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Integrin signalling: a new Cas(t) of characters enters the stage

Geraldine M. O’Neill, Sarah J. Fashena and Erica A. Golemis

Cellular morphology is determined by the organization of the intracellular actin cytoskeleton, which is influenced by external and internal cues. Focal adhesions are sites at which the actin cytoskeleton is linked to the extracellular matrix by integrin receptor complexes. In addition to providing structural tethering points for cells, integrin receptor complexes transduce signals that influence a broad range of cellular processes, including migration, proliferation, transformation and apoptosis. The Cas proteins (p130Cas, HEF1/Cas-L and Efs/Sin), a family of docking proteins containing multiple interaction domains, are important components of integrin receptor signalling and have been implicated in all of these processes.

It has long been known that cell shape regulates growth control and anchorage dependence in normal (untransformed) cells, and that transformed cells exhibit altered morphology and matrix attachment properties[1,2]. The shape of a cell is determined primarily by its internal actin cytoskeletal architecture and influenced by focal adhesions that constitute points of linkage between the extracellular matrix (ECM), the integrin receptor system and actin scaffolds (reviewed in Refs 3 and 4). In response to external adhesive signals or internally generated cues, cells rearrange their actin cytoskeletal networks, thereby altering assembly of integrin-associated signal-transducing networks. Biological processes affected by the combined changes in cell shape and signal transduction mediated through integrin receptors include cell migration, proliferation, transformation and apoptosis[4,5], making the elucidation of the mechanisms involved of considerable interest.

The Cas group is a recently identified family of proteins that might serve as key bridge proteins for actin cytoskeleton-dependent signalling networks. There are currently three defined Cas family members. The first described, p130Cas, was identified as a 130-kDa protein that is highly tyrosine phosphorylated in v-Src-[6] and v-Crk-transformed cells[7], providing the basis of the family name, Cas (Crk-associated substrate). The second member, HEF1/Cas-L (hereafter referred to as HEF1, human enhancer of filamentation 1), was isolated in a screen for human proteins that confer morphoregulatory changes leading to filamentous budding in yeast[8], and was independently isolated based on homology to p130Cas[9]. The third member, Efs/Sin (embryonal Fyn substrate/Src-interacting – for simplicity, here

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BOX 1 – CAS PROTEINS AND RECEPTOR SIGNALLING

Cas proteins have been implicated in many different receptor signalling pathways. This review is restricted mainly to roles in integrin signalling, but further reading related to different receptor systems is listed here.

Antigen receptor stimulation

T-cell receptor[1].

B-cell receptor[2].

Growth factor receptor stimulation

Nerve growth factor receptor[3].

Epidermal growth factor receptor[4].

Insulin receptor[5].

G-protein-coupled receptor (GPCR) signalling

p130Cas in neuropeptide stimulation of GPCR signalling[6].

Calcitonin receptor[7].

Neuropeptide stimulation[8,9].

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The authors are in the Fox Chase Cancer Center, Division of Basic Science, 7701 Burholme Avenue, Philadelphia, PA 19111, USA.
E-mail: EA_Golemis@fccc.edu

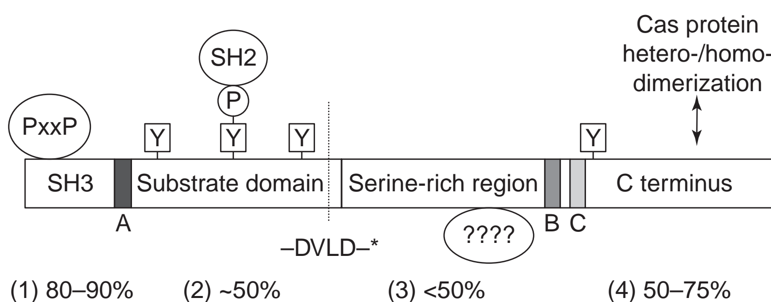

FIGURE 1

Schematic representation of the overall conserved domain structure of the three Cas proteins. The overall percentage amino acid identity of the domains among the three family members is indicated: (1) the Src homology 3 (SH3) domain that mediates interaction with proteins containing poly-proline motifs (PxxP); (2) the substrate domain that, upon phosphorylation (P) of the multiple tyrosines (Y) within this region, mediates interactions with SH2-containing proteins; (3) the serine-rich region; and (4) the C-terminus that mediates homo- and heterodimerization. Also indicated are the poly-proline regions that are found in both p130Cas and Efs; the mitotic caspase cleavage site found in HEF1; and a conserved C-terminal site whose phosphorylation by focal adhesion kinase (FAK) recruits Src-family kinases to subsequently phosphorylate the substrate domain.

referred to as Efs), was characterized initially as a Fyn SH3-domain-binding protein[10,11].

