[1–4]. In every case repeated amino acid stretches present in the proteins have been indicated to be the binding sites for tubulin. Similar (almost identical) sequences have been found for the tubulin binding site on MAP2 and tau, while a different tubulin binding motif has been observed in MAP1B. A main structural difference between both types of binding sequences is that the MAP2/tau sequence contains a high proportion of glycine and proline residues, (α -helix breakers), while the possible domains for tubulin interaction on MAP1B have the potential for forming an α -helix (Fig. 1), as determined by the prediction methods of Chou–Fasman [5] and Garnier–Osguthorpe–Robson [6], and, also, as previously suggested by Noble *et al.* [4].

One important observation from these analyses is that the putative tubulin-binding α -helices on MAP1B may have an amphipathic character. The helices show a polarity based on the difference between their two surfaces, one containing a high proportion of acidic residues and the other containing a high proportion of basic residues. The cationic surface of these α -helices could bind to the C-terminal region of tubulin, a domain highly enriched in acidic amino acids which also has the potential to become an α -helix (Fig. 1).

The C-terminal acidic sequence of tubulin had actually been described as the one involved in its binding to MAP1 peptides [7]. Thus it can be proposed that the binding of MAP1B to tubulin could take place by the ionic interaction of two α -helices. However, the binding of MAP2 to tubulin has not the same structural constraints as the interaction between two α -helices. It remains to be established whether these differences might have consequences for the polarity of microtubules, if brain MAPs are involved in microtubule nucleation *in vitro*, for example in the axonal growth cone or in dendrites. The fact that microtubules have uniform polarity in axons [8], in which MAP1 is a major constituent and MAP2 is nearly absent, whereas microtubules with opposite polarities are found within dendrites [8], in which MAP2 is a major constituent, might support this view.

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- Lewis, F. A., Wang, D. & Cowan, N. J. (1988) *Science* **242**, 936–939
- Lee, G., Cowan, N. & Kirschner, M. (1988) *Science* **239**, 285–288
- Goedert, M., Wischik, C., Crowther, R., Walker, J. & Klug, A. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4051–4055
- Noble, M., Lewis, S. A. & Cowan, N. J. (1989) *J. Cell Biol.* **109**, 3367–3376
- Chou, P. Y. & Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47**, 251–276
- Garnier, J., Osguthorpe, D. J. & Robson, B. (1978) *J. Mol. Biol.* **120**, 97–120
- Serrano, L., Montejo, E., Hernandez, M. A. & Avila, J. (1985) *Eur. J. Biochem.* **153**, 595–600
- Baas, P. W., Deitch, J. S., Black, M. M. & Banker, G. A. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8335–8339
- Wang, D., Villasante, A., Lewis, S. A. & Cowan, N. J. (1986) *J. Cell Biol.* **103**, 1903–1910
- Schiffer, M. & Edmundson, A. B. (1967) *Biophys. J.* **7**, 121–130

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Inositol 1,4,5-trisphosphate 3-kinase activity in high-speed supernatants from rat pancreatoma cells, AR4-2J

AR4-2J is a stable cell line derived from a rat pancreatoma which has been found to retain many of the functional

Fig. 1. $[^3\text{H}]\text{Ins}(1,4,5)\text{P}_3$ metabolism in AR4-2J cell homogenate and soluble subcellular fraction

Cell homogenate and soluble fraction were prepared in an intracellular-like solution containing ATP and an ATP-regenerating system (Horstman *et al.*, 1988) and incubated with $[^3\text{H}]\text{Ins}(1,4,5)\text{P}_3$ (100 nM final concentration). The duration of the incubations shown were 1 min for homogenate (panel a) and 5 min for soluble fraction (panel b). $[^3\text{H}]\text{Ins}(1,4,5)\text{P}_3$ and metabolites were determined by h.p.l.c. according to the method of Dean & Moyer (1987) and Horstman *et al.* (1988). The unlabelled peak eluted at approx. 37 min was an unidentified contaminant present regardless of assay condition. Similar results were obtained in a separate experiment.

characteristics of pancreatic acinar cells (Longnecker *et al.*, 1979; Jessop & Hay, 1989; Womack *et al.*, 1985). However, Horstman *et al.* (1988), in an investigation of $\text{Ins}(1,4,5)\text{P}_3$ metabolism and Ca^{2+} mobilization after substance P receptor stimulation, found that the formation of $\text{Ins}(1,4,5)\text{P}_3$ was transient in these cells and that no $\text{Ins}(1,3,4,5)\text{P}_4$ accumulated. Furthermore, phosphorylation of $\text{Ins}(1,4,5)\text{P}_3$ to $\text{Ins}(1,3,4,5)\text{P}_4$ was not detected in AR4-2J cell homogenates. These results suggested that the AR4-2J cells may be deficient in the $\text{Ins}(1,4,5)\text{P}_3$ 3-kinase found in normal pancreas and other tissues. Subsequently, we have observed an accumulation of $\text{Ins}(1,3,4,5)\text{P}_4$ when AR4-2J cells were stimulated with bombesin (Nogimori *et al.*, 1990; Menniti *et al.*, 1990). It was suggested that the accumulation of $\text{Ins}(1,3,4,5)\text{P}_4$ after bombesin receptor stimulation, but not after substance P receptor stimulation, is a consequence of differences in the efficacy and duration of response to these agonists (Nogimori *et al.*, 1990; Menniti *et al.*, 1990). Nevertheless, it is paradoxical that $\text{Ins}(1,4,5)\text{P}_3$ 3-kinase activity was not previously detected in AR4-2J cell homogenates. This issue is resolved by the finding described below that the 3-kinase activity in AR4-2J cell extracts is unmasked during cell fractionation.

