PLZF Outreach: A Finger in Interferon's Pie

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DOI 10.1016/j.immuni.2009.06.003

In this issue of *Immunity*, Xu et al. (2009) find that the transcription factor PLZF activates interferon-stimulated genes and facilitates natural killer cell functions. Interferon-induced PLZF phosphorylation and histone deacetylase 1 recruitment probably mediates the repressor-to-activator conversion.

PLZF (ZBTB16) belongs to a transcription factor family that carries the Pox virus and Zinc finger-Bric-a-brac Tramtrack Broad complex (POZ-BTB) domain and Kruppel type C_2H_2 zinc fingers in the N- and C-terminal regions, respectively. PLZF has been known as a transcriptional repressor and regulates many target genes through the promoter elements recognized by the zinc fingers. Like other family members, PLZF recruits nuclear receptor corepressors 1 and 2 (NCoR1 and NCoR2) and histone deacetylases (HDACs) to achieve repression. PLZF controls development of skeletal elements and spermatogenesis, and PLZF mutations in humans are associated with abnormalities related to these functions, such as skeletal defects, genital hypoplasia, and mental retardation. PLZF is expressed in hematopoietic stem cells and in peripheral lymphoid and myeloid cells, with probable roles in regulating their growth and differentiation. PLZF regulates growth control genes such as *MYC* and *CDC6*, partly by interacting with the retinoblastoma protein (McConnell et al., 2003). It also mediates lymphocyte apoptosis and is thought to take part in B cell chronic lymphocytic leukemia. PLZF, in addition, is involved in acute promyelocytic leukemia, where it is fused to retinoic acid receptor α (RARα). In this issue of *Immunity*, Xu et al. (2009) report that PLZF stimulates transcription of a subset of interferon (IFN)-stimulated genes (ISGs) and contributes to IFN's antiviral activity. Moreover, it commands natural killer cell function.

Xu et al. (2009) microarray analysis indicates that a relatively large fraction of ISGs (perhaps more than 100) are regulated by PLZF. These genes presumably contain PLZF binding sites, in addition to the IFN-stimulated response element in their promoters, through which PLZF and the ISGF3 complex (STAT1, STAT2, IRF9) cooperatively activate transcription. The *cis*-acting enhancement of transcription has been described for a number of ISGs that have binding sites for other transcription factors, including proteins of the NF-κB and Ets families. These *cis*-acting proteins create diversity and complexity to IFN responses (Hiscott et al., 2003). For example, NF-κB, IRF, and AP1 proteins are assembled on the IFN-β promoter upon stimulation to form a hypothetical structure called the "enhanceosome," leading to efficient transcription. Some ISGs targeted by PLZF, such as CXCL10, also carry an NF-κB site, suggesting an additional layer of diversity. Ets family proteins such as PU.1, expressed highly in macrophages and dendritic cells, also contribute to the combinatorial diversity and cell-type-dependent effects of IFNs. Xu et al. (2009) show that PLZF-regulated ISGs include those genes involved in antiviral defense, such as RSAD2, OAS1, and TRIM22, and accordingly PLZF-deficient mice are susceptible to infection by Semliki Forest virus and Encephalomyocarditis virus, despite the fact that these mice produced IFNs in normal amounts. Xu et al. (2009) made a notable discovery that NK cells in PLZF-deficient mice were not properly activated upon IFN stimulation and were deficient in tumor cell killing and granzyme B production, highlighting the requirement of PLZF in IFN-induced NK cell activation. Combined with two recent studies showing that PLZF regulates development of NKT cells, this work by Xu et al. (2009) firmly establishes the role for PLZF in shaping innate and adaptive immune responses (Kovalovsky et al., 2008; Savage et al., 2008).

NK cells express surface receptors that recognize virus-infected cells as well as tumor cells (Caligiuri, 2008). NK cells are activated in response to interferons and other cytokines such as IL-12 and IL-15 to release the pore-forming proteins granzyme B and perforin, which prompts target cell apoptosis. Through the potent cytotoxic activity, NK cells help to contain viral infection, an important aspect of innate immune responses. Accordingly, deficiency in NK cells is associated with susceptibility to herpes viruses and cytomegalovirus infection in human and mice. It may be anticipated that PLZF controls additional inducible activities of NK cells beyond those found in this study. Because NK cells are activated not only by IFN but also by other cytokines, and because PLZF activation seems to be induced by signals not solely dependent on IFNs (see below), PLZF may play a broader role in NK cell activation not limited to those linked to IFN signals.

