Integrin-linked Kinase Regulates the Nuclear Entry of the c-Jun Coactivator α-NAC and Its Coactivation Potency*

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Overexpression of the integrin-linked kinase (ILK) was shown to increase c-Jun-dependent transcription. We now show that this effect of ILK involves the c-Jun transcriptional coactivator, nascent polypeptide-associated complex and coactivator α (α-NAC). ILK phosphorylated α-NAC on residue Ser-43 upon adhesion of cells to fibronectin. Co-expression of constitutively active ILK with α-NAC led to the nuclear accumulation of the coactivator. Conversely, α-NAC remained in the cytoplasm of cells transfected with a dominant-negative ILK mutant, and a mutated α-NAC at phosphoacceptor position Ser-43 (S43A) also localized outside of the nucleus. The S43A α-NAC mutant could not potentiate the effect of ILK on c-Jun-dependent transcription. We conclude that ILK-dependent phosphorylation of α-NAC induced the nuclear accumulation of the coactivator and that phosphorylation of α-NAC by ILK is required for the potentiation of c-Jun-mediated responses by the kinase. The results represent one of the rare examples of a transcriptional coactivator shuttling between the cytosol and the nucleus.

Integrin-mediated interactions of cells with components of the extracellular matrix affect many aspects of cell function, including survival, proliferation, differentiation, and migration, by ultimately regulating gene transcription (1, 2). Following engagement with extracellular matrix components, integrin receptors signal via multiple downstream effectors (3), including integrin-linked kinase (ILK)4 (4, 5). The kinase activity of ILK is stimulated in a phosphatidylinositol 3,4,5-trisphosphate-dependent manner (6) following binding of extracellular matrix components to integrin receptors (5). Unregulated ILK expression promotes anchorage-independent growth, fibronectin matrix assembly, and tumorigenesis (4, 7, 8). These results confirm the key role of ILK as an effector of downstream integrin signaling and as an important regulator of cellular activity.

The net effect of stimulating the ILK signaling cascade is to modulate gene transcription. ILK activation or constitutive ILK expression has been shown to stimulate cyclin D1 transcription (7, 9, 10). Upon activation, ILK phosphorylates its downstream effectors, which include protein kinase B/Akt and glycerogen synthase kinase 3 (6). Activation or overexpression of ILK leads to translocation of β-catenin to the nucleus, where it functions as a coactivator of lymphoid enhancer factor-1/T-cell factor-dependent transcription (11). Overexpression of ILK has also been shown to potentiate homodimeric c-Jun activating protein-1 (AP-1)-dependent transcription (12).

The Jun proteins, members of the AP-1 family of transcription factors, regulate a wide variety of cellular processes including cell proliferation, differentiation, apoptosis, and onco-genesis (13). Jun proteins function as dimeric transcription factors that bind AP-1 regulatory elements in the promoter and/or enhancer regions of numerous genes (14). Jun family members (c-Jun, JunB, JunD) can homodimerize as well as form heterodimers among themselves or with partners of the Fos or activating transcription factor family members (15, 16). The dimeric complexes bind DNA on AP-1 sites with high affinity and cAMP response elements with low affinity (16).

Jun proteins interact with coactivators to potentiate transcription. The following proteins were characterized as coactivators of AP-1-mediated transcription: CBP (cAMP-response element-binding protein (CREB)-binding protein) (17), JAB-1 (jun-activation domain-binding protein 1) (18), SRC-1 (steroid receptor coactivator-1) (19), ASC-2 (activating signal cointegrator-2) (20), and α-NAC (nascent polypeptide associated complex and coactivator α) (21–23). The α-NAC protein, first described as involved in translational control (24), was also shown to function as a transcriptional coactivator by potentiating the activity of the chimeric Gal4-VP16 activator (25) and of c-Jun homodimers (21–23). α-NAC provides a protein bridge between sequence-specific DNA binding transcription factors and the basal transcriptional machinery by contacting the general transcription factor TATA binding protein (25). This stabilizes the transcription factors on their cognate response elements and results in enhanced transcription rates (23). To exert its coactivation function, α-NAC enters the nucleus (21, 22, 25, 26), and the subcellular localization of the protein is regulated at several levels. Phosphorylation by GSK3β impacts on the half-life of α-NAC, and inhibition of the kinase leads to stabilization and nuclear accumulation of the coactivator (21) in a manner analogous to β-catenin (27). The export of α-NAC from...
the nucleus also appears phosphorylation-dependent. Until now, the signal(s) that initiate the α-NAC cascade and tag the coactivator for entry into the nucleus have remained unidentified.

