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Genome-wide analysis of mRNA decay in resting and activated primary human T lymphocytes

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ABSTRACT

We used microarray technology to measure mRNA decay rates in resting and activated T lymphocytes in order to better understand the role of mRNA decay in regulating gene expression. Purified human T lymphocytes were stimulated for 3 h with medium alone, with an anti-CD3 antibody, or with a combination of anti-CD3 and anti-CD28 antibodies. Actinomycin D was added to arrest transcription, and total cellular RNA was collected at discrete time points over a 2 h period. RNA from each point was analyzed using Affymetrix oligonucleotide arrays and a first order decay model was used to determine the half-lives of approximately 6000 expressed transcripts. We identified hundreds of short-lived transcripts encoding important regulatory proteins including cytokines, cell surface receptors, signal transduction regulators, transcription factors, cell cycle regulators and regulators of apoptosis. Approximately 100 of these short-lived transcripts contained ARE-like sequences. We also identified numerous transcripts that exhibited stimulus-dependent changes in mRNA decay. In particular, we identified hundreds of transcripts whose steady-state levels were repressed following T cell activation and were either unstable in the resting state or destabilized following cellular activation. Thus, rapid mRNA degradation appears to be an important mechanism for turning gene expression off in an activation-dependent manner.

INTRODUCTION

In diverse eukaryotic organisms ranging from yeast to humans, control of mRNA turnover plays a key role in regulating cellular responses to environmental stimuli (1,2). Following transcriptional activation, for example, the regulated decay of mammalian immediate early response gene transcripts, including c-fos, c-jun and c-myc, is crucial for normal cellular functions such as cell cycle progression, proliferation and apoptosis (3). Aberrant regulation of decay leads to oncogenic activation and malignancy (3–7). In T lymphocytes, T cell receptor (TCR) stimulation induces the expression of numerous early response genes. Many of these genes, including cytokine genes and proto-oncogenes, produce mRNA transcripts that exhibit rapid degradation, but subsets of these short-lived transcripts can undergo differential regulation. For example, CD28 co-stimulation of TCR-activated T lymphocytes leads to specific stabilization of cytokine transcripts, including interleukin-2 (IL-2), granulocyte-macrophage colony stimulating factor (GM-CSF), tumor necrosis factor (TNF)-α and interferon (IFN)-γ, while proto-oncogene transcripts such as c-myc remain unstable (8). Thus, the decay of an individual mRNA transcript can exhibit gene-specific, stimulus-dependent regulation that impacts the overall expression of the gene.

Although increasing information suggests that mRNA degradation is an important control point for regulating T lymphocyte gene expression, mRNA decay rates have been measured for only a small number of T lymphocyte mRNA transcripts. Recently developed microarray technology has revolutionized gene expression research, allowing the expression of thousands of genes to be simultaneously profiled in different cell types or different treatment conditions. The vast majority of experiments involving microarray technology have evaluated only steady-state mRNA levels. Recent work, however, suggested that microarray technology can be used to categorize mRNA transcripts based on their mRNA decay rates (9,10). In the present study, microarray technology was used to quantitatively measure on a genome-wide basis the decay rates of mRNA transcripts in resting and activated primary human T lymphocytes following transcriptional arrest. The half-life and 95% confidence interval (CI) was determined for each of approximately 6000 transcripts expressed in T lymphocytes. This approach allowed the identification of hundreds of T lymphocyte genes that are regulated at the level of mRNA degradation.

MATERIALS AND METHODS

Purification of human T lymphocytes

Human T lymphocytes were purified as described previously (11). Briefly, human peripheral blood mononuclear cells were

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isolated through a Ficoll-Hypaque (Amersham Biosciences) cushion from buffy coat white blood cell packs (American Red Cross) and were then passed through T cell enrichment columns (R&D Systems). Purified cells consisted of 90–95% $CD3^+$ T lymphocytes based on flow cytometry analysis.

T lymphocyte stimulation and RNA isolation following actinomycin D treatment

Purified T lymphocytes were cultured overnight in RPMI 1640 (Life Technologies Inc.) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin G and 100 µg/ml streptomycin. Cells (25 000 000–50 000 000 cells/group) were then stimulated for 3 h with medium alone or with immobilized monoclonal antibodies (1.0 µg/ml) directed against the CD3 component of the TCR complex (αCD3) (R&D Systems) or a combination of αCD3 and a monoclonal antibody directed against the CD28 co-stimulatory molecule (αCD28) (R&D Systems) as described previously (11). Actinomycin D (Act D) (Sigma Corp.) was then added to a final concentration of 10 µg/ml and total cellular RNA was isolated at discrete time points over a 2 h period using Trizol reagent (Life Technologies). Four independent experiments were performed. After addition of Act D, RNA was isolated at 0, 45, 90 and 120 min time points in two experiments, at 0 and 120 min time points in one experiment and at 0 and 90 min time points in one experiment.

Microarray hybridizations

cDNA was synthesized from 10–15 µg total RNA using the Superscript II RT cloning kit (Life Technologies). This cDNA was used to synthesize biotin-labeled cRNA in an *in vitro* transcription reaction using a commercially available kit (Enzo Diagnostics). cRNA was purified with the RNeasy Mini-kit (Qiagen) and 15 µg cRNA was used for hybridization to human U95Av2 arrays (Affymetrix Inc.), according to the manufacturer's protocol. Quantitative scanning of arrays was done on an HP Agilent 2200 confocal scanner.

Microarray data analysis

Affymetrix Microarray Software Suite (v.4.0) was used to calculate hybridization intensities, referred to as average difference (AD), for each gene present on the microarray and to determine if transcripts corresponding to these genes were 'present' or 'absent'. After scaling the average intensity of all arrays to 1000, the AD values for each target transcript on each array was normalized to values for GAPDH on the same array. The scaled, normalized AD values at each time point following addition of Act D were used to estimate the transcript half-life ($t_{\frac{1}{2}}$) based on a first order decay model using the equation, $y = \beta_0 e^{\beta_1 t} + \varepsilon$, where y is the normalized AD value at time t following the addition of Act D, β_0 is the initial intensity, β_1 is a decay parameter related to half-life ($t_{\frac{1}{2}} = -\ln 2/\beta_1$) and ε is an error term. This first order decay model, combining data from all four experiments, was used to determine probability distributions for the initial hybridization intensity (β_0) and for the half-life of each transcript under each stimulation condition. A detailed description of the statistical analysis can be found in Supplementary Material.

