Integrin–protein kinase C relationships

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Abstract

The integrins have an ability to interact with extracellular matrix proteins to confer adhesive and motile properties on cells. The means by which these activities operate and the manner in which they are integrated with cell functions is of particular relevance to many biological processes. In the present paper, the developing understanding of the bi-directional relationship between the protein kinase C family of signal transducers and integrins is discussed.

Introduction

Cell–matrix interactions are essential elements in maintaining a variety of cellular properties including polarity, migration, survival and growth-factor responsiveness. These interactions are supported by a family of heterodimeric (α/β), cell surface, matrix receptors – the integrins [1]. Combinations of distinct α- and β-integrins determine binding specificity for the different matrix proteins (fibronectin, laminin, vitronectin, collagen, gelatin), the basis of which has become more clearly defined through structural analysis [2]. The ability to bind matrix and the consequent assembly of cellular signalling complexes is determined not only by cell-surface expression, but also through ‘inside-out’ signalling that can confer high affinity/avidity on α/β-integrins [3,4].

Notable among agents that can effect this inside-out signalling are phorbol esters and, although there are now multiple classes of phorbol-ester-binding proteins established, it is thought that the protein kinase C (PKC) family has an important role in this integrin control [3]. Reciprocally, there is accumulating evidence that integrins have a key role in the function of PKC family members [5]. The inter-relationship of these two families of proteins is reviewed in the present paper. The particular aspects considered below relate to the permissive control of PKCs and their influence on the traffic of, and migratory responses to, integrins.

Integrin control of PKC

Although members of the PKC family have long been considered to be under the direct allosteric control of lipid second messengers, a recent study has emphasized the importance of a series of conserved phosphorylations that are important for optimum catalytic function [5] (Figure 1). One key reason underlying the generally slow appreciation of the importance of these phosphorylations is that the PKC proteins, as isolated from cells or tissues, are usually highly phosphorylated and hence have a respectable specific activity. This clearly contrasts with more conventional mitogen-activated protein kinases such as protein kinase B (PKB; also known as Akt) and extracellular-signal-related kinase (ERK)1/2, where acute stimulation is needed to trigger robust phosphorylation and activity. In part, this high degree of phosphorylation for the novel PKCs (nPKCs) δ and ε is a function of cell–cell and/or cell–matrix contacts, since, in some, but not all, cell types, phosphorylation is readily maintained while cells remain adherent to substrate. The importance of integrins in this pattern of behaviour is clearly shown by replating experiments that demonstrate matrix-dependent rephosphorylation of these nPKCs [6]. In fact, recent evidence indicates that release from, and replating on, matrix can have a profound effect upon the PKCδ phosphorylation state, with the accumulation of dephosphorylated species [7]. The precise underlying mechanisms in this pattern of behaviour remain unresolved, although the acute nature of this response indicates that this is a protein-phosphatase-dependent process, as opposed to the accumulation of de novo unphosphorylated PKCε. Interestingly, this response to cell–matrix disengagement is not a universal one for all PKC isoforms. For example, PKCα does not appear to become acutely dephosphorylated at its three priming sites on disengagement of integrins (P. Whitehead and P.J. Parker, unpublished work). Interestingly, sustained maintenance of cells in suspension has shown that both PKCε and PKCδ accumulate as dephosphorylated forms [6].

One well-established element in the control of PKC phosphorylation is the phosphoinositide 3-kinase (PI3K)/phosphoinositide-dependent kinase-1 (PDK1) pathway [8]. PDK1 has been shown to form complexes with many PKC isoforms and will phosphorylate the conserved activation loop TFCG motif (where underlining indicates the site of phosphorylation) [9]. The involvement of PI3K is indicated, by the effects of inhibition and the established properties of PDK1, as

Key words: adherence; migration; phosphorylation.

Abbreviations used: ERK, extracellular-signal-related kinase; nPKC, novel PKC; PAR, protease-activated receptor precursor; PDK1, phosphoinositide-dependent kinase-1; PI3K, phosphoinositide 3-kinase; PI(PLC), phosphoinositide-specific phospholipase C; PKC, protein kinase C; P13K, phosphatase and tensin homologue deleted on chromosome 10.

