The pharmacology of cell differentiation
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# Role of MAP kinases in the control of cell proliferation

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## INTRODUCTION

Mitogen Activated Protein Kinases (MAP kinases) also described as extra cellular signal regulatory kinases (ERKs), belong to a group of protein serine/threonine kinases that are activated in response to extracellular stimuli in virtually all cell types (see references 1-4 for reviews). Two highly related mammalian MAP kinases, $p44^{mapk}$ (ERK1), and $p42^{mapk}$ (ERK2) have been cloned and found ubiquitously expressed in cultured cells (5-8) and highly homologous to the yeast kinases SLT2 (9), KSS1 (10) and FUS3 (11). Dual phosphorylation of these MAP kinases on both tyrosine and threonine residues has been shown to be required for full activity of the enzyme (12). The sites of phosphorylation, identified in $p42^{mapk}$ (13) and conserved in all members of the family, were found to reside on a single phosphopeptide separated by only one residue T*EY*. Recently a MAP kinase activator was purified, cloned (14, Pages, G., Brunet, A. and Pouysségur, J., in preparation), and shown to phosphorylate *in vitro* a kinase-deficient mutant of $p44^{mapk}$ and re-activate a dephosphorylated wild type MAP kinase in the presence of ATP. This MAP kinase activator referred now to as MAP kinase kinase could be phosphorylated and activated *in vitro* by v-Raf or constitutively active forms of c-Raf (15, 16). Thus, c-Raf appears to be, as we previously proposed (17), one transducer integrating mitogenic and differentiating signals leading to activation of the MAP kinase cascade.

Here we bring direct evidence that this kinase cascade plays an essential role in transmitting the proliferative response. We show (i) that synergistic mitogens synergistically activate MAP kinases, (ii) that the persistent phase of MAP kinase activation correlates with G0 to S-phase progression, (iii) that MAP kinases rapidly translocate to the nuclei in response to growth factors and (iv) that suppression of MAP kinase activation inhibits cell proliferation.

## SYNERGISTIC ACTIVATION OF MAP KINASES

Reinitiation of DNA synthesis in resting cells is the result of cooperation between multiple signalling pathways. For example in CCL39 cells, the strong

mitogen α-thrombin stimulates phospholipases (PIP2-PLC (18), PC-PLD, PLA2 (19)) and inhibits adenylyl cyclase (20), implicating activation of pertussis toxin-sensitive and -insensitive G proteins (17). On the other hand, serotonin which is not a mitogen on its own although it activates G protein-coupled effectors systems, synergizes with FGF to induce mitogenesis (21). We therefore analyzed the magnitude and time course of MAP kinase activation in response to individual growth factor or to the synergistic combination serotonin/FGF.

