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Reovirus infection induces stabilization and up-regulation of cellular transcripts that encode regulators of TGF-β signaling

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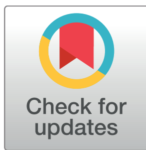

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Abstract

Reovirus infection induces dramatic changes in host mRNA expression. We utilized oligonucleotide microarrays to measure cellular mRNA decay rates in mock- or reovirus-infected murine L929 cells to determine if changes in host mRNA expression are a consequence of reovirus-induced alterations in cellular mRNA stability. Our analysis detected a subset of cellular transcripts that were coordinately induced and stabilized following infection with the reovirus isolates c87 and c8, strains that led to an inhibition of cellular translation, but not following infection with Dearing, a reovirus isolate that did not negatively impact cellular translation. The induced and stabilized transcripts encode multiple regulators of TGF- β signaling, including components of the Smad signaling network and apoptosis/survival pathways. The coordinate induction, through mRNA stabilization, of multiple genes that encode components of TGF-β signaling pathways represents a novel mechanism by which the host cell responds to reovirus infection.

Introduction

Viral infection leads to changes in cellular steady state mRNA levels within infected cells. Some of these alterations represent the cell's innate antiviral response, while others are induced by the invading virus in an attempt to sequester host antiviral responses and usurp the cellular machinery for viral replication. Virus-induced changes in cellular gene expression are often regulated through transcriptional mechanisms. For example, infection with many viruses increases the transcription of genes involved in antiviral responses including the type I interferons (IFN) as well as numerous IFN-stimulated genes (ISGs) (reviewed in [1, 2]). Although

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transcriptional regulation is important for mammalian cells to respond to their environment, numerous mammalian genes are also regulated at the level of mRNA decay in response to a variety of external signals (reviewed in [3, 4]. Virus-induced changes in cellular steady state mRNA levels have also been shown to be regulated at the level of mRNA decay [5, 6].

In this study, we utilized reovirus infection to evaluate the effect of viral infection on host cellular gene expression at the level of mRNA decay. Reovirus, a prototypic member of the *Reoviridae* family, is a non-enveloped double-stranded RNA virus that has been studied extensively as a model of viral infection [7]. This virus was a valuable model for us to examine the effect of viral infection on mRNA stability for several reasons: i) the consequences of reovirus infection are well documented and include induction of type I IFN, initiation of apoptosis, inhibition of cellular translation, and a G1/S cell cycle arrest (reviewed in [7]); ii) global studies on the impact of reovirus infection on cellular gene expression have been published [8–11]; and iii) reovirus isolates vary in their effects on infected cells [10, 12–14]. Since reovirus isolates induce distinct changes in cellular gene expression, as well as distinct cellular responses to infection, alterations in cellular gene expression following reovirus infection can be correlated to specific phenotypes [10]. For example, in murine L929 cells, reovirus isolates Dearing and c87 induce high levels of type I IFN, whereas cells infected with isolate c8 have a poor IFN response [10]. As a consequence, numerous ISG transcripts are induced following infection with isolates Dearing or c87, but not isolate c8 [10]. Additionally, infection with c87 or c8 lead to an inhibition of cellular translation, whereas infection with Dearing does not [10, 12, 14]. Mechanisms for inhibition of cellular translation in response to reovirus infection involve phosphorylation and inactivation of the alpha subunit of eukaryotic initiation factor-2 (eIF2α) by the dsRNA-dependent protein kinase (PKR) or the ER-stress-induced kinase PERK [10]. We previously identified a specific subset of cellular transcripts that were induced following infection with c8 and c87, which inhibit cellular translation, but were not induced following infection with isolate Dearing, which does not inhibit cellular protein synthesis [10]. The mechanisms for increased steady state expression of these cellular transcripts could involve transcriptional and/or posttranscriptional mechanisms.

In this report, we investigate the role that mRNA decay plays in regulating host cellular gene expression following reovirus infection. We used oligonucleotide microarrays to measure mRNA decay rates in L929 cells that were mock-infected or infected with reovirus isolates Dearing, c8, or c87. We detected a subset of transcripts that were coordinately induced and stabilized upon infection with reovirus strains that induced host translational shutoff, i.e. strains c8 and c87. The induced and stabilized transcripts encoded multiple regulators of transforming growth factor-beta (TGF-β) signaling, including components of the Smad signaling network (SSN) and apoptosis/survival pathways. TGF-β is a cytokine that has multiple activities including immune modulation, promotion of fibrosis, control of cellular growth, and regulation of apoptosis [15–20]. TGF-β production is activated following infection with a variety of viruses [21–28], including reovirus [29, 30], suggesting that the TGF-β signaling cascade plays a role in viral pathogenesis. In particular, regulation of apoptosis through TGF-β signaling may be part of a host response to viral infection. Thus, the coordinate stabilization and up-regulation of transcripts that encode components of TGF-β signaling pathways likely represent a cellular anti-viral response to reovirus infection.