In a search for ILK targets, an interaction between ILK and α-NAC was observed. The similarities between the signal transduction pathways involving both molecules, namely the GSK3β-intermediate and the potentiation of c-Jun-mediated transcription, prompted us to evaluate whether signaling by ILK impacts on the subcellular localization of α-NAC and its coactivating function. We report that ILK phosphorylates α-NAC on residue serine 43 in vitro and in living cells. This serves as the signal for entry of α-NAC into the nucleus and leads to maximal coactivation of c-Jun-dependent transcription by the α-NAC protein.

EXPERIMENTAL PROCEDURES

Plasmids and Constructs—The FLAG epitope was inserted into the pSI mammalian expression vector (Promega, Madison, WI) to yield the pSI-Flag plasmid. The cDNAs encoding wild-type or mutant α-NAC (see Fig. 2A) were inserted in-frame into pSI-Flag to yield the pSI-NAC-Flag expression vectors.

Full-length α-NAC (wild type) and mutant cDNAs were also subcloned in-frame at their C termini with the intein-chitin binding domain of the pT7B, expression vector (New England Biolabs Ltd., Mississauga, ON, Canada) to give pT7B, NAC plasmids.

Coinmunoprecipitation—COS-7 cells were transfected with the pSINAC-Flag and pDNA3.1/V5-His-ILK (12) expression vectors in the combinations indicated in the legend to Fig. 1. Forty-eight hours post-transfection, the cells were lysed in 2× lysis buffer (100 mM Tris-Cl, pH 7.4, 500 mM NaCl, 2 mM EDTA, 2 mM EGTA, 2% Triton X-100) with inhibitors of proteases (5 μg/ml leupeptin, aprotinin, pepstatin A, and 1 μM phenylmethylsulfonyl fluoride). The cell lysates were diluted with H2O to reach 1× lysis buffer. They were incubated overnight at 4 °C with Sepharose beads conjugated to an anti-Flag antibody (anti-Flag M2 affinity gel, Sigma) or to an unrelated anti-glutathione-S-transferase antibody. The beads were extensively washed in 1× lysis buffer and resuspended in SDS-sample buffer. Immunoprecipitates were run on 12% SDS-PAGE and transferred to a polyvinylidene difluoride membrane.

In Vitro Kinase Assays—Wild-type His-tagged ILK was purified from COS-7 cells stably transfected with the pdCNA3.1/V5-His-ILK expression vector (12). Briefly, confluent cells were rinsed in phosphate-buffered saline, lysed in MCAC-0 buffer (50 mM NaH2PO4, pH 8.0, 500 mM NaCl, 10% glycerol, 1 μM each of the aprotinin, pepstatin, and leupeptin antiproteases, 1 mM phenylmethylsulfonyl fluoride), and sonicated following 30 min of incubation on ice. The total cell extract was incubated for 2.5 h at 4 °C with 0.5 μl of nickel-nitrilotriacetic acid slurry (Qiagen Canada Inc., Mississauga, ON) in an Econocolumn (Bio-Rad). The nickel-nitrilotriacetic acid resin was then washed with 6 ml of MCAC-0 buffer, followed by 3.5 ml of MCAC-20 buffer (MCAC buffer with 20 mM imidazole). The His-ILK protein was eluted from the column with 1× MCAC-20 buffer (200 mM imidazole). It was then pooled, dialyzed against dialysis buffer (20 mM Hepes, pH 7.9, 100 mM KCl, 10% glycerol, 1 μM each of the aprotinin, pepstatin, and leupeptin antiproteases, 1 mM phenylmethanesulfonyl fluoride), and concentrated to 10 μg/μl using a Centricon 10 column (Amicon, Inc., Beverly, MA).

The recombinant α-NAC proteins from pT7B-NAC plasmids were produced and purified in Escherichia coli following the manufacturer's procedure (New England Biolabs Ltd.). For in vitro kinase assays, 2 μg of the recombinant α-NAC proteins were incubated for 15 min at 30 °C in ILK buffer (pH 7.9, 100 mM NaCl, 10 mM MgCl2, 2 mM NaF) with 200 ng of purified ILK and 5 μCi of [γ-32P]ATP, and then resolved by SDS-PAGE on a 12% gel. The dried gel was autoradiographed on X-AR film (Eastman Kodak Co.).

