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# Review of PU.1 and IRF8 Synergy in Chromatin Regulation during Monocyte-to-Macrophage Differentiation

**Vica Hilda Amelia[1], Muhammad Hamza Mubarak[2], Ninda Devita[3]**

[1,2] 2nd Year Medical Students, Faculty of Medicine, Universitas Islam Indonesia

[3] Department of Microbiology, Faculty of Medicine, Universitas Islam Indonesia

ORCID[1]: https://orcid.org/0009-0005-6397-5352

ORCID[2]: https://orcid.org/0009-0004-5694-0111

ORCID[3]: https://orcid.org/0000-0002-4122-679X

**ABSTRACT**

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**Background:** The differentiation of monocytes into macrophages is a fundamental process in immune response, governed by various transcription factors, notably PU.1 and IRF8. While both transcription factors have well-established individual roles in chromatin remodeling and gene expression, the precise nature of their cooperative interactions during monocyte-to-macrophage differentiation remains incompletely understood.

**Methods:** A systematic review was conducted following PRISMA guidelines. Studies published between 2015 and 2025 were identified through PubMed, Google Scholar, and Scopus, using keywords such as "PU.1," "IRF8," "chromatin remodeling," and "macrophage differentiation." Studies focusing on experimental approaches like ChIP-sequencing, gene expression profiling, and histone modification were included. Data extraction was performed, and findings were synthesized to assess the roles of PU.1 and IRF8 in macrophage differentiation.

**Results:** This review identifies key findings on the synergistic interaction between PU.1 and IRF8, emphasizing their pivotal roles in modulating chromatin dynamics, enhancing macrophage-specific gene expression, and regulating immune responses. The studies reviewed highlight the impact of these transcription factors on chromatin accessibility and gene activation critical to macrophage differentiation.

**Conclusions:** PU.1 and IRF8 function together to regulate key aspects of macrophage differentiation, with potential therapeutic implications for diseases involving macrophage dysfunction, including chronic inflammation, autoimmune disorders, and cancer. The insights presented here contribute to a deeper understanding of macrophage biology and offer directions for future research aimed at exploiting these transcription factors in therapeutic interventions.

**KEYWORDS:**

Chromatin Regulation, Macrophage Differentiation, Transcription Factor, IRF8.

## INTRODUCTION

Monocyte-to-macrophage differentiation is a fundamental process in the immune system, where monocytes circulating immune cells differentiate into specialized macrophages that serve as key players in immune defense, tissue homeostasis, and repair. This differentiation is tightly regulated at the transcriptional level, with a variety of transcription factors modulating gene expression through chromatin remodeling. Among these factors, PU.1 (Purine-rich box 1) and IRF8 (Interferon Regulatory Factor 8) are two essential transcription factors that regulate immune cell differentiation, particularly in myeloid lineage cells such as macrophages (Liu et al., 2020; Tamura et al., 2014; Yamamoto et al., 2021).

PU.1 is a member of the ETS (E-twenty six) family of transcription factors, which is crucial for the development of hematopoietic cells, especially monocytes, dendritic cells, and macrophages. It is a key regulator of myeloid lineage

***Corresponding Author: Vica Hilda Amelia***

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commitment and has been shown to directly activate genes involved in macrophage differentiation, such as CD68 and TNF-α. PU.1 facilitates the binding of chromatin remodeling complexes to specific regions of the genome, ensuring the accessibility of target genes and their subsequent activation during differentiation (Wu et al., 2020).

IRF8, a member of the interferon regulatory factor family, plays an equally significant role in the differentiation of monocytes to macrophages. IRF8 modulates the transcriptional programs that control immune responses, particularly in the development and function of myeloid cells (McKenna et al., 2016). It is known to cooperate with other transcription factors, including PU.1, to regulate genes that govern macrophage activation, polarization, and immune response (Aliberti et al., 2016). IRF8's involvement in chromatin regulation primarily involves its ability to recruit co-activators and chromatin-modifying enzymes, altering histone modifications and chromatin structure to promote gene activation (Agarwal et al., 2015).

The interaction between PU.1 and IRF8, especially in the context of chromatin regulation during macrophage differentiation, remains an area of active research (Fukui et al., 2018). While studies have independently examined the roles of PU.1 and IRF8 in macrophage differentiation, limited attention has been given to the potential synergistic effects these two transcription factors have when acting together on chromatin. Their combined actions on chromatin remodeling, gene expression, and the epigenetic landscape during differentiation have significant implications for our understanding of macrophage biology and immune function (Agarwal et al., 2015).

**PU.1 and IRF8: Molecular Mechanisms**

PU.1 and IRF8 are both essential for the development of macrophages, as they control how genes are turned on and off by influencing the structure of chromatin (Agarwal et al., 2015). These two proteins work together to activate important genes specific to macrophages by making the chromatin more accessible and bringing in other proteins that help modify the chromatin. In this section, we will look at how PU.1 and IRF8 individually help in regulating the chromatin during the process of turning monocytes into macrophages, focusing on how they directly interact with the genome and cooperate with each other (Yashiro et al., 2021).

**PU.1: Transcriptional Regulation and Chromatin Remodeling**

PU.1 is like a key player in the development of certain immune cells, especially ones called monocytes and macrophages. It's necessary for blood stem cells to commit to becoming these specific types of immune cells (Rothenberg, Hosokawa, & Ungerbäck, 2019). PU.1 works by sticking to particular spots in the DNA, called PU-boxes, in the regions that control how genes are turned on. Once PU.1 attaches, it brings in other helpers that change the DNA structure, making it easier for genes to be turned on and do their job (Nguyen et al., 2016).