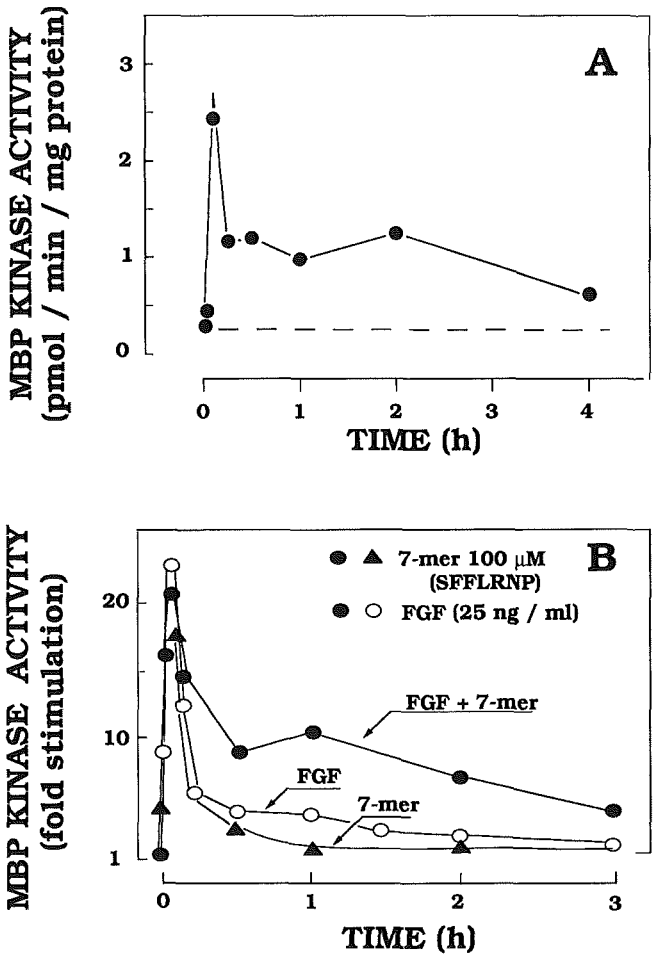


Figure 1. *Time course of p44 MAP kinase activation by growth factors in quiescent hamster fibroblasts . A) Stimulation with α-thrombin (10nM). B) Stimulation with either FGF, 7-mer thrombin receptor peptide or FGF + 7-mer peptide. Assay conditions were as previously described (8, 22).*

Figure 1A shows that α-thrombin induces a biphasic activation of $p44^{mapk}$ in CCL39 cells: a rapid and sharp phase appearing at 5-10 min was followed by a late and sustained phase lasting at least 4 hours (22).
A set of experiments strongly suggest that the second and persistent phase of activation is required for G0-arrested cells to enter the S-phase. The non-mitogenic agents for CCL39 cells: TPA, serotonin, thrombin-receptor 7-mer peptide (SFFLRNP) activate only the first peak (Figure 1B, 23). Early interruption of the α-thrombin signal with hirudin completely abolished the late phase of $p44^{mapk}$ activation as well as DNA synthesis reinitiation (22). Furthermore, pretreatment of the cells with pertussis toxin, which inhibits more than 95% α-thrombin-induced mitogenicity (24), resulted in the complete loss of late $p44^{mapk}$ activation phase, while the early peak was partially attenuated. Finally, serotonin and the thrombin-receptor peptide that cannot induce mitogenicity nor the sustained $p44^{mapk}$ activation phase on their own, do so when they are associated with a suboptimal concentration of FGF. Figure 1B illustrates the synergistic effects obtained with the thrombin receptor peptide on MAP kinase activation. Identical results were obtained with serotonin.

Taken together, these results point to a very close correlation existing between the ability of a growth factor to induce late and sustained $p44^{mapk}$ activation and its mitogenic potential. Similar results have also been obtained for the activation of $p42^{mapk}$ (Wang, Y., Pouysségur, J. and Dunn, M., unpublished results).

## MAP KINASES TRANSLOCATE TO THE NUCLEI

At the present time the respective roles of the two isoforms of MAP kinases ($p44^{mapk}$ and $p42^{mapk}$) have not been identified. Both isoforms are phosphorylated on conserved threonine and tyrosine residues *via* the action of the MAP kinase kinase and their kinetics of activation are identical reflecting the action of a common activator. In addition, both isoforms seem to possess the same substrate specificity, at least *in vitro*. In an attempt to determine whether each isoform exerts a specific function, we examined their subcellular distribution by indirect immuno-fluorescence microscopy in G0-arrested and serum-stimulated CCL39 cells. Antisera directed against individual MAP kinases were raised with 16 and 14 amino-acid synthetic peptides corresponding respectively to the predicted carboxyl terminus of rat $p44^{mapk}$ and $p42^{mapk}$. The 837 antisera (6) was made $p44^{mapk}$ specific by absorption with the p42 carboxy peptide. With this antisera we detected the endogenous $p44^{mapk}$ mainly in the cytoplasm of resting CCL39 cells (Figure 2A). Interestingly a clear nuclear translocation appeared within 15 min following serum stimulation (Figure 2B). The signal became predominantly nuclear after one hour (Figure 2C) and reached its maximum three hours following stimulation (Figure 2D), then six hours later, the $p44^{mapk}$

