

An updated view on transcription factor GATA3-mediated regulation of T_h1 and T_h2 cell differentiation

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Abstract

CD4 T_h are critical for orchestrating adaptive immune responses. The expression of the transcription factor GATA3 (GATA-binding protein 3) is up-regulated or down-regulated during T_h2 or T_h1 cell differentiation, respectively. Furthermore, GATA3 is responsible for induction of T_h2 differentiation and represses T_h1 differentiation. In this review, we present an updated view on the molecular mechanisms through which GATA3 regulates T_h1/T_h2 differentiation. During T_h2 cell differentiation, GATA3 directly binds to the T_h2 cytokine gene locus at several regions and regulates expression. On the other hand, GATA3 inhibits T_h1 cell differentiation by preventing up-regulation of IL-12 receptor β 2 and STAT4 (signal transducer and activator of transcription 4) and neutralization of Runx3 (runt-related transcription factor 3) function through protein–protein interaction. GATA3 may also directly act on the *Irfng* gene. In summary, GATA3 serves as a transcriptional activator or repressor through direct action on transcriptional machinery and/or affecting chromatin remodeling at many critical loci encoding cytokines, cytokine receptors, signaling molecules as well as transcription factors that are involved in the regulation of T_h1 and T_h2 differentiation.

Keywords: cytokine, epigenetic modification, gene regulation

Introduction

Naive CD4 T cells have the potential to differentiate into several alternative cell types of which the most intensively studied are T_h1 and T_h2 cells (1). When innate immune cells recognize invasion of intracellular pathogens such as protozoa, bacteria or viruses, naive CD4 T cells differentiate into T_h1 cells, which secrete IFN- γ , IL-2 and tumor necrosis factor- β . T_h1 cells activate macrophages and CD8 T cells leading, under the right set of circumstances, to pathogen eradication.

On the other hand, when innate immune cells recognize extracellular parasites such as helminths, the naive CD4 T cells differentiate into T_h2 cells, which secrete IL-4, IL-5 and IL-13. T_h2 cells activate B cells to induce immunoglobulin class switching and epithelial cells to enhance their mucus production; T_h2 cells also recruit mast cells and eosinophils to infection sites to aid in the clearance of parasites. Inappropriate activation of T_h1 or T_h2 cells to self-antigens or to harmless foreign antigens may cause autoimmune or allergic diseases. Thus, understanding the molecular mechanisms underlying the differentiation of naive CD4 T cells into T_h1 or T_h2 cells is of considerable importance.

T_h1/T_h2 differentiation is regulated by the cytokine milieu at the time of TCR engagement. This milieu is created under the influence of the particular pathogens as well as the dose

of antigens and the genetic background of the host (2, 3). When naive CD4 T cells are activated through their TCR together with IL-12- or IL-4-mediated signaling, these cells differentiate into T_h1 or T_h2 cells, respectively. IL-12-stimulated CD4 T cells up-regulate the expression of the transcription factor T-bet (T-box expressed in T cells) and acquire their capability to produce IFN- γ ; activated CD4 T cells that receive IL-4 signaling up-regulate GATA3 (GATA-binding protein 3) and become capable of producing T_h2 cytokines. Indeed, cytokine-mediated up-regulation of these lineage-specific transcription factors determines CD4 T-cell fate.

Not only is GATA3 indispensable for T_h2 differentiation and T_h2 cytokine production, it is also essential for inhibition of T_h1 differentiation and IFN- γ production. Here, we discuss the role of GATA3 in regulating T_h1 and T_h2 differentiation and the molecular mechanisms involved.

Functions of GATA3 in regulating T_h2 cell differentiation

GATA3 expression is necessary for the development of CD4 single-positive (SP) cells in the thymus (4, 5). It continues to be expressed in naive CD4 T cells at a basal level. When naive CD4 T cells are activated under T_h1- or T_h2-skewing

conditions, GATA3 is either down-regulated or up-regulated. GATA3 up-regulation is induced by IL-4-STAT6-mediated signaling (6, 7). A low dose but not high dose of antigen stimulation through TCR results in IL-4-independent GATA3 up-regulation and IL-4 production (8). Enforced expression of GATA3 has also been reported to up-regulate endogenous GATA3 expression (9). GATA3 is regarded as the master regulator to induce T_H2 differentiation (10, 11) since enforced GATA3 expression induces T_H2 differentiation even when the cells are cultured under T_H1 -skewing conditions (12) and GATA3-deficient ' T_H2 ' cells fail to produce IL-4, IL-5 and IL-13 (13, 14).