In Vivo Phosphorylation Assays—COS-7 African green monkey kidney cells were maintained in low glucose Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37 °C in 5% CO2 and transiently transfected with the Geneporter transfection reagent (5 μg/μl DNA) according to the manufacturer's procedure (Gene Therapy System, San Diego, CA). Cells were transfected with the wild-type pSI-NAC-Flag vector alone or in combination with constitutively active (pSI S343D) or dominant-negative (ILK S343A) ILK expression vectors (12). One sample was transfected with the α-NAC phosphoacceptor mutant S43A, and one sample was transfected with pSI-NAC-Flag and treated for 1 h prior to permeabilization with 50 μM of the specific ILK inhibitor, FP929 (56). At 48 h post-transfection, the transfected COS-7 cells were permeabilized with 0.6 unit/ml streptolyisin O (Sigma-Aldrich) and labeled for 1 h with 50 μCi of [γ-32P]ATP (Amersham Biosciences). The signal was quantified by phosphoimager. The radiolabeled cell extracts were incubated overnight at 4 °C with anti-Flag M2 affinity gel (Sigma). The affinity gels were washed extensively in 1× lysis buffer and resuspended in SDS-sample buffer in the absence of dithiothreitol. Immunoprecipitates were run on 12% SDS-PAGE. The gel was subsequently dried and exposed at −80 °C. The intensity of the signals was quantified using a Storm phosphoimager (Amersham Biosciences). The signal was revealed with the ECL Plus kit (Amersham Biosciences) and quantified with the Typhoon phosphorimager. Phosphorylation signals were normalized to the protein expression levels, and the relative signal calculated for wild-type α-NAC-Flag was arbitrarily assigned the value of 100%. Normalized phosphorylation signals for the other samples were expressed as a percentage of the α-NAC-Flag signal.

Phospho-Ser-43-specific Antibody—A peptide corresponding to α-NAC residues 35–46 was synthesized with a phosphoserine residue at relative position 43, coupled to ovalbumin, and used to raise rabbit polyclonal antibodies following standard protocols. The antiserum was depleted of nonspecific immunoglobulins by purification against the corresponding unphosphorylated peptide (57) coupled to SulfoLink gel (Pierce).

Adhesion Assays—Confluent plates of COS-7 cells were trypsinized, and the trypsin was inhibited with 1 mg of trypsin inhibitor (Sigma). Cells were resuspended in serum-free Dulbecco's modified Eagle's medium at 9 × 105 cells/ml. Tissue culture plates (100 mm) were coated for 15 h with 2 ml of a fresh solution of fibronectin (2 μg/ml). The fibronectin solution was then aspirated and the plates were left to air dry. Cells (2 ml) were added and left to adhere for the indicated times. Control cells were left in suspension for the duration of the experiment. Plates were washed three times with phosphate-buffered saline, and the adherent cells were recovered in 0.4 ml of 2× lysis buffer. The samples were subsequently diluted to 1× with water. An equal volume of SDS-PAGE sample buffer was added, and the samples were migrated on a 12% SDS-PAGE gel, transferred to polyvinylidene difluoride, and probed with the phospho-Ser-43-specific antibody (1:100 dilution). After washes, the membrane was incubated with the anti-rabbit secondary antibody conjugated to horseradish peroxidase (Amersham Biosciences). The signal was revealed with the ECL Plus kit (Amersham Biosciences). The membrane was stripped according to the manufacturer's protocol and probed with the anti-NAC antibody (55).

Immunofluorescence—COS-7 cells were transfected as described above with the constitutively active (ILK S343D) or dominant-negative (ILK S343A) ILK expression vectors (12) alone or in combination with α-NAC-Flag or an empty-FLAG expression vector (pSI-NAC-Flag-epitope-tagged α-NAC phosphoacceptor mutant S43A). At 24 h post-transfection, the cells were fixed in 4% paraformaldehyde and permeabilized with 0.2% Triton X-100. Following blocking with 1% blocking reagent (Roche Molecular Biochemicals) supplemented with 0.2% Tween 20, the cells were incubated with the anti-Flag M2 antibody (1:200; Sigma) and a monoclonal anti-V5 tag antibody (1:200; Invitrogen). The cells were then incubated for 1–2 h at room temperature with a fluorescein isothiocyanate-conjugated anti-rabbit IgG secondary antibody (dilution 1:500) to reveal the V5-tagged ILK molecules and a rhodamine-conjugated anti-mouse IgG secondary antibody (dilu-
were transfected with α-NAC when ILK was not immunoprecipitated when an unrelated antibody was used (lane 3). The anti-Flag antibody did not immunoprecipitate ILK when α-NAC-Flag was transected alone (lane 2). These results show that α-NAC interacts with ILK in mammalian cells.

