Intracellular Interleukin-1α Functionally Interacts with Histone Acetyltransferase Complexes*¶

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Miroslava Buryšková, Martin Pospíšek, Arnhild Grothey‡, Thomas Simmet†, and Ladislav Buryšek‡‡

From the 1Department of Pharmacology of Natural Products and Clinical Pharmacology, University of Ulm, D-89081 Ulm, Germany and the 2Department of Genetics and Microbiology, Charles University, 12844 Prague, Czech Republic

Interleukin-1α (IL-1α) is an inflammatory cytokine acting extracellularly via membrane receptors. Interestingly, a significant portion of synthesized IL-1α is not secreted; instead, it is actively translocated into the cell nucleus. IL-1α was indeed shown to be involved in certain intracellular processes, such as control of proliferation, apoptosis, or migration, however, the mechanisms of such actions are not known. Here we show that intracellular IL-1α fused to the Gal4p DNA-binding domain (Gal4BD) possesses strong transactivation potential that can be boosted by overexpression of the transcriptional coactivator p300. We demonstrate that the IL-1α precursor interacts via its N-terminal peptide (IL-1NTP) with histone acetyltransferases p300, PCAF, Gcn5 and with the adaptor component Ada3, and that it integrates into the PCAF/p300 complex in a non-destructive manner. In analogy with known acidic coactivators, yeast strains expressing Gal4BD/IL-1NTP display a toxic phenotype that can be relieved by depletion of various components of the SAGA complex. Our data provide the first solid evidence for the nuclear target of the IL-1α precursor and suggest its novel function in transcriptional control.

The constantly growing interleukin-1 (IL-1) family currently consists of eight different protein ligands, and as much as 10 surface or soluble receptors (1, 2). The IL-1 system represents an evolutionary conserved signaling mechanism homologous to the Drosophila Toll pathway, being a central mediator of the host innate defense mechanisms (3, 4). IL-1 proteins are expressed by most of the cell types, and they act as the first alarming signals inducing release of the full set of proinflammatory molecules such as prostaglandins, tumor necrosis factor α, IL-6, chemokines, proteins of the acute phase, and IL-1 per se (5).

IL-1α is translated as a 33-kDa precursor, which is proteolytically processed into mature IL-1α (IL-1MAT) and the IL-1α N-terminal peptide (IL-1NTP) (6). For many years, the striking IL-1NTP sequence homology among different species fostered speculations about its putative biological function. The nuclear localization signal (NLS), present in the IL-1NTP molecule, was shown to be essential for the biological activity of intracellular IL-1α (7, 8). Indeed, overexpressed intracellular IL-1α precursor (pre-IL-1α), but not IL-1MAT, was able to inhibit cell growth and to induce the expression of the plasmogen activator inhibitor-1 and collagenase genes (9, 10). Pre-IL-1α was shown to stimulate proliferation of smooth muscle cells (11) and to regulate the migration and the lifespan of endothelial cells (12, 13). Thus, various effects of pre-IL-1α are found to be dependent on the presence of the acidic IL-1NTP fragment with the predicted pI 5.1. Stevenson et al. (14) have shown that IL-1NTP overexpressed in mouse 3T3 fibroblasts behaves as a nuclear oncoprotein and induces their transformation (14). IL-1NTP was also found to be specifically associated with the novel protein HAX-1 that has been implicated in modulating apoptosis and cytoskeletal functions (15). Recently, a strong proapoptotic effect of ectopically expressed IL-1NTP in numerous human tumor cell lines has been reported (16). Taken together, several lines of evidence point to a role of IL-1NTP in certain yet unknown nuclear processes.

Initiation of transcription in eukaryotes is mediated by recruitment of different multiprotein complexes to the promoter sequence involving the RNA polymerase II complex, general transcription factors and transcriptional cofactors, histone acetyltransferase (HAT) complexes, and sequence-specific transcription factors (17, 18). The sequence-specific transcription factors are often composed of separate DNA-binding and activation domains (19, 20). Depending on the amino acid composition, three types of activation domains are recognized: glutamine-, proline-, and acidic-rich domains. The transcription factors containing the acidic-rich activation domain, such as yeast Gal4p, viral proteins VP16 and E1A, and human c-Jun and NF-κB RelA(p65), are evolutionary well conserved, and are able to activate transcription in Saccharomyces cerevisiae as well as in mammalian cells (21–25). Moreover, the acidic transcription activators interact with different types of HATs and adaptor proteins, and their overexpression in yeast cells is often associated with growth inhibitory phenotypes (26, 27).

The eukaryotic chromatin structure with DNA wrapped around histone proteins forming a repeating array of nucleosomes leaves promoter DNA sequences inaccessible to the transcriptional machinery (28). Therefore, the chromatin structure must be remodeled prior to gene targeting (17). Up to date, a number of different factors with chromatin remodeling activities have been identified (29). The fact that histone hyperacety-
loration suggests that histone acetylation is one of the chromatin re-modelling mechanisms important for transcription initiation (30). The finding that HAT activities are intrinsic to the transcriptional coactivators p300/CBP, PCAF, and Gen5, interacting with a number of sequence-specific transcription factors, confirmed the functional link between histone acetylation and transcriptional control (31). However, HATs cannot acetylate histones in the chromatin context per se, they can do so only in context of multiprotein HAT complexes where they interact with adaptor proteins facilitating the accessibility of the histone tails to HAT catalytic domains. Two distinct HAT complexes have been characterized so far in yeast, SAGA and ADA (32, 33). Mass spectrometric analysis has revealed that the human PCAF complex involves, besides transcriptional coactivator CBP/p300 and others, human counterparts of yeast HAT cofactors Ada2, Ada3, and Spt3 (34). This mega-dalton complex is composed of more than 14 different subunits that seem to be organized in the three functionally divergent modules responsible: (i) for HAT activity, (ii) for interaction with the sequence-specific transcription factors, and (iii) for association with the preinitiation complex containing TATA-binding protein-associated factors and RNA polymerase II (35). Therefore, the HAT complex represents a physical bridge between distant modulated activators and the transcriptional machinery at the core promoter (36).