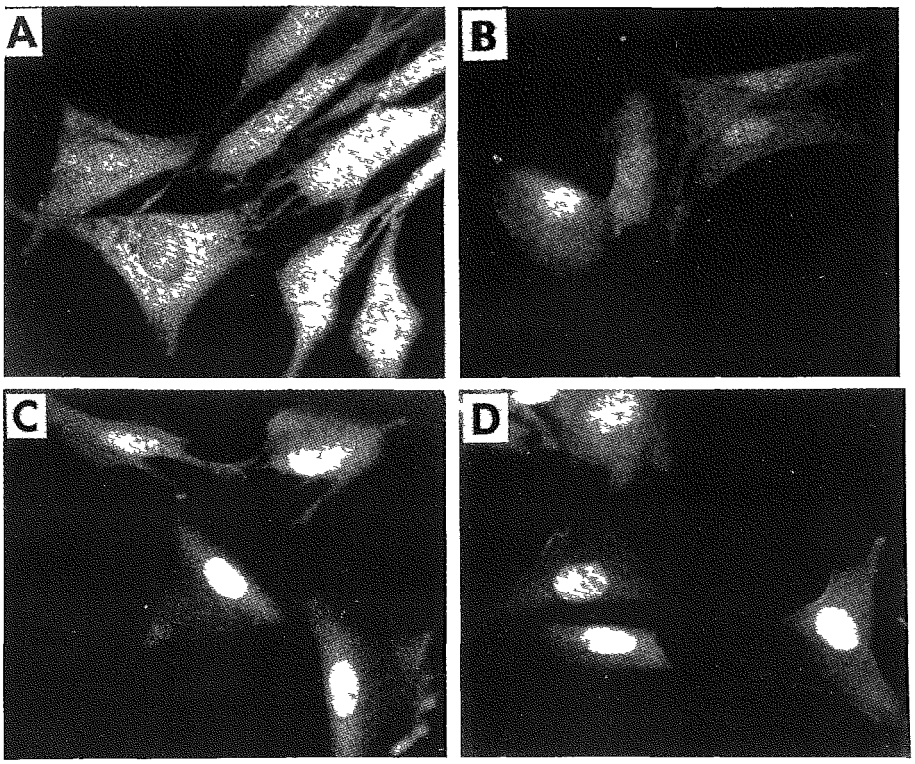


Figure 2. *Immunofluorescence labeling of p44$^{mapk}$ in CCL39 fibroblasts with 837 antiserum showing rapid nuclear translocation following serum stimulation. G0-arrested CCL39 fibroblasts (A), stimulated with 10% fetal calf serum for 15 min (B), 60 min (C) or 180 min (D).*

specific signal returned to the cytoplasm. The nuclear accumulation of MAP kinase was not permanent and required the continuous presence of serum to be maintained. Removal of serum induced a rapid return of MAP kinases to the cytoplasm (almost complete within 30 min). This rapid serum-dependent MAP kinase translocation to the nuclei also occurred in response to potent mitogens such as α-thrombin or FGF but not in response to non-mitogenic agents such as α-thrombin receptor peptides or phorbol esters. α-Thrombin and FGF are less potent than 10% serum in their ability to trigger MAP kinase translocation when they are added individually, yet almost equivalent to serum when added together. Interestingly, the intensity and percentage of nuclei that were labelled correlated well with the relative potency of mitogens to reinitiate DNA synthesis. It is

worthy to note that the nuclear translocation of $p44^{mapk}$ coincides with the second phase of MAP kinase activation. Thus the MAP kinase molecules that translocate to the nucleus are potentially active. It has been shown *in vitro* that MAP kinases were able to phosphorylate nuclear proteins such as c-Jun, c-Myc (25-27) and $p62^{TCF}$ (28). In the latter case, the phosphorylation of $p62^{TCF}$ by MAP kinase has been correlated with increased binding of a multiprotein complex on the transcriptional response element SRE (Serum Responsive Element) of the *c-fos* gene.

