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REVIEW

RNA Editing Catalyzed by ADAR1 and Its Function in Mammalian Cells

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Abstract—In mammalian cells two active enzymes, ADAR1 and ADAR2, carry out A-to-I RNA editing. These two editases share many common features in their protein structures, catalytic activities, and substrate requirements. However, the phenotypes of the knockout animals are remarkably different, which indicate the distinct functions they play. The most striking effect of ADAR1 knockout is cell death and interruption of embryonic development that are not observed in ADAR2 knockout. Evidences have shown that ADAR1 plays critical roles in the differentiating cells in embryo and adult tissues to support the cell's survival and permit their further differentiation and maturation. However, our knowledge in understanding of the mechanism by which ADAR1 exerts its unique effects is very limited. Many efforts had been made trying to understand why ADAR1 is so important that it is indispensible for animal survival, including studies that identify the RNA editing substrates and studies on non-editing mechanisms. The interest of this review is focused on the question why ADAR1 and not ADAR2 is required for cell survival. Therefore, only the data, published and unpublished, potentially connecting ADAR1 to its cell death effect is selectively cited and discussed here. The features of cell death caused by ADAR1 deletion are summarized. Potential involvement of interferon and protein kinase RNA-activated (PKR) pathways is proposed, but obviously more experimental evaluations are needed.

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Hydrolytic deamination on adenosine in RNA transcripts converts adenosine to inosine. This is the biochemical principle of A-to-I RNA editing [1, 2]. A-to-I editing is carried out by a group of adenosine deaminases (adenosine deaminase that act on RNA, ADAR) that present in animals from *Caenorhabditis elegans* [3], *Drosophila melanogaster* [4, 5], squid fish [6], aves [7], up to mammals [8, 9] including humans [10, 11]. Three ADARs exist in mammalian cells, ADAR1-3, but only ADAR1 and ADAR2 were shown to be active for RNA editing [12, 13]. While ADAR1 and ADAR2 share many common features in protein structure, expression pattern, and requirements of double stranded RNA structures for their substrates, they are obviously different in their biological functions, especially in sustaining cell survival in mammalian cells demonstrated in knockout animals [14-17]. Reviews of A-to-I RNA editing have been very well updated to include the latest progress in understanding of this fundamental posttranscriptional process [18-22]. In mechanistic studies of A-to-I RNA editing and in most reviews, ADAR1 and ADAR2 are usually considered indistinctively as the general enzymes generating inosines in RNA molecules, although significant differences exist. The reasons might be due, as least partially, to the insufficient knowledge in understanding of the seemingly unrelated effect of ADAR1 and ADAR2 in animals. This review tries to bring up the unique characteristics of ADAR1 and discuss the questions remaining in ADAR1 study, emphasizing its critical roles in cell survival.

BRIEF DESCRIPTION OF ADAR1 AND A-to-I RNA EDITING

ADAR1 was first identified in bovine liver extract as a double stranded RNA unwinding protein [8, 23] that had been observed earlier in oocytes [24, 25]. Soon it was also found in a placenta library [26], as well as in fibroblasts as an interferon-inducible protein [27]. In its cDNA sequence three double stranded RNA binding motifs and a highly conserved deamination domain were found [23]. Meanwhile, the unwinding activity was found to result from the A-to-I conversion due to the deamination of adenosines [24]. Therefore ADAR1, named DRADA

originally, was identified as the first A-to-I RNA editing enzyme [9, 23]. The cDNA cloning of ADAR1 quickly led to the identification of ADAR2 [28] and ADAR3 [29, 30]. Although ADAR3 has not been demonstrated to be an active editing enzyme, it is highly homologous to the other ADARs [30]. ADAR1 and ADAR2 act on double stranded RNAs *in vitro* without specific selection on the adenosine residuals within the RNA strands and efficiently convert them into inosines [31, 32]. This contrasts with the high specificities of editing sites *in vivo*. In cells, the editing sites reside in the imperfect, with wobbles, double stranded structures formed intramolecularly. Some well studied sites are solely or preferentially edited by one of the ADARs, although some other sites are edited by both ADAR1 and ADAR2 [2, 33].

As inosine is recognized as guanine by the protein translation machinery and it pairs with cytosine instead of uracil in double stranded RNAs, A-to-I conversion can change their sequence information and structures [2]. The significance of A-to-I RNA editing was primarily observed in neuron receptors, such as GluR-B ion channels [34-39] and 5-hydroxytryptamine receptors 2C ($5HT_{2C}R$ or SR2c) [40-42]. Identification of proteins that their coding mRNA subject to editing was the central focus in A-to-I RNA editing studies [2, 12, 43, 44]. A-to-I editing can also generate or eliminate splicing sites, for example, ADAR2 edits its own coding RNA generating alternative splicing [45]. Editing on microRNAs modifies the Drosha/Dicer processing sites, which affects the efficacy of microRNA production [46]; the edited microRNA also shifts its targets to an alternative spectrum of RNAs [18]. To date, editing of these particular sites and the knowledge of the functional modification caused by editing of these limited number of RNA targets represent most of our understanding of the molecular mechanisms of A-to-I RNA editing. Failure in identification of a new editing target that explains the dramatic animal phenotype, as well as the recent finding of a large number of editing sites on non-coding RNA regions, have gradually led the focus of studies to drift from protein codons to non-coding regions [18, 19].

RNA editing exerts its biological and/or pathological effects through substrate RNA molecules — the edited forms convey different functions from the unedited, genome coded forms. The differences in the ADAR1 and ADAR2 knockout phenotypes indicate discrepant functions of these two enzymes, especially regarding relevance to cell death [16, 17]. Therefore, to understand the specific function of ADAR1 in the regulation of cell death will require the identification of the RNA spectrum that is uniquely edited by ADAR1. However, both ADAR1 and ADAR2 exhibit the same editing activity biochemically [2], and most of the thousands of newly discovered editing sites in non-coding regions have not been well characterized [19, 47]. This makes the general understanding of the function of A-to-I RNA editing difficult, and it is even more difficult to distinguish the functions of ADAR1 from ADAR2. Nevertheless, emerging evidence indicates that ADAR1 plays fundamental roles not limited to neuron cells, but in much broader field including cell survival [17], stem cell differentiation [48, 49], and embryonic development [15-17, 50], as well as in pathological conditions, such as virus infection [51-58], tumor development [59-62], autoimmune disease [63], and disruption of skin pigmentation [64-67]. More specific reviews have been published that summarize the relevance of RNA editing to infections [52, 68], malignancies [69], and other diseases [70]. It is conceivable that understanding the molecular mechanism of this RNA editing enzyme will significantly enhance our understanding of some of the fundamental biological questions in cell survival and differentiation. It will also help to understand the molecular mechanisms in the occurrence and development of the diseases involving abnormal cell proliferation and death.

ADAR1 PROTEIN STRUCTURE AND EXPRESSION

Within the small family of ADAR proteins, ADAR1 has the most distinguishable structure compared to the other ADARs in mammalian cells or to lower organisms [19, 47]. *Drosophila melanogaster* and *C. elegans* have one or two ADAR enzymes, respectively, with low conservativeness with the mammalian proteins [3, 5]. Their function seems to be limited to the neural cells, i.e. there is not of much relevance to the function of ADAR1 to be discussed here [71, 72]. In squids two ADAR enzymes are expressed from a single *ADAR* gene with alternative splicing [6]. These ADARs are more homologous to mammalian ADAR2 than ADAR1, and their functions are less studied than in mammalian cells. Therefore, the specific function of ADAR1 is mainly studied in mice due to the availability of cell and animal models.