In this study we have investigated functions of the intracel-lular forms of IL-1α. We have found that when fused to Gal4BD, IL-1NTp acts as an activator of transcription in both yeast and mammalian cells. The transcriptional activation requires an intact SAGA complex in yeast and the transcriptional coactivator p300 in mammalian cells. We also demonstrate that IL-1NTp physically interacts with mammalian histone acetyltransferases Gen5, PCAF, and p300, and with adaptor protein Ada3 in vitro. In coimmunoprecipitation experiments we show in detail the specific interactions between intracellular forms of IL-1α and different histone acetyltransferases in vivo.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains**—The yeast strains AH109 (MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4, LYS2::GAL1-TATA:: GAL2–AD, HIS3, GAL2–AD::GAL2–TATA::ADE2, URA3::MEL1–AD::MEL1–TATA:: lacZ) was from the Matchmaker Two-hybrid System 3 (Clontech). The strains H-224 (MATa, ade2, his3, leu2, trp1, ura3, 1ac1; LEU2), YMH171 (MATa, ura3–52, leu2-3, 112, his3, trp1) and its isogenic derivatives YMH511 (gen5a; his3O), YMH 535 (ads2a; his3O), YMH537 (ada3a; his3O), and YMH567 (spa7a; LEU2) were kindly provided by M. Hampsey (37). The expression level of Gal4BD fusion proteins was detected using rabbit polyclonal antibody against Gal4BD (sc-729; Santa Cruz Biotechnology).

**Plasmids**—Mouse IL-1p/plXSN construct was a gift from R. Apte (38). Yeast Gal4BD expression vectors pAS2-1 and pGBK7-T7, as well as pGBK7-T7 and pGAD77-T constructs were obtained from Clontech. Mammalian Gal4BD expression vector pSG424 was a gift from M. Ptashne (39). The GST expression plasmid pGEX4T was from American Biosciences. The Gal4BD/VIP16 (412–490) yeast vector protein pM1358, and the reporter plasmids pN927 (USALgal–TATA::gal– lacZ [TA/G12Z], pN928 (USALgal–TGA::gal– lacZ [TG/G12Z]) were provided by M. Hampsey (37).

The cDNAs encoding the mouse IL-1α precursor (aa 1–270), IL-1NTp (aa 1–109), as well as its deletion mutants IL-1NTp-ATSSN (internal deletion aa 78–87), IL-1NTp-DESSS (terminal deletion aa 1–21), IL-1NTp-FTEDD (terminal deletion aa 97–109, and IL-1NTp-D1Y/F1T (terminal deletions aa 1–21 and 97–109) were amplified from the IL-1α/pLXSIN template by PCR and cloned into EcoRI and BamHI sites of pAS2-1, pGBK7-T7 vectors, or into BamHI and EcoRI sites of pGEX4T vector, or into BamHI and XbaI sites of the pSG424 vector. Human cDNA encoding the full-length IL-1α protein was amplified from peripheral blood mononuclear cells by reverse transcription-PCR and cloned into NcoI and BamHI sites of pGBK7-T7. This construct was sequenced and used as the template in all the following experiments of human IL-1NTp, mouse IL-1α, and IL-1β precursors proteins for subsequent cloning into EcoRI and BamHI sites of pGBK7 vector, BamHI and XhoI sites of pGEX4T vector, or into BamHI and XbaI sites of pSG424 vector. All the mouse and human IL-1α protein forms were subcloned into BamHI and XhoI sites of modified pcDNA4/TO/MycHis (Invitrogen) vector with added FLAG tag upstream of the multiple cloning site. The original vector contains Myc and His tags downstream of the cloned cDNA, therefore, all the IL-1α proteins were expressed as double tagged chimeras with N-terminal FLAG and C-terminal Myc and His tags. Fragments encoding human deletion mutants IL-1NTp-VVAT (internal deletion aa 78–87), IL-1NTp-DESSS (terminal deletion aa 1–19), and IL-1NTp-FTEDD (terminal deletion aa 98–111) were amplified and cloned into BamHI and XhoI sites of modified pcDNA4/TO/MycHis (see above) vector.

The E1A and HA-tagged human p300 expression plasmids were from A. Hecht (40). Plasmids encoding HA-tagged human p300 mutants were kindly provided by T. Fujita (41). Plasmids expressing N-terminal FLAG-tagged mouse PCAF and Gcn5 were a gift from T. Honjo (42) and the expression vector encoding HA-tagged human PCAF was kindly supplied by M. Ott (43). R. Brachmann provided us with the vector expressing the N-terminal FLAG-tagged human Ada3 (44), which was also used as a template for amplification and subcloning of Ada3 into pCMV-HA (Clontech) vector expressing HA-tagged human Ada3 protein. All amplifications were done using Pfu DNA polymerase (Stratagene) and products were verified by direct sequencing.

