Cordycepin Inhibits Protein Synthesis and Cell Adhesion through Effects on Signal Transduction*

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3’-Deoxyadenosine, also known as cordycepin, is a known polyadenylation inhibitor with a large spectrum of biological activities, including anti-proliferative, pro-apoptotic and anti-inflammatory effects. In this study we confirm that cordycepin reduces the length of poly(A) tails, with some mRNAs being much more sensitive than others. The low doses of cordycepin that cause poly(A) changes also reduce the proliferation of NIH3T3 fibroblasts. At higher doses of the drug we observed inhibition of cell attachment and a reduction of focal adhesions. Furthermore, we observed a strong inhibition of total protein synthesis that correlates with an inhibition of mammalian target of rapamycin (mTOR) signaling, as observed by reductions in Akt kinase and 4E-binding protein (4EBP) phosphorylation. In 4EBP knock-out cells, the effect of cordycepin on translation is strongly reduced, confirming the role of this modification. In addition, the AMP-activated kinase (AMPK) was shown to be activated. Inhibition of AMPK prevented translation repression by cordycepin and abolished 4EBP1 dephosphorylation, indicating that the effect of cordycepin on mTOR signaling and protein synthesis is mediated by AMPK activation. We conclude that many of the reported biological effects of cordycepin are likely to be due to its effects on mTOR and AMPK signaling.

Cordycepin (3’-deoxyadenosine) is found in the parasitic fungus Cordyceps militaris and has been proposed as the active component of traditional medication that is reputed to alleviate a large variety of ailments (1–3). It has been reported to have numerous biological activities, including the inhibition of cell proliferation (4–6), induction of apoptosis (7–10), inhibition of platelet aggregation (11), inhibition of cell migration and invasiveness (4, 12), and inhibition of inflammation (13). In mice, cordycepin can reduce tumor formation in a model of metastasis (12) and has therefore been proposed as a cancer drug. The effect of cordycepin on RNA polymerases has been shown to be relatively minor. In contrast, cordycepin strongly inhibits mRNA polyadenylation, presumably by causing chain termination after it has been incorporated as cordycepin triphosphate (14). At high doses cordycepin inhibits incorporation of labeled uridine into mRNA, but not into its precursor hnRNA, indicating that the export, processing, or stability of transcribed mRNA is inhibited, rather than primary synthesis (15).

In this study, we confirm that cordycepin causes a decrease in the poly(A) tail size of specific mRNAs with some mRNAs being much more sensitive to cordycepin than others. Low doses of cordycepin cause a decrease in cell proliferation. At high doses, however, cordycepin prominently affects cell adhesion and indirectly reduces protein synthesis to very low levels. It shuts down a signal transduction pathway, the mTOR5 pathway, which is known to control proliferation, cell adhesion, and protein synthesis (16–18). In contrast to rapamycin, cordycepin inhibits the activities of both the mTORC1 and the mTORC2 complexes, affecting the activity of the protein kinase Akt. Adenosine is a cordycepin antagonist, and inhibitors of adenosine import and phosphorylation prevent the effect of cordycepin on protein synthesis, indicating that this drug is acting intracellularly and needs to be converted to cordycepin monophosphate. Cordycepin was also shown to function as an activator of the AMPK pathway. An inhibitor of AMPK blocked cordycepin-mediated inhibition of translation and Akt dephosphorylation, indicating the effects of cordycepin on translation and mTOR signaling are mediated by its activation of AMPK. These effects of cordycepin explain most of the observations reported in tissue culture experiments and provide a mechanistic explanation for the action of this agent as an anti-proliferative and anti-inflammatory drug.

**EXPERIMENTAL PROCEDURES**

Reagents—Cordycepin, cordycepin triphosphate, LY294,002, rapamycin, nitrobenzylthioinosine and iodotubericidin were purchased from Sigma and dissolved in DMSO at 1000-fold concentrated stock to obtain the indicated final concentrations. Compound C (6-[4-(2-piperidin-1-ylethoxy)phenyl]-3-pyridin-4-ylpyrazolo[1,5-a]pyrimidine) was purchased from Sigma and dissolved in DMSO at 5 mM.