Northern blot and real time RT–PCR

Purified human T lymphocytes were stimulated for 3 h with medium or αCD3+αCD28, Act D was added, and total cellular RNA was harvested at 0, 45, 90 and 120 min time points. For northern blot analysis, 10 µg total RNA from each sample was separated by electrophoresis on a 1% glyoxal agarose gel using the NorthernMax™-Gly Glyoxal-Based System (Ambion). The RNA was blotted onto Brightstar-Plus membranes (Ambion), and membranes were crosslinked with UV energy and hybridized for 16–18 h with ^{32}P-labeled GAPDH, MAD-3, TNF superfamily member 14 (TNFSF14) and p27kip1 probes. The GAPDH probe was generated using the DECAprime™ II Random Priming DNA Labeling Kit (Ambion) according to the manufacturer's protocol. The MAD-3 probe was generated by end-labeling a DNA oligonucleotide containing the sequence 5′-GCCCCTTTGCACTCATAACGTCAGACGCTGGCCTCCAAACACACAGTCATCATAGGGC-3′) and the TNFSF14 probe was generated by end-labeling the DNA oligonucleotide 5′-GGCACCCTCTGAGTTCTCCACGTGTCAGACCCATGTCCAATGCACCACGCTCC-3′. End-labeling reactions were performed using a KinaseMax™ 5′ End-Labeling Kit (Ambion) according to the manufacturer's directions. The p27kip1 probe was generated by RT–PCR. cDNA was generated using the ProSTAR™ Ultra HF RT–PCR System (Stratagene) using total RNA from purified human T cells. PCR was then performed using p27kip1 specific forward and reverse primers (5′-TTCAGACGGTTCCCCAAAT-3′ and 5′-AACGCTTTTAGAGGCAGATCA-3′). The PCR product was gel purified and used as a template for an additional PCR in the presence of [α-^{32}P]ATP. The hybridized blots were washed, and then quantified using a phosphorimager (Molecular Dynamics). The hybridization intensity of each transcript was normalized to GAPDH and the normalized values were used to calculate half-lives.

For real time RT–PCR, cDNA was synthesized from total cellular RNA and used for PCR using the Brilliant™ Two-Step Quantitative RT–PCR Core Reagent Kit (Stratagene) according to the manufacturer's instructions. Dye-labeled TaqMan probes were synthesized by PE Applied Biosystems and the oligonucleotide primers were synthesized by Integrated DNA Technologies Inc. The probe and primer sequences for each gene evaluated are listed below in the following order: sense primer, probe, antisense primer. IL-2: GAATCCCAAACTCACCAGGA, ACCTCTGGAGGAAGTGCTGAATTTAGCTCA, ATGGTTGCTGTCTCATCTGC; GM-CSF: CAGCCTCACCAAGCTCAAG, ACTTCCTGTGCAACCCAGATTATCACCTTT, AAGGGGATGACAAGCAGAAA; tat response element DNA-binding protein (TARDBP): GGGGATGTGATGGATGTCTT, TCATATATCCAATGCCGAACCTAAGCACAA, CCACCTGGATTACCACCAAA; c-myc: TCGGATTCTCTGCTCTCCTC, AGCGACTCTGAGGAGGAACAAGAAGATGAG, CTCTGACCTTTTGCCAGGAG; phospholipase C β2 (PLCB2): ACAACTCCCACATCCAGGAA, GAACAGATACGGGAGATGGAAAAGCAGTTC, CTTCACCTCTGCCTCCAGAC; hypoxanthine phosphoribosyl transferase (HPRT): GGTGAAAAGGACCCCACGAA, TGTTGGATTTGAAATTCCAGACAAGTTTGT, AGTCAAGGGCATATCCAACA. The internal oligonucleotides for IL-2, GM-CSF, TARDBP and PLCB2 TaqMan® probes were

Table 1. Comparison of T lymphocyte transcript half-life values obtained by microarray analysis or northern blot

Probe ID	Accession no.	Gene	Microarray analysis			Northern blot			Ref.
			Stimulus	Half-life (min.) [95% CI]	Change (*P*-value)	Stimulus	Half-life (min)	Change	
1574_s_at	M13982	IL-4	αCD3	136 [100,>360]		αCD3	144		(28)
1365_at	M26062	IL-2Rβ	Medium	62 [50,108]		αCD28	60		(23)
1779_s_at	M16750	pim-1	αCD3	60 [40,>360]		ConA	35		(29)
33238_at	U23852	p56[lck]	Medium	>360 [176,>360]		Medium	240		(58)
1263_at	M20137	IL-3	αCD3+αCD28	76 [62,>360]		ConA	45		(22)
37645_at	Z22576	CD69	αCD3	45 [36,49]		PMA	<60		(27)
32861_s_at	M37815	CD28	Medium	>360 [95,>360]		Medium			(25)
			αCD3	95 [58,>360]	D (0.08)[a]	Peptide+MHC		D	
40738_at	M16366	CD2	Medium	74 [71,87]		Medium			(26)
			αCD3	101 [75,>360]	S (0.03)	αCD3		S	
1973_s_at	V00568	c-myc	αCD3	18 [2,36]		αCD3	8[b]		(8)
			αCD3+αCD28	19 [2,32]	NSC	αCD3+αCD28	10[b]	NSC	
						αCD3	10		(48)
1401_g_at	M13207	GM-CSF	αCD3	18 [10,22]		αCD3	11[b]		(8)
			αCD3+αCD28	39 [33,43]	S (0.01)	αCD3+αCD28	82[b]	S	
						αCD3	20[b]		(48)
						ConA	20		(22)
1852_at	X02910	TNF-α	αCD3	25 [15,31]		αCD3	10[b]		(8)
			αCD3+αCD28	31 [17,42]	S (0.15)[a]	αCD3+αCD28	60[b]	S	
1538_s_at	X00695	IL-2	αCD3	34 [29,58]		αCD3	23[b]		(8)
			αCD3+αCD28	150 [79,>360]	S (0.01)	αCD3+αCD28	150[b]	S	
						αCD3	30		(24)
						αCD3+αCD28	90	S	
1611_s_at	J00219	IFN-γ	αCD3	77 [40,>360]		aCD3	23[b]		(8)
			αCD3+αCD28	134 [67,>360]	S (0.13)[a]	αCD3+αCD28	150[b]	S	