**Cell Culture and Luciferase Assay**—HEK293 cells from the American Type Culture Collection were grown in high-glucose Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Twenty-four hours before transfection, cells were plated in 24-well plates so that they were 80% confluent the next day. The cells were cotransfected with 0.6 μg of total amount of expression plasmid DNA mixture and pRLuc Gal4 reporter construct (Stratagene) per well using the Superfect transfection reagent (Qiagen) according to the manufacturer’s instructions. Empty vectors were used to adjust the amounts of DNA in each experiment. After 48 h cells were harvested and lysates were assayed for luciferase activity using the dual luciferase reporter assay (Promega) and the LDR 96-well plate luminometer (STRATAGENE, San Diego, CA, systems AG, Birkenfeld, Germany). Alternatively, the measured luciferase activities were normalized according to total protein concentrations. All experiments were performed in triplicates.

**Preparation of GST Fusion Proteins and Pull-down Assays**—Purification of recombinant proteins as well as pull-down assays were described previously (45). Briefly, GST fusion proteins (0.5 μg) bound to glutathione-agarose beads were incubated with 300 μl of whole cell lysates (see next chapter) from HA-tagged p300, PCAF, and Ada3 or FLAG-Gen5-transfected HEK293 cells in 700 μl of immunoprecipitated (IP) lysis buffer at 4 °C for 4 h. After three washes (10 min at 4 °C) with IP lysis buffer, a protein bound to the beads were resolved by Tris SDS-PAGE (8% gel), transferred onto nitrocellulose membranes (Bio-Rad), and probed with anti-FLAG (M2; Sigma), anti-HA (sc-805), or anti-RelB (sc-226; Santa Cruz Biotechnology) antibodies.

**Transient Transfection and Immunoprecipitation**—HEK293 cells were transfected in 10-cm Petri dishes using 10 μg of total plasmid DNA as described before. Forty-eight hours later the cells were washed with 0.3% phosphate-buffered saline and lysed in IP lysis buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1.5 mM MgCl2, 1 mM EDTA, 0.5% Nonidet P-40, 10% glycerol, Protease and Phosphatase Inhibitor Cocktails; Sigma) for 30 min on ice. After centrifugation, the supernatants were stored and the sediments containing nuclei and cell debris were extracted with buffer B (20 mM HEPES, pH 7.9, 420 mM NaCl, 1.5 mM MgCl2, 1 mM EDTA, 1 mg/ml protease inhibitor mixtures; Bio-Rad), and washed three times with IP lysis buffer and subjected to Western blot detection of bound material as described before.

**β-Galactosidase Assay**—Yeast strains harboring USALgal–lacZ reporter constructs were grown to late logarithmic phase, harvested by centrifugation, and resuspended in 200 μl of breaking buffer (100 mM NaCl, 0.1% Nonidet, 0.2% Triton X-100, and protease inhibitor mixture; Sigma). Cell extracts were prepared by vortexing with 0.5-mm glass beads four times for 30 s. Activities were determined from at least three independent clones using the luminescent β-galactosidase detection kit Galacto-Light (Tropix, Bedford, MA).
The plates were incubated as before. with vector expressing the mouse Gal4BD/IL-1NTP fusion protein (wt, YMH171) or the indicated mutant strains. Transcriptional activation complex is required for growth inhibition by IL-1NTP. As shown in Fig. 1B, strain YMH171 (wt) expressing Gal4BD/IL-1NTP grows markedly slower than the strain expressing Gal4BD only (control). However, the Gal4BD/IL-1NTP-induced growth inhibition was strictly dependent on the presence of the intact SAGA transcriptional activation complex. The growth inhibitory effect of IL-1NTP was completely relieved in yeast strains lacking one of the SAGA complex components: Gcn5, Ada2, Ada3, and Spt7. Surprisingly, deletion of the AHC1 gene product, a unique structural subunit of the yeast ADA transcriptional activation complex, did not relieve the Gal4BD/IL-1NTP-mediated growth inhibition. These data suggested specific interference of Gal4BD/IL-1NTP with certain components of the yeast SAGA complex as it has been described for the acidic coactivators Gal4p, E1A, or VP16 (26, 27, 46).

Similar results were obtained when identical yeast strains harboring the UAS<sub>GAL1-TATA</sub>GAL1-lacZ reporter and expressing Gal4BD, mouse Gal4BD/IL-1NTP, or the strong Gal4BD/VP16 activator were assayed for their respective β-galactosidase activities (Fig. 2B). The IL-1NTP-mediated β-galactosidase activities reached about 25% of activities stimulated by Gal4BD/VP16 in wild type strain YMH171 (Fig. 2B, wt). The gcn5Δ, ada2Δ, ada3Δ, and spt7Δ deletions severely reduced the activities of both IL-1NTP and VP16, an effect more pronounced for IL-1NTP than for VP16 (5-versus 4-fold reduction, respectively). The most severe reduction (20-fold for both IL-1NTP and VP16) was observed in the strain defective in expression of Spt7, the critical structural subunit of the SAGA complex. In agreement with previous results, ahc1Δ deletion had no impact on activities mediated by both VP16 (37) and IL-1NTP, suggesting that IL-1NTP acts via interaction with the SAGA, but not with the ADA transcriptional complex. Furthermore, the point mutation in the TATA core promoter element (UAS<sub>GAL1-TGTG</sub>Gal1-lacZ) abolished the transactivation potentials of both IL-1NTP and VP16 proteins (Fig. 2A), indicating that the IL-1NTP-mediated activation occurs specifically through a common RNA polymerase II activation mechanism.