**RNA Isolation and Poly(A) Tests**—RNA was isolated according to Chomczynski and Sacchi (19). RNA ligation-mediated poly(A) tests were performed as described by Rassa et al. (20).

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5 The abbreviations used are: mTOR, mammalian target of rapamycin; AMPK, AMP-activated kinase; PBS, phosphate-buffered saline; TRITC, tetramethylrhodamine isothiocyanate; 4EBP, 4E-binding protein.
and Klenow priming poly(A) tests as described by Di Penta et al. (21). Oligonucleotides were purchased from Invitrogen. The primers used for Rps4x and Actg1 have been reported previously (22). Other primer sequences were as follows: Cdkn1a-1, GTCTGGACTGTCTACCCCTTAA; Cdkn1a-2, CAGGACACTGAGCAAATGGCT; Hif1a, CCCACCGTGGTGTATAAGG; Atf4, CCGAGTGTAGGAGCTAGA; and Rpl28, GCCAC-TTCTTATGTG.

**Tissue Culture, Protein Synthesis, Polysome Profiles, and Cell Adhesion Assays**—NIH3T3 cells were cultured in Dulbecco’s modified Eagle’s medium (Sigma) with 10% fetal bovine serum, 4.5 g/liter glucose and passaged every 2–3 days. For each experiment, the cells were plated at a density of 20,000 cells/cm² the day before use.

For determination of protein synthesis rates in NIH3T3 cells, cells were plated in 4 replicates in 24-well plates at 25,000 cells/well the day before use. After the indicated treatments, the medium was removed, the wells were washed twice with PBS, and 5–15 μCi/ml Tran35S label (MP biologicals) was added in cysteine- and methionine-free Dulbecco’s modified Eagle’s medium. The cells were incubated at 37 °C for 10–15 min, the medium was removed, and the plate was placed on ice. After two PBS washes, the cells were extracted with 50 μl of passive lysis buffer (Promega) per well. Incorporation was measured by trichloroacetic acid precipitation on Whatman 3MM paper, and protein content was determined using Coomassie Reagent (Thermo/Pierce). The incorporation was corrected for the protein content, and the average incorporation of the control was set at 100%. An error bar representing one standard deviation is shown in each graph.

For polysome profiling, cells were grown as described above. They were treated with 100 μg/ml cycloheximide for 15 min at 37 °C, washed with ice-cold PBS, scraped, and pooled on ice. The cell pellet was lysed in 1% Triton X-100, 300 mM NaCl, 15 mM MgCl₂, 100 μg/ml cycloheximide, 1 mg/ml heparin, 15 mM Tris/HCl, pH 7.5, and centrifuged. The supernatant was loaded on a 10–60% sucrose gradient in the same buffer without Triton and centrifuged at 38,000 × g for 2 h. 1-ml fractions were collected, and RNA was isolated and transferred to a Northern blot. The methylene blue stain of such a blot is shown.

To characterize cell spreading, cells were cultured as described above, detached with trypsin/EDTA, resuspended in medium with serum, and washed once with serum-free medium (Dulbecco’s modified Eagle’s medium with 10 mM HEPES, pH 7), before being resuspended in serum-free medium at ~1 million cells/ml. The resuspended cells were incubated with shaking at 37 °C for 1 h to dissolve all focal adhesions, diluted in serum-containing medium, and plated on glass coverslips in the presence of the indicated drugs for 5 h. Fixing and staining with phalloidin and Hoechst was as described below.

