# Hic-5, an adaptor-like nuclear receptor coactivator

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**In recent years, numerous nuclear receptor-interacting proteins have been identified that influence nuclear transcription through their direct modification of chromatin. Along with coactivators that possess histone acetyltransferase (HAT) or methyltransferase activity, other coactivators that lack recognizable chromatin-modifying activity have been discovered whose mechanism of action is largely unknown. The presence of multiple protein-protein interaction motifs within mechanistically undefined coactivators suggests that they function as adaptor molecules, either recruiting or stabilizing promoter-specific protein complexes. This perspective will focus on a family of nuclear receptor coactivators (i.e., group III LIM domain proteins related to paxillin) that appear to provide a scaffold to stabilize receptor interactions with chromatin-modifying coregulators.**

Received January 19th, 2006; Accepted May 9th, 2006; Published July 7th, 2006 | **Abbreviations:** **AR:** androgen receptor; **CBP:** cAMP response element binding protein; **ChIP:** chromatin immunoprecipitation; **FAK:** focal adhesion kinase; **GR:** glucocorticoid receptor; **HAT:** histone acetyltransferase; **LD motif:** leucine aspartic acid motif; **MMTV:** mouse mammary tumor virus; **NCoR:** nuclear receptor corepressor; **PPARγ:** Peroxisome proliferation activating receptor γ; **RAC3:** receptor associated coactivator 3; **TBL1:** transducin β-like 1; **TIF-2:** transcription intermediary factor-2 | 

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## Group III LIM domain proteins

Hic-5 and its closely related family member, paxillin [Kasai et al., 2003], are nuclear receptor coactivators that lack histone acetyltransferase (HAT) or methyltransferase activity. These proteins are members of the group III LIM domain containing family of proteins, which are characterized by their localization to both focal adhesions and within the nucleus [Dawid et al., 1998]. Along with four carboxyl-terminal LIM domains, Hic-5 also has four LD motifs within its amino terminus. LIM proteins are well recognized for their roles as molecular adaptors, functioning to stabilize higher order protein complexes at focal adhesion complexes [Dawid et al., 1998] (See Figure 1). Within focal adhesion complexes, Hic-5 as well as paxillin links various intracellular signaling modules to plasma membrane receptors that respond to extracellular signals including growth factors and the extracellular matrix [Nishiya et al., 1998]. For example, Hic-5 and paxillin interact with multiple focal adhesion-associated proteins such as vinculin and focal adhesion kinase (FAK) [Thomas et al., 1999].

While much work on Hic-5 has focused on its action at focal adhesions, yeast two hybrid screens revealed the association of this protein with the androgen receptor (AR), the glucocorticoid receptor (GR), and peroxisome proliferator-activated receptor gamma (PPARγ) [Fujimoto et al., 1999; Yang et al., 2000]. In fact, the ability of Hic-5 to function as an AR coactivator led to its alternative naming as ARA55. The tau2 transactivation domain of GR has been delineated as a minimal Hic-5/ARA55 interaction region, but Hic-5/ARA55 binding domains of AR and PPARγ have only been broadly localized to receptor ligand binding domains [Drori et al., 2005; Fujimoto et al., 1999; Yang et al., 2000]. Furthermore, in addition to various nuclear receptors, Hic-5/ARA55 also interacts with other transcription factors such as SP-1 and Smad3, upregulating or inhibiting their transcriptional activation properties, respectively [Shibanuma et al., 2004; Wang et al., 2005].

## Hic-5/ARA55 adaptor function

Recent work from our laboratory has revealed the mechanism responsible for Hic-5/ARA55 coactivation of GR [Heitzer and DeFranco, 2006]. In the A1-2 derivative of T47D breast cancer cells which possess an integrated mouse mammary tumor virus (MMTV) promoter, single and sequential chromatin immunoprecipitation (ChIP) assays revealed an association of Hic-5/ARA55 with GR and various coactivators on the MMTV as well as p21 and c-fos promoters. Because Hic-5/ARA55 does not possess HAT or methyltransferase activity, it most likely does not modify histones directly. However, Hic-5/ARA55 may be involved in recruiting other chromatin modifying coactivators. In fact, sequential ChIPs revealed that Hic-5/ARA55 interacts with TIF-2, RAC3, CBP, and p300 coactivators at glucocorticoid responsive promoters [Heitzer and DeFranco, 2006]. Although Hic-5/ARA55 is only one of several coactivators that associates with the glucocorticoid-regulated MMTV promoter in A1-2 cells, its ablation severely limits glucocorticoid induction of this viral promoter and an endogenous glucocorticoid-regulated gene (i.e., p21). However, Hic-5/ARA55 ablation does not effect glucocorticoid induction of the c-fos gene in A1-2 cells, demonstrating selectivity of Hic-5/ARA55 effects. Furthermore, after siRNA-mediated ablation of Hic-5/ARA55, ligand-dependent TIF-2 and p300 recruitment to the MMTV promoter was reduced. Thus, Hic-5/ARA55 has a role in maintaining the assembly of coactivator complexes required for efficient glucocorticoid-induced transcription at the MMTV promoter. Hic-5/ARA55 may function as an adaptor protein, recruiting or stabilizing HAT-containing complexes at steroid responsive promoters (Figure 1). The corresponding reduction of GR transactivation and coactivator recruitment that results