Methods and materials

Cells, viruses and viral infection

Murine L929 cells were maintained as suspension cultures as described previously [31]. Reovirus isolates Dearing and c87/Abney are prototypic laboratory strains [32], and isolate c8 was

previously described [31, 33]. Purified virions were prepared by CsCl density gradient centrifugation of extracts from cells infected with third-passage L929 cell lysate stocks [34]. In order to analyze three independent infections, each set of infections was initiated on separate days. L929 cells were plated in 150 x 25 mm tissue culture plates and were allowed to incubate at 37˚C for 4 h, after which time the medium was removed and cells were mock-infected or infected with purified virions (Dearing, c87 or c8) at a multiplicity of infection (MOI) of 80 plaque forming units (PFU)/cell. After a 1.5 h viral adsorption at 37˚C, medium was added and samples were incubated at 37˚C for an additional 18 h.

RNA isolation and microarray hybridization

At 19.5 h post-infection (p.i.), actinomycin D (Sigma, MO) was added at a final concentration of 10 μg/ml to stop transcription by RNA polymerase II and total RNA was isolated at 0, 45, 90 and 120 min post-actinomycin D treatment using Trizol reagent (Invitrogen, CA) according to the manufacturer's instructions. Total RNA was purified with the RNeasy column (QIAGEN, CA); 15 μg of RNA was converted to cDNA using the Superscript custom kit (Invitrogen) with an oligo dT-T7 primer (Geneset, CO). The purified cDNA was used for an *in vitro* transcription reaction using T7 RNA polymerase and biotinylated nucleotides following the manufacturer's protocol (ENZO Bioarray, NY). Biotinylated anti-sense cRNA was purified with the RNeasy column; 15 μg was fragmented according to Affymetrix instructions' and hybridized to Affymetrix murine U74Av2 oligonucleotide microarrays (Affymetrix Inc., CA). Microarrays were scanned on a Hewlett Packard Agilent 2200 confocal scanner (Bio-Rad Laboratories, CA) and normalized signal intensities were obtained using Affymetrix MAS 5.0 software as described previously [10].

Microarray data analysis

Expressed transcripts levels were determined as the average signal values with 95% confidence intervals (95% CI) across three replicate arrays. Fold changes (FC) in expression between two infection conditions were determined as the ratio of average signal values. P values were calculated using a two-sample t test assuming equal variance. We fit the log signal values over time following actinomycin D treatment to a linear regression model over the linear portion of the decay curves to calculate transcript half-lives as described in detail in a previous statistical supplement [35]. A p-value of ≤ 0.05 was used to identify differences in mRNA decay rates in mock-infected cells compared to reovirus-infected cells.

Reverse transcription real-time PCR

Reverse transcription real time PCR (RT-PCR) was used to validate changes in transcript level and mRNA decay rates of three transcripts that were found to be up-regulated and stabilized following infection with certain reovirus isolates: Gdf15, Tgif, and Myc. Total cellular RNA from the same reovirus infections as described above was used for real time RT-PCR. RNA was converted to cDNA by using StrataScript™ reverse transcriptase (Stratagene) and gene specific primers. PCR amplifications were performed in a BioRad iCycler thermocycler by using the QuantiTect™ SYBR Green PCR Kit (QIAGEN) with the following cycling conditions: initial heating at 95˚C for 13.5 min, followed by 40 cycles of 3-step temperature cycling at 95 ˚C for 10 s, 55.6 ˚C for 10 s, and 72 ˚C for 30 s. Data was analyzed using the iCycler software and standard curves were generated to measure transcripts levels, which were normalized to the level of HPRT transcript. The normalized values at each point were then used to generate mRNA decay curves. Oligonucleotide primers (Integrated DNA Technologies Inc) were: `Gdf15 5′ CCG AGA GGA CTC GAA CTC AG 3′,5′ GTA GGC TTC GGG GAG ACC 3′;Hprt`

5′ GGT GAA AAG GAC CTC TCG AA 3′,5′ AGT CAA GGG CAT ATC CAA CA 3′; c-Myc 5′ TGA AGG CTG GAT TTC CTT TG 3′,5′ TTC TCT TCC TCG TCG CAG AT 3′;Tgif 5′ TCC TAG AAA CCC CAG CTT CA 3′,5′ GCT GCT GAT GAG GAA AGG TC 3′.

