**JAK2 Is Required for Induction of the M urine DUB-1 Gene**

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Received 17 December 1996; returned for modification 12 February 1997; accepted 20 March 1997

Cytokine receptors activate multiple signal transduction pathways, resulting in the induction of specific target genes. We have recently identified a hematopoietic cell-specific immediate-early gene, DUB-1, that encodes a growth-regulatory deubiquitinating enzyme. The DUB-1 gene contains a 112-bp enhancer element that is specifically induced by the βc subunit of the interleukin-3 (IL-3) receptor. To investigate the mechanism of DUB-1 induction, we examined the effects of dominant-negative forms of JAK kinases, STAT transcription factors, and Raf-1 in transient transfection assays. In Ba/F3 cells, IL-3 induced a dose-dependent activation of DUB-1-luciferase (luc) and GAS-luc reporter constructs. A dominant-negative form of JAK2 (truncated at amino acid 829) inhibited the induction of DUB-1-luc and GAS-luc by IL-3. A dominant-negative form of STAT5 (truncated at amino acid 650) inhibited the induction of GAS-luc but not DUB-1-luc. A dominant-negative form of Raf-1 inhibited the induction of DUB-1-luc but had no effect on the induction of GAS-luc by IL-3. The requirement for JAK2 in the stimulation of the DUB-1 enhancer was further supported by the suppression of DUB-1 induction in Ba/F3 cells stably expressing the dominant-negative JAK2 polypeptide. We hypothesize that IL-3 activates a JAK2/Raf-1 signaling pathway that is required for DUB-1 induction and is independent of STAT5.

Interleukin-3 (IL-3) is a glycoprotein hormone that regulates the growth and differentiation of hematopoietic progenitor cells (17). IL-3 activates a specific receptor (IL-3R) which is expressed on the surface of target cells. The IL-3R complex consists of an IL-3-specific α chain and a common β chain, βc, shared by the granulocyte-macrophage colony-stimulating factor receptor and the IL-5 receptor (20, 37). The IL-3R activates multiple signal transduction pathways, including the Ras/Raf/mitogen-activated protein (MAP) kinase pathway and the JAK (janus kinase)/STAT (signal transducer and activator of transcription) pathway, resulting in the induction of immediate-early genes. How these immediate-early genes couple receptor activation to the biochemical machinery of cell growth and cell cycle progression is poorly understood.

Discrete domains of the cytoplasmic region of the βc subunit activate the Ras/Raf/MAP kinase pathway and the JAK/STAT pathway. The distal cytoplasmic domain of βc, for example, activates the Ras/Raf/MAP kinase pathway (33, 34). This region is also required for the induction of the immediate-early genes c-fos and c-jun (34). The membrane-proximal domain of βc, in contrast, is required for activation of the JAK/STAT pathway (45).

The IL-3R, like other cytokine receptors, activates a combination of JAK and STAT proteins (7, 15). JAK kinases are cytoplasmic proteins that constitutively associate with the membrane-proximal region of cytokine receptors and become activated upon receptor-ligand interaction. STAT proteins are latent cytoplasmic transcription factors that are phosphorylated by JAK kinases. Seven STAT proteins are known at present. Phosphorylated STAT proteins form homo- and hetero-oligomeric complexes and translocate to the nucleus, where they activate transcription of specific genes. The specific set of genes induced by cytokine receptors is determined, at least in part, by the specific combination of JAK kinases and STAT proteins that are activated (16, 18).

We have recently identified an IL-3-inducible immediate-early gene, DUB-1, that encodes a growth-regulatory deubiquitinating enzyme (49). The DUB-1 gene is induced by receptors that share the βc receptor subunit, including the receptors for IL-3, granulocyte-macrophage colony-stimulating factor, and IL-5. DUB-1 is not induced by the receptor for erythropoietin (EPO) or IL-4. Furthermore, the membrane-proximal region of the βc subunit is required for IL-3 induction, suggesting a role for the JAK/STAT pathway (51). Interestingly, DUB-1 is a member of a novel family of immediate-early genes encoding deubiquitinating enzymes (50). This family includes DUB-2, an IL-2-inducible immediate-early gene. How DUB genes modulate cytokine-dependent growth signals is not understood.