As far as the $p42^{mapk}$ localization is concerned, unfortunately, all the antisera that we raised recognized both isoforms *in vivo* as judged by immunofluorescence of ectopic expression of each MAP kinase isoforms (see next section). We circumvented this problem by introducing at the extreme carboxylic end of each cloned MAP kinase isoforms a specific epitope recognized by a monoclonal antibody. We added 17 residues corresponding to the Vesiculo Stomatitis Virus Glycoprotein (VSVG) (29) and a steric spacer between the body of the kinase and the epitope. We showed that both $p44^{mapk}$ and $p42^{mapk}$ VSVG-tagged isoforms were able to translocate to the nucleus upon growth factor stimulation. This signal was specific as the VSVG monoclonal antibody did not elicit any significant immuno-fluorescence in non-transfected cells. This result confirms similar work performed with HeLa cells showing nuclear localization of both isoforms of MAP kinases (30).

In order to shed light on the mechanism of MAP kinase translocation, we transfected several point mutants of the cloned $p44^{mapk}$. We transfected both kinase-deficient (T192A Y194F) and mutants for which the phosphorylation sites were mutated to glutamic acid in order to mimic the negative charges brought by the phosphorylations: T192E, Y194E and TY-EE. All of these kinase-deficient mutants showed nuclear localization, and for those studied in detail (T192A, Y194F and TY-EE) the nuclear translocation in response to serum was not affected. We thus conclude that MAP kinase translocation is independent of its intrinsic activity (Lenormand, P., Sardet, C., Pagès, G. and Pouysségur J. in preparation).

## CLONING AND ECTOPIC EXPRESSION OF MAP KINASES

We cloned a near full length cDNA of the $p44^{mapk}$ from Chinese hamster lung fibroblasts (CCL39) model system (8). The hamster $p44^{mapk}$ revealed 98.6% homology with the rat protein. To analyze the expression and the biological function of the cloned $p44^{mapk}$, an artificial initiation codon and 9 aminoacid corresponding to an epitope of the HA1 influenza hemagglutinin were added at the amino terminal end of the MAP kinase (8). The chimeric kinase under transcriptional control of the cytomegalovirus promoter, was stably expressed in CCL39 cells in a functional form. We showed that its basal activity, measured by phosphorylation of the substrate myelin basic protein, is activated several-fold (up to 25) by the mitogens α-thrombin, platelet-derived growth factor, fibroblast

growth factor and fetal calf serum. In response to α-thrombin, this ectopically expressed $p44^{mapk}$ shows two peaks of activation that parallels increase phosphorylation of tyrosine and threonine residues. From all criteria (hormonal activation, phosphorylation, time-course of activation, nuclear localization) this expressed form of $p44^{mapk}$ has apparently retained all the regulatory features of the endogenous form. We therefore decided to use this cloned isoform to see whether expression of the wild type or mutated forms of the kinase could alter growth control.

First, we isolated different clones of CCL39 cells stably expressing the recombinant $p44^{mapk}$ to levels representing up to 5 times the endogenous one. All these clones remained growth factor-dependent with no sign of increased sensitivity to mitogens. This result suggested that MAP kinase basal level is thighly regulated. Indeed, in spite of an increased level of $p44^{mapk}$, MAP kinase basal level was not elevated by overexpression of the enzyme. We then expressed $p44^{mapk}$ mutants in which the two key phosphorylation sites,T192 and Y194, were replaced by glutamic acid, hoping to partially mimick the negative charges of the phosphate groups. The simple or double mutant T192E/Y194E did not show any 'constitutive'activity when expressed in CCL39 cells. Therefore, to interfere with the endogenous MAP kinases, our second approach was to overexpress $p44^{mapk}$ antisense RNA or kinase-deficient mutants of $p44^{mapk}$.