ADARs are highly conserved in mammals with the common features of a catalytic domain in its C terminal and double stranded RNA binding motifs (DRBMs) next to the catalytic domain at the N terminal side [23, 26, 73]. There are three DRBMs in ADAR1, locate in the middle of the protein sequence, compare to two DRBMs in ADAR2 and ADAR3 [28, 29]. In addition, two Z-DNA binding domains, Z-α and Z-β, reside in its N terminus, which are unique to ADAR1 [74, 75] (Fig. 1; see color insert).

It has been well characterized in both mouse and human that *ADAR1* expression is driven by multiple promoters that link to alternative exons in the *ADAR1* gene [73, 76-82]. Two major isoforms of ADAR1, P150 and P110, named following their molecular weights, are yielded by these different RNA splicings (see Fig. 2). The 150 kDa isoform, translated from exon 1a, is the full

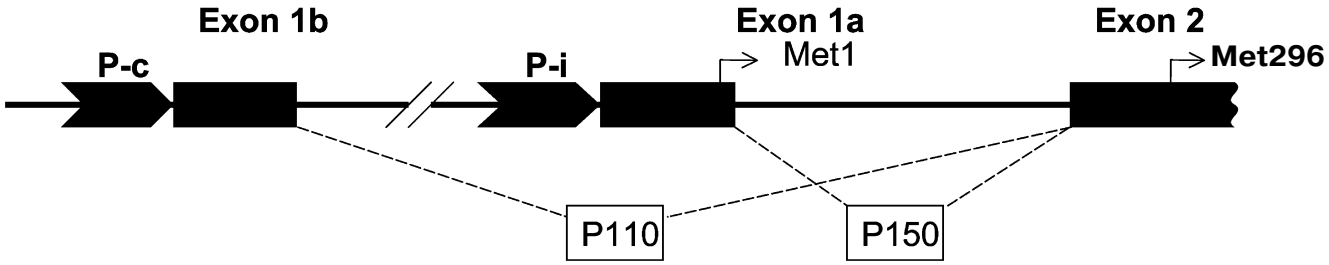

Fig. 2. Promoters and alternative exons in the *ADAR1* gene locus. Two major protein isoforms are expressed from the *ADAR1* gene resulting from variant splicing and the initial Met codon to be used for translations. Either exon 1a or 1b can be spliced to exon 2. Exon 1a is transcribed driven by an interferon inducible promoter, Pi. Exon 1b is located upstream of exon 1a and is under the control of a constitutively active promoter, Pc. Exon 1a codes the Met1 translation initial codon for the translated protein P150 isoform. Transcript of exon 1b is translated from Met296, which locates at exon 2. P110 can be translated from both mRNAs containing either exon 1a or exon 1b.

length protein that is mainly distributed in the cytosol; the 110 kDa isoform, coded by other alternative first exons and lacking the Z-DNA binding domains, is mainly located in the cell nucleus. Exon 2 can be spliced to either exon 1a or 1b. Exon 1a, 5.4 kb upstream of exon 2, is driven by an inducible promoter (Pi). Exon 1b, 14.5 kb upstream of exon 2, is driven by a consistently activated promoter (Pc) [76]. The Pi promoter of ADAR1 has an interferon (IFN)-stimulated response element, ISRE, and KCS-like elements associated with IFN responsiveness [80, 83]. IFN stimulation in cultured cells significantly increases P150 expression, but not that of P110 [73, 76]. As IFN production is a defensive mechanism again infection by microbial pathogens [84], and ADAR1 P150 level is elevated in infectious conditions [79, 81, 85, 86], ADAR1 was presumed to play roles in the courses of infections [52, 87]. However, ADAR1 is not simply an anti-infection protein; it also plays proviral roles in some cases [52, 55, 56].

P110 is constitutively expressed in cells [73, 76]. In early mouse embryos, P110 expression could be detected on day 10.5 [81], but P150 was not expressed until day 15 [81]. ADAR1 knockout embryos die at day 11.5 to 12 [16, 17], so P110 is theoretically sufficient for embryo survival around the mid-gestation stage. Regulation of P110 expression, however, has not been well characterized.

The unique structure of ADAR1, the Z-DNA binding domains, only exist in the P150 isoform. It binds specifically to the left-handed Z-DNA conformation with high affinity (K_d = 4 nM) [74, 88, 89]. Z-DNA is a high energy conformer of B-DNA that occurs 5′ to or behind a moving RNA polymerase, the result of torsional strain generated by a moving polymerase during transcription [90]. It was proposed that its Z-DNA binding activity allows ADAR1 to act on the nascent RNA so that editing occurs before splicing [74, 91, 92]. ADAR1 was also shown to associate with splicing factors in lnRNP particles [93], so the Z-DNA binding activity of ADAR1 connects RNA transcription, splicing, and editing together. It was recently reported that Z-DNA formation is involved in gene suppression [94], and inhibitors for Z-DNA binding activity have been proposed as therapeutic reagents [95]. Nevertheless, as the P150 is not expressed in the early stage embryos, by then the embryos die in ADAR1 knockout, the activity of the Z-DNA binding domain that exists only in P150 is not likely to be a major factor related to the cell death phenomenon in the ADAR1 knockout cells. Interestingly, a recent report [96] showed in a particular knockout mouse model that deletion of exon 1a specifically removes P150 ($P150^{-/-}$) and should not affect P110, result in embryonic lethality at 11 days post coitus (d.p.c.). The phenotype is similar to or even severer than that previously observation in both P150 and P110 knockout mice [16, 17]. It seems that P150, not the P110, is essential for the embryo to survive. However, the conclusion may not be easily made from the observation of this animal model. It raised the question why P150 not expressed in day 11 embryos is required for their survival. Whether the P110 protein is expressed normally in the $P150^{-/-}$ cells needs to be examined. Somehow this critical data was not presented in the publication.

EDITING ACTIVITY OF ADAR1

Both ADAR1 and ADAR2 edit RNA transcripts *in vivo* at selected adenosine resides. The editing sites can be very specific for ADAR1 or ADAR2, while it can be completely random at other sites [18]. Traditionally, RNA editing sites were discovered accidently through aligning different functional cDNA sequences to the same genomic locus [38, 39, 41]; therefore, only a very limited number of these sites were identified. The specific editing enzymes responsible for these sites were well defined. An incomplete list of these traditional editing sites can be found in Table 1, which represents most editing sites under biologic conditions and their corresponding editors. Notably, in the most extensively studied editing sites, the GluR-B Q/R site and $5HT_{2C}R$ A, B, C, D, and E

sites, ADAR1 specifically or preferentially edits $5HT_{2C}R$ A and B sites, while ADAR2 is responsible for GluR-B Q/R and $5HT_{2C}R$ D and E sites. In general, editing catalyzed by ADAR2 is more critical for the functions of glutamine receptors, while both ADAR1 and ADAR2 are required for the function of $5HT_{2C}R$. It has been demonstrated that improper editing on $5HT_{2C}R$ could lead to unusual behaviors similar to depression or cause Prader–Willi like syndrome in animals [97-99].

During the last several years, variant methodologies were developed trying to systematically find editing sites, including chemical modification of the editing sites [3, 100], bioinformatic analysis of the RNA database [101-106], and new generation high throughput RNA sequencing technologies [107-109]. As a consequence, several thousands of new editing sites have been found, most of them being in non-coding regions, and their function has not been well characterized yet. More detailed information on editing of these non-coding sequences can be found in recent review articles [18, 19, 101, 110, 111]. However, these highly efficient methods are not able to distinguish the editing substrates specific for ADAR1 or ADAR2. To date, no method or experimental system has been reported that permits systematic discovery of the ADAR1 or ADAR2 specific substrate. Only a few of the newly found editing sites are known for their responsible editase. Because the non-coding regions usually form long hairpin structures, it is likely that they can be acted on by both ADAR1 and ADAR2, instead of being specific substrates. It is difficult to explain the specific function of ADAR1 without knowing the unique spectrum of its substrates.