D, destabilized; S, stabilized; NSC, no significant change; ConA, concanavalin A; PMA, phorbol myristate acetate; MHC, major histocompatability complex molecule.
[a]Not statistically significant.
[b]Calculated from data shown.

labeled with a 5′ reporter dye, 6-carboxyfluorescein (6FAM), and a 3′ quencher dye, 6-carboxytetramethyl rhodamine (TAMRA). The control HPRT probe was labeled with a 5′ reporter dye, tetrachloro-6-carboxyfluorescein (TET), and a 3′ quencher dye, TAMRA. PCR amplification was carried out using a Cepheid® Smart Cycler thermocycler and analyzed using Smart Cycler software. Standard curves for each gene were generated to determine the relative concentrations of amplified transcripts. The concentration of each transcript was then normalized to HPRT and the normalized values were used to calculate half-lives.

RESULTS

Steady-state mRNA levels in resting and activated T lymphocytes

Purified human T lymphocytes were stimulated in four independent experiments with medium alone (resting), with immobilized αCD3 or with a combination of immobilized αCD3 and αCD28 (αCD3+αCD28) antibodies. αCD3 activates the TCR complex, providing partial cellular activation, and αCD28 provides additional co-stimulation, allowing complete T cell activation. After 3 h of stimulation, Act D was added to arrest transcription and total cellular RNA was isolated at discrete time points over a 2 h period. This time course was designed to evaluate early changes in gene expression and the role of mRNA degradation in regulating early gene expression events. Transcripts were considered to be present at the initial time point (at the time of Act D addition) under each stimulation condition, if the Affymetrix software determined them to be present in three of the four experiments. Of 12 625 genes represented on the arrays, transcripts from 5296, 5541 and 5497 genes were present at this initial time point in the medium, αCD3 and αCD3+αCD28 groups, respectively. A total of 6112 transcripts were present in one or more of these stimulation conditions. The correlation coefficient comparing initial AD values between two different experiments exceeded 0.98 within each of the stimulation groups.

For each expressed transcript, an initial hybridization intensity parameter called β_0 was computed based on our first order decay model using data from all four experiments (see Materials and Methods). The correlation between the β_0 median values and the initial Affymetrix AD values within an experiment exceeded 0.96. The initial hybridization intensity values (β_0) for each stimulation condition were used to identify transcripts that were induced or repressed following cellular activation. The number of transcripts induced greater than 5-fold by αCD3 and αCD3+αCD28 were 81 and 98, respectively ($P < 0.05$ for each), whereas the number of transcripts repressed at least 5-fold by αCD3 and αCD3+αCD28 were 107 and 95, respectively ($P < 0.05$ for each). We also identified 80 transcripts that were induced by CD28 co-stimulation ($P < 0.05$) by comparing the initial hybridization intensities (β_0) after αCD3 versus αCD3+αCD28 stimulation. The transcripts induced by CD28 co-stimulation included cytokine genes (see Table 1), in accord with previously reported results (8). Interestingly, we found almost no transcripts whose expression was repressed by CD28

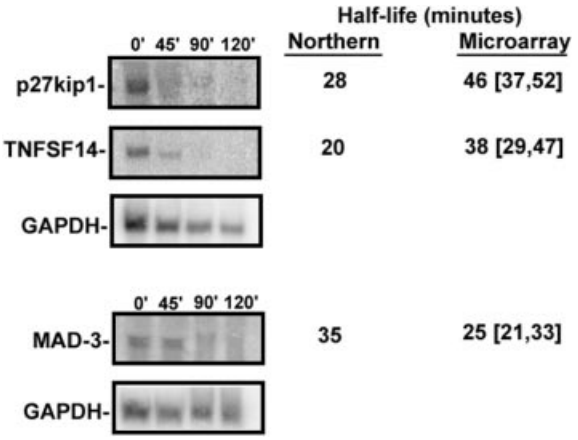

Figure 1. Comparison of transcript half-lives determined by northern blot or microarrays. Purified human T lymphocytes were stimulated for 3 h with medium or αCD3+αCD28. Act D was added and total cellular RNA was then isolated at the 0, 45, 90 and 120 min time points. Expression of TNFSF14, MAD-3 and p27kip1 was evaluated by northern blot. Each plot was also probed for GAPDH expression. The blots were quantified using a phosphorimager and the intensity of each band was normalized to the intensity of the GAPDH band. mRNA decay curves were derived for each transcript and were used to calculate transcript half-lives. Transcript half-life values derived using microarrays are also shown. The Affymetrix probe IDs for TNFSF14, MAD-3 and p27kip1 are 31724_at, 1461_at and 33847_s_at, respectively.

co-stimulation in a statistically significant manner. Table 3 shows a subset of the transcripts induced or repressed by αCD3+αCD28 stimulation ($P < 0.05$ for each). More complete listings of transcripts that were induced or repressed following T lymphocyte activation are included in Supplementary Material. The complete data set from this study can be found on the web at http://web.ahc.umn.edu/~bohjanen/.

Identification of short-lived transcripts and stimulus-induced changes in mRNA decay

We next computed mRNA decay rates for the transcripts found to be present under each stimulation condition. The median half-life and 95% CI were calculated for each expressed transcript under each stimulation condition and can be found at http://web.ahc.umn.edu/~bohjanen/. We compared T lymphocyte transcript half-lives determined using microarrays to half-lives reported in the literature that were obtained using northern blots (see Table 1) and found a good correlation. We also used northern blots to measure half-lives following Act D treatment of three transcripts that had not been previously reported in the literature and again found good correlation with our microarray data (Fig. 1). In addition, we analyzed five transcripts by real time RT–PCR and found that the half-lives correlated well with half-lives determined using microarrays (Table 2). Figure 2A shows the percentage of transcripts expressed under each stimulation condition that had median half-lives falling within the indicated ranges as determined using microarrays. The largest proportion of transcripts had long half-lives (>6 h) with a much smaller proportion of transcripts having very short half-lives. Partial listings of transcripts with short half-lives are shown in Tables 3 and 4, and a more complete listing is shown in Supplementary Material. Short-lived transcripts exhibited three dominant patterns of gene expression: activation-induced transcripts with short half-lives, short-lived transcripts that were repressed by activation, and stable transcripts that were destabilized and repressed by activation.

