What scales the T cell response?

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Abstract

T cells are known to scale their clonal expansion and effector cytokine response according to the dose and strength of antigenic signal in order to balance their role of affecting protection with the intertwined, immunologically driven tissue damage. How T cells achieve this is now beginning to be understood. We underscore temporal integration of digital TCR signaling as the basis for achieving scaled response by means of accumulating critical mediators over time. We also discuss the role of temporally integrated cross-talk between TCR and IL2 signaling, in mediating a scaled, coherent, collective response by T cells. Finally, we highlight numerous known and putative regulatory interactions in the transcriptional program that are expected to quantitatively scale the T cell response and also offer new mechanisms to hitherto unexplained observations.

Scaling of the T cell response

An optimal immune response is critical to nullify the pathogenic threat while ensuring that the host is not irreversibly disadvantaged by the various effector mechanisms. Innate cells evaluate the level and type of microbial threat for a scaled anti-microbial response [1]. This is translated to the adaptive arm of the immune system by the dendritic cells. Naïve T cells for any antigen are present in very low frequency and need to undergo clonal expansion by 10-20 rounds of cell division upon activation by dendritic cells [2, 3]. T cell expansion scales with respect to the initial inoculum of the pathogen and the level of expression of the antigen [4-6]. Similarly cytokine expression scales with the dose of the antigen *ex vivo* and *in vivo* [5, 7]. Such scaling is also observed in response to infection by a panel of recombinant *L. monocytogenes* that give rise to altered peptide ligands (APL) representing a wide range of TCR-MHCp affinity and in response to direct APL immunization [5, 8-10]. It must be noted that the scaling can plateau at very high antigenic dose or strength, but the collective evidence points to scaling occurring over a wide range [5, 6]. A sub-optimal T cell response, be it in clonal expansion or in effector molecule expression, can result in failed or delayed clearance, chronic infection or escape of a tumor [6], for example. On the other hand, an over-blown response, especially clonal expansion, is wasteful and is expected to put strain on the resources of an already distressed host. More importantly, an overt T cell response can cause excessive tissue damage and potentially exacerbate or initiate autoimmune reactions [9, 11]. These observations argue for scaled response being an important functional feature of the immune system.

Temporal integration for scaling the T cell response

Recognition of a cognate MHCp by the TCR leads to a signaling cascade followed by a transcriptional cascade resulting in the expression of cytokines, and master regulators of cell cycle, anabolic processes, and differentiation [12, 13]. Examination of activation status of multiple enzymes and transcription factors in stimulated cell populations has revealed that the TCR signaling system functions as a digital switch with very limited dynamic range in their levels among the cells activated with varying doses of antigen (Supplementary Note 1) [14]. *Ex vivo* investigations have shown that escalating fraction of cells respond with increasing dose or

1 affinity of antigen (Supplementary Note 1) [14]. Thus, varying the fraction of cells recruited to
2 the response can theoretically give up to 100-fold dynamic range. However, in response to an
3 infection, essentially all the cognate T cells are recruited irrespective of the pathogen, antigen
4 dose and affinities for the MHCp [4, 6, 8]. This near complete recruitment is thought to
5 breakdown only if affinity comes close to that of self-peptides or if the subdominant epitope is
6 expressed or presented at very low levels [6, 8, 15]. Then how do T cells achieve scaling of their
7 response according to the dose and strength of the antigens they encounter?

8 There is a large body of evidence to conclude that the T cell activation process is reliant on
9 temporal integration of signaling [14]. This can be classified into four groups: 1) Firstly, the pre-
10 requisite for temporal integration, i.e. continuous or prolonged signaling, is satisfied during T
11 cell activation [16]. Although there is ligand-induced degradation of TCR, de novo synthesis and
12 receptor recycling ensures that a lower steady state level of surface TCR is maintained during
13 continuous signaling [16-18]. Since this steady state level of TCR is still in vast excess compared
14 to cognate MHCp, time-integrated TCR-MHCp is expected to scale linearly with MHCp [14, 16,
15 19]. 2) T cells have been shown to integrate from discontinuous signals due to hysteresis and
16 stability of signaling intermediates and transcription factors [20-23]. 3) The commitment to
17 proliferation or production of cytokines is delayed when T cells are presented weaker signals [5]
18 by means of lower dose [16] or affinity of antigen [24] or withdrawal of co-stimulation [16]. 4)
19 Finally, the extent of proliferation scales with the experimentally manipulated duration of
20 antigenic signaling *in vivo*, which has been demonstrated by multiple groups by multiple
21 approaches [3]. This forms the most crucial set of evidence from the standpoint of scaling. But,
22 how does temporal integration lead to scaling?

