**Nramp1-mediated Innate Resistance to Intraphagosomal Pathogens Is Regulated by IRF-8, PU.1, and Miz-1**

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Natural resistance-associated macrophage protein 1 (Nramp1) is a proton/divalent cation antiporter exclusively expressed in monocyte/macrophage cells with a unique role in innate resistance to intraphagosomal pathogens. In humans, it is linked to several infectious diseases, including leprosy, pulmonary tuberculosis, visceral leishmaniasis, meningococcal meningitis, and human immunodeficiency virus as well as to autoimmune diseases such as rheumatoid arthritis and Crohn’s disease. Here we demonstrate that the restricted expression of Nramp1 is mediated by the macrophage-specific transcription factor IRF-8. This factor exerts its activity via protein-protein interaction, which facilitates its binding to target DNA. Using yeast two-hybrid screens we identified Myc Interacting Zinc finger protein 1 (Miz-1) as a new interacting partner. This interaction is restricted to immune cells and takes place on the promoter Nramp1 in association with PU.1, a transcription factor essential for myelopoiesis. Consistent with these data, IRF-8 knockout mice are sensitive to a repertoire of intracellular pathogens. Accordingly, IRF-8−/− mice express low levels of Nramp1 that can not be induced any further. Thus, our results explain in molecular terms the role of IRF-8 in conferring innate resistance to intracellular pathogens and point to its possible involvement in autoimmune diseases.

Macrophages are essential components of innate immunity but also provide important links between innate and adaptive immunity. Mature macrophages differentiate from a bipotent myeloid progenitor stem cell that can also differentiate to granulocyte in a regulated process in which many transcription factors are involved. Macrophages are an essential component of the host defense mechanisms against invading pathogens. These invading foreign bodies activate the macrophages, which in response engulf and subsequently entrap the pathogens in the phagosome/lysosome compartment. In this specialized compartment, the pathogens are subjected to massive attack by reactive oxygen and nitrogen intermediates (1). This is achieved by two macrophage specific enzymatic pathways, phagocyte oxidase (phox) (2) and inducible nitric-oxide synthase (iNOS) (3). In addition, macrophages mediate innate resistance to host infection by intracellular pathogens such as Mycobacterium, Salmonella, and Leishmania. This is controlled by a single dominant gene termed natural resistance-associated macrophage protein 1 (Nramp1) also known as solute carrier family 11 member a1 (Slc11a1) or Iiy/Lsh/Bcg. Nramp1 demonstrates high similarity to Nramp2, which encodes a membrane-bound protein that functions as iron, and other divalent cations that are transporters into the cell cytoplasm (4). Based on the high identity between these two Nramp members, it was shown that Nramp1 is also a proton/divalent cation antiporter with a unique role in innate resistance to intraphagosomal pathogens and autoimmune disease (for review see Refs. 5–7). The exact function of Nramp1 as iron and divalent cation transporter is still controversial. It either functions to increase transphagosomal Fe2+ catalyzing the Haber-Weiss/Fenton reaction to generate the highly toxic hydroxyl radical essential for macrophage bactericidal activity. On the other hand, it is thought to deprive the intraphagosomal bacteria of availability of Fe2+ and other divalent cations, which are critical for the ability of the invading pathogens to survive the phagosomal damage (5). Unlike the ubiquitous expression of Nramp2, Nramp1 is exclusively expressed in monocyte/macrophage cells (8). In addition, in response to the invading pathogen, activated macrophages secrete proinflammatory cytokines such as IL-12 and IL-18 that recruit the cell-mediated adaptive immunity, e.g. Th1 cells (9–11). It is, therefore, not surprising that, among the many transcription factors that participate in these well coordinated macrophage activities against intracellular pathogens, IRF-8 is alternatively termed as interferon consensus sequence binding protein (ICSBP).