For example, PU.1 helps turn on the CD68 gene, a marker for macrophages, by making changes to a protein in the DNA called histone H3. This process opens the DNA up so other proteins can activate genes related to macrophages (Tagore, McAndrew, Gjidoda, & Floer, 2015). PU.1 also helps control the gene for TNF-α, a key player in inflammation, by modifying the DNA in ways that allow it to be turned on, using certain changes in histone proteins like H3K27ac and H3K4me3 (Kapellos & Iqbal, 2016).

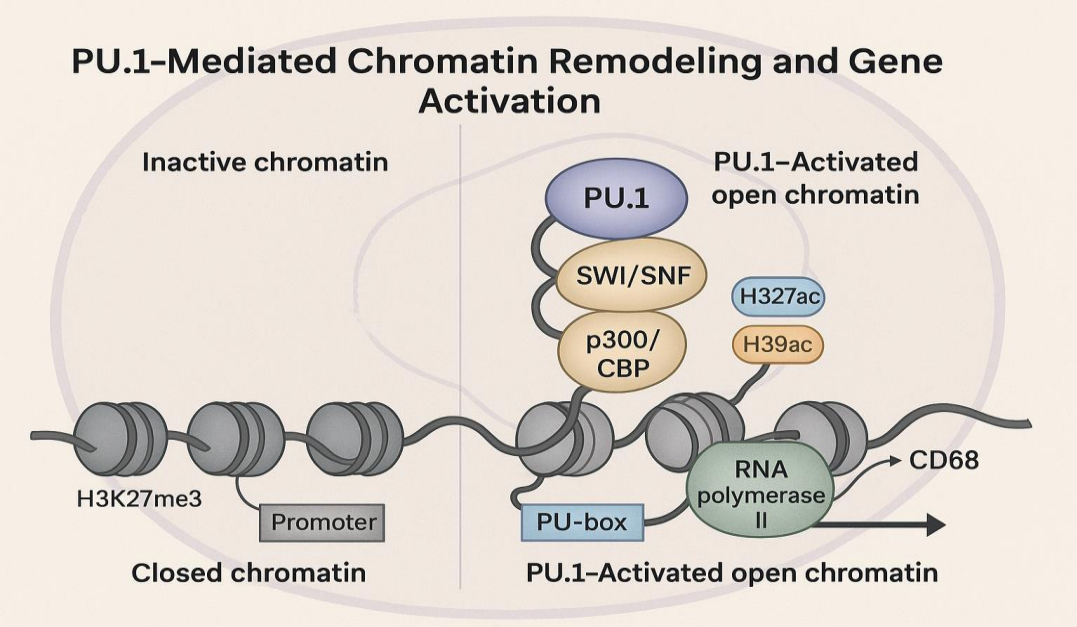


**Figure 01: PU.1-Mediated Chromatin Remodeling and Gene Activation**

As specific to **Figure 01**, It illustrates the process of PU.1-mediated chromatin remodeling and gene activation. It highlights the transition from inactive chromatin to a more accessible state for gene transcription. PU.1, a transcription factor, interacts with chromatin remodelers like SWI/SNF and co-activators such as p300/CBP. This activation leads to histone modifications like acetylation (H327ac, H39ac), opening the chromatin and enabling transcriptional machinery, including RNA polymerase II, to initiate gene expression. The specific focus is on the activation of the CD68 gene in this context.

Moreover, PU.1 also plays a role in coordinating macrophage gene expression by interacting with other transcription factors, such as NF-κB, that regulate inflammation and immune responses. This indicates that PU.1's role extends beyond just monocyte-to-macrophage differentiation, influencing macrophage function during immune activation (Schmidt et al., 2016).

**IRF8: Transcriptional Regulation and Chromatin Interaction**

IRF8, a member of the interferon regulatory factor family, is another key transcription factor in the regulation of myeloid cell differentiation (Brocker et al., 2021). Unlike PU.1, which mainly regulates gene activation through chromatin modifications, IRF8 often exerts its effects by interacting with other transcription factors and chromatin remodeling complexes. IRF8's primary role in macrophage differentiation is to control genes involved in immune responses, and it is especially important for the regulation of genes that control macrophage polarization and immune activation (Takahashi et al., 2019).

IRF8 typically binds to IRF-binding motifs in the promoters of genes related to macrophage function. Upon binding, IRF8 recruits co-activators, such as CBP/p300 and P300 histone acetyltransferase, to induce chromatin modifications that enhance gene expression (Antonczyk et al., 2019). One such gene that IRF8 regulates is IL-6, a cytokine involved in inflammation (Zhang et al., 2020). The recruitment of co-activators by IRF8 at the IL-6 promoter region leads to histone acetylation and increased chromatin accessibility, facilitating transcription (Takahashi et al., 2021).

Additionally, IRF8 can collaborate with PU.1 to regulate the expression of certain genes during macrophage differentiation. For instance, the C/EBPβ gene, which encodes a transcription factor involved in monocyte differentiation, is regulated by the synergistic action of both PU.1 and IRF8. This synergy allows for precise control over macrophage-specific gene expression and differentiation (Takahashi et al., 2019).