## INHIBITION OF MAP KINASES STOPS CELL PROLIFERATION

In order to assess the biological roles of MAP kinases, we decided to specifically suppress MAP kinase activation in fibroblasts by transiently expressing either the entire $p44^{mapk}$ antisense RNA, or $p44^{mapk}$ kinase-deficient mutants (T192A or Y194F) (31). We set up a transient transfection assay to markedly overexpress either the active and inactive forms of $p44^{mapk}$ and to investigate the incidence of the transgene on the activation of endogenous MAP kinases. Thus, we co-transfected PS120, a $Na^+/H^+$ antiporter-deficient derivative of CCL39 (32) cells, with either of the $p44^{mapk}$ constructs along with a plasmid bearing the antiporter gene (33). Two days after transfection, application of an acid-load selection specifically enriched the population with cells which had been efficiently transfected. Under these conditions, the transfected mutant or wild type $p44^{mapk}$ were highly overexpressed ( Figure 3) and the activation of endogenous $p42^{mapk}$ activity by serum was analyzed. We found that expression of the mutant forms specifically inhibited by up to 80% growth factor-stimulated endogenous MAP kinases (31). In an alternative approach to reduce endogenous MAP kinase activation, but using the same transient transfection assay, we expressed $p44^{mapk}$ antisense RNA. Several antisense constructs specifically reduced, in a dose-dependent manner, the expression of both endogenous isoforms of MAP kinase. As a consequence the antisense constructs suppressed growth factor activation of

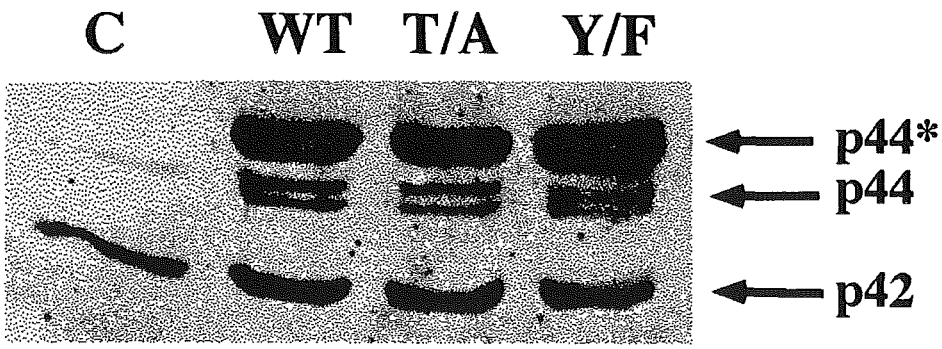


Figure 3. *Transient expression in PS120 cells of the wild type (WT) and mutated forms of p44$^{mapk}$ (T/A; Y/F). Cell extract proteins were separated on SDS-PAGE and immunoblotted with an antiserum that recognizes both MAP kinase isoforms. Note the overexpression of the epitope-tagged p44$^{mapk}$ isoform (p44*). (C), cells transfected with an empty vector.*

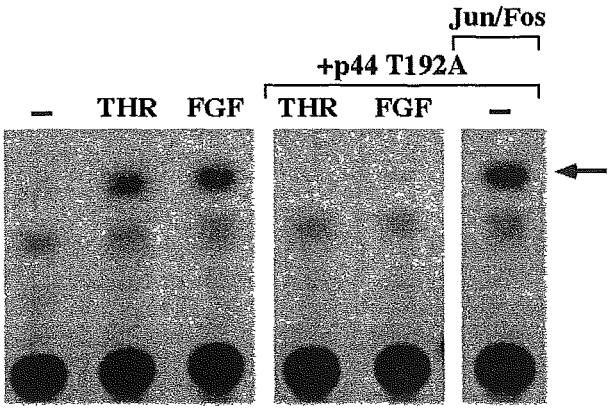


Figure 4. *Dominant-negative inhibition of growth factor-stimulated AP1 transcriptional activity (collagenase promoter CAT assay) by expression of T192A p44$^{mapk}$. Quescient CCL39 cells were stimulated with either thrombin (THR) or FGF. Note that cotransfection of jun/fos antagonizes the negative effect of T192A.*

MAP kinases, whereas the sense construct did not. Interestingly, the inhibition of MAP kinase activation upon growth factor stimulation had striking effects on cell proliferation: it reduced the number of colonies obtained one week after transfection of either the antisense or the kinase-deficient forms of p44$^{mapk}$. The

growth inhibitory effects are specific since for example, p44$^{mapk}$ sense RNA expression reverted the effect of the p44$^{mapk}$ antisense RNA expression. Similar effects were observed on the transcription of a reporter gene under the control of the collagenase minimal promoter (34). Growth factor-induced transcriptional activity of the reporter gene was inhibited by expression of the p44$^{mapk}$ kinase-deficient mutants (Figure 4).