Mice with null mutation to IRF-8 are defective in the differentiation of bone marrow myeloid progenitor cells toward mature macrophages (12). Therefore, these defective mice over-produce granulocytes, which eventually lead to lymphadenopathy and hepatosplenomegaly resembling chronic myelogenous leukemia syndrome in humans (13). Thus, IRF-8 is an essential
factor for the differentiation of bipotential myeloid progenitor cells toward mature macrophages while inhibiting the differentiation pathway toward granulocytes (14). In addition, it was shown that IRF-8 is an essential factor for proper functioning of mature macrophages. For example, it is essential for the regulated expression of specific phagosome components like phagocyte oxidase complex (gp91^{phox} and p67^{phox} (15), iNOS (16), and Nramp1 (as shown here)). Furthermore, it is an essential regulatory element for the production of the proinflammatory cytokines IL-12 (17), IL-18 (18), and IL-1β (19).

IRF-8 belongs to a family of nine cellular members all sharing significant similarity at the N-terminal 115 amino acids, which comprise the DNA binding domain (DBD). This DBD binds to specific DNA sequence motif termed IFN-stimulated response element, which mediates in part IFN type I signaling (20). Unlike other IRF members, IRF-8 is capable of binding to target DNA sequence only following association with other IRF or non-IRF transcription factors. The interaction with non-IRF members, such as PU.1, leads to the binding of the heterocomplex to a DNA composite element of which half is an IRF binding site and half is the DNA binding site for the interacting partner. The domain essential for these protein-protein associations was mapped and found to be conserved among all other IRF members, excluding IRF-1 and IRF-2, which associate with IRF-8. This module was termed IRF association domain (IAD) and demonstrates structural similarity with the MH2 domain of transcription factors, which mediate transforming growth factor-β signaling also through protein-protein interactions (21). This region has several α helix structures of which the α helix structure surrounding leucine 331 of IRF-8 is highly conserved and is essential for protein-protein interaction. The association modules of IRF-1, IRF-2 and PU.1, which interact with IRF-8, were identified and found to be a PEST domain, which is enriched with proline, glutamic acid, serine and threonine (for review see Ref. 20).

The interacting partner with IRF-8 dictates not only the DNA binding site but also the transcriptional activity, e.g. activation or repression. Interaction with non-IRF factors primarily leads to transcriptional synergy, whereas interaction with IRF members such as IRF-1 or IRF-2 primarily leads to transcriptional repression (20). The stoichiometry between the interacting partners was not determined, and it is possible that several PEST domains can interact with an IAD as observed for several PEST domains can interact with an IAD as observed for IRF-1, IRF-2 and PU.1, and IRF-8 were reported.

Yeast two-hybrid screen with the IAD of IRF-8 as bait was used to search for new interacting partners for IRF-8. This search led to the identification of the association of IRF-8 with subunit 2 of the COP9/signalosome complex (22). The interaction with COP9/signalosome leads to the phosphorylation of serine 260 within the IAD of IRF-8. This phosphorylated residue within the IAD is essential for efficient interaction between IRF-8 and IRF-1 or IRF-2. The data collected from such screen suggested that IRF-8 is engaged in numerous interactions with additional transcription factors.

In this communication, we report the cloning of Myc interacting zinc-finger protein 1 (Miz-1) as a new interacting partner with IRF-8 identified in yeast cells. This interaction could be demonstrated only in mammalian cells of hematopoietic origin. Furthermore, Nramp1 was identified as the target gene synergistically activated by Miz-1 and IRF-8. This synergistic activation is further enhanced by PU.1. Thus, our results lay the molecular basis for the regulated expression of Nramp1 in macrophages and explain in molecular terms the role of IRF-8 in conferring innate resistance to intracellular pathogens.
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Experimental Procedures.