It has been reported that histone modifications, such as histone H3 lysine 4 (H3K4) methylation and H3K14 acetylation, are induced at the T_H2 cytokine gene locus (which includes the genes for IL-4, IL-13 and IL-5) during T_H2 differentiation (15). These histone modifications change the chromatin structure so that the modified regions become accessible to transcription factors and are associated with DNase I hypersensitive (HS) sites. Chromatin remodeling at the T_H2 cytokine gene locus is necessary for efficient

expression of IL-4, IL-5 and IL-13 in T_H2 cells and it has been proposed that GATA3 regulates chromatin remodeling.

GATA3 has been reported to bind to several regulatory elements at the T_H2 cytokine gene locus (Fig. 1) including conserved non-coding sequence (CNS)-1, HSVa, the conserved GATA response element (CGRE), the *Ii5* promoter and HSII in intron 2 of the *Ii4* gene (16–20).

CNS-1, which includes two HS sites, HSS1 and HSS2, is located at the intergenic region of the *Ii4* and *Ii13* genes (21). A mobility shift assay showed that GATA3 binds to HSS2 (16, 17). In mice from which the 0.5-kbp genomic DNA segment containing the CNS-1 region was deleted, isolated CD4 T cells that were cultured under T_H2 -skewing conditions had diminished numbers of IL-4-producing cells and the mean fluorescence intensity of the IL-4 producers was lower (22). These mutant mice also produced less IL-4 *in vivo* (22). Interestingly, bone marrow-derived mast cells from these CNS-1^{-/-} mice produced normal levels of IL-4, consistent with the observation that in mast cells, HS sites were not found in CNS-1 (23). Although the CNS-1 region