For immunohistochemistry and phalloidin staining, cells were plated on glass coverslips in the presence of serum. After treatment, cells were fixed with 4% paraformaldehyde. Vinculin antibody was applied at 1:200 in 3% bovine serum albumin in PBS. The secondary antibody was anti-mouse–coupled to Alexa 546 (Molecular Probes), also at 1:200 in 3% bovine serum albumin in PBS. Phalloidin stains were performed with fluorescein isothiocyanate- or TRITC-coupled phalloidin from Sigma at 2.5 μg/ml in PBS. Hoechst (Sigma) stains were performed at 5 μg/ml in PBS. Imaging was performed on a Zeiss LSM510 Meta confocal microscope, and the images were processed using the manufacturer’s software.

Mouse embryonic fibroblasts were cultured as for NIH3T3 cells. Knock-in mutant cells in which Ser51 of elf2α was replaced by Ala (S51A cells) and their matched controls were a gift from Dr. R. J. Kaufman (23). Cells from mice with disruptions in the genes for 4EBP1 and 4EBP2 (double knockouts) and their matched controls were a gift from Dr. N. Sonenberg (24). Protein synthesis was determined by incubation of the cells in complete medium containing [³⁵S]methionine (7 μCi/ml) for 1 h. The cells were washed with PBS, then dissolved in 0.3 M NaOH, and protein was precipitated with 10% (w/v) trichloroacetic acid and filtered through glass fiber paper. Protein content was determined, and incorporation of radioactivity was analyzed as described above for NIH3T3 cells.

**In Vitro Translation**—The sensitivity of in vitro translation to cordycepin and cordycepin triphosphate was determined in reticulocyte lysate that had not been treated with nuclease, essentially as described previously (25). The nucleosides and nucleotides indicated were added to a final concentration of 200 μM. No ATP was added to the reaction unless otherwise indicated.

**Antibodies and Western and Northern Blots**—For Western blots, cells cultured as described above were scrapped in cold PBS on ice and collected by centrifugation. After washing with cold PBS, the cell pellet was lysed in radioimmune precipitation assay buffer (PBS, 0.1% SDS, 0.5% Igepal, 0.5% sodium deoxycholate), and the protein concentration was determined. Equal amounts of protein, ~25–40 μg/sample, were analyzed by SDS gel electrophoresis and blotting on Hybond-C membranes (Amersham Biosciences). Antibodies were used for Western blotting according to the manufacturers’ recommendations. Vinculin and β-actin antibodies were purchased from Sigma. Antibodies against total and phosphorylated elf2α (Ser51), total and phosphorylated Akt1 (Ser473), phospho and total AMPKβ1 (Ser108), total and phosphorylated Acetyl-CoA Carboxylase (Ser79), and total and phosphorylated 4EBP1 (Thr37/46) were purchased from Cell Signaling Technology. Northern blots for β-actin and Cdkn1a were performed as described previously (22).

**RESULTS**

To assess if cordycepin does indeed influence the poly(A) tail length of individual mRNAs in mammalian cells, we incubated NIH3T3 fibroblasts with 10, 50, or 200 μM of the drug for 2 h and examined the poly(A) tails of a set of mRNAs for which we had RNA ligation based polyadenylation test assays available. As can be seen in Fig. 1A, the mRNAs encoding Hif1a and Atf4 showed reduced polyadenylation already at the 10 μM dose. Actg1 (γ-actin), Rps4x, and Rpl28 mRNAs were much less sensitive to cordycepin. To investigate the effect of cordycepin on the poly(A) tails of different mRNAs in more detail, we used a Klenow priming-based polyadenylation test assay on RNA from cells treated for different times with 50 μM cordycepin (Fig. 1B). Again different mRNAs showed different sensitivities to cordycepin, with Hif1a, Cdkn1a, and Atf4 being sensitive,
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A

| RNaseH+ oligo(dT) | μM Cordycepin |
|-------------------|---------------|
| +                 | 0  | 10 | 50 | 200 |
| Primease         | 916| 790| 600| 500 |
| Hif1a             | 916| 790| 600| 500 |
| Atf4              | 916| 790| 600| 500 |
| Actg1             | 600| 500| 400| 300 |
| Rps4x             | 600| 500| 400| 300 |
| Rpl28             | 600| 500| 400| 300 |