## CONCLUSIONS

The results presented herein indicate that the HA1-tagged p44$^{mapk}$ transfected into CCL39 cells behaves similarly to the endogenous MAP kinase. For example stimulation of quiescent cells by mitogenic growth factors induced a biphasic stimulation of MAP kinase activity, the first peak occurring 5 min after stimulation and the second peak between 1 and 3 hours. Non mitogenic signals induced the first peak of activation but not the second one. This correlation indicated for the first time a potential involvement of MAP kinase in the transmission of mitogenic signalling.

We then established that both p42$^{mapk}$ and p44$^{mapk}$ translocate to the nucleus upon growth factor activation. This nuclear localization was rapid, being detectable 15 min after agonist addition and maximal after 3 hours. This nuclear translocation was transient since MAP kinase returned to the cytoplasm 6 hours after stimulation, and the nuclear accumulation was not permanent since removal of serum induced a rapid efflux of MAP kinase to the cytoplasm. Furthermore, the nuclear translocation occurred only in response to strong mitogenic agonists.

Finally, we were able to specifically suppress MAP kinase activation in fibroblasts by transiently overexpressing either the antisense MAP kinase RNA or kinase-deficient mutants of MAP kinase. We found that either of these two means of markedly reducing growth factor-stimulated MAP kinase activation, inhibited cell growth and the expression of a reporter gene under the control of the collagenase minimal promoter. ***Therefore, we conclude that growth factor activation of p44$^{mapk}$ and p42$^{mapk}$ is an absolute requirement for triggering the proliferative response.*** We predict that these MAP kinases are also essential for establishing differentiating programs. For example, we expect to suppress NGF-induced differentiation of PC12 cells if we specifically block MAP kinase activation. If this happen to be the case, then this kinase cascade, conserved in yeast (35), should be considered as a *master signalling route* activating multiple cellular targets, in particular, transciption factors essential for growth and differentiation.

## ACKNOWLEDGEMENTS

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## REFERENCES

1. Sturgill, T. W. and Wu, J. (1991): Biochim.Biophys.Acta. 1092, 350-357.
2. Cobb, M.H., Boulton, T.G. and Robbins, D.J. (1991): Cell. Regul. 2, 965-978.
3. Thomas, G. (1992): Cell. 68, 3-6.
4. Pelech, S.L. and Sanghera, J.S. (1992): TIBS 17, 233-238.
5. Boulton, T., Yancopoulos, G., Gregory, J., Slaughter, C., Moomaw, C., Hsu, J., Cobb, M. (1991): Science 249, 64-67.
6. Boulton, T. G. and Cobb, M. H. (1991): Cell Regul. 2, 357-371.
7. Her, J.-H., Wu, J., Rall, T. B., Sturgill, T. and Weber, M. J. (1991): Nucl. Ac. Res. 19, 3743.
8. Meloche, S., Pagès, G. and Pouysségur, J. (1992): Mol. Biol. Cell. 3, 63-71.
9. Torres, L., Martin, H., Garcia-Saez, I., Arroyo, J., Molina, M., Sanchez, M. and Nombela, C. (1991): Mol. Microbiol. 5, 2845-2854.
10. Courchesnes, W.E., Kunisawa, R. and Thorner, J. (1989): Cell 58, 1107-1119.
11. Elion, E.A., Grisafi, P.L.and Fink, G.R. (1990): Cell, 64, 649-664.
12. Anderson, N. G., Maller, J. L., Tonks, N. K. and Sturgill, T. W. (1990): Nature. 343, 651-653.
13. Payne, D. M., Rossomando, A. J., Martino, P., Erickson, A. K., Her, J. H., Shabanowtiz, J., Hunt, D. F., Weber, M. J. and Sturgill, T. W. (1991): EMBO J. 10, 885-892.
14. Crews, C. M., Alessandrini, A. and Erikson, R. L. (1992): Science. 258, 478-480.
15. Dent, P., Haser, W., Haystead, T., Vincent, L. A., Roberts, T. M. and Sturgill, T. W. (1992): Science. 257, 1404-1407.
16. Kyriakis, J.M., App, H., Zhang, X-F, Banerjee, P., Brautigan, D.L., Rapp, U.R. and Avruch, J. (1992): Nature 358, 417-421.
17. Pouysségur, J., Kahan, C., Vouret, V., L'Allemain, G., Van Obberghen-Schilling, E. and Seuwen, K. (1991): In "Origins of Human Cancer", Cold Spring Harbor Laboratory Press. 285-295,
18. L'Allemain, G., Paris, S., Magnaldo, I. and Pouysségur, J. (1986): J. Cell. Physiol. 129, 167-174.
19. McKenzie, F. R., Seuwen, K. and Pouysségur, J. (1992): J. Biol. Chem. 267, (in press),
20. Magnaldo, I., Pouysségur, J. and Paris, S. (1988): Biochem.J. 253, 711.719.