The mechanism by which DUB-1 modulates IL-3-dependent growth signals remains unknown. Recent evidence implicates the ubiquitin-dependent proteolysis pathway in multiple cellular processes, including cell cycle regulation (9), transcriptional activation (38), and receptor metabolism (13). Regulatory proteins destined for degradation are initially conjugated to the 76-amino-acid peptide tag, ubiquitin. Polyubiquitinated proteins are next targeted to the proteasome, where protein degradation occurs (12). Deubiquitinating enzymes function by removing ubiquitin and thereby rescuing target proteins from degradation (14). The ubiquitin-mediated proteolytic pathway can be modified by cytokine stimulation (8). Recent evidence for ubiquitination of STAT1 (19), Cbf (40), and cytokine receptors (36) underscores the importance of ubiquitin-mediated proteolysis in signal transduction. DUB-1 may therefore modulate cellular growth by altering the degradation or ubiquitination state of signal transducing proteins downstream of the IL-3R. In this way, the induction of DUB-1 protein may pos-

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Stable transfection of Ba/F3 cells with WT-JAK2 or DN-JAK2. Ba/F3 cells growing in IL-3 were transfected by electroporation as described above. The cDNA constructs applied were either pBOS (empty vector), pBOS-WT-JAK2, or pBOS-DN-JAK2 (10 µg) along with pSV2 neo (1 µg). After electroporation, cells were diluted in 20 ml RPMI 1640 with 10% WEHI conditioned medium and grown for 48 h prior to the addition of G418 (1 mg/ml). G418-resistant Ba/F3 subclones were isolated by limiting dilution in 96-well microtiter plates. Stable expression of WT-JAK2 or DN-JAK2 was confirmed by immunoblot analysis.

Northern analysis. RNA samples (20 µg) were electrophoresed on denaturing formaldehyde gels and blotted onto Duralon-UV membranes (Stratagene, La Jolla, Calif.). The indicated cDNA inserts were purified from agarose gels (Qiagen, Calif.), radiolabeled as previously described, and hybridized with a probe to the human 28S rRNA at 68°C. Hybridized filters were washed for 15 min in 2 x SSC (0.15 M NaCl plus 0.015 M sodium citrate) with 0.1% sodium dodecyl sulfate (SDS) and for 30 min in 0.1% SSC–0.1% SDS at room temperature.

Electrophoretic mobility shift assay. Nuclear extracts were prepared from unstimulated and IL-3-stimulated Ba/F3 cells as previously described (51). 3P-labeled double-stranded DNA fragments were separated from unincorporated nucleotides with a G-50 column (Pharmacia, Uppsala, Sweden). The probe from the DUB-1 enhancer (5-TAACAAGAAATTATGCTAGAG-3') corresponds to nucleotides -1512 to -1494 of the DUB-1 gene (51). A probe from the b-casein promoter (5'-AGATTGCCTGAGACTTT-3') was used to analyzes STAT binding. Binding reaction mixtures (20 µl) contained nuclear extract from 2 x 106 cells, 10% FCS, 10% conditioned medium from WEHI-2 cells, 0.5 µM EDTA, 8% glycerol, 1 µg of poly(dI-dC), and end-labeled DNA. Binding was measured on a Molecular Dynamics 400A phosphorimager (Molecular Dynamics, Sunnyvale, Calif.).

RESULTS

IL-3 and IFN-α-dependent growth characteristics of Ba/F3 cells. Ba/F3 cells are an IL-3-dependent murine pro-B cell line (28). We initially analyzed Ba/F3 cells for IL-3-dependent and IFN-α-dependent growth characteristics (Fig. 1). IL-3 stimulated Ba/F3 cell growth within the concentration range of 0.01 to 100 pM (Fig. 1A). Half-maximal growth of Ba/F3 cells was observed at 0.1 pM, consistent with previous studies (6). IFN-α (2.2 x 103 U/ml) inhibited Ba/F3 cell growth across a concentration range of 1 to 10,000 U/ml, consistent with the known growth-suppressing effects of IFN-α (30). Half-maximal inhibition was observed at approximately 10 U of IFN-α per ml.

To further demonstrate the presence of functional IL-3- and IFN-α receptors on Ba/F3 cells, cells were stimulated with cytokine and JAK kinase activation was examined (Fig. 1B). IL-3 activated the tyrosine phosphorylation of JAK2 (Fig. 1B, top), as previously described (5, 32). IL-3 also weakly activated JAK1 tyrosine phosphorylation in these cells, though the functional relevance of JAK1 activation remains unclear (35). IFN-α activated the tyrosine phosphorylation of JAK1 and Tyk2 (Fig. 1B, bottom), as previously described (31).

