c-Abl Tyrosine Kinase Selectively Regulates p73 Nuclear Matrix Association*

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p73 is a structural and functional homologue of the p53 tumor-suppressor protein. Like p53, p73 is activated in response to DNA-damaging insults to induce cell cycle arrest or apoptosis. Under these conditions p73 is tyrosine-phosphorylated by c-Abl, a prerequisite modification for p73 to elicit cell death in fibroblasts. In this study we report that in response to ionizing radiation, p73 undergoes nuclear redistribution and becomes associated with the nuclear matrix. This association is c-Abl-dependent because it was not observed in cells that are defective in c-Abl kinase activation. Moreover, STI-571, a specific c-Abl kinase inhibitor, is sufficient to block significantly p73 nuclear matrix association. The observed c-Abl dependence of nuclear matrix association was recapitated in the heterologous baculovirus system. Under these conditions p73a but not p53 is specifically tyrosine-phosphorylated by c-Abl. Moreover, the phosphorylated p73a is predominantly found in association with the nuclear matrix. Thus, in response to ionizing radiation p73 is modified in a c-Abl-dependent manner and undergoes nuclear redistribution and translocates to associate with the nuclear matrix. Our data describe a novel mechanism of p73 regulation.

The p73 polypeptide is a member of the p53 family of proteins (1, 2). There are several p73 isoforms that differ at their N and C termini (3–5). p73a is the longest isoform containing at its C terminus a sterile-α motif domain, a domain known to mediate protein-protein interactions (6–8). The p73 AN isoforms lack the transactivation domain and display dominant negative activity (9–12). Thus it is very likely that each of the isoforms has a unique role whose nature remains to be clarified.

Like p53, p73 can induce apoptosis in a variety of cell lines and support transcription of genes containing p53-response elements (1, 2). In response to DNA damage signals, these proteins undergo covalent modifications. However, the identity of the upstream effectors was not fully resolved. The c-Abl non-receptor tyrosine kinase was identified as an upstream effector of p73 both in vitro and in vivo (13, 14). The level of p73 tyrosine phosphorylation is elevated when cells are exposed to ionizing radiation, a condition whereby c-Abl kinase is activated (13, 14). Disruption of c-Abl-p73 interaction rescues cells from irradiation-induced apoptosis (13, 14). Interestingly, a p73 point mutant at tyrosine 99 (T99F), a site that is phosphorylated by c-Abl, behaves as a dominant negative mutant and blocks the apoptotic response to IR1 (14).2

Accumulating evidence indicates that p73 is essential for apoptosis induced by many chemotherapeutic agents and IR. These treatments often lead to p73 accumulation. However, some cell lines are refractory to certain agents (1, 15, 16) suggesting the involvement of additional effectors in this process. A recent study showed that the induction of apoptosis by p53 requires the presence of p73 (17). However, p73 can induce apoptosis in the absence of p53 (15). An interesting interplay between p73 and p53 mutants became recently evident. Several p53 mutants, in particular of the p53Arg72 variant, are potent inhibitors of p73 function (15, 16). Although not directly demonstrated, it is possible that their physical interaction (18–20) accounts for the inhibitory function of p53 mutants.

Although an enzyme-substrate relationship between c-Abl and p73 has been established, it is still unclear how p73 modification by c-Abl influences p73 ability to induce apoptosis. It has been demonstrated that the p73 half-life is prolonged by treatment with the DNA-damaging agent cisplatin, and the accumulated p73 activates certain downstream targets to induce apoptosis (21). However, sole p73 accumulation might not be sufficient in this process. Recently, it has been reported that DNA damage induces p73 acetylation by the acetyltransferase p300, a modification that gives rise to selective transactivation of proapoptotic target genes (22). This process requires functional c-Abl, suggesting that p73 tyrosine phosphorylation may trigger the whole pathway. This is also the case in other p73 pathways. For example, p73 threonine phosphorylation by p38 mitogen-activated protein kinase by p38 mitogen-activated protein kinase requires functional c-Abl (23). It appears therefore that p73 tyrosine phosphorylation by c-Abl sensitizes p73 to undergo additional modifications.