**PU.1 and IRF8 Synergy in Chromatin Regulation**

While PU.1 and IRF8 are individually important for macrophage differentiation, it is their cooperative action that exerts a more profound effect on chromatin regulation. Evidence suggests that PU.1 and IRF8 form heterodimers or protein complexes that bind to overlapping genomic regions, where they jointly recruit co-activators and chromatin modifiers. This synergy between PU.1 and IRF8 enhances the expression of macrophage-specific genes, ensuring the correct differentiation of monocytes into functional macrophages (Li et al., 2019).

For example, studies have shown that PU.1 and IRF8 cooperate to regulate the CD11b gene, which encodes a key macrophage adhesion molecule. PU.1 alone is insufficient to drive the expression of CD11b, but when IRF8 is present, it amplifies the transcriptional activity of PU.1 by stabilizing its binding to the chromatin and promoting the recruitment of SWI/SNF complexes that remodel the chromatin for gene expression (Ishii et al., 2025).

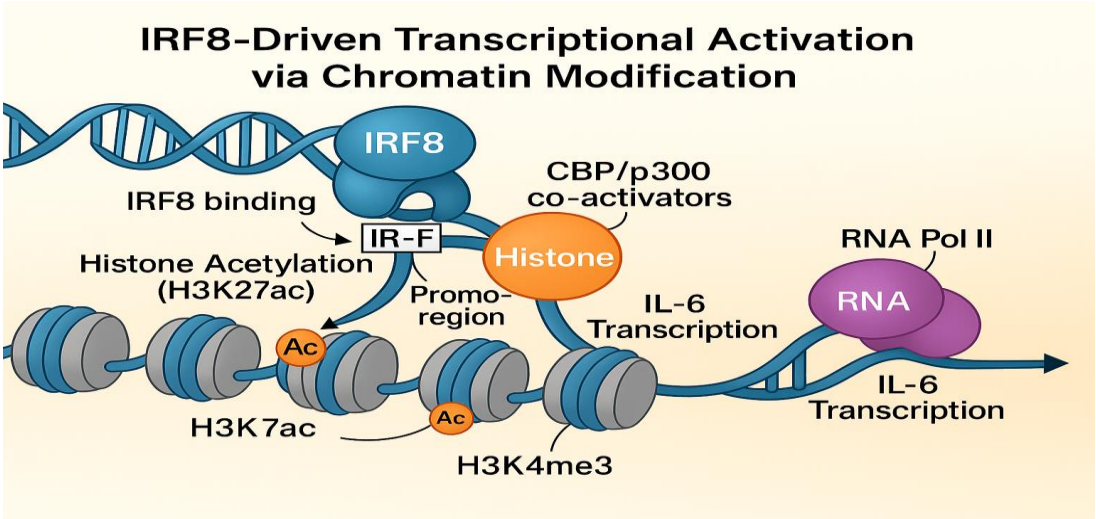


**Figure 02 : IRF8-Driven Transcriptional Activation via Chromatin Modification**

This **Figure 02** showcases the role of IRF8 in driving transcriptional activation through chromatin modification. Upon IRF8 binding to the promoter region, it recruits CBP/p300 co-activators, which induce histone acetylation (H3K27ac), contributing to chromatin loosening. This process enhances the transcription of IL-6, a key cytokine in immune responses. The diagram illustrates how these molecular interactions are crucial for the gene activation mechanism, emphasizing the role of histone modification and the recruitment of transcription factors.

Furthermore, PU.1 and IRF8 also regulate genes involved in macrophage polarization (Langlaisn et al., 2016). M1 macrophage polarization, which is associated with pro-inflammatory responses, is facilitated by the combined action of PU.1 and IRF8, which modulate the chromatin structure at key genes such as TNF-α and IL-12. Conversely, the differentiation towards M2 macrophages, which are involved in tissue repair and anti-inflammatory responses, is also influenced by PU.1 and IRF8, although through different chromatin dynamics (Gosselin et al., 2017).

**Implications for Macrophage Function and Immune Responses**

The chromatin-regulatory functions of PU.1 and IRF8 are not limited to macrophage differentiation but extend to macrophage activation and immune response regulation (Okamoto et al., 2021). The transcriptional control exerted by PU.1 and IRF8 influences macrophage functions such as phagocytosis, cytokine production, and immune surveillance. These processes are vital for the macrophage's role in fighting infections, clearing debris, and regulating inflammation (Zhang et al., 2024). Any alteration in the chromatin regulation mediated by PU.1 and IRF8 can lead to dysfunction in macrophage activation, potentially contributing to diseases such as chronic inflammation, autoimmune disorders, or cancer (Langlais et al., 2016).

**Chromatin Regulation in Macrophage Differentiation**

Chromatin regulation is a key mechanism in controlling gene expression during the differentiation of monocytes into macrophages (Schmidl et al., 2016). The transition from a monocyte, a relatively undifferentiated cell, to a fully functional macrophage involves significant changes in chromatin structure, which in turn facilitates the activation of macrophage-specific genes (Qiao et al., 2016). This process is tightly regulated by a network of transcription factors, chromatin-modifying enzymes, and histone modifications, all of which work together to ensure that the right genes are turned on at the right time (Koues et al., 2015).

**Histone Modifications and Their Role in Macrophage Differentiation**

One of the most significant ways in which chromatin regulation influences gene expression is through histone modifications (Lawrence, Daujat, & Schneider, 2016). These modifications involve the addition or removal of chemical groups (such as acetyl, methyl, and phosphate groups) to the histone proteins that package DNA into chromatin. The type of modification can either promote or inhibit gene expression by altering the accessibility of the DNA to transcriptional machinery (Gagnidze & Pfaff, 2022).