The finding that p130Cas is hyperphosphorylated in Src- or Crk-transformed cells, the notion that integrin-based signalling pathways are constitutively active in such oncogenically transformed cells and the presence of multiple protein-interaction motifs in p130Cas gave the first hint of a role for Cas proteins as docking intermediaries in integrin receptor signalling. Research from multiple laboratories in the past five years has firmly established this role, and has provided links between Cas family functions and the integrin-dependent processes of migration, cell-cycle control and transformation. In addition, recent work makes it clear that Cas proteins play a role in signalling through other receptor systems, including the G-protein-coupled calcitonin receptor[12] and costimulation by the T-cell receptor[13,14] (see Box 1); however, as the role for Cas proteins is most firmly established in adhesion signalling, this review will primarily address this subject.

Structure, localization and posttranslational processing

The Cas proteins have a conserved amino acid secondary structure (see Fig. 1) with numerous protein–protein interaction domains, including a

Src-homology 3 (SH3) domain, numerous SH2-binding sites in a 'substrate domain' (see identified consensus binding sites in the footnote to Table 1), proline-rich motifs (in p130Cas and Efs), and a novel C-terminal dimerization module. This structure has indicated a role for Cas proteins as docking molecules, and numerous interacting proteins have been identified for distinct members of the family, as summarized in Table 1. To date, the most intensively studied interactive partners are focal adhesion kinase (FAK), the FAK-related protein RAFTK/Cakβ/Pyk2 (for simplicity hereafter referred to as RAFTK), the Src family protein tyrosine kinases Lyn and Fyn, members of the Crk adaptor protein family, and the phosphatases PTP1B and PTP-PEST.

Subcellular-localization studies in interphase cells have indicated that p130Cas and HEF1 localize mainly to focal adhesions and proximal stress fibres, with some protein appearing diffuse in the cytosol[7,15,16]. The SH3 domain of p130Cas is necessary for the observed localization to focal adhesions[17]. Considering the high degree of sequence homology among the SH3 domains of Cas family members[8] and their reported interactions with FAK[8,18], it is likely that SH3-domain-dependent interactions with FAK contribute significantly to the localization of all Cas proteins. As noted above, Cas family proteins are modified extensively by both tyrosine and serine/threonine kinases in response to oncogenic transformation[7,19], integrin ligation and cellular adhesion[9,15,20–25], and cell-cycle progression[26,27]. The phosphorylation status of Cas proteins also contributes to their localization properties. Nonphosphorylated p130Cas localizes mainly to the cytosol, whereas phosphorylated p130Cas is found in membranous, nuclear[7] and insoluble cytoskeletal fractions[28], and is thought to be more tightly associated with focal adhesions. By contrast, Liu *et al.* have reported that p130Cas co-sediments with the endoplasmic reticular (ER) fraction of v-Crk-transformed cells, suggesting that p130Cas might also be recruited to an ER signalling complex[29], although the functional significance of this localization remains to be determined. Strikingly, the localization of at least one Cas family member, HEF1, is highly dynamic during the cell cycle and is accompanied by substantial posttranslational modifications. The HEF1 protein is specifically cleaved at the G2–M transition, resulting in the elimination of the full-length

TABLE 1 – SUMMARY OF PROTEINS THAT HAVE BEEN DEMONSTRATED TO INTERACT WITH CAS PROTEIN DOMAINS

Domain	Cas protein	Interactor	Refs
Protein interactions demonstrated *in vivo*			
SH3	p130Cas	FAK, RAFTK, PTP-1B	16, 20, 32
	HEF1	FAK, RAFTK	8, 20
Substrate	p130Cas and HEF1	Crk	20, i, ii 20
Poly-proline	p130Cas	Lyn	20[a]
	Efs	Fyn, Yes	11
Serine-rich	p130Cas	14-3-3, Grb2	iii, 43
Other protein interactions demonstrated *in vitro*			
SH3	p130Cas	FAK, FRNK, PTP-PEST, C3G PTP1B, CMS	15, 16, 29, 38, iv, v, vi
	HEF1	FAK, RAFTK, PTP-PEST	8, 18, 38
	Efs	RAFTK, FAK, PTP-PEST	18, iii
Substrate domain[b,c]	p130Cas	Crk	vii, viii
	HEF1	Abl, Crk, Nck, Lck, SHPTP2, Csk Dim1p, Crk	9, 13, 26
Src binding[b,d]	p130Cas	Src (YDYVHL)	32, 33, vii
	HEF1	Src (YDYVHL)	32, 33
Poly-proline			
(see C, Fig. 1)	p130Cas	Fyn, Lyn, Hck, Src	20
(see B, Fig. 1)	Efs	Fyn, Src	10, 11
C terminus	HEF1	HEF1, p130Cas, Id2	8, ix