Results

We profiled the changes in L929 cellular gene expression and mRNA decay rates that occurred as a consequence of infection with reovirus isolates c87, c8 and Dearing. At 19.5 h p.i., a time point where reovirus-induced host translational shutoff is well established [10], ongoing transcription was arrested by addition of actinomycin D and global mRNA expression levels were measured after 0, 45, 90 and 120 min of actinomycin D treatment using Affymetrix U74Av2 microarrays as described previously [35, 36]. This experiment was performed three separate times for each reovirus isolate and the mRNA half-life with 95% confidence interval (95% CI) was calculated for over 6500 expressed transcripts based on a model of first order decay. The transcript levels and mRNA decay rates for all transcripts expressed under at least one condition are shown in S1 Table and the complete set of raw data has been deposited into the NCBI GEO Database (accession # GSE119061). The numbers of transcripts that were up-regulated and/or stabilized in reovirus-infected cells compared to mock-infected cells are shown in Table 1.

We observed the stabilization ($p \leq 0.05$) of 349, 253, and 51 cellular transcripts after infection with reovirus isolates c87, c8, and Dearing, respectively. A complete list of the stabilized transcripts and their decay rates are displayed in S2 Table. Of the stabilized transcripts, 172 were stabilized following infection with both c87 and c8 isolates, whereas only 24 transcripts were stabilized after infection with all three isolates. We also noted the destabilization ($p \leq 0.05$) of a small number of transcripts in L929 cells following infection with these same reovirus isolates, but there was poor correlation between isolates; only four transcripts were destabilized following infection with both c87 and c8 and no destabilized transcripts were common to all three isolates.

We evaluated the steady state mRNA levels of stabilized cellular transcripts to determine whether or not stabilization following reovirus infection correlated with changes in overall levels of these transcripts. Following c87 infection, 70 cellular transcripts were up-regulated ($p \leq 0.05$) and stabilized ($p \leq 0.05$); 40 transcripts were up-regulated and stabilized following c8 infection (Table 1). Of these, 26 transcripts were up-regulated and stabilized following infection with both of these reovirus isolates. In contrast, only two transcripts were up-regulated and stabilized following infection with Dearing, the reovirus isolate that did not inhibit cellular translation. Using more strict criteria to identify transcripts that were up-regulated ($FC \geq 2.0$ and $p \leq 0.05$) in reovirus-infected cells, we identified 31 transcripts that were up-regulated and stabilized following c87 infection and 41 transcripts that were up-regulated and stabilized following c8 infection; 13 were up-regulated and stabilized following infection with both isolates that led to an inhibition of host protein synthesis. We did not identify any transcripts that were up-regulated $\geq$ 2-fold and stabilized following infection with strain Dearing.

A subset of the transcripts that were up-regulated ($p \leq 0.05$) and/or stabilized ($p \leq 0.05$) following infection of L929 cells with c87 or c8 are listed in Table 2. A complete list is found in S3 Table. These transcripts encode various components of the TGF-β signaling pathway (Fig 1), including cell cycle inhibitors and regulators of transcription, apoptosis and stress responses. In particular, many encode protein components of the SSN, which regulates transcription associated with cell cycle arrest, differentiation and apoptosis [37–41]. This suggests that the SSN is involved in a coordinated cellular response to infection with reovirus isolates

Table 1. Number of cellular transcripts that were stabilized and up-regulated following reovirus infection.

	Strain c87	Strain c8	Strain Dearing	Strains c87 and c8	All 3 Strains
Up-regulated ($p<0.05$)	709	481	590	305	205
Up-regulated (FC > 2-fold, $p<0.05$)	347	200	210	143	88
Stabilized ($p < 0.05$)	349	253	52	172	24
Stabilized and Up-regulated ($p < 0.05$)	70	49	2	26	0
Stabilized and Up-regulated (FC > 2-fold, $p < 0.05$)	31	41	0	13	0

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Table 2. Subset of transcripts that were stabilized or up-regulated following reovirus infection.

