Immediate Early Gene X1 (IEX-1) Is Organized in Subnuclear Structures and Partially Co-localizes with Promyelocytic Leukemia Protein in HeLa Cells*

Received for publication, February 10, 2005, and in revised form, April 15, 2005
Published, JBC Papers in Press, April 26, 2005, DOI 10.1074/jbc.M501571200

Marie-Luise Kruse‡, Alexander Arlt‡, Alexander Sieke§, Frauke Grohmann, Maike Grossmann, Jörg Minkenberg, Ulrich R. Fölsch, and Heiner Schäfer‡

From the Laboratory of Molecular Gastroenterology and Hepatology, First Department of Medicine, Christian-Albrechts-University of Kiel, Schittenhelmstrasse 12, D-24105 Kiel, Germany

Immediate early gene X1 (IEX-1) represents a stress response gene involved in growth control and modulation of apoptosis. Here, we report a detailed analysis of IEX-1 with respect to its intracellular localization. By means of confocal laser scanning microscopy, a green fluorescent protein-IEX-1 fusion protein translocated into HeLa cells, as well as endogenous IEX-1, could be detected in distinct subnuclear structures. This particular subnuclear localization of IEX-1 was not observed with a green fluorescent protein-IEX-1 fusion protein lacking a putative nuclear localization sequence, along with a decreased effect on apoptosis. Double immunofluorescence staining revealed a partial co-localization of endogenous promyelocytic leukemia protein (PML) and IEX-1 in these subnuclear structures. Nuclear localization of IEX-1 is also enhanced upon treatment of cells with leptomycin B, an inhibitor of the nuclear exporter CRM1. These observations indicate that IEX-1 is specifically shuttled to and from the nucleus. Overexpression experiments using PML isoforms III and IV revealed distinct intranuclear interaction of IEX-1 and PML. Co-precipitation experiments showed physical interaction between IEX-1 and PML. The close structural relation of IEX-1-containing nuclear subdomains and PML nuclear bodies suggests a function of IEX-1 related to the multiple functions of these unique subnuclear regions, particularly during stress response and growth control.

Immediate early gene X1 (IEX-1) is a growth- and stress-associated early response gene (1) implicated in the actions of growth factors (1–4), cytokines (1, 4–6), and several stress signals, such as virus infection (7), UV- and γ-irradiation (1, 3, 8), or biomechanical strain (9). IEX-1, as well as its rodent homologues PRG1 and gly96 (10, 11), is a short lived protein without a putative nuclear localization sequence, such as virus infection (7), UV- and γ-irradiation (1, 3, 8), or biomechanical strain (9). IEX-1, as well as its rodent homologues PRG1 and gly96 (10, 11), is a short lived protein lacking a putative nuclear localization sequence, along with a decreased effect on apoptosis. Double immunofluorescence staining revealed a partial co-localization of endogenous promyelocytic leukemia protein (PML) and IEX-1 in these subnuclear structures. Nuclear localization of IEX-1 is also enhanced upon treatment of cells with leptomycin B, an inhibitor of the nuclear exporter CRM1. These observations indicate that IEX-1 is specifically shuttled to and from the nucleus. Overexpression experiments using PML isoforms III and IV revealed distinct intranuclear interaction of IEX-1 and PML. Co-precipitation experiments showed physical interaction between IEX-1 and PML. The close structural relation of IEX-1-containing nuclear subdomains and PML nuclear bodies suggests a function of IEX-1 related to the multiple functions of these unique subnuclear regions, particularly during stress response and growth control.

Materials—Cell culture medium was purchased from PAA-Labs (Linz, Austria), fetal bovine serum from Seromed (Berlin, Germany), recombinant IFNγ, leptomycin-B, and tubulin monoclonal antibodies from Sigma, and secondary antibodies from Molecular Probes (Eugene, OR) or from Dianova (Hamburg, Germany). Goat IEX-1 antibodies, monoclonal and polyclonal PML antibodies, rabbit HSP90, PARP-1 (89 kDa), and GFP antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). A different monoclonal PML antibody was purchased from MBL (Medical & Biological Laboratories, Nagoya, Japan). A monoclonal GFP antibody and the EGFP control vector were from Clontech.

