Published in final edited form as:
Science. 2012 November 16; 338(6109): 891–892. doi:10.1126/science.1231310.

Cooperative Transcription Factor Complexes in Control

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Abstract

The cooperative binding of transcription factors regulates the differentiation of T cells as well as the generation and function of B cells and dendritic cells.

The cornerstone of transcriptional regulation is the binding of transcription factors to regulatory elements in DNA. The fine-tuning of this process is almost universally achieved through the formation of "enhanceosomes," assemblies of DNA-binding proteins that are stabilized by an intricate network of protein-protein and DNA-protein contacts. The power of such cooperative interactions is now highlighted by four recent studies (1–4), including the report by Glasmacher *et al.* on page 975 in this issue. Together, these studies demonstrate that a cooperative complex formed by a monomer of the interferon regulatory factor (IRF) family of transcription factors and a heterodimer of the activator protein–1 (AP-1) Jun and B cell activating transcription factor (BATF) has a major role in lineage specification and function in cells of the immune system.

The elegance of the enhanceosome strategy lies in its flexibility. Because the affinities of individual protein-protein and protein-DNA interactions tend to be rather low, any given regulatory region in DNA can potentially assemble multiple factors whose recruitment is influenced by whether they can bind to nucleosomal DNA and recruit chromatin-remodeling complexes ("pioneering" transcription factors) or whether they prefer nucleosome-depleted DNA. The formation and stability of such a multiprotein-DNA complex depends on the concentrations of the DNA-binding proteins in the nucleus, their affinities for DNA sequences located within the regulatory region, and their ability to make cooperative interactions with other transcriptional regulators bound to adjacent DNA sequences (5).

In cells of the immune system—B cells, dendritic cells, and macrophages—IRF4 and IRF8 drive lineage-specific gene expression through cooperative interactions with the Ets transcription factor family on Ets-IRF composite elements (EICEs) (1, 6). These complexes are less likely to form in T cells, which express low amounts of Ets factors. IRF4 facilitates the differentiation of $CD4^+$ T helper (T_H) cells into distinct functional subsets: T_H17 cells that produce the proinflammatory cytokine interleukin-17 (IL-17); follicular helper T cells required for antibody production by B cells; and T_H2 cells that mediate immune responses to parasites (7–9). Because IRF4 binds to DNA with relatively weak affinity (6), its activity in $CD4^+$ T cells suggests a requirement for cooperative interactions with other transcription factors. The identification of the BATF-Jun heterodimer as a cooperative factor has now been established through chromatin immunoprecipitation followed by next-generation sequencing (ChIP-Seq) (1, 2, 4). Bioinformatic analyses of the ChIP-Seq data identified two DNA motifs, referred to as AP-1–IRF composite elements (AICEs) (see the figure).

To experimentally confirm the bioinformatic analyses, the studies show that binding of BATF to composite sites decreased in T cells lacking IRF4, and vice versa (1, 2, 4). Similarly, electrophoretic mobility shift assays (EMSAs) demonstrated the cooperative binding of BATF-Jun heterodimers with IRF4 (or IRF8) to composite elements. Thus, IRF4

and IRF8 are distinct from the other more widely expressed IRF family members, not only in their expression pattern in immune cells but also in their cooperative binding to AP-1 and Ets family members on AICE and EICE motifs, respectively (1, 3).

The transcription factor signal transducer and activator of transcription 3 (STAT3) may also be part of an extended IRF-BATF-Jun complex. STAT3 binds to Jun (10) and the BATF-Jun-IRF heterotrimer could potentially recruit activated STAT3 to relevant target genes. There was no enrichment for STAT3 binding sites in the immediate vicinity of AICE composite elements, suggesting that STAT3-Jun association could occur between proteins bound to spatially separated sites. Indeed, STAT3 and IRF4 both bind to the *Prdm1* promoter element (11), but at nonadjacent sites.