Previous studies have demonstrated that IL-3 activates STAT5A and STAT5B (25), resulting in the induction of genes containing an upstream GAS consensus sequence (47, 48). Ba/F3 cells were transiently transfected with various reporter constructs, and dose-dependent luciferase activity was measured (Fig. 1C). IL-3 induced a dose-dependent increase in GAS-luc and DUB-1-luc activity, with half-maximal activation at approximately 0.5 pM IL-3. IL-3-induced GAS-luc and DUB-1-luc activity therefore correlated well with IL-3-induced mitogenesis (Fig. 1A). IFN-α induced a dose-dependent increase in ISRE-luc, with half-maximal activation at approximately 1,000 U of IFN-α per ml. IFN-α-induced ISRE-luc activity therefore correlated well with IFN-α inhibition of cell growth (Fig. 1A).

JAK2 is required for the induction of the DUB-1 gene. Previous studies have demonstrated that the IL-3R activates JAK2

MATERIALS AND METHODS

Cells and cell culture. Ba/F3 cells are an IL-3-dependent murine pro-B cell line (28). Ba/F3 cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) and 10% conditioned medium from WEHI-2B cells as a source of murine IL-3. For induction studies, Ba/F3 cells were washed three times in RPMI 1640 (without FCS or supplemental growth factor) and resuspended in RPMI 1640–10% FCS without IL-3. Recombinant murine IL-3 (Kirin) was used to stimulate the cells where indicated.

IL-3 and IFN-α-dependent growth characteristics of Ba/F3 cells. Ba/F3 cells, growing in IL-3-supplemented medium, were deprived of IL-3 for 8 h. Cells were next plated in 96-well microtiter plates at 2 x 104 cells/well and incubated continued for an additional 4 h. Afterwards, cells were harvested onto filters and washed, and incorporation of [3H]thymidine was quantified by liquid scintillation counting. In parallel, cell viability was assessed by the trypan blue exclusion test (54).

Immunoprecipitation analysis and immunoblotting. Immunoprecipitation and immunoblotting of proteins derived from Ba/F3 cells were performed as previously described (2), using the ECL detection system (Amer sham, Arlington Heights, Ill.). A rabbit antibodies to JAK1, JAK2, and phosphotyrosine (4G10) were purchased from Upstate Biotechnology, Inc. (Lake Placid, N.Y.). Antibodies to JAK1, JAK2, and phosphotyrosine (4G10) were used to analyze the tyrosine phosphorylation of JAK1 and JAK2 in Ba/F3 cells, as well as JAK kinases (5) and JAK kinases (32). The use of these antibodies was confirmed by immunoblot analysis.

Transient-transfection and transactivation experiments. All plasmid DNAs were purified by using QiaGen columns (QiaGen Inc., Chatsworth, Calif.). Ba/F3 cells were plated in 96-well microtiter plates (2 x 104 cells/well) and incorporated [3H]thymidine was quantified by liquid scintillation counting. In parallel, cell viability was assessed by the trypan blue exclusion test (54).

Preparation of Ba/F3 nuclear extracts. Nuclear extracts were prepared from unstimulated and IL-3-stimulated Ba/F3 cells as previously described (51). 3P-labeled double-stranded DNA fragments were separated from unincorporated nucleotides with a G-50 column (Pharmacia, Uppsala, Sweden). The probe from the DUB-1 enhancer (5-TAACAAGAAATTATGCTAGAG-3') corresponds to nucleotides -1512 to -1494 of the DUB-1 gene (51). A probe from the b-casein promoter (5'-AGATTGCCTGAGACTTT-3') was used to analyzes STAT binding. Binding reaction mixtures (20 µl) contained nuclear extract from 2 x 106 cells, 10% FCS, 10% conditioned medium from WEHI-2B cells, 0.5 µM EDTA, 8% glycerol, 1 µg of poly(dI-dC), and end-labeled DNA. Binding was measured on a Molecular Dynamics 400A phosphorimager (Molecular Dynamics, Sunnyvale, Calif.).