24 **Scaled accumulation of critical mediators by temporal integration**

25 From a mechanistic viewpoint, transcription and translation are inherently time-integrated
26 processes as the products can continue to accumulate over prolonged periods of signaling. When
27 prolonged or continuous signaling is coupled to transcription, small quantitative differences in
28 the signaling intermediates, as would be the case for the TCR signaling cascade, can faithfully be
29 read into the build-up of translated products over time. This should provide convenient means to

1 a large dynamic range and a linear response, an assertion best exemplified by studies from Dario
2 Vignali's group on manipulating the multiplicity of ITAMs in the CD3 complex. The extent of
3 proliferation, as measured by thymidine incorporation, was linear with respect to the number of
4 ITAMs in the CD3 complex, despite no significant difference in immediate cytokine production
5 [25]. Proliferation scaling in relation to ITAMs was later shown to be due to marginal increase in
6 Vav1 association with CD3 resulting in increased Notch1 activation and subsequent scaling in
7 Myc transcription, while the activation status of other signaling intermediates such as Zap70 and
8 Erk were indistinguishable [26]. Myc leads to global transcriptional amplification and also drives
9 essentially the entire biosynthetic and cell cycle machinery [27-29]. Similarly, notch1 and notch2
10 together contribute to nearly half of the gene expression changes in activated T cells due to their
11 ability to target the biosynthetic and cell cycle machinery like Myc [30, 31].

12 There is a second set of evidence supporting the accumulation of critical mediators of the T cell
13 responses due to temporal integration. Continuous TCR signaling for ~24-48 hours is sufficient
14 to program the cells to then undergo a set number of divisions in an antigen-independent manner,
15 but as per the strength of the initial antigenic signal [3, 10]. Inflammatory and co-stimulatory
16 signals are additive in this early period of signaling, which further increase the number of
17 divisions the cells undergo later [10]. This is possible if critical regulators of the biosynthetic and
18 mitotic machinery accumulate proportional to the sum of all three signals and then get diluted
19 during each round of division due to lack of any additional accumulation. This assertion is also
20 supported by the observation that time taken for later divisions increases [32]. Two recently
21 described transcription factors necessary for CD8 T cell clonal expansion and effector
22 differentiation, BATF and IRF4, have the suitable characteristics to enable scaled response in the
23 context described above: 1) Gradual accumulation of IRF4 over the first 24-48 hours scales
24 according to antigenic dose and strength ex vivo [33, 34]. 2) IRF4 drives majority of the affinity-
25 dependent gene expression, which includes hundreds of metabolic and biosynthetic enzymes
26 [33]. 3) Haploid status for IRF4 and BATF results in concordant reduction in clonal expansion
27 and expression of immune effector molecules in vivo [33, 35]. 4) Knock-down of BATF 72
28 hours after LCMV infection by IPTG-inducible shRNA expression does not impact the T cell
29 response [36]. This would mean that BATF attains the maximal required accumulation within
30 ~60 hours. 5) IRF4 and BATF are not required to initiate the first ~4 divisions [33, 35, 37].

1 However they are important for sustained clonal expansion by means of driving cellular growth,
2 repressing CDK inhibitors and preventing apoptosis.

3 Amplification arising out of temporal integration can convert heterogeneity in expression of
4 signaling proteins into a large diversity in functional outcome in individual cells [38]. Indeed,
5 recent data from single-cell-centric experiments have revealed a diversity of individual and
6 clonal behavior in T cells responding to infection [39]. How do we then observe consistent
7 responses despite the large diversification? Experimental and theoretical analyses independently
8 reported by three laboratories have shown that population averaging of fates from as few as 50
9 naïve cells is sufficient to obtain consistent responses between mice [39]. Theoretical analysis of
10 fates of B cells that were activated *ex vivo* pointed out that stochastic choice between competing,
11 mutually exclusive outcomes in a cell autonomous manner is sufficient for reproducible,
12 averaged responses in a small population of cells [40]. Nonetheless, T cell expansion and
13 differentiation does not occur in a fully autonomous manner and is known to be plastic and
14 shaped by several cytokines [2, 3, 41, 42]. This suggests that responding T cells attempt to
15 reduce divergent responses by means of paracrine effects of cytokines and do not rely solely on
16 the statistical action of the central limit theorem [43]. Recent studies have shed light onto how
17 the interplay between cytokine and TCR signaling facilitated by temporal integration leads to
18 scaled, coherent, collective responses.