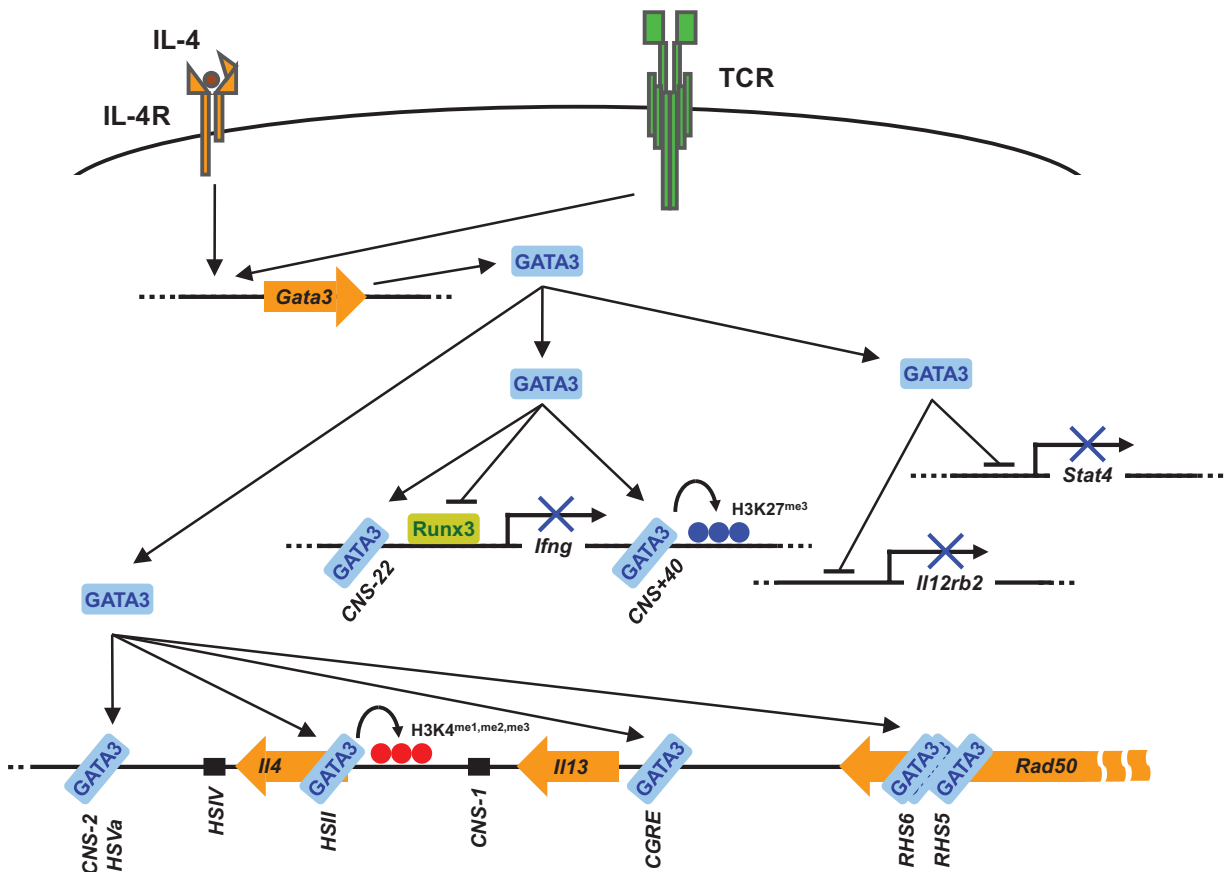


Fig. 1. Molecular mechanisms of GATA3-mediated regulation of T_H1 and T_H2 cell differentiation. GATA3 expression can be induced by TCR and/or IL-4-mediated signaling. GATA3 induces T_H2 cytokine expression through its direct binding to the *Ii4/Ii13* gene locus at several sites including HSII, HSVa, CGRE as well as RHS5 and RHS6 in the LCR. GATA3 can remodel the chromatin structure of this gene locus such as induction of H3K4 methylation; GATA3 may also directly act on the *Ii13* gene to induce transcription. GATA3 inhibits T_H1 cell differentiation by at least three distinct mechanisms: inhibition of IL-12R β 2 and STAT4 expression, suppression of Runx3-mediated IFN- γ production and silencing the *Ifng* gene by adding H3K27^{me3} suppressive marks. Our GATA3 ChIPseq data suggest that GATA3 directly binds to *Stat4*, *Ii12rb2* and *Ifng* loci (24).

appears to be critical for IL-4 production in T_H2 cells, our genome-wide GATA3 ChIPseq (chromatin immunoprecipitation followed by high-throughput sequencing) data did not reveal significant GATA3 binding to CNS-1 (24), suggesting that this region recruits other critical transcription factors that promote IL-4 production in T_H2 cells.

HSV α is located 5-kbp downstream of the 3' end of the *Il4* coding region; its DNase I HS is induced in T_H2 cells upon re-stimulation (18). Both GATA3 and NFAT1 (nuclear factor of activated T cells 1) bind to HSV α in T_H2 but not T_H1 clones (18). ' T_H2 ' cells generated from mice in which a 3.7-kbp region including HSV α and HSV (CNS-2) was deleted had reduced IL-4 production (25). Our GATA3 ChIPseq data confirmed GATA3 binding to HSV α (24), implying that HSV α is an important regulatory element through which GATA3 induces IL-4 production in stimulated T_H2 cells.

CGRE (a 71-bp sequence) is located 1.6-kbp upstream of the *Il13* gene and contains four putative GATA-binding sequences conserved across species (19). GATA3 binding to CGRE is confirmed by our GATA3 ChIPseq analysis. Since CGRE corresponds to the 5' edge of the region of histone hyperacetylation in the T_H2 cytokine gene locus and to the site to which RNA polymerase II and CBP/p300, containing histone acetyltransferase activity, bind (19), it may play an important role in *Il13* transcription and in chromatin remodeling at the *Il13* locus. Actually T_H2 cells generated from CGRE-deleted mice have diminished IL-13 but not IL-4 and IL-5 production (20).