Fig. 1. Coimmunoprecipitation studies and mammalian two-hybrid assays reveal specific interaction between IRF-8 and Miz-1. COS-7 cells were transfected, and coimmunoprecipitation studies were performed as described under “Experimental Procedures.” Briefly, cells were transfected with expression vectors encoding to either ICSBP (7 µg) or Miz-1 (20 µg) or both as indicated. 48 h later the equivalence of ~10^6 cells was lysed for each lane. The cleared lysates were either used immediately (A) or incubated for 20 min at 25 °C with 100 µl of cleared lysate from 3 × 10^7 HL-60 cells (C). 30 µl of the lysates were taken for direct Western blot analysis with antibodies directed against ICSBP (A and C, lane 1) or antibodies directed against Miz-1 (A and C, lanes 5 and 6). For coimmunoprecipitation (IP) the lysates were incubated for 16 h at 4 °C with 25 µl of packed protein A-Sepharose beads, which were prewashed with 3 µg of antibodies against Miz-1. Following the incubation period, the beads were washed extensively, and all samples were loaded on 10% SDS-PAGE and subjected to Western blot (WB) analysis with antibodies against ICSBP (A and C, lanes 2–4). NS, nonspecific band. Mammalian two-hybrid assays were performed in NIH3T3 cells (B) or U937 cells (D). NIH3T3 were cotransfected with 2 µg of pGAL4 × 5-Luc, 0.1 µg of GAL4 Miz-1 (lane 1), 0.36 µg of ICSBP-VP16 (lane 2), or both (lane 3). U937 cells were cotransfected with 20 µg of pGAL4 × 5-Luc, 5 µg of GAL4 Miz-1 (lane 1), 18 µg of ICSBP-VP16 (lane 2), or both (lane 3). 24 h later cells were lysed, and relative luciferase activities were determined and calculated as fold of activation as detailed under “Experimental Procedures.” The fold of synergism observed in U937 in comparison to NIH3T3 was calculated as described under “Experimental Procedures” with a statistical significance of p < 0.05 using Student’s t test.

Northern Blot Analysis—Total RNA was extracted from CL2 and J477A.1 cell lines using Tri-Reagent (Sigma) according to the manufacturer’s instruction before and 5 h after exposure to 100 units/ml IFN-γ (PeproTech Inc., Rocky Hill, NJ) and 50 ng/ml LPS (Sigma). Northern blot analysis was performed as previously described (32) with 32P-labeled probes corresponding to a 600-bp HindIII and BamHI fragment from the coding region of murine Nramp1 and a 262-bp PvuII-BglII fragment corresponding to the C-terminal half of human ICSBP (33).
RESULTS

Yeast Two-hybrid Screens Demonstrated a Specific Interaction between IRF-8 and Miz-1—IRF-8 interacts with different transcription factors such as IRF-1, IRF-2, PU.1, and E47 through the IAD (for review see Ref. 20). To identify other possible interacting factors with IRF-8, yeast two-hybrid screens were employed. The full-length IRF-8 missing only the first 33 amino acids and the IAD of IRF-8 (amino acids 201–377) were fused to the DNA binding domain of the yeast transcription factor GAL4 and used as baits against a human B-cells cDNA library fused to the activation domain of GAL4 (for details see “Experimental Procedures”). Yeast HFT7C cells were transformed with the bait constructs, and the resulting cells were transformed with the human B-cells library (26). Four interacting clones were identified among which was a truncated form of Miz-1 (accession number Y09723) (34). This truncated Miz-1, missing its 357 N-terminal amino acids (Miz1ΔN357), demonstrated strong association with the full-length IRF-8 and moderate association with just its IAD in the yeast cells. Support for the specificity of interaction between IRF-8 and Miz1ΔN357 stems from the fact that a mutated IAD of IRF-8, in which leucine 331 was mutated to proline (L331P), was incapable of interacting in this assay. This mutation is at a conserved leucine in a predicted α-helix structure in the IAD, essential for the interaction with other factors (28, 35). No interaction with Miz1ΔN357 was observed if either the IAD of IRF-8 or the full factor were swapped with the corresponding segments of IRF-4, the closest homologue of IRF-8, and IRF-7. These data further demonstrate the specificity of interaction between IRF-8 and Miz-1 in yeast cells.