20 **Cross-talk with IL2 signaling for collective scaling**

21 Interleukin-2 (IL2) is a cytokine that is critical for sustained clonal expansion of responding T
22 cells [2]. Scaled production and secretion of IL2 is therefore likely to be important to drive
23 scaled expansion. IL2 signaling is known to repress further transcription of *Il2* locus, which
24 would set a ceiling on the extracellular accumulation of IL2 [44, 45]. Thus it remained an enigma
25 as to how scaled production of IL2 and subsequent expansion is achieved. Altan-Bonnet and
26 colleagues have addressed this enigma in their recent study by finding that TCR signaling
27 inhibits Stat5 phosphorylation (Figure 1a) [7]. Tyrosine phosphorylation of some of the Stat
28 transcription factors is prevented by serine phosphorylation and subsequent sumoylation at
29 adjacent residues, which may be enforced on Stat5 by TCR signaling [46, 47]. Regardless of the

mechanism, inhibition of Stat5 acts against rapid and robust repression of transcription of the *Il2* locus by IL2 signaling. Thus, IL2 continues to accumulate for ~48 hours of continuous TCR signaling (Figure 1b). Altan-Bonnet and colleagues have identified two additional features that enhance the scaling in time-integrated, collective production of IL2 over the first ~48 hours: 1) Gradual increase in the number of cells secreting IL2. 2) Non-linear increase in rate (i.e. acceleration) of secretion of IL2 on a per cell basis [7] (Figure 1b). These ultimately enable over 3 orders of magnitude scaling in IL2 accumulation within ~48 hours in response to 4 orders of magnitude difference in antigen dose, irrespective of 2 orders of magnitude variations in the starting number of antigen-specific T cells [7]. The authors have postulated the presence of an intermediate step with a positive feedback for driving the accelerated accumulation of IL2 in response to continuous TCR signaling (Figure 1a).

Beyond the first ~48 hours, time-integrated accumulation of a critical amount of IL2 receptor (IL2R) due to TCR signaling kick-starts the positive feedback of further transcription of IL2R subunits due to IL2 signaling (Figure 1a) [44]. This positive feedback is mediated directly by Stat5 and indirectly by Notch expression and activation [31, 48]. Accumulated IL2R quickly begins to consume available IL2 due to internalization and degradation of the IL2R-IL2 complex. Further, dominant role of IL2 signaling will also suppress transcription of the *Il2* locus leading to gradual depletion of IL2 (Figure 1a, 1b). This regulatory network enables accumulated IL2 to drive a concordant number of divisions after 24-48 hours, which can proceed in an antigen-independent manner [3, 44]. However, the presence of antigen beyond ~48 hours is expected to prolong the availability of IL2 and IL2R and thus drive additional expansion, as observed *in vivo* [3].

IL2 is known to have paracrine effects with an effective range of few tens of μm , which will impact the immediate surrounding cells [45]. Paracrine messengers secreted from cells that produce them in larger quantities can either trigger secretion of a secondary messenger from non-producers and/or trigger responses in all the cells in vicinity due to diffusive spread [49, 50]. Thus, a paracrine messenger can be a shared messenger for a collective, coherent response. IL2 has also been shown to be a shared messenger: 1) primed T cells form homotypic aggregates with IL2 production and consumption occurring in a polarized fashion in these aggregates [51]. 2) Cells responding to a weaker antigen can co-opt IL2 produced by cells responding to a

stronger antigen to divide more times in co-culture settings and thus reduce the disparity with cells responding the stronger antigen [52]. This was shown to be due to the growth effects of IL2 via PI3K signaling that triggers entry into cell cycle (Figure 1c). Additional critical nodes of integration can be gleaned from recently reported studies: 1) Notch1 is activated by T cell signaling *in cis* and additional Notch1/2 expression is triggered by IL2 signaling [26, 53]. 2) Further, TCR signaling induces digital expression of Myc, which is subsequently scaled by the level of IL2 signaling [54]. Surprisingly, integration of TCR and IL2 signaling starts even within the first 12 hours [52]. Thus, IL2 works to reduce heterogeneity from the earliest stages of T cell activation and clonal expansion. This may be the case of additional cytokines produced by innate cells such as IL12, IL1 β , IL6 and IFN α that are known to shape the early stages of T cell proliferation and differentiation [55].