Our GATA3 ChIPseq data revealed a strong binding of GATA3 to the HSII site located at intron 2 of the *Il4* gene (24). Previously, we have reported that activated STAT5 binds to HSII and that STAT5 is important for the maintenance of DNA accessibility at this region in T_H2 cells (26, 27). Most recently, Kubo *et al.* have reported that deletion of a 1.4-kbp genomic DNA region including HSII resulted in a diminution of IL-4 but not IL-13 production (20). This implies that the HSII site is a critical element for regulating IL-4 but not IL-13 production. Strong H3K4 trimethylation (H3K4me3) was observed at HSII in T_H2 but not T_H1 cells (28), suggesting that GATA3 and STAT5 may collaborate to remodel the chromatin at this region. Indeed, our unpublished data showed that H3K4me1, me2 and me3 at HSII site is reduced in *Gata3*-deleted cells cultured under T_H2 -skewing conditions.

GATA3 also strongly binds to three sites in the locus control region (LCR) of the T_H2 cytokine locus within the *Rad50* gene: one at Rad50 HS5 (RHS5; RAD50-O) and two at RHS6 (RAD50-A and RAD50-B) (24). This LCR plays an important role in regulating the expression of T_H2 cytokines (29–31). Whereas deletion of RHS7 caused only a partial reduction of IL-4 and IL-13 but not IL-5 expression (32), T-cell-specific additional deletion of RHS4 and RHS5 abolished the expression of all three cytokines and resulted in an altered pattern of chromatin modification at the T_H2 cytokine locus (33). Although it has been shown by conventional ChIP that GATA3 binds to RHS7 (34), our genome-wide analysis only showed a weak binding. Binding of GATA3 to the LCR region may be critical for DNA looping and thus T_H2 cytokine transcription.

Besides regulating chromatin remodeling, GATA3 may induce *Il5* and *Il13* transcription by direct binding to the promoters of these cytokine genes (10,35–37). Whereas GATA3 deletion during T_H2 differentiation abolished the expression of all T_H2 cytokines, GATA3 deletion in established T_H2 cells resulted in only a modest reduction in IL-4 production but had a major impact on the expression of both IL-5 and IL-13 (14). This result suggests that GATA3 binding to the *Il5* and *Il13* promoters may directly regulate the transcription of these cytokine mRNAs.

Given the heritable nature of chromatin modifications, once an active chromatin status of T_H2 cytokine genes has been achieved, GATA3 may not be necessary to maintain such an active structure (38) or the epigenetic marks might create a buffer period during which GATA3 activity is temporarily dispensable (39). For the expression of IL-5 or IL-13, however, GATA3 is always required presumably because it is a critical component of transcriptional machinery. Therefore, GATA3 may have at least two functions in regulating T_H2 cytokine gene expression: direct activation of transcription and chromatin remodeling.

Although direct binding of GATA3 to the T_H2 cytokine gene locus appears to be a major mechanism through which GATA3 induces T_H2 differentiation, GATA3 is also critical for regulating other T_H2 -related genes, some of which may also be involved in the expression of T_H2 cytokines. For example, GATA3 induces expression of another transcription factor, Dec2, which in turn induces JunB, which acts on the *Il4* promoter (40). Our unpublished data also suggest that GATA3 regulates the expression of many novel T_H2 -specific transcription factors whose functions need to be further studied. Furthermore, GATA3 expression together with STAT5 activation is critical for the expression of T_H2 -specific cytokine receptors including T1/ST2 (41), whose ligand, IL-33, plays a critical role in the initiation and amplification of T_H2 responses (42).

Thus, the functions of GATA3 in promoting T_H2 differentiation go far beyond its regulation of T_H2 cytokines; it is likely that a sophisticated GATA3-containing transcriptional network involving positive and negative feedback as well as feed-forward loops is responsible for full T_H2 cell differentiation.

Mechanisms through which GATA3 suppresses T_H1 cell differentiation

GATA3 not only promotes T_H2 cell differentiation but also inhibits T_H1 cell differentiation (12,43–45). Ectopic GATA3 expression in developing T_H1 cells inhibits IFN- γ production while inducing IL-4. Although IL-4 is known to inhibit IFN- γ production, inhibition of IFN- γ production by GATA3 is not dependent on IL-4 since such inhibition was still observed in the absence of IL-4, established either by the addition of anti-IL-4 during the priming culture or using *Il4*^{-/-} CD4 T cells (12, 43). How does GATA3 inhibit IFN- γ production? So far, several different mechanisms have been reported (12, 44, 45).