B

| Unseparated | 50 μM Cordycepin |
|-------------|-------------------|
| 15 min      | 1000              |
| 30 min      | 500               |
| 45 min      | 200               |
| 60 min      | 100               |

C

50 μM Cordycepin

| Time (minutes) | Cdkn1a mRNA | Actb1 mRNA |
|----------------|-------------|-------------|
| 0              |             |             |
| 15             |             |             |
| 30             |             |             |
| 45             |             |             |

D

| Concentration | Million cells per plate |
|---------------|--------------------------|
| DMSO          | 3.5 ± 0.5                |
| 5 μM Cordycepin | 3.0 ± 0.2               |
| 10 μM Cordycepin | 2.5 ± 0.1              |

Size in basepairs

Relative intensity (%)
Cordycepin affects poly(A) tail length and cell proliferation at low concentrations. A, RNA ligation poly(A) tests on RNA from NIH3T3 cells treated with different doses of cordycepin. RNase H + Oligo(dT) indicates RNA from untreated cells digested with RNase H and oligo(dT) to remove the poly(A) tail as a control. The GenBank™ gene name abbreviations indicate which mRNAs were tested: Hif1a (hypoxia-inducible factor 1α), Cdkn1a (p21/Waf/Cip), Atf4 (activating transcription factor 4), Actg1 (γ-actin), Rps4X (X-linked ribosomal protein S4), and Rpl28 (ribosomal protein L28). B, Klenow priming poly(A) tests on a time course of cordycepin treatment. On the right side the panels shows the distribution of the intensity in each lane as a percentage of the maximum intensity in that lane on the vertical axis and the size of the poly(A) test products in base pairs on the horizontal axis. Black, oligo(dT) RNase A-treated sample; purple, no treatment; green, 15-min cordycepin; dark blue, 30-min cordycepin; light blue, 45-min cordycepin; orange, 60-min cordycepin. C, Northern blot for Cdkn1a and β-actin (Act1b) on total RNA isolated from cells treated with 50 μM cordycepin for the indicated times. D, cell numbers after 72 h of treatment with cordycepin (medium refreshed daily, including drug).
vitro translation system. As can be seen in Fig. 4, neither cordycepin nor cordycepin triphosphate inhibited in vitro translation, indicating that the effect is indirect. Cap analogue, a dinucleotide known to inhibit translation initiation, did markedly repress in vitro translation.

Cellular protein synthesis rates are predominantly downregulated by the modification of translation initiation factors and their interacting proteins, especially via the phosphorylation of eIF2α and the dephosphorylation of 4EBP (18). We therefore performed Western blots for these proteins and their phosphorylated forms on cells treated with different doses of cordycepin for 2 h. As can be seen in Fig. 5A, an increase in eIF2α phosphorylation was detectable at 20 μM, whereas a decrease in 4EBP1 phosphorylation was first detected with 50 μM cordycepin. Because 4EBP phosphorylation is mediated by mTOR, we also investigated the mTOR-mediated phosphorylation of Akt1. Akt1 is a kinase that is involved in cell adhesion and proliferation, and its phosphorylation by the mTORC2 complex is known to be upstream of activation of mTORC1, the complex that phosphorylates 4EBP, as summarized in Fig. 8 (27). Akt1 phosphorylation was very strongly inhibited by 50 μM cordycepin, and the levels of the protein kinase were also reduced, indicating that the block of mTOR signaling is at the level of Akt or upstream. Time courses of these changes indicated that Akt and 4EBP1 were dephosphorylated within 30 min of treatment of cells with 200 μM cordycepin (Fig. 5B), coinciding with the decrease in overall protein synthesis (Fig. 4D), whereas the increased phosphorylation of eIF2α only occurred at later times.

