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Movin' on through with Cdc2

Rudy Juliano

Proteins that control the cell cycle would seem to have little to do with cell migration. However, a recent report indicates that Cdc2, a key cyclin-dependent kinase, can also enhance cell migration. Levels of Cdc2 are upregulated in cells that express high levels of the $\alpha_v\beta_3$ integrin, a protein that has long been implicated in enhanced migration and invasion of tumour cells.

The ability of malignant tumours to invade surrounding tissues requires that the tumour cells be motile. Cell motility involves a highly complex spatiotemporally orchestrated series of events that includes extension of a membrane protrusion, formation of stable cell–matrix attachments near the leading edge of the protrusion, movement of the cell body forwards, and release and retraction at the rear of the cell. Both polymerization of actin networks involving the Arp2/3 complex and the development of contractile forces by actinomyosin are vital to this process¹. Adhesion receptors, particularly those of the integrin family, are clearly important in cell migration and tumour invasion, as they provide a transmembrane linkage between the extracellular matrix and the actinomyosin cytoskeleton, as well as contributing to signalling². Cell migration is regulated through the orchestration of multiple signal transduction pathways. Rho-family GTPases, particularly RhoA, Rac and Cdc42, and their downstream effectors have a key function in these events³, but other signalling components are also involved. Not surprisingly, a number of protein kinases contribute to cell migration. Focal adhesion kinase seems to function upstream of Rac⁴, whereas PAK family kinases function downstream of Rac/Cdc42 and impinge on actin and myosin through LIM kinase and myosin light chain kinase, respectively⁵. The Rho-activated kinases⁶ and the Erk mitogen-activated protein kinase⁷ also contribute to actinomyosin contractility. Now, as a result of a study⁸ by Manes *et al.* in

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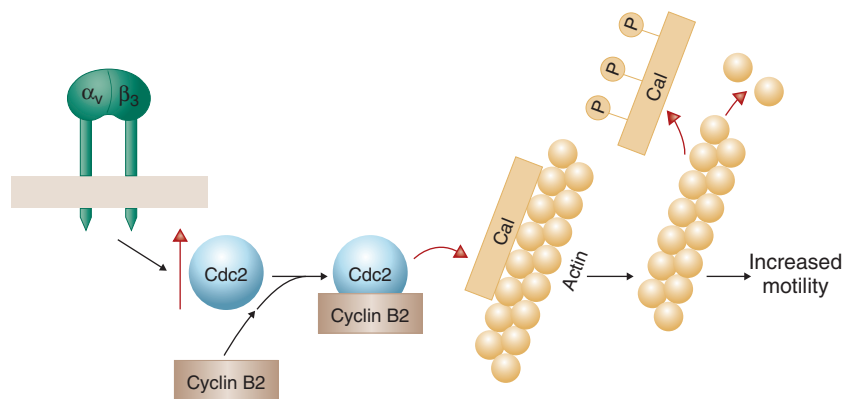


Figure 1 A tentative model for Cdc2 in tumour cell migration. Increased levels of the $\alpha_v\beta_3$ integrin upregulate levels of the cyclin-dependent kinase Cdc2. A Cdc2–cyclin B2 active kinase complex forms, which may then phosphorylate caldesmon (Cal) bound to actin. This could result in the displacement of caldesmon from actin, destabilization of actin filaments and altered interaction with myosin. These events contribute to increased cell migration.

a recent issue of *Journal of Cell Biology*, a surprising new factor — the cell-cycle regulator Cdc2 — has joined the crowded cast of the cell motility road show.

Manes *et al.* report that increased levels of Cdc2 function through cyclin B2 and the actin-stabilizing protein caldesmon to promote cell migration. Cdc2/Cdk1 is, of course, a prototypical cyclin-dependent kinase that regulates the mitotic phase of the cell cycle⁹. I was just beginning to grapple with the unfamiliar concept of a cell-cycle regulator being involved in cell migration when another report appeared¹⁰, indicating that cyclin D1 contributes to motility. Is a pattern emerging?