Cell Culture—HeLa cells were cultured in RPMI 1640 supplemented with 1% glutamine, 10% fetal bovine serum, and 1% penicillin-streptomycin. Cells were grown on glass coverslips or in six-well plates and used after 50–90% confluence. For a more detailed description of the cell culture conditions, see the original manuscript.
gentamicin (0.1 mg/ml). Cells were split twice a week and incubated at 37 °C with 5% CO₂ at 85% humidity.

**Generation of GFP Fusion Protein Expression Vectors**—Full-length cDNA of IEX-1 (20) was cloned into the pCR3.1-NT-GFP vector (Invitrogen), producing a fusion protein of GFP joined to the N terminus of IEX-1 (GFP-IEX-1). By means of PCR, an N-terminal-deleted (position 5–35) fragment of IEX-1 was amplified and cloned into pCR3.1-NT-GFP (GFP-(ΔNLS)-IEX-1). Using composite SaII primers for PCR, an IEX-1 construct was generated lacking the putative nuclear localization sequence (HRKK, positions 65–68). After subcloning, this IEX-1 variant was cloned into pCR3.1-NT-GFP (GFP-(ΔNLS) IEX-1). All constructs were DNA sequenced.

**Cell Transfection**—Cells were plated directly in 12-well plates or on coverslips in 12-well plates, were grown overnight and then incubated with 1.0 ml of OptiMEM I (Invitrogen). Transfection (1 μg of plasmid) was done under serum-free conditions for 6 h with 500 μl of ExGen500 (Fermentas) according to the manufacturer’s protocol. Further treatment was usually commenced the next day. For immunoprecipitation, cells were seeded at a density of 750,000 cells/plate (9 cm) and submitted to the same transfection procedure (HRKK, positions 65–68). After subcloning, this IEX-1 variant was cloned into pCR3.1-NT-GFP (GFP-(ΔNLS) IEX-1). All constructs were DNA sequenced.

**GFP Fusion Protein Transfectants**—Cells were transfected with these GFP fusion proteins or with EGFP as control protein exhibit strong expression of all these proteins, as verified by Western blotting with a GFP antibody (data not shown). GFP fusion protein transfectants were investigated by confocal LSM. As shown in Fig. 1, transfection of HeLa cells with pGEX4T3-GST-IEX-1 (a generous gift from Dr. Porteu, Paris, France) or pGEX4T2-GST were expressed in Escherichia coli BL21(DE3)pLyS5 (Invitrogen) as described (23). Bacteria were harvested by centrifugation and sonicated in TBS (6 min) before adjusting to 1% Triton X-100. After lysis (30 min, room temperature) and centrifugation (3000 x g, 15 min), the resulting supernatants were incubated with glutathione-Sepharose 4B (Amersham Biotechnologies) overnight at 4 °C. Beads were washed in TBS containing 150 and 500 mM NaCl and then resuspended in 50 mM Tris, pH 8.0. For pulldown assays, cell lysates were prepared and precleared with bovine serum albumin-Sepharose 4B as described for immunoprecipitation. Supernatants were then incubated with glutathione-Sepharose-coupled GST-IEX-1 or GST, or with glutathione-Sepharose 4B only as control. Further handling and Western blot analysis were carried out as described above.

**RESULTS**

**N-terminal GFP-IEX-1 Fusion Protein Is Accumulated in Distinct Subnuclear Domains in HeLa Cells**—To analyze the cellular distribution of IEX-1 in more detail, independent of available antibodies, various GFP fusion proteins were generated encompassing the GFP moiety at the N terminus. Besides full-length IEX-1 (GFP-IEX-1), fusion proteins of an N-terminal-shortened (positions 5–35) IEX-1 variant, GFP-(ΔNT)-IEX-1, and IEX-1 lacking a putative NLS (HRKK; positions 65–68), GFP-(ΔNLS)-IEX-1, were generated. HeLa cells transfected with these GFP fusion proteins or with EGFP as control protein exhibit strong expression of all these proteins, as verified by Western blotting with a GFP antibody (data not shown). GFP fusion protein transfectants were investigated by confocal LSM. As shown in Fig. 1, transfection of HeLa cells with GFP-IEX-1 produced an intensive and quite distinct nuclear staining after 24 h of expression. In particular, the accumulation of this fusion protein in dense subnuclear compartments was striking. To exclude that overexpression-induced DNA damage accounts for the unique staining pattern seen with GFP-IEX-1, we performed terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) staining to ensure that the accumulated GFP-IEX-1 was not localized to apoptotic bodies, but we did not find DNA damage in transfected cells (data not shown). Addressing the specificity of this nuclear localization of IEX-1, we next elucidated whether two structurally modified IEX-1 variants exhibit an altered intracellular distribution. As shown in Fig. 1B, a quite similar nuclear staining compared with GFP-IEX-1 variants was observed when the N-terminal-shortened IEX-1ΔNT variant (GFP-(ΔNT)-IEX-1) was transfected into HeLa cells. In contrast, if the putative nuclear localization signal (NLS) of IEX-1...
was deleted, the corresponding GFP fusion protein (GFP-(ΔNLS)IEX-1) was predominantly expressed in the cytoplasm and no accumulation in the nucleus was seen (Fig. 1C). For comparison, HeLa cells transfected with a vector encoding EGFP only, which lacks an NLS, exhibit a mostly cytoplasmic staining with some diffusion into the nucleus (Fig. 1D).