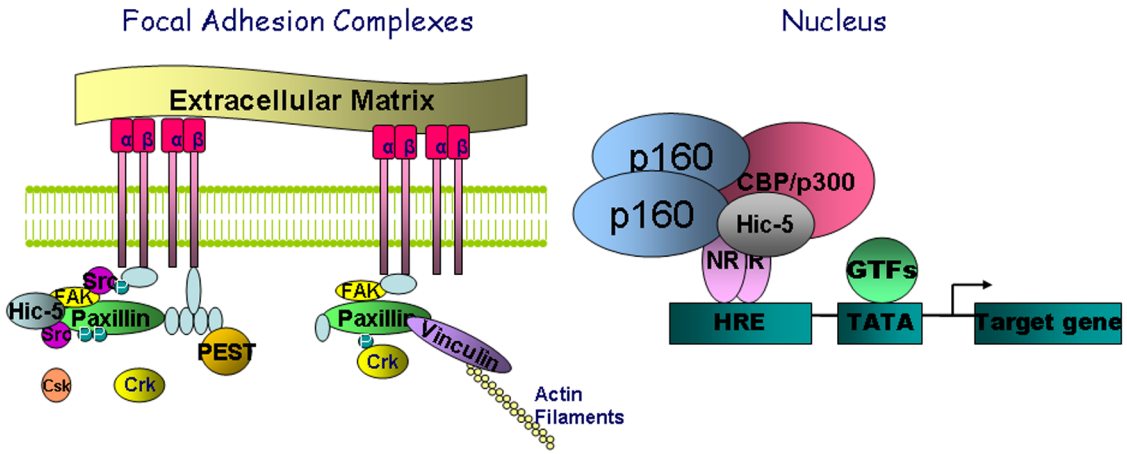


**Figure 1. Hic-5-containing complexes at focal adhesions and in the nucleus** At focal adhesion complexes, Hic-5/ARA55 interacts with multiple proteins such as focal adhesion kinase (FAK) and Src, thereby functioning as an adaptor molecule that coordinates multiple protein-protein interactions. Similarly, in the nucleus, Hic-5/ARA55 serves as an adaptor coregulator, interacting with coactivator containing complexes on nuclear receptor target promoters.

from partial Hic-5/ARA55 ablation demonstrates the critical role of Hic-5/ARA55 in maintaining the assembly of coactivator complexes required to bring about efficient glucocorticoid-induced transcription.

## Hic-5/ARA55-NCoR interactions

Hic-5/ARA55 not only interacts with various coactivator complexes, but it also associates with NCoR-containing corepressor complexes in the absence of hormone at nuclear receptor-responsive promoters [Heitzer and DeFranco, 2006]. This suggests that Hic-5/ARA55 is capable of directly interacting with other coregulators and not necessarily via nuclear receptors. Furthermore, because Hic-5/ARA55 is present on GR-responsive promoters in the absence and presence of glucocorticoids [Heitzer and DeFranco, 2006], it may function in coordinating corepressor release and coactivator recruitment upon glucocorticoid stimulation.

Although Hic-5/ARA55 was localized to GR-responsive promoters in the absence of ligand and detectable promoter-bound GR, the mechanism by which Hic-5/ARA55 is bound to the promoter in the apparent absence of the receptor is unclear. In addition to receptor-independent binding of Hic-5/ARA55 to the MMTV promoter, we also detected receptor-independent localization of NCoR to the MMTV promoter in the absence of ligand [Heitzer and DeFranco, 2006]. Direct binding of either Hic-5/ARA55 or NCoR to the MMTV promoter has not been analyzed.

Interestingly, Hic-5/ARA55 does display zinc-dependent DNA binding [Nishiya et al., 1998]. However, a specific DNA sequence for which Hic-5/ARA55 is capable of binding has yet to be identified. If Hic-5/ARA55 is capable of directly binding the MMTV promoter, it may function in tethering the NCoR-containing complex to the MMTV promoter in the absence of ligand. However, it is also possible that both Hic-5/ARA55 and NCoR are binding the MMTV promoter through another, yet unidentified DNA binding protein.