[a]Authors also show HEF1 and Lyn co-IP, even though HEF1 has no poly-proline region.
[b]Tyrosine phosphorylation (P-Tyr)-dependent interactions.
[c]The following consensus binding sites have been located: HEF1 – fgr, grb2, crk, abl and less conserved sites for Src, Fes, Abl and Vav; p130Cas – Fgr, Crk, Grb2, Abl, 3BP2 and less conserved sites for Src, Fes, Abl and Vav. Alexandropoulos and Baltimore[10] have described SH2-binding sites on Efs/Sin.
[d]Efs also contains the consensus Src SH2 binding site (see Fig. 1 and the tyrosine residue closest to the C terminus): however, studies of Efs have been focused on the SrcSH3/Efs poly-proline interaction.

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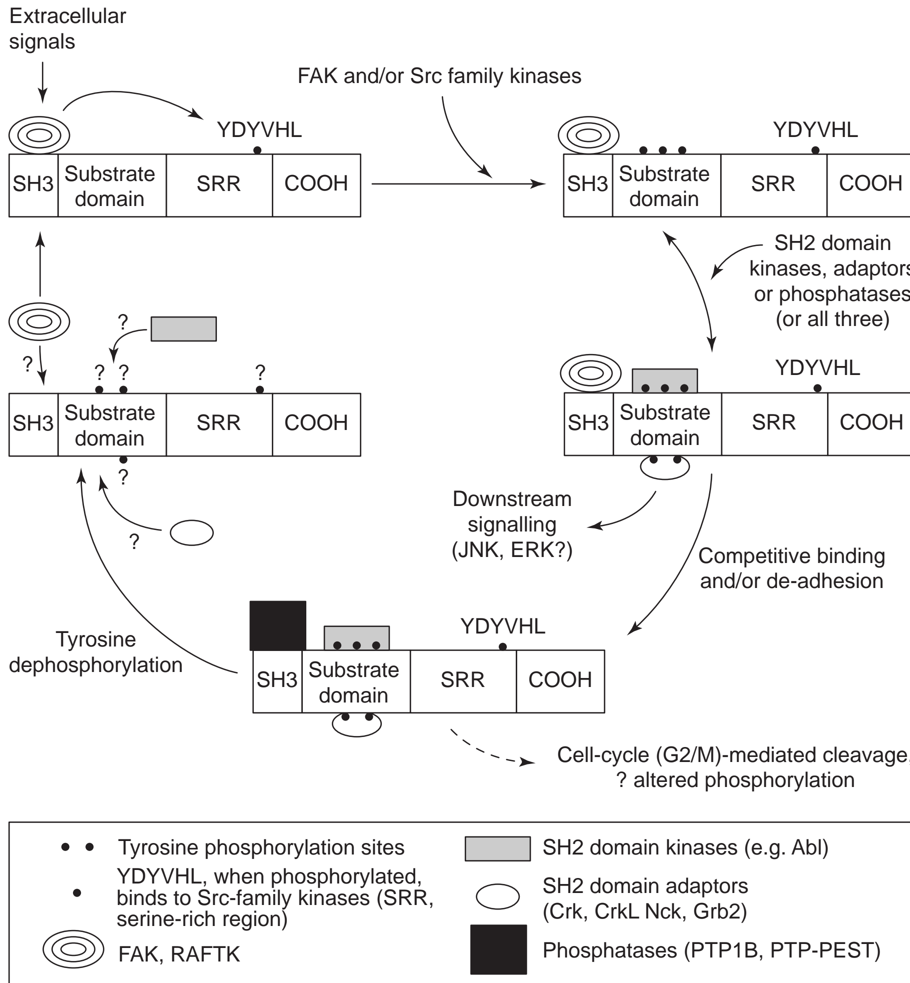

FIGURE 2

Schematic representation of the cycle of protein phosphorylations and resultant signal transduction at sites of focal adhesion. Upon integrin receptor engagement and clustering induced by ligand binding, Cas proteins use their Src homology 3 (SH3) domains to bind to the C-terminal focal adhesion kinase (FAK)/FAK-related protein (RAFTK) poly-proline region, where they are tyrosine phosphorylated either directly by FAK/RAFTK or by cooperative interaction between Src and FAK/RAFTK (p130Cas and Efs). Following tyrosine phosphorylation, the Cas proteins assemble additional SH2-domain-containing proteins. Depending on specific extracellular signals, phosphatases (e.g. PTP1B and PTP-PEST) might also be recruited by the Cas family members, potentially leading to the dephosphorylation of the Cas proteins and other colocalized proteins. Cyclical alteration of the Cas protein phosphorylation state provides a potential mechanism to modulate the stable assembly of signalling complexes at focal adhesions.

hyperphosphorylated species (p105 and p115) and producing a nontyrosine-phosphorylated 55-kDa N-terminal species encompassing the SH3 domain and the majority of the SH2-binding-site motifs[26]. This species migrates to the mitotic spindle[26] despite the presence of residual FAK-interactive sequences, and might act as a signal connoting readiness for cell division (see below). The varied subcellular localization of Cas proteins following posttranslational modification and the numerous interacting protein partners suggest that the Cas proteins play an important role in the coordination and control of different cellular processes.

