proteins at the *Glt2 ICR*, although in this case their analyses did not allow them to determine whether these interactions occur on the maternal or on the paternal chromosome. The *Dlk1/Glt2* locus is somewhat analogous to the *Igf2/H19* locus in gene organization and regulation (Wan and Bartolomei, 2008). An *ICR* adjacent to the *Gtl2* promoter regulates *Gtl2* and the far upstream *Dlk1*. The results of Kernohan et al. support the idea that interactions of cohesin, MeCP2, and ATRX proteins are of general importance and not restricted to *H19/Igf2*. However, several differences between the two loci suggest that the nature of these interactions may not be straightforward. At *Dlk1/Glt2*, the proteins each bind to distinct parts of the *ICR* and not to a single region, like they do at the *H19* locus. Furthermore, MeCP2 binding to the *ICR* is ATRX dependent at *Glt2*, but ATRX independent at *H19*.

By several criteria, *Igf2*, *H19*, *Dlk1*, and *Glt2* are part of a network of at least 10 imprinted genes (Varrault et al., 2006). These genes all share developmental and tissue-specific patterns of expression and respond similarly to mutations at the *Zac1* locus. Curiously this network shows almost no overlap with imprinted genes involved in interchromosomal interactions with the *H19 ICR*. Kernohan et al. provide good evidence that ATRX is required for the downregulation of expression of each of these genes in late embryonic or in postnatal development. The key question remains whether this downregulation is important in the ATR-X syndrome.

Evolutionary theory and the analysis of many knockout mouse strains both support the idea that the primary effect of imprinting is on fetal and early neonatal growth. However, some experiments have suggested a role for imprinted genes in brain development and function (Wilkinson et al., 2007). Chimeric animals generated by mixtures of wild-type and gynogenetic (maternal chromosomes only) cells, or by mixtures of wild-type and androgenetic (paternal chromosomes only) cells, show divergent phenotypes with gynogenetic and androgenetic cells each contributing to distinct brain structures (Keverne et al., 1996). These experiments are hard to interpret on a molecular level. Gynogenetic cells not only lack any paternal-specific transcripts, but also have 2-fold overexpression of all maternal-specific RNAs. Nonetheless the results are intriguing. Mammalian cells go to great effort to carefully regulate the doses of imprinted genes. Whether misexpression of *H19* or of any of the imprinted genes plays a clinically important role in brain development and function is an important and difficult question that remains to be addressed.

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A PAK-Activated Linker for EGFR and FAK

Alok Tomar[1] and David D. Schlaepfer[1,*]
[1]Department of Reproductive Medicine, University of California, San Diego, Moores Cancer Center, La Jolla, CA 92093, USA
*Correspondence: dschlaepfer@ucsd.edu
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Transmembrane growth factor and integrin matrix receptors form multiprotein signaling complexes with FAK, a cytoplasmic cell motility-associated kinase. In a recent issue of *Molecular Cell*, Long et al. now show that a PAK-phosphorylated alternate-spliced isoform of the steroid receptor coactivator-3 (SRC-3Δ4) bridges EGFR and FAK, enhancing breast carcinoma cell migration and metastasis.

Cell movement results from the coordination of actin cytoskeletal and cell adhesion site formation-turnover alterations generating shape and traction force changes. Focal adhesion kinase (FAK) is a cytoplasmic tyrosine kinase that colocalizes with and is activated by integrin matrix receptors at adhesion sites. For a cell to process motility-promoting stimuli correctly, there must be essential proteins that function as "integrators" in the coordination of signals regulating cell shape, adhesion, and cell motility. FAK is one such integrator linking transmembrane integrin, growth factor, and G protein-linked receptors to the cell motility machinery (Mitra et al., 2005). FAK is required for efficient epidermal growth factor (EGF)-stimulated cell motility and this connection is facilitated through FAK FERM (band 4.1, ezrin, radixin, moesin