Transcript Description	Gene Symbol	Mock		Strain c87			Strain c8		
		HL(min)	95% CI	FC	HL(min)	95% CI	FC	HL(min)	95% CI
Up-regulated ($p \leq 0.05$) and Stabilized ($p \leq 0.05$); c87 and c8									
*growth differentiation factor 15	Gdf15	69	[47,124]	18.24	>480	[101,>480]	12.07	>480	[115,>480]
*MAD homolog 7 (Drosophila)	Smad7	67	[52,95]	4.03	197	[89,>480]	1.87	199	[90,>480]
*dual specificity phosphatase 1	Dusp1	50	[39,68]	3.65	>480	[112,>480]	3.65	104	[57,>480]
*myelocytomatosis oncogene	Myc	44	[37,55]	2.59	366	[108,>480]	2.20	183	[83,>480]
*vascular endothelial growth factor A	Vegfa	119	[79,240]	2.44	>480	[226,>480]	3.49	>480	[156,>480]
*TG interacting factor	Tgif	94	[76,124]	2.33	>480	[217,>480]	2.59	468	[174,>480]
*Kruppel-like factor 5	Klf5	115	[82,192]	2.18	>480	[149,>480]	1.78	>480	[179,>480]
coagulation factor III	F3	77	[55,130]	7.79	>480	[115,>480]	5.16	>480	[128,>480]
nuclear receptor subfamily 1, group D, member 1	Nr1d1	125	[89,207]	4.97	>480	[294,>480]	3.79	>480	[174,>480]
nuclear factor, interleukin 3, regulated	Nfil3	65	[49,100]	2.77	171	[75,>480]	2.85	184	[77,>480]
B-cell translocation gene 1, anti-proliferative	Btg1	152	[109,250]	1.68	>480	[398,>480]	2.15	>480	[209,>480]
CDC like kinase 4	Clk4	78	[59,115]	3.29	>480	[128,>480]	2.99	>480	[174,>480]
Up-regulated ($p \leq 0.05$) and Stabilized ($p \leq 0.05$); c87									
*dual specificity phosphatase 2	Dusp2	91	[60,180]	3.31	>480	[120,>480]	1.57	371	[96,>480]
*growth arrest and DNA-damage-inducible 45 beta	Gadd45b	53	[40,79]	2.18	139	[63,>480]	3.21	80	[48,259]
*MAD homolog 2 (Drosophila)	Smad2	216	[153,368]	1.28	>480	[286,>480]	1.15	>480	[335,>480]
immediate early response 3	Ier3	49	[37,71]	4.22	230	[75,>480]	2.17	113	[56,>480]
nucleoporin 62	Nup62	146	[101,264]	1.73	>480	[196,>480]	1.06	188	[104,>480]
seven in absentia 2	Siah2	72	[56,100]	1.78	>480	[174,>480]	1.29	454	[129,>480]
Up-regulated ($p \leq 0.05$) and Stabilized ($p \leq 0.05$); c8									
*signal transducing adaptor molecule 1	Stam	222	[142,>480]	1.45	>480	[341,>480]	1.81	>480	[340,>480]
*MAD homolog 1 (Drosophila)	Smad1	163	[123,242]	1.16	>480	[>480,>480]	1.36	>480	[252,>480]
B-cell leukemia/lymphoma 6	Bcl6	49	[41,61]	1.01	>480	[130,>480]	1.86	273	[105,>480]
cyclin G2	Ccng2	93	[71,134]	0.67	>480	[215,>480]	2.41	>480	[229,>480]
TGFB inducible early growth response	Tieg / Klf10	61	[51,75]	0.69	287	[126,>480]	1.49	145	[88,397]
E4F transcription factor 1	E4f1	373	[216,>480]	1.24	>480	[443,>480]	1.58	>480	[>480,>480]
Stabilized ($p \leq 0.05$); c87 and c8									
*mitogen activated protein kinase kinase kinase 1	Map3k1	77	[59,108]	0.48	>480	[157,>480]	1.27	399	[128,>480]
*Cbp/p300-interacting transactivator, CITED2	Cited2	71	[56,97]	0.69	>480	[138,>480]	1.47	264	[110,>480]
*BCL2-like 11	Bcl2l11	67	[52,94]	0.86	348	[112,>480]	0.79	261	[102,>480]
*Son of sevenless homolog 2, (Drosophila)	Sos2	93	[63,174]	0.97	>480	[120,>480]	1.57	>480	[121,>480]
*B-cell leukemia/lymphoma 10	Bcl10	374	[239,>480]	1.10	>480	[>480,>480]	1.18	>480	[455,>480]
*TANK-binding kinase 1	Tbk1	121	[85,210]	1.04	>480	[142,>480]	1.20	>480	[170,>480]

*Transcripts shown in Fig 2.

