Crystal Structure of the P38α-MAPKAP Kinase 2 Heterodimer

Ernst ter Haar¹, Prakash Prabakhar, Xun Liu, and Christopher Lepre

From Vertex Pharmaceuticals Incorporated, Cambridge, Massachusetts 02139

The p38 signaling pathway is activated in response to cell stress and induces production of proinflammatory cytokines. P38α is phosphorylated and activated in response to cell stress by MKK3 and MKK6 and in turn phosphorylates a number of substrates, including MAPKAP kinase 2 (MK2). We have determined the crystal structure of the unphosphorylated p38α-MK2 heterodimer. The C-terminal regulatory domain of MK2 binds in the docking groove of p38α, and the ATP-binding sites of both kinases are at the heterodimer interface. The conformation suggests an extra mechanism in addition to the regulation of the p38α and MK2 phosphorylation states that prevents phosphorylation of substrates in the absence of cell stress. Addition of constitutively active MK6-DD results in rapid phosphorylation of the p38α-MK2 heterodimer.

In this report, we present a structural analysis of the p38α-MK2 heterodimer. Crystal structures were determined for both the full-length p38α-MK2 heterodimer and the p38α-MK2 peptide (residues 370–400) complex. The structures reveal the heterodimer conformation buries the substrate binding grooves of both p38α and MK2a, thus preventing both kinases from phosphorylating their respective substrates. The structure suggests that substrate phosphorylation is dependent on the phosphorylation states of both kinases and the heterodimer conformation.

EXPERIMENTAL PROCEDURES

Protein Purification and Generation of Protein-Protein Complex—Human p38α was expressed and purified as described (20). Full-length MK2 was expressed and purified according to the protocol used for N-terminal-truncated MK2 (residues 46–400) (21). P38α-MK2 heterodimer samples were prepared by incubating the purified proteins in equimolar concentrations (25 µM each) for 1 h on ice. The p38α-MK2 complex was separated from the monomers by Q-Sepharose anion exchange chromatography using a linear NaCl gradient (100–500 mM), and the fractions containing the p38α-MK2 heterodimer were pooled. Finally, the buffer was exchanged into 20 mM Tris-Cl, pH 7.8, 50 mM NaCl, and 2 mM dithiothreitol, and the protein sample was concentrated to 20 mg/ml for crystallization set-ups.
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Crystalization and Data Collection—Crystals were grown with the hanging drop method over a reservoir containing 3.75% polyethylene glycol 2000, 100 mM Hepes, pH 7.5. Prior to the crystallization set up, 1-s-nonyl-1-β-D-thiogluco side (1x critical micelle concentration) and heptanetriol (1.5%) were added to the protein sample. The crystallization drop was prepared by mixing the reservoir solution with the protein solution in a 1:1 ratio. Crystals grew overnight and were typically harvested after 72 h. The crystals were transferred for 1 min to a solution consisting of reservoir solution and 30% glycerol before being flash frozen in liquid nitrogen. Data collection at beamline 5.0.2 and the Advanced Light Source of Lawrence Berkeley National Laboratory, Berkeley, CA, yielded a data set to 4.0 Å resolution. The crystals of full-length p38α-MK2 heterodimer belonged to spacegroup P41 with unit cell dimensions of 103.09 Å, 103.09 Å, 231.42 Å, 90.0°, 90.0°, 90.0°. Crystals of p38α-MK2 peptide (residues 370–400) were grown with the hanging drop method over a reservoir containing 25% polyethylene glycol 3350 and 250 mM sodium formate. The crystallization drop was prepared by mixing the protein solution with the reservoir solution in a 1:1 ratio. The crystals grew in a matter of hours and were harvested after 24 h. The crystals were transferred for 1 min to a solution consisting of reservoir solution and 30% glycerol before being flash frozen in liquid nitrogen. Diffraction data collected at beamline 5.0.2 of Advanced Light Source D*trek. The structure was solved using only the rigid body protocol of BUSTER, and building of the MK2 activation loop.