The $\alpha_v\beta_3$ integrin has long been known to be important for angiogenesis and for increased invasiveness of certain carcinoma cells². In addition to their structural role, integrins can contribute to signalling pathways

that stimulate gene induction¹¹. Thus, Manes *et al.* used cDNA array analysis to probe for genes that were upregulated in prostate tumour cells in response to overexpression of the β_3 subunit. They found that one of the most prominent genes so regulated was Cdc2. Additional studies showed that the Cdc2 response was selective in that overexpression of another integrin β -subunit that pairs with α_v integrin failed to elicit Cdc2 upregulation. Increased expression of Cdc2 correlated with enhanced cell migration, whereas overexpression of a dominant-negative Cdc2 mutant or use of chemical inhibitors that block Cdc2 kinase activity inhibited migration.

During their established function in the cell cycle, cyclin-dependent kinases function together with particular cyclin partners, and Cdc2 has been reported to pair with cyclins A, B1 and B2. Manes *et al.* went on to test

whether Cdc2 also requires a cyclin partner for its effects on cell migration and found that cyclin B2 seems most important for migration. They observed that overexpression of cyclin B2, but not cyclins A or B1, enhanced migration of prostate tumour or HeLa cells, whereas mouse fibroblasts lacking cyclin B2 showed reduced migration when compared with their wild-type counterparts.

So how might Cdc2–cyclin B2 affect the actinomyosin machinery that mediates cell motility? Manes *et al.* focused on actin and the calmodulin-binding protein caldesmon as possible candidates. The usual role of caldesmon is to stabilize actin filaments and to inhibit actinomyosin-mediated contractility¹². Previous reports have indicated that Cdc2 can phosphorylate several serine and threonine residues on caldesmon¹³, and that phosphorylation of these sites inhibited the effects of caldesmon on actin and myosin¹², thereby interfering with entry into M phase and with cytokinesis. In addition, caldesmon has been found to localize in focal adhesions and to affect their organization¹⁴, thus increasing the plausibility of a role for caldesmon in cell migration. Pursuing this, Manes *et al.* showed that overexpression of a dominant-negative form of caldesmon could block the increase in migration that is caused by Cdc2 expression and, furthermore, that Cdc2 and caldesmon colocalize in membrane ruffles of actively migrating cells. So, Manes *et al.* have indicated a novel and interesting connection between an M-phase cyclin-dependent kinase, the cytoskeletal protein caldesmon, and the control of cell migration, particularly in invasive tumour cells (Fig. 1).

As with many novel studies, this work poses important questions, as well as providing interesting insights. First, there is the question of exactly how increased $\alpha_v\beta_3$ integrin expression results in induction of Cdc2. Integrins connect to many signalling pathways and identification of the specific factors that link $\alpha_v\beta_3$ integrin to Cdc2 will not be easy. An intriguing question is why cyclin B2 has been singled out to partner with Cdc2 for this new function. This molecule is somewhat atypical in that it is associated primarily with the Golgi apparatus and, in contrast with cyclin B1, does not relocalize to the nucleus at prophase¹⁵. Manes *et al.* implicate cyclin B2 specifically in the control of cell migration, but what is the underlying mechanism? There is little indication of substrate specificity for different cyclin B–Cdc2 complexes that would suggest that caldesmon is a specific target for the Cdc2–cyclin B2 kinase¹⁶; rather, the unique localization of cyclin B2 with membranes might be the key.

At first glance, the most obvious interpretation of the data is that Cdc2 regulates the role of caldesmon in the actinomyosin machinery at the leading edge of motile cells. However, cell motility also requires the trafficking of membrane constituents into areas of membrane protrusion, such as membrane ruffles¹⁷; furthermore, it is clear that vesicle traffic can involve actin¹⁸. Thus, an alternative interpretation, based on the membrane localization of cyclin B2, is that the Cdc2–cyclin B2 complex may regulate caldesmon that is involved in actin-based vesicle traffic from the Golgi to sites of membrane extension. A final question is whether the observations presented by

Manes *et al.* represent physiology or pathophysiology. The accepted role of Cdc2–cyclin B complexes is to regulate multiple events around and during M phase¹⁹. Among these events are actin-based processes involved in cell rounding at the onset of M phase and cytokinesis later in the cell cycle. Rather than Cdc2 normally having a function in cell migration, perhaps this kinase is ‘hijacked’ by certain tumour cells that overexpress $\alpha_v\beta_3$ integrin and is pressed into service to accelerate tumour invasion. In any case, the work of Manes *et al.* opens up a number of interesting avenues for further investigation. □

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