A large proportion of transcripts that were induced by T lymphocyte activation had very short half-lives (Fig. 2B). We identified 81 and 98 activation-induced transcripts with half-lives of <60 min following αCD3 and αCD3+αCD28 stimulation, respectively (see Supplementary Material). The pattern of gene induction and mRNA decay of a subset of these short-lived transcripts is shown in Figure 3 (Induced). Overall, αCD3 stimulation and αCD3+αCD28 stimulation induced similar sets of transcripts that exhibited rapid decay. Many αCD3+αCD28-induced transcripts were more abundant, however, and some exhibited longer half-lives (Fig. 3 and Supplementary Material). Half-life data for several short-lived transcripts that were induced by αCD3+αCD28 stimulation are shown in Table 3 (top). This set of transcripts included transcripts encoding important regulatory proteins such as cytokines, signal transduction regulators, transcription factors and regulators of apoptosis.

A second predominant pattern of expression included short-lived transcripts whose steady-state levels were repressed following αCD3 or αCD3+αCD28 stimulation (see Fig. 3, Repressed). Stimulation with αCD3 or αCD3+αCD28 led to a 2.5-fold or greater repression of 114 or 100 transcripts with half-lives of <60 min, respectively ($P < 0.05$ for each). The set of genes repressed by αCD3 and αCD3+αCD28 were very similar. A subset of the short-lived transcripts whose steady-state levels were repressed by αCD3+αCD28 stimulation is shown in Table 3 (bottom). Many of these short-lived transcripts also encode important regulatory proteins, including transcription factors and cell cycle regulators. The rapid mRNA decay exhibited by these transcripts may be essential for their decreased expression upon cellular activation.

By comparing mRNA decay rates in resting and activated T lymphocytes, we identified 212 transcripts that were

Table 2. Comparison of mRNA decay rates determined using microarrays or real time RT–PCR

Probe ID	Accession no.	Description	Microarray data		RT–PCR data	
			Medium Half-life (min) [95% CI]	αCD3+αCD28 Half-life (min) [95% CI]	Medium Half-life	αCD3+αCD28 Half-life
1401_g_at	M13207	GM-CSF	Absent	39 [33,43]	28	39
1538_s_at	X00695	IL-2	Absent	150 [79,>360]	17	232
1973_s_at	V00568	c-myc	61 [31,>360]	19 [2,32]	<45	<45
210_at	M95678	PLCB2A	>360 [>360]	Absent	>360	Absent
32241_at	AL050265	TARDBP	117 [77,>360]	42 [36,>360]	>360	19

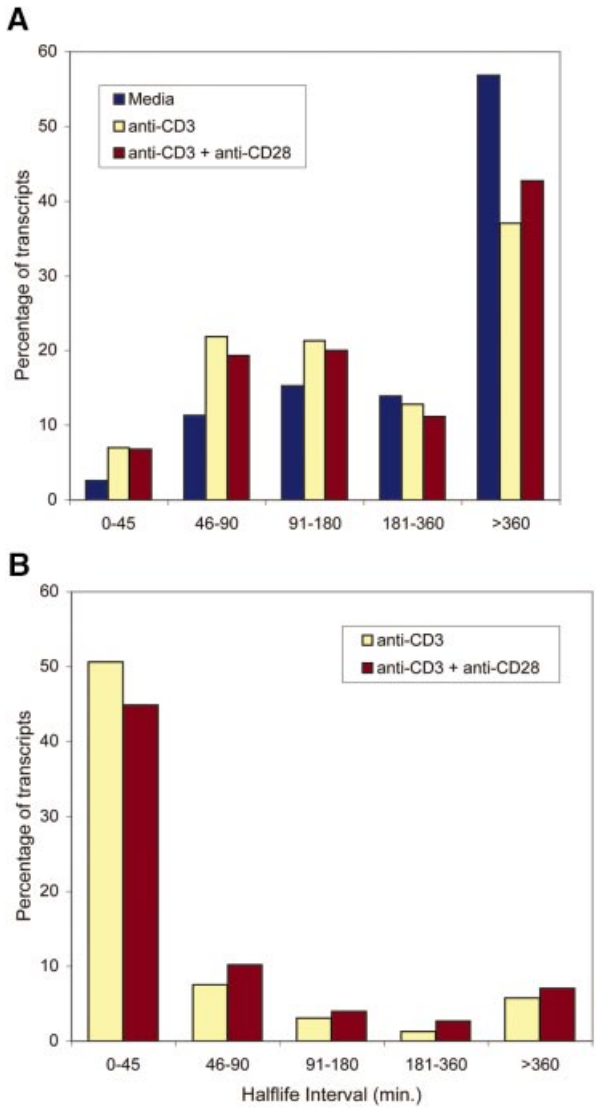

Figure 2. Profile of T lymphocyte transcript half-lives. (**A**) Purified human T lymphocytes were stimulated for 3 h with medium, αCD3 or αCD3+αCD28. Act D was added and total cellular RNA was isolated at discrete time points over a 2 h period. This RNA was used to probe Affymetrix microarrays in order to calculate mRNA half-lives. Transcripts with an Affymetrix 'present' call in at least three of four experiments under each stimulation condition were categorized by their median half-life value into five intervals. The median half-life values were calculated based on data from four independent experiments. The data is shown as a percentage of transcripts expressed under each stimulation condition. (**B**) The subset of transcripts that exhibited 5-fold or greater induction upon stimulation with αCD3 and αCD3+αCD28 were profiled by median half-life values.

repressed at least 2-fold ($P < 0.05$) and also destabilized ($P < 0.05$) by αCD3+αCD28 stimulation. A very similar set of transcripts was also repressed and destabilized by αCD3 stimulation. In addition, 166 transcripts were identified that were relatively stable (half-life > 120 min) in resting cells but presumably became unstable following cellular activation because they became absent after αCD3 or αCD3+αCD28 stimulation (see Supplementary Material). Table 4 shows a subset of the transcripts that appeared to be destabilized by αCD3+αCD28 stimulation. This group of transcripts also encoded important regulatory proteins, including cell surface receptors, signal transduction mediators, transcription regulators, and regulators of cell growth or death.