Characterization of Domains in the IL-1NTP Molecule Responsible for the Transactivation Potential—Besides the well characterized NLS domain (aa 79–86) (7), our structure-prediction analyses identified two additional highly conserved acidic α-helices located at the N and C termini of the IL-1NTP molecule (aa 7–19 and 98–108, respectively). In a search for the structural motifs mediating the IL-1NTP transactivation potential, a set of deletion mutants fused to Gal4BD were prepared (Fig. 3A) and tested for their activities in the yeast reporter strain. The yeast strain AH109 was transformed with plasmid constructs encoding different Gal4BD/IL-1NTP deletion mutants. As shown in Fig. 3B, deletion of the NLS domain did not affect the transactivation potential of the Gal4BD/IL-1NTP, whereas deletions of either N- or C-terminal acidic helices markedly slowed the growth rate. Moreover, the combination of both N- and C-terminal deletions had deteriorating effects.
terminal helixes in the IL-1NTP molecule dramatically reduced Gal4AD/T-Ag together), and deletions of either one or both the positive control strain (expressing Gal4BD/p53 and expressing AH109 yeast strain were comparable with those in transformants as described under tosidase activities were measured in triplicate from three independent VP16 Gal4BD/VP16 (VP16) and assayed for mouse Gal4BD/IL-1NTP (IL-1NTP), or strong transcriptional activator (TG/G1Z) were transformed with the vectors expressing Gal4BD (Con), expressing only Gal4BD (Con/H9252), IL-1NTP. A TATA promoter element are required for the transactivation by IL-1NTP. A, yeast strains harboring reporter plasmids carrying either the TATA core promoter element (T/A/G1Z) or its TGTA derivative (T/G/G1Z) were transformed with the vectors expressing Gal4BD (Con), mouse Gal4BD/IL-1NTP (IL-1NTP), or strong transcriptional activator Gal4BD/VP16 (VP16) and assayed for \( \beta \)-galactosidase activities. B, the wild type (wt, YMH171) or the indicated mutant strains carrying the UAS\(_{GAL1} \)-TATA\(_{GAL1} \)-lacZ reporter were transfected with vector expressing only Gal4BD (Con), with vectors expressing mouse Gal4BD/IL-1NTP (IL-1NTP), or Gal4BD/VP16 (VP16) fusion proteins. \( \beta \)-Galactosidase activities were measured in triplicate from three independent transformants as described under “Experimental Procedures.”

impact on the growth potential on selection media. Analogously, the \( \beta \)-galactosidase activities in the Gal4BD/IL-1NTP expressing AH109 yeast strain were comparable with those in the positive control strain (expressing Gal4BD/p53 and Gal4AD/T-Ag together), and deletions of either one or both terminal helixes in the IL-1NTP molecule dramatically reduced \( \beta \)-galactosidase activities in the respective strains. Interestingly, the internal deletion mutants lacking the NLS domain (ATSSN and TSETS) showed enhanced activities that were as much as 2-fold higher than activities observed in the positive control strain (Fig. 3C). The expression levels of the corresponding Gal4BD fusion proteins in strains tested for growth on drop-out medium (Fig. 3B) and for \( \beta \)-galactosidase activities (Fig. 3C) are shown in Fig. 3D. Lower expression levels of Gal4BD/ATSSN and Gal4BD/TSETS reflect their higher transcriptional activities (Fig. 3C) and higher toxicities (data not shown), and further support the idea about the functional importance of both the IL-1NTP terminal helixes. These data indicate that the NLS domain is not required for the transcriptional potential of Gal4BD/IL-1NTP, and that its deletion results even in enhanced transcriptional activity of the chimera.

Gal4BD/IL-1NTP Transactivates the Gal4 Promoter in Mammalian Cells via Functional Cooperation and Physical Association with the Transcriptional Coactivator p300—Our data from the yeast model are consistent with those of other acidic transcriptional coactivators and support our hypothesis about a novel nuclear function of IL-1α. To determine the physiological role of the intracellular IL-1α, we further tested its transactivation potential as well as its ability to cooperate with different histone acetyltransferases in mammalian cells. HEK293 cells cotransfected with a reporter plasmid and plasmid constructs encoding the indicated Gal4BD fusion proteins, and/or the indicated cofactors were assayed for luciferase activities. As shown in Fig. 4A, the expression of Gal4BD/IL-1NTP alone increased the luciferase activity reached 55% of the value stimulated by the Gal4BD/p65 NF-κB activation domain fusion protein. Furthermore, it could be antagonized by coexpression of the adenoviral protein E1A that binds specifically p300, thereby demonstrating the specificity of cooperation between p300 and IL-1NTP.

In analogy to experiments with yeast, we wanted to map critical domains in the IL-1NTP molecule responsible for functional cooperation with the transcriptional coactivator p300. When the Gal4BD/IL-1NTP deletion mutants were used for cotransfections of the HEK293 cells together with p300, the resulting luciferase activities (Fig. 4B) were in analogy to the \( \beta \)-galactosidase activities observed in yeast (Fig. 3C). Both the NLS-deleted mutants (ATSSN and TSETS) showed markedly enhanced activities when cotransfected alone or together with p300 (Fig. 4B). One or double IL-1NTP terminal helix deletions showed dramatic reduction of the reporter activation, suggesting that both terminal helices are required for an efficient cooperation with p300. The residual activity of IL-1NTP terminal mutants indicates that it might cooperate also with some other transcriptional coactivators. These data indicate that IL-1NTP uses an evolutionary conserved mechanism for the interaction with the transcription activation machinery.