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a PtdIns(3,4,5)P$_3$-binding protein [10]. There is, however, some question concerning the in vivo requirement of PtdIns(3,4,5)P$_3$ in the PDK1 phosphorylation of some PKC isoforms [11]. Despite this, however, the prolonged suspension culture accumulation of dephosphorylated PKCε and PKCδ is influenced by the status of PTEN (phosphatase and tensin homologue deleted on chromosome 10) [6]. This tumour suppressor gene encodes a PtdIns(3,4,5)P$_3$ 3-phosphatase [12] and its loss of function is associated with elevated PtdIns(3,4,5)P$_3$ and signal output through the PI3K pathway. Whether this particular adherence-dependent PTEN–PKC relationship underlies the association of PTEN loss with advanced, metastatic tumours is of significant interest and may relate to the roles that PKC isoforms have in cell migration (see below).

The coupling of integrins to cellular signals is effected through the recruitment of various signalling and structural/scaffold proteins. Among these, the protein tyrosine kinases FAK (focal adhesion kinase), PYK2 (proline-rich tyrosine kinase 2) and c-Src seem to have key roles in signal propagation, whereas the cytoskeletal proteins such as α-actinin, talin and paxillin contribute changes in actin organization [13,14]. With respect to the control of PKC, the key transducers are phosphoinositide-specific phospholipase C (PI-PLC) and PI3K. These transducers have been reported to become associated with certain activated α/β integrins. In the case of PI-PLC$_{γ1}$, this may be through direct interaction with the β$_1$–integrin tail [15], although in the context of the activated α$_1$β$_1$–integrin complex, PI-PLC was not identified [16]. In relation to these PKC pathways, evidence for integrin-dependent spreading involving interaction with Rac, Ras, PKCε and PI3K has also been presented [17]. The specificity and details of integrin complex formation with PI-PLC, PI3K etc. and hence their signalling capacity via diacylglycerol and PtdIns(3,4,5)P$_3$ to PDK and PKC requires further analysis.

**PKC control of integrins**

Adhesive properties of integrins that control cell-spreading properties, and the dynamic properties of integrin–matrix engagement that control cell movement, are under the influence of a number of different intracellular regulators. These inside-out signals include the actions of small G-proteins (Ras, Rac and cdc42), PI3K and PKC [18]. A significant element of the literature on the influence of PKC on integrins derives from the use of phorbol esters. These PKC activators have long been known to affect integrin affinity/avidity. Although it is now well established that PKCs are not the only phorbol ester receptors [19], studies demonstrate that PKCs do indeed regulate integrin properties.
A general theme arising out of the recent studies on integrins and PKC is the demonstration of co-localization. This is evident in spreading responses, migration, traffic and, more particularly, in direct or indirect complex formations. Such observations of co-localization are important in assessing the actions of signal transducers and it is certainly the case for PKCs where a plethora of binding proteins are considered to be important in determining PKC function through localization [20]. The direct association of PKCa with β1-integrin [21] and of classical or conventional PKCs (cPKCs) with CD81 (an integrin-associated tetraspan protein) [22] provides clear evidence for a role ‘by association’. For CD81, the region of interaction has been partly refined to exclude the small internal loop, the transmembrane regions 3 and 4 and the large extracellular loop. In case of β1-integrin interaction with PKCa, the binding occurs through the association of the β1-integrin tail with the V3 variable region between the regulatory and catalytic domains [23]. This latter association was originally shown to correlate with β1-integrin dependent migration [21]. More recent studies exploiting the mapping of this binding site through the use of a competitive binding peptide and deletion mutants have provided direct evidence for this role of PKC [23]. Catabolically, there is no evidence for a role of PKCa in the direct phosphorylation of β1-integrin in vivo. In contrast, several serine/threonine sites in the β3-integrin cytoplasmic domain have been shown to be phosphorylated by PKC in vitro [24]; one of these PKC sites, Thr-758, could lead to the recruitment of 14-3-3 proteins to the activated integrin following phosphorylation in situ. However, the assembly of the β1-integrin–PKC complex is correlated with recruitment of the ERM (ezrin–radixin–moesin) family protein ezrin and its phosphorylation on the Thr-567 site [25]. This phosphorylation is associated with stabilizing the open conformation of ezrin with the capacity for interactions through its tail domain (with actin) and its N-terminal FERM domain (with various membrane localized partners) [26]. The phosphorylation of ezrin is essential for supporting integrin dependent migration and in vitro studies, alongside pharmacological ones, indicating that this is PKC-mediated phosphorylation [25].