RESULTS

General Description of the Structure—P38α and MK2 are both serine/threonine protein kinases. The structures of both P38α and MK2 have been determined previously as monomers (20, 21, 25). Both structures have typical kinase folds with a small N-terminal domain dominated by β-strands, a larger C-terminal domain consisting of α-helices, and an ATP-binding site wedged between the two domains. The ATP-binding site is formed by the flexible glycin-rich loop, the hinge that connects the small N-terminal domain with the large α-helical domain, and the catalytic loop. The kinase domains of p38α and MK2 also have a substrate binding groove. This groove is at the interface of the N- and C-terminal domains and binds the part of the substrate protein that contains the phosphorylation site. Both p38α and MK2 have additional unique features. P38α has an extra docking groove for activating kinases, substrate kinases, and inactivating phosphatases. The docking groove contains the previously identified glutamate-aspartate (ED) and common docking (CD) regions and is conserved among other MAP kinases such as ERK1/2 and JNK, but not in other kinases.

The kinase domain of MK2a is preceded by a proline-rich sequence (residues 1–44) that is not present in the published crystal structures (26). Beyond the kinase domain it has a C-terminal regulatory domain (residues 338–400) that consists of helices αJ and αK and a long loop. The C-terminal regulatory domain contains an NES and the bipartite NLS (17, 18). The αK-helix occupied the MK2a substrate binding groove in an autoinhibitory conformation in one of the crystal structures of the monomer (21).

We have determined the crystal structure of a p38α-MK2 heterodimer that contains full-length p38α and MK2a, and we have also determined a complementary structure of p38α complexed with only the docking peptide (residues 370–400) of MK2. The p38α and MK2a molecules that make up the heterodimer are positioned "face to face" so that the ATP-binding sites of both kinases are at the heterodimer interface (Fig. 1). The C-terminal segment of MK2 (residues 368–400), which follows the αK-helix, wraps around p38α and inserts into the docking groove. Residues 382–390, including the C-terminal part of the NLS, fold into an α-helix (αL), which occupies the CD region of the p38α docking groove (Fig. 1). The αK-helix (residues 344–367) of the C-terminal regulatory domain is sandwiched between the p38α and MK2a kinase domains. It occupies the MK2a substrate binding groove, as does it in the crystals of the MK2a kinase domain alone (21). The C-terminal end of the αK-helix (Fig. 1, A and C) that contains the NES of MK2a (residues 356–365) is buried between the two kinase domains in close proximity to the p38α ATP-binding site. The MK2a activation loop (residues 207–233) also adapts to binding with p38α. In the monomer crystal forms, the activation loop is disordered (not shown), whereas in the p38α-MK2 heterodimer the complete MK2a activation loop backbone can be traced through the electron density map, as it is stabilized by
interactions with the α-helical domain of p38α (supplemental Fig. S1). The phosphorylation site in the MK2a activation loop (Thr222) does not make contact with p38α.

The C-terminal regulatory domain and the activation loop of MK2a form most of the contacts between p38α and MK2a in the heterodimer (Fig. 2). The C-terminal regulatory domain contacts both the N-terminal β-strand and the α-helical domains of p38α. The C-terminal end of the αK-helix (residue Glu354) touches the tip of the glycine-rich loop (Ala34) and the β1L0–β2L0 loop, but only Glu354 (MK2a) and Ala34 (p38α) are less than 4 Å apart. The C-terminal loop of the C-terminal regulatory domain occupies the docking groove of p38α and is responsible for the high affinity interaction between p38α and MK2a (16). This interaction will be discussed in more detail below. The activation loop of MK2a makes contacts with p38α in the heterodimer and a neighboring MK2a molecule. The conformation of the C-terminal portion of the activation loop (residues 232–237) is stabilized by the loop between the αD- and αE-helices in p38α, but only the backbone density is visible in the electron density map. There is an additional contact between the MK2a loop of αH and α1 and the p38α loop between αF and αG. The solvent-accessible surface area buried in the heterodimer is 1373 Å² for MK2a and 1477 Å² for p38α (Fig. 2).

The p38α activation loop contains two phosphorylation sites (Thr180 and Tyr182) that when modified stabilize its activated conformation and open the substrate binding groove. In the heterodimer described here, p38α is not phosphorylated and the p38α activation loop is largely disordered. Residues 174–184 were omitted from the model due to lack of electron density. This disordered region was in close proximity to a disordered loop region from MK2a (residues 265–283). The MK2a residues 264–281 are included in a published, mercury-derivatized crystal structure (1KWP) (21), but their positions are not compatible with the p38α–MK2 heterodimer conformation.