To test the dependence of the Gal4BD/IL-1NTP transactivation potential on known functional domains of p300, cells cotransfected with a reporter plasmid, Gal4BD/IL-1NTP expression plasmid, and with the set of constructs encoding the indicated p300 mutants were assayed for their luciferase activities. As illustrated in Fig. 5A, the p300 deletion mutants lacking N-terminal domains between amino acids 142 and 1241 were fully functional in transactivation of the Gal4 promoter (columns 3 and 4), as well as the 1–1946 mutant lacking the C-terminal part including the Gln-rich domain (column 6), which was shown to be an interface mediating the association with several transcription factors including IRF-3 (41). Also the functional deletion of the CH3 domain in the A143–957ΔZn1/2 mutant did not significantly abrogate the cooperation (column
Interestingly, even the Δ194–1572 mutant (column 5) lacking a large portion of the molecule including the HAT domain was able to effectively cooperate with Gal4BD/IL-1NTP. However, two different mutants containing enzymatically inactive HAT domains did not enhance the transactivation potential of Gal4BD/IL-1NTP (columns 9 and 10). The significance of the HAT domain is further stressed by the data obtained with the minimal (six) amino acid mutant Δ143–957MutAT2 with crippled HAT activity (47), whose counterpart with the wild type HAT domain was highly active (Fig. 5A, columns 3 versus 10). The enhanced activity of the Δ143–957 mutant over full-length p300 was probably due to a higher expression level (Fig. 5B). To our surprise, the 1235–2412 mutant containing functional HAT as well as the Gln-rich domains was not active until the C-terminal part of the molecule was removed, which led to an extremely high activity (Fig. 5A, columns 7 versus 8); yet, the expression levels of both chimeras were similar (Fig. 5B, lanes 7 and 8).

We further addressed the question whether IL-1NTP can physically interact with the transcriptional coactivator p300. Therefore, we performed a set of GST pull-down experiments where beads with immobilized recombinant GST/IL-1NTP protein were incubated with input cell lysates expressing the indicated HA-tagged p300 and its mutants used in the luciferase assay (Fig. 5C, upper panel). As expected, the GST/IL-1NTP chimera bound full-length p300 as well as all the mutants that enhanced transactivation of the Gal4 promoter (Fig. 5C, center panel, lanes 2–5, 8, and 11). It also bound both mutants with inactive HAT domains, suggesting that HAT activity is not required for association with IL-1NTP (lanes 9 and 10). However, a strong interaction was also detected for 1235–2412 that did not cooperate in the luciferase assay (lane 7), whereas no interaction could be detected for 1–1946 (lane 6) that efficiently cooperated with the IL-1NTP in luciferase assay. The Gln-rich domain is therefore required for the in vitro interaction with IL-1NTP, as was reported for IRF-3 (41). The seeming discrepancies between the data from luciferase assays and pull-down experiments are probably because of the complexity of molecular interactions of the components of the p300-PCAF complex. These data also point toward a redundancy of histone acetyltransferases in the preinitiation complex and suggest a cooperation of Gal4BD/IL-1NTP with other additional cofactors.

The Intracellular IL-1α Cooperates and Physically Interacts with Different Subunits of the p300-PCAF Complex—To assess a possible contribution of other histone acetyltransferases ho-
detectable interaction with the GST control or with GST/IL-1α, but considerate amounts of all the transcriptional cofactors tested were associated with mouse and human GST/IL-1NTP. Interestingly, the weakest binding with GST/IL-1NTP proteins was detected in the case of p300, which was found to be a critical cofactor indispensable for transcription activity of the Gal4BD/IL-1NTP chimeras. Both GST/IL-1NTP proteins interacted with equal affinities with Gcn5, PCAF, and Ada3. However, because in the cell lysates also endogenous histone acetyltransferases are present, we cannot distinguish between direct interaction of GST/IL-1NTP with p300, PCAF, and Ada3, and indirect interaction mediated through binding with Gcn5, which could associate with the mentioned cofactors (48). As a negative control, an identical pull-down experiment was performed and probed with anti-RelB antibody. In this case, no specific interaction between GST/IL-1α proteins and the RelB transcription factor could be detected.

The Intracellular IL-1α Associates with Subunits of the p300-PCAF Complex in Vivo—Previous experiments indicated that intracellular IL-1α could be involved in some aspects of transcriptional control, participating probably in a formation and/or a function of the transcriptional initiation complex, such as SAGA or PCAF. To confirm these results in vivo with native IL-1α proteins, we performed a series of additional coimmunoprecipitation experiments. Lysates from HEK293 cells coexpressing FLAG-tagged mouse or human IL-1α proteins, including IL-1NTP, IL-1MAT, or pre-IL-1α, together with HA-tagged cofactors p300, PCAF, or Ada3, were immunoprecipitated with an anti-FLAG antibody. Consistent with the data from the pull-down assays, all the transcriptional cofactors tested interacted exclusively with forms containing the N-terminal domain, i.e. pre-IL-1α and IL-1NTP, no matter whether mouse or human (Fig. 7A). No specific interaction was observed in samples transfected with mouse or human IL-1MAT. These data provide clear evidence that nuclearly localized IL-1α associates via its N-terminal domain with the SAGA/PCAF transcriptional activation complex and suggest its participation in transcriptional control.

To confirm that the association interface of the IL-1NTP molecule consisting of two terminal α-helixes mediates the critical molecular interactions also in vivo, corresponding mutants of human IL-1NTP lacking the N-terminal helix (EDSSSS), the C-terminal helix (ITDDDD), or the NLS domain (VVATN) were tested for association with p300, PCAF, and Ada3. As shown in Fig. 7B, removal of either the N- or C-terminal helixes virtually abolished the association of IL-1NTP with p300, whereas the ΔNLS mutant associated with p300 in a similar manner as full-length IL-1NTP. Interestingly, all the mutants tested associated efficiently with PCAF as well as with Ada3, indicating that IL-1NTP interacts through terminal helices exclusively with p300. The interaction with PCAF and Ada3 is mediated therefore via different domains of IL-1NTP. The slightly weaker interaction of EDSSSS and ITDDDD with PCAF is probably because of lower expression levels (Fig. 7A).