The Nuclear Localization of IEX-1 Relates to Its Apoptosis-sensitizing Effect—To elucidate whether the loss of nuclear localization affects the function of IEX-1 with respect to its cell death-enhancing activity, HeLa cells transfected with EGFP, GFP-IEX-1, or GFP-(ΔNLS)IEX-1 were treated with DNA-damaging apoptotic stimuli. A homogeneous luminescent assay for caspase-3 and caspase-7 activity revealed that EGFP-transfected HeLa cells exhibit a 12- and 7-fold increase of caspase-3/-7 activity if subjected to etoposide treatment and ultraviolet-B radiation, respectively (Fig. 2A). This caspase-3/-7 activation was significantly enhanced in GFP-IEX-1-transfected HeLa cells, exhibiting a 20- and 12-fold increase of caspase-3/-7 activity in response to both apoptotic stimuli, whereas no such enhancement was seen in HeLa cells transfected with GFP-(ΔNLS)IEX-1. Moreover, Western blot analysis of the caspase-dependent cleavage product of PARP-1 (89 kDa) revealed higher levels of cleaved PARP-1 in HeLa cells transfected with GFP-IEX-1 than in EGFP- or GFP-(ΔNLS)IEX-1-transfected cells (Fig. 2B). These data indicate that the sensitizing effect of IEX-1 on DNA damage-induced apoptosis is impaired if its nuclear localization has ceased.

IEX-1 Is Partially Co-localized with PML Protein in the Nuclei of HeLa Cells—As the nuclear localization pattern of IEX-1 resembled nuclear dots, we explored co-localization with PML protein, well known for appearance in subnuclear domains termed PML nuclear bodies (PML-NBs) (28, 29). Mock- or IEX-1-transfected HeLa cells were incubated with 50 ng/ml IFNγ for 24 h, inducing accumulation of PML in PML-NBs (30). Double immunofluorescence staining with a rabbit IEX-1 antiserum (4) revealed that in mock-transfected, uninduced cells, endogenous IEX-1 protein is found with dominant cytoplasmic staining and in distinct nuclear dots (Fig. 3A, red staining). A similar staining pattern holds true for the PML protein (green), and occasionally, co-localization is seen by yellow staining in...
IFNγ treatment of mock-transfected (A, B) or transfected with native IEX-1 (C, D), either left untreated (A, C) or treated with IFNγ for 24 h at 50 ng/ml (B, D). Transfectants were analyzed by confocal LSM, original magnification ×400. A and B, immunostainings for endogenous IEX-1 (red) and endogenous PML (green) are shown. C and D, overexpressed IEX-1 (red) and endogenous PML staining (green) are shown. Co-localization is indicated by yellow coloring.

the overlay (see single spot in Fig. 3A). IFNγ treatment of mock-transfected HeLa cells resulted in an enhanced staining for PML in the nucleus (Fig. 3B), and endogenous IEX-1 was found partially co-localized with larger PML dots (Fig. 3B). Upon overexpression of native IEX-1 protein and in the absence of IFNγ, co-localization is seen in a few spots as a partial overlap (Fig. 3C). These dots consisted of pure PML staining (green) and pure IEX-1 staining (red), with distinctly co-localized contact sites as demonstrated by yellow coloring. In the presence of IFNγ, overexpressed native IEX-1 was found more completely co-localized with PML nuclear dots (Fig. 3D). Under any circumstances regarding IFNγ-induced endogenous IEX-1
or overexpression of IEX-1, individual single stained nuclear dots for IEX-1 and PML were found. Each protein appears to exist in its own nuclear subdomain, and the proteins only partially co-localize.

