acid killer sequence EWEMPVMP-
DISAH did not reveal a novel protein module. However,
a core motif (underlined) was found for Drosophila, C.
elegans, and mammalian proteins in GenBank. The pro-
teins that contained this motif included C. elegans
CED-9 [the histidine is replaced with a lysine] and hy-
pothetical protein C09H10.9 [accession no. T19165], hu-
man and murine Hect2/rs protein [accession no.
AAC31433], Drosophila CG11958 and CG9906 gene
products [accession nos. AAF57631 and AAF48618]. The
mechanism by which the killer sequence QKI-7 commu-
nicates to the apoptotic machinery is unknown.

The suppression of apoptosis by the coexpression of
QKI-5 or QKI-6, but not QKI:1–311 suggests that het-
erodimerization with QKI-7 is not sufficient for the sup-
pression. It has been shown that QKI-5 is capable of nu-
cleoplasmic shuttling [Wu et al. 1999]. Thus, QKI-5 and
other QKI isoforms such as QKI-6 and QKI-G may
shuttle into the nucleus as a heterodimer with QKI-7.
Because QKI-1–311 has an exclusive cytoplasmic local-
ization, QKI-7/QKI:1–311 heterodimers would remain
cytoplasmic resulting in apoptosis. Our data suggest that
cytoplasmic but not nuclear QKI-7 signals to the apot-
otic machinery.

The qk mice have been shown to contain an en-
hancer/promoter deletion in the qk gene [Ebersole et al.
1996]. It has been shown that the qk OLs express QKI-5,
but not QKI-6 and QKI-7 [Hardy et al. 1996]. How an en-
hancer/promoter deletion prevents the production of the
QKI-6 and QKI-7 isoforms in OLs is unknown. It is
thought that the absence of QKI-6 and QKI-7 prevents
the proper maturation of the OLs and/or the process of
myelination [Hardy et al. 1996]. Our findings suggest that
the balance in the QKI isoforms is critical for the
normal function of the QKI proteins and cell viability.
Based on our findings, the loss of QKI-6 and QKI-7 should
not affect the viability of the OLs in the qk mice and
indeed normal quantities and hyperplasia of OLs have
been reported [Friedrich 1975]. We believe that the
loss of QKI-6 and QKI-7 would provide an imbalance in
the QKI isoforms that would impair the differentiation
and maturation of the OLs resulting in the dysmyelina-
tion phenotype observed in the qk mice. A balance be-
tween QKI isoforms (HOW, held-out-wings) has been
reported for the export of the stripe mRNA in Drosophila
melanogaster [Nabel-Rosen et al. 1999]. This supports the
hypothesis that the balance in the QKI isoforms
regulates their function.

The ethynitrosourea-induced point mutation altering
glutamic acid 48 to glycine prevents homo- and het-
erodimerization of all QKI isoforms [Fig 7; Chen and
Richard 1995, Wu et al. 1999]. Moreover, we showed that
all QKI isoforms containing the E48G amino acid sub-
stitution are able to induce apoptosis, regardless of
whether they contained the killer sequence. These data
suggest that the QKEE48G represents a gain-of-function
amino acid substitution that is separate from the QKI-7
killer sequence. Thus, the QKI proteins have multiple
regions that can induce apoptosis: QKI-7 has the killer
sequence and all isoforms have an NK region that can be
altered to induce cell death. These findings further sup-
port our hypothesis that the QKI isoforms are critical
proteins for maintenance of cell viability and cell death.