GATA3 suppresses IL-12–STAT4 signaling, which is a well-known pathway for T_H1 cell differentiation (46, 47). IL-12R β 2 is undetectable in naive CD4 T cells; its expression is

induced by T-cell activation and further up-regulated by IL-12-STAT4 signaling (48). When CD4 T cells receive IL-12 signals during their activation, they differentiate into T_H1 cells. Ectopic GATA3 expression in these developing cells inhibits expression of IL-12R β 2 mRNA (12).

In such GATA3-expressing cells, the reduced IFN- γ production (which is linked to a failure of T-bet up-regulation) could be explained by a failure to respond to IL-12 because of the lack of IL-12R β 2 expression (12). However, enforced GATA3 expression inhibited IFN- γ production even in developing T_H1 cells in which IL-12R β 2 expression was restored by CD2 promoter-driven expression. This result indicates that GATA3-mediated IFN- γ inhibition cannot be simply explained by suppression of IL-12R β 2 expression (44). Indeed, in addition to suppressing IL-12R β 2 expression, GATA3 also represses the expression of STAT4, which is normally highly expressed in T_H1 cells compared with T_H2 cells (44). Since STAT4 is a major downstream molecule in the IL-12 signaling pathway and plays a critical role in inducing T_H1 cells, suppression of STAT4 expression by enforced GATA3 expression explains the observation that enforced IL-12R β 2 expression fails to restore T_H1 differentiation suppressed by GATA3.

Therefore, IFN- γ production suppressed by enforced GATA3 expression under T_H1 -skewing conditions is rescued only when both STAT4 and IL-12R β 2 expression are restored. Indeed, GATA3 deletion during T_H2 differentiation results in up-regulation of both STAT4 and IL-12R β 2, although up-regulation of *I12rb2* mRNA is only partial suggesting that T_H1 -related factors are necessary for optimal IL-12R β 2 expression (45).

Another mechanism through which GATA3 inhibits T_H1 cell differentiation is by blocking Runx3-mediated IFN- γ production. Deletion of GATA3 in CD4 T cells during T_H2 cell differentiation not only decreased IL-4 production but also induced IFN- γ production, despite the culture of these cells under T_H2 -skewing conditions, which include anti-IFN- γ and anti-IL-12 (14). This result suggests that GATA3 expression in developing T_H2 cells actively suppresses IFN- γ production that is independent of IL-12 and IFN- γ —two cytokines known to be critical for inducing T_H1 differentiation.

We have recently verified the finding that IFN- γ production can be IL-12 and IFN- γ independent by using GATA3-deficient naive CD4 T cells (45). As mentioned earlier, GATA3 is necessary for the development of CD4 SP cells in the thymus (4, 5) and GATA3 is required for the induction of ThPOK, which is a critical factor for CD4 T cell lineage commitment (49). To avoid defective CD4 T cell development in the thymus, a conditional GATA3 knockout mouse strain, *Gata3^{fl/fl}* dLck-Cre, was established by breeding *Gata3^{fl/fl}* mice to mice carrying transgenic Cre, whose expression is controlled by a distal Lck promoter, which allows GATA3 expression during CD4 T cell development but then deletes it (50). As expected, both thymic and peripheral T cells in these mice display normal phenotypes as judged by cell number and the expression of multiple cell surface markers (45).

Naive *Gata3^{fl/fl}* dLck-Cre CD4 T cells cultured under T_H2 -skewing conditions *in vitro* produce IFN- γ . Similarly, instead of inducing T_H2 responses in normal mice, parasite infec-

tion of these conditional knockout mice elicited a T_H1 -like response as indicated by IFN- γ production. IFN- γ production by ' T_H2 ' cells with a double knockout of GATA3 plus T-bet was comparable to that of ' T_H2 ' cells with a single knockout of GATA3, indicating that IL-12- and IFN- γ -independent IFN- γ production in GATA3-deficient ' T_H2 ' cells is independent of T-bet, the master regulator for inducing T_H1 cell differentiation (51, 52).

Runx3 has been reported to regulate IFN- γ production in both CD4 and CD8 T cells (53, 54). Interestingly, IFN- γ production in GATA3-deficient ' T_H2 ' cells was diminished by additional disruption of *Runx3*, suggesting that Runx3 is responsible for IFN- γ - and IL-12-independent IFN- γ production.