Leptomycin-B Treatment of HeLa Cells Leads to Enrichment of IEX-1 Protein in the Nucleus—One explanation for the strong enrichment of GFP-IEX-1 in the nucleus might be an inefficient nuclear export, either because of the covering of a potential nuclear export signal by the GFP moiety of the fusion protein or the exhaustion of the respective carrier because of insufficient degradation of the fusion protein. To investigate whether IEX-1 is subject to specific nuclear export, HeLa cells, either mock- or IEX-1-transfected, were treated with leptomycin-B (LMB), an inhibitor of the CRM1 nuclear export protein, leading to the nuclear enrichment of shuttling proteins that depend on CRM1-mediated nuclear export. Compared with untreated cells that show little, though distinct, nuclear staining for IEX-1 (Fig. 4A), HeLa cells treated with 10 ng/ml LMB for 20 h exhibit strongly enhanced nuclear staining, and some cells show an enlargement of IEX-1-positive nuclear subdomains (Fig. 4B) reminiscent of the GFP fusion protein aggregates (compare Fig. 1A). As only endogenous IEX-1 is detected in mock-transfected cells (Fig. 4B), this clearly underlines that inhibition of transport leads to enrichment of IEX-1 in the nucleus.

The particular pattern of IEX-1 and PML in partially overlapping nuclear substructures was also seen after LMB treatment (Fig. 4C and D), suggesting that enhanced nuclear staining for IEX-1 is not only seen during IFNγ treatment but also when nuclear export is blocked by LMB, indicating that the appearance of partial co-localization is a specific process.

Overexpression of PML in HeLa Cells Reveals Isoform-dependent Nuclear Dot Structures Serving as a Matrix for Distribution of Overexpressed IEX-1—To explore the co-localization of IEX-1 and PML in more detail, PML isoforms III and IV were independently overexpressed in HeLa cells co-transfected with GFP-IEX-1. Whereas PML-III overexpression (Fig. 5, A–D) resulted in numerous small round dot structures (Fig. 5B, red), PML-IV overexpression (Fig. 5, E–H) was generally weaker, and the protein, as detected by anti-PML antibodies, was distributed more diffusely in patch-like structures (Fig. 5F, red). Interestingly, the expression pattern for GFP-IEX-1 fusion proteins in these double transfected cells followed suit. In PML-III-overexpressing cells, GFP-IEX-1 was seen in numerous small round dots (Fig. 5A, green) and almost totally co-localized with PML-III (Fig. 5, C and D). In PML-IV-overexpressing cells, GFP-IEX-1 expression was weaker and again was distributed like PML-IV in patch-like assemblies (Fig. 5E). This expression pattern for PML isoforms was also found in HeLa cells after individual overexpression of PML-III or -IV (data not shown), and co-localization with GFP-IEX-1 was reproducible in a different, mesenchymal cell line, U2OS (data not shown).

Double transfection experiments with both PML isoforms and native IEX-1 revealed a perfect co-localization between PML-IV and IEX-1 (Fig. 6, E–H), showing the same patch-like distribution for IEX-1 (Fig. 6E, green) and PML-IV (Fig. 6F, red), and overexpressed proteins were fully co-localized (Fig. 6, G and H). Native IEX-1 was found more diffuse in the nucleus (Fig. 6A, green) when co-expressed with PML-III (Fig. 6B, red), and co-localization was seen at the rim of PML-III nuclear dots (Fig. 6, C and D).

Co-immunoprecipitation Experiments Reveal Direct Interaction of IEX-1 and PML—To detect the physical interaction of IEX-1 with PML, the PML isoforms PML-III and PML-IV as well as IEX-1 were expressed in single or double transfected HeLa cells. Western blotting with antibodies against IEX-1 (C20; Santa Cruz) revealed three different bands of IEX-1 at a size of 20–30 kDa in 4–20% gradient gels. Monoclonal anti-PML antibodies (Santa Cruz) revealed a broader band of PML-IV at a size of 90 kDa and a more distinct band of PML-III at a size of 100 kDa (Fig. 7). Compared with single transfection, the amount of PML-III protein strongly increased in HeLa cells co-transfected with IEX-1, whereas IEX-1 levels were slightly elevated. Co-transfection of PML-IV and IEX-1 did not alter the amount of PML-IV protein in comparison with single transfected cells, but IEX-1 protein levels significantly decreased in the presence of PML-IV (Fig. 7).