Although the Cas proteins have a conserved overall domain structure, they have different tissue distribution. p130Cas is expressed uniformly[7], whereas the highest HEF1 expression is noted in epithelial cells[8,26], T cells[9] and B cells[9,20], and Efs expression is restricted mostly to placenta, embryo, muscle and brain[11]. The restricted tissue distribution of HEF1 and Efs could reflect somewhat specialized functions of these two molecules, whereas the ubiquitous nature of p130Cas suggests a vital role for this molecule in normal cell physiology.

Cas proteins in integrin-mediated signalling

Based on current knowledge, the following general pathway for Cas proteins in integrin receptor signalling can be proposed (Figs 2 and 3): following integrin receptor ligation by means of binding to the extracellular matrix, the Cas-protein-associated kinase FAK/RAFTK undergoes autophosphorylation, creating a binding site for Src-family kinases (reviewed in Refs 30 and 31) and other proteins. Cas proteins bind to the C-terminal FAK/RAFTK poly-proline region via their SH3 domains[8,16,18,20,32] and are tyrosine phosphorylated either directly by FAK/RAFTK[32,33] or through cooperative interaction between Src and FAK/RAFTK (p130Cas and Efs)[32,34,35]. Following tyrosine phosphorylation, the Cas proteins act as docking molecules, assembling additional SH2-domain-containing proteins such as Crk[9,20,25] along their cognate phosphorylated SH2-binding sites. This assembly might lead to recruitment of additional kinase molecules and subsequent 'processive phosphorylation' (e.g. by Abl[36]), providing binding sites for further SH2-domain-containing proteins, thereby creating a network of signalling proteins that transduce integrin signals to the nucleus. Conversely, depending on specific extracellular signals, phosphatases (PTP1B, PTP-PEST and SHP2) might also be recruited by the Cas family members[29,37–39], potentially leading to the dephosphorylation of the Cas proteins and other colocalized proteins. Cyclical alteration between phosphorylated and dephosphorylated states of the Cas proteins provides a potential mechanism to modulate the stable assembly of signalling complexes at focal adhesions.

Co-transfection of cells with Efs and c-Src has been shown to cause increased Src activation[10]. One recent report confirms a role for p130Cas mediation of Src-induced activation of serum response elements[40]. Furthermore, Erk2 activation was shown to be a requisite intermediate step in this process, which was promoted by the p130Cas serine-rich domain[40]. Several studies have demonstrated that integrin receptor ligation stimulates Cas proteins to interact with RAFTK[32,41] and recruit Crk[9], leading to activation of ERK2 and JNK. However, coexpression

of RAFTK with either a p130Cas mutant lacking the SH2-binding-site-rich substrate domain or a Crk SH2-domain mutant results in decreased JNK activity, whereas ERK2 activity is unaffected. This suggests that p130Cas association with Crk links RAFTK with JNK activation directly, and is consistent with data demonstrating that Crk expression causes activation of JNK[42] but not ERK2[43]. Dolfi *et al.*[43] have proposed that the Crk SH3 domain is likely to signal through DOCK180 to Rac and finally JNK. Interestingly, they demonstrated that p130Cas lacking the substrate domain blocks integrin-induced, but not v-Src- and EGF-induced, JNK activity[43]. At present, it appears likely that Cas family proteins signal under some circumstances via both ERK and JNK kinase cascades, but that the degree of interaction differs according to cell type or to other undetermined variables.

The Cas proteins get things moving

As cell motility is intimately dependent upon the ability to form and release extracellular attachments, the association of Cas proteins with FAK at focal adhesions suggests a physiological role for this protein family in cell movement. Indeed, it has been demonstrated that FAK overexpression stimulates cell migration in a Src/Fyn-dependent manner[44]; this study was followed by the demonstration that FAK-dependent p130Cas–Crk coupling is required for induction of cell migration[45–47]. Cas–Crk co-overexpression was shown to cause enhanced cellular motility, in a process requiring the function of Rac GTPase and the Crk SH2 domain. In a proposed model, association of p130Cas–Crk induces the Crk SH2 domain to recruit DOCK180 to the cell membrane and activate Rac, causing the induction of membrane ruffling; this collaborates with the induction, along a separate signalling pathway, of mitogen-activated protein (MAP) kinases ERK1 and ERK2 that control actin–myosin assembly and cell contraction of collagen matrix[47].