Cytokine transcripts were previously reported to be stabilized by CD28 co-stimulation compared to αCD3 stimulation alone (8). Our data showed that transcripts from αCD3-stimulated cells had similar mRNA decay profiles to αCD3+αCD28-stimulated cells, with notable exceptions. For example, IL-2 and GM-CSF transcripts were stabilized by CD28 co-stimulation in a statistically significant manner (Table 1), in agreement with previous reports (8). CD28 co-stimulation also led to statistically significant ($P < 0.05$) stabilization of 18 other activation-induced genes, including the chemokines exodus-1 and lymphotactin, the signal transduction regulators JAK-binding protein/TIP3 and Ras-like protein Tc4, the transcription factors NF-κB, ERF and CREM and the apoptosis regulator IPL (Table 5). Transcripts encoding other cytokines, such as IFN-γ and TNF-α, also showed a trend toward stabilization by CD28 co-stimulation, but this effect did not reach statistical significance (Table 1).

Identification of short-lived transcripts that contain AU-rich element-like sequences

Perhaps the best characterized example of a *cis*-element that controls mRNA degradation is the AU-rich element (ARE). AREs present in the 3′-untranslated regions (3′-UTRs) of cytokine and proto-oncogene transcripts mediate their rapid decay (1,2,12–15). By comparing sequences from known ARE-containing transcripts, a minimal consensus ARE was recently defined as WWWUAUUUAUWWW (W = U or A) (16). These researchers searched GenBank for human transcripts that contained this consensus sequence in their 3′-UTRs and found 897 transcripts that were compiled into an ARE database (16). The ARE-like sequences present in >90% of the transcripts in the ARE database, however, have not been evaluated for function. We searched for the intersection between transcripts that we found to be expressed in T cells and transcripts present in the ARE database, and we found approximately 400 transcripts that were expressed in T cells and contained ARE-like sequences. Of these, ~25% (~100 transcripts) exhibited rapid decay (half-life <60 min) under at least one stimulation condition, 45% exhibited intermediate decay (half-life 120–180 min) under at least one condition but did not exhibit rapid decay under any condition, and 30% exhibited relatively slow decay (half-life >180 min). Half-life data for a subset of the transcripts that contained ARE-like sequences and exhibited rapid decay is shown in Table 6, and a more complete listing is shown in Supplementary Material. Interestingly, some of these transcripts were induced by T cell activation while others were repressed. The ARE-like sequences present in these short-lived transcripts are good candidates for functional *cis*-elements that control decay, but experiments, including mutational analysis or expression of the *cis*-elements in a heterologous context, would need to be performed to verify their function. Overall, our findings suggest that a major subset of short-lived transcripts contain ARE-like sequences. Many other short-lived transcripts, however, do not contain defined ARE or ARE-like sequences and many transcripts that contain ARE-like sequences do not exhibit rapid decay, suggesting that other signals that have not been identified also regulate decay. A complete listing of the transcripts expressed in T cells that contain ARE-like

Table 3. Short-lived transcripts that were induced ($P < 0.05$) or repressed ($P < 0.05$) upon αCD3+αCD28 stimulation

Probe ID	Accession no.	Description	Function	Half-life (min) [95% CI]
Induced transcripts				αCD3+αCD28
259_s_at	M16441	Lymphotoxin α (TNF superfamily)	Ligand	36 [22,47]
31742_at	AF064090	TNF superfamily, member 14	Ligand	38 [29,47]
37645_at	Z22576	CD69 antigen (p60)	Cell surface receptor	47 [39,56]
33513_at	U33017	Signaling lymphocytic activation molecule (SLAM)	Cell surface receptor	42 [29,63]
224_at	S81439	TGFβ inducible early growth response	Signal transducer	29 [15,35]
41592_at	AB000734	JAK-binding protein (TIP3/JAB)	Signal transducer	18 [12,29]
34770_at	Z14138	MKKK8	Protein kinase	36 [16,53]
973_at	Y10032	Serum/glucocorticoid regulated kinase	Protein kinase	37 [9,54]
2049_s_at	M29039	jun B proto-oncogene	Transcription factor	11 [1,28]
37627_g_at	D78261	Interferon regulatory factor 4 (IRF-4)	Transcription factor	32 [26,40]
287_at	L19871	Activating transcription factor 3 (ATF-3)	Transcription factor	28 [12,38]
1916_s_at	V01512	c-fos	Transcription factor	20 [2,35]
1519_at	J04102	ets-2	Transcription factor	44 [18,53]
1851_s_at	U11821	Fas ligand (FasL)	Apoptosis	24 [5,33]
2002_s_at	U27467	Bcl-2-related protein Bfl-1	Apoptosis	42 [28,60]
Repressed transcripts				Medium
1062_g_at	U00672	Interleukin 10 receptor α	Transmembrane receptor	37 [29,48]
40646_at	U20350	Chemokine (C-X3-C) receptor 1	Transmembrane receptor	49 [43,>360]
39753_at	X06256	Integrin, α5	Transmembrane receptor	39 [31,51]
38045_at	U96136	Catenin, δ2	Cell adhesion	23 [1,55]
1857_at	AF010193	SMAD7, MAD homolog 7 (*Drosophila*)	Signaling protein	25 [18,47]
36741_at	D63482	GPCR kinase-interactor 2	Signaling protein	39 [36,53]
761_g_at	Y09216	Dual-specificity kinase 2	Protein kinase	59 [44,63]
1202_g_at	D14889	RAB33A, member RAS oncogene family	GTPase	24 [9,33]
40511_at	X58072	GATA 3	Transcription factor	32 [26,48]
33113_at	U65093	CBP/p300-interacting transactivator	Transcription factor	33 [29,48]
35319_at	U25435	CCCTC-binding factor (Zn finger protein)	Transcription factor	60 [45,83]
40727_at	AL080090	Anaphase-promoting complex subunit 10	Cell cycle control	36 [22,102]
38822_at	AB011420	Ser/Thr kinase 17a (DRAK1)	Apoptosis	46 [43,49]
35588_at	AB011414	Kruppel-type zinc finger (C_2H_2)	Apoptosis	34 [13,61]
39013_at	Y11588	APG5 autophagy 5-like (*S.cerevisiae*)	Apoptosis	27 [10,44]