α-NAC Is an ILK Substrate—To determine whether ILK could phosphorylate α-NAC, the wild-type α-NAC protein, deletion and point mutants (Fig. 2A) were produced and purified in E. coli using pTYB2-based expression vectors, which yield recombinant proteins devoid of an associated fusion moiety. The recombinant proteins were used for in vitro kinase assays with wild-type His-tagged ILK purified from COS-7 cells stably transfected with the pcDNA3.1/V5-His-ILK expression vector (12). The purified ILK preparation was devoid of contaminants that could be phosphorylated by ILK (Fig. 2B, lane 1), and ILK did not phosphorylate the negative control maltose-binding protein purified by the same method as the α-NAC mutants (lane 9). The results of the kinase assay show that α-NAC was a substrate of ILK in vitro (Fig. 2B, lane 2). Deleting residues 179–215 (mutant Δ179–215, lane 3), 4–25 (mutant Δ4–25, lane 4), 46–69 (mutant Δ46–69, lane 6), or 69–80 (mutant Δ69–80, lane 7) did not affect phosphorylation of the recombinant proteins by ILK (Fig. 2B). Deleting residues 4–45 from the α-NAC protein, however, resulted in an almost complete lack of phosphorylation of mutant Δ4–45 by ILK (lane 5), suggesting that the ILK phosphoacceptor site within the α-NAC protein was located between residues 26 and 45. Edman sequencing of recombinant α-NAC kinase by ILK in the presence of [γ-32P]ATP showed that the radioactive tracer eluted at cycle 43, a serine residue (not shown). A point mutation was engineered to replace residue Ser-43 by an alanine residue (mutant S43A). The recombinant S43A point mutant was poorly phosphorylated by ILK in the in vitro kinase assay (Fig. 2B, lane 8), confirming that it is the main ILK phosphoacceptor site within the α-NAC protein sequence.

We next tested whether α-NAC was a substrate of ILK in cells (Fig. 2C). Wild-type or mutant α-NAC-Flag in COS-7 cells was transfected in the presence or absence of ILK expression vectors or ILK inhibitors, and metabolic labeling of the intact cells was performed with [35S]methionine (data not shown). Wild-type α-NAC is phosphorylated in intact cells (21), and this level of phosphorylation was arbitrarily set at 100% (Fig. 2C). Co-transfecting a constitutively active ILK molecule (12) with α-NAC increased the phosphorylation of α-NAC (Fig. 2C, ILK S343D). Mutating residue Ser-43 to an alanine (mutant S43A) significantly reduced the phosphorylation level of the protein in cells. The reduction in phosphorylation observed in mutant S43A matched the inhibition observed in the presence of an ILK inhibitor (KP392) or in the presence of a dominant-negative ILK mutant (ILK S343A) (12) (Fig. 2C). Taken together, these data confirm that α-NAC was a substrate of ILK in living mammalian cells.

Induction of α-NAC Phosphorylation upon Cell Adhesion—We raised an antibody directed against the ILK-phosphorylated form of α-NAC (Ser-43-phosphorylated) and used it to assay the phosphorylation status of the coactivator following engagement of integrin receptors by extracellular matrix components. Upon adhesion of the cells to fibronectin, there was a transient increase in Ser-43-phosphorylated α-NAC that peaked at 40 min postadhesion (Fig. 3, lanes 2–5). The total amount of α-NAC did not vary whether the cells were in suspension or adhered to fibronectin (Fig. 3, lower panel). These results show...
ILK Phosphorylates α-NAC

Fig. 2. ILK phosphorylates α-NAC in vitro and in vivo. A, schematic representation of wild-type α-NAC (WT) and deletion mutant proteins. B, in vitro kinase assay. Purified ILK was incubated with recombinant α-NAC wild type, deletion or point mutants, or negative control (maltose-binding protein (MnBP)), in the presence of [γ-32P]ATP. The 32P-phosphorylated substrates were detected by autoradiography. Mr, molecular size markers. C, permeabilization assay. COS-7 cells were transfected with the wild-type pSI-NAC-Flag vector alone or in combination with constitutively active (ILK S343D) or dominant-negative (ILK S343A) ILK expression vectors (12). One sample was transfected with the α-NAC phosphoacceptor mutant S43A, and that integrin-mediated adhesion of cells to an extracellular matrix component, which activates ILK (6), induced the phosphorylation of the α-NAC coactivator on the ILK phosphoacceptor site.