Regulatory mechanisms facilitating a scaled response

The detailed characterization of scaling in IL2 accumulation exemplifies that multiple regulatory mechanisms act in concert to enable a scaled response. We recognize and propose additional regulatory mechanisms that promote a scaled response based on: 1) parallels drawn from other signal-response systems; 2) accumulated functional, gene-expression and promoter/enhancer occupation data on transcription factors involved in T cell differentiation and clonal expansion, and 3) temporal gene-expression profile of TCR transgenic T cells responding to antigen *in vivo*. We provide additional credence to these regulatory mechanisms by highlighting theoretical and quantitative analyses that demonstrate their contribution to a scaled response.

We can draw on design principles learned from the study of receptor tyrosine kinase signaling that links signal integration to cell division in multiple cell types. Messenger RNA of immediate early genes (IEGs), such as Fos, Jun and Myc, are transcribed in the first wave of the response to growth factors like platelet derived growth factor. These have high content of AU-rich elements that target them to degradation. Thus, messages of IEGs do not accumulate appreciably. Accumulation of protein products of IEGs and cyclinD is contingent on prolonged Erk and Akt signaling downstream of growth factor signaling to prevent their degradation and initiate entry in to S-phase [56, 57]. This constitutes a “type I coherent feed-forward interaction with AND

logic”, which acts as a ‘persistence detector’ and decodes the duration of signaling [58]. IEG and cyclinD accumulation during the priming stage of T cell activation should also be mediated by the same feed-forward interaction through prolonged signaling (Figure 2a). AP4, a transcription factor induced by Myc, has also been shown to be dependent on continuous TCR and IL2R signaling to prevent its degradation and enable continuation of metabolic and biosynthetic reprogramming [59].

Acceleration in the secretion of IL2 has been shown to contribute to scaling of IL2 accumulation according to antigen dose [7]. The authors used the phenomenology of an intermediary positive feedback to model acceleration in the production of IL2. We propose that this positive feedback manifests due to the generic action of mTor and Myc. Myc leads to transcriptional amplification of all active promoters in B cells [60]. mTor also increases the activity of all three classes of RNA polymerases. Further, both Myc and mTor are master regulators of ribosome biogenesis. mTor is expected to be continuously active during T cell priming due to continuous PI3K signaling. Additional gradual increase in mTor activity is expected due to the accumulation of the transporters responsible for uptake of amino acids from the extracellular milieu. With Myc also accumulating due to continuous signaling (Figure 2a), there is a global non-linear increase in the transcriptional and translational output [60, 61]. We posit that this phenomenon during the priming stage of T cell activation enables scaling of every aspect of the response and not just that of IL2.

Detailed examination of targets of master regulators involved in clonal expansion revealed to us several instances of two master regulators acting synergistically, with one of them also inducing the other master regulator (Figure 2b) (Supplementary Note 2). This type of interaction is called a “Type I coherent feed-forward with SUM logic” [62]. Such a regulatory interaction imparts two functional features: 1) accumulation of the end-product can accelerate over time and reach levels not possible due to a single transcription factor due to the SUM logic. 2) Transcription of the end-product can continue well after the initiating transcription factor activity has ceased [62]. The first feature contributes to scaling of the response. The second feature provides an explanation for how clonal expansion and differentiation can proceed well after the TCR signaling has ceased [3, 10, 59]. It is worth noting that the gleaned examples also include cases of cascading feed-forwards, which further pronounce the above described functional features.