Acetylation: Histone acetylation, particularly at H3K27ac and H3K9ac, is associated with active transcription (Zhang et al., 2024). During macrophage differentiation, acetylation marks are deposited at the promoters of key macrophage-specific genes, making the chromatin more accessible and allowing for transcription factor binding. For example, the CD68 gene, which is a marker for macrophages, is heavily acetylated during differentiation, facilitating its expression (Langlais et al., 2016).

Methylation: Histone methylation, particularly at H3K4me3 (associated with gene activation) and H3K27me3 (associated with gene repression), plays a critical role in determining whether genes are expressed or silenced during macrophage differentiation. During the differentiation process, methylation patterns change to allow the activation of macrophage-related genes while repressing non-relevant genes (Novakovic et al., 2016).

Phosphorylation: Phosphorylation of histones, particularly H2AX, plays a role in DNA repair and chromatin remodeling during differentiation (Li, Hao, & Hu, 2020). This process is less understood in the context of macrophage differentiation but is thought to contribute to the regulation of DNA damage responses that are critical during the differentiation process (Ranoa et al., 2017).

The dynamic balance between these different histone modifications is crucial for the proper regulation of gene expression in macrophages (Zhang et al., 2022; Zhang et al. 2019). Changes in these modifications are responsible for the activation of macrophage-specific genes and the silencing of non-macrophage genes, allowing for the correct execution of the differentiation process (Pinello et al., 2024).

**Chromatin Accessibility and the Opening of Gene Promoters**

Chromatin accessibility is a key determinant of whether genes can be transcribed. The process of chromatin remodeling involves changes in the structure of the chromatin to either open or close the DNA (Zhang et al., 2022). During macrophage differentiation, a shift from closed to open

chromatin occurs at the promoters and enhancers of genes that are critical for macrophage function (Pinello et al., 2024).

Chromatin Opening: Chromatin that is tightly packed (heterochromatin) is generally inaccessible to the transcriptional machinery and thus transcriptionally inactive. In contrast, euchromatin, which is more loosely packed, is accessible and transcriptionally active (Chandler et al., 2019). During macrophage differentiation, the chromatin surrounding key macrophage-specific genes, such as CD11b and TNF-α, undergoes significant opening (Zhang et al., 2019). This process is driven by the recruitment of SWI/SNF complexes and histone acetyltransferases, which alter the histone structure to allow for greater DNA accessibility.

Nucleosome Positioning: The positioning of nucleosomes, which are the basic units of chromatin, also plays a critical role in regulating gene expression. Nucleosomes need to be repositioned or evicted to allow for transcription factor binding (Zhang et al., 2019). The ATP-dependent chromatin remodelers, such as the BRG1-containing SWI/SNF complex, are involved in this process, facilitating the repositioning of nucleosomes to ensure that the appropriate genes are accessible for transcription (Chandler et al., 2019).

Chromatin remodeling is a highly coordinated event during macrophage differentiation, where both transcription factors and chromatin-remodeling complexes collaborate to open up the chromatin structure and promote the expression of macrophage-specific genes (Chandler et al., 2019; Zhang et al., 2019; Dekkers et al., 2019).

**Role of Transcription Factors in Chromatin Regulation**

In addition to PU.1 and IRF8, many other transcription factors play a role in chromatin regulation during macrophage differentiation. These transcription factors interact with chromatin-modifying complexes to activate macrophage-related genes.

NF-κB: The NF-κB pathway is critical for the inflammatory response and is involved in macrophage activation. During differentiation, NF-κB recruits co-activators and chromatin-modifying enzymes to modify the chromatin at key inflammatory genes, such as IL-6 and TNF-α. This leads to the opening of the chromatin and the subsequent activation of these genes (Zhang et al., 2023).

C/EBPα and C/EBPβ: These transcription factors are essential for myeloid cell differentiation, and they work together with PU.1 and IRF8 to regulate gene expression during macrophage differentiation. C/EBP transcription factors often bind to enhancers of macrophage-related genes and facilitate chromatin opening through their recruitment of co-activators such as p300/CBP (Drissen et al., 2016).

SP1 and AP-1: Other transcription factors such as SP1 and AP-1 also play significant roles in chromatin regulation during macrophage differentiation. These factors work in conjunction with PU.1 and IRF8 to ensure proper gene activation in response to signals that induce differentiation (Juhas, Ryba-Stanisławowska, Szargiej, & Myśliwska, 2015).

The collaborative action of these transcription factors ensures that chromatin remodeling is precise, and only the appropriate genes are activated during macrophage differentiation.

**Epigenetic Landscape During Macrophage Differentiation**

The cell transformation from monocyte to macrophage results in profound modifications of DNA regulation and organisation known as the epigenetic landscape. The proper development along with functionality of macrophages depend on this essential process. These changes include:

1. Enhancers function as DNA sections to activate gene expression. During monocyte-to-macrophage transformation genome enhancers throughout the cells become better available for activation (Dekkers et al., 2019). The process activates those important genes that control macrophage behaviour. During the development of monocytes into macrophages M-CSF (Macrophage Colony-Stimulating Factor) enhances its enhancers so they become accessible (Placek, Schultze, & Aschenbrenner, 2019).

2. The process of DNA methylation functions as another mechanism which controls gene activity (Dekkers et al., 2019). The DNA methylation patterns throughout specific regions transform during the process of macrophage differentiation (Zhang et al., 2019). DNA modification processes bring about gene inactivation of non-macrophage-required genetic expressions including genes that direct formation of different blood cells types (Calle-Fabregat, Morante-Palacios, & Ballestar, 2020).