Table 4. Transcripts that were repressed ($P < 0.05$) >2.5-fold and were destabilized ($P < 0.05$) or became absent upon stimulation with αCD3+αCD28

Probe ID	Accession no.	Description	Function	Half-life (min) [95%CI]	
				Medium	αCD3+αCD28
32158_at	U53174	Cell cycle checkpoint control protein	Cell cycle control	>360 [>360]	Absent
34217_at	AB015132	Ubiquitous Kruppel-like factor	Cell cycle control	>360 [200,>360]	Absent
748_s_at	D63940	Max interacting protein 1	Cell cycle control	196 [106,>360]	Absent
38639_at	AF040963	Mad4	Cell cycle control	>360 [269,>360]	Absent
32596_at	W25828	Retinoblastoma-like 2 (p130)	Cell cycle control	>360 [188,>360]	Absent
1794_at	M92287	Cyclin D3	Cell cycle control	213 [121,>360]	100 [74,176]
1189_at	X85753	Cyclin-dependent kinase 8	Cell cycle control	>360 [177,>360]	68 [54,110]
35647_at	U20536	Caspase 6, isoform β	Apoptosis	>360 [52, >360]	Absent
38010_at	AF002697	Bcl-2-binding protein Nip3	Apoptosis	125 [93,>360]	56 [50,95]
32212_at	AL049703	Programmed cell death 8	Apoptosis	>360 [343,>360]	57 [23,348]
38398_at	AB002356	MAP-kinase activating death domain	Apoptosis	127 [103,204]	73 [64,137]
36473_at	AB023220	Ubiquitin-specific protease 20	Protease	>360 [290,>360]	77 [64,>360]
40961_at	X72889	SWI/SNF-related, subfamily a, member 2	Transcription	324 [136,>360]	81 [67,97]
34689_at	AJ243797	3′ repair exonuclease 1	DNA repair	>360 [129,>360]	Absent
1919_at	X16316	vav1 proto-oncogene	Receptor signaling	>360 [>360]	Absent
34416_at	X57110	c-cbl proto-oncogene	Receptor signaling	>360 [121,>360]	Absent
2004_at	U29671	MEK kinase	Receptor signaling	141 [74,>360]	Absent
33238_at	U23852	$p56^{lck}$	Receptor signaling	>360 [176,>360]	115 [94,157]
210_at	M95678	Phospholipase C β2	Receptor signaling	>360 [>360]	Absent
35980_at	AB011153	Phospholipase C β1	Receptor signaling	>360 [45,>360]	Absent
36499_at	Z16411	Phospholipase C β3	Receptor signaling	126 [104,>360]	Absent
37468_at	AF058925	Janus kinase 2	Receptor signaling	124 [57,>360]	Absent
33410_at	S66213	Integrin α chain, α6	Cell adhesion	>360 [92,>360]	Absent
33228_g_at	AI984234	Interleukin 10 receptor β	Transmembrane receptor	>360 [101,>360]	87 [70,117]

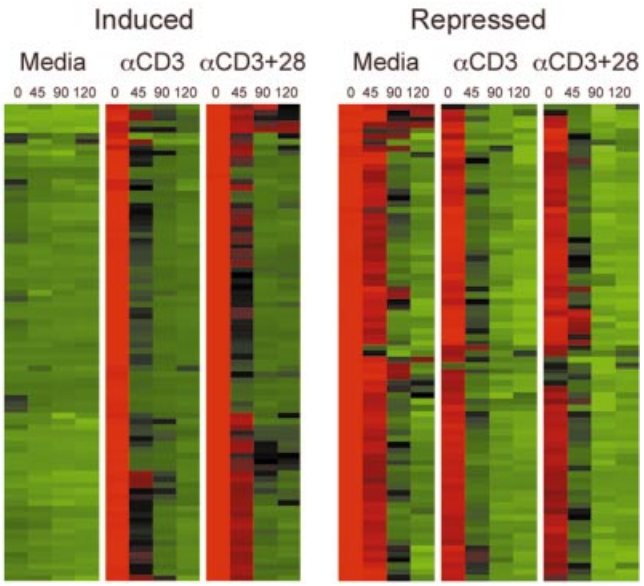

Figure 3. Short-lived transcripts whose steady-state levels were induced or repressed upon T cell activation. Purified human T lymphocytes were stimulated for 3 h with medium or αCD3+αCD28. Act D was added and total cellular RNA was isolated at the 0, 45, 90 and 120 min time points. This RNA was used to probe Affymetrix microarrays. The data shown is from an individual experiment and shows raw hybridization intensity (AD) data for 200 short-lived transcripts that were induced or repressed following αCD3+αCD28 stimulation. The intensity data is represented by a color scale, showing low intensity in green and high intensity in red.

sequences, along with data regarding their expression and half-lives, can be found at http://web.ahc.umn.edu/~bohjanen/.

DISCUSSION

We used microarray technology to measure decay rates of approximately 6000 T lymphocyte mRNA transcripts, allowing us to identify hundreds of genes that are regulated at the level of mRNA decay. In particular, we identified important regulatory genes that produce unstable transcripts or transcripts that were stable in resting cells but were destabilized in a stimulus-dependent manner. Since steady-state mRNA levels are determined by the rate of transcription as well as the rate of mRNA degradation, rapid mRNA degradation provides the cell with a general mechanism for rapidly turning off the expression of regulatory genes in response to changes in transcription. In addition, activation-induced mRNA destabilization provides another mechanism for turning gene expression off.