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Stable transfection of Ba/F3 cells with WT-JAK2 or DN-JAK2. Ba/F3 cells growing in IL-3 were transfected by electroporation as described above. The cDNA constructs applied were either pBOS (empty vector), pBOS-WT-JAK2, or pBOS-DN-JAK2 (10 µg) along with pSV2 neo (1 µg). After electroporation, cells were diluted in 20 ml RPMI 1640–10% FCS, divided into pools, and cultured for 2 h in the incubator. Next, cultures were restimulated with cytokine at variable concentrations. Luciferase levels were assayed 16 h after cytokine stimulation according to the vendor’s specifications (Luciferase A assay Kit; A nalysis and interpretation Laboratory, San Diego, Calif.). Each construct was tested at least three times by independent transfection, with similar results each time.
A dominant-negative form of JAK2, lacking the carboxy-terminal tyrosine kinase domain, partially inhibits IL-3-induced mitogenesis (4, 52). To test the effects of DN-JAK2 on DUB-1 induction, Ba/F3 cells were cotransfected with the cDNAs encoding either WT-JAK2 or DN-JAK2, along with various luciferase reporters (Fig. 2A). DN-JAK2 inhibited the induction of GAS-luc by IL-3, consistent with the role of JAK2 in STAT5 activation. In contrast, cotransfection with the cDNA encoding WT-JAK2 caused a small increase in GAS-luc induction.

To ensure that DN-JAK2 polypeptide was expressed in Ba/F3 cells in these transient assays, lysates from mock-transfected or DN-JAK2-transfected Ba/F3 cells were analyzed by immunoblotting (Fig. 2B). Ba/F3 cells transfected with the DN-JAK2 cDNA expressed relatively equal levels of endogenous JAK2 polypeptide and DN-JAK2 polypeptide (lane 2). STAT5 is not required for induction of the DUB-1 gene. IL-3 and other hematopoietic cytokines activate JAK2, which, in turn, phosphorylates STAT5A and STAT5B. Protein complexes containing STAT5 homodimers bind to GAS elements (7), found upstream of several cytokine-inducible genes (47, 48). Previous studies have demonstrated that carboxy-terminal truncations of STAT5 result in dominant inhibition of IL-3-induced mitogenesis (24, 26). Naturally occurring dominant-negative forms of STAT5A have also been identified (24, 39).

To test the potential role of STAT5 in DUB-1 induction, we generated a deletion mutant of STAT5A, truncated at amino acid 650. Ba/F3 cells were transfected with the cDNAs encoding the Myc epitope-tagged wild-type (full-length) or truncated DN-STAT5A (Fig. 3A). DN-STAT5 inhibited activation of GAS-luc by IL-3 but did not inhibit the activation of DUB-1–luc by IL-3 or ISRE-luc by IFN-α. Transfection with the cDNA encoding WT-STAT5A had little effect on GAS-luc or DUB-1–luc. STAT5A activation is therefore not required for the induction of the DUB-1 gene.

To ensure expression of WT-STAT5A and DN-STAT5A in transfected Ba/F3 cells, cell lysates were analyzed by anti-Myc immunoblotting (Fig. 3B). Transfected cells expressed the Myc epitope-tagged wild-type (full-length) or truncated DN-STAT5A (Fig. 3A). DN-STAT5A inhibited activation of GAS-luc by IL-3 but did not inhibit the activation of DUB-1–luc by IL-3 or ISRE-luc by IFN-α. Transfection with the cDNA encoding WT-STAT5A had little effect on GAS-luc or DUB-1–luc. STAT5A activation is therefore not required for the induction of the DUB-1 gene.
Vol. 17, 1997 JAK2 IS REQUIRED FOR DUB1 INDUCTION

JAK2 and the Ras/Raf-1 MAP kinase pathway contribute to the induction of DUB1. We next tested the effects of DUB1 induction of JAK2 in the absence of JAK2 and Raf-1 expression. The data show that JAK2 and Raf-1 are required for DUB1 induction.

DNA-JAK2 or DUB1 LUCIFERASE reporter activity was measured in Ba/F3 cells expressing JAK2 or DUB1. The results show that JAK2 is required for DUB1 induction.

The data suggest that both JAK2 and the Ras/Raf-1 MAP kinase pathway contribute to the induction of DUB1. The role of JAK2 in DUB1 induction is further supported by the observation that JAK2 is critical for the induction of DUB1 in the absence of Raf-1 or the Ras/Raf-1 MAP kinase pathway.