The Interaction between IRF-8 and Miz-1 Is Identified Only in a Cell Line of Hematopoietic Origin—To show that the interaction between IRF-8 and Miz-1 takes place in mammalian cells, commounprecipitation assays were performed in COS-7 cells that allow overexpression of these two factors. Both full-length IRF-8 and Miz-1 were cloned under the cytomegalovirus promoter, and their expression in transfected cells was readily detected by Western blotting (Fig. 1, A and C, lanes 1 and 6, respectively). However, when Miz-1 was immunoprecipitated, no coprecipitation of IRF-8 could be detected (Fig. 1A, lane 2). In addition, lanes 3 and 4 served as controls for the specificity of the commounprecipitations, and lane 5 demonstrates that coexpression of IRF-8 had no effect on ectopic expression of Miz-1. Expression of just the truncated form of Miz-1 (Miz1ΔN357) did not lead to coprecipitation of IRF-8 as was expected from the yeast two-hybrid assays (data not shown).

Therefore, we decided to detect possible interaction between these two factors using mammalian two-hybrid assay. For that purpose, the C terminus of IRF-8 was fused to the herpes simplex virus VP16 transactivation domain (IRF-8-VP16) (30), and the mammalian expression vector encoding to this chimeric factor was cotransfected with a luciferase reporter gene driven by five repeats of the GAL4 binding site (pGAL4×5-Luc) into the fibroblast cell line NIH3T3. As expected, this chimeric IRF-8 was not able to induce the reporter gene (Fig. 1B, column 2). In addition, the DBD of the yeast transcription factor GAL4 was fused to the N terminus of Miz-1 and was cotransfected with the same reporter plasmid. This lead to a 25-fold increase in the level of the reporter gene indicating that as expected this GAL4-Miz-1 chimeric construct acted as a transcriptional activator (Fig. 1B, column 1). When both GAL4-Miz-1 and IRF-8-VP16 were cotransfected together with the reporter gene, no further induction of the reporter activity was observed, strongly suggesting that the two factors do not interact in the transfected cells (Fig. 1B, column 3). Because IRF-8 is a myeloid-specific factor, we repeated these experiments in the promyelocytic cell line U937. In this cell line, the GAL4-Miz-1 chimeric construct induced the reporter gene expression by 19-fold, but together with IRF-8-VP16 the induction was almost 50-fold (Fig. 1D, columns 1 and 3, respectively). These results strongly suggest that Miz-1 and IRF-8 lead to a statistically significant synergistic effect in this cell line as calculated in Fig. 1E.

Thus, interaction between IRF-8 and Miz-1 might occur only in cells of hematopoietic origin. This suggests that these factors either have undergone specific modifications that occur only in immune cells or interact with a third-party element present only in such cells. To test this, communprecipitation experiments were performed as described in Fig. 1A except that, prior to the precipitation step, the COS-7 cell extracts were incubated first with cell extract from the promyelocytic cell line HL-60, which does not express detectable levels of IRF-8. As seen in Fig. 1C, under these conditions a weak yet reproducible band of IRF-8 coprecipitating with Miz-1 is detected (Fig. 1C, lane 2).

IRF-8 Represses Miz-1 Activity on a Synthetic Promoter—Because no target promoter for both IRF-8 and Miz-1 was previously reported, we decided to test the effect of these factors on a synthetic promoter composed of five GAL4 binding sites driving the expression of the firefly luciferase reporter (pGAL4×5-Luc). The full-length Miz-1 and IRF-8 were fused to the DBD of GAL4 (see schematic in Fig. 2A), and their effect on the reporter gene activity was tested. It is clear that GAL4-Miz-1 strongly activated this synthetic promoter (Fig. 2, B and C, lanes 4 and 2, respectively), whereas increasing amounts of GAL4-IRF-8 (Fig. 2B, columns 5–9) or just GAL4 DBD alone (Fig. 2B, columns 2 and 3) had no significant effect. However, cotransfection of increasing amounts of GAL4-IRF-8 with GAL4-Miz-1 leads to repression of the reporter gene that was not observed when increasing amounts of just GAL4DBD were cotransfected (Fig. 2B, compare columns 10–14 to 15–19, respectively). Similar results were observed with the truncated form of Miz-1, Miz1ΔN357, although the reporter gene activity was significantly and reproducibly stronger (data not shown). This repression effect of GAL4-IRF-8 could not be attributed to a squelching effect due to competition between the two chimeric factors on the same DNA binding sites, because the repression effect was noted even under very low transfection ratios of IRF-8 versus Miz-1. The actual level of expression of the various transfected factors was determined by Western blot analyses with antibodies directed against the GAL4 DBD (data not shown). To test if this inhibitory effect of IRF-8 is due to interaction with Miz-1, a mutant IRF-8, in which leucine residue 331 was mutated to proline, was used. As shown in Fig. 2C, similar amounts of GAL4-IRF-8-L331P did not suppress the Miz-1-mediated activation of the reporter gene (Fig. 2C, columns 15–17). On the other hand, mutation of serine 260 to alanine (S260A) had no effect on the ability of this factor to suppress Miz-1 activity (Fig. 2C, columns 18–20). This mutation affects the ability of IRF-8 to interact with IRF-1 and IRF-2 but not with PU.1 and E47 (22). All together, these results imply that IRF-8 is capable of specific repression of Miz-1 probably through protein-protein interaction when the factors are artificially forced to interact on the same synthetic promoter through the GAL4 DBD.