Rapamycin can inhibit the activity of the mTORC1 complex and prevent the phosphorylation of 4EBPs in cells stimulated with growth factors (28). However, as shown in Fig. 5C, a 2-h treatment with even quite high doses of rapamycin did not reduce translational activity in our cells, even though it did somewhat reduce 4EBP1 and Akt1 phosphorylation (Fig. 5D). In contrast, LY294,002, an inhibitor of the growth signal transducer phos- phatidylinositol 3-kinase, did reduce translation and abolished 4EBP1 and Akt1 phosphorylation (Figs. 5C, 5D, and 8). These data are consistent with the effect of cordycepin being mediated by a severe reduction in Akt/mTORC2 signaling.

To determine the relative importance of 4EBP dephosphorylation and eIF2α phosphorylation for the inhibition of protein synthesis by cordycepin, we employed mutant mouse embryo fibroblasts in which either Ser51 of eIF2α was replaced by Ala.
S51A cells (23) or in which the genes for 4EBP1 and 4EBP2 were disrupted (24). As can be seen in Fig. 6, both mutant cell lines showed some resistance to cordycepin, compared with their corresponding wild-type controls. The reduced sensitivity to the inhibitor was particularly marked in the 4EBP double knock-out cells, suggesting that 4EBP dephosphorylation is an important mechanism by which cordycepin inhibits translation.

We sought to further clarify the mechanism by which cordycepin inhibits protein synthesis. To confirm that cordycepin is acting as an adenosine antagonist, we added an equal amount of adenosine to the cell cultures. As can be seen in Fig. 7A, this completely abolished the repression of protein synthesis. This shows that cordycepin is primarily mediating its effect on protein synthesis through AMPK.

FIGURE 3. Cordycepin inhibits cell spreading. NIH3T3 cells were detached, suspended for 1 h in serum-free medium, and allowed to re-attach to coverslips with serum for 5 h in the presence or absence of cordycepin, cycloheximide, or actinomycin D at the concentrations indicated. After fixation, cells were stained with phalloidin to visualize the actin cytoskeleton. A, images of typical control and cordycepin-treated cells. B and C, quantitation of the percentage of unspread cells (largest diameter, 25 μm or less) in cells incubated with the indicated doses of drugs.

DISCUSSION

In this study we have shown that at low doses cordycepin affects the poly(A) tails of specific mRNAs, and this correlates with a reduction in cell proliferation. Surprisingly, at higher doses cordycepin also inhibits cell adhesion and virtually abolishes protein synthesis, probably through its effects on Akt and 4EBP phosphorylation. The most upstream target of cordycepin at higher doses appears to be AMPK, which it activates, leading to the observed reduction in mTOR signaling. Therefore the two main effects of cordycepin appear to be the inhibition of polyadenylation at low doses and the activation of AMPK at higher doses.