The role of p130Cas in migration has been further supported by experimental data from p130Cas(–/–) fibroblasts that display reduced migration[48]. It is instructive to compare the phenotypes of cell lines derived from homozygous knockouts of p130Cas[48] and its interactors FAK[49] and PTP-PEST[50]. Compared with wild type, fibroblasts derived from knockout animals have increased numbers of focal adhesions, dense peripheral actin stress fibres [p130Cas(–/–) and FAK(–/–)], microspikes around the perimeter (characteristic of the early stages of nonpolar spreading) [PTP-PEST(–/–) and FAK(–/–)], disorganization of the cortical cytoskeleton and reduced mobility on fibronectin. The presence of increased numbers of focal adhesions and commensurate greater degree of cell attachment is associated with reduced motility as migratory cells have fewer focal adhesions and those that are present are located in or just behind the leading edge. In addition, stress fibres and movement are reportedly inversely correlated (reviewed in Ref. 3). One notable point to take from these combined studies is that the interactions of FAK, PTP-PEST and p130Cas are not required for the formation of focal adhesions as more adhesions are found in their absence; rather, the crucial activity required for these proteins must be related to the sequential turnover of focal adhesions and formation of appropriate connections between adhesions and the internal actin cytoskeleton.

In all of the migration studies regarding FAK and p130Cas, migration is never abolished completely, neither when the gene is knocked out nor in the presence of loss-of-function dominant–negative constructs. There are several possible explanations for the residual migration. First, there might be partial redundancy among the different Cas family members, as has been shown for FAK and RAFTK[51]. Second, integrin–FAK–Src–p130Cas signalling might not represent the only migration signalling pathway. Recently, it has been proposed that FAK is involved in two types of migration signalling[52]: the FAK–Src–p130Cas connection already described and, second, a pathway involving the adaptor protein Shc, interacting with FAK and signalling through MEK1. The p130Cas pathway has been linked to directional (persistent) movement, whereas the Shc-mediated pathway has been proposed to account for random cell migration[52]. As FAK(–/–) and p130Cas(–/–) cells retain some degree of motility, the presumption is that migration is not completely dependent on FAK or p130Cas activity either because of functional redundancy or because alternative inducer proteins can stimulate movement. The relative contributions of these distinct pathways to cell migration remain to be determined.

Cas proteins and cell shape

In an early antisense study, treatment of ornithine-decarboxylase-transformed cells to either reduce p130Cas phosphorylation or ablate p130Cas expression resulted in reversion from an altered morphology to a flat, 'normal' cell phenotype with a restored actin cytoskeleton[53]. As mentioned above, fibroblasts from the p130Cas mouse knockout model displayed impaired actin stress fibre formation[48]. Morphologically, the cells were 'flat, thin and round-shaped', reminiscent of FAK(–/–) fibroblasts, which were described as rounded and poorly spread with altered actin organization[49]. Recently, it has also been reported that p130Cas is involved in actin organization of osteoclasts (bone-resorbing cells)[54]. Activation of osteoclasts by integrin-mediated adhesion is characterized by the formation of a ring-like structure of actin at the cell periphery. The authors demonstrate that p130Cas is tyrosine phosphorylated during actin rearrangement and colocalizes with the actin ring structure. This is apparently dependent on the presence of Src as Src(–/–) osteoclasts show no actin ring formation, p130Cas phosphorylation or colocalization in a ring structure. Induced expression of the HEF1 protein has also been found to alter cell shape, causing MCF-7 cells overexpressing the protein to assume a crescent-like shape with extended lamellipodia (Fig. 4). Taken together, these results indicate a role for Cas family proteins in the reorganization of the actin cytoskeleton in response to various external