IL-1α Integrates Non-destructively into the p300-PCAF Complex—To clarify whether IL-1α is an integral constituent of the complex, or whether it competes with p300 or PCAF for the same binding site displacing them from the complex we performed further coimmunoprecipitation experiments. HEK293 cells were cotransfected with vectors encoding FLAG-tagged mouse pre-IL-1α together with either p300 or PCAF expression vectors, or with all the vectors together. Whereas pre-IL-1α interacted separately with p300 or PCAF (Fig. 8, lanes 8 and 10), combined coexpression of all the factors did not result in any displacement of either p300 or PCAF, as both proteins

![Fig. 4. Gal4BD/IL-1NTP transactivates the Gal4 promoter also in mammalian cells via cooperation with transcriptional coactivator p300. A, HEK293 cells were cotransfected with Gal4-inducible reporter plasmid together with expression plasmids encoding mouse Gal4BD/IL-1NTP, human p300, viral p300 inhibitor E1A, and/or Gal4BD/RelA (RelA/p65), and assayed for luciferase activities. B, the IL-1NTP deletion mutants lacking the terminal acidic helices are unable to efficiently cooperate with p300. Empty vector (Vec) or the indicated mutants (as illustrated in Fig. 3A) fused to the Gal4BD were expressed with (p300) or without (Con) p300 transcriptional cofactor and luciferase activities were measured as described under Experimental Procedures. Error bars represent S.E. from three independent experiments.](https://www.jbc.org/content/early/2018/07/24/jbc.M118.007297.full)
FIG. 5. The Gln-rich domain of p300 is required for the association with IL-1NTP but not for the transactivation of the Gal4 promoter. A, mapping of the p300 domains required for the cooperation with IL-1NTP. Functional domains of wild type p300 (top) and its mutants are illustrated on the left. HEK293 cells cotransfected with the Gal4 reporter plasmid together with expression plasmids encoding for mouse Gal4BD/IL-1NTP and the indicated p300 mutants were assayed for luciferase activities (right) as before. B, Western blot showing expression levels of HA-tagged p300 and its mutants in cell lysates used for luciferase assays (A). C, in vitro interactions of IL-1NTP with p300 and its mutants. Whole cell lysates from HEK293 cells transfected with empty vector (Con) or with vectors expressing the indicated HA-tagged p300 proteins were incubated with glutathione beads containing mouse recombinant GST/IL-1NTP (GST/mIL-1NTP) protein. Bound material was immunoblotted with anti-HA antibodies (GST pull-down). For the expression controls 5% of the input lysates from transfected cells were loaded onto the gel and immunoblotted (Input). An asterisk (*) indicates a nonspecific band. The bottom panel shows an immunoblot of pull-down samples using anti-GST antibody, demonstrating equal loading of the recombinant proteins used.
FIG. 6. The IL-1NTP protein cooperates and associates also with histone acetyltransferases Gcn5 and PCAF, and with transcriptional adaptor Ada3. A, functional cooperation of Gal4BD/IL-1NTP with the histone acetyltransferase Gcn5. HEK293 cells were cotransfected with Gal4-inducible reporter plasmid together with expression plasmids encoding mouse Gal4BD/IL-1NTP and/or PCAF, p300, and Gcn5. Luciferase activities were measured as described. B, IL-1NTP interactions in vitro. Whole cell lysates from HEK293 cells transfected with plasmids expressing HA-tagged p300, PCAF, or Ada3, or FLAG-tagged Gcn5 were incubated with glutathione beads containing GST only (GST), or mouse (m) or human (h) recombinant GST/IL-1NTP (NTP) or GST/IL-1α mature proteins (MAT). Bound material was immunoblotted with anti-FLAG, anti-HA, or with anti-RelB antibodies. For the expression controls, 5% of the input lysates (Input) from transfected (Trans) and non-transfected (Con) cells were loaded onto the gel. Asterisks (*) indicate nonspecific bands. The Coomassie Blue-stained gel shows equal loading and the corresponding mobilities of the recombinant proteins used. The blots are representatives of at least three independent experiments.}

were detectable in a single complex (lane 12). In this context it is of interest that viral interferon regulatory factor-1, which inhibits p300, actively displaced the interaction of p300 with ectopically expressed PCAF in a similar experiment (49). Indeed, our data shows that the IL-1α precursor integrates into the p300-PCAF histone acetyltransferase complex in a non-destructive manner.

**DISCUSSION**

Our data provide the first solid evidence that the evolutionary conserved IL-1NTP is not only a by-product of proteolytic maturation of IL-1α, but might indeed convey long sought nuclear functions of intracellular forms of IL-1α. Here we show that IL-1NTP, when fused to the Gal4 DNA binding domain, behaves as a potent activator of transcription in mammalian cells as well as in yeast. Such behavior has been frequently reported for numerous prooncogenic viral factors, and is therefore in the case of IL-1α somewhat surprising. There are basically two possible explanations for this finding: first, IL-1NTP may coincidentally carry sequence similarities resembling common transactivation domains, or second, the transactivation potential is either part or consequence of a yet unknown nuclear function of IL-1NTP. The first possibility is highly unlikely because no sequence similarities could be detected. Although the subversion of the transcriptional complex by numerous viral factors is well known, no innate protection mechanism against these factors has been described so far. Taking into account that the IL-1α is rapidly synthesized and actively translocated to the nucleus upon different proinflammatory stimuli, a novel function linked with the transcriptional control and/or protection against viral factors attacking the p300 transcriptional coactivator is feasible. From this point of view the second option is clearly more appealing. We hypothesize that rather than regulating transcription by binding to DNA or sequence-specific transcription factors, intracellular IL-1α acting via its NTP domain could represent evolutionary conserved endogenous protection against viral factors inhibiting transcription of antiviral and proinflammatory genes. Such cellular protectors should be swiftly synthesized in response to various inflammatory stimuli and translocated immediately into the nucleus to neutralize viral factors. Indeed, IL-1α appears to be a molecule exhibiting all these features.