Activation-induced genes in T lymphocytes tend to be expressed during precise periods of time, and then their expression is turned off (17–21). Our results, as well as the results of others (8,22–29), suggest that many activation-induced genes produce transcripts with short half-lives. The precise program of transient gene expression following cellular activation requires precise coordination between transcription and mRNA decay. For example, certain T lymphocyte early response genes, including cytokine genes and proto-oncogenes, are induced transcriptionally but appear to be turned off, at least in part, through rapid mRNA decay (8). Our data suggest that many other regulatory transcripts are also induced transcriptionally and then turned off through mRNA degradation. We cannot, however, exclude the possibility that mRNA stabilization contributes to the induction of these transcripts since many are expressed at low or undetectable levels in the resting state and their half-lives cannot be measured. A combination of decreased transcription, rapid mRNA decay and rapid protein turnover may all contribute to turning off the expression of specific genes.

Turning off expression of activation-induced genes through rapid mRNA decay may be part of a homeostatic mechanism to stop T cell activation and thereby down-modulate an immune response. For example, turning off expression of cytokine genes (see Table 1) through rapid mRNA decay

Table 5. Transcripts that were induced by activation ($P < 0.05$) and were stabilized by αCD3+αCD28 stimulation in comparison to αCD3 stimulation alone

Probe	Accession no.	αCD3 Half-life (min)	[95% CI]	αCD3+αCD28 Half-life (min)	[95% CI]	Description
1538_s_at	X00695	34	[29,58]	150	[79,>360]	Interleukin-2
1401_g_at	M13207	18	[10,22]	39	[33,43]	GM-CSF
40385_at	U64197	73	[50,121]	>360	[258,>360]	Chemokine exodus-1
39652_at	AL031736	121	[86,227]	185	[129,>360]	Lymphotactin/lymphotaxin
1840_g_at		239	[210,>360]	>360	[>360]	Ras-like protein Tc4
41592_at	AB000734	8	[1,15]	18	[12,29]	JAK-binding protein/TIP3
1242_at	U15655	33	[24,43]	47	[42,69]	ets domain protein ERF
545_g_at	S76638	46	[43,50]	>360	[46,>360]	p50-NF-κB homolog
40362_at	X61498	54	[50,63]	>360	[52,>360]	NF-κB subunit
32067_at	S68271	70	[64,77]	97	[76,>360]	cAMP response element modulator (CREM)
32065_at	S68134	57	[50,96]	106	[84,121]	CREM β isoform
31888_s_at	AF001294	88	[80,110]	125	[109,>360]	Imprinted in liver and placenta (IPL)
36602_at	D21064	59	[50,64]	72	[59,>360]	
36313_at	M55267	74	[66,77]	97	[84,>360]	EV12 protein
36685_at	W63793	75	[71,102]	136	[111,174]	
37699_at	U29607	27	[15,98]	145	[91,>360]	Methionine aminopeptidase mRNA
262_at	M21154	77	[69,101]	204	[96,>360]	*S*-adenosylmethionine decarboxylase
32571_at	X68836	241	[231,>360]	>360	[>360]	*S*-adenosylmethionine synthetase
37275_at	U13045	26	[18,41]	39	[36,43]	Nuclear respiratory factor-2 subunit β1
39432_at	AF038662	63	[49,80]	83	[73,94]	β-1,4-galactosyltransferase
32227_at	X17042	265	[174,360]	>360	[>360]	Hematopoetic proteoglycan core protein

Table 6. Examples of short-lived T cell transcripts that contain ARE-like sequences and were either induced ($P < 0.05$) or repressed ($P < 0.05$) upon αCD3+αCD28 stimulation

Probe	Accession no.	Description	Function	Half-life (min) [95% CI]
Induced transcript				αCD3+αCD28
1237_at	S81914	Immediate early response 3	Apoptosis	20 [2,32]
1461_at	M69043	MAD-3 (IκB-like)	Apoptosis	25 [21,33]
279_at	L13740	Nuclear receptor subfamily 4, group A, member 1	Nuclear receptor	25 [15,43]
37310_at	X02419	Plasminogen activator, urokinase	Signal transducer	29 [20,47]
1852_at	X02910	Tumor necrosis factor (TNF superfamily, member 2)	Ligand	31 [17,42]
41447_at	AB023207	Carbohydrate (chondroitin) synthase 1	Metabolic enzyme	38 [29,43]
1401_g_at	M13207	Colony stimulating factor 2 (granulocyte-macrophage)	Ligand	39 [33,43]
190_at	U12767	Nuclear receptor subfamily 4, group A, member 3	Nuclear receptor	45 [36,51]
38692_at	AF045451	NGFI-A-binding protein 1 (EGR1-binding protein 1)	Signaling protein	45 [36,57]
40074_at	X16396	Methylene tetrahydrofolate dehydrogenase (NAD^+ dependent)	Metabolic enzyme	49 [36,62]
Repressed transcript				Medium
35659_at	U00672	Interleukin 10 receptor α	Transmembrane receptor	25 [22,>360]
32541_at	S46622	Protein phosphatase 3 (formerly 2B), (calcineurin A γ)	Signaling protein	43 [29,224]
37312_at	D50917	Transcriptional regulator interacting with the PHS bromodomain 2	Transcription factor	43 [29,111]
33249_at	M16801	Nuclear receptor subfamily 3, group C, member 2	Nuclear receptor	45 [32,56]
38822_at	AB011420	Serine/threonine kinase 17a (apoptosis-inducing)	Apoptosis	46 [43,49]
40570_at	AF032885	Forkhead box O1A (rhabdomyosarcoma)	Transcription factor	48 [40,58]
38526_at	U02882	Phosphodiesterase 4D, cAMP-specific	Signaling protein	52 [36,187]
40320_at	AF000367	CDC14 cell division cycle 14 homolog A (*S.cerevisiae*)	Cell cycle control	53 [50,95]
36690_at	M10901	Nuclear receptor subfamily 3, group C, member 1	Nuclear receptor	59 [50,128]
36979_at	M20681	Solute carrier family 2 (facilitated glucose transporter), member 3	Glucose transporter	60 [50,95]

would serve to down-modulate an immune response and prevent excessive inflammatory destruction. Down-modulation of co-stimulatory cell surface receptors such as CD69 (30) or signaling lymphocytic activation molecule (31) may contribute to limiting an immune response (see Table 3, top). Also, turning off expression of induced pro-apoptotic regulators such as Fas ligand (32) would prevent excessive killing of target or bystander cells. Induction of the anti-apoptotic Bcl-2-related protein Bfl-1 (33,34) may allow T cells to initially survive and perform their effector functions and then turning off this gene at a later point through mRNA decay would promote apoptotic death of T cells and thereby limit further inflammation. Also, activation-induced genes (21,35) encoding signal transducers such as JAK-binding protein/TIP3, and MKKK8 or transcription factors such as c-Myc, c-Fos, Jun B, interferon regulatory factor-4 and activating transcription factor-3 (Tables 1 and 3) appeared to be turned off, in part, through rapid mRNA degradation. Turning off expression of these genes may bring an activated T cell back toward an inactive state, further limiting an immune response. Failure to turn off activation-induced gene expression through mRNA degradation could lead to severe intracellular consequences. For example, abnormal stabilization of activation-induced transcripts such as c-fos or c-myc has been associated with malignancy (4–7).