Interestingly, microRNA has been demonstrated to be a new category of RNA editing substrate. In human brain, approximately 16% of pri-miRNAs are subject to A-to-I editing [112]. As microRNA has emerged to be a potent class of small RNAs that silence gene activities and may target a broad range of molecules, editing on miRNAs could therefore have a large impact on miRNA-mediated gene silencing. Studies of microRNA editing have been carried out mainly by Nishikura's group [18, 46, 112-114]. With the advantage of using ADAR1 and ADAR2 specific knockout materials, they determined that some of the sites are specific for ADAR1 or ADAR2. In Table 2 the microRNA editing sites found in mice are

Table 1. Protein coding mRNA editing sites and corresponding enzymes

RNA substrate	Editing site	Editing efficiency, %	ADAR1*	ADAR2*	Reference
GluR-B	Q/R	98-100		+++	[14, 17]
	+60 hotspot	~30-60	+++		[14, 17]
	R/G	37-75	+	++	[14, 17]
GluR-C	R/G	90	++	+	[14]
GluR-D	R/G	45	+	++	[14]
GluR5	Q/R	23-64	++	+	[14, 17]
GluR6	Q/R	41-86	+	++	[14, 17]
	I/V	87	+	++	[14]
	Y/C	90		+++	[14]
$5\text{-}HT_{2C}R$	A	75-90	+++		[14, 16, 17, 41]
	B	56-90	+++		[14, 16, 17, 41]
	C	13-40	+	++	[14, 16, 17, 41]
	D	40-70		+++	[14, 16, 17, 41]
	E	0-7		+++	[14, 16, 17]
ADAR2	–1	15-~33		+++	[14, 136]
	+23	~13(?)-25		+++	[14, 45]
	+24	~13(?)-45		++	[14, 45]
Kv1.1	I/V	~50		+++	[105]
Gabra-3	I/M	100	++	++	[137]

* Relative specificity of the editing on the indicated site edited by ADAR1 or ADAR2:
+++, exclusively or almost exclusively edited by ADAR1 or ADAR2;
++, dominantly edited by ADAR1 or ADAR2;
+, contribute to editing on the indicated site at low efficiency.

Table 2. Editing sites in microRNAs and corresponding enzymes

RNA substrate	Editing site	Editing efficiency*, %	ADAR1**	ADAR2**	Reference
Mir-142	−12	<1	?	?	[45]
	+4	~4	+		[45]
	+5	~4	+		[45]
	+6	<1	?	?	[45]
	+9	<1	?	?	[45]
	+12	~4-5	+		[45]
	+19	<1	?	?	[45]
	+40	~10	+	+	[45]
	+50	<1	?	?	[45]
	+55	<1	?	?	[45]
	+62	<1	?	?	[45]
Mir-376a	+4	H: 40-100 M: <5-70		+++	[46]
Mir-376b	+8			+++	[46]
Mir-376b	+44	M: 50-60	++	+	[46]
Mir-376c	+44	M: 55-70	+++		[46]
Mir-151	−1		+	++	[108]
	+3		+++		[108]
	49, UAG	H: 40 M: 30	+++		[107]
let-7g	14, UAG	H: 30 M: 20		+++	[107]
miR-27a	10, CAG	H: 50 M: 20		+++	[107]
miR-99a	13, AAA	H, M: 20		+++	[107]
miR-99b	44, CAC	H, M: 10	+++		[107]
miR-99b	47, AAG	H: 50 M: 10			[107]
miR-203	85, UAG	H: 60 M: 30		+++	[107]
miR-376a	9, UAG	H, M: 50		+++	[107]
	15, UAG	H: 90 M: 50		+++	[107]
miR-376b	67, UAG	H: 95 M: 50	+++		[107]
miR-379	10, UAG	H: 60 M: 20		+++	[107]
miR-411	20, UAG	H: 80 M: 60	+++		[107]
miR-423	13, UAG	H: 40 M: 20	+++		[107]

* MicroRNA substrates are listed in this table only when mouse microRNA data is available. If the human version is also available, they are included for references. H, human microRNA; M, mouse microRNA.

** Relative specificity of the editing on the indicated site edited by ADAR1 or ADAR2:

+++, exclusively or almost exclusively edited by ADAR1 or ADAR2;

++, mainly edited by ADAR1 or ADAR2;

+, contribute to editing on the indicated site at low efficiency;

?, data is not available.

summarized, and their corresponding editing enzymes are also added to this table according to the published data.

In addition, RNA editing patterns are different in different species, and they are also different in the same species in different tissues. Studies employing particular cells or tissues may fail to find some of the editing events that happen in other tissues. Therefore, it will be very difficult to identify all the editing sites and evaluate their functions. Unquestionably, applying new material and more advanced techniques in future studies will discover more substrates and editing sites. However, identification of the specific editing sites for ADAR1 and ADAR2 will not be achieved solely using these high throughput methods. Understanding the specific function of ADAR1 remains challenging.

ADAR1 AND CELL SURVIVAL

The most prominent function of ADAR1 demonstrated in the gene knockout animals is to support cell survival and embryonic development. ADAR1$^{-/-}$ embryos could not survive beyond 11.5-12 d.p.c. [16, 17]. The severe phenotype was quite a surprise compared to ADAR2 knockout [14]. ADAR2 was thought to be more important than ADAR1 [43], but embryonic lethality was not observed in ADAR2 knockout, in which the defect was mainly related to neuron functions. The knockout mice lived to three weeks old, and the postnatal death was directly linked to over-excitation of the GluR-B receptor. The edited form of GluR-B expressed from the genomically engineered gene loci rescued ADAR2 knockout animals [14], indicating the lethality of ADAR2 is mainly related to the GluR-B editing site [115]. In contrast, ADAR1 knockout lead to embryonic death at an early developmental stage, and by then neuron function was not essential for embryo survival. Massive cell death in the absence of ADAR1 revealed an essential function of this editing enzyme in cell survival [17]. Experiments using the ADAR1 knockout mouse model provided the first evidence that A-to-I editing exerts its function beyond neural tissues in normal biological conditions [15, 17], although the ubiquitous expression pattern of ADAR1 had been observed when the gene was cloned [23].

The most vulnerable cells to ADAR1 loss were the hematopoietic cells and hepatocytes [15-17], while other cells including the cells in brain, heart, lung, blood vessels, kidney, and placenta in ADAR1$^{-/-}$ embryos did not appear particularly abnormal. A defect in embryonic hematopoiesis was the major site affected by ADAR1 deletion, although it is not known whether it is the only or predominant cause for the death of the embryo. Interestingly, our recent work [48], together with the data of another group [49], showed that ADAR1 was also required for adult hematopoiesis. ADAR1 deletion completely blocks the development of all the hematopoietic lineages. Differentiating progenitors depend on ADAR1 for their survival and further differentiation. However, the self-renewal and homing of hematopoietic stem cells were intact in the knockout conditions [48].

ADAR1 specific knockout in liver [17], as well as in other tissues (unpublished data) also resulted into cell death in the adult stage. This revealed a broad effect of ADAR1 in supporting cell survival. Although embryonic and mature tissues might be different in the mechanisms by which ADAR1 exerts its functions to support cell viability, no solid evidence has been obtained to explain either of the cell death pathways.