It is evident from the study of p53 that, in addition to protein modification and stabilization, subcellular localization plays a critical role in regulation of p53 function (24–26). Little is known about p73 subcellular localization, but it appears that this may play an important role in p73 function as well. Both p73 and c-Abl colocalize in the cytoplasm of spermatogonia and spermatocytes suggesting a tissue-specific regulation of these proteins by the mechanism of subcellular localization (27). The Yes-associated protein is a p73 transcriptional coactivator (28). It has been reported recently (29) that cytoplasmic sequestration of Yes-associated protein reduces p73-mediated induction

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1 The abbreviations used are: IR, ionizing radiation; CIP, calf intestinal alkaline phosphatase; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; PBS, phosphate-buffered saline; PIPES, 1,4-piperazinediethanesulfonic acid; Gy, gray; PML, promyelocytic leukemia.

2 M. Ben-Yehoyada, I. Ben-Dor, and Y. Shaul, unpublished data.
of Bax expression and p73-mediated apoptosis, following DNA damage. However, the possibility that under this condition p73 translocates to the cytoplasm was not investigated. Finally, HIPK2 is a member of a novel family of nuclear serine/threonine kinases previously found to bind and activate p53 (31, 32) via direct p53 phosphorylation at Ser-46 (33). HIPK2 localizes with p55 and PML-3 in the nuclear bodies (also named promyelocytic leukemia (PML) bodies). Interestingly, similar to p55, p73 colocalizes with HIPK2 in PML. These data define functional interaction between p73 and HIPK2 that results in the targeted subcellular localization of p73.

In this study we followed p73 cellular distribution under conditions where p73 is tyrosine-phosphorylated by c-Abl. We provide evidence for p73α translocation to the nuclear matrix fraction in a c-Abl-dependent manner. This process requires a functional c-Abl kinase domain. Interference with c-Abl kinase activity either by using c-Abl kinase-incompetent cells or STI-571, a c-Abl kinase pharmacological inhibitor, disrupts p73α nuclear matrix association. Finally, we utilized the insect cells to overexpress p73 and c-Abl. Under these conditions p73α is efficiently tyrosine-phosphorylated with its concomitant translocation to the nuclear matrix fraction. These data provide evidence for a novel mechanism of p73 regulation.

**EXPERIMENTAL PROCEDURES**

**Cell Cultures and Baculovirus Infection—**Human colon carcinoma lines HCT116, HCT116-3(6) (34), and monkey kidney fibroblast COS-1 were cultured in standard medium that was supplemented with 10% fetal bovine serum and antibiotics. H5 insect cells were grown in Grace’s insect medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum, 50 units/ml penicillin, 50 μg/ml streptomycin, 100 μg/ml gentamicin at 27°C.

Expression of c-Abl and p73 in insect cells was performed as described in the Bac-to-Bac baculovirus expression system manual (Invitrogen). c-Abl cDNA (nucleotides 1–4113) was ligated into the BamHI-StuI sites of the pFASTBAC HTb expression vector encoding an N-terminal histidine tag. Simian p73 cDNA (nucleotides 1–1887) was introduced into the BamHI-HindIII sites of pFASTBAC HTc also encoding a histidine tag. Bacmid corresponding to each plasmid was isolated and amplified and the viruses were amplified for three additional times. The p53 baculovirus was kindly provided by Dr. V. Rotter.

**Materials and Antibodies—**Propidium iodide and micrococcal nuclease were purchased from Sigma; genistein was from Calbiochem; c-Abl was from ABIC Israel; c-terminal alkaline phosphatase (CIP) was from New England Biolabs, and DNase I was from Roche Applied Science. STI-571 was kindly provided by Novartis Pharmaceuticals. IR was done with Co60 source. Antiserum to simian p73 was raised in rabbits immunized with bacterially produced, SDS-PAGE-purified p73 (amino acids 1–667). PY99 antibodies were raised in rabbits immunized with phosphorylated peptide (CVPTSHSPY(PO3H2)AQP-NH2). Rabbit antiserum was further purified on a protein-A/G column. The phosphorylated antibody was characterized, and it recognizes specifically phosphorylated p73α at tyrosine 99 (data not shown). Antibody 1801, a p53 monoclonal antibody (35), was kindly provided by Dr. M. Oren. Goat polyclonal anti-c-Abl (M-20), rabbit polyclonal anti-c-Abl (K-12), mouse monoclonal p73 (H-79), and mouse monoclonal anti-phosphotyrosine (PY20) were purchased from Santa Cruz Biotechnology.