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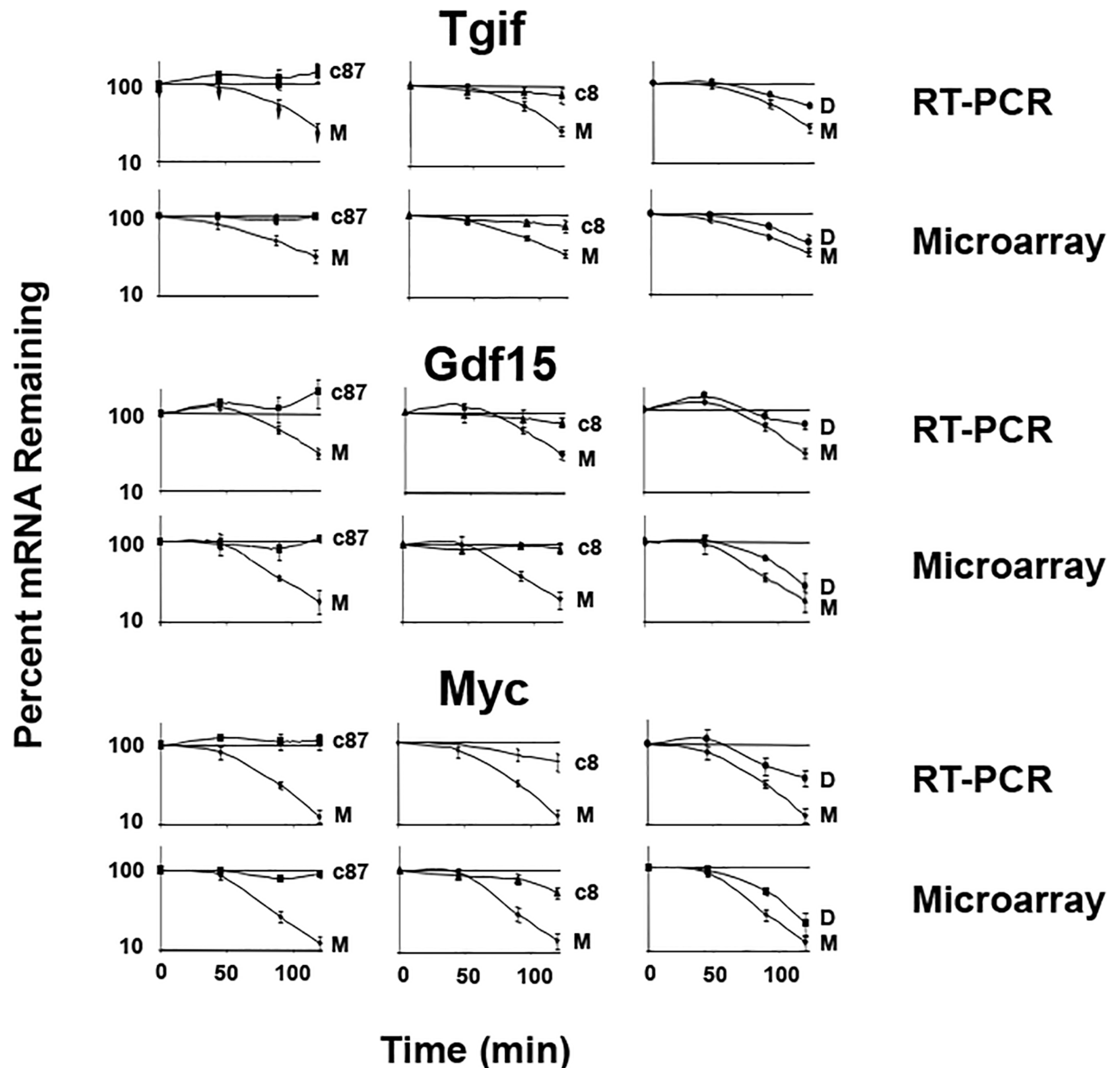

Fig 1. Real time RT-PCR validation of transcript up-regulation and stabilization. Murine L929 cells were infected for 19.5 h with reovirus isolates c87, c8 and Dearing or mock (M) infected. Actinomycin D was added to stop transcription and total cellular RNA was purified 0, 45, 90, and 120 min post-actinomycin D treatment. The same RNA was used for both microarry and real time RT-PCR. Tgif, Gdf-15 and c-Myc mRNA levels were measured by real-time RT-PCR using gene specific primers and transcript levels were normalized to the level of the HPRT transcript. Data shown are from three independent experiments. Each point represents the mean ± standard error of the mean.

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c8 and c87. In contrast, none of the transcripts encoding components of this pathway were up-regulated and/or stabilized following infection with isolate Dearing, suggesting that reovirus isolates differ in their ability to induce changes in the decay of cellular transcripts encoding SSN components. The finding that strains that induced stabilization of transcripts encoding components of the SSN network also induced cellular translational inhibition suggests that the SSN network and inhibition of translation could be linked.

To validate our microarray mRNA decay data, we utilized real time RT-PCR to measure mRNA levels from three selected genes: Tgif, Gdf15 and c-Myc. These transcripts encode

Table 3. Comparison of transcript expression and half-life data obtained using real time RT-PCR or microarrays.