A high amount of IEX-1 protein was precipitable (Fig. 8a,
From HeLa cells co-transfected with IEX-1 and PML-III, and a significant portion of IEX-1 co-precipitated together with PML-III in anti-PML immunoprecipitation experiments (Fig. 8a, lane 3). In contrast, very little amount of IEX-1 protein was precipitable (Fig. 8a, lane 8) from HeLa cells co-transfected with IEX-1 and PML-IV, and no IEX-1 protein co-precipitated together with PML-IV (Fig. 8a, lane 9). To ensure optimal PML expression (31), co-transfectants were further treated with arsenic oxide (1 μM, 4 h), strongly stabilizing the PML protein, and with MG132 (1 μM, 4 h), inhibiting degradation of PML by the proteasome. Under these conditions, elevated amounts of IEX-1 protein (Fig. 8a, lane 5) as well as PML-III and PML-IV were immunoprecipitated. In addition, an increased portion of IEX-1 protein co-precipitated together with PML-III (Fig. 8a, lane 6). Of note, a high portion of the increased amount of immunoprecipitable IEX-1 protein (Fig. 8a, lane 11) was now co-precipitated with PML-IV (Fig. 8a, lane 12). However, no co-precipitated PML-III or PML-IV protein could be detected under these conditions if IEX-1 was immunoprecipitated. We further performed immunoprecipitation experiments using GFP-IEX-1 fusion protein co-transfected in HeLa cells together with PML-III or PML-IV. As shown in Fig. 8b, immunoprecipitation of PML-III co-precipitated significant amounts of GFP-IEX-1 detected by antibodies directed against the GFP moiety of the fusion protein. Immunoprecipitation of PML-IV from co-transfection with GFP-IEX-1 showed less material precipitated with anti-PML antibodies, similar to the results with control cells shown in Fig. 8a, lane 9. Obviously, there is no
restriction of interaction between the stabilized GFP-IEX-1 fusion protein and PML-III, as seen before for perfect co-localization of these proteins by LSM analysis.

**Pulldown Experiments Using GST-IEX-1 Fusion Protein Precipitate PML Proteins—**As shown above, we barely observed PML reactivity in anti-IEX-1 precipitates. This finding suggested that the antibodies used for precipitating IEX-1 could not access IEX-1 bound to PML. To test this hypothesis, we used pulldown experiments with HeLa cells, overexpressing PML-III or PML-IV. Cellular lysates were incubated with GST-IEX-1 coupled to glutathione-Sepharose or for control purposes with either glutathione-Sepharose 4B or GST protein coupled to glutathione-Sepharose. As shown in Fig. 9, from PML-III-transfected HeLa cells a strong PML reactive band in GST-IEX-1 pulldowns was detectable, whereas pulldowns from PML-IV-transfected HeLa cells brought about a rather weak PML reactive band. In contrast, no such bands were seen in the two controls using either glutathione-Sepharose alone or GST-Sepharose. These results confirm the interaction of IEX-1 with PML that makes IEX-1 less accessible to the antibody described above. Again, this interaction of IEX-1 is more pronounced with PML-III than with PML-IV.

**DISCUSSION**

The exact molecular mechanisms by which IEX-1 exerts its role as a widely expressed stress- and proliferation-associated early response gene are still not very well known. IEX-1 and its rodent counterparts gty96 and PRG1 are part of growth and survival signals on the one hand (2, 4, 11, 17, 19, 22, 23) and death-inducing signals on the other hand (14, 17, 18, 20, 21, 24–27).