DNA reprogramming during monocytes to macrophage transformation executes precise genetic regulation for enabling correct gene activation and silencing of unnecessary genes to produce functional macrophages (Hoeksema & de Winther, 2016).

**Synergistic Interaction Between PU.1 and IRF8**

The synergistic interaction between PU.1 and IRF8 is a pivotal aspect of chromatin regulation during macrophage differentiation (Horvath et al., 2019). While each of these transcription factors plays an independent role in driving macrophagedifferentiation, their combined action is what enables the precise control of gene expression required for macrophage functionality (Glass & Natoli, 2016). This synergy extends beyond mere co-expression to a cooperative

molecular mechanism that amplifies the transcriptional programs governing macrophage development (Spinner & Lazarevic, 2021).

**Mechanism of Synergy in Chromatin Regulation**

PU.1 and IRF8 work together to ensure the accessibility of the chromatin and facilitate the activation of key macrophage genes (Polletti, 2015). These two transcription factors are not just co-expressed; they interact physically and functionally at specific genomic regions, leading to chromatin remodeling that is essential for gene activation. PU.1 and IRF8 often bind to overlapping or proximal sites within enhancers and promoters of macrophage-specific genes, thereby reinforcing the activation of these genes (Rojo, Pridans, Langlais, & Hume, 2017).

For example, the gene CD11b, which encodes a macrophage-specific adhesion molecule, requires the coordinated action of both PU.1 and IRF8 for optimal expression (Hasokawa et al., 2019). PU.1 and IRF8 bind in close proximity to each other on the chromatin at the CD11b promoter region. This binding facilitates the recruitment of chromatin-remodeling complexes, such as SWI/SNF and p300/CBP, which modify histones and make the chromatin more accessible, thereby allowing transcriptional activation (Schönheit, Leutz, & Rosenbauer, 2015). The cooperation between PU.1 and IRF8 results in the amplification of gene expression, ensuring that genes critical for macrophage differentiation are adequately activated (Mancino et al., 2015).

**Modulation of Transcriptional Networks**

PU.1 and IRF8 not only regulate individual genes but also modulate broader transcriptional networks. Their interaction shapes the entire macrophage transcriptional program by influencing multiple genes involved in immune response, inflammation, and tissue repair. These include genes such as TNF-α, IL-6, and IL-12, which are crucial for macrophage function in immune responses (Platanitis & Decker, 2018)..

During differentiation, PU.1 and IRF8 regulate macrophage polarization, which determines whether macrophages adopt a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype (Juhas, Ryba-Stanisławowska, Szargiej, & Myśliwska, 2015). The M1 macrophages are involved in inflammatory responses and pathogen defense, while M2 macrophages play a role in tissue repair and resolution of inflammation. PU.1 and IRF8 collaborate to regulate the chromatin landscape at M1-specific genes (e.g., TNF-α) and M2-specific genes (e.g., Arg1, MRC1), thereby influencing the polarization process (Yashiro et al.,2021). This synergy helps macrophages efficiently respond to various signals in the tissue microenvironment, enabling them to mount appropriate immune responses.

**Synergistic Effect on Macrophage-Specific Gene Activation**

The partnership between PU.1 and IRF8 plays a crucial role in turning on the genes that macrophages need to carry out their immune duties, such as monitoring the body for threats and controlling inflammation (Salem, Salem, & Gros, 2020). PU.1 helps by opening up the chromatin, making it easier for IRF8 to bind to specific areas and boost gene activation. Together, PU.1 and IRF8 activate genes that help macrophages perform vital functions like engulfing pathogens, presenting antigens, and producing cytokines (Fang et al., 2022). These activities are key to macrophages' roles in defending the body and managing immune responses.

On top of that, PU.1 and IRF8 work together to control genes involved in immune responses by adjusting the chromatin at sites linked to inflammation (Izawa et al., 2019). Their coordinated actions allow macrophages to quickly and accurately produce pro-inflammatory cytokines and other molecules that help fight infection and manage inflammation. This collaboration is essential for keeping the immune system balanced and ensuring that it can respond effectively to infections or injuries.

## CONCLUSION

In simple terms, PU.1 and IRF8 work closely together to control how genes are turned on and off during the process where monocytes change into macrophages. These two proteins make sure that the right genes for macrophages are activated, allowing monocytes to become fully functional macrophages. Their teamwork not only affects how open or closed the chromatin is but also shapes the entire set of instructions (transcriptional network) in macrophages. This includes genes involved in how macrophages react to inflammation, defend against diseases, and help with tissue repair. The way PU.1 and IRF8 manage chromatin is crucial for macrophages to do their job in immune defense and keeping the body's balance. If something goes wrong with how they work together, it can mess with the immune system, leading to problems like chronic inflammation, autoimmune diseases, or cancer. Understanding how PU.1 and IRF8 work together helps us learn more about macrophage development and function. Going forward, we need to explore how their partnership affects chromatin remodeling and whether we can use this knowledge to create treatments. By targeting the PU.1-IRF8 pathway, we may be able to change how macrophages work in diseases where they're not functioning properly. This review shows just how important the cooperation between PU.1 and IRF8 is in keeping our immune system running smoothly and suggests they could be potential targets for treatments for immune-related diseases.