That ADAR1 deficiency causes cell death is very obvious. There are three features of cell death in ADAR1 knockout. (a) It is related to cell differentiation. Cell death happened in embryos only when the embryos progressed to mid-gestation stage. At this stage embryo are fast-growing, and organs expand in size rapidly and specialized functions start to be established. ADAR1 deficient embryonic stem cell lines were also raised successfully [16, 17]. Obviously, ADAR1 is dispensable in these pluripotent cells for their survival and proliferation. In addition, the knockout embryos developed to mid-gestation stage in the uterus. It did not show abnormality in early embryos. The embryo sizes were not evidently different from the wild type and heterozygous embryos in the same litter at day 11.5, the time death occurred. Pathologic study of the embryos did not reveal noticeable difference in major organs. The only notable change was in the embryonic liver in which cell death in hepatocyte and hematopoietic cells was observed. (b) Not every cell type requires ADAR1 for their survival. The brain, lung, muscle, kidney, and placenta tissues are not dependent on ADAR1 or are at least less sensitive to ADAR1 deletion ([17] and unpublished data). (c) Cell lines derived from knockout embryos through natural immortalization grow normally [17], while the primary cells are prone to dying in the same culture condition as the wild type cells (unpublished data).

These characteristics more or less reflect a mechanism in which ADAR1 exerts its function in cell survival. First, ADAR1 is not completely necessary for cell survival because some cells survive without ADAR1. It is conceivable that ADAR1 is not an essential protein in basic metabolism, such as energy production, DNA replication, RNA transcription, or other fundamental mechanisms required for cell survival. Second, at least one other molecular event has to coexist in ADAR1 deficiency to trigger the cell death pathway, and this event did not happen in undifferentiated cells, but only in certain committed lineages. Finally, the *in vitro* immortalization of the knockout cells likely caused epigenetic modifications that compensated the deficiency of ADAR1 or eliminated the negative effect that enhances ADAR1 deficiency. This epigenetic modification does not happen in primary cells.

These characteristics will need to be considered to elucidate the specific function of ADAR1 in cell death regulation.

RNA editing and ADAR proteins were reported to potentially regulate gene activities at chromosome, RNA transcript, and protein translation levels. Some of these effects seemed to be mediated by the inosine residues generated by A-to-I RNA editing, such as the retention of edited RNA in the nucleus [116], edited RNA involved in heterochromatin formation [117], hyper-edited dsRNA subject to cleavage [118], and multiple IU pair-containing RNAs suppressing interferon induction [119]. Such mechanisms are unlikely to be unique for cell survival effects of ADAR1 as inosine containing RNA also can be produced by ADAR2. On the other hand, some other findings are probably tied to ADAR1 specific functions, although it is not known if they are the major factors for the cell death, such as upregulated interferon signaling in ADAR1 knockout embryos [49], inhibition of PKR activation by ADAR1 [87], or potential formation of protein complexes [54, 120]. RNA editing at particular sites by ADAR1, such as the specific sites on microRNAs [112], as well as the non-editing activities of ADAR1, e.g. the Z-DNA and RNA binding domain functions [94] might underlie its role in sustaining cell viability.

PKR PATHWAY AND PROTEIN TRANSLATION

During mRNA translation in mammalian cells, a rate-limiting step, binding of eukaryotic initiation factor 2 (eIF2) to Met-tRNAi, is regulated by the phosphorylation of the α-subunit of eIF2 [121, 122]. The phosphorylated eIF2-α inhibits eIF5, which catalyzes conversion of eIF2-GDP to eIF2-GTP required for the binding with Met-tRNAi and transfers Met-tRNAi to the 40S ribosomal subunit [123, 124]. Therefore, phosphorylation of eIF2-α prevents formation of the eIF2-GTP-Met-tRNAi complex and inhibits global protein synthesis [125-127]. Protein kinase activated by RNA (PKR) is one of the kinases that phosphorylate eIF2-α [126, 127]. Under condition of viral infection, PKR can be activated, which leads to the shutting down of protein synthesis and cell death [126, 128].

ADAR1 was found to form a protein complex with PKR and inhibit its catalytic activity [52, 54]. ADAR1-inhibited eIF2-α phosphorylation had been observed by independent laboratories [52, 54, 129]. Loss of the inhibition of ADAR1 may lead to the deterrence of global protein synthesis through the phosphorylation of eIF2-α as it does in viral infection. For that reason this pathway is potentially involved in ADAR1 knockout cell death. However, some evidences exist against this hypothesis. First, ADAR1 knockout cells grow well in cultured conditions [17] and in early embryos, indicating global protein synthesis is not inhibited by the absence of ADAR1. Second, in order to test if the PKR pathway is a critical player in ADAR1 knockout cell death, we prepared ADAR1 and PKR double knockout mice. The double knockout did not rescue the embryonic lethal phenotype [17]. Nevertheless, it is still possible that eIF2-α is involved in the cell death pathway independent of PKR regulation. It will be interesting to find out whether other eIF2-α kinases are highly activated in ADAR1 deficiency that leads to eIF2-α phosphorylation.

IFN AND APOPTOTIC PATHWAYS

ADAR1 knockout embryos die in a very narrow time window. In different animal models the embryos survived up to 11.5 [17] and 12 d.p.c. [16]. At day 11.5, the size and morphology did not show obvious difference between the wild type and the knockout embryos, indicating that once the death pathway is activated, it kills the animal very rapidly. If this is true, it is difficult to catch the right time point for cell death pathway analysis, as the death does not occur at exactly the same time point in different embryos even in the same litter. The half-day difference might be a big difference in the cell death processes, and it is difficult to determine if the death signaling had just initiated or it was near its end. We tried to analyze the gene expression in knockout embryos by RNA microarray analysis, but the results turned out to vary in different embryos. We could not distinguish whether the changes were a direct effect of ADAR1 deficiency or were secondary reactions.

Recently, a sophisticated microarray analysis was conducted in a particular cell population, $CD150^{+}CD48^{-}CD244^{-}$ hematopoietic stem cells (HSCs) sorted by flow cytometry from the livers of early 11 d.p.c. embryos [49]. Dramatic increases (up to 300-fold) of gene expressions were found in interferon (IFN) regulated genes, including *STAT1*, *STAT2*, *IRF1*, *IRF7*, *IRF9*; the GTPases Mx1, Mx2; the RNA-activated protein kinase PKR (EIF2AK2); the 2′,5′-oligoadenylate synthetases OAS1, OAS2, OAS3; the ubiquitin-like modifiers Isg15, Isg20; the interferon-induced proteins with tetratricopeptide repeats Ifit1-Ifit3. Therefore, the crucial function of ADAR1 as a suppressor of interferon signaling was reasoned. These upregulated genes probably influence many cellular and molecular events in the embryos. However, the IFN levels in the extracellular fluid of $ADAR1^{-/-}$ embryos were over 13-fold (IFN-α) and over 80-fold (IFN-β) more than the control embryos. It is not known whether the upregulated IFN target genes were the results of increased IFN levels or ADAR1 deficiency boosted the signal transduction downstream to IFN receptors. Three questions were raised from this observation. First, if IFN regulated genes were also upregulated in other tissues of the deficient embryos, just like in the HSCs, which potentially contributed to the

embryo's death. Second, if the increased IFN target gene expression was the major reason for the embryo and embryonic cell death. Third, if the elevated IFN level and its regulated gene expression were the direct effect of ADAR1 deficiency or a secondary response. In other words, how ADAR1 deficiency led to the elevated IFN signaling. As discussed earlier in this review, it is well defined that expression of ADAR1 P150 is under the regulation of IFN through direct IFN binding to the KCS-like element in its promoter sequence. How ADAR1 feedbacks and inhibits IFN expressions is not known. Although questions exist, the upregulation of IFN responsive genes is an obvious signature of ADAR1 knockout. We also observed these changes in our microarray analysis (unpublished data).