From these divergent effects of IEX-1 on cellular viability it could be speculated that IEX-1 is implicated in a more general cellular signaling network adapting a cell to governing stress and growth conditions. To gain new insights into the mode of actions exerted by IEX-1, we characterized its intracellular localization in HeLa cells by various means. We investigated the localization of different IEX-1-GFP fusion proteins, overexpressed native IEX-1, and endogenous IEX-1 protein. Although the majority of IEX-1 protein resides in the cytoplasm of HeLa cells, we found it was also present in distinct subnuclear compartments. Expression of an N-terminal-fused GFP-IEX-1 protein resulted in its strong accumulation in the nucleus, particularly in quite unique, mostly rounded nuclear subdomains with clear margins. This finding led us to investigate the impact of the putative NLS found in IEX-1. As we could show, the accumulation of IEX-1 in the nucleus depends on this NLS for nuclear import because the GFP-ΔNLS/IEX-1 fusion protein lacking this NLS was not found in the nucleus anymore, whereas an N-terminal-shortened IEX-1-GFP fusion protein retaining the NLS was still detectable in nuclear subdomains. Notably, along with the abolished nuclear localization, the proapoptotic activity of IEX-1 decreased, suggesting that the recruitment of IEX-1 into the nucleus is related to its role in apoptosis. However, the question arose as to whether further factors might influence nuclear accumulation of IEX-1. Using leptomycin-B, an inhibitor of the nuclear exporter CRM1 (34), we found evidence that IEX-1 not only enters the nucleus directed by its NLS but also depends on CRM1 for nuclear export. In the case of the N-terminal fusion to GFP, this CRM1-dependent export may be prevented, leading to strong accumulation of GFP-IEX-1 in the nucleus.

These data indicated that IEX-1 is specifically localized in particular subnuclear regions, and it was attractive to test for co-localization of IEX-1 with PML, which is known to reside in defined so-called PML nuclear bodies. These unique PML-NBs (28, 29) are extensively investigated but still not fully understood protein complexes consisting of the PML protein and other important regulatory proteins (28–32). In response to viral stress, DNA damage, and various other stress conditions, PML, in part accompanied by its sumoylation, accumulates in PML-NBs (31, 35, 36), thereby recruiting proteins such as Sp100, CBP/p300, Daxx, Mdm2, HIPK2, p53, Rb, or HDAC (33, 37–45). The modulated actions of these proteins then account for the PML-dependent changes in cellular growth, including cell cycle arrest, senescence, apoptosis, or differentiation (46–51). Consequently, disruption of PML-NB function can result in higher susceptibility to tumor formation (49, 51, 52). Because a great variety of regulatory proteins seems to be involved in the actions of PML, it has been suggested that PML is required for the assembly and functional orchestration of these proteins. It is therefore tempting to speculate that IEX-1 contributes to the modulatory action of these unique nuclear structures.

We could show a very distinct co-localization pattern of PML with endogenous IEX-1 protein and overexpressed native IEX-1 protein in HeLa cells. This co-localization pattern shows a partial overlap of PML-NBs and IEX-1 nuclear dots, which share a common region occupied by both proteins. A partial overlap in a quite similar manner has been also shown for PLZF and PML in leukemia cell lines, indicating a differential architecture within nuclear dots (33).

As further shown by co-transfection experiments with PML-III or PML-IV, these isoforms showed totally different expression/accumulation patterns in the nucleus of HeLa cells. Whereas PML-III was found in the expected well defined dense small round nuclear dots, PML-IV expression showed a patch-like accumulation in the nucleus. Most notably, co-transfected GFP-IEX-1 fusion protein followed these individual PML expression patterns, showing perfect overlap with PML-III in small round nuclear dots and a patchy expression co-localized with PML-IV. It is tempting to speculate that the different C termini of PML proteins are responsible for their distribution. The finding that IEX-1 overexpression follows suit in distribution when co-expressed with PML isoforms suggests that PML isoforms generate a matrix for other associated proteins to attach to or to use as a guideline or scaffold. Furthermore, besides the neat co-localization of PML and IEX-1, immunoprecipitation experiments revealed coprecipitation of IEX-1 and PML. Interestingly, the distinct positive feedback for PML-III and IEX-1 expression, as well as the negative co-regulation of PML-IV and IEX-1 expression, hints toward functional associations yet to be investigated.

Altogether, the finding that IEX-1 is associated with PML-NBs provides a new idea about the role of IEX-1 as stress-responsive gene in so far as it may participate in a complex signaling network that connects distinct subnuclear compartments and modulates various nuclear processes such as gene
transcription, DNA repair, or the storage, modification, and degradation of nuclear proteins. In this way, the dual role of IEX-1 in the regulation of cellular growth and survival (reviewed in Ref. 53) might be related to its interaction with the PML-NBs and their multiple functions in apoptosis and stress response. This conclusion would also fit the recent view that IEX-1 may possess tumor-suppressive potential (54–56).