Transcript	Mock		Strain c87			Strain c8			Strain Dearing		
	HL(min)	95% CI	FC	HL(min)	95% CI	FC	HL(min)	95% CI	FC	HL(min)	95% CI
Tgif											
RT-PCR	83	[71,103]	3.49	>480	[>480]	3.69	>480	[>480]	2.78	171	[147,195]
Microarray	94	[76,124]	2.33	>480	[217,>480]	2.59	468	[174,>480]	1.55	113	[80,191]
Gdf15											
RT-PCR	124	[85,174]	20.45	>480	[>480]	18.24	>480	[458,>480]	14.19	226	[151,>480]
Microarray	69	[47,124]	18.24	>480	[101,>480]	12.07	>480	[115,>480]	3.83	63	[40,149]
Myc											
RT-PCR	54	[47,61]	1.43	>480	[>480]	2.60	339	[223,443]	1.85	86	[75,98]
Microarray	44	[37,55]	2.59	366	[108,>480]	2.20	183	[83,>480]	1.89	56	[41,88]

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important proteins that interface with the SSN and, based upon our microarray data, were up-regulated and stabilized following infection with c87 and c8, but not Dearing. We generated decay curves for the three transcripts by real time RT-PCR using the same RNA that was used for the microarray analysis. Decay curves generated for these transcripts by real time RT-PCR were very similar to the mRNA decay curves generated from the microarray analysis (Fig 1). These data confirm that Tgif, Gdf-15, and c-Myc were up-regulated and stabilized following infection with reovirus isolates c87 and c8 (Table 3). In contrast, infection with strain Dearing led to little or no stabilization of these transcripts (Fig 1 and Table 3). We also performed western blotting using antibodies against the Tgif, Gdf-15 and c-Myc proteins and found that the level of these proteins did not increase followinginfection with reovirus isolate c87 (S1 Fig). Thus, the upregulation and stabilization of these transcripts did not correlate with increased protein levels. This result is not surprising, as infection with this reovirus isolate led to an inhibition of cellular protein synthesis.

Discussion

We found that multiple cellular transcripts encoding components of the SSN are coordinately up-regulated and stabilized following reovirus infection. We hypothesize this is a cellular response to reovirus infection meant to induce the apoptosis of infected cells, particularly following infection with reovirus isolates that lead to the inhibition of cellular translation. Numerous viruses, including reovirus, induce increased expression of TGF-β as part of the host response to viral infection. Depending upon the integration of signals through the TGF-β receptor and other receptors, the SSN regulates the balance between apoptosis or cell growth and survival (reviewed in [42]). In virus-infected cells, TGF-β signaling may play an antiviral role by promoting the apoptosis of virus-infected cells, whereas in uninfected cells, signaling through the SSN may play a role in protecting against apoptosis [29]. In addition to activating the SSN, the TGF-β family of receptors interfaces with several other signaling pathways, including NF-κB, MAPK/ERK, p38 and JNK pathways [43–46]. These pathways influence the SSN by regulating the phosphorylation of Smad proteins, which in turn, control cell proliferation, differentiation and migration through their role as transcription factors [39, 41, 47–49]. Smad transcription complexes are activated by TGF-β receptors 1 and 2. Following receptor activation, Smad 2,3 complexes or Smad 1,5,8 complexes become phosphorylated and interact with Smad 4, creating activated transcription complexes [38, 50]. These newly formed Smad 4-containing complexes translocate into the nucleus, bind DNA, and activate target gene transcription. Depending on other signals, however, Smad 6 and Smad 7 can repress

phosphorylation and prevent activation of Smad transcription complexes by blocking their translocation into the nucleus [39, 51, 52]. Smad 7 can also act in a feedback loop to repress TGF-β signaling by inducing receptor ubiquitylation and protein degradation [53–55]. Thus, depending on the integration of multiple signals, the SSN can activate or repress transcription of a specific subset of cellular genes.

Transcripts that were up-regulated and stabilized following reovirus infection included SMAD 1, 2, 6 &7, Tgif, c-Myc, CITED2 and KLF5, which encode components of the SSN that control transcription of genes that regulate apoptosis and cell growth [47, 50, 51, 53, 54]. Of note, reovirus has been shown to preferentially infect and induce lysis of cells that express high levels of c-Myc or other oncoproteins, suggesting that reovirus might exploit this oncogenesis signaling pathways to preferentially kill cancer cells [56]. For this reason, reovirus infection is a potential treatment of cancer [57]. Other up-regulated and stabilized transcripts encode growth regulatory cytokines that impact the SSN (see Fig 2). For example, the transcripts encoding Gdf15, a TGF-β superfamily cytokine, and Vegfa, an angiogenic endothelial cell