Inflammatory and stress response systems are characterized by digital signaling leading to graded transcriptional response [63-65]. These systems contain regulatory features that enable ‘dose-to-duration’ encoding [66]. If dose scales the duration of digital signaling, then temporal integration during transcription/translation is theoretically sufficient for scaling the response. Thus, ‘dose-to-duration’ encoding is arguably the most important feature of digital signaling systems. There is emerging evidence that T cells contain an analogous encoding mechanism to translate antigenic dose and strength to duration of signaling. Higher antigen dose and strength increase the net T-DC contact duration *in vivo* [67-69], which will automatically increase the duration of signaling and thus enable scaling by temporal integration. However, it is not known how T cells achieve this. We propose a mechanism for ‘dose/strength-do-duration’ encoding in the next section.

Positive and negative feedbacks for scaling the response

As part of the efforts of the Immgen Consortium, we have previously reported gene expression profiling of OT1 TCR transgenic T cells undergoing priming *in vivo* due to infection with ovalbumin expressing *Listeria* [12]. Functional classification of the transcripts with decreased expression during the first 48 hours revealed system-wide reduction in the transcripts for the motility apparatus and the TCR signaling machinery (Supplementary Figure S1-S4). Based on these system-wide changes we propose transcriptional positive and negative feedbacks and explain how they can contribute towards scaling the response.

Balance between the opposing ‘stop’ and ‘go’ signals determine the duration of contact with the antigen-presenting dendritic cells [70]. The current view based on collective evidence is that receptor proximal signalling constitutes the ‘stop’ signal in T cells. Continuous TCR triggering is known to reduce the receptor proximal signalling output due to activation induced degradation of tyrosine phosphorylated signalling proteins [71]. This is expected to gradually reduce the ‘stop’ signal until a lower steady state signaling is approached after ~6 hours. Unless there is a reduction in the ‘go’ signals as well, we anticipate that T cells will not be able to maintain and prolong the interaction with the engaged dendritic cells after the first ~6 hours. In fact, Jens Stein’s lab has recently documented a T-cell intrinsic suppression of motility behavior in the first 2-3 days after antigen encounter in CD4 T cells [72]. We have found that the entire motility

1 apparatus, encompassing chemokine receptors, integrins, modulators and effectors of Rho family
2 GTPases, adhesion components and actin binding proteins, had ~50% decrease in message
3 levels during the priming stage (Supplementary Figure S1 and S2). We propose that system-wide
4 suppression of both amoeboid and mesenchymal motility modes disables both the cell-intrinsic
5 and extrinsic 'go'-signals and enables prolonged TCR signalling as a result of extended contact
6 with engaged DCs. Since initial TCR signaling transcriptionally suppresses the motility
7 apparatus, a slow positive-feedback is formed to increase the duration of TCR signaling (Figure
8 3a, Key Figure). If the extent of suppression in the motility apparatus is proportional with TCR
9 signaling strength, the observed phenomenon can cause the response to a stronger signal to
10 widen further from a weaker signal. It is well-established, through studies of TNF α response and
11 efforts to build analog synthetic circuits, that transcriptional positive feedback can extend the
12 dynamic range of the response [73, 74]. Thus, we offer a mechanistic explanation for how
13 'dose/strength-to-duration' encoding occurs to scale the T cell response.

14
15 We have also observed ~50% reduction in the messages of nearly all TCR proximal signaling
16 proteins as well as that of proteins constituting every major pathway of signaling downstream of
17 TCR, including calcium, MAPK, NF- κ B, Akt and mTor (Supplementary Figure S3 and S4).
18 Over prolonged or continuous TCR signaling beyond ~6 hours, this phenomenon is likely to
19 operate as a negative feedback, wherein the TCR signaling output is gradually reduced by a
20 sizeable fraction (Figure 3b). A well-characterized transcriptional negative feedback is that of
21 Erk induced production of its phosphatases, which was also the first case of negative feedback to
22 have been demonstrated to linearize the response [75]. This specific negative feedback is also
23 expected to be operational during T cell priming. Multiple other natural and synthetic signal
24 response systems are benefited by transcriptional negative feedbacks in increasing the dynamic
25 range by linearizing the response (Supplementary Note 3). We anticipate that the signal-
26 dependent reduction in the TCR signaling output due to system-wide decrease in the expression
27 of pathway components also contributes to linearizing and scaling the T cell response. How is
28 the negative feedback on the TCR signaling apparatus enforced? We propose two parallel
29 mechanisms (Figure 3b). It is known that several miRNAs are induced during the T cell priming
30 stage [76]. Interestingly, signaling proteins were highly enriched among the predicted targets of