REFERENCES

1. Kondratyev, A. D., Chung, K. N., and Jung, M. O. (1996) Cancer Res. 56, 1498–1502
2. Kobayashi, T., Pettelkow, M. R., Warner, G. M., Squillace, K. A., and Kumar, R. (1996) Biochem. Biophys. Res. Commun. 231, 868–873
3. Kumar, R., Kobayashi, T., Warner, G. M., Wu, Y., Salisbury, J. L., Lingle, W., and Pettelkow, M. R. (1996) Biochem. Biophys. Res. Commun. 233, 336–341
4. Schafer, H., Lettau, P., Trauzold, A., Bansch, M., and Schmidt, W. E. (1996a) FEBS Lett. 343, 139–143
5. Domachowski, J. B., Bonville, C. A., Mortelliti, A. J., Celella, C. B., Kim, U., and Rosenberg, H. P. (2000) J. Infect. Dis. 181, 824–830
6. Schafer, H., Trauzold, A., Schens, T., Deppert, W., Fulsch, U. R., and Schmidt, W. E. (1998) Oncogene 16, 2479–2487
7. De Keulenaer, G. W., Yang, Y., Fang, Y., Muangman, S., Yamamoto, K., Thompson, J. P., Turi, T. G., Landschutz, K., and Lee, R. T. (2002) Circ. Res. 90, 690–696
8. Schafer, H., Trauzold, A., Siegel, E. G., Fulsch, U. R., and Schmidt, W. E. (1996) Cancer Res. 56, 2641–2648
9. Charles, C. H., Yoon, J. K., Simske, J. S., and Lau, L. F. (1993) Mol. Cell. Biol. 13, 998–1001
10. Schafer, H., Yoon, J. K., Simoke, J. S., and Lau, L. F. (1993) Oncogene 8, 797–801
11. Pietzsch, A., Buchler, C., and Schmitz, G. (1997) Biochem. Biophys. Res. Commun. 235, 4–9
12. Pietzsch, A., Buchler, C., Schmitz, G., and Schmitz, G. (1997) Biochem. Biophys. Res. Commun. 235, 4–9
13. Im, H. J., Craig, T. A., Pittelkow, M. R., and Kumar, R. (2002) FEBS Lett. 494, 196–200
14. Arlt, A., Minkenberg, J., Kocs, B., Grossmann, M., Kruse, M. L., Fulsch, U. R., and Schafer, H. (2004) Leukemia 18, 1646–1655
15. Im, H. J., Pettelkow, M. R., and Kumar, R. (2002) J. Biol. Chem. 277, 14612–14621
16. Huang, Y.-H., Wu, J. Y., Zhang, Y., and Wu, M. X. (2002) Oncogene 21, 6819–6828
17. Grobe, O., Arlt, A., Ungefroren, H., Krupp, G., Fulsch, U. R., Schmidt, W. E., and Schafer, H. (2001) FEBS Lett. 494, 196–200
18. Segev, D. L., Ha, T. U., Tran, T. T., Kenneally, M., Harkin, P., Jung, M., MacLaughlin, D. T., Donahoe, P. K., and Maheswaran, S. (2000) J. Biol. Chem. 9, 28371–28379
19. Wu, M. X., Ao, Z., Prasad, K. V., Wu, R., and Schlossman, S. F. (1998) Science 281, 998–1001
20. Arlt, A., Grobe, O., Sieke, A., Kruse, M. L., Fulsch, U. R., Schmidt, W. E., and Schafer, H. (2001) Oncogene 20, 69–76
21. Schilling, D., Pettelkow, M. R., and Kumar, R. (2001) Oncogene 20, 7992–7997
22. Zang, Y., Schlossman, S. F., Edwards, R. A., Ou, C. N., Gu, J., and Wu, M. X. (2002) Nat. Acad. Sci. U.S.A. 99, 878–883
23. Garcia, J., Ye, Y., Arranz, V., Letournoux, C., Pezeron, G., and Porteu, F. (2002) EMBO J. 21, 5151–5163
24. Schafer, H., Arlt, A., Trauzold, A., Huermann-Jansen, A., and Schmidt, W. E. (1999) Biochem. Biophys. Res. Commun. 262, 139–145
25. Segev, D. L., Hoshiya, Y., Stephen, A. E., Hoshiya, M., Tran, T. T., MacLaughlin, D. T., Donahoe, P. K., and Maheswaran, S. (2001) J. Biol. Chem. 276, 26799–26806