**Protein Extraction and Immunoprecipitation—**Forty eight hours post-infection, H5 insect cells were collected and resuspended in RIPA buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, 1% Nonidet P-40 (v/v), 0.1% SDS (v/v), 0.05% Triton X-100, 1 μg/ml each of leupeptin, aprotinin, pepstatin, and 1 mM PMSF (Sigma mixture)). After 5 min at 4°C, the cytoskeletal framework was separated from soluble proteins by centrifugation at 5,000 × g for 3 min. Chromatin was digested with 1 mg/ml DNase I in 2 volumes of CSK buffer for 5 min at 37°C. Then ammonium sulfate was added to final concentration in CSK buffer to a final concentration of 0.25M and after 5 min at 4°C and spun down. The pelot was further extracted with 2 μl NaCl in CSK buffer for 5 min at 4°C and centrifuged. This treatment removed the entire DNA and histones from the nucleus. The remaining pelot was solubilized in urea buffer (8 M urea, 0.1 M NaH2PO4, and 0.01 M Tris, pH 8), and regarded as the nuclear matrix containing fraction.

**Chromatin Fractionation—**Chromatin fractionation was performed as described (37). In brief, for chromatin isolation, cells were resuspended in buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM MgCl2, 0.34 M sucrose, 10% glycerol, 1 mM DTT, 1 μg/ml each of leupeptin, aprotinin, pepstatin, and 1 mM PMSF (Sigma mixture)). Triton X-100 (0.1%) was added, and the cells were incubated for 5 min on ice. Nuclei were collected by low speed centrifugation (4 min for 1,300 × g). The supernatant was further clarified by high speed centrifugation (10 min for 20,000 × g) to remove cell debris and insoluble aggregates. Nuclei were washed once in buffer B and then lysed in buffer B (3 mM EDTA, 0.2 mM EGTA, 1 mM DTT, protease inhibitors as described above). Insoluble chromatin was collected by centrifugation (4 min for 1,700 × g), washed once in buffer B, and centrifuged again under the same conditions. The final chromatin pellet was resuspended in Laemmli buffer and sonicated for 15 s. To release chromatin-bound proteins by nuclease treatment, cell nuclei were resuspended in buffer A plus 1 mM CaCl2 and 0.5 units of micrococcal nuclease. After incubation for 5 min at 37°C, the nuclease reaction was stopped by the addition of 1 mM EDTA. Nuclei were collected by low speed centrifugation and lysed according to the chromatin isolation protocol described above.

**Cell Cycle Analysis—**For fluorescence-activated cell sorter analysis cells were harvested 2–40 h post-IR, washed twice with PBS, and fixed in 70% ethanol. After fixation, the cells were washed with PBS and resuspended in 50 μg/ml RNase A and 25 μg/ml propidium iodide in PBS. In each assay 10,000 cells were collected by FACScan and analyzed with the Cellquest program (BD Biosciences).

**RESULTS**

p73α Associates with the Nuclear Matrix in Irradiated Cells—Protein post-translational modifications such as phosphorylation and sumoylation regulate protein spatial compartmentalization (38–40). To investigate the effect of IR and cisplatin on p73 nuclear redistribution, we employed the high salt nuclear matrix fractionation procedure to separate the various nuclear fractions (36). First, soluble proteins were extracted using non-ionic detergent (fraction I) followed by DNase I digestion and ammonium sulfate precipitation of the chromatin associated proteins (fraction II). Fraction III was obtained by washing the pellet with 2 μl NaCl. The remaining pelot was solubilized in 8 M urea to obtain fraction IV which is contained with the structural nuclear matrix and the associated proteins (Fig. 1A) (41). The obtained fractions were SDS-PAGE separated and immunoblotted with the indicated antibodies. In the non-treated COS-1 cells p73α is detected predominantly in the nucleocytoplasm fraction (Fig. 1B). Interestingly, following exposure to IR, a significant increase in p73 level was detected in the nuclear matrix fraction (fraction IV). The effectiveness of the fractionation protocol was verified by specific detection of the lamin B nuclear matrix protein in this fraction. In COS-1 cells cisplatin did not increase p73 levels, and no nuclear matrix accumulation was observed. As a control we monitored the level of p53 in the obtained fractions. Notably, under these conditions the amount of p53 remained unchanged in response
The nuclear matrix fraction is c-Abl kinase-dependent.