We next tested the effects of DUB1 induction of JAK2 in the absence of JAK2 and Raf-1 expression. The data show that JAK2 and Raf-1 are required for DUB1 induction.
shown that DUB-1 is an immediate-early gene, induced by signaling pathways downstream of the IL-3R βc subunit. Three Ba/F3 subclones, stably expressing DN-JAK2, exhibited diminished IL-3 induction of DUB-1 mRNA, consistent with the requirement of JAK2 in the DUB-1 induction pathway (Fig. 8A, lanes 3 to 8). Stable expression of WT-JAK2 had little effect on DUB-1 induction (Fig. 8A, lanes 9 to 12). Interestingly, DN-JAK2 did not inhibit induction of c-myc mRNA in these clones. To rule out the possibility of a delay in DUB-1 induction rather than an absolute reduction, one Ba/F3 subclone expressing DN-JAK2 was induced with IL-3 over several time points (Fig. 8B). Again, DN-JAK2 inhibited DUB-1 mRNA induction without affecting c-myc mRNA levels. Furthermore, c-fos mRNA induction was not affected in DN-JAK2-expressing cells. The inhibition of DUB-1 expression by DN-JAK2 was also confirmed at the level of DUB-1 polypeptide (Fig. 8C; compare lanes 2 and 4).

DISCUSSION

DUB-1 encodes a deubiquitinating enzyme and is specifically induced by the IL-3R βc subunit. In this study, we used dominant-inhibitory forms of JAK kinases and STAT proteins in order to identify signaling components required for DUB-1 induction. We have exploited an advantage of the Ba/F3 cell system, namely, the existence of two nonoverlapping signaling pathways, one for the IL-3R and one for the IFN-α receptor. Stimulation of the IL-3R results in activation of the JAK2/STAT5 pathway; complexes containing activated STAT5 bind to GAS elements. Stimulation of the IFN-α receptor results in activation of the JAK1/Tyk2 and STAT1/STAT2 pathways; complexes containing activated STAT1, STAT2, and p48 bind to ISREs. Our results demonstrate that DUB-1 induction requires a functional JAK2 signal. An increasing number of immediate-early genes require JAK2. Some genes, such as the OSM gene (47), CIS (48), and
PIM-1 (26), require both JAK2 and STAT5. Other genes, such as c-myc, require JAK2 but do not require STAT5 activation (26). DUB-1 is therefore a new member of this latter group of STAT-independent, inducible genes. DUB-1 is the only known member of this group that modulates the ubiquitin pathway and has growth-suppressing activity (49).

Recent studies have suggested that JAK2 acts upstream of Raf-1 in signal transduction. For instance, following growth hormone stimulation, JAK2 activates the Ras/Raf/MAP kinase pathway (43, 44). Following EPO stimulation, the Ras/Raf adapter protein, Shc, is recruited to tyrosine-phosphorylated JAK2 (11). A complex of Raf-1 and JAK2 has also been identified (46), suggesting that JAK2 activates Raf-1 kinase activity directly. Our current data demonstrate that both JAK2 and Raf-1 are required for DUB-1 induction. According to one model, the constitutively activated Ras mutant partially overrides the inhibitory effects of DN-JAK2, suggesting that JAK2 is upstream of the Ras/Raf/MAP kinase pathway in DUB-1 induction. Alternatively, the DN-JAK2 may override the effect of activated Ras.

Activated MAP kinase is known to phosphorylate Elk-1, a member of the ets family of transcription factors (29, 42). Interestingly, the minimal functional enhancer of the DUB-1 gene contains an ets site. Mutation of this site ablates IL-3-dependent DUB-1 enhancer activity (51). In contrast, mutation of the AP-1 site of the DUB-1 enhancer only partially inhibits enhancer activity (18a). Taken together, these data suggest that Elk-1, or some other ets family member, is activated by IL-3 and is required for DUB-1 induction. The specific ets family member(s) that bind to the DUB-1 enhancer remains unknown.

The induction of DUB-1 differs from the induction of other JAK2-dependent immediate-early genes, c-fos and c-myc (41). In transient-transfection assays, DN-JAK2 inhibits the induction of DUB-1-luc, as well as fos-luc (data not shown), confirming the requirement of JAK2 in both DUB-1 and fos transcription. Previous studies, with transient transfection assays in fibroblasts, confirm the requirement of JAK2 in βc subunit-modulated induction of c-fos (41). In contrast, in the stably transfected cells, DN-JAK2 inhibits DUB-1 induction but fails to inhibit induction of c-fos and c-myc mRNAs. Taken together, these data suggest that JAK2 is more critical for DUB-1 induction than for c-fos or c-myc induction. This observation suggests that during the selection of stable clones expressing DN-JAK2, c-fos and c-myc remain essential for cellular growth. Other kinases may substitute for JAK2 in pathways upstream of c-myc and c-fos, as previously suggested (22). The viability of DN-JAK2 stable clones lacking DUB-1 polypeptide suggests that DUB-1 is not required for cell growth and supports a model of DUB-1 as a growth suppressor (49).