Translocation inactivates the QKI apoptotic inducer
[Chen and Richard 1998]. The corresponding GFP plasmids were obtained by digesting the myc-Bluescript plasmids with EcoRI and subcloning in the EcoRI site of pEGFP-C1 (Clontech). The plasmids GFP–QKI-5, GFP–QKI-6, and GFP–QKI-G were constructed by amplifying plasmid myc–QKI-7 with the T7 promoter primer and the following reverse primers: 5'-CAAGAATTCTATAACACACACTGGGTTC-3' (QKI-1–311), 5'-CGTGAATTCATCTTTTCTTTCCTTTGGATGGCCTGAATATATGGGAGGATCATGA-3' (QKI-7:NLS), 5'-TTTGGAAATCCACCTTTGTCGGAAAGACCATCCTAACACACACTGGTGTCAATACT-3' (QKI-7:NLS), 5'-GACGAATTCACATGTTGGTGTGAC-3' (QKI-G). Plasmid GFP–GLD1:14 was constructed by a two-step subcloning strategy: The GLD-1 DNA fragment was first subcloned into pEGFP-C1 using PCR amplification of myc–GLD-1 as a DNA template with the T7 promoter primer and the oligonucleotide 5'-TACAAGCTTGAAGAGG-GTGGTTGTGAC-3' as primer. The amplified DNA fragment was then digested with BglII and HindIII and subcloned into the corresponding sites of pEGFP-C1, generating GFP–GLD-1. The plasmid GFP-GLD-1:14 was constructed by inverse PCR using GFP–QKI-7 as a DNA template with the T7 promoter primer and the following reverse primers containing a BglII site: 5'-GGTAGATCTGAGTGGATTGAAATGCCAATC-3' and a GFP reverse primer. The amplified sequence was first subcloned into pEGFP-C1 using PCR amplification of myc–Bluescript QKI isoforms into HeLa cells was carried out with the T7 vaccinia virus system as described previously (Chen et al. 1997). For the experiments with the caspase-3 inhibitor, 50 µM Z-DEVD.fmk (Calbiochem) or DMSO was added to the media after the LipofectAMINE transfection.

**Apoptosis assays, transfection efficiency, and immunostaining**

After transfection (12 or 36 h), the cells were fixed with 4% paraformaldehyde in 1× PBS for 10 min and permeabilized with 1% Triton X-100 in 1× PBS for 5 min, and the nuclei were stained with 3 µg/mL 4,6-diamidino-2-phenylindole (DAPI). The morphology of transfected cells and the localization of QKI isoforms were examined by fluorescence microscopy. Cells with characteristic morphological features such as nuclear condensation and fragmentation were considered apoptotic. The transfection efficiency was calculated as a percentage of transfected cells (green cells/total cells). For immunostaining, the fixed cells were incubated with the anti-myc 9E10 antibody (1:1000) at room temperature for 1 h and then followed by incubation of a rhodamine-conjugated goat anti-mouse secondary antibody (Jackson Laboratories; 1:200) for 30 min. The nuclei were stained with DAPI.

**Protein expression**

For protein expression, the cells were lysed in Laemmli buffer and the proteins were separated by SDS-PAGE, transferred to nitrocellulose, and immunoblotted. Immunoblotting was performed using anti-myc 9E10, or anti-hemagglutinin (HA), anti-pan QKI (Chen and Richard 1998), anti-QKI-5, anti-QKI-6, and anti-QKI-7 (Hardy et al. 1996) rabbit polyclonal antibodies, followed by a horseradish peroxidase-conjugated secondary antibody and developed using chemiluminescence. Immunoprecipitations were performed as described previously (Chen and Richard 1998).

**Primary rat OLs cultures and cell viabilities**

OLs progenitor cells were purified from newborn Sprague-Dawley rats essentially as described using a Percoll gradient (Lubetzkii et al. 1991). These cells were incubated for 2 d in media supplemented with 10% fetal calf serum, 2.5 ng/mL platelet-derived growth factor-AA, 2.5 ng/mL basic fibroblast growth factor, and 10 nM triiodothyronine. These OLs were infected with the indicated QKI expressing adenovirus and an adenovirus AdCMV–TTA that expresses the TTA. Each virus was added at an M.O.I. of 10.
OL cells survival assays were measured by staining with annexin V–phycoerythrin as described by the manufacturer [Pharmingen]. Doxycyclin (1 μg/mL) was added at the same time as the adenoviruses to represent the uninduced cultures. The doxycyclin binds to the tTA and represses the tTA resulting in a noninduced culture. The induced and noninduced OLs were gated for GFP green expression and for annexin V–phycoerythrin red. The annexin V positive cells represented the cells undergoing early signs of programmed cell death and infection was calculated as the percentage of GFP positive cells.