AR4-2J cell homogenates were prepared in an intracellular-like solution containing ATP and an ATP-regenerating system as described by Horstman *et al.* (1988). The final concentration of cell protein was 50–100 mg/ml. Soluble and particulate fractions were prepared by centrifugation of homogenates at 100 000 g (TLA 100.3 rotor in a Beckman mini-ultracentrifuge) for 1 h at 3 °C. Homogenate and subcellular fractions (each representing 100 μl of original homogenate) were preincubated for 5 min at 37 °C prior to addition of 20 μl of $[^3\text{H}]\text{Ins}(1,4,5)\text{P}_3$ (sp. radioactivity 5.6 Ci/mmol; NEN Research Products, E.I. du Pont de Nemours and Co., Boston, MA, U.S.A.) to initiate the reaction.

The final [^3H]Ins(1,4,5) P_3 concentration was 100 nM, adjusted by addition of non-radioactive Ins(1,4,5) P_3 (Amersham Corp., Arlington Heights, IL, U.S.A.). Reactions were stopped after 1–5 min by addition of 120 μl of ice-cold 15% trichloroacetic acid containing 250 mg of phytate. After a further incubation at 4 °C for 20 min, precipitated protein was removed by centrifugation and the supernatants were neutralized by ether extraction as previously described (Horstman *et al.*, 1988). The [^3H]inositol phosphates in the neutralized samples were quantified by h.p.l.c. according to the method of Dean & Moyer (1987) as previously described (Horstman *et al.* 1988).

In whole homogenates of AR4-2J cells, exogenous [^3H]Ins(1,4,5) P_3 was dephosphorylated to [^3H]Ins(1,4) P_2 and [^3H]Ins(4) P (Fig. 1a). As previously reported (Horstman *et al.*, 1988), no [^3H]Ins(1,3,4,5) P_4 or its metabolites were formed. This pattern of [^3H]Ins(1,4,5) P_3 metabolism was similar in the particulate fraction (results not shown). However, [^3H]Ins(1,3,4,5) P_4 was recovered after incubation of [^3H]Ins(1,4,5) P_3 with the supernatant fraction (Fig. 1b). The [^3H]Ins(1,3,4,5) P_4 formed was further metabolized to [^3H]Ins(3,4) P_2 , and both [^3H]Ins(1 or 3)- and (4) P ; [^3H]Ins(1,3,4) P_3 did not accumulate. These findings are consistent with a cytosolic localization for the Ins(1,4,5) P_3 3-kinase in AR4-2J cells, as found in other tissues (Irvine *et al.*, 1986; Shears, 1989).

Although previous and present results now indicate that the AR4-2J cells contain an Ins(1,4,5) P_3 3-kinase, it has been pointed out that the agonist-stimulated accumulation of Ins(1,3,4,5) P_4 in intact AR4-2J cells is very low as compared to non-tumour acinar cells (Nogimori *et al.*, 1990; Menniti *et al.*, 1990). In addition to the previously raised possibility, that these cells express only low levels of the Ins(1,4,5) P_3 3-kinase (Horstman *et al.*, 1988), the AR4-2J cells may over-express the 5-phosphatase which degrades Ins(1,4,5) P_3 to Ins(1,4) P_2 . Alternatively, the

3-kinase may be chronically inhibited in the AR4-2J cells. These latter suggestions stem from the present finding that 3-kinase activity was readily detected only in the soluble fraction prepared from these cells. This unmasking of 3-kinase activity may result from a purification away from an endogenous inhibitor of the enzyme or a separation from phosphatase activities which compete for Ins(1,4,5) P_3 substrate in the assay of the homogenate fraction. Resolution of these various possibilities may shed light on the regulation of this component of Ins(1,4,5) P_3 metabolism in this cell line as well as in non-tumour acinar cells.

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- Dean, N. M. & Moyer, J. D. (1987) *Biochem. J.* **242**, 361–366
 Horstman, D. A., Takemura, H. & Putney, J. W., Jr. (1988) *J. Biol. Chem.* **263**, 15297–15303
 Irvine, R. F., Letcher, A. J., Heslop, J. P. & Berridge, M. J. (1986) *Nature (London)* **320**, 631–634
 Jessop, N. W. & Hay, R. J. (1989) *In Vitro* **16**, 212
 Longnecker, D. S., Lilja, H. S., French, J. I., Kuhlmann, E. & Noll, W. (1979) *Cancer Lett.* **7**, 197–202
 Menniti, F. S., Oliver, K. G., Nogimori, K., Obie, J. F., Shears, S. B. & Putney, J. W., Jr. (1990) *J. Biol. Chem.* **265**, 11167–11176
 Nogimori, K., Menniti, F. S. & Putney, J. W., Jr. (1990) *Biochem. J.* **269**, 195–200
 Shears, S. B. (1989) *Biochem. J.* **260**, 313–324
 Womack, M. D., Hanley, M. R. & Jessel, T. M. (1985) *J. Neurosci.* **5**, 3370–3378

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