21. Seuwen, K., Magnaldo, I. and Pouysségur, J. (1988): Nature. 335, 254-256.
22. Meloche, S., Seuwen, K., Pagès, G. and Pouysségur, J. (1992): Mol. Endocrinol. 638, 845-854.
23 Vouret-Craviari, V., Van Obberghen-Schilling, E., Scimeca, J-C., Van Obberghen, E. and Pouysségur, J. (1993): Biochem. J. (in press)
24. Chambard, J. C., Paris, S., L'Allemain, G. and Pouysségur, J. (1987): Nature. 326, 800-803.
25. Alvarez, E., Northwood, I. C., Gonzalez, F. A., Latour, D. A., Seth, A., Abate, C., Curran, T. and Davis, R. J. (1991): J. Biol. Chem. 266, 15277-15285.
26. Baker, S.J., Kerppola, T.K., Luk, D., Vandenberg, M.T., Marshak, D.R., Curran T., Abate, C. (1992) Mol. Cell. Biol. 12, 4694-4705
27 Pulverer, B., Kyriakis, J., Avruch, J., Nikolakaki, E., and Woodgett, J. (1991): Nature 353, 670-673.
28. Gille, H., Sharrocks, A.D. and Shaw, P.E. (1992): Nature 358, 414-421.
29. Kreis, T. (1986): EMBO. J. 5,931-941.
30. Chen, R. H., Sarnecki, C. and Blenis, J. (1992): Mol. Cell. Biol. 12, 915-927.
31. Pagès, G., Lenormand, P., L'Allemain, G., Chambard, J. C., Meloche, S. and Pouysségur, J. (1993) (submitted).
32. Pouysségur, J., Sardet, C., Franchi, A., L'Allemain, G. and Paris, S. (1984): Proc.Natl.Acad.Sci.USA. 81, 4833-4837.
33. Sardet, C., Franchi, A. and Pouysségur, J. (1989): Cell. 56, 271-280.
34. Angel, P., Baumann, I., Stein, B., Delius, H. and Rahmsdorf, H. J. (1987): Mol. Cell. Biol. 7, 2256-2266.
35 Sprague, G. (1992): Current Biol. 2, 587-589.

## Discussion - ROLE OF MAP KINASES IN THE CONTROL OF CELL PROLIFERATION

M. Oren

Do you have any idea of what is driving the MAP kinases to the nucleus? Is it just the threonine phosphorylation or do you need something else? When you have this early event in the first few minutes, do you see any translocation in the immediate response or is it only in the delayed response that anything translocates into the nucleus?

J. Pouysségur

The translocation appears to be very rapid because we can detect it as soon as fifteen minutes after the addition. Now when you use a partial agonist like the thrombin receptor peptide that is not mitogenic, we do not see the translocation, or perhaps in very few percent of cells you see the translocation. We made many mutations in the tyrosine and threonine phosphorylation sites to see whether that kinase can also translocate. The answer is yes. So whatever the kinase is active or not the translocation to the nucleus is not affected. So we postulate that there is a machinery for the translocation and that this machinery is what responds to the phosphorylation, because we need a persistent occupation of the receptor and the kinase activation in order to maintain the nuclearization. This will be another way to eventually antagonise perhaps only p42 or p44, if we can make peptides that will recognise the specific sequence that is needed for the translocation. Then we could antagonise the nuclearization and see whether we can also stop proliferation. This, perhaps, is one way in which we could ask what will be the role of p42 or p44, and if the mechanisms are distinct.