Recently, transducin β-like 1 (TBL1), an adaptor-like protein, has been reported to mediate the exchange of corepressors for coactivators on nuclear receptor-responsive promoters in response to ligand [Perissi et al., 2004]. TBL1 was initially isolated as part of the NCoR corepressor complex [Li et al., 2000]. ChIP analysis of nuclear receptor target promoters revealed prolonged TBL1 promoter association in the presence of ligand [Perissi et al., 2004]. Furthermore, TBL1 recruited components of the proteosome machinery to nuclear receptor target promoters, leading to degradation of the corepressor complex followed by association of the coactivator complex [Perissi et al., 2004]. However, interactions between Hic-5/ARA55 and components of the proteosome machinery have not yet been analyzed.

## Regulation of Hic-5/ARA55 and paxillin activity and subcellular localization

In addition to Hic-5/ARA55, other members of the group III LIM domain containing family such as Trip6, and zyxin, also function in the nucleus [Kadrmas and Beckerle, 2004; Kassel et al., 2004; Nix et al., 2001], but a detailed understanding of their function in these contexts remains undefined. Furthermore, it is unclear whether the nuclear activity of group III LIM domain proteins is regulated under any physiological or pathophysiological conditions.

Although regulation of Hic-5/ARA55 and paxillin coactivator activities has yet to be analyzed, both Hic-5/ARA55 and paxillin are differentially phosphorylated by FAK, Pyk2 and Fyn downstream of various growth factor and integrin signaling pathways [Ishino et al., 2000; Nishiya et al., 2001]. Future analysis may illustrate subtle requirements for group III LIM domain-containing protein activation as well as subcellular localization.

Although many LIM domain containing proteins have been detected in the nucleus and at focal adhesions and their nuclear export sequences have been identified, the precise signals that induce their translocation are mostly

unknown [Labalette et al., 2004]. However, FHL2, another LIM domain containing AR coactivator, translocates to the nucleus in response to activation of Rho, a small GTPase [Labalette et al., 2004; Muller et al., 2000]. Furthermore, it has been reported that nuclear accumulation of Hic-5/ARA55 occurs in response to oxidants such as $H_2O_2$ [Shibanuma et al., 2003]. Deciphering the signals which are responsible for Hic-5/ARA55 activation and/or subcellular trafficking may highlight a putative role for Hic-5/ARA55 in mediating signals originating from plasma membrane-associated focal adhesion complexes to the nucleus, thereby affecting gene expression driven by nuclear receptors.

## Hic-5/ARA55 tissue distribution

Although regulatory mechanisms governing Hic-5/ARA55 and paxillin action are largely unknown, analysis of their limited tissue distribution illustrates yet another unique feature of this family of nuclear receptor coactivators. For example, selective expression of Hic-5/ARA55 in smooth muscle and myoepithelial cells has been revealed by immunohistochemistry [Yuminamochi et al., 2003]. Hic-5/ARA55 was not detected in epithelial cells of the tissues examined, including the stomach, colon, liver, skin, and mammary gland, whereas paxillin was [Yuminamochi et al., 2003]. Additionally, within an individual organ, Hic-5/ARA55 and paxillin are expressed in a cell-type specific manner. For example, in the prostate, Hic-5/ARA55 is localized to the stromal, while paxillin is largely found within the epithelial compartment [Li et al., 2002] (and unpublished results).

Thus, this unique family of nuclear receptor coactivators including Hic-5/ARA55 and paxillin, may act as “adaptors” or scaffolds at distinct compartments in the cell (i.e. focal adhesions and the nucleus) regulating multiple signal pathways in a cell type-specific manner at sites both proximal and distal to the initiating signal.

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## References

Dawid, I. B., Breen, J. J. and Toyama, R. (1998) LIM domains: multiple roles as adapters and functional modifiers in protein interactions *Trends Genet* **14**, 156-62.

Drori, S., Girnun, G. D., Tou, L., Szwaya, J. D., Mueller, E., Xia, K., Shivdasani, R. A. and Spiegelman, B. M. (2005) Hic-5 regulates an epithelial program mediated by PPARgamma *Genes Dev* **19**, 362-75.

Fujimoto, N., Yeh, S., Kang, H. Y., Inui, S., Chang, H. C., Mizokami, A. and Chang, C. (1999) Cloning and characterization of androgen receptor coactivator, ARA55, in human prostate *J Biol Chem* **274**, 8316-21.

Heitzer, M. D. and DeFranco, D. B. (2006) Mechanism of action of Hic-5/androgen receptor activator 55, a LIM domain-containing nuclear receptor coactivator *Mol Endocrinol* **20**, 56-64.