This paper makes it amply clear that when stimulated by IFN, PLZF acts as a bona fide transcriptional activator, rather than a repressor as it was previously defined. The authors' mechanistic investigation suggests that phosphorylation may be a key to the repressor-to-activator switch: PLZF was phosphorylated within the BTB domain, likely through the c-Jun amino-terminal kinase (JNK) cascades, rather than the JAK and TYK kinases of the main IFN sinaling pathway. This phosphorylation was necessary for ISG induction. Previously, another domain of PLZF was shown to be phosphorylated by cyclin-dependent kinase CDK2, which lessened transcriptional repression, suggesting that phosphorylation can antagonize repression (Costoya et al., 2008). Additionally, Xu et al. (2009) found that IFN facilitates PLZF to bind to HDAC1, in a manner dependent on the phosphorylation. The recruitment of a HDAC by PLZF
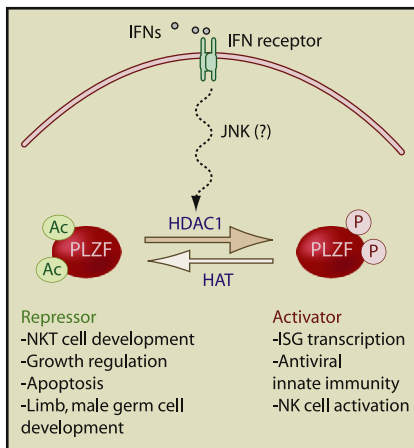
Figure 1. Dynamic Interchange of PLZF Function: A Model

IFN triggers PLZF phosphorylation, which converts PLZF from a transcriptional repressor to an activator, a possible switch dependent on its HDAC1-mediated deacetylation. The PLZF in this state regulates antiviral innate immunity and affects NK cell activity. Conversely, when unphosphorylated and acetylated by a nearby HAT, PLZF acts as a classic repressor and regulates development, cell growth, and apoptosis. The repressor activity of PLZF has been described before.

brings up an unsolved enigma of IFN-stimulated transcription, where ISG transcription depends, for the most part, on HDAC activity. A series of HDAC inhibitors are known to block ISG induction and some HDACs are found on the ISG promoters. The requirement of HDAC activity in IFN-stimulated transcription has been puzzling, because IFN stimulation causes recruitment of histone acetylases (HATs), increasing chromatin acetylation in the ISG genes. Does the PLZF-HDAC interaction explain the IFN enigma? Not quite, because ISGs not regulated by PLZF, such as IFIT1, nevertheless depend on

HDAC activity. There appear to be other mechanisms. A clue to the IFN enigma may partly lie in the recently described STAT1 acetylation (Kramer et al., 2009). It was shown that STAT1, when phosphorylated after IFN stimulation, was then acetylated by the CREB binding protein (CBP), one of many HATs. Acetylated STAT1 was then sequestered in the cytoplasm, unable to stimulate transription. The role of HDACs was to deacetylate STAT1, restoring the transcriptionally activate state. Thus, the HDAC requirement for IFN-stimulated transcription may not be at the level of chromatin, but may represent a dynamic acetylation-deacetylation switch that finely tunes the function of an activator important for ISG transcription. Dynamic, reciprocal action of HATs and HDACs has been proposed to explain the requirement of HDAC activity for other genes. Further supporting a dynamic acetylation-deacetylation exchange, some HDACs and HATs are shown to be in very close physical proximity (Yamagoe et al., 2003). The STAT1 acetylation regulated by the HAT-HDAC dynamics is intriguing, because PLZF is also acetylated by p300, a HAT closely related to CBP (Guidez et al., 2005). Furthermore, it has been shown that this acetylation is necessary for PLZF to act as a transcriptional repressor. Is there a parallel between STAT1 and PLZF in this regard? Possibly: the transcriptional status of PLZF might be dynamically exchanged by acetylation and phosphorylation, given that PLZF is phosphorylated upon IFN stimulation, analogous to STAT1 (Figure 1).

The study by Xu et al. (2009) demonstrates PLZF as a factor important for antiviral host defense and which commands

NK cell innate immunity. The dual behavior of PLZF as a transcriptional repressor and activator and the dynamic posttranslational modifications evoke many interesting thoughts and will no doubt open new avenues of research in years to come.

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