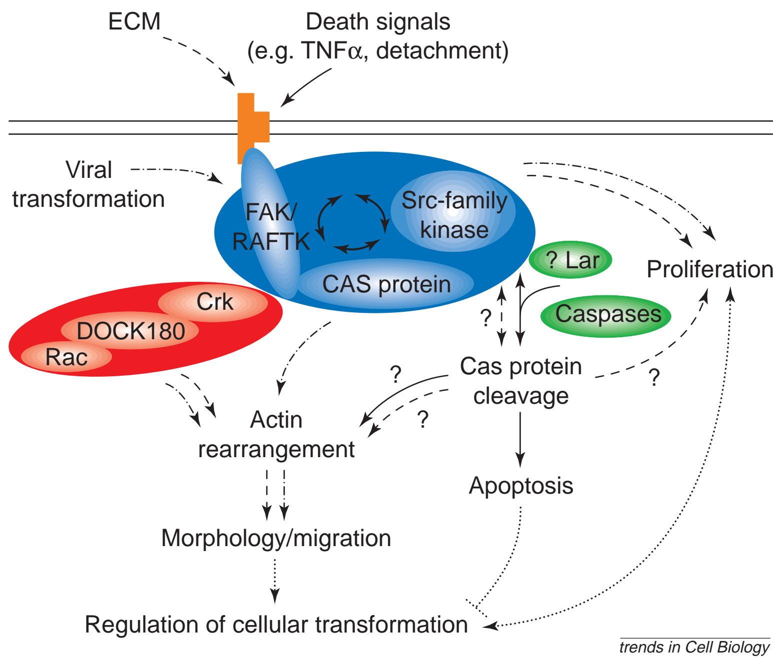

FIGURE 3

Schematic representation of the interconnecting physiological pathways impacted by Cas proteins. Signal-transduction pathways affected by viral transformation (dashed/dotted lines), integrin receptor ligation (dashed lines) and following apoptosis signals (black lines) are shown. Many external signals are transduced internally by cooperative interaction among FAK/RAFTK, Src-family kinases and Cas proteins. Upon the recruitment of Crk to this signalling complex, cells are stimulated to migrate; additionally, there is actin rearrangement as a result of signals transduced through the complex that impact both cellular morphology and migration. In response to transformation, Cas proteins appear to be constitutively tyrosine phosphorylated and might play a role in the development of cancer. The formation of neoplasias might also be affected by upregulated/constitutive pro-proliferative signals via as-yet-undetermined intermediates. The downstream mediators of the different outlined pathways have not been noted as these molecules have so far not been well defined. Although it has been noted, for example, that JNK is activated following p130Cas expression and Crk recruitment, to date it is not clear what role, if any, this plays in migration. Note that pathways marked with a question mark represent recently emerging potential novel effects of Cas proteins. The observation that HEF1 is cleaved in a cell-cycle-related manner suggests that cleavage of this molecule might play a role in proliferation, either by direct action or by acting on an intermediary product. Pro-apoptotic signals might be transduced through the complex, potentially by destabilization of the complex through dephosphorylation, providing accessibility for caspases. Caspase cleavage could then result in the production of either pro-apoptotic Cas protein peptides or loss-of-function molecules that additionally impact on the rearrangement of the actin cytoskeleton observed during apoptosis.

environmental triggers. To date, signalling through the epidermal growth factor (EGF) receptor has been implicated as one stimulus co-regulating actin polymerization status and p130Cas[55], but much work remains to be done on this topic. Potentially, this function of the Cas proteins might be hyperactivated during transformation, contributing to the production of the altered cell morphologies typical of cancerous cells.

Life, death and transformation?

In normal cell division, mitosis is marked by cells breaking down focal adhesions, reducing contact with the ECM and rounding up prior to undergoing cytokinesis, which is followed by reattachment with the return to G1 phase. The process of apoptosis is also marked by the loss of cell contacts as moribund attached cells detach from the matrix. Finally, the process of transformation and the acquisition of cellular metastatic potential is also generally accompanied by downregulation of normal cellular attachments. As the study of Cas proteins has advanced, it has become of interest to investigate their functions in the important biological processes of cell cycle, cell viability and transformation, given the implied similar mechanistic underpinning of altered attachment through integrins involved. Although at an earlier stage than the work discussed above, some provocative findings have emerged.

Recent studies of the HEF1 and p130Cas proteins have implicated their function in the loss of cell attachment observed in cell-cycle progression. Although caspases are best known as enzymes activated during apoptosis[56], roles in cytokine processing[57], cell differentiation[58] and cytoskeleton integrity[59] have been proposed recently. HEF1 has been shown to be cleaved at a DLVD motif by caspases specifically at the start of mitosis, eliminating full-length forms of the protein associated with focal adhesions but producing a 55-kDa peptide that localizes to the mitotic spindle[26]. This p55 species is lost as cells complete cytokinesis and is replaced with newly synthesized full-length protein that relocalizes to assembling focal adhesions upon entry into G1 phase[26]. Although p130Cas is apparently not subject to cell-cycle-dependent cleavage, recent work by Yamakita *et al.* has shown altered phosphorylation of p130Cas and FAK during mitosis, which has been proposed to block FAK and Cas association and separately block integrin signalling[27]. The combined loss of HEF1 and p130Cas signalling potential at mitosis indicates that clearance of Cas-dependent signalling complexes might be an essential control point in the process of cell division. Some data suggest that Cas family members might play additional roles in control of cell growth. Expression and phosphorylation of the HEF1 protein is induced when cells are stimulated with serum or released from thymidine block, whereas HEF1 levels are low in quiescent cells[26]. Furthermore, a requirement for p130Cas and FAK interaction to activate the Jun N-terminal kinase (JNK) and allow progression of the cells through the G1 phase of the cell cycle has been reported recently[60]. Currently, the roles of Cas family members in cell-cycle progression are an open issue.