G.E. Francis

One could even put the site of the synergy somewhat earlier than MAP kinase itself. Have you looked at the kinase kinase?

J. Pouysségur

No, the antibodies are not yet available, they will be within a month. We expect that the synergy will be also at the level of the MAP kinase kinase and also perhaps at the level of Raf. Raf has been a big problem for many investigators, because there were no adequate substrates for the Raf kinase. Now the real substrate, if this model is correct, will be the dephosphorylated MAP kinase kinase.

G.E. Francis

Is there also the possibility of it acting at the MAP kinase level by on-rates and off-rates both being tampered with at the same time for the phosphate groups?

J. Pouysségur

It was a very appealing hypothesis at the beginning, when we had a dual phosphorylation, because many people thought and we thought also that this could be the nice way where you get, by different routes, both signals and this would be the basis of synergy. This turned out to be wrong because the MAP kinase kinase, by itself has a dual specificity to phosphorylate threonine and tyrosine, so it looks like for the moment there is no additional phosphorylation site on the MAP kinase in which you could explain a synergy by a different route beside the channelling via the kinase kinase.

G.E. Francis

Has anyone looked to see if you can modulate the phosphorylation at the two sites via MAP kinase kinase independently?

J. Pouysségur

No, because the stoichiometry is 1-1. It seems that both the threonine and tyrosine phosphorylation appear to go in parallel.

M. Crescenzi

How tight is the need for MAP kinase in order to get cell proliferation. In other

words, is it necessary only to transduce all known signals to the nucleus or is it really necessary for the proliferation apparatus? Can you get an oncogene - transformed cell which proliferates in the absence of MAP kinase?

J. Pouysségur

We have not done the experiments, but now with the antisense nucleic acids we have the possibility of a very potent and specific blockade of p42 and p44. What we expect if we antagonise the MAP kinase in Raf transformed cells is that we also will block proliferation. Whether we need the nuclearisation, we still do not know, we have to find tools to antagonise the translocation to the nuclei to see whether we also attenuate the proliferative response or the differentiating response.

J. Massagué

For how long should MAP kinase be active in order to mediate the mitogenic effects of thrombin?

J. Pouysségur

We have shown that in order to get mitogenicity with thrombin or PDGF or many mitogens you need persistent occupation of the receptors for at least six to eight hours. If you remove the mitogen at any time the cells back up to $G_0$, and we know that all this persistent activation, including MAP kinase, is required in order to make the progression and to reach "start" or the Restriction point where the cells decide to traverse S-phase.

J. Massagué

Do you need continued occupancy of the receptors to obtain the extended kinase activation phase? How is this compatible with what we were learning this morning from Moolenaar in terms of receptor desensitization by prolonged exposure to ligand?

J. Pouysségur

Sure, you get the desensitisation of the LP or the thrombin receptor. Even when the thrombin receptor is cleaved it is constitutively on, because the hormone is attached covalently to the receptor. There is very efficient desensitisation of the thrombin receptor, but always there is still ten to twenty percent of activity of the thrombin receptor. So desensitisation is never hundred percent and this little amount of signalling after the desensitisation is absolutely required. If you remove the mitogen then the cells stop to activate early and some late events.

J. Massagué

What is then the purpose of the desensitisation if it does not desensitise the system, that is, it desensitises the receptor but it does not desensitise the response? What is the role for the process if it does not accomplish the elimination of the response?

J. Pouysségur

Well, the way we measure the desensitisations like many people, is by the use of massive amounts of the hormone. We saturate the system in order to see a beautiful desensitisation. In vivo you never work with such a high hormone concentration, and in this case the desensitisation response is more modest and even sometimes one does not see it.