Nramp1 but Not p15ink4b Is a Target Gene for Both Miz-1 and IRF-8—Two genes, p15ink4b (36) and Nramp1 (25), were recently reported as target genes for Miz-1. We tested if these genes are also coregulated by IRF-8. For that purpose, the promoter regions of these two genes were fused to the firefly luciferase reporter gene. Because IRF-8 is hematopoietic-specific the reporter gene assays were performed in the promyelo-
cyclic cell line U937. We first tested the p15<sup>ink4b</sup> promoter and in contrast to Miz-1, which induced this promoter following cotransfection, IRF-8 had no effect on the reporter gene activity in U937 cells (Fig. 3A). Furthermore, cotransfection of IRF-8 and Miz-1 had no additional effect on the transactivation of p15<sup>ink4b</sup> reporter gene by Miz-1 alone suggesting that IRF-8 does not interact with Miz-1 on this promoter.

We next tested the Nramp1 promoter in U937 cells. As seen in Fig. 3B, transfected Miz-1 activated this promoter, whereas IRF-8 alone had no effect. However, cotransfection of both Miz-1 and IRF-8 lead to synergistic activation of the reporter gene (Fig. 3, column 5). No synergistic activation of the reporter gene was noted when IRF-8 L331P mutant was used instead of IRF-8 expression vector. Furthermore, when a GAL4 IRF-8 defective in its DBD was cotransfected, no synergistic activation of Miz-1 was observed. This suggests that intact DBD and IAD of IRF-8 are essential for the association with Miz-1.

This synergistic effect between IRF-8 and Miz-1 was specific to U937 cells and was not detected in NIH3T3 cells (Fig. 3C).

Taken together, our data presented thus far indicate that synergistic interaction between Miz-1 and IRF-8 takes place in cells of hematopoietic origin and lays the basis for the macrophage-restricted and IFN-γ/H9253-induced expression of Nramp1.
essential for the regulated expression of NRAMP1 with the hematopoietic-specific factors IRF-8 and PU.1 are expected, each factor alone had moderate effect on the reporter activity (Fig. 4A). However, when all three factors were cotransfected, a strong synergistic activation of the reporter gene was observed (Fig. 4A, column 7). This suggested that Miz-1 together with the hematopoietic-specific factors IRF-8 and PU.1 are essential for the regulated expression of NRAMP1.

These results also suggest that, in addition to IRF-8, the missing component in non-hematopoietic cells, such as NIH3T3, might have been PU.1. To test this, the same cotransfection experiments were performed in NIH3T3 cells. As expected, NIH3T3 cells (B) were cotransfected with pGL3-NRAMP1 (1.5 µg), and expression vectors encoding for Miz-1 (0.25 µg) or ICSBP (1.5 µg) or PU.1 (1.5 µg) as indicated. Luciferase activity was determined and normalized to transfection efficiency as described under “Experimental Procedures.”