In addition, p53 unlike p73, is not preferentially associated with the nuclear matrix and not with the chromatin fraction. In contrast to p73, p53 was accumulated upon treatment but not the nuclear matrix fraction, because it was solubilized almost completely by micrococcal nuclease (Fig. 2C, lower panel, lanes 4 and 5). These data indicate that in response to IR, p73α, but not p53, translocates from soluble nuclear fraction to the nuclear matrix.

**Inhibition of c-Abl Kinase Activity Blocks p73α Association with the Nuclear Matrix**—To substantiate the role of c-Abl kinase in p73α nuclear matrix translocation, we used the c-Abl kinase inhibitor STI-571 (42, 43). The proficient c-Abl HCT116-3(6) cells were irradiated in the presence or absence of STI-571, and nuclear fractions were analyzed. Here again, irradiation of HCT116-3(6) cells triggered p73α association with nuclear matrix (Fig. 3, lane 3). Interestingly, no p73α accumulation in the nuclear matrix fraction was evident when c-Abl kinase activity was inhibited by STI-571 (lane 4). The proficiency of the fractionation protocol was confirmed by using anti-lamin B antibody, a nuclear matrix protein. This finding indicates that in response to IR c-Abl kinase regulates p73α association with the nuclear matrix.

**p73α Nuclear Redistribution Is a Slow, Gradual, and IR Dose-dependent Process**—By having demonstrated that in response to IR, p73α becomes associated with the nuclear matrix in a c-Abl kinase-dependent manner, we next measured IR dose response and time kinetics of this process. For IR dose response the paired HCT cells were used. A significant accumulation of p73 in the nuclear matrix was obtained only with the c-Abl proficient HCT116-3(6) line (Fig. 4A). This process is IR dose-dependent and responds to as low as 4 Gy irradiation. To study the time kinetic of this process, c-Abl-proficient HCT cells were irradiated with 20 Gy, and the p73α level in fractions I and IV was compared at the indicated time points (Fig. 4B). Analysis of the p73α levels in fraction IV revealed that p73 is slowly accumulated to reach maximal level 40 h after irradiation. A number of p73 bands were detected of which the slowest migrating one is selectively eliminated from the soluble nucleoplasm (fraction I) and became associated with the nuclear matrix. The nature of the detected various p73 bands was not resolved. The level of the slowest migrating p73α was quantified, and the ratio between the two nuclear fractions was determined and plotted versus time post-exposure to IR (Fig. 4C). Following IR, the p73 level in nuclear matrix slowly but gradually increases from about 25% at the basal level to about 80% 40 h post-irradiation. The cell cycle pattern of the irradiated cells was determined at these time points. 20 h after irradiation a substantial cell fraction displayed G2-M cell cycle arrest and after 40 h cells began to undergo apoptosis as determined by an increase in the level of sub-G1 fraction (Fig. 4D). However, similar cell cycle profiles were obtained with the c-Abl activation-deficient HCT116 cells, indicating that p73α association with the nuclear matrix is c-Abl kinase-dependent.

To substantiate the finding that p73α associates with the nuclear matrix and to rule out the possibility that this is the direct outcome of the high salt extraction procedure, we used an alternative chromatin fractionation protocol (37). In this procedure, cells are lysed with non-ionic detergent in a sucrose-rich buffer, and nuclei are then collected by low speed centrifugation, washed, and lysed in no salt buffer. A second centrifugation step separates the remaining soluble nuclear proteins from the insoluble fraction that contains proteins bound to chromatin or to the nuclear matrix. To distinguish between the chromatin and the nuclear matrix-bound proteins, micrococcal nuclease treatment was employed to release the chromatin fraction (Fig. 2B). In the untreated HCT116-3(6) cells, p73α is predominantly found in the fraction containing the soluble proteins (Fig. 2C, lane 1) with a minute amount in the soluble nuclear protein fraction (lanes 2 and 3) but not in the chromatin fraction. Upon IR treatment, the p73α level in the soluble protein fraction decreased with concomitant accumulation in the chromatin and nuclear matrix fractions. Because p73α was not solubilized after micrococcal nuclease treatment, we concluded that p73α is preferentially associated with the nuclear matrix and not with the chromatin fraction.
nuclear matrix is not the outcome of either G2-M arrest or apoptosis but rather a more direct effect of an active c-Abl.

