Phosphorylation of full-length Paxillin

Tuesday, June 28, 2005

4 Reactions set up in 0.65 mL eppendorfs:

- 0.6 mg GST-Pax (1-SSS7)-FLAG (2 μL of elution #2 on p. 126 at 300 μg/μL. Cells for prot. pur. had been lysed (6,23.)
  ~intended to use 2 mg of substrate ~
- 4 μL of a 1 mM ATP solution (50 μM final conc.)
- 4.5 μL of a 226 mM MgCl₂ solution (15 mM final conc.)
- 0.8 μL of a 1 mM ZnCl₂ soин. (10 μM final conc.)
- 2 μL Src, or 2 μL JNK, or 4 μL ERKII, or no kinase
- 66.5 (or 64.5) μL of TBS

= 80 μL total

The above reagents were pipetted up and down 7× and flicked once to mix, then incubated at 30°C for 15 min. The reactions were stopped w/ the addition of 80 μL 2× SDS reducing gel loading buffer (to give 3.75 ng/μL) A 1/2 dilution of this was made with more loading buffer (1.9 ng/μL).

4 gels were loaded w/ 4 μL (7.5 μg) / lane with 10 μL (19 ng) loaded for the ERK and JNK lanes. (intended to load 20 ng for Src no enzyme and 50 ng for the others ~ less needed w/ the minimally degraded substrate.

5H 3 2 1
1* ab 1/10,000 mouse anti-Paxillin
2* 1/10,000 goat anti-mouse HRP
rxn with:

1 7.5 ng no kinase
3 7.5 ng Src
4 19 ng ERKII
5 19 ng JNK

5H 3 2 1
1* ab 1/120,000 rabbit anti-pY31 Pax
2* 1/120,000 goat anti-rabbit HRP
Full-length Pax: GST-Pax(1-557)-FLAG

1° 1:1,000 mouse anti-paxillin
2° 1:1,000 goat anti-mouse HRP

1° 1:2,000 rabbit anti-pS126
2° 1:20,000 goat anti-rabbit HRP

Summary of Full-length Pax phosphorylation (still confusing results w/ pS178...)

anti-paxillin (pan)
(20 ng/well)

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<thead>
<tr>
<th></th>
<th>anti-pY31 Pax (20 ng/well)</th>
<th>anti-pY118 Pax (20 ng/well)</th>
<th>anti-pS126 Pax (200 ng/well)</th>
<th>anti-pS178 Pax (50 ng/well)</th>
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5 4 3 2 1
20 ng/well loaded

In 1 ladder
2 src rxn no kinase
3 Src rxn.
4 ERK rxn.
5 JNK rxn.

1° 1:2,000 rabbit anti-pY31
2° 1:20,000 goat anti-mouse HRP