Phosphorylation activities of partial phospho-forms of Erk

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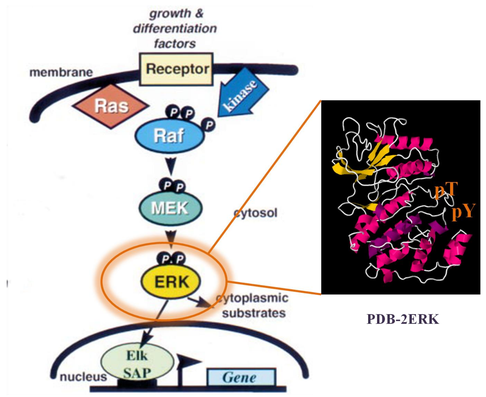
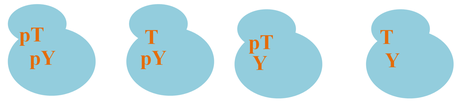
Background

Erk is a signalling protein that signals many different responses in the cell.



Adapted fig 1. Kolch Biochem. J. (2000) *351, 289±305*

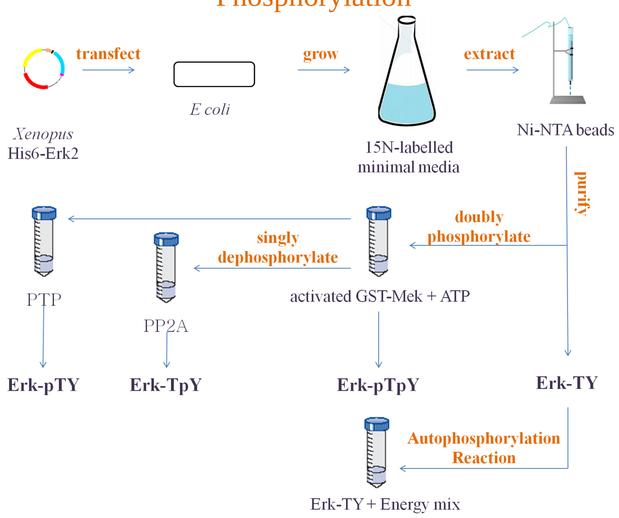
We believe that multisite post translational modifications, specifically phosphorylation, of Erk may play a key role in it's activity.
Erk has 2 phosphorylation sites – on Threonine (T) and on Tyrosine (Y), this gives rise to 4 possible phospho-forms.



Previous experiments in the lab have led us to believe that partially phosphorylated *pTY* form has catalytic efficiency and this led us to question the abilities of other phosho-forms. We have speculated that *TpY* form has autophosphorylation capabilities. The aim of my work was to test the autocatalytic activity of *TpY* form, as well as other catalytic activities of these 4 phospho-forms of Erk.

Approach

Generation of Samples in the different states of Phosphorylation



Autophosphorylation Reaction

Results

Erk-TY Sample

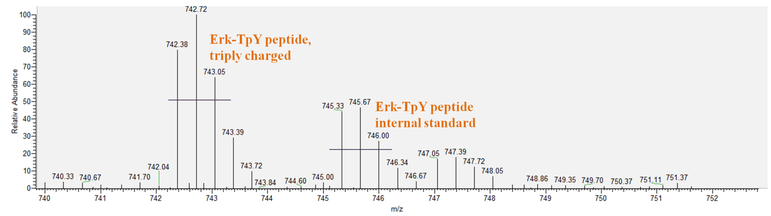
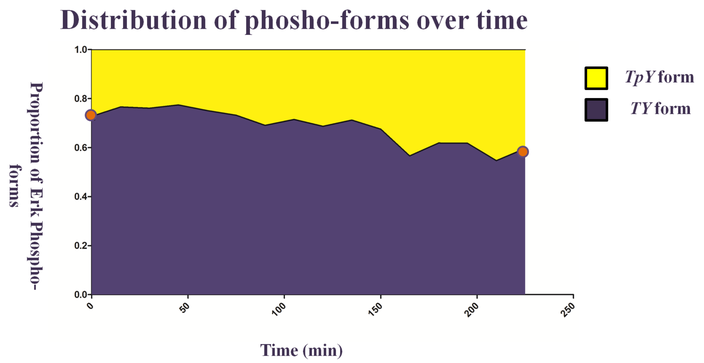


Figure 1. Relative intensities of Erk-TpY peptide, along with its internal standard.



The initial distribution of *TpY* form was 24% and *TY* form was 76% while the final distribution of *TpY* form was 36% and *TY* was 64%

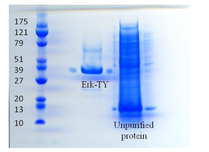


Figure 2
One band at the appropriate molecular weight on protein gel indicates purified wild type Erk is present.

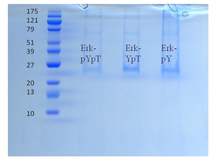


Figure 3.
After the different phosphorylation reactions 3 identical bands correspond to the different phospho-forms of Erk.

Conclusion

Using the bacterial purified Erk that contained 24% of *TpY* form I was able to show that *TpY* form has autophosphorylation activity. Also, from the purified protein I was able to obtain samples of Erk in 3 states of phosphorylation; **Erk-pTpY, Erk-TpY** and **Erk-pTY**. Given more time the catalytic activities of the different samples would be investigated.

References

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2. Prabakaran S, Everley Robert, Landrieu Isabelle, Wieruszeski Jean-Michel, Lippens Guy, Steen Hanno, Gunawardena J (2011) Comparative analysis of Erk phosphorylation suggests a mixed strategy for measuring phospho-form distributions. *Molecular Systems Biology* 7:482
3. Prabakaran S, Lippens Guy, Steen Hanno, Gunawardena J (2012) Post – translational modification: nature's escape from genetic imprisonment and the basis for dynamic information encoding. *WIREs Syst Biol Med 2012*

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