**p73α and c-Abl Overexpression and Modification in Insect Cells**—To investigate the relationship between p73α and c-Abl in a heterologous system, we expressed both proteins in H5 insect cells infected with recombinant baculovirus stocks. Cells infected with c-Abl or p73α recombinant baculoviruses express 140- and 80-kDa histidine-tagged c-Abl and p73α proteins, respectively (Fig. 5A). Interestingly, in cells coexpressing p73α and c-Abl, additional and up-shifted p73α bands that may correspond to the phosphorylated p73α forms were detected. In contrast, expression of p53 with or without c-Abl showed no change in p53 mobility on the SDS-PAGE. By using CIP, the upper band was selectively lost (Fig. 5B). Anti-Tyr(P)-specific antibody confirmed CIP efficient activity. To verify this finding, we then prepared a specific polyclonal antibody, termed anti-PY99, that selectively detected tyrosine-phosphorylated p73α at position 99 (data not shown). This position corresponded to c-Abl phosphorylation site. As expected anti-PY99 specifically detected the upper and slower migrating bands (Fig. 5C). Thus, we concluded that the upper p73α band corresponded to phosphorylated p73 mostly at position Tyr-99.

**p73α but Not p53 Physical Interaction with c-Abl**—In animal cells p73 and c-Abl are in physical interaction (13, 14). To examine whether physical interaction also takes place under heterologous environment, extracts of H5 cells expressing p73α...
alone or in combination with c-Abl were subjected to coimmunoprecipitation, followed by Western blot analysis with anti-p73 and anti-phosphotyrosine antibodies. Interestingly, p73 was coimmunoprecipitated with anti-c-Abl antibody suggesting their direct physical interaction (Fig. 6A). Moreover, we found that p73 was efficiently immunoprecipitated using anti-phosphotyrosine antibodies (Fig. 6A). In a reciprocal experiment anti-p73 antibody immunoprecipitated tyrosine-phosphorylated p73, confirming the previous finding that p73 is a genuine c-Abl substrate (Fig. 6B). Significantly, despite the efficient p53 expression in H5 cells (Figs. 5 and 6C) no physical interaction of p53 with c-Abl was obtained, and p53 was not tyrosine-phosphorylated (Fig. 6B, left panel). Collectively, these data indicate that p73α and c-Abl interaction is highly specific and can be recapitulated in heterologous insect cells.

**Preferential Association of Tyrosine-phosphorylated p73α with the Nuclear Matrix**—By having demonstrated that p73 is tyrosine-phosphorylated by c-Abl in H5 insect cells, we next asked whether modified p73 translocates to the nuclear matrix. Western blot analysis of the subcellular fractions described above revealed that p73α accumulates in the nuclear matrix fraction (Fig. 7A, lane 4). Moreover, nuclear matrix associated p73α is preferentially tyrosine-phosphorylated as was determined by employing the anti-phosphotyrosine antibodies. Under these conditions a minor portion of c-Abl was found in the nuclear matrix fraction possibly due to its association with p73 (Fig. 7A, lanes 1 and 4). To test whether the phosphorylation status of p73α governs its sub-nuclear localization, cells were treated with genistein (40 μM for 48 h), an inhibitor of tyrosine kinases. As a result the level of tyrosine phosphorylation was markedly reduced with a significant reduction in the amount of p73 in the nuclear matrix fraction.

For the more direct role of c-Abl kinase activity in this process, we treated H5 cell with STI-571 (10 μM for 48 h), a specific inhibitor of c-Abl tyrosine kinase. Under this condition a remarkable reduction in the level of phosphotyrosine proteins

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**Fig. 4.** p73α association with the nuclear matrix is irradiation dose-dependent and a gradual process. A, the paired HCT116 and HCT116-3(6) were irradiated with different IR doses and harvested 40 h post-treatment. The nuclear matrix fraction was subjected to SDS-PAGE and immunoblotted (IB) with the indicated antibodies. B, HCT116-3(6) cells were irradiated (20 Gy), and nuclear matrix fractionation and analysis were performed at the indicated time points. C, the relative p73α levels in the nuclear matrix versus total p73α expression were quantified and plotted versus time. D, cell cycle analysis of the paired cells at different time points after irradiation.