Interestingly, a very recent report showed that inosine-containing double stranded RNA (c-IU dsRNA) suppressed both the interferon and apoptotic pathways [119]. Specific binding of I-U dsRNA to the cytosolic sensor proteins for poly(IC), melanoma differentiation-associated protein 5 (MDA-5), and retinoic acid inducible gene I (RIG-I) inhibited the ligand binding to poly(IC). MDA-5 and RIG-1 binding to poly(IC) will lead to activation of a transcription factor interferon regulatory factor 3 (IRF3). Subsequent phosphorylation of multiple residues on IRF3 is followed by dimerization and nuclear translocation. Then activated IRF3 triggers production of type I interferons and ultimately induces a transcriptional cascade. Activation of IRF-3 is also required for virus- or dsRNA-induced apoptosis, both MDA-5 and RIG-I interact with and initiate apoptotic pathways. This finding potentially connects ADAR1 deficiency to the cell death consequence through an IFN pathway. This hypothesis is illustrated in Fig. 3 (see color insert). This MDA-5/RIG-I and IRF3 pathway is interesting but needs further assessments. As shown in this study [119], there was a minimum content of I-U RNA required to suppress IRF3 activation. It is not yet known if ADAR1produces sufficient inosine-containing RNA in cells under the biologic conditions; and if inosine-containing RNA produced by A-to-I RNA editing works similarly as the chemically synthesized I-U RNA used in the study. ADAR1 will need to produce enough inosine-containing RNAs to surpass the threshold for the suppression of MDA-5 and RIG-I in order to prevent the cell death. In addition, this pathway to our current knowledge does not explain well why only ADAR1 knockout but not ADAR2 knockout causes cell death.

EDITING VERSUS NON-EDITING ACTIVITY OF ADAR1

As an active RNA editing enzyme, ADAR1 likely acts on many more editing sites than that in the characterized substrates. However, only a limited number of RNA editing sites have been identified in protein coding RNAs, and they have not been demonstrated to link to cell survival mechanisms. The finding that microRNA is subjected to RNA editing is interesting. A total of 47 primiRNAs were found highly edited in human brain [112]. The editing has significant impact on microRNA genesis [46] and on specific target gene selections [18]. However, it has not yet been well studied how efficient microRNA editing occurs in other tissues besides the brain, and if it also occurs in cells of embryonic tissues. A particular microRNA editing that relates to the cell death pathway has not been demonstrated.

RNA editing, mainly through ADAR1 that regulates DNA repair enzyme Neil1, was just reported [130]. The edited Neil1 showed dramatic difference from the genomic encoded protein in its DNA damage repair activities. Tested by *in vitro* assay, purified ADAR1 can edit up to 40% of Neil1 mRNA, indicating that ADAR1 might be involved in DNA damage repair through its editing activities. As DNA damage happens in biological and stressed conditions, and leads to arrest of cell proliferation and apoptosis, Neil1 editing might be the candidate that links ADAR1 and its cell survival effect if efficient ADAR1 editing on Neil1 can be confirmed in the cells that are vulnerable to ADAR1 deletion.

While ADAR1 was mainly studied as one of the A-to-I editases, emerging evidences have shown that it also acts independently from its catalytic activity. Through an unknown mechanism, multiple mutations in *ADAR1* gene loci cause a mild human skin pigmentation disease, Dyschromatosis symmetrica hereditaria (DSH) [65, 66, 131, 132]. More than 90 mutations have been reported in different families [64, 67] that scatter from the N terminus to the C terminus. It is not certain that all of these mutations abolish the editing activity of ADAR1, but the symptoms in patients with different types of mutation do not appear to be different. It is conceivable that a non-editing function of ADAR1 relates to the pigmentation in human skin.

Through protein–protein interactions, ADAR1 forms protein complexes with other proteins with or without double strand RNA bridges, such as NF90 [133], $P54^{nrb}$ [116], lnRNP [93], and hUpf1 [134]. These complexes potentially convey ADAR1 effects into a broad range of regulations in gene transcription, RNA posttranscriptional processes, and RNA degradations, and therefore have impact on cell viability. However, no evidence has hitherto been found that these interactions play a particular role in the mechanism for ADAR1 in cell death regulation.

Comprising three RNA binding motifs is a unique feature of ADAR1. It might contribute to high affinity RNA binding, including short interfering RNAs (siRNA) [33]. We have demonstrated that ADAR1 binding to siRNA dramatically reduces the gene silencing effects of siRNAs that is independent from its RNA editing activity

[33]. It was also found that through RNA binding but not the editing activity, ADAR1 inhibits RNAi through the siRNA pathway and affects microRNA processing independently of the enzymatic activity as demonstrated with mir-376a2 [135].

In summary, ADAR1 exerts its effects through different functional domains responsible for RNA editing and non-editing activities. It is not yet known whether one of these functions or both are required for cell survival and embryonic development. In the available knockout mouse models, the gene deletions lead to the eradication of the entire ADAR1 protein molecule. The truncated proteins theoretically coded by the remaining *ADAR1* gene were not detected in the knockout tissues/cells, this likely being due to instabilities of the modified mRNA and/or the truncated proteins. Therefore, the functions of different domains of ADAR1 are not amenable to study with the animal models. To dissect the specific functions of each domain of ADAR1 and to determine if they contribute to the cell death phenotype, new experimental systems will need to be established.

CHALLENGES IN ADAR1 STUDIES AND FUTURE DIRECTIONS

It is more than ten years since ADAR1 was found to be essential for embryonic development and cell survival. Mechanistic study of ADAR1 proved to be very challenging. First of all, limited by technologic developments, only a few examples of A-to-I RNA editing sites were identified whose functions were studied in the first several years. Recently a large number of editing sites have been found employing new methodologies that were not available earlier. In general, the biologic function of RNA editing has not been well elucidated. As one of the major editases, ADAR1 is yet to be extensively studied. Second, the study of ADAR1 has been hindered by the shortage of appropriate material that allows molecular studies to dissect the cell death pathway. Early embryonic lethality and the heterogeneous pattern of cell death in ADAR1 knockout make embryo tissues not ideal for biochemical studies. In addition to the tiny size of embryos at 9 to 10 d.p.c., each embryo needs to be genotyped for phenotype analysis as only 25% of embryos are homozygous within the progenies of heterogeneous mouse breeding. Also, different organs and tissues show different cell death phenomena. Microarray analysis of the un-dissected embryo tissues yielded very ambiguous data (unpublished data). The cell lines derived from the knockout embryos lost the cell death feature after they were cultured *in vitro* and subjected to immortalization. Conditional knockout was also achieved, but it is limited by the cre/lox system, the efficiency of cre recombination being not very high. This gave rise to mosaic tissues consisting of ADAR1 gene deleted and non-deleted cells. Again it did not produce a homogenous cell population for analysis. In addition cells die rapidly once the gene is deleted, and this makes the tissue even complicated for its components. Besides these, ADAR1 interacts with the silencing small RNAs, small interfering RNA and microRNAs, that makes small RNA-based knockdown strategies more knotty to be explained if they are used for ADAR1 studies. Furthermore, cell death after ADAR1 gene deletion happens kinetically. The initial and secondary molecular events caused by ADAR1 depletion are difficult to be distinguished in the studies with available animal and cell models. To illuminate the specific roles of ADAR1 in biological and pathological processes in the future studies, it might be wise to explore both RNA editing and non-editing activities of ADAR1. For its RNA editing significance, we will need to identify its specific spectrum of RNA targets. There is not a clear clue what its non-editing function could be. However, for a convincing conclusion we will need to overcome the material limitation for biochemical analysis. In a cell or animal model, if ADAR1 protein expression or gene deletion can be controlled temporarily and it yields a homogenous cell population for analysis, it will accelerate the identification of the direct target of ADAR1 or the downstream molecules.

To demonstrate if the RNA editing or the non-RNA editing activity is more important for ADAR1 function, a new animal model carrying incompetent ADAR1, for example a knock-in mouse of a mutation in its catalytic domain, will need to be prepared.

