

## Alternative Control: What's WASp Doing in the Nucleus?

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**Wiskott-Aldrich syndrome (WAS) is a rare X-linked recessive immunodeficiency disorder of childhood that is caused by mutations in the WAS gene. WAS encodes WASp, a protein that is known to function in the cytoplasm of hematopoietic cells and is required for the induced differentiation of CD4<sup>+</sup> T helper type 1 (T<sub>H</sub>1) lymphocytes. Now, a paper in *Science Translational Medicine* describes another mechanism for impaired immunity in WAS by showing that WASp localizes in the nucleus and regulates histone modifications and chromatin structure, thereby modulating expression of the T<sub>H</sub>1 master gene *TBX21* (*TBET*).**

More than 50 years ago, Wiskott and Aldrich separately described features of an X-linked recessive disorder of blood cell development during childhood that is characterized, in affected males, by a low number of small platelets (microthrombocytopenia), bleeding, skin rash (eczema), recurrent infections, and early death (1, 2). The mutated gene that gives rise to this disease—known today as Wiskott-Aldrich syndrome (WAS)—was positionally cloned in 1994 and is referred to as WAS (3). Clinical symptoms of WAS vary among patients and families and, in addition to the original phenotypical observations, now include defects in adaptive and innate immunity that involve CD4<sup>+</sup> T helper type 1 (T<sub>H</sub>1) cells, T regulatory cells, antibody-producing B cells, antigen-presenting dendritic cells, natural killer cells, and monocytes (4–6). In the most severe cases, autoimmunity, pre-malignant myelodysplastic syndrome (MDS) (a bone marrow stem cell disorder associated with defective myeloid lineage development), and malignancies (usually Epstein-Barr virus-positive B cell lymphoma) may arise. Specific types of defects in the WAS-encoded protein WASp are often but not invariably associated with the severity of patient phenotypes. For example, the lack of WASp expression causes the most severe phenotype, termed classic WAS; whereas inactivating WASp missense mutations cause less severe X-linked thrombocytopenia; and activating WASp alterations generate milder X-linked neutropenia (5, 7). The underlying molecular mechanisms for some of the

disease manifestations, including thrombocytopenia, autoimmunity, eczema, and most types of immune cell dysfunction, are incompletely characterized. In contrast, extensive studies combine to suggest a mechanism for T<sub>H</sub>1 cell dysfunction via a defect in WASp-dependent cortical actin polymerization in the cytoplasm of developing T<sub>H</sub>1 cells during T cell activation (4). In the current issue of *Science Translational Medicine*, a paper by Taylor *et al.* challenges this purely cytoplasmic model of WASp function in T<sub>H</sub>1 cell differentiation. The authors show that WASp exists in the nucleus as part of two distinct histone-modifying complexes at the promoters of key T<sub>H</sub>1 genes, including the master regulator gene *TBX21*. This new work suggests a nuclear mechanism for the disruption in WASp-dependent T<sub>H</sub>1 cell development observed in WAS patients (8).

Normally, T cell receptor–antigen interactions and T cell costimulation result in the formation of an immune synapse between a T cell and an antigen-presenting cell. During this process, inactive WASp is recruited to plasma membrane lipid rafts in stimulated T<sub>H</sub>0 cells by the WASp-interacting protein and the NCK adaptor protein; this is followed by WASp activation, which occurs as a result of WASp binding to the Rho guanosine triphosphatase CDC42 and subsequent WASp phosphorylation (9–11). WASp-regulated actin cytoskeleton remodeling is thought to be essential for relaying signals to the nucleus that activate the T<sub>H</sub>1 gene expression program. Defective WASp fails to induce filamentous actin (F-actin) formation, which inhibits expression of the T<sub>H</sub>1 master regulator gene *TBX21* (*TBET*), resulting in a block of T<sub>H</sub>1 cell differentiation and the absence of T<sub>H</sub>1 cytokines, such

as interferon- $\gamma$  (IFN- $\gamma$ ) (12). WASp also regulates hematopoietic cell chemotaxis, adhesion, phagocytosis, and trafficking through its role in organizing the immune synapse by actin cytoskeleton remodeling (13).

Antigen-stimulated CD4<sup>+</sup> T<sub>H</sub>1 cells secrete cytokines (IFN- $\gamma$ , tumor necrosis factor- $\beta$ , and interleukin-2) that activate the proliferation and killing activities of macrophages and CD8<sup>+</sup> cytotoxic T cells during an effective cellular immune response. In WAS, the development of T<sub>H</sub>1 immunity is impaired by a block in the differentiation of T<sub>H</sub>1 cells from naïve CD4<sup>+</sup> T<sub>H</sub>0 cells, providing a plausible explanation for the recurrent infections observed in WAS patients. WASp is a 502–amino acid, multidomain, nonenzymatic member of a five-member family of scaffold proteins that includes N-WASp and three SCAR/WAVE proteins, all of which regulate actin polymerization into F-actin (14). WASp is activated by a conformational change, which unfolds the protein to permit its interaction with the actin-related protein complex (ARP2/3) through a small WASp VCA (verprolin, cofilin, and acidic region) homology domain, thereby initiating actin filament nucleation in the cytoplasm (15, 16).