**Fig. 5.** p73α is phosphorylated by c-Abl in insect cell culture. A, H5 insect cells were coinfected with equal titers of c-Abl, p73, and p53 recombinant baculovirus stocks, as indicated on top of the lanes. Western blot analysis of total cell extract of infected H5 insect cells was performed using either anti-p73 or anti-p53 and anti-c-Abl (K12) antibodies. B, cell extracts from H5 insect cells coinfected with p73 and c-Abl viruses were treated with 2 units of CIP for 15 min at 37 °C. C, PY99 phospho-p73 protein-specific antibodies recognized a specific band only in the cells coinfected with p73 and c-Abl recombinant baculovirus stocks. IB, immunoblotted.
over, this sequence of events is unique to p73

perform the relevant experiments in these cells as well.

enough to detect the endogenous p73 in animal cells in order to

nuclear matrix. This antibody was found not to be efficient

position 99 plays an essential role in translocation of p73 to

indicate that in insect cells p73 tyrosine phosphorylation at po-

As a control we fractionated H5 cells coexpressing p53 and

was detected (Fig. 7B). In addition anti-PY99 antibody that

reacts specifically with the tyrosine-phosphorylated p73 at position 99 detected the modified p73 exclusively in the nuclear

matrix fraction (Fig. 7B). Following STI-571 treatment no ty-

rosine-phosphorylated p73α could be detected. These data in-

dicate that in insect cells p73 tyrosine phosphorylation at po-

sition 99 plays an essential role in translocation of p73 to nuclear matrix. This antibody was found not to be efficient
d

to detect the endogenous p73α in animal cells in order to

perform the relevant experiments in these cells as well.

As a control we fractionated H5 cells coexpressing p53 and

c-Ab. Under these conditions p53 was accumulated mainly in the
nucleocytoplasm and chromatin fractions, namely fractions I and II but not in the nuclear matrix fraction. These data
dicate that tyrosine phosphorylation of p73α by c-Ab is sufficient
to trigger p73α association with nuclear matrix. Moreover,

this sequence of events is unique to p73α as p53, and the other member of the protein family was not affected by c-Ab in any of the measured parameters.

DISCUSSION

p73, a member of the p53 expanded family, is a downstream

substrate of c-Ab in an apoptotic pathway that is activated in response to ionizing radiation and DNA-damage agents (13,

14). In this study, we attempted to investigate this process by
dissecting the most immediate and downstream molecular se-

dquence of events that are triggered by p73 tyrosine phosphorylation. Collectively, our results indicate that p73α undergoes

nuclear redistribution and becomes associated with the nuclear

matrix in response to IR. This was not the case with cisplatin-
treated cells. Two different fractionation protocols were used to substantiate this finding. Furthermore, this behavior was ob-

served in different cell lines, including the heterologous insect

H5 cells. However, we could not detect p73 nuclear matrix

association in MCF7 cells (data not shown), suggesting certain
cell type specificity. The difference between MCF7 and HCT

response to IR is also evident at the level of cell cycle arrest,

and although the former is blocked at the G1/S phase the latter
displays G2 arrest (data not shown).

In response to IR, p73α is destined to the nuclear matrix,

provided that the cells bear a functional c-Ab kinase and that the

c-Ab kinase domain is not pharmacologically inhibited. Given the fact that p73 acetylation is c-Ab-dependent (22) one
can argue for acetylation to play a role in this process as well.

To address this point we used the insect cells where sole coex-

pression of c-Ab and p73 was sufficient to recapitulate the

process of p73 association with the nuclear matrix. These data

determine the role of p73 modification apart from tyrosine phos-

phorylation in p73α nuclear matrix association. Finally, it has

been reported that p73 is sumoylated at KXX (44). The p73α
clone that we have expressed in the insect cells is truncated at the C terminus and lacks 7 amino acids including the sumoylated residue; nevertheless, association with the nuclear matrix was not compromised. Thus, p73a sumoylation appears not to be essential in the process of p73a nuclear matrix association.

