*Inhibition of Myosin Light Chain Kinase by p21-Activated Kinase

Figure 1A. Phosphorylation by PAK1 decreases MLCK activity.
Figure 1B. PAK1 phosphorylates MLCK ***in vitro***

⇨ Cellular effects of PAK could be mediated through phosphorylation and inactivation of MLCK and possibly a decrease in MLC phosphorylation.

Figure 2A. Cells expressing constitutively active PAK1 attached normally, but cell spreading was reduced (27% spreading of PAK1 T423E expressing cells vs 80% spreading of WT PAK1 expressing cells.)

⇨ Activation of PAK1 inhibits cell spreading

Figure 2B. BDM, an inhibitor of myosin II ATPase activity, inhibits cell spreading.

⇨ Myosin II participates in cell spreading

Figures 3. Cells expressing constitutively active PAK1 (T423E) show decreased MLCK activity as compared to cells expressing WT PAK1 or nontransfected cells.

⇨ PAK1 inhibits MLCK activity (***in vivo***)

Figure 4. MLC phosphorylation of Ser^{19} increases in control cells allowed to spread, but was reduced at all time points in cells expressing PAK1 T423E or the upstream PAK activator Rac.

⇨ PAK1 inhibits MLC phosphorylation on Ser^{19} .

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PAK1 inhibits cells spreading by inhibiting MLCK activity which, in turn, is no longer able to phosphorylate MLC on Ser^{19} rendering it inactive and unable to stimulate the ATPase activity of myosin II and to regulate its force-generating ability.

*Adhesion to the extracellular matrix regulates the coupling of the small GTPase Rac to its effector PAK

Figure 1A. Rac binding to PAK1 p21-binding domain is increased by cell adhesion,
Figure 1B. as well as serum (growth factors.)
Figure 1C. Effects of adhesion and serum are additive.

⇨ Adhesion and growth factors (serum) contribute independently and approximately equally to Rac GTP loading (a requirement for Rac/ PAK1 binding)

Figure 2A. PAK kinase activity is stimulated by serum in attached cells, but is nearly undetectable in suspended cells.
Figure 2B . Replating cells on antibody to the integrin β1 subunit (but not CD44) as opposed to fibronectin, restores PAK activation in response to serum.
⇨ Effect of cell attachment is mediated by integrins
Figure 2C. PAK activity is stimulated by Cdc42-GTPγS
⇨ PAK from suspended cells can be stimulated in vitro by an active GTPase

Figure 3A. Levels of V12 versus endogenous Rac are comparable in the presence of tet; without tet V12 Rac is expressed at a five-fold higher level than endogenous.
Figure 3B,C. Increasing levels of ac results in PAK activity becoming partially adhesion-dependent.
⇨ Adhesion and GTP loading regulate PAK at distinct and different steps.

Figure 4A, B. Rac only translocates to the membrane fraction in adherent cells.
Figure 4C. Activated (V12) Rac still translocates to the membrane in adherent cells.
⇨ Cell adhesion targets Rac to the membrane, and this step is independent of GTP loading.

Figure 5A, B. Membrane-targeted Rac, but not the C189S mutant, increased PAK activity.
⇨ Membrane association of Rac is required for PAK activation.

Figure 6A. Rac mutants with stronger membrane-targeting sequences.
Figure 6B. These mutants have enhanced membrane localization.
Figure 6C. Suspended cells expressing mutant Rac showed a five-fold incraese in PAK activation.
⇨ Membrane localization of Rac is sufficient to restore PAK activation in non-adherent cells.

Figure 7. Cytoplasmic Rac from both adherent and suspended cells showed increased binding to membranes of attached cells.
⇨ Adhesion regulates Rac translocation via and effect on its membrane binding sites.

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* Rac GTP loading is regulated by adhesion to ECM.
* Adhesion regulates the ability of active Rac to stimulate PAK kinase activity
* A difference in compartmentalization is responsible for these effects.
* Rac, and not PAK, is the major target of regulation by adhesion.
* Adhesion regulates the membrane binding sites of Rac
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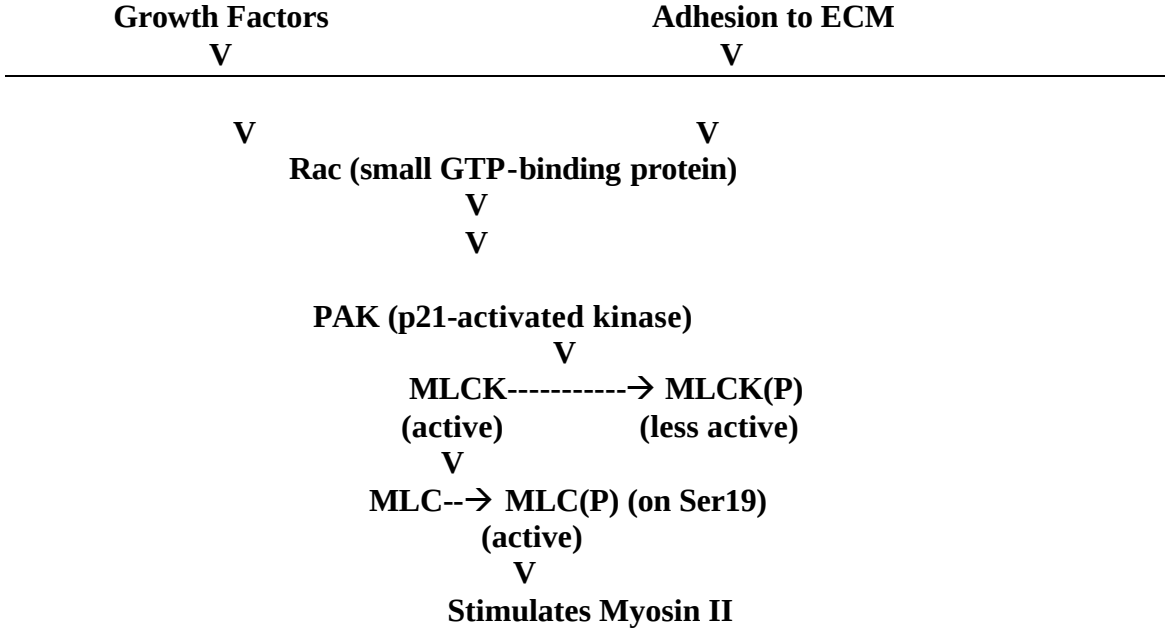
Growth Factors
Adhesion to ECM
Rac (small GTP-binding protein)
PAK (p21-activated kinase)
MLCK----------→ MLCK(P)
(active)
(less active)
MLC--→ MLC(P) (on Ser19)
(active)
Stimulates Myosin II