Addressing the issue of apoptosis, Bannerman *et al.* have demonstrated caspase cleavage of p130Cas and other cell–cell and cell–matrix proteins in endothelial cells induced to die by treatment with bacterial lipopolysaccharide[61]. Furthermore, overexpression of the tyrosine phosphatase LAR has been proposed to cause apoptosis by dephosphorylation, destabilization and subsequent degradation of p130Cas[62], although this has not been demonstrated directly. Finally, induction of apoptosis by treatment of cells with TNFα was found to cause HEF1 cleavage by caspases, and overexpression of HEF1 was found to induce apoptosis robustly (S. F. Law, G. M. O'Neill, S. J. Fashena

and E. A. Golemis, unpublished). Although these points remain under investigation, it is likely that loss of full-length p130Cas and HEF1 and their ability to assemble signalling complexes are significant factors in the disruption of integrin-dependent attachment signals. Over the past few years, it has become clear that FAK plays a significant role in cell death mediated by loss of adhesion[63–66], termed anoikis[67]. The involvement of Cas proteins in integrin signalling mediated by FAK therefore suggests that they might also play a role in anoikis, and potentially in other processes related to loss of cell–substrate contacts.

Finally, some data imply that Cas proteins might be involved in transformation and cancer. The way that p130Cas was first identified[7,19], as a target of hyperphosphorylation by oncogenic Src and Crk, suggested that the function of the Cas protein group might be significant in transformed cells. p130Cas (–/–) cells cannot be transformed by Src, apparently because of the actin-bundling defect in p130Cas knockout cells[48]. However, arguing to the contrary, a recent study has found that disruption of Src–Cas interactions had no effect on cellular transformation by Src[68], complicating the interpretation of the knockout finding. In support of a more generalized role for Cas proteins in cancer, it has been demonstrated that p130Cas is highly phosphorylated in ornithine decarboxylase- and Ras-transformed cells[53,69]. Reversion of v-Crk-transformed cells to an untransformed phenotype by PTP1B overexpression is accompanied by a decrease in p130Cas tyrosine phosphorylation and decreased association with Crk[69], whereas associated p130Cas–Crk complexes are found at higher levels in cell lines with enhanced metastatic and invasive properties[46]. As yet, no *in vivo* experiments have been performed to test directly whether misexpression of Cas proteins modifies transformation potential.

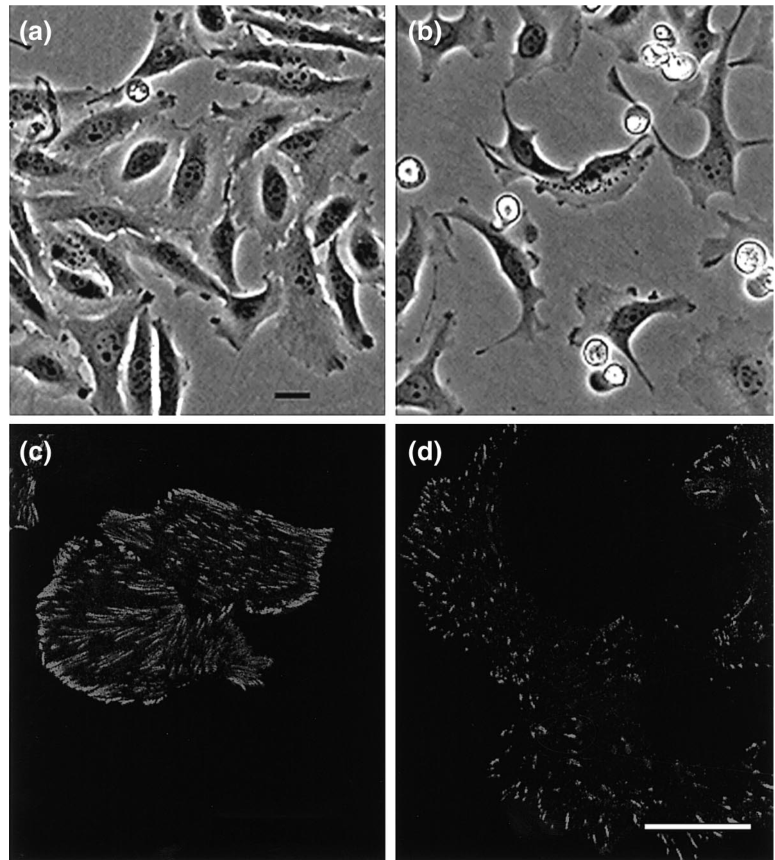

FIGURE 4

HEF1 induces morphological changes characterized by the formation of large leading-edge lamellipodia in which focal adhesion sites are concentrated. Stable MCF-7-derived cell lines were generated in which HEF1 expression is regulated by tetracycline levels. Phase contrast images of these cells, either (a) uninduced or (b) induced to express HEF1, illustrate dramatic HEF1-mediated morphological changes. Immunofluorescent staining to visualize paxillin in either (c) uninduced or (d) induced cells reveals that focal adhesion sites are distributed uniformly about the perimeter of the two uninduced cells (c), whereas they are concentrated at the leading-edge lamellipodia of the large crescent-shaped HEF1 expressing cell (d). Bar in (a) and (d), 25 μm.