Hundreds of transcripts were identified whose steady-state levels decreased following T lymphocyte activation. A subset of these repressed transcripts had short half-lives in the resting state (Table 3, bottom). The finding that steady-state levels of these transcripts were maintained in unstimulated cells, despite their rapid rate of decay, suggests that the rapid decay was balanced by rapid transcription. Apparently, the cell expends considerable metabolic energy to constantly synthesize and degrade these transcripts in order to maintain a steady state. This balanced state may exist so that these transcripts are poised to be regulated quickly. For example, cellular activation may lead to decreased transcription of these transcripts, shifting the balance toward decreased steady-state levels. Such a mechanism would allow genes to be turned off rapidly under the appropriate stimulation conditions. Another subset of activation-repressed transcripts exhibited activation-dependent mRNA destabilization (Table 4). Expression of these transcripts could potentially be turned off rapidly, even in the face of continued transcription.

It appears that cellular quiescence is an actively maintained state and turning off the expression of genes that actively maintain quiescence is a critical component of normal cellular activation (36,37). We found that transcripts encoding many important regulators of cell proliferation or cell fate were turned off following cellular activation, at least in part, through rapid mRNA degradation (see Table 3, bottom, and Table 4). These transcripts encoded cell growth regulators such as the ubiquitous Kruppel-like factor, cell cycle checkpoint control protein, anaphase promoting complex, cyclin D3, cyclin T2, cyclin-dependent kinase 8 and retinoblastoma-like protein 2, as well as apoptosis regulators such as autophagy 5-like protein (APG5), Kruppel-type zinc finger protein, Drak1 and caspase-6. We also found that transcripts encoding components of receptor signaling pathways (21,35,38), including vav1, janus kinase 2, phospholipase C β1, phospholipase C β3, MEK kinase and $p56^{lck}$, were destabilized and repressed after activation, perhaps as part of a homeostatic feedback loop to shut off TCR-mediated signaling. Rapid mRNA degradation also contributed to the activation-induced shut-off of transcripts encoding cell surface molecules, including α5 integrin, α6B integrin, δ2 catenin, chemokine C-X3-C receptor-1, IL-10 receptor α and IL-10 receptor β. Perhaps at the site of inflammation, activated T cells no longer require signals from these receptors for homing, chemotaxis or differentiation (39–42). Alternatively,

turning off expression of these receptors could prevent additional T cell signaling and thereby help to down-modulate an immune response.

Although we identified approximately 100 transcripts that were stabilized by αCD3+αCD28 stimulation, few of these transcripts displayed a significant increase in steady-state level at the time point studied. We speculate that activation-induced stabilization could have effects on steady-state mRNA levels at later time points following αCD3+αCD28 stimulation. At least at the early time point studied, activation-induced mRNA destabilization produced much more dramatic effects on steady-state mRNA levels than did mRNA stabilization. We also identified numerous short-lived transcripts whose steady-state levels did not change after 3 h of activation with αCD3 or αCD3+αCD28. Perhaps these transcripts are poised to be rapidly regulated at other time points or under environmental conditions not examined in our experiments. Our experiments evaluated mRNA decay at only a single point in time, 3 h after T cell activation, and therefore dynamic changes in mRNA decay that occur at other time points could have been missed. Future experiments will be performed at a variety of time points following T cell activation in order to better profile the dynamic changes in mRNA decay that may occur.

TCR-mediated stimulation without CD28 co-stimulation results in defective proliferation and decreased survival (43,44). CD28 exerts its effect in large part by inducing increased expression of IL-2, a critical T cell growth factor (45,46). CD28 co-stimulation leads to increased IL-2 transcription as well as stabilization of IL-2 mRNA (8,47). We also found that CD28 co-stimulation led to stabilization of IL-2 mRNA (see Table 1). In addition, we identified several other transcripts that were stabilized by CD28 co-stimulation, including transcripts encoding the chemokines exodus-1 and lymphotactin, the signal transduction regulators JAK-binding protein/TIP3 and Ras-like protein Tc4, the transcription factors NF-κB, ets-domain protein ERF, cAMP response element modulator, and the apoptosis regulator imprinted in liver and placenta (IPL). Stabilization of these transcripts could contribute to CD28-mediated cellular activation and proliferation.

Many of the short-lived transcripts that we identified contain AREs, well characterized mRNA sequences that target transcripts for rapid degradation (1,2,12–15). AREs, found at the 3′ end of many rapidly degraded transcripts, function through their interaction with ARE-binding proteins to promote the deadenylation and degradation of mRNA (11,48–56). ARE-binding proteins may regulate mRNA degradation through their interaction with the exosome, a multi-protein complex that has 3′→5′ exonuclease activity (57). A database of transcripts containing ARE or ARE-like sequences has been compiled (16) and many of the short-lived transcripts that we identified contain these sequence motifs (Table 6). Many short-lived transcripts, however, do not contain characterized ARE sequences, therefore other sequences in these transcripts appear to regulate their rapid decay. Also, many transcripts that contain ARE-like sequences do not exhibit rapid decay, suggesting that the presence of an ARE-like sequence is not sufficient for mediating rapid mRNA decay and that additional signals may be required. Identification of transcripts that are regulated at the level of mRNA decay is a first step toward identifying additional novel regulatory sequence elements and *trans*-acting proteins, with the goal of understanding the biochemical mechanisms that regulate rapid or stimulus-dependent mRNA decay.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at NAR Online.

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