The physical and functional relationships between PKC, β1 integrin and the ERM proteins have important implications in biological settings other than integrin-mediated migration. For instance, a constitutive PKCa–β1-integrin complex has also been found in multiple myeloma cells and has a significant role in the development of a vascular endothelial growth factor (VEGF)-responsive migratory phenotype [27]. In addition, CD44-mediated motility upon phorbol ester stimulation is dependent on β1- and not αV-integrin (28); M. Parsons and T. Ng, unpublished results). It is possible that the dissociation of ezrin from other surface receptors such as CD44, L-selectin or intracellular adhesion molecule-1 (ICAM-1) may be a prerequisite for ezrin association with PKCa and its subsequent phosphorylation, which is essential for supporting integrin-dependent migration.

The role of PKC described above in mediating this characteristic integrin-dependent event is perhaps more reflective of integrin control of PKC than vice versa. However, there is an additional element, more poorly understood, that suggests there is, in fact, a reciprocal relationship. The association of PKCs with β1-integrin in cells, as determined by FRET (fluorescence resonance energy transfer), is characterized by a greater interaction between the ‘activated’ conformer (12G10-positive) than with the ligand-unoccupied conformer (Mab13-positive) [21]. Since the interaction is promoted by PKC activation, it is reasonable to consider that the binding of PKCa to β1-integrin shifts the conformer equilibrium towards the ‘activated’ conformer. This is also associated with increased cell-surface expression of the integrin. The overall working hypothesis would be that the open conformer–PKCa interaction promotes the accumulation of the ‘activated’ conformer of β1-integrin and its delivery to the cell surface, thereby regulating matrix interactions. This correlates with the many observations of phorbol-ester-induced up-regulation of integrins and integrin–matrix association.

A further aspect of this relationship relates to the cellular traffic of integrins. For β1-integrin, it appears that the catalytic activity of PKCa contributes to the rate of internalization from the cell surface, a process critical to the dynamic migratory process. Intracellular traffic associated specifically with directional movement has been shown to be associated with PKCs function. In this instance, inhibition of PKC activity leads to the reversible accumulation of β1-integrin that is internalized from the cell surface within an intracellular vesicular compartment alongside PKCs [29]. Loss of PKCs from these isolated β1-integrin-positive vesicles requires PKC activity alongside as yet unidentified cytosolic component(s). How this process confers directionality to β1-integrin traffic (i.e. trailing edge to leading edge movement) awaits further analysis, as indeed does understanding where and when PKCs functionally interact with other integrins.

**Perspectives**

The bi-directional relationship between integrins and PKC, although on a firm foundation, requires a deeper level of understanding. The individual elements that contribute to functional complexes that exert the mutual controls outlined above, as well as the proximal outputs, are only partially documented. Furthermore, within a broader perspective, the polarity of events associated with localized cell surface, a process critical to the dynamic migratory process. Intracellular traffic associated specifically with directional movement has been shown to be associated with PKCs function. In this instance, inhibition of PKC activity leads to the reversible accumulation of β1-integrin that is internalized from the cell surface within an intracellular vesicular compartment alongside PKCs [29]. Loss of PKCs from these isolated β1-integrin-positive vesicles requires PKC activity alongside as yet unidentified cytosolic component(s). How this process confers directionality to β1-integrin traffic (i.e. trailing edge to leading edge movement) awaits further analysis, as indeed does understanding where and when PKCs functionally interact with other integrins.

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Received 6 September 2002