To understand the regulatory network driving T_H17 cell differentiation, Ciofani *et al.* (4) used a comprehensive systems biology approach in which they combined ChIPSeq for several transcription factors implicated in T_H17 differentiation with transcriptional profiling of cells expressing or lacking these factors. Among the factors tested, expression of BATF and IRF4 was induced by T cell receptor stimulation alone, whereas the expression/activation of transcription factors Maf, STAT3, and retinoic acid receptor–related orphan receptor gamma t (RORγt) was induced only if the T cell receptor was stimulated in the presence of the T_H17-polarizing cytokines transforming growth factor β(TGFβ) and IL-6. In nonpolarizing conditions, BATF and IRF4 bound to DNA cooperatively even in the absence of the other three T_H17 transcription factors. The authors propose that BATF and IRF4 function as pioneer transcription factors that nucleate the binding of lineage-specific transcription factors to DNA by promoting chromatin remodeling and nucleosome repositioning (4). In addition, expression of the AP-1 family member Fosl2 was induced in T_H17 cells and repressed IL-17 production (1, 4). T cells lacking Fosl2 showed increased IL-17 production and expression of Foxp3, the transcription factor essential for regulatory T cell function. Ciofani *et al.* demonstrate substantial overlap between BATF and Fosl2 binding sites in DNA; Glasmacher *et al.* found that the Fosl2-JunB heterodimer is unable to form a cooperative complex with IRF4 on AICE motifs. Together, these data suggest that BATF and Fosl2 might compete for DNA binding to AICE motifs, and that Fosl2 may antagonize BATF and repress IL-17 production by binding to the motifs but preventing IRF4 recruitment. Thus, the balance of BATF versus Fosl2 occupancy at AICE sequences would dictate the amount of IL-17 produced.

The in vivo importance of the BATF-IRF4 interaction was addressed in depth by Tussiwand *et al.* (3). The authors show in mice that BATF1, BATF2, and BATF3 display redundant functions, as might have been expected based on similar DNA target sequences. BATF-IRF4 complexes regulate dendritic cell development, B cell antibody class switching, and T_H17 cell differentiation in vitro and in vivo. In EMSA assays and reconstitution experiments in vitro, four residues in BATF were identified as crucial for BATF-Jun-IRF4 complex formation (1, 3).

The four independent studies reemphasize the physiological importance of cooperative interactions between unrelated transcription factors on DNA. The pairing of strong (or moderately strong) and weak binding sites in DNA is typical of composite elements. For example, the transcription factor nuclear factor of activated T cells (NFAT) binds to a relatively strong consensus site whereas the AP-1 site is typically nonconsensus. Nevertheless, the cooperative NFAT–AP-1 complex binds with high affinity to DNA (12, 13). The advantages for gene regulation and signal integration are obvious: The activity of cooperating transcription factors can be regulated at both transcriptional and posttranscriptional levels in response to signaling inputs, allowing enormous flexibility and control over gene expression. Future research will illuminate the structural basis for

cooperative BATF-Jun-IRF binding to the two classes of AICE composite sites, and define by affinity and kinetic measurements the precise increase in the stability of the cooperative complex over that of the individual AP-1 and IRF components alone.

Acknowledgments

We thank A. Gudlur for help with the figure. G.J.M. is supported by the Jane Coffin Childs Memorial Fund for Medical Research. This work was supported by U.S. National Institutes of Health grant R01 CA42471 (to A.R.).