Regulation of the Subcellular Localization of α-NAC by ILK—Cells were transfected with ILK and α-NAC expression vectors to determine the effect of ILK activity on the subcellular localization of α-NAC. In all cells that expressed both the constitutively active S343D ILK mutant (12) and α-NAC-Flag, the coactivator predominantly located to the nucleus (Fig. 4, panels A–C). This was particularly evident in fields containing cells that had incorporated the α-NAC-Flag vector without the S343D ILK expression vector. In those cells, α-NAC-Flag localized to the cytoplasm while it accumulated in the nucleus of cells that had incorporated both expression vectors (Fig. 4, B and C). On the contrary, in cells that co-expressed α-NAC-Flag and the dominant-negative S343A ILK mutant (12), α-NAC was excluded from the nucleus (Fig. 4, D–F). Similarly, mutating the Ser-43 phosphoacceptor site in the α-NAC protein prevented the protein from entering the nucleus even in the presence of the constitutively active ILK (Fig. 4, G–I). Expression of the constitutively active S343D ILK mutant radically altered the cellular distribution of α-NAC; under steady state conditions, α-NAC is located in the cytoplasm in most cells (58%), and very few cells exhibit an exclusive nuclear sublocalization of the protein (8%). Co-localization in both the cytosol and nucleus was observed in 34% of cells under steady state conditions. On the contrary, in S343D ILK-transfected cells, α-NAC predominantly localized to the nucleus (27% exclusively nuclear and 66% nuclear and cytoplasmic). α-NAC remained in the cytoplasm of only 7% of constitutive ILK-transfected cells. The Ser-43-mutated α-NAC protein never localized to the nucleus (not shown). We conclude that ILK-dependent phosphorylation of α-NAC on residue Ser-43 induced the nuclear accumulation of the coactivator.

Phosphorylation by ILK Regulates the Coactivating Function of α-NAC—Because constitutive ILK activity resulted in the relocalization of α-NAC to the nucleus, we next examined the effect of mutating the ILK phosphoacceptor site on the coactivating function of α-NAC. One sample was transfected with pSI-NAC-Flag and treated for 1 h prior to permeabilization with 50 μM of the specific ILK inhibitor, KP382 (56). The transfected cells were radiolabeled with [γ-32P]ATP following permeabilization. After immunoprecipitation with the anti-Flag M2 beads, the phosphorylation status of the Flag-tagged proteins was measured using a Typhoon PhosphorImager and controlled for protein expression levels. Phosphorylation of the wild-type α-NAC protein was arbitrarily set at 100%.
rhodamine detection of /H9251 presence of wild-type /H9251 nuclear stain. These results suggest that phosphorylation of (Fig. 5, the reporter gene (Fig. 5). As previously reported (12), consti-

ulated Gal4-cJun activity over a range of concentrations (data

localization of the coactivator. 

transfected with constitutively active ILK (ILK S343D) and the S43A phoshoacceptor mutant α-NAC protein. Bar, 100 μm.

Discussion

We have identified a physiological substrate of the ILK kinase activity. ILK phosphorylated the α-NAC coactivator on residue Ser-43 in test tubes and in intact cells. Phosphorylation of α-NAC on Ser-43 was induced upon adhesion of cells to an extracellular matrix component. ILK activity and the ILK phoshoacceptor residue on α-NAC impacted the subcellular localization of the coactivator. α-NAC potentiated the ILK-dependent increase in c-Jun-mediated transcription, and this effect required an intact ILK phoshoacceptor residue on the α-NAC molecule.