Ishino, M., Aoto, H., Sasaski, H., Suzuki, R. and Sasaki, T. (2000) Phosphorylation of Hic-5 at tyrosine 60 by CAKbeta and Fyn *FEBS Lett* **474**, 179-83.

Kadrmas, J. L. and Beckerle, M. C. (2004) The LIM domain: from the cytoskeleton to the nucleus *Nat Rev Mol Cell Biol* **5**, 920-31.

Kasai, M., Guerrero-Santoro, J., Friedman, R., Leman, E. S., Getzenberg, R. H. and DeFranco, D. B. (2003) The Group 3 LIM domain protein paxillin potentiates androgen receptor transactivation in prostate cancer cell lines *Cancer Res* **63**, 4927-35.

Kassel, O., Schneider, S., Heilbock, C., Litfin, M., Gottlicher, M. and Herrlich, P. (2004) A nuclear isoform of the focal adhesion LIM-domain protein Trip6 integrates activating and repressing signals at AP-1- and NF-kappaB-regulated promoters *Genes Dev* **18**, 2518-28.

Labalette, C., Renard, C. A., Neuveut, C., Buendia, M. A. and Wei, Y. (2004) Interaction and functional cooperation between the LIM protein FHL2, CBP/p300, and β-catenin *Mol Cell Biol* **24**, 10689-702.

Li, J., Wang, J., Nawaz, Z., Liu, J. M., Qin, J. and Wong, J. (2000) Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3 *Embo J* **19**, 4342-50.

Li, P., Yu, X., Ge, K., Melamed, J., Roeder, R. G. and Wang, Z. (2002) Heterogeneous expression and functions of androgen receptor co-factors in primary prostate cancer *Am J Pathol* **161**, 1467-74.

Muller, J. M., Isele, U., Metzger, E., Rempel, A., Moser, M., Pscherer, A., Breyer, T., Holubarsch, C., Buettner, R. and Schule, R. (2000) FHL2, a novel tissue-specific coactivator of the androgen receptor *Embo J* **19**, 359-69.

Nishiya, N., Tachibana, K., Shibanuma, M., Mashimo, J. I. and Nose, K. (2001) Hic-5-reduced cell spreading on fibronectin: competitive effects between paxillin and Hic-5 through interaction with focal adhesion kinase *Mol Cell Biol* **21**, 5332-45.

Nishiya, N., Sabe, H., Nose, K. and Shibanuma, M. (1998) The LIM domains of hic-5 protein recognize specific DNA fragments in a zinc-dependent manner in vitro *Nucleic Acids Res* **26**, 4267-73.

Nix, D. A., Fradelizi, J., Bockholt, S., Menichi, B., Louvard, D., Friederich, E. and Beckerle, M. C. (2001) Targeting of zyxin to sites of actin membrane interaction and to the nucleus *J Biol Chem* **276**, 34759-67.

Perissi, V., Aggarwal, A., Glass, C. K., Rose, D. W. and Rosenfeld, M. G. (2004) A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors *Cell* **116**, 511-26.

Shibanuma, M., Kim-Kaneyama, J. R., Sato, S. and Nose, K. (2004) A LIM protein, Hic-5, functions as a potential coactivator for Sp1 *J Cell Biochem* **91**, 633-45.

Shibanuma, M., Kim-Kaneyama, J. R., Ishino, K., Sakamoto, N., Hishiki, T., Yamaguchi, K., Mori, K., Mashimo, J. and Nose, K. (2003) Hic-5 communicates between focal adhesions and the nucleus through oxidant-sensitive nuclear export signal *Mol Biol Cell* **14**, 1158-71.

Thomas, S. M., Hagel, M. and Turner, C. E. (1999) Characterization of a focal adhesion protein, Hic-5, that shares extensive homology with paxillin *J Cell Sci* **112 ( Pt 2)**, 181-90.

Wang, H., Song, K., Sponseller, T. L. and Danielpour, D. (2005) Novel function of androgen receptor-associated protein 55/Hic-5 as a negative regulator of Smad3 signaling *J Biol Chem* **280**, 5154-62.

Yang, L., Guerrero, J., Hong, H., DeFranco, D. B. and Stallcup, M. R. (2000) Interaction of the tau2 transcriptional activation domain of glucocorticoid receptor with a novel steroid receptor coactivator, Hic-5, which localizes to both focal adhesions and the nuclear matrix *Mol Biol Cell* **11**, 2007-18.

Yuminamochi, T., Yatomi, Y., Osada, M., Ohmori, T., Ishii, Y., Nakazawa, K., Hosogaya, S. and Ozaki, Y. (2003) Expression of the LIM proteins paxillin and Hic-5 in human tissues *J Histochem Cytochem* **51**, 513-21.