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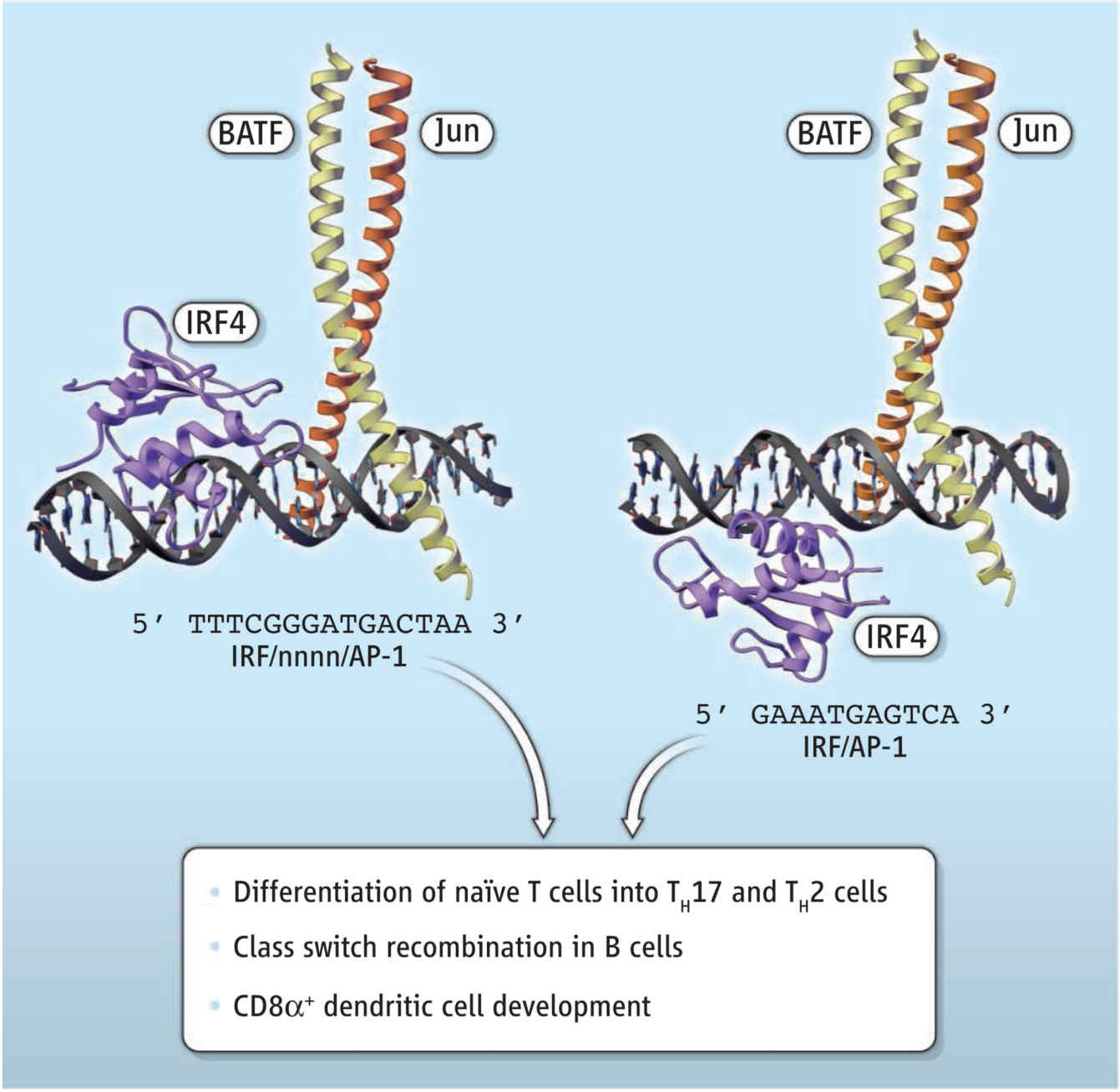

Figure. The structure of cooperation

A model of the BATF-Jun-IRF4 complex (1) is shown, based on the known structures of the Jun-ATF2-IRF3B complex in the interferon-β-enhanceosome (14) and the PU.1-IRF4 complex on the *IgL* λgene enhancer (15). The model was built using AICE motifs from *Ctla4* (4 bp spacing) and *Bcl11b* (0 bp spacing) loci. The sequences of the *Ctla4* (left), and *Bcl11b* (right) AICE elements are shown. The different outcomes of cooperation between IRF4 and BATF-Jun in the immune system are listed.