Although an impairment in cytosolic actin polymerization is the current leading hypothesis for the molecular basis of WAS, it remains unclear how a general defect in cortical actin polymerization causes a selective defect in *TBX21* gene activation and T<sub>H</sub>1 cell differentiation. Also, mice that harbor a form of WASp that is constitutively active for enhanced cortical actin polymerization still display a defect in the induction of *T-bet* gene expression, which results in WAS-type immunodeficiency (17). For these and other reasons, including the fact that many actin-binding proteins, such as the widely expressed N-WASp, shuttle between the cytoplasm and nucleus (18–20), Taylor and colleagues (8) sought to determine whether WASp also functions in the nucleus of T cells.

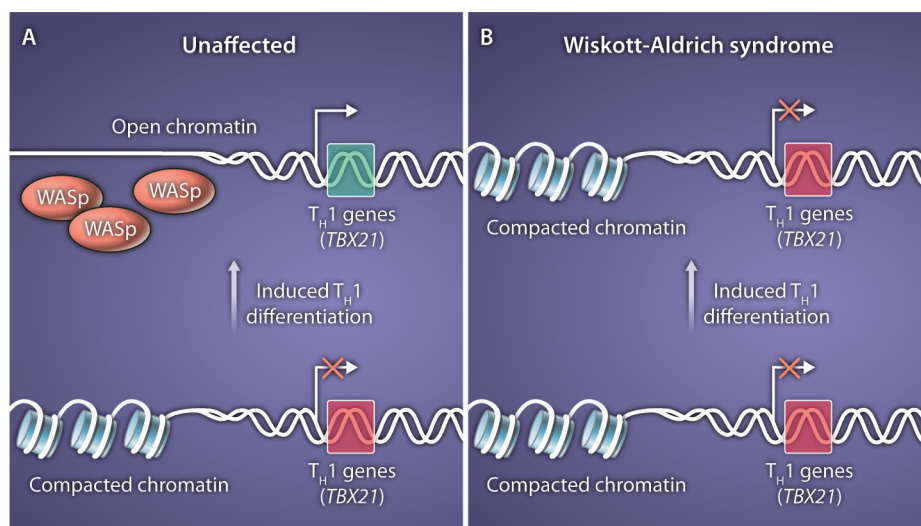
The authors showed that WASp is localized in both the cytoplasm and nucleus of primary human T<sub>H</sub>0 cells incubated in T<sub>H</sub>1-polarizing culture conditions. In the nucleus, Taylor *et al.* (8) found that WASp associates with the histone-modifying complexes H3K4-trimethyltransferase (RBBP5) and H3K9-tridemethylase (JMJD2A), which are recruited to T<sub>H</sub>1 gene promoters (*TBX21*, *RUNX3*, and *IFN- $\gamma$* ) but not T<sub>H</sub>2 (*GATA-3*) or T<sub>H</sub>17 (*RORc*) gene promoters, to generate open, transcription-competent euchroma-

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tin during  $T_H1$  cell differentiation (Fig. 1A). The transcription factors STAT1, T-BET, and SP1, and general transcription pre-initiation complexes that contain Mediator TRAP220/MED1 and RNA polymerase II, are also present at the *TBX21* core promoter, along with WASp and histone marks associated with regions of active transcription [including histone 3-lysine 4 trimethylation (H3K4me3), histone 3-lysine 9 acetylation (H3K9ac), and the histone 2A Z-variant, H2A.Z].

In contrast, primary human T cells in culture that lacked WASp expression showed decreased RBBP5 enrichment at the *TBX21* promoter, which maintains a  $T_H0$ -like inactive, condensed heterochromatin state with predominantly repressive H3K9me3 and H3K27me3 histone marks (Fig. 1B). The inhibition of  $T_H1$  gene transcription and  $T_H1$  cell differentiation in T cells that lacked WASp expression was ameliorated when the authors reconstituted WASp expression in a WAS patient-derived  $T_H1$  cell line (human T cell leukemia virus-immortalized by retroviral transduction) and incubated these transduced cells with  $T_H1$ -differentiating cytokines. Differentiating  $T_H1$  cells also showed an enrichment in F-actin at the *TBX21* promoter, which is consistent with a role for nuclear ARP2/3 and F-actin in RNA polymerase II-dependent gene transcription (21). However, only two of three WASp-deficient patient T cell samples showed reduced F-actin at the *TBX21* promoter with  $T_H1$  differentiating cytokines, suggesting that WASp epigenetic activity may be separate from its actin nucleation activity and more relevant for regulating *TBX21* transcription and  $T_H1$  cell differentiation. This idea requires further testing, although a normal amount of F-actin at the *TBX21* promoter in one WASp-deficient T cell sample could be generated by other actin-binding proteins in the nucleus, including nonredundant N-WASp (18).