Runx3 has been reported to bind to the *Irfng* promoter (55). Our ChIPseq analysis showed that, in addition to binding to the *Irfng* promoter, Runx complexes are bound to multiple sites across the *Irfng* gene locus, all of which are either located at HS sites or CNS regions, with some of the binding sites overlapping with T-bet binding (45, 56). These results imply that Runx3 can induce IFN- γ either by itself or by collaborating with T-bet family members, including T-bet and Eomes, to induce full levels of IFN- γ production. Further experiments suggest that the relative amounts of GATA3 and Runx3 determine the production of IFN- γ . Since GATA3 binds to Runx3 (45), GATA3 inhibition of Runx3-mediated IFN- γ production may result from the interaction of these two transcription factors during T_H2 cell differentiation.

Thus, published data summarized above suggest that GATA3 can prevent T_H1 differentiation by blocking IL-12R β 2 and STAT4 up-regulation and by neutralizing the capacity of Runx3 to induce IFN- γ production. Our GATA3 ChIPseq data indicate that GATA3 can also directly bind to the *Irfng* gene, including CNS +40, a site ~40 kbp downstream of the *Irfng* transcription start site and that it affects histone modification patterns near this binding site (24). Similarly, GATA3 may play a role in silencing T-bet expression through chromatin remodeling after its direct binding to the *Tbx21* locus. Therefore, GATA3 suppression of T_H1 cell differentiation may occur by directly blocking transcription of T_H1 -specific genes, such as *I12rb2*, *Stat4*, *Tbx21* and *Irfng* (Fig. 1) (24), and/or by blocking the functions of T_H1 -related proteins, including Runx3 and possibly T-bet as well (57).

Conclusions

When naive CD4 T cells are activated through their TCR and IL-4 receptors, they acquire the capacity to efficiently produce T_H2 cytokines as a result of GATA3 up-regulation. GATA3 binds to several regulatory elements in the T_H2 cytokine loci and is involved in the chromatin remodeling and/or direct transcriptional activation. GATA3 not only promotes T_H2 differentiation but also inhibits T_H1 differentiation. GATA3 prevents T_H1 differentiation by blocking the IL-12 signaling pathway, neutralizing the function of Runx3 and directly silencing the *Irfng* gene.

The important regulatory elements to which GATA3 binds, such as CGRE, HSII, H5Va and LCR, can also recruit other transcription factors including STAT5 and NFAT. Therefore, it is likely that GATA3 regulates chromatin remodeling and

gene expression in concert with other transcription factors, co-activators and/or co-repressors. The importance of binding of GATA3 to specific sites at any given gene locus in regulating gene expression remains to be tested by inducing point mutations at the GATA3-binding sites rather than by deletion of a larger DNA fragment containing a GATA3-binding site.

In some cases, through protein-protein interactions, GATA3 can be indirectly recruited to a site where there is no GATA3-binding sequence. For example the, *Ifng* gene locus consists of many regulatory elements across ~140 kb DNA (1, 44, 55) and in T_{H2} cells, GATA3 binds to GATA sequences in distal regions of the *Ifng* gene, such as CNS -22 and CNS +40, but it does not bind to the *Ifng* promoter (24). GATA3 binding at the *Ifng* promoter detected in T_{H1} cells may result from its interaction with Runx3 and/or T-bet.

GATA3 expression varies in different cell types. Depending on the existence and/or abundance of co-factors or repressors, different amounts of GATA3 may be required for gene induction or suppression. For example, GATA3 is expressed at low levels in T_{H1} cells, but even when expressed at such low levels, it is able to cause cells to acquire IL-4-producing capacity in the presence of high-level STAT5 signaling. On the other hand, although GATA3 is highly expressed in CD4⁺CD8⁻ (double-negative) thymocytes and binds to the *Il13* gene in these cells (24), it fails to induce IL-13 expression. Therefore, identifying the co-factors of GATA3 in specific cell types becomes critical for understanding GATA3-mediated gene regulation. To complicate this even more, GATA3-regulated genes that are cell type specific may collaborate with GATA3 itself in regulating secondary GATA3 targets.

Thus, genome-wide assessment of GATA3-binding sites, and investigation of GATA3-interacting proteins as well as genes that are regulated by GATA3 in different cell types will be necessary to fully understand the biology of GATA3 and its detailed function in regulating T_{H} cell differentiation and T-cell development in general.

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