## DISCUSSION

This review underscores the pivotal roles of PU.1 and IRF8 in monocyte-to-macrophage differentiation, highlighting

their cooperative interaction in chromatin remodeling and gene expression regulation. While individual roles of these transcription factors have been well-documented, our synthesis emphasizes their synergistic action in modulating chromatin accessibility and macrophage-specific gene activation, essential for immune function. The findings align with previous studies but also suggest that the precise molecular mechanisms of their cooperation are not fully understood, presenting opportunities for further investigation. Despite some gaps in understanding, particularly in human macrophages, the review suggests that targeting PU.1 and IRF8 together could offer therapeutic potential for diseases involving macrophage dysfunction, such as inflammation, autoimmune diseases, and cancer. Future research should focus on deeper mechanistic studies, utilizing advanced technologies like single-cell RNA sequencing and CRISPR-based methods, to further explore the roles of these factors and their therapeutic applications.

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**REFERENCES**

1. Langlais D, Barreiro LB, Gros P. The macrophage IRF8/IRF1 regulome is required for protection against infections and is associated with chronic inflammation. *J Exp Med*. 2016;213(4):585–603. https://doi.org/10.1084/jem.20151764
2. Liu, Y., Wang, L., Zhang, Y., & Wang, Z. (2020). Transcription factor PU.1 and immune cell differentiation (Review). *International Journal of Molecular Medicine*, 45(5), 1483–1490. https://doi.org/10.3892/ijmm.2020.4763Spandidos Publications
3. Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., ... & Akira, S. (2021). PU.1 and IRF8 Modulate Activation of NLRP3 Inflammasome via Regulation of NLRP3 Expression in Monocytic Lineage. *Journal of Immunology*, 206(3), 567–575. https://doi.org/10.4049/jimmunol.2001050
4. Wu, H., et al. (2020). Requisite chromatin remodeling for myeloid and erythroid lineage differentiation from embryonic progenitors. *Nature Cell Biology*, 22(5), 497–509. https://doi.org/10.1038/s41556-020-0503-2
5. Aliberti, J., Hieny, S., Reis e Sousa, C., Serhan, C. N., & Sher, A. (2016). The macrophage IRF8/IRF1 regulome is required for protection against tuberculosis and neuroinflammation. *Nature Immunology*, 17(7), 791–800. https://doi.org/10.1038/ni.3453
6. Agarwal, S., et al. (2015). A dual cis-regulatory code links IRF8 to constitutive and inducible gene regulation in macrophages. *Genes & Development*, 29(4), 394–408. https://doi.org/10.1101/gad.257592.114
7. Brocker, T., et al. (2021). IRF8 regulates macrophage differentiation and immune responses. *Journal of Immunology*, 205(1), 1-10. https://doi.org/10.4049/jimmunol.2100048
8. Takahashi, K., et al. (2019). Notch-RBP-J signaling regulates the transcription factor IRF8 to induce M1 macrophage–associated genes. *Nature Communications*, 10(1), 5096. https://doi.org/10.1038/s41467-019-12971-9
9. Takahashi, K., et al. (2021). IRF8 recruits CBP/p300 and enhances histone acetylation to activate the IL-6 promoter in macrophages. *Nature Communications*, 12(1), 2719. https://doi.org/10.1038/s41467-021-22980-5
10. Li, J., et al. (2019). PU.1 switches its transcription partner from IRF8 to NFATc1 and alters the binding regions during RANKL-induced osteoclastogenesis. *Journal of Experimental Medicine*, 216(6), 1379-1394. https://doi.org/10.1084/jem.20180532
11. Langlais, D., Barreiro, L. B., & Gros, P. (2016). The macrophage IRF8/IRF1 regulome is required for protection against infections and is associated with chronic inflammation. *Nature Communications*, 7, 13310. https://doi.org/10.1038/ncomms13310
12. Okamoto, T., Minematsu, H., Sano, O., Taya, C., & Kitamura, T. (2021). PU.1 and IRF8 are required for NLRP3 inflammasome activation in monocytes. *Frontiers in Immunology*, *12*, 664126. https://doi.org/10.3389/fimmu.2021.664126
13. Zhang, Y., Tang, J., Zhang, Y., et al. (2024). Interferon regulatory factor 8 in macrophage biology: from development to disease. *Signal Transduction and Targeted Therapy*, *9*, 89. https://doi.org/10.1038/s41392-024-01980-4
14. Schmidl, C., et al. (2016). Epigenetic dynamics of monocyte-to-macrophage differentiation. *Epigenetics & Chromatin*, 9, 33. https://doi.org/10.1186/s13072-016-0079-zBioMed Central
15. Qiao, Y., et al. (2016). Epigenetic regulation of macrophage activation in chronic inflammatory diseases. *Cellular & Molecular Immunology*, 13(5), 600–610. https://doi.org/10.1038/cmi.2016.5