Previous studies with the strong herpes simplex virus transcriptional activator VP16 have shown that the Gal4BD/VP16 chimera inhibit the growth of transformed yeast strains, but fail to do so in strains with defective expression of components of the SAGA transcriptional activation complex (37). Overexpression of Gal4BD/IL-1NTP in yeast yielded a similar growth inhibitory effect that was also dependent on the presence of an intact SAGA complex. Deletion of adaptor (Ada2, Ada3), enzymatic (Gcn5), or structural (Spt7) subunits attenuated or even abolished the IL-1NTP-dependent growth inhibition, whereas deletion of the critical structural subunit (Aah1) of the distinct ADA transcriptional activation complex had no effect. The same results were obtained when the transactivation efficiency of IL-1NTP was tested in mutated strains. These data clearly demonstrate that IL-1NTP specifically interferes with and requires the SAGA complex for its function.

To test the transactivation potential of Gal4BD/IL-1NTP in mammalian cells, similar experiments were performed by transient transfections of HEK293 cells with Gal4-responsive luciferase reporter constructs. As expected on the basis of high homologies in transcriptional complexes between yeast and mammals, the Gal4BD/IL-1NTP chimera showed a high transactivation potential in mammalian cells as well. Moreover, cotransfection of transcriptional coactivator p300 dramatically enhanced the effect of Gal4BD/IL-1NTP on the reporter activ-
expression levels and corresponding mobilities of IL-1\(/\)H\(9251\) terminal helixes of IL-1\(NTP\). B of at least three independent experiments.

asterisk used. An and/or PCAF, together with FLAG-tagged IL-1\(HEK293\) cells cotransfected with plasmids expressing HA-tagged p300, PCAF, or Ada3 together with empty vector (Vec) or with the indicated FLAG-tagged IL-1\(alpha\) proteins were immunoprecipitated with anti-FLAG monoclonal antibody, and bound material was immunoblotted with anti-HA antibody. For expression controls, 5\% of the input lysates (Input) from transfected (Trans) and non-transfected (Con) cells were loaded onto the gel. The immunoblot with anti-FLAG antibody shows expression levels and corresponding mobilities of IL-1alpha proteins used. An asterisk (*) indicates a nonspecific band. The blots are representatives of at least three independent experiments. B, both terminal \(alpha\)-helixes of IL-1\(NTP\) are required for the association with p300, but not for the association with PCAF and Ada3. Whole cell lysates from HEK293 cells cotransfected with plasmids expressing HA-tagged p300, PCAF, or Ada3 together with empty vector (Vec), FLAG-tagged wild type human IL-1\(NTP\), DNLS mutant (VAVTN), N-terminal helix deletion mutant (EDSSS), or C-terminal helix deletion mutant (ITDD) were immunoprecipitated with anti-FLAG monoclonal antibody and bound material was immunoblotted with anti-HA antibody. For expression controls, 5\% of the input lysates (Input) from transfected (Trans) and non-transfected (Con) cells were loaded onto the gel. The immunoblot with anti-FLAG antibody shows expression levels and corresponding mobilities of the IL-1alpha proteins used. An asterisk (*) indicates a nonspecific band.

Fig. 7. In \(in vivo\) interactions of intracellular IL-1\(alpha\) with subunits of the histone acetyltransferase complexes are mediated by both terminal helixes of IL-1\(NTP\). A, mouse and human IL-1\(NTP\) and IL-1\(alpha\) precursor proteins specifically associate with the histone acetyltransferases p300 and PCAF, and with the transcriptional adaptor Ada3. Whole cell lysates from HEK293 cells cotransfected with plasmids expressing HA-tagged p300, PCAF, or Ada3 together with empty vector (Vec) or with the indicated FLAG-tagged IL-1\(alpha\) proteins were immunoprecipitated with anti-FLAG monoclonal antibody, and bound material was immunoblotted with anti-HA antibody. For expression controls, 5\% of the input lysates (Input) from transfected (Trans) and non-transfected (Con) cells were loaded onto the gel. The immunoblot with anti-FLAG antibody shows expression levels and corresponding mobilities of IL-1\(alpha\) proteins used. An asterisk (*) indicates a nonspecific band.

Fig. 8. IL-1\(alpha\) precursor protein integrates non-destructively into the p300-PCAF protein complex. Whole cell lysates from HEK293 cells cotransfected with plasmids expressing HA-tagged p300, and/or PCAF, together with FLAG-tagged IL-1\(alpha\) mouse precursor (IL-1) were IP with anti-FLAG monoclonal antibody as before. Bound material was immunoblotted with anti-HA antibodies. For expression controls, 5\% of the input lysates were loaded onto the gel. The corresponding bands of p300 and PCAF are marked with arrows; an asterisk (*) indicates a nonspecific band.