REFERENCES

1. Polson, A. G., Crain, P. F., Pomerantz, S. C., McCloskey, J. A., and Bass, B. L. (1991) *Biochemistry*, **30**, 11507-11514.
2. Bass, B. L. (2002) *Ann. Rev. Biochem.*, **71**, 817-846.
3. Morse, D. P., and Bass, B. L. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 6048-6053.
4. Palladino, M. J., Keegan, L. P., O'Connell, M. A., and Reenan, R. A. (2000) *Cell*, **102**, 437-449.
5. Hanrahan, C. J., Palladino, M. J., Bonneau, L. J., and Reenan, R. A. (1999) *Ann. NY Acad. Sci.*, **868**, 51-66.
6. Palavicini, J. P., O'Connell, M. A., and Rosenthal, J. J. (2009) *RNA (N.Y.)*, **15**, 1208-1218.
7. Herbert, A., Lowenhaupt, K., Spitzner, J., and Rich, A. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 7550-7554.
8. Kim, U., Garner, T. L., Sanford, T., Speicher, D., Murray, J. M., and Nishikura, K. (1994) *J. Biol. Chem.*, **269**, 13480-13489.
9. O'Connell, M. A., and Keller, W. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 10596-10600.
10. Mittaz, L., Antonarakis, S. E., Higuchi, M., and Scott, H. S. (1997) *Hum. Genet.*, **100**, 398-400.
11. Mittaz, L., Scott, H. S., Rossier, C., Seeburg, P. H., Higuchi, M., and Antonarakis, S. E. (1997) *Genomics*, **41**, 210-217.
12. Nishikura, K. (2004) *Nature Biotechnol.*, **22**, 962-963.
13. Nishikura, K. (2006) *Nature Rev.*, **7**, 919-931.

14. Higuchi, M., Maas, S., Single, F. N., Hartner, J., Rozov, A., Burnashev, N., Feldmeyer, D., Sprengel, R., and Seeburg, P. H. (2000) *Nature*, **406**, 78-81.
15. Wang, Q., Khillan, J., Gadue, P., and Nishikura, K. (2000) *Science*, **290**, 1765-1768.
16. Hartner, J. C., Schmittwolf, C., Kispert, A., Muller, A. M., Higuchi, M., and Seeburg, P. H. (2004) *J. Biol. Chem.*, **279**, 4894-4902.
17. Wang, Q., Miyakoda, M., Yang, W., Khillan, J., Stachura, D. L., Weiss, M. J., and Nishikura, K. (2004) *J. Biol. Chem.*, **279**, 4952-4961.
18. Kawahara, Y., Zinshteyn, B., Sethupathy, P., Iizasa, H., Hatzigeorgiou, A. G., and Nishikura, K. (2007) *Science*, **315**, 1137-1140.
19. Hundley, H. A., and Bass, B. L. (2010) *Trends Biochem. Sci.*, **35**, 377-383.
20. Zinshteyn, B., and Nishikura, K. (2009) *Wiley Interdiscip. Rev.*, **1**, 202-209.
21. Bass, B. L. (2006) *Cold Spring Harbor Symp. Quant. Biol.*, **71**, 285-292.
22. Morabito, M. V., and Emeson, R. B. (2009) *Neuropsychopharmacology*, **34**, 246.
23. Kim, U., Wang, Y., Sanford, T., Zeng, Y., and Nishikura, K. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 11457-11461.
24. Bass, B. L., and Weintraub, H. (1988) *Cell*, **55**, 1089-1098.
25. Bass, B. L., and Weintraub, H. (1987) *Cell*, **48**, 607-613.
26. O'Connell, M. A., Krause, S., Higuchi, M., Hsuan, J. J., Totty, N. F., Jenny, A., and Keller, W. (1995) *Mol. Cell. Biol.*, **15**, 1389-1397.
27. Patterson, J. B., Thomis, D. C., Hans, S. L., and Samuel, C. E. (1995) *Virology*, **210**, 508-511.
28. Melcher, T., Maas, S., Herb, A., Sprengel, R., Seeburg, P. H., and Higuchi, M. (1996) *Nature*, **379**, 460-464.
29. Melcher, T., Maas, S., Herb, A., Sprengel, R., Higuchi, M., and Seeburg, P. H. (1996) *J. Biol. Chem.*, **271**, 31795-31798.
30. Chen, C. X., Cho, D. S., Wang, Q., Lai, F., Carter, K. C., and Nishikura, K. (2000) *RNA* (*N.Y.*), **6**, 755-767.
31. Lai, F., Chen, C. X., Lee, V. M., and Nishikura, K. (1997) *J. Neurochem.*, **69**, 43-52.
32. Lai, F., Drakas, R., and Nishikura, K. (1995) *J. Biol. Chem.*, **270**, 17098-17105.
33. Yang, W., Wang, Q., Howell, K. L., Lee, J. T., Cho, D. S., Murray, J. M., and Nishikura, K. (2005) *J. Biol. Chem.*, **280**, 3946-3953.
34. Geiger, J. R., Melcher, T., Koh, D. S., Sakmann, B., Seeburg, P. H., Jonas, P., and Monyer, H. (1995) *Neuron*, **15**, 193-204.
35. Lomeli, H., Mosbacher, J., Melcher, T., Hoger, T., Geiger, J. R., Kuner, T., Monyer, H., Higuchi, M., Bach, A., and Seeburg, P. H. (1994) *Science*, **266**, 1709-1713.
36. Egebjerg, J., Kukekov, V., and Heinemann, S. F. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 10270-10274.
37. Kohler, M., Burnashev, N., Sakmann, B., and Seeburg, P. H. (1993) *Neuron*, **10**, 491-500.
38. Higuchi, M., Single, F. N., Kohler, M., Sommer, B., Sprengel, R., and Seeburg, P. H. (1993) *Cell*, **75**, 1361-1370.
39. Sommer, B., Kohler, M., Sprengel, R., and Seeburg, P. H. (1991) *Cell*, **67**, 11-19.
40. Rueter, S. M., Burns, C. M., Coode, S. A., Mookherjee, P., and Emeson, R. B. (1995) *Science*, **267**, 1491-1494.
41. Burns, C. M., Chu, H., Rueter, S. M., Hutchinson, L. K., Canton, H., Sanders-Bush, E., and Emeson, R. B. (1997) *Nature*, **387**, 303-308.
42. Niswender, C. M., Sanders-Bush, E., and Emeson, R. B. (1998) *Ann. NY Acad. Sci.*, **861**, 38-48.
43. Seeburg, P. H. (2002) *Neuron*, **35**, 17-20.
44. Seeburg, P. H., and Hartner, J. (2003) *Curr. Opin. Neurobiol.*, **13**, 279-283.
45. Rueter, S. M., Dawson, T. R., and Emeson, R. B. (1999) *Nature*, **399**, 75-80.
46. Yang, W., Chendrimada, T. P., Wang, Q., Higuchi, M., Seeburg, P. H., Shiekhattar, R., and Nishikura, K. (2006) *Nature Struct. Mol. Biol.*, **13**, 13-21.
47. Nishikura, K. (2010) *Ann. Rev. Biochem.*, **79**, 321-349.
48. XuFeng, R., Boyer, M. J., Shen, H., Li, Y., Yu, H., Gao, Y., Yang, Q., Wang, Q., and Cheng, T. (2009) *Proc. Natl. Acad. Sci. USA*, **106**, 17763-17768.
49. Hartner, J. C., Walkley, C. R., Lu, J., and Orkin, S. H. (2009) *Nature Immunol.*, **10**, 109-115.
50. Jacobs, M. M., Fogg, R. L., Emeson, R. B., and Stanwood, G. D. (2009) *Dev. Neurosci.*, **31**, 223-237.
51. Osenberg, S., Paz Yaacov, N., Safran, M., Moshkovitz, S., Shtrichman, R., Sherf, O., Jacob-Hirsch, J., Keshet, G., Amariglio, N., Itskovitz-Eldor, J., and Rechavi, G. (2010) *PloS ONE*, **5**, e11173.
52. Toth, A. M., Li, Z., Cattaneo, R., and Samuel, C. E. (2009) *J. Biol. Chem.*, **284**, 29350-29356.
53. Ward, S. V., George, C. X., Welch, M. J., Liou, L. Y., Hahm, B., Lewicki, H., de la Torre, J. C., Samuel, C. E., and Oldstone, M. B. (2011) *Proc. Natl. Acad. Sci. USA*, **108**, 331-336.
54. Nie, Y., Hammond, G. L., and Yang, J. H. (2007) *J. Virol.*, **81**, 917-923.
55. Doria, M., Neri, F., Gallo, A., Farace, M. G., and Michienzi, A. (2009) *Nucleic Acids Res.*, **37**, 5848-5858.
56. Phuphuakrat, A., Kraiwong, R., Boonarkart, C., Lauhakirti, D., Lee, T. H., and Auewarakul, P. (2008) *J. Virol.*, **82**, 10864-10872.
57. Hartwig, D., Schutte, C., Warnecke, J., Dorn, I., Hennig, H., Kirchner, H., and Schlenke, P. (2006) *J. Viral Hepat.*, **13**, 150-157.
58. Jayan, G. C., and Casey, J. L. (2002) *J. Virol.*, **76**, 3819-3827.
59. Cenci, C., Barzotti, R., Galeano, F., Corbelli, S., Rota, R., Massimi, L., Di Rocco, C., O'Connell, M. A., and Gallo, A. (2008) *J. Biol. Chem.*, **283**, 7251-7260.
60. Galeano, F., Leroy, A., Rossetti, C., Gromova, I., Gautier, P., Keegan, L. P., Massimi, L., Di Rocco, C., O'Connell, M. A., and Gallo, A. (2010) *Int. J. Cancer*, **127**, 127-137.
61. Martinez, H. D., Jasavala, R. J., Hinkson, I., Fitzgerald, L. D., Trimmer, J. S., Kung, H. J., and Wright, M. E. (2008) *J. Biol. Chem.*, **283**, 29938-29949.
62. Gandy, S. Z., Linnstaedt, S. D., Muralidhar, S., Cashman, K. A., Rosenthal, L. J., and Casey, J. L. (2007) *J. Virol.*, **81**, 13544-13551.
63. Laxminarayana, D., Khan, I. U., O'Rourke, K. S., and Giri, B. (2007) *Immunology*, **122**, 623-633.
64. Linnstaedt, S. D., Kasprzak, W. K., Shapiro, B. A., and Casey, J. L. (2009) *RNA* (*N.Y.*), **15**, 1177-1187.
65. Zhang, X. J., Gao, M., Li, M., Li, M., Li, C. R., Cui, Y., He, P. P., Xu, S. J., Xiong, X. Y., Wang, Z. X., Yuan, W. T., Yang, S., and Huang, W. (2003) *J. Invest. Dermatol.*, **120**, 776-780.