Our observations strongly suggest that α-NAC mediates the previously reported stimulation of c-Jun-dependent transcription by ILK (12). The kinetics of Ser-43 phosphorylation of α-NAC upon adhesion to fibronectin closely match the kinetics of ILK induction (6). Because all cell lines tested express α-NAC 2 it is surmised that the published effect of ILK on c-Jun-dependent transcription (12) was mediated through endogenous α-NAC activity. It will be interesting to test the impact of ILK overexpression on c-Jun activity in cells deficient for α-NAC when they become available, or in cells in which α-NAC expression would have been inhibited using small interfering RNA or similar strategies.

Nuclear accumulation of α-NAC was induced by ILK overexpression. It can be hypothesized that ILK-mediated phosphorylation of α-NAC unmasked an unidentified nuclear localization sequence (NLS) or activated a cryptic NLS. Our attempts to identify an NLS within the α-NAC protein were unsuccessful. The basic sequence between amino acids 70 and 80 that resembles an NLS did not confer nuclear localization to a heterologous protein, and it could be deleted from the α-NAC sequence without affecting nuclear entry of the coac-
tivator.2 On the other hand, we have shown that the inter-

action between α-NAC and c-Jun is increased when the co-

activator is phosphorylated by ILK (30). We propose that the nuclear translocation of α-NAC induced by ILK is mediated through interaction with c-Jun. Interestingly, nuclear translocation of the coactivator of lymphoid enhancer factor-1/c-fos protein activity, β-catenin, also occurs in an NLS-independent manner (31).

We propose the following model for the potentiation of c-Jun transcription by α-NAC: the constitutive phosphorylation of α-NAC on residue Thr-159 by GSK3β targets the coactivator for degradation by the proteasome (21). The inactivation of GSK3β in response to adhesion and ILK activation (6) would then result in a Thr-159-hypophosphorylated α-NAC that would become unavailable for proteasome degradation but would become a substrate for the ILK kinase activity on resi-

due Ser-43. The Ser-43-phosphorylated α-NAC would preferen-\ntially interact with c-Jun (30), translocate to the nucleus, and potentiate transcription (23).

A characteristic of AP-1 responses is that they are transient (32–34). The nuclearcytoplasmic shuttling of an AP-1 coactivator 

such as α-NAC could contribute to the tight regulation of AP-1-mediated responses. This hypothesis implies that α-NAC exits the nucleus in a regulated manner. Preliminary results show that differential phosphorylation at alternate sites controls nuclear export of α-NAC.4 Once returned to the cytosol, the phosphorylation status of α-NAC must be returned to neutral for the cycle to initiate again. Phospho-α-NAC is most certainly dephosphorylated by serine/threonine phosphatase(s) (35, 36) that remain to be identified.

The results reported herein represent one of the rare examples of a transcriptional coactivator shuttling between the cytosol and the nucleus. The β-catenin coactivator also transits between both cellular compartments (37–39). It is interesting to note the many similarities between the mechanism of action of α-NAC and β-catenin. First, both molecules interact with TATA binding protein (25, 40) and activate gene transcription when located in the nucleus (23, 39) but serve a different function when residing in the cytoplasm (24, 41). Second, both proteins are phosphorylated by GSK3β, and this post-transla-
tional modification regulates their degradation through the

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proteasome pathway (21, 27). Finally, translocation of β-catenin to the nucleus is induced upon cell adhesion and ILK activation (11) in a manner similar to what we observed for α-NAC.

The tight, intricate control of the subcellular localization of α-NAC and β-catenin suggests that perturbations of this regulated process could have severe consequences for cellular function. Indeed, mutations that impact β-catenin stability or transcriptional activity lead to oncogenic transformation (42–44). Similarly, a defect in the nuclear translocation of the major histocompatibility complex class II transactivator coactivator causes a form of type II bare lymphocyte syndrome (45). Interestingly, nuclear staining for α-NAC was consistently observed in human osteosarcomas (46), suggesting that perturbation of the subcellular localization of the coactivator may be involved in the malignant transformation of osteoblasts.

Integrin-mediated binding of extracellular matrix components regulates AP-1 activity in many cell types, including osteoblasts (47, 48). A key role for AP-1 family members in osteoblast function (49, 50) and osteoblast-specific gene expression (51–53) has been described. Osteoblasts express α-NAC during development (23). It will be interesting to characterize the functional relevance of the ILK-α-NAC signaling cascade in the regulation of osteoblast-specific gene expression. The conditional ILK mutant mice that we have recently engineered (54) will represent a useful genetic tool for this purpose.

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