DISCUSSION

NRAMP1 encodes a divalent ion transporter expressed exclusively in macrophages and confers resistance to intracellular pathogens. This divalent iron transporter is expressed in late endosomes/lysosomes of these cells and constitutes an important component of innate immunity. NRAMP1 is induced by these pathogens that can be experimentally mimicked by treating cells with IFN-γ and LPS (Fig. 5, lanes 1 and 2, respectively). On the other hand, induction of aberrant mRNA corresponding to NRAMP1 was noted in the CL-2 cells indicating that these cells responded to IFN-γ and LPS stimulation. However, the open reading frame of this message is disrupted, thus no protein is translated (13). Altogether, our results strongly suggest that IRF-8 is a key player in the expression of NRAMP1 in mature macrophages and its activation in response to IFN-γ and LPS.

NRAMP1 Expression Is Not Induced by IFN-γ and LPS in Macrophage Cell Line Derived from IRF-8 Null Mice—The data presented here suggest that the regulated expression of NRAMP1 in hematopoietic cells is due to a synergistic activation mediated by the macrophage-specific transcription factors PU.1 and IRF-8 together with the ubiquitous transcription factor Miz-1. Because IRF-8 is an essential factor in macrophage maturation, and because NRAMP1 expression is restricted to these cells, we wanted to see if IRF-8 is essential for NRAMP1 expression. Total RNA was extracted from the macrophage cell line J774A.1 representing a wild type mice and from a CL-2 macrophage cell line that was derived from IRF-8 null mice (17) before and after 5 h of treatment with 100 units/ml of IFN-γ and 10 mM LPS. As seen in Fig. 5, basal levels of NRAMP1 were detected in J774A.1 cells that were further induced by IFN-γ and LPS treatment. Similarly, and as previously reported, mRNA corresponding to IRF-8 was also induced (Fig. 5, lanes 3 and 4, respectively) (39). In contrast, the mRNA levels corresponding to NRAMP1 were very low in the CL-2 cell line derived from IRF-8−/− mice and that low basal expression was not further induced by treating the cells with IFN-γ and LPS (Fig. 5, lanes 1 and 2, respectively).
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IRF-8 is able to compete for this synergistic interaction. Furthermore, a dominant negative construct of which do not interact with any tested factor, do not synergize transfected. In addition, mutant IRF-8 L331P within the IAD, takes place in NIH3T3 cells only when both IRF-8 and PU.1 are non-hematopoietic cells is PU.1. In support of this is the fact these mice are devoid of IL-12 and thus defective in the induction of this gene suggest an additional mechanism for the enhanced sensitivity of these mice. This sensitivity to intraphagosomal pathogens is in addition to the fact that these mice are devoid of IL-12 and thus defective in Th1-mediated immunity.