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References

Adams, J.M. and Cory, S. 1998. The Bcl-2 protein family: Arbi-
ters of cell survival. Science 281: 1322–1326.
Ahnem, E.S., Fernandes-Alnemri, T., and Litwack, G. 1995.
Cloning and expression of four novel isoforms of human
interleukin-1 β converting enzyme with different apoptotic
activities. J. Biol. Chem. 270: 4312–4317.
Ashkenazi, A. and Dixit, V.M. 1998. Death receptors: Signaling
and modulation. Science 281: 1305–1308.
Baehrecke, E.H. 1997. who encodes a KH RNA binding protein
that functions in muscle development. Development 124:
1323–1332.
Boise, L.H., Gonzales-Garcia, M., Postema, C.E., Ding, L., Lind-
sten, T., Turka, L.A., Mao, X., Nunez, G., and Thompson,
C.B. 1993. bcl-x, a bcl-2-related gene that functions as a
dominant regulator of apoptotic cell death. Cell 74:
597–608.
Chen, T. and Richard, S. 1998. Structure-function analysis of
QKI. A lethal point mutation in mouse quaking prevents
homodimerization. Mol. Cell. Biol. 18: 4863–4871.
Chen, T., Damaj, B., Herrerra, C., Lasko, P., and Richard, S.
1997. Self-association of the single-KH domain family mem-
ers Sam68, GRP33, GLD-1 and Qki1: Role of the KH do-
main. Mol. Cell. Biol. 17: 5707–5718.
Cox, R.D., Hugill, A., Shedlovsky, A., Noveroske, J.K., Best, S.,
Justice, M.J., Lehrach, H., and Dove, W.F. 1999. Contrasting
effects of ENU induced embryonic lethal mutations of the
quaking gene. Genomics 57: 333–341.
Di Fruscio, M., Chen, T., Bonyadi, S., Lasko, P., and Richard, S.
1998. The identification of two Drosophila KH domain pro-
teins: KEP1 and SAM are members of the Sam68 family of
GSG domain proteins. J. Biol. Chem. 273: 30122–30130.
Ebersole, T.A., Chen, Q., Justice, M.A., and Artzt, K. 1996. The
quaking gene unites signal transduction and RNA binding in
the developing nervous system. Nat. Genet. 12: 260–265.
Evan, G. and Littlewood, T. 1998. A matter of life and cell death.