66. Miyamura, Y., Suzuki, T., Kono, M., Inagaki, K., Ito, S., Suzuki, N., and Tomita, Y. (2003) *Am. J. Hum. Genet.*, **73**, 693-699.
67. Suzuki, N., Suzuki, T., Inagaki, K., Ito, S., Kono, M., Horikawa, T., Fujiwara, S., Ishiko, A., Matsunaga, K., Aoyama, Y., Tosaki-Ichikawa, H., and Tomita, Y. (2007) *J. Invest. Dermatol.*, **127**, 309-311.
68. George, C. X., Gan, Z., Liu, Y., and Samuel, C. E. (2011) *J. Interferon Cytokine Res.*, **31**, 99-117.
69. Gallo, A., and Galardi, S. (2008) *RNA Biol.*, **5**, 135-139.
70. Maas, S., Kawahara, Y., Tamburro, K. M., and Nishikura, K. (2006) *RNA Biol.*, **3**, 1-9.
71. Hanrahan, C. J., Palladino, M. J., Ganetzky, B., and Reenan, R. A. (2000) *Genetics*, **155**, 1149-1160.
72. Domeier, M. E., Morse, D. P., Knight, S. W., Portereiko, M., Bass, B. L., and Mango, S. E. (2000) *Science*, **289**, 1928-1931.
73. Patterson, J. B., and Samuel, C. E. (1995) *Mol. Cell. Biol.*, **15**, 5376-5388.
74. Herbert, A., Alfken, J., Kim, Y. G., Mian, I. S., Nishikura, K., and Rich, A. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 8421-8426.
75. Herbert, A., Schade, M., Lowenhaupt, K., Alfken, J., Schwartz, T., Shlyakhtenko, L. S., Lyubchenko, Y. L., and Rich, A. (1998) *Nucleic Acids Res.*, **26**, 3486-3493.
76. George, C. X., and Samuel, C. E. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 4621-4626.
77. George, C. X., and Samuel, C. E. (1999) *Gene*, **229**, 203-213.
78. Kawakubo, K., and Samuel, C. E. (2000) *Gene*, **258**, 165-172.
79. Shtrichman, R., Heithoff, D. M., Mahan, M. J., and Samuel, C. E. (2002) *Infect. Immun.*, **70**, 5579-5588.
80. Markle, D., Das, S., Ward, S. V., and Samuel, C. E. (2003) *Gene*, **304**, 143-149.
81. George, C. X., Wagner, M. V., and Samuel, C. E. (2005) *J. Biol. Chem.*, **280**, 15020-15028.
82. George, C. X., Das, S., and Samuel, C. E. (2008) *Virology*, **380**, 338-343.
83. Ward, S. V., Markle, D., Das, S., and Samuel, C. E. (2002) *J. Interferon Cytokine Res.*, **22**, 891-898.
84. Samuel, C. E. (2001) *Clin. Microbiol. Rev.*, **14**, 778-809, table of contents.
85. Rabinovici, R., Kabir, K., Chen, M., Su, Y., Zhang, D., Luo, X., and Yang, J. H. (2001) *Circ. Res.*, **88**, 1066-1071.
86. Yang, J. H., Nie, Y., Zhao, Q., Su, Y., Pypaert, M., Su, H., and Rabinovici, R. (2003) *J. Biol. Chem.*, **278**, 45833-45842.
87. Li, Z., Wolff, K. C., and Samuel, C. E. (2010) *Virology*, **396**, 316-322.
88. Schwartz, T., Rould, M. A., Lowenhaupt, K., Herbert, A., and Rich, A. (1999) *Science* (*N. Y.*), **284**, 1841-1845.
89. Kim, Y. G., Lowenhaupt, K., Maas, S., Herbert, A., Schwartz, T., and Rich, A. (2000) *J. Biol. Chem.*, **275**, 26828-26833.
90. Herbert, A., and Rich, A. (1999) *Genetica*, **106**, 37-47.
91. Berger, I., Winston, W., Manoharan, R., Schwartz, T., Alfken, J., Kim, Y. G., Lowenhaupt, K., Herbert, A., and Rich, A. (1998) *Biochemistry*, **37**, 13313-13321.
92. Eckmann, C. R., and Jantsch, M. F. (1999) *J. Cell Biol.*, **144**, 603-615.
93. Raitskin, O., Cho, D. S., Sperling, J., Nishikura, K., and Sperling, R. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 6571-6576.
94. Ray, B. K., Dhar, S., Shakya, A., and Ray, A. (2011) *Proc. Natl. Acad. Sci. USA*, **108**, 103-108.
95. Kim, D., Lee, Y. H., Hwang, H. Y., Kim, K. K., and Park, H. J. (2010) *Current Drug Targets*, **11**, 335-344.
96. Ward, S. V., George, C. X., Welch, M. J., Liou, L. Y., Hahm, B., Lewicki, H., de la Torre, J. C., Samuel, C. E., and Oldstone, M. B. (2011) *Proc. Natl. Acad. Sci. USA*, **108**, 331-336.
97. Kawahara, Y., Grimberg, A., Teegarden, S., Mombereau, C., Liu, S., Bale, T. L., Blendy, J. A., and Nishikura, K. (2008) *J. Neurosci.*, **28**, 12834-12844.
98. Mombereau, C., Kawahara, Y., Gundersen, B. B., Nishikura, K., and Blendy, J. A. (2009) *Neuropharmacology*, **59**, 468-473.
99. Morabito, M. V., Abbas, A. I., Hood, J. L., Kesterson, R. A., Jacobs, M. M., Kump, D. S., Hachey, D. L., Roth, B. L., and Emeson, R. B. (2010) *Neurobiol. Disease*, **39**, 169-180.
100. Sakurai, M., Yano, T., Kawabata, H., Ueda, H., and Suzuki, T. (2010) *Nature Chem. Biol.*, **6**, 733-740.
101. Levanon, E. Y., and Eisenberg, E. (2006) *Brief. Funct. Genom. Proteom.*, **5**, 43-45.
102. Levanon, E. Y., Eisenberg, E., Yelin, R., Nemzer, S., Hallegger, M., Shemesh, R., Fligelman, Z. Y., Shoshan, A., Pollock, S. R., Sztybel, D., Olshansky, M., Rechavi, G., and Jantsch, M. F. (2004) *Nature Biotechnol.*, **22**, 1001-1005.
103. Kim, D. D., Kim, T. T., Walsh, T., Kobayashi, Y., Matise, T. C., Buyske, S., and Gabriel, A. (2004) *Genome Res.*, **14**, 1719-1725.
104. Blow, M., Futreal, P. A., Wooster, R., and Stratton, M. R. (2004) *Genome Res.*, **14**, 2379-2387.
105. Bhalla, T., Rosenthal, J. J., Holmgren, M., and Reenan, R. (2004) *Nature Struct. Mol. Biol.*, **11**, 950-956.
106. Athanasiadis, A., Rich, A., and Maas, S. (2004) *PLoS Biol.*, **2**, e391.
107. Graveley, B. R., Brooks, A. N., Carlson, J. W., Duff, M. O., Landolin, J. M., Yang, L., Artieri, C. G., van Baren, M. J., Boley, N., Booth, B. W., Brown, J. B., Cherbas, L., Davis, C. A., Dobin, A., Li, R., Lin, W., Malone, J. H., Mattiuzzo, N. R., Miller, D., Sturgill, D., Tuch, B. B., Zaleski, C., Zhang, D., Blanchette, M., Dudoit, S., Eads, B., Green, R. E., Hammonds, A., Jiang, L., Kapranov, P., Langton, L., Perrimon, N., Sandler, J. E., Wan, K. H., Willingham, A., Zhang, Y., Zou, Y., Andrews, J., Bickel, P. J., Brenner, S. E., Brent, M. R., Cherbas, P., Gingeras, T. R., Hoskins, R. A., Kaufman, T. C., Oliver, B., and Celniker, S. E. (2011) *Nature*, doi:10.1038/nature09715.
108. Picardi, E., Horner, D. S., Chiara, M., Schiavon, R., Valle, G., and Pesole, G. (2010) *Nucleic Acids Res.*, **38**, 4755-4767.
109. Rosenberg, B. R., Hamilton, C. E., Mwangi, M. M., Dewell, S., and Papavasiliou, F. N. (2011) *Nature Struct. Mol. Biol.*, **18**, 230-236.
110. Wulff, B. E., Sakurai, M., and Nishikura, K. (2011) *Nat. Rev. Genet.*, **12**, 81-85.
111. Levanon, K., Eisenberg, E., Rechavi, G., and Levanon, E. Y. (2005) *EMBO Rep.*, **6**, 831-835.