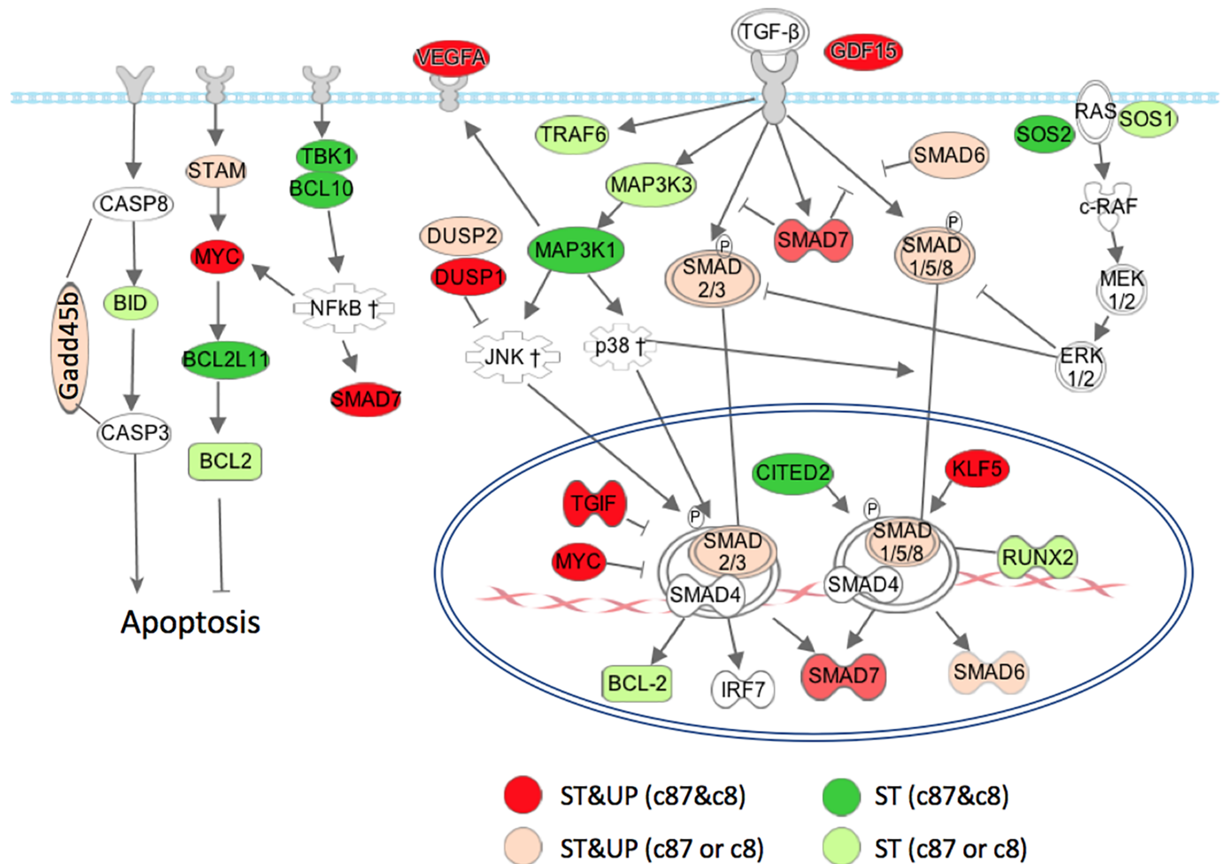

Fig 2. Transcripts that encode components of the SSN or related proteins were up-regulated and/or stabilized following reovirus infection. Signaling through the TGF-β family of receptors activate several pathways, including NF-κB, MAPK/ERK, p38, and JNK pathways. These pathways regulate phosphorylation of Smad proteins, which in turn regulate cell survival and apoptosis. Transcripts shown in red were up-regulated and stabilized following infection of L929 cells with reovirus isolates c87 and c8, transcripts shown in light orange were up-regulated and stabilized following infection with isolate c87 or c8, transcripts shown in dark green were stabilized (but not up-regulated) following infection with isolates c87 and c8, and transcripts shown in light green were stabilized (but not up-regulated) following infection with isolate c87 or c8. This figure was created using Ingenuity Pathway Analysis software starting with the canonical pathway related to TGF-β signaling (right side of the figure), which was combined with transcripts related to apoptosis (left side of the figure). GDF15, VEGFA, DUSP1/2, KLF5, and CITED2 transcripts were added manually based on their relevance to TGF-β or apoptosis signaling pathways.

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growth factor, were dramatically stabilized and up-regulated following infection with reovirus isolates c8 and c87. Interestingly, Vegfa is induced by TGF-β and acts in concert with TGF-β to induce the apoptosis of endothelial cells [58, 59]. In addition to growth factors, transcripts encoding downstream regulators of kinase pathways that can also impact the SSN, including Sos1, Sos2, Map3k3, Map3k1, Dusp1, and Dusp2, were also up-regulated and/or stabilized. The transcript encoding Stam, a cytokine signaling protein that interfaces with the SSN by activating c-Myc [60], was also up-regulated and stabilized in cells infected with reovirus isolates c87 or c8.