Science 281: 1317–1321.
Francis, R., Maine, E., and Schedl, T. 1995. Gld-1: A cell-type
specific tumor suppressor gene in C. elegans. Genetics
139: 607–630.
Friedrich, V.L.J. 1975. Hyperplasia of oligodendrocytes in quak-
ing mice. Anat. Embryol. 147: 259–271.
Gibson, T.J., Thompson, J.D., and Heringa, J. 1993. The KH
domain occurs in a diverse set of RNA-binding proteins that
include the antiterminator NusA and is probably involved
in binding to nucleic acid. FEBS Letts. 324: 361–366.
Gross, A., McDonnell, J.M., and Korsmeyer, S.J. 1999. BCL-2
family members and the mitochondria in apoptosis. Genes
& Dev. 13: 1899–1911.
Hardy, R.J., Loushlin, C.L., Friedrich, Jr., V.L., Chen, Q., Eber-
sole, T.A., Lazzarini, R.A., and Artzt, K. 1996. Neural cell
type-specific expression of QKI proteins is altered in the
quaking viable mutant mice. J. Neurosci. 16: 7941–7949.
Hogan, E.L and Greenfield, S. 1984. Animal models of genetic
disorders of myelin. In Myelin [ed. P. Morell], pp. 489–534.
Plenum, New York.
Jan, E., Motzny, C.K., Graves, L.E., and Goodwin, E.B. 1999. The
STAR protein, GLD-1, is a translational regulator of sexual
identity in Caenorhabditis elegans. EMBO J. 18: 258–269.
Jiang, Z.-H., Zhang, W.-J., Rao, Y., and Wu, J.Y. 1998. Regulation
of Ichi-1 pre-mRNA alternative splicing and apoptosis by
mammalian splicing factors. Proc. Natl. Acad. Sci. 95:
9155–9160.
Jones, A.R. and Schedl, T. 1995. Mutations in GLD-1, a female
germ cell-specific tumor suppressor gene in C. elegans, affect
a conserved domain also found in Sam68. Genes & Dev.
9: 1491–1504.
Justice, M. and Bode, V. 1986. Induction of new mutations in a
mouse t-haplotype using ethynitrosourea mutagenesis.
Genet. Res. 47: 187–192.
Justice, M.J. and Bode, V.C. 1988. Three ENU-induced alleles of
the murine quaking locus are recessive embryonic lethal
mutations. Genet. Res. 51: 95–102.
Ko, L.J. and Prives, C. 1996. p53: Puzzle and paradigm. Genes
& Dev. 10: 1054–1072.
Kondo, T., Furuta, T., Mitsunaga, K., Ebersole, T.A., Shichiri,
M., Wu, J., Artzt, K., Yamamura, K., and Abe, K. 1999. Ge-
nomic organization and expression analysis of the mouse
qki locus. Mamm. Genomene 10: 662–669.
Livingstone, L.R., White, A., Sprouse, J., Livanos, E., Jacks, T.,
and Tisty, T.D. 1992. Altered cell cycle arrest and gene am-
plification potential accompany loss of wild-type p53. Cell
70: 923–935.
Lubetzki, C., Goujet-Zale, C., Ganssmuller, A., Mone, M., Bril-
lat, A., and Zale, B. 1991. Morphological, biochemical, and
function characterization of bulk isolated glial progenitor
cells. J. Neurochem. 56: 671–680.
Massie, B., Couture, F., Lamoureux, L., Mosser, D.D.,
Guilbauld, C., Jolicoeur, P., Belanger, F., and Langelier, Y.
1998. Inducible overexpression of a toxic protein by an
denovirus vector with a tetracycline-regulatable expression
cassette. J. Virol. 72: 2289–2296.
Massie, B., Mosser, D.D., Koutroumanis, M., Vitét-Mony, L,
Lamoureux, L., Couture, F., Paquet, L., Guilbauld, C,
Dionne, J., Chahla, D., et al. 1999. New adenovirus vectors
for protein production and gene transfer. Cytotechnology
28: 1–12.
Mezquita, J., Pau, M., and Mezquita, C. 1998. Four isoforms of
the signal-transduction and RNA-binding protein Qki1 ex-
pressed during chicken spermatogenesis. Mol. Reprod.
Dev. 50: 70–78.
Nabel-Rosen, H., Dorevitch, N., Reuvény, A., and Volk, T.

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1999. The balance between two isoforms of the *Drosophila* RNA-binding protein How controls tendon cell differentiation. *Mol. Cell* 4: 573–584.

Nicholson, D.W., Ali, A., Thornberry, N.A., Vaillancourt, J.P., Ding, C.K., Gallant, M., Gareau, Y., Griffin, P.R., Labelle, M., and Lazebnik, Y.A. 1995. Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature* 376: 37–43.

Porter, A.G. 1999. Protein translocation in apoptosis. *Trends Cell Biol.* 9: 394–401.

Reed, J.C. 1998. Bcl-2 family proteins. *Oncogene* 17: 3225–3236.

Porter, A.G. 1999. Protein translocation in apoptosis. *Trends Cell Biol.* 9: 394–401.

Shaham, S. and Horvitz, H.R. 1996. An alternatively spliced *C. elegans* ced-4 RNA encodes a novel cell death inhibitor. *Cell* 86: 201–208.

Wan, L., Dockendorff, T.C., Jongens, T.A., and Dreyfuss, G. 2000. Characterization of dFMR1, a *Drosophila* melanogaster homolog of the fragile X mental retardation protein. *Mol. Cell. Biol.* 20: 8536–8547.

Zorn, A.M. and Krieg, P.A. 1997. The KH domain protein encoded by *quaking* functions as a dimer and is essential for notochord development in *Xenopus* embryos. *Genes & Dev.* 11: 2176–2190.

Zorn, A.M., Grow, M., Patterson, K.D., Ebersole, T.A., Chen, Q., Artzt, K., and Krieg, P.A. 1997. Remarkable sequence conservation of transcripts encoding amphibian and mammalian homologues of *quaking* a KH domain RNA-binding protein. *Gene* 188: 199–206.
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