the induced miRNAs. Thus, we propose that miRNAs expressed in response to TCR signaling target the entire TCR signaling apparatus. A second set of evidence supports this hypothesis. Dicer-deficient CD8 T cells, which don't have mature miRNAs, exhibit faster initial response and also produce more effector molecules [77, 78]. More importantly, Dicer-deficient CD4 T cells secrete IL2 without co-stimulatory signals and thus cannot distinguish between anergic and immunogenic signals [79]. In other words, absence of critical miRNAs reduces the dynamic range of the T cell response, which as per our hypothesis is due to the absence of the proposed negative feedback in Dicer-deficient T cells. A second mechanism may also contribute towards reducing the expression of TCR signaling proteins. Bcl11b and Tcf1 are important drivers of the T-cell lineage program and contribute to the expression of proteins that participate in TCR signaling [80]. We have found that priming of OT1 T cells leads to reduction in the levels of Bcl11b and Tcf1, which is expected to reduce the expression of TCR signaling proteins.

Concluding remarks

Scaling of the T cell response as per the antigenic dose and strength is crucial for an optimal immune response to resolve the infection with minimal damage to the host tissues. In this article we have attempted to build a unifying conceptual framework to explain scaling in T cell response. Scaled response is made possible by a 'dose/strength-to-duration' encoding, wherein the duration of digital TCR signaling is scaled according to the antigenic dose and strength. Scaled accumulation of critical transcriptional intermediates as per the duration of digital TCR signaling, then programs the T cell response. Numerous feedforward interactions in the transcriptional program allow for decoding the duration of signaling, and for increasing and extending the response well beyond the cessation of signaling. The proposed feedback regulatory interactions in the transcriptional program improve the linearity of the response and extend the dynamic range of the T cell response. The shared nature of cytokine production and consumption is expected to reduce cellular heterogeneity to enable a coherent, collective T cell response.

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Figure legends:

Figure 1: Regulatory interactions between TCR and IL2R signaling for a collective, coherent, scaled response. a) TCR signaling leads to IL2 and IL2R transcription. IL2 accumulation occurs due to suppression of Stat5 phosphorylation by TCR signaling and because of a putative positive feedback. Beyond ~48 hours, IL2R signaling leads to further IL2R transcription, but suppression

1 of IL2 transcription. Further, IL2 is degraded after internalization. b) Profiles of IL2 and IL2R as
2 dictated by the regulatory interactions. c) PI3K, Notch, and Myc respond to both TCR and IL2R
3 signaling, acting as nodes of integration for a coherent growth program in responding cells. Note
4 that the effect of mTOR on Myc and cell growth and cell-cycle machinery is not just by
5 transcriptional means.

6
7 **Figure 2:** Coherent feed forward interactions promote a scaled T cells response. a) Erk and Akt
8 signaling downstream of TCR and IL2R signaling ensure accumulation of the protein products
9 by preventing their degradation for the duration of signaling. This feed-forward interaction with
10 AND logic is highly sensitive to loss of upstream signaling, leading to sudden drop in the output.
11 Accumulation of the secondary products, cyclin D and AP4, is even more sensitive to the
12 duration of signaling due to the cascading induction. b) Individual examples of feed-forward
13 interactions with SUM logic in the transcriptional programs during T cell activation. These were
14 inferred based on the collective knowledge on these transcription factors, especially in the
15 context of T cell activation (Supplementary Note 2). These increase the dynamic range of the
16 response due to continuous increase in the rate of production of the end-product and also extend
17 the response well after the cessation of TCR signaling.

18
19 **Figure 3:** Proposed positive and negative feedbacks in scaling the T cell response. a) Contact
20 duration with DCs is known to be influenced by the dose and strength of antigen presented by
21 the DCs. The initial arrest is due to the TCR mediated ‘stop’ signal, represented as the ‘fast’
22 double negative feedback. However, over time the TCR signal diminishes and the stop signal is
23 reinforced by a slow transcriptional feedback that reduces the expression of the entire motility
24 apparatus as per the signaling output. This transcriptional positive feedback increases the
25 duration of signaling and enables scaling of the response. b) TCR signaling also leads to
26 reduction in the messages of the signaling machinery. This negative feedback is enforced via
27 microRNAs and lineage determining factors Bcl11b and Tcf1. Transcriptional negative feedback
28 is known to linearize the response.