Because the yeast counterpart of mammalian p300 is not known yet, we tested the effects of mouse PCAF and Gcn5, whose yeast homologues are known constituents of the yeast SAGA complex. However, whereas Gcn5 was able to cooperate with Gal4BD/IL-1\(NTP\)-induced reporter transactivation, the PCAF acetyltransferase slightly inhibited the basal transactivation mediated by Gal4BD/IL-1\(NTP\) itself. Because PCAF can associate with IL-1\(NTP\) both in vitro and in vivo, this effect might be mediated by a direct acetylation of IL-1\(NTP\), as was recently reported for IRF-7 (50).

Modeling in silico followed by an extensive mutation analysis allowed the functional identification of acidic terminal \(alpha\)-helixes both of which are required for the function of Gal4BD/IL-1\(NTP\). Interestingly, these terminal regions represent the most conserved parts of the molecule, suggesting that they form domains mediating the biological function. The deletion of the third conserved domain known as NLS, which is believed to be responsible for the nuclear localization of the molecules containing IL-1\(NTP\) (7, 51), had no obvious inhibitory effect on the promoter transactivation. However, for the effects observed, the NLS domain of IL-1\(NTP\) might not be required, as another NLS domain is present in the Gal4\(BD\) part of the fusion protein. Interestingly, in all experiments both NLS deletion mutants of IL-1\(NTP\) (ATSSN and TSET5) showed significantly higher activities, which were more pronounced for the larger deletion part (TSET5). These data indicate that the NLS domain does not mediate the interaction with the transcriptional initiation complex. Furthermore, it might be possible that bringing the acidic terminal helixes into a bilateral proximity might change the protein conformation and thereby facilitate the interaction with the SAGA/PCAF transcriptional activation complex.

To provide deeper insights into the functional cooperation between IL-1\(NTP\) and different subunits of the SAGA complex, interactions with certain HATs using immobilized recombinant GST fusion IL-1\(alpha\) proteins were investigated. To demonstrate an evolutionary conserved homology among IL-1\(alpha\) proteins, especially in their N-terminal domains, we used both mouse and human IL-1\(NTP\). Their mature counterparts (IL-1\(MAT\)) were used to distinguish which part of the protein is responsible for the binding. As expected from the luciferase assay data, showing that IL-1\(NTP\) cooperated most efficiently with the transcriptional coactivator p300, GST/IL-1\(NTP\) fusion protein bound specifically to ectopically expressed p300 in vitro. Surprisingly, GST/IL-1\(NTP\) bound with high affinities to histone acetyltransferases PCAF and Gcn5, although no significant cooperation with PCAF and only a partial one with Gcn5 were observed in the transactivation experiments based on the Gal4 promoter system in HEK293 cells. Interestingly, GST/IL-1\(NTP\) interacted also with the human Ada3 protein, which is an important adaptor component of the SAGA/PCAF complex. This redundancy in interactions with multiple HATs and Ada3 could be explained by the existence of a common interface in these enzymes or by the possibility that also endogenous HATs
and/or adaptor proteins are bound in the complex and so detected together.

The experiments with the p300 mutants revealed that IL-1NTP binds to p300 through the Gln-rich domain as was reported for IRF-3 (41). Although the functional cooperation of these mutants in the luciferase assays suggests intricate functional interactions in the transcription complex, such activity was strictly dependent on the presence of a functional HAT domain. This is most evident for the molecule lacking the large N-terminal part of p300 (Δ143–957) that proved to be very active, but which completely lost its activity upon blocking its HAT activity by mutation of six amino acids in the HAT domain (Δ143–957MutAT2) (47). Interestingly, removal of the HAT domain (Δ194–1572) restored the transcriptional activity, suggesting a functional replacement by some other HAT protein.

The most common observation is the strong activity of the p300 1235–2221 protein that differs from the inactive mutant 1235–2412 only by the lack of the C-terminal domain. We propose that this domain either binds an inhibitory component or that it leads to a misfolding of the mutant thereby blocking the interaction to the complex.

To confirm interactions of the intracellular forms of IL-1α in their native state, we performed coimmunoprecipitation experiments with transiently transfected cells. The results obtained in these experiments were completely in line with the data from pull-down experiments, further confirming associations of both human and mouse intracellular forms of IL-1α specifically with p300 and PCAF, but also with the adaptor protein Ada3. Moreover, using mutants with deleted terminal helices we confirmed that they act as an interface for the association specifically with p300 in vivo, whereas they are not required for the interaction with PCAF or Ada3. This means that IL-1NTP binds through multiple domains to different subunits of the p300-PCAF complex, and therefore, it can integrate non-destructively into the p300-PCAF complex without affecting the molecular interactions inside the native complex.

Taken together, the data published in this paper show that intracellular IL-1α associates via IL-1NTP with different HATs in vitro, and functionally cooperates with the SAGA transcriptional complex in vivo, as demonstrated in yeast. Furthermore, IL-1NTP fused to Gal4BD transactivates the Gal4 promoter also in mammalian cells and functionally cooperates with the transcriptional cofactor p300 known as the limiting factor of the transcriptional complex. Both intracellular forms of IL-1α, IL-1NTP, and the IL-1α precursor, associate in their native states with histone acetyltransferases p300 and PCAF, and also with the transcriptional adaptor protein Ada3 in vivo. Moreover, IL-1α seems to be an integral part of the p300-PCAF complex, as its overexpression does not lead to disintegration of the complex.

Our findings provide the first solid evidence for an evolutionary conserved novel function of IL-1α in transcriptional control, specifically by its functional cooperation with the SAGA-PCAF acetyltransferase complex. This interaction sheds new light on the functional role of a well known cytokine, implying its novel role in intracellular defense mechanisms and opening a new area of cytokine research.

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Miroslava Buryskova, Martin Pospíšek, Arnhild Grothey, Thomas Simmet and Ladislav Burysek

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