112. Kawahara, Y., Megraw, M., Kreider, E., Iizasa, H., Valente, L., Hatzigeorgiou, A. G., and Nishikura, K. (2008) *Nucleic Acids Res.*, **36**, 5270-5280.
113. Kawahara, Y., Zinshteyn, B., Chendrimada, T. P., Shiekhattar, R., and Nishikura, K. (2007) *EMBO Rep.*, **8**, 763-769.
114. Habig, J. W., Dale, T., and Bass, B. L. (2007) *Mol. Cell*, **25**, 792-793.
115. Rula, E. Y., and Emeson, R. B. (2007) *Methods Enzymol.*, **424**, 333-367.
116. Zhang, Z., and Carmichael, G. G. (2001) *Cell*, **106**, 465-475.
117. Wang, Q., Zhang, Z., Blackwell, K., and Carmichael, G. G. (2005) *Curr. Biol.*, **15**, 384-391.
118. Scadden, A. D., and O'Connell, M. A. (2005) *Nucleic Acids Res.*, **33**, 5954-5964.
119. Vitali, P., and Scadden, A. D. (2010) *Nature Struct. Mol. Biol.*, **17**, 1043-1050.
120. Clerzius, G., Gelinas, J. F., Daher, A., Bonnet, M., Meurs, E. F., and Gatignol, A. (2009) *J. Virol.*, **83**, 10119-10128.
121. Pain, V. M. (1994) *Biochimie*, **76**, 718-728.
122. Pain, V. M. (1986) *Biochem. J.*, **235**, 625-637.
123. Jefferson, L. S., and Kimball, S. R. (2003) *J. Nutr.*, **133**, 2046S-2051S.
124. Kimball, S. R. (2001) *Progr. Mol. Subcell. Biol.*, **26**, 155-184.
125. Clemens, M. J., Bushell, M., Jeffrey, I. W., Pain, V. M., and Morley, S. J. (2000) *Cell Death Differ.*, **7**, 603-615.
126. Pain, V. M. (1996) *Eur. J. Biochem./FEBS*, **236**, 747-771.
127. Kimball, S. R. (1999) *Int. J. Biochem. Cell Biol.*, **31**, 25-29.
128. Samuel, C. E., Kuhen, K. L., George, C. X., Ortega, L. G., Rende-Fournier, R., and Tanaka, H. (1997) *Int. J. Hematol.*, **65**, 227-237.
129. Wang, Y., and Samuel, C. E. (2009) *J. Mol. Biol.*, **393**, 777-787.
130. Yeo, J., Goodman, R. A., Schirle, N. T., David, S. S., and Beal, P. A. (2010) *Proc. Natl. Acad. Sci. USA*, **107**, 20715-20719.
131. Zhang, X. J., He, P. P., Li, M., He, C. D., Yan, K. L., Cui, Y., Yang, S., Zhang, K. Y., Gao, M., Chen, J. J., Li, C. R., Jin, L., Chen, H. D., Xu, S. J., and Huang, W. (2004) *Human Mutation*, **23**, 629-630.
132. Oyama, M., Shimizu, H., Ohata, Y., Tajima, S., and Nishikawa, T. (1999) *Br. J. Dermatol.*, **140**, 491-496.
133. Nie, Y., Ding, L., Kao, P. N., Braun, R., and Yang, J. H. (2005) *Mol. Cell. Biol.*, **25**, 6956-6963.
134. Agranat, L., Raitskin, O., Sperling, J., and Sperling, R. (2008) *Proc. Natl. Acad. Sci. USA*, **105**, 5028-5033.
135. Heale, B. S., Keegan, L. P., McGurk, L., Michlewski, G., Brindle, J., Stanton, C. M., Caceres, J. F., and O'Connell, M. A. (2009) *EMBO J.*, **28**, 3145-3156.
136. Feng, Y., Sansam, C. L., Singh, M., and Emeson, R. B. (2006) *Mol. Cell. Biol.*, **26**, 480-488.
137. Ohlson, J., Pedersen, J. S., Haussler, D., and Ohman, M. (2007) *RNA* (*N. Y.*), **13**, 698-703.