Finally, WAS missense mutation-containing T cells from patients showed more chromatin-accessible histone modifications and a more open chromatin configuration (by a DNase hypersensitivity assay) at the *TBX21* locus with  $T_H1$  differentiation, as compared to T cells that completely lacked WASp. This finding suggests a link between WAS disease severity and the extent of aberrant epigenetic control seen with specific defects in WAS, although more samples need to be examined to validate such an interpre-



**Fig. 1. Nuclear WASp opens chromatin.** (A) Healthy T cells induced to undergo  $T_H1$  cell differentiation contain nuclear WASp and actively transcribe  $T_H1$ -related genes, including the  $T_H1$  master gene, *TBX21* (*TBET*). (B) When WASp is missing, the chromatin in  $T_H0$  cells remains compact.  $T_H1$ -related gene expression and thus  $T_H1$  cell differentiation are blocked.

ation. Taken together, the results of Taylor *et al.* (8) suggest a crucial function for WASp in selective  $T_H1$  chromatin regulation,  $T_H1$  gene expression, and  $T_H1$  cell differentiation that may predict WAS disease severity based on the epigenetic profile at  $T_H1$  gene promoters.

These exciting new findings raise many interesting questions about the role of nuclear WASp in the regulation of  $T_H1$  gene transcription and  $T_H1$  cell differentiation. How WASp is selectively recruited by or assembled into histone-modifying complexes on  $T_H1$  target genes remains unresolved, as is the role of nuclear WASp in regulating F-actin. The mechanism behind the localization of WASp in the nucleus of T cells also is unclear. Furthermore, it is unknown whether WASp arrives in the nucleus in an active conformation for actin polymerization or assembly into histone-modifying complexes, or whether further nuclear activation of WASp is required. Answers to these questions await additional mechanistic investigations to decipher essential details behind the nuclear role of WASp in healthy and WAS T cells.

Early diagnosis is essential for prophylaxis (against infections) and treatment of WAS patients. Currently, the most effective treatment is hematopoietic stem cell (HSC) transplantation, which has yielded robust results thus far when HSCs are taken from antigen-matched family or unrelated donors, or even from partially matched umbilical cord donors (22–26). Early gene therapy trials are under

way that use harvested HSCs transduced with a retroviral vector that expresses a functional WAS cDNA. Furthermore, researchers are in the process of developing a lentiviral WAS gene replacement strategy that uses the endogenous WAS gene promoter, which has the potential to be safer than retroviral transduction (4). However, patient management when HSC transplantation is not an option remains prophylaxis or targeted treatment of specific disease symptoms. For example, male infants with classic WAS receive prophylactic protection from opportunistic lung infections caused by *Pneumocystis carinii* and intravenous immunoglobulin infusions to combat additional bacterial, viral, and fungal infections. Platelet transfusions and removal of the spleen are performed to stem active bleeding caused by low platelet numbers and to help block WAS platelet destruction, although splenectomy is contraindicated in a young patient who is expected to undergo potentially curative HSC transplantation (27). Steroids and other immunosuppressants are used to treat eczema and a broad spectrum of WAS autoimmune manifestations. Intriguingly, WASp nuclear activity in chromatin-modifying complexes suggests the possibility that agents that stimulate chromatin-activating histone and possibly DNA demethylating modifications could have a previously unappreciated role in managing WAS patients, particularly with respect to  $T_H1$  cell immunodeficiency. Although nonspecific, the pan-

histone deacetylase (HDAC) inhibitor suberoylanilide hydroxamic acid (SAHA, vorinostat) and the DNA methyltransferase (DNMT) inhibitors 5-azacytidine and 5-aza-2'-deoxycytidine are U.S. Food and Drug Administration–approved for the treatment of MDS, which can be part of the clinical spectrum for classic WAS. The development of drugs that control histone methylation could one day have an impact on the accessibility of inactive  $T_H1$  gene promoters, such as *TBX21*, allowing  $T_H1$  cell differentiation and the recovery of defective cellular immune function. Today, augmenting open chromatin by increasing H3K9ac and/or DNA demethylation with HDAC and DNMT inhibitors in WASp-deficient T cells from mice (28) or harvested from WAS patients could be evaluated preclinically for  $T_H1$  gene activation and the correction of  $T_H1$  immune deficiency. For poorly understood reasons, pan-HDAC inhibitors induce responses that include cell cycle arrest, apoptosis, and terminal differentiation, with the latter outcome being desirable for WAS  $T_H1$  cell differentiation. With more specific epigenetic modifying drugs to be discovered, validated, and tested for safety and efficacy, altered chromatin from defective nuclear WASp could provide a surprising and interesting new target in the future management of the immunodeficiency seen in WAS patients.

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