The failure of DN-JAK2 and DN-STAT5 to block IFN-α signal transduction is consistent with previous studies.
translation of the IFN-α receptor results in activation of the tyrosine kinase JAK1 and Tyk2. In our studies, DN-JAK2 had no effect on the activation of JAK1/Tyk2 pathway, implying that this pathway is independent and parallel to JAK2. Subsequently, activated Tyk2 and JAK1 tyrosine phosphorylate STAT1 and STAT2, which, in turn, interact with the ISRE (7, 15). DN-STAT5 does not disrupt this transcriptional complex.

The mechanism of βc-specific induction of DUB-1 remains unclear. For instance, both IL-3 and EPO activate JAK2 and Ras/Raf/MAP kinase pathways. Still, DUB-1 is selectively induced by IL-3, not by EPO. While the major pathway necessary for DUB-1 induction is JAK2/Raf-1, this pathway is not sufficient for the specificity of DUB-1 induction. These data suggest the existence of additional signaling pathways activated by the βc subunit but not by the EPO receptor.

The DUB-1 gene encodes a deubiquitinating enzyme with growth-regulatory activity (49). Expression of DUB-1 mRNA in Ba/F3 cells is highly regulated. DUB-1 is induced as an immediate-early gene, and DUB-1 mRNA levels rapidly decline following cytokine induction. Furthermore, DUB-1 protein levels are also highly regulated. The DUB-1 protein has a half-life of 10 min and is itself degraded by ubiquitin-mediated proteolysis (6a). Recent evidence demonstrates that multiple proteins involved in signal transduction, including growth factor receptors (36), Cbl (40), and STAT1 (19), are regulated by ubiquitin-mediated proteolysis. DUB-1 may modulate signal transduction, by controlling the degradation or ubiquitination state of proteins required for signal transduction. Proof of this hypothesis is contingent upon the identification of specific substrates of the DUB-1 enzyme.

**FIG. 6.** Growth characteristics of Ba/F3 cells stably expressing DN-JAK2. (A) Ba/F3 cells were stably cotransfected with pSV2 neo plus pBOS (empty vector) (open symbols) or pBOS-DN-JAK2 (closed symbols). Cells were selected in G418 (1 mg/ml) and subcloned by limiting dilution. IL-3-dependent growth response of the indicated cells was quantified by detecting the incorporation of [3H]thymidine into DNA as described for Fig. 1. For both transfections, the growth responses of two typical subclones are shown. Data are averaged values ± standard deviations of triplicate samples. (B) Stable Ba/F3 subclones above were analyzed for IL-3-dependent JAK2 tyrosine phosphorylation. The indicated stable cell lines (10⁴ cells per sample) were cultured for 8 h in the absence of IL-3 before restimulated with the growth factor (10 pM) as indicated. Proteins were immunoprecipitated (Ip) with an anti-JAK2 antibody (Ab) (Upstate Biotechnology) and blotted with the 4G10 antiphosphotyrosine (pTyr) antibody (top). Afterward, the blot was stripped and reprobed with anti-JAK2 (bottom). For DN-JAK2, two typical subclones are shown.

**FIG. 7.** DN-JAK2 inhibits the formation of gel shift complexes bound to GAS elements and DUB-1 enhancer elements. The indicated stable Ba/F3 subclones were deprived of cytokine for 8 h and restimulated with IL-3 as indicated (10 pM). (A) Nuclear extracts were subjected to gel mobility shift analysis using a labeled β-casein GAS oligonucleotide probe. For competition analysis (lane 3), a 100-fold molar excess of the unlabeled probe was added to the binding reaction. In lane 4, supershift analysis was performed by incubating the extract with an anti-STAT3A antibody (Ab) (0.5 μg). (B) Gel mobility shift analysis was performed with a DUB-1 enhancer probe (probe 1 from reference 51). Competition analysis (lane 3) was performed as described for panel A.


17a. Jaster, R. Unpublished data.