Nramp1 is mainly expressed in macrophages, and our data imply that in addition to IRF-8 the missing component in non-hematopoietic cells is PU.1. In support of this is the fact that strong synergistic induction of the Nramp1 promoter takes place in NIH3T3 cells only when both IRF-8 and PU.1 are transfected. In addition, mutant IRF-8 L331P within the IAD, which do not interact with any tested factor, do not synergize with Miz-1. Furthermore, a dominant negative construct of IRF-8 is able to compete for this synergistic interaction. Finally, the constitutive level of Nramp1 in macrophage cell line from IRF-8−/− mice is very low and could not be induced by IFN-γ and LPS. These results together with our communoprecipitation and mammalian two-hybrid studies point to the role of IRF-8 in the basal expression level of Nramp1 in macrophages and its inducibility following activation by pathogens. IRF-8 exerts its activity through the association with other transcription factors. This is mediated via the conserved IAD that leads to the formation of heterocomplexes with other IRF members such as IRF-1 and IRF-2 and non-IRF members such as PU.1 and E47. These factors harbor a PEST domain, which facilitates protein-protein interaction (20). Following protein-protein association the formed heterocomplex can bind to the target DNA sequence, which is a bipartite DNA binding site. Half of it is an IRF-8 binding site, and half is the DNA binding site of the interacting partner (45). In many of the reported cases, the interaction is mediated between the IAD of IRF-8 and a PEST domain of the interacting partner. The stoichiometry of these interactions is not characterized, and multicomponent complexes composed of IRF-8, IRF-1, and PU.1 and in some cases also IRF-4 were reported (46). Here we show that IRF-8 can interact with Miz-1 in yeast cells and that this interaction can be demonstrated in a hematopoietic cell line or in extracts of COS-7 cells transfected with expression vectors encoding for the two factors only if supplemented with extract from promyelocytic cell line HL-60, which do not express IRF-8 (32). According to the algorithm developed by Rogers and Rechsteiner (47), Miz-1 harbors two PEST domains at positions 198–228 and 251–285. However, these two PEST motifs were missing in the original Miz-1 clone identified by yeast two-hybrid screening. This truncated Miz-1, missing its first 357 amino acids, still interacts with IRF-8 using reporter gene assay. In this system the truncated factor alone acts as a very strong activator. This suggests that the region encompassing the POZ domain, the PEST domains, and the first 3 zinc fingers (out of 12 in a row) exert an inhibitory effect on Miz-1 activity. The data imply that the association of Miz-1 with IRF-8 and PU.1 leads to conformational change that relieves this inhibitory effect of the N-terminal part of Miz-1 leading to a maximal transcriptional activation. Similar conformational changes were speculated for IRF-4 following the interaction with PU.1 thus alleviating some of its autoinhibitory activities (48, 49). It is possible that the PEST domains are involved in multicomponent protein-protein interactions that also include PU.1 leading to the alleviation of the partial autoinhibitory effect of the N terminus of Miz-1 and subsequent strong activation.

Nramp1 promoter is TATA box-deficient and instead it harbors two initiator elements to which Miz-1 can bind. Such elements were reported for other TATA box-deficient genes such as p15ink4b (24), p21cip1 (50), Mad4 (51), and more. The basal expression of such promoters is mediated by Miz-1, which can be repressed through protein-protein interaction with the proto-oncogene c-myc. Here we show that the interaction of IRF-8 with Miz-1 is dependent upon the promoter context through upstream elements. Consequently, a Miz-1 basal promoter reporter construct, pHB8, containing only the two initiator sites and the adjacent upstream SP1 site (25) does not respond significantly to either IRF-8 or PU.1.2 Fitting with this is the fact that IRF-8 has no effect on the induced expression of the p15ink4b promoter in U937 cells. Furthermore, when both Miz-1 and IRF-8 were fused to the GAL4 DBD, the chimeric GAL4 Miz-1 was able to induce the reporter gene as expected. However, coexpression of GAL4 IRF-8 lead to the repression of this synthetic promoter. This repression was not due to squelching effect exerted by the two fusion factors competing on the same DNA binding sites on the synthetic promoter but rather through cooperative interaction that leads to a strong repression. This is supported by the fact that coexpression of either GAL4 DBD alone or IRF-8 harboring the L331P point mutation were not able to repress the GAL4 Miz-1-mediated activation. The GAL4 synthetic promoter can not only avoid of PU.1 binding sites, and this might be the reason for the repression exerted by IRF-8. On the other hand, the Nramp1 promoter, unlike p15ink4b promoter, harbors a computer-predicted PU.1 binding site that leads to the assembly of transcriptional activation complex. Thus, the promoter context and the cellular milieu of transcription factors dictate which type of enhanceosome is assembled that is engaged in either repression or activation. Taken together, our results lay the molecular basis for the restricted expression of Nramp1 suggesting that in macrophages the assembled enhanceosome recruits specific transcription factors such as IRF-8 and PU.1, whereas in non-immune cells the assembled enhanceosome lacks these factors and thus repression occurs that might be mediated by other IRF members such as IRF-2.

In this communication, we provide evidence for the restricted regulation of Nramp1 by IRF-8. In humans, this divalent iron transporter is linked to several infectious diseases, including leprosy, pulmonary tuberculosis, visceral leishmaniasis, meningococcal meningitis, and human immunodeficiency virus as well as to autoimmune diseases such as rheumatoid arthritis and Crohn’s disease (7). Interestingly, all these diseases manifest type IV granulomatous hypersensitivity also know as de-

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