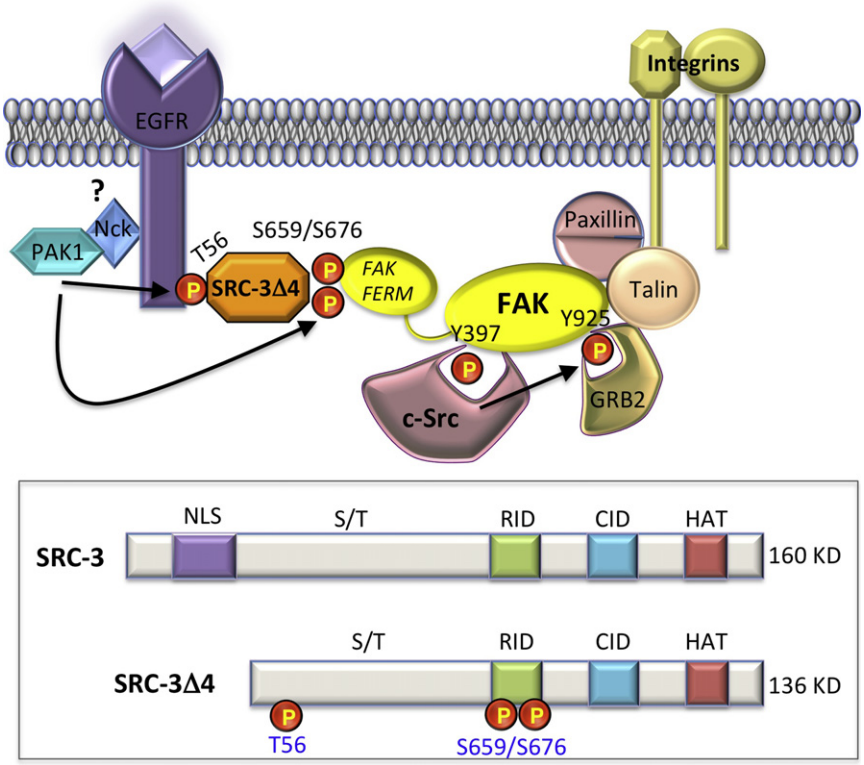

Figure 1. Model for SRC-3Δ4-Mediated EGFR and FAK Interaction

EGF-stimulated EGFR activation results in PAK activation, potentially via Nck adaptor protein binding to EGFR. PAK-mediated SRC-3Δ4 phosphorylation at T56 and S659/S676 promotes its binding to EGFR and the FERM domain of FAK, respectively. FAK activation and autophosphorylation at Y397 occurs after integrin clustering (via paxillin and talin binding) or via SRC-3Δ4-mediated changes in FAK FERM conformation. SRC-3Δ4 enhances FAK phosphorylation at Y925, potentially via enhanced activation of c-Src within a FAK-c-Src integrin signaling complex. FAK Y925 phosphorylation promotes Grb2 adaptor protein binding to FAK. (Inset) SRC-3 consists of a nuclear localization sequence (NLS) region, a serine-threonine rich domain (S/T), a nuclear receptor interacting domain (RID), a CBP interacting domain (CID), and a histone acetyltransferase (HAT) domain. SRC-3Δ4 lacks the NLS region and is cytoplasmically distributed. SRC-3Δ4 S659/S676 phosphorylation sites lie within the RID that binds FAK FERM.

homology) domain association with activated EGF receptor (EGFR) signaling complexes. Simplistically, FAK activation triggers its autophosphorylation at tyrosine 397 (Y397), allowing c-Src tyrosine kinase to bind to phosphorylated Y397 FAK and generating a FAK-c-Src signaling complex. Although FAK FERM may bind directly to other growth factor receptors (Chen and Chen, 2006) and various studies have connected EGFR-FAK-c-Src signaling to tumor cell invasiveness and metastasis (Mitra and Schlaepfer, 2006), FAK association with EGFR is indirect and the molecular details of this linkage have remained elusive.

Reporting in a recent issue of *Molecular Cell*, Long et al. (2010) have now identified the alternate-spliced isoform of steroid receptor coactivator-3 (SRC-3)—termed SRC-3Δ4 (deletion of exon 4)—as an EGFR-FAK bridging protein. Full-length SRC-3/AIB1 (amplified in breast cancer-1) is a member of the p160 family of cotranscriptional regulators of hormone-bound nuclear receptors (Lahusen et al., 2009). Interestingly, inhibition of SRC-3 expression altered FAK localization and prevented ovarian carcinoma cell motility (Yoshida et al., 2005), and SRC-3 overexpression enhanced FAK activation and prostate carcinoma invasion (Yan et al., 2008). However, no direct connection between SRC-3 and FAK was established and these effects may have been related to transcriptional modulation of cell-matrix interactions. SRC-3Δ4 is produced from a second translational start site, does not contain a nuclear localization sequence, and is cytoplasmically distributed; SRC-3Δ4 expression is also elevated in breast cancer (Reiter et al., 2004). Long et al. (2010) now show that SRC-3Δ4 colocalizes with FAK at the leading edge of motile MDA-MB231 breast carcinoma cells and that SRC-3Δ4 forms a complex with FAK. Direct binding was confirmed between the FAK FERM domain and the central receptor interacting domain (RID) of SRC-3Δ4. Notably, SRC-3Δ4 was required for efficient EGF-stimulated MDA-MB231 cell motility. The knockdown of SRC-3Δ4 decreased EGFR-FAK association, whereas EGF stimulation enhanced SRC-3Δ4 association with FAK. These results support a role for SRC-3Δ4 in linking EGFR to FAK.