We demonstrate that cordycepin reduces the poly(A) tail length of a subset of mRNAs (Fig. 1). These mRNAs are likely to represent unstable mRNAs that need constant cotranscriptional polyadenylation to maintain their levels. Conversely, other mRNAs are resistant to both cordycepin and actinomycin D, suggesting that these mRNAs do not require transcription to maintain their poly(A) tail length and are protected from deadenylation and degradation in the cytoplasm. Even low doses of cordycepin have effects on the polyadenylation of sensitive mRNAs, and for one such mRNA (Cdkn1a) a rapid drop in mRNA levels in response to cordycepin was observed. This indicates that cordycepin acts at least in part by reducing the important negative regulator of mTOR signaling and protein synthesis (29, 30). We therefore checked if AMPK was activated by cordycepin treatment. As can be seen in Fig. 7C, phosphorylation of AMPK on its autophosphorylation site in the β subunit (Ser108) increased within 30 min of treatment with cordycepin (31). Effects on AMPK activity coincided with the dephosphorylation of Akt1 and 4EBP1. To test if the activation of AMPK is required for the inhibition of protein synthesis by cordycepin, we added the AMPK inhibitor Compound C 15 min before a 1-h treatment with cordycepin and determined the effect on protein synthesis. As can be seen in Fig. 7D, Compound C completely cancelled the effect of cordycepin on protein synthesis. In addition, Compound C repressed the phosphorylation of AMPKβ and enhanced phosphorylation of Akt1 (Fig. 7E). Compound C also prevented the cordycepin-induced dephosphorylation of 4EBP1. These data indicate that cordycepin is primarily mediating its effect on protein synthesis through AMPK-mediated inhibition of mTOR signaling.
FIGURE 4. Cordycepin inhibits protein synthesis. A, protein synthesis rates of NIH3T3 cells as measured by $^{35}\text{S}$ incorporation into protein, corrected for total protein concentration. The effects of treatment with 200 μM cordycepin or adenosine or with 10 μg/ml actinomycin D (AcD) are shown. B, polyribosome profiles of control cells and cells treated with cordycepin (200 μM) for 2 h. 40S and 60S indicate the dissociated free ribosomal subunit peaks, polysomes indicates ribosomes translating mRNA. The inset shows the RNA isolated from a control gradient, proving the identification of the 40S peak. C, dose response of the effect of cordycepin on protein synthesis rates in NIH3T3 cells treated for 2 h. D, time course of the response of protein synthesis rates to three doses of cordycepin. E, dose response of the effect of cordycepin on protein synthesis rates in HeLa cells treated for 2 h. F, incorporation of radioactive methionine into protein in vitro in reticulocyte lysate supplemented with cordycepin, adenosine, cordycepin triphosphate (cordyTP), ATP, or cap analogue (m7GpppG) (all at 200 μM).
levels of sensitive mRNAs. This is in agreement with recent findings in yeast, where mutations in the mRNA degradation machinery convey resistance to cordycepin (32).

We have also shown clear effects of cordycepin on cell adhesion, both in attached cells and in cells adhering from suspension (Figs. 2 and 3). These effects appear to be at least partially independent of protein synthesis, because the strong translation inhibitor cycloheximide has little influence on attached cells and a much less pronounced effect on adhering cells. It is therefore likely that the effect of cordycepin on cell adhesion is mediated through its inhibition of Akt, which is a well known regulator of the actin cytoskeleton (33, 34).

Partial inhibition of translation by cordycepin in L5178Y cells and mouse L-cells was reported over 30 years ago (14, 35). In agreement with this, we now have also observed inhibition of protein synthesis in NIH3T3 and HeLa cells (Fig. 4) and in a human colon carcinoma cell line (results not shown). This suggests that protein synthesis is sensitive to cordycepin in a variety of cell lines. In contrast to this finding is the report that cordycepin enhances the translation of ribosomal protein mRNAs (36). Ribosomal proteins contain a 5’ oligonucleotide pyrimidine tract, which mediates serum-stimulated translation in a manner dependent on mTOR, but not on the mTORC1 or mTORC2 complexes (37). In serum-starved HeLa cells, cordycepin and actinomycin D were found to stimulate the polyribosome association of these mRNAs without influencing general polyribosome assembly (36). We have done experiments with serum-starved NIH3T3 cells, and we still see a cordycepin-induced reduction in translational activity as determined by amino acid incorporation (results not shown), but we have not examined the polyribosomes under these conditions. Intriguingly, we consistently see that ribosomal protein mRNAs have relatively homogeneous poly(A)
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FIGURE 7. Cordycepin activates AMPK. A, adenosine blocks cordycepin-mediated repression of protein synthesis. NIH3T3 cells were treated with cordycepin and/or adenosine for 2 h, and protein synthesis rates were measured by $^{35}$S incorporation, corrected for total protein concentration. B, import and phosphorylation of cordycepin are required for inhibition of protein synthesis. Cells were treated with nitrobenzylthioinosine (NBTI, 10 $\mu$M) or iodotubericidin (ITu, 0.1 $\mu$M) for 15 min before treatment with cordycepin (200 $\mu$M) for 1 h. Protein synthesis rates were determined as before. C, cordycepin induces AMPK activation. Western blots are shown for AMPK $\beta1$ and its autophosphorylation site (Ser108), acetyl-CoA carboxylase and its AMPK phosphorylation site (Ser79) as well as for the proteins listed in the legend to Fig. 5. Treatment with cordycepin was for the indicated times and dose. D, protein synthesis in cells pretreated with Compound C or DMSO for 15 min prior to the addition of 200 $\mu$M cordycepin and incubation for 1 h. E, cells were treated as in D, and Western blotting was performed for the proteins indicated as described above.
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![Summary of the AMPK and mTOR signaling pathways](image)