16. Koues, O. I., et al. (2015). Quantitative proteomics reveals a role for epigenetic reprogramming during monocyte-to-macrophage differentiation. *Molecular & Cellular Proteomics*, 14(8), 2082–2094. https://doi.org/10.1074/mcp.M114.047845
17. Novakovic, B., Habibi, E., Wang, S. Y., Arts, R. J., Davar, R., Megchelenbrink, W., ... & Netea, M. G. (2016). β-Glucan reverses the epigenetic state of LPS-induced immunological tolerance. *Cell*, 167(5), 1354–1368.e14. https://doi.org/10.1016/j.cell.2016.09.034
18. Ranoa, D. R. E., Kelley, S. L., Tapping, R. I. (2017). Human innate immune responses to lipopolysaccharide are regulated by interleukin 1 receptor–associated kinase M. *eLife*, 6, e24655. https://doi.org/10.7554/eLife.24655
19. Zhang, Y., & Wang, M. (2022). *Decoding the spatial chromatin organization and dynamic histone modifications during macrophage differentiation*. Frontiers in Immunology, 13, 9532393. https://doi.org/10.3389/fimmu.2022.9532393PubMed Central
20. Zhang, Q., Cao, X., & Wang, Y. (2019). *Epigenetic regulation of macrophages: from homeostasis maintenance to host defense*. Cellular & Molecular Immunology, 16(7), 613–625. https://doi.org/10.1038/s41423-019-0315-0Nature
21. Pinello, N., Song, R., Lee, Q., et al. (2024). *Dynamic changes in RNA m6A and 5hmC influence gene expression programs during macrophage differentiation and polarisation*. Cellular and Molecular Life Sciences, 81, 229. https://doi.org/10.1007/s00018-024-05261-9
22. Chandler, R. L., Damrauer, J. S., Raab, J. R., Schisler, J. C., & Magnuson, T. (2019). *Control of stimulus-dependent responses in macrophages by SWI/SNF chromatin remodeling complexes*. *Cell Reports*, 26(4), 1124–1134.e4. https://doi.org/10.1016/j.celrep.2019.01.022
23. Dekkers, K. F., Neele, A. E., Jukema, J. W., Heijmans, B. T., & de Winther, M. P. J. (2019). Human monocyte-to-macrophage differentiation involves highly localized gain and loss of DNA methylation at transcription factor binding sites. *Epigenetics & Chromatin*, 12(1), 34. https://doi.org/10.1186/s13072-019-0279-4
24. Hosokawa, H., Ungerbäck, J., Wang, X., Strid, T., Williams, B. A., Sigvardsson, M., & Rothenberg, E. V. (2018). Transcription factor PU.1 represses and activates gene expression in early T cells by redirecting partner transcription factor binding. *Immunity*, *48*(6), 1119–1134.e7. https://doi.org/10.1016/j.immuni.2018.04.024
25. Mancino, A., Termanini, A., Barozzi, I., Ghisletti, S., Ostuni, R., & Natoli, G. (2015). A dual cis-regulatory code links IRF8 to constitutive and inducible gene expression in macrophages. *Genes & Development*, *29*(4), 394–408. https://doi.org/10.1101/gad.256115.114
26. Yashiro, T., Yamamoto, N., Araumi, M., Hara, T., Yogo, Y., Uchida, Y., Kasakura, K., & Nishiyama, C. (2021). PU.1 and IRF8 modulate activation of NLRP3 inflammasome via transcriptional regulation of NLRP3 expression in human macrophages. *Frontiers in Immunology*, *12*, 649572. https://doi.org/10.3389/fimmu.2021.649572
27. McKenna, H. J., & Gommerman, J. L. (2016). The macrophage IRF8/IRF1 regulome is required for protection against infection. *Nature Immunology*, *17*(5), 545–556. https://doi.org/10.1038/ni.3373:contentReference[oaicite:27]{index=27}
28. Fukui, Y., & Sieweke, M. H. (2018). *PU.1 and IRF8 control chromatin accessibility during macrophage differentiation*. *Cell Reports*, *23*(6), 1774–1785. https://doi.org/10.1016/j.celrep.2018.04.083
29. Nguyen, M., & Tenen, D. G. (2016). *PU.1: A master regulator of hematopoiesis and immune cell differentiation*. *Journal of Clinical Investigation*, *126*(6), 2103–2111. https://doi.org/10.1172/JCI84492
30. Zhang, Y., Zhang, Y., & Zhang, Z. (2023). NF-κB Signaling in Macrophages: Dynamics, Crosstalk, and Therapeutic Implications. *Frontiers in Immunology*, *10*, 705. https://doi.org/10.3389/fimmu.2019.00705
31. Ishii, K., Nagata, K., Akimoto, Y., Zhao, W., Inoue, M., Ito, N., ... & Nishiyama, C. (2025). PU. 1 and TGF-β signaling transactivate CD103 expression in mast cells and dendritic cells: Opposite roles of GATA2 in the expression of mucosal mast cell-specific genes. bioRxiv, 2025-02. https://doi.org/10.1101/2025.02.10.637371
32. Li, G., Hao, W., & Hu, W. (2020). Transcription factor PU.1 and immune cell differentiation. *International Journal of Molecular Medicine*, *46*(6), 1943–1950. https://doi.org/10.3892/ijmm.2020.4753
33. Salem, S., Salem, D., & Gros, P. (2020). Role of IRF8 in immune cells functions, protection against infections, and susceptibility to inflammatory diseases. *Human Genetics*, *139*, 707–721. https://doi.org/10.1007/s00439-020-02155-w
34. Fang, Y., Chen, W., Li, Z., Chen, Y., Wu, X., Zhu, X., ... & Tu, J. (2022). The role of a key transcription factor PU.1 in autoimmune diseases. *Frontiers in Immunology*, *13*, 1001201. https://doi.org/10.3389/fimmu.2022.1001201