This bridge model was further support by the fact that SRC-3Δ4 also bound to EGFR via the amino-terminal domain of SRC-3Δ4. As EGF stimulation enhanced the formation of a complex between EGFR, SRC-3Δ4, FAK, and the serine-threonine kinase PAK1, Long et al. (2010) explored the hypothesis that PAK1 phosphorylation of SRC-3Δ4 may strengthen the EGFR, SRC-3Δ4, and FAK linkage. PAK1 is a cytoskeletal-associated kinase activated by small GTP binding proteins and functions downstream of FAK signaling (Bokoch, 2003). However, PAK1 can also be proximally recruited to activated EGFR signaling complexes and possibly function upstream of FAK. Although the temporal nature of PAK1 activation was not addressed, Long et al. (2010) found that PAK1 directly phosphorylated three sites on SRC-3Δ4: threonine 56 (T56) within the SRC-3Δ4 amino-terminal (NT) domain, and serines 659 (S659) and 676 (S676) within the SRC-3Δ4 RID. These are the domains that mediate SRC-3Δ4 binding to EGFR and FAK, respectively. Accordingly, mutation of T56 disrupted EGFR association with the SRC-3Δ4 NT domain and mutation of S659/S676 disrupted binding of the SRC-3Δ4 RID to FAK. Combined triple T56/S659/S676 mutations prevented SRC-3Δ4 complex formation with both EFGR and FAK and also blocked SRC-3Δ4 effects on EGF-stimulated HeLa cell migration. Because low-level SRC-3Δ4 binding to FAK or EGFR can also occur independently of PAK1 phosphorylation, future studies will likely need to focus on the molecular details of these interactions.

Nevertheless, the findings made by Long et al. (2010) provide support for an intriguing bridging model (Figure 1)

wherein EGF-stimulated PAK activation facilitates SRC-3Δ4 phosphorylation at T56, resulting in EGFR binding. PAK-mediated phosphorylation of SRC-3Δ4 at S659 and S676 promotes its binding to the FERM domain of FAK. Interestingly, EGF or modulation of SRC-3Δ4 expression did not affect FAK phosphorylation at Y397, but SRC-3Δ4 knockdown was associated with decreased FAK Y925 phosphorylation, c-Src activation, and signaling to the ERK/mitogen-activated protein (MAP) kinase. Phosphorylation of FAK Y925 is mediated by c-Src and promotes the binding of the Grb2 adaptor protein to FAK, leading to ERK/MAP kinase activation (Mitra and Schlaepfer, 2006). Although not directly tested, these results imply that the SRC-3Δ4 linkage enhances EGF-stimulated FAK activation via binding to the FAK FERM domain, leading to conformational FAK activation and the enhanced formation of a FAK-Src signaling complex (determined by changes in FAK Y925 phosphorylation). Although FAK Y925 is not essential for normal fibroblast motility, this site is required in promoting an angiogenic switch in tumors (Mitra and Schlaepfer, 2006; Tomar et al., 2009). Interestingly, when Long et al. (2010) injected MDA-MB231 cells overexpressing SRC-3Δ4 (which show enhanced motility-invasion in vitro associated with elevated FAK Y925 phosphorylation) into mouse breast fat pads, these cells exhibit enhanced lymph node and lung metastasis without alterations in primary tumor growth. Because increased levels of SRC-3Δ4 cells were found circulating in the blood, Long et al. proposed that this may reflect increased motility or extravasation of tumor cells from primary tumor sites.

Overall, this study provides intriguing results supporting a new signaling connection for a cytoplasmically distributed, alternate-spliced isoform of SRC-3. Although this study provides valuable steps forward in resolving some of the mysteries surrounding the linkage between EGFR and FAK, several questions remain. What are the SRC-3Δ4 binding sites on EGFR or FAK FERM, and how does phosphorylation of SRC-3Δ4 influence binding? Does SRC-3Δ4 link FAK to other receptors such as the platelet-derived growth factor receptor known to promote PAK activation and cell motility? Because SRC-3Δ4 expression is generally low in noncancerous cell types, do different mechanisms promote FAK association with EGFR in normal versus cancer cells? What is the connection between tumor-associated SRC-3Δ4 expression, FAK Y925 phosphorylation, and the invasive cell phenotype? Clearly, the identification of SRC-3Δ4 as a bridging protein raises many exciting new questions whose answers are needed for understanding the molecular mechanisms initiating and controlling cell movement.

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This preview is dedicated to the memory of Gary Bokoch, Ph.D. (1954–2010).

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Alternative Polyadenylation Blooms

Emanuel Rosonina[1] and James L. Manley[1,*]
[1]Department of Biological Sciences, Columbia University, New York, NY 10027, USA
*Correspondence: jlm2@columbia.edu
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Alternative polyadenylation generates mRNAs with 3′ untranscribed regions of different lengths, often affecting transcript stability. Hornyik et al., in this issue of *Developmental Cell*, and Liu et al. now demonstrate a role for alternative polyadenylation in gene silencing and the regulation of flowering time in *Arabidopsis thaliana*.

Messenger RNA (mRNA) 3′ end processing defines the end of the transcript through endonucleolytic cleavage of the precursor transcript, provides a protective polyadenylate tail, and enables subsequent termination of transcription by RNA polymerase II. Just as alternative splicing allows greater diversity of mRNA products from a limited number of genes, in animals and plants it is estimated that >50% of genes have alternative polyadenylation (polyA) sites, the majority of