AMPK activates mTOR through the following pathways:
- AMPK downstream of mTOR
- mTOR downstream of AMPK

- AMPK activates mTOR through the following pathways:
  - AMPK downstream of mTOR
  - mTOR downstream of AMPK

mTOR inhibitor Tsc2. In Tsc2 knock-out cells, AMPK was still activated by metformin, but no down-regulation of mTOR signaling was observed. However, the antagonism between AMPK and Akt appears to be bi-directional, at least in some cases, with Akt activation repressing AMPK activation and Akt down-regulation increasing AMPK activity. We cannot therefore completely exclude the possibility that the induction of Akt1 phosphorylation by Compound C is blocking a direct effect of cordycepin on Akt, with AMPK activation by cordycepin being a secondary effect of the reduction in Akt signaling.

One possible mechanism for the activation of AMPK by cordycepin is that cordycepin monophosphate is a stronger activator of AMPK than AMP. Another possibility is that cordycepin may activate AMPK through the induction of a change in the AMP:ATP ratio. We propose that cordycepin activates AMPK by an unknown mechanism, and this inhibits
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mTORC2 as well as mTORC1 activity, leading to a double block of the mTOR signaling pathway as depicted in Fig. 8.

As a polyadenylation inhibitor, cordycepin has been used as a tool to investigate the role of cytoplasmic polyadenylation in a variety of systems (e.g. 46–48). In most cases, the doses employed are high and therefore likely to activate AMPK and repress mTOR signaling and total protein synthesis. Fortunately, in these studies due care was taken to include other methods to verify that cytoplasmic polyadenylation is taking place. However, we have to conclude that an observation of effects of cordycepin on a biological process can no longer be used as an indication of a role of cytoplasmic polyadenylation without rigorous checks on the effects on the AMPK and mTOR signaling pathways.

Cordycepin has been under investigation as an anti-proliferative drug for nearly 50 years (49). However, the instability of the molecule in the body has been problematic so far, due to the presence of adenosine deaminases. A combination therapy with an adenosine deaminase inhibitor as a treatment for TdT-positive leukemia is currently in Phase I/II clinical trials (Onco-vista, Inc.). Recently, more stable prodrugs have been synthesized, but their potential therapeutic effects have not yet been assessed (50). Two aspects of the current study indicate that cordycepin continues to be an interesting lead compound for cancer therapy as well as a potentially useful tool to identify therapeutic targets.

Firstly, at low doses, at which the only detectable effect of cordycepin is on poly(A) tail length, the target is likely to be one or more of the mRNAs that have a poly(A) tail with a high sensitivity to cordycepin. These can now be identified using a poly(A) fractionation microarray screen (22). These low doses of cordycepin are likely to be achievable in patients with only moderate modifications in the drug delivery.

Secondly, the mTOR and AMPK pathways are currently under intense scrutiny as targets in cancer therapy (16, 51–55). As described above, the effects of cordycepin on translation and mTOR signaling are similar to those observed for metformin. In addition, the anti-inflammatory effect of cordycepin can be explained by its effects on mTOR and AMPK signaling, because cordycepin-mediated repression of inflammation has been reported to involve the inhibition of Akt (13). As an inhibitor of Akt and an activator of AMPK, cordycepin therefore is a candidate drug for type II diabetes, a potential anti-inflammatory, and a putative cancer drug. Perhaps tradition was in the right after all.

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