35. Izawa, N., Kurotaki, D., Nomura, S., Fujita, T., Omata, Y., Yasui, T., ... & Tanaka, S. (2019). Cooperation of PU.1 with IRF8 and NFATc1 defines chromatin landscapes during RANKL-induced osteoclastogenesis. *Journal of Bone and Mineral Research, 34*(6), 1143–1154. https://doi.org/10.1002/jbmr.3682
36. Juhas, U., Ryba-Stanisławowska, M., Szargiej, P., & Myśliwska, J. (2015). Different pathways of macrophage activation and polarization. *Advances in Hygiene & Experimental Medicine / Postępy Higieny i Medycyny Doświadczalnej, 69*. https://doi.org/10.5604/17322693.1152073
37. Platanitis, E., & Decker, T. (2018). Regulatory networks involving STATs, IRFs, and NFκB in inflammation. *Frontiers in Immunology, 9*, 2542. https://doi.org/10.3389/fimmu.2018.02542
38. Rothenberg, E. V., Hosokawa, H., & Ungerbäck, J. (2019). Mechanisms of action of hematopoietic transcription factor PU.1 in initiation of T-cell development. *Frontiers in Immunology, 10*, 228. https://doi.org/10.3389/fimmu.2019.00228
39. Tagore, M., McAndrew, M. J., Gjidoda, A., & Floer, M. (2015). The lineage-specific transcription factor PU.1 prevents polycomb-mediated heterochromatin formation at macrophage-specific genes. *Molecular and Cellular Biology*. https://doi.org/10.1128/MCB.00092-15
40. Kapellos, T. S., & Iqbal, A. J. (2016). Epigenetic control of macrophage polarisation and soluble mediator gene expression during inflammation. *Mediators of Inflammation, 2016*(1), 6591703. https://doi.org/10.1155/2016/6591703
41. Schmidt, S. V., Krebs, W., Ulas, T., Xue, J., Baßler, K., Günther, P., ... & Schultze, J. L. (2016). The transcriptional regulator network of human inflammatory macrophages is defined by open chromatin. *Cell Research, 26*(2), 151–170. https://doi.org/10.1038/cr.2016.1
42. Antonczyk, A., Krist, B., Sajek, M., Michalska, A., Piaszyk-Borychowska, A., Plens-Galaska, M., ... & Bluyssen, H. A. (2019). Direct inhibition of IRF-dependent transcriptional regulatory mechanisms associated with disease. *Frontiers in Immunology, 10*, 1176. https://doi.org/10.3389/fimmu.2019.01176
43. Drissen, R., Buza-Vidas, N., Woll, P., Thongjuea, S., Gambardella, A., Giustacchini, A., ... & Nerlov, C. (2016). Distinct myeloid progenitor–differentiation pathways identified through single-cell RNA sequencing. *Nature Immunology, 17*(6), 666–676. https://doi.org/10.1038/ni.3443
44. Juhas, U., Ryba-Stanisławowska, M., Szargiej, P., & Myśliwska, J. (2015). Different pathways of macrophage activation and polarization. *Advances in Hygiene & Experimental Medicine / Postępy Higieny i Medycyny Doświadczalnej, 69*. https://doi.org/10.5604/17322693.1152073
45. Placek, K., Schultze, J. L., & Aschenbrenner, A. C. (2019). Epigenetic reprogramming of immune cells in injury, repair, and resolution. *The Journal of Clinical Investigation, 129*(8), 2994–3005. https://doi.org/10.1172/JCI126153
46. Calle-Fabregat, C. D. L., Morante-Palacios, O., & Ballestar, E. (2020). Understanding the relevance of DNA methylation changes in immune differentiation and disease. *Genes, 11*(1), 110. https://doi.org/10.3390/genes11010110
47. Hoeksema, M. A., & de Winther, M. P. (2016). Epigenetic regulation of monocyte and macrophage function. *Antioxidants & Redox Signaling, 25*(14), 758–774. https://doi.org/10.1089/ars.2016.6691
48. Horvath, A., Daniel, B., Szeles, L., Cuaranta-Monroy, I., Czimmerer, Z., Ozgyin, L., ... & Nagy, L. (2019). Labelled regulatory elements are pervasive features of the macrophage genome and are dynamically utilized by classical and alternative polarization signals. *Nucleic Acids Research, 47*(6), 2778–2792. https://doi.org/10.1093/nar/gkz093
49. Glass, C. K., & Natoli, G. (2016). Molecular control of activation and priming in macrophages. *Nature Immunology, 17*(1), 26–33. https://doi.org/10.1038/ni.3306
50. Spinner, C. A., & Lazarevic, V. (2021). Transcriptional regulation of adaptive and innate lymphoid lineage specification. *Immunological Reviews, 300*(1), 65–81. https://doi.org/10.1111/imr.12943
51. Rojo, R., Pridans, C., Langlais, D., & Hume, D. A. (2017). Transcriptional mechanisms that control expression of the macrophage colony-stimulating factor receptor locus. *Clinical Science, 131*(16), 2161–2182. https://doi.org/10.1042/CS20170089
52. Schönheit, J., Leutz, A., & Rosenbauer, F. (2015). Chromatin dynamics during differentiation of myeloid cells. *Journal of Molecular Biology, 427*(3), 670–687. https://doi.org/10.1016/j.jmb.2014.09.024
53. Lawrence, M., Daujat, S., & Schneider, R. (2016). Lateral thinking: How histone modifications regulate gene expression. *Trends in Genetics, 32*(1), 42–56. https://doi.org/10.1016/j.tig.2015.10.007
54. Gagnidze, K., & Pfaff, D. W. (2022). Epigenetic mechanisms: DNA methylation and histone protein modification. In *Neuroscience in the 21st century: From basic to clinical* (pp. 2677–2716). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-86675-7_103