The p73 tyrosine phosphorylation by c-Abl in response to IR is a relatively fast event and could be detected within 3–4 h (13, 14), whereas association with the nuclear matrix progresses rather slowly. This lag in time cannot be explained by slow p73 intra-nuclear trafficking because a large body of evidence indicates that nuclear proteins are highly dynamic and can be spatially relocated within seconds to minutes (45). Given the fact that the whole process is recapitulated in insect cells by overexpressing c-Abl and p73 without exposure to IR, the involvement of other limiting factors in this process is ruled out. We are therefore left with the possibility that the lag in time is needed in order for the modified p73 to accumulate to reach a threshold level necessary for translocation to the nuclear matrix. Furthermore, we found that p73a is accumulated and nuclear matrix associated in cells that were treated with MG132, an inhibitor of proteasomal degradation (data not shown). Interestingly, both estrogen and glucocorticoid receptors display similar behavior of translocation to the nuclear matrix in the presence of their cognate ligands. However, MG132 induces a similar effect in the absence of the ligands (46, 47). Thus, protein accumulation may be a general requirement for nuclear matrix association of these nuclear proteins. However, the sole p73 accumulation does not guarantee nuclear matrix association because in cisplatin-treated cells p73 level was increased but no translocation took place.

The fact that p73a-c-Abl physical interaction was confirmed in heterologous insect cells rules out the involvement of a third auxiliary protein in this process. Furthermore, the observed efficient tyrosine phosphorylation of p73a by c-Abl under these conditions provides direct evidence for their enzyme-substrate relationship. Previously, the possible p53 and c-Abl cross-talk was reported by a number of groups (48–52). Interestingly, under conditions whereby p73 interacts and becomes phosphorylated by c-Abl, we failed to detect a significant p53-c-Abl interaction and p53 tyrosine phosphorylation. It is therefore possible that the cross-talk between these proteins is indirect and is mediated by some other players that are absent in insect cells. The recent finding that Mdm2, a p53-associated protein, is tyrosine-phosphorylated by c-Abl (53) is consistent with this possibility.

Nuclear matrix plays both direct and indirect roles in several cellular processes including cell cycle, DNA replication, repair, transcription, and apoptosis (36, 54–56). Furthermore, in a number of cases the process of association with the nuclear matrix is regulated by phosphorylation (36, 57, 58). For example, the retinoblastoma protein undergoes cell cycle-dependent phosphorylation and dephosphorylation with concomitant alteration of its subnuclear distribution. The former is found in the nucleoplasm, whereas the latter is mostly associated with nuclear matrix (57). Although nuclear matrix might provide a supportive scaffold for the associated proteins, it is possible that the destined proteins undergo inactivation by sequestration. The fact that, at least in the cases of p73, the nuclear matrix association of the estrogen and glucocorticoid receptors is positively correlated with the amount of proteins lends support to this model.

IR and many chemotherapeutic agents induce apoptosis in a p73-dependent manner. In fact induction of apoptosis by p53 requires the presence of p73 (17), whereas p73 induces apoptosis in the absence of p53 (15). Here we show that these two proteins in response to IR behave distinctively. p73 undergoes nuclear matrix association, at least under conditions examined by us, whereas p53 failed to do so. Thus, the selective p73 elimination from the nucleoplasm fraction is expected to have an impact on the capacity of p53 to function. Interestingly, recent findings suggest that under certain cases the engagement between the two proteins becomes too intimate for p73 to escape p53. Seven p53 mutant variants, in particular p53Arg–72 variant, are potent inhibitors of p73 function (15, 16) possibly via their physical interaction (18–20). Under these conditions p73 is functionally inhibited. Whether in the presence of the appropriate p53, mutants that inhibit p73 function, the nuclear matrix translocation of p73 is compromised is an open and interesting question to be examined in the future.

Recently, it has been reported that a number of nuclear proteins, such as p53 (59, 60) and Rad51 (61), when they are activated become accumulated in extra-nuclear particles that structurally resemble micronuclei. We have detected similar p73 positive micronuclei in cells that were exposed to IR. It has been proposed that micronuclei can be formed by nuclear budding, a mechanism that requires association with the nuclear matrix (62). We would like to propose that nuclear matrix association of p73 provides an early step in micronucleation. According to this model, excess of active forms of nuclear proteins are sequestered out of the nucleus by a mechanism of nuclear budding and micronucleation formation.

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