Since the SSN regulates the balance between apoptosis and survival, it is possible that the coordinate induction of SSN components through mRNA stabilization represents an attempt by the virus-infected cell to undergo apoptosis. Some up-regulated and stabilized transcripts encode components of receptor-mediated apoptosis pathways including: Bcl-10, which activates NF-κB [61]; Tbk1, which promotes anti-viral responses [62] and activates NF-κB [63]; Gadd45b, a NF-κB-inducible mediator of apoptosis [64]; and Bid, an important component of caspase-induced apoptosis [65]. TGF-β signaling also leads to activation of NF-κB and promotion of apoptosis through the signaling protein TGF-β activating kinase 1 (Tak1) [45, 66, 67]. The NF-κB signaling network interfaces with the SSN by activating Smad 7, which feeds back to repress TGF-β-induced transcription of genes that promote cell growth and survival [45, 68]. Thus, the outcome of the SSN—cell growth and survival versus growth inhibition and cell death—is controlled by the coordinate integration of several signals. Following reovirus infection, not all cellular anti-viral responses lead to apoptosis; rather, a balance between death and survival occurs [69, 70]. Cells attempt to avoid viral infection, but if unsuccessful, cell death pathways are frequently activated. Meanwhile, viruses need to prevent cell death for a period of time to ensure viral replication. Thus, the interplay between host anti-viral responses to promote death of infected cells and viral evasion mechanisms determines the fate of the cell.

We have previously demonstrated that reovirus isolates that led to an inhibition of cellular translation (c87 and c8) also induced stress granule formation [8]. Others have shown that reovirus particles are recruited to stress granules during infection, and the stress response induced by reovirus may be necessary for viral replication [71]. Here we demonstrate that infection with the reovirus isolates that induced stress granule formation also caused the stabilization of numerous cellular transcripts, including transcripts encoding components of the SSN and regulators of apoptosis. Perhaps, stress granule formation leads to the stabilization and sequestration of certain cellular transcripts, such as the transcripts that encode the regulators of the SSN and apoptosis pathways we identified here. Thus, the stabilization and up-regulation of transcripts that encode components of the SSN and associated apoptosis pathways may be part of a cellular stress response in which these transcripts are stabilized within stress granules while the cell determines its fate (cell death or survival).

Numerous other viruses have developed mechanisms to modulate or usurp TGF-β signaling pathways, perhaps to prevent cell death and promote viral replication. For example, Kaposi sarcoma herpes virus produces viral homologues of human interferon response factors that function to regulate TGF-β signaling [72, 73]. Herpes simplex virus 1 down-regulates TGF-β and Smad 3 expression in infected cells [74, 75]. Although this effect was reported to be due to an HSV-1-encoded microRNA [74], other groups were unable to reproduce those results [76]. Human papillomavirus E6 and E7 proteins bind to specific Smad proteins, thereby inhibiting the SSN [77, 78], and the human T cell lymphotropic virus 1 tax protein inhibits TGF-β signaling through c-jun activation [79]. Other viruses, such as cytomegalovirus and BK virus, usurp TGF-β signaling to promote viral replication [80, 81]. The fact that numerous viruses have developed specific mechanisms to manipulate or evade the SSN suggests this pathway is important for host anti-viral responses.

Our findings demonstrate that transcripts encoding numerous components of the SSN are coordinately up-regulated and stabilized following reovirus infection, suggesting that cells have mechanisms to selectively recognize and stabilize specific subsets of cellular transcripts. These transcripts may contain specific regulatory sequence(s) in common that allow them to be selectively recognized by RNA-binding proteins or microRNAs. The finding that after reovirus infection, only a specific subset of transcripts undergo stabilization, rather than all transcripts, suggests that alterations in general pathways for mRNA decay cannot explain our results. It is possible that certain transcripts that are targets for translation-dependent mRNA decay under normal conditions are stabilized when translation is inhibited following reovirus infection. Nonsense-mediated mRNA decay, which is translation-dependent [82, 83], has been shown to regulate the decay of transcripts involved in the TGF-β signaling pathway [84, 85]. For example, many of the transcripts involved in TGF-β signaling depicted in Fig 1 have been shown to be targeted by nonsense-mediated decay, such as Smad7 [85], DUSP1/3 [86], GADD45B [87], and Myc [88]. Further work is needed to define the mechanism for the up-regulation and stabilization following reovirus infection of the transcripts we identified which encode specific components of the SSN.

Supporting information

S1 Table. Expression intensity and half-lives of all transcripts in mock, c87, c8, or Dearing infected cells.
(XLS)

S2 Table. Transcripts that were stabilized following reovirus infection.
(XLS)

S3 Table. Transcripts that were up-regulated and stabilized following reovirus infection.
(XLS)

S1 Fig. Western blot analysis of total cell lysates from mock and reovirus-infected L929 cells.
(PDF)

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