A multiplicity of roles

Although numerous roles for the Cas proteins have been proposed in this review, all of these cellular functions are intimately connected and interdependent (see Fig. 3). Migration, cell shape, cell death and proliferation are impacted by messages received from the external environment via cell-surface receptors, whereas oncogenesis usually represents a circumvention of these normal cellular pathways. It remains to be demonstrated to what extent each Cas protein contributes individually or redundantly to the different functions described and indeed the manner with which the proteins are able to assemble different downstream signalling molecules. Furthermore, the observation that Cas proteins are cleaved into discrete peptides that constitute different protein–protein interaction domains begs the question as to whether these peptides might have a specific cellular function. Finally, the characterization of the Efs protein is currently at an early stage, raising the possibility that Efs might differ in key respects from p130Cas and HEF1. The multiplicity of roles implicated to date suggests that the Cas proteins are important docking molecules that coordinate cell-signalling cascades with the physical processes of attachment and movement. Further studies should illuminate the role of this new Cas(t) of characters in integrin signalling.

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Interaction blues: protein interactions monitored in live mammalian cells by β-galactosidase complementation

Fabio M.V. Rossi, Bruce T. Blakely and Helen M. Blau

In mammalian cells, protein–protein interactions constitute essential regulatory steps that modulate the activity of signalling pathways and many other intracellular processes. Although efficient genetic methods for identifying interacting partners exist and have been successfully applied by a number of laboratories, there has been a need for a technique that allows protein interactions to be monitored in real time in the cellular compartment in which they normally take place. The β-galactosidase-based intracistronic complementation methodology described here is the first technology that might fulfil these requirements and can be applied to live mammalian cells. In addition, it holds promise for applications in high-throughput screens for agonists and antagonists of specific interactions and for the development of a 'mammalian two-hybrid' screen for novel protein partners.

Cell physiology and development is controlled by well-regulated cascades of protein–protein interactions. For example, the activation and subsequent auto-phosphorylation of growth-factor receptors is often dependent on their ligand-induced homo- or heterodimerization[1]. Such protein interactions result, in turn, in the creation of docking sites for downstream components of the relevant signalling pathways and thus in additional protein–protein interactions[2]. Several systems have been developed for identifying and studying protein–protein interactions, including the yeast two-hybrid system[3,4], the split-ubiquitin system[5,6], the Sos-recruitment system[7,8] and dihydrofolate reductase (DHFR) complementation[9,10]. As shown in Table 1, each of these systems has inherent advantages, as well as disadvantages, including lack of utility in mammalian cells or with membrane proteins, or lack of rapid quantitative analysis of the interaction owing to the absence of signal amplification, indirect readouts or other assay limitations.

We have developed a novel assay for monitoring protein–protein interactions based on intracistronic β-galactosidase complementation. There are several advantageous properties of the intracistronic β-galactosidase complementation method:

- it works in live mammalian cells;
- it monitors interactions in the compartment in which they normally take place (e.g. membrane or cytoplasm);
- rapid sensitive assays are available that are amenable to high-throughput screening methods;
- it provides a quantitative readout, allowing the monitoring of interaction kinetics;
- it provides signal amplification, allowing physiological interactions to be monitored in the absence of overexpression.

Properties of β-galactosidase intracistronic complementation for monitoring protein–protein interactions

Intracistronic β-galactosidase complementation is a phenomenon whereby two mutants of the bacterial enzyme β-galactosidase that harbour inactivating mutations in different crucial domains are capable of recreating an active enzyme by sharing their intact domains[11,12]. It has long been known that, in *Escherichia coli*, specific mutants can complement one another more or less efficiently, depending on the nature of the mutations[13]. We have shown that the same holds true in mammalian cells[14]. Our protein-interaction detection method capitalizes on the expression of low levels of chimeric proteins incorporating weakly complementing β-galactosidase mutants. β-galactosidase activity is recreated only when physical interaction of the mutants is forced by the non-β-galactosidase components of the hybrids. Under these conditions, the complementation of β-galactosidase mutants does not drive, but rather monitors, the interaction of other proteins.

To monitor the interaction between two proteins, each of the proteins is

The authors are in the Dept of Molecular Pharmacology, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305-5332, USA.
E-mail: hblau@cmgm.stanford.edu.

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