The p38α Docking Groove—p38α and the other MAP kinases have a docking groove for binding with their activating kinases, inactivating phosphatases, and substrates (23, 27–36). The docking groove is not to be confused with the substrate binding groove at which the phosphorylation reaction takes place. The groove was first identified by mutational studies as two separate regions (Fig. 3), the CD region and the ED region (27). The CD region is part of a shallow groove formed by the acidic residues Asp313, Asp316, Glu81, and the aromatic residues Phe129 and Tyr311. The latter is part of a deeper groove formed by residues 159–163 (159-VNEDCE-163) at one side and residues Gln120, His126, and Phe129 at the opposite side.

The C-terminal end of the regulatory domain of MK2a (residues 369–384) binds in the docking groove of p38α and makes key interactions with the ED and CD regions (Fig. 4). These interactions are a combination of hydrophobic and polar contacts. Earlier studies identified the MAP kinase binding motif as rich in positively charged residues, and because the ED and CD regions of p38α are mainly negatively charged, it was assumed that these motifs contact each other. Our structure shows that this is indeed the case.
noticed that the binding of the MK2a to p38 contribute most to the formation of the heterodimer. They also calorimetry, they observed that the docking groove interactions onance, stopped flow fluorescence, and isothermal titration calorimetry. The N terminus of the peptides are colored blue to red spectrum. The binding orientation of MK2 is reversed when compared with the MEF2c (16). Using surface plasmon resonance, stopped flow fluorescence, and isothermal titration calorimetry, they observed that the docking groove interactions contribute most to the formation of the heterodimer. They also noticed that the binding of the MK2a to p38α depends on the ionic strength of the buffer, consistent with the many electrostatic interactions between p38α and MK2a that we see in the heterodimer.

The sequence of the MAP kinase binding motif is well conserved among proteins that bind p38α, ERK, or JNK. The main difference is the location of the motif with respect to the catalytic domain. In activating kinases and inactivating phosphatases, the sequences of the MAP kinase binding motif come before the catalytic domains, whereas for the substrate kinases such as MK2a the binding motif comes after the kinase domain. Two crystal structures of p38α in complex with short peptides have been determined previously. One peptide is derived from the p38α activator MKK3b, and the second peptide is from the transcription activator MEF2c (23). These short peptides also bind in the p38α docking groove, but the major difference with the p38α-MK2 complex is the reversed direction of the peptide backbones (Fig. 4). The peptides employed in these studies have very little sequence homology with each other or with MK2a, but the interactions between the peptide side chains and p38α at the ED domain are conserved despite the reversed direction of the peptide backbone. The residue pattern is similar but reversed: hydrophobic, polar, hydrophobic, polar. For instance, at the position of MK2a residue Ile<sup>370</sup>, both peptides also have an isoleucine with the side chains pointing in the same direction. Lys<sup>371</sup> overlaps with an arginine of the MKK3b peptide and both side chains extend into the solvent, away from p38α. The peptides also have a hydrophobic residue at the position of Ile<sup>370</sup>, that is buried by the loop of p38α consisting of residues 158–363, and a polar residue that contacts Asp<sup>161</sup>.

Constitutively Active MKK6-DD Phosphorylates the p38α-MK2 Heterodimer—The ATP-binding sites and substrate binding grooves of p38α and MK2a are at the interface of the heterodimer, making them inaccessible to substrates. Thus, the conformation of the heterodimer seen here does not represent a p38α phosphorylation complex but rather a conformation for which the catalytic activity of p38α and MK2a are each sequen- tered from potential substrates. It probably represents the unactivated complex and reflects the state of both kinases when the cell does not need their phosphorylation capacity, i.e. when the cell does not receive any stress signals. As soon as stress signals do arrive at the cell, however, we expect the p38α-MK2 heterodimer to alter such that both kinases are accessible to their substrates.

The p38α-MK2 heterodimer is a stable, high affinity complex with a <i>K<sub>d</sub></i> of 6 nM (16). When the p38 signaling pathway is activated due to cell stress, the activating kinases MKK6 or MKK3 phosphorylate p38α. If the p38α-MK2 heterodimer is the preferred conformation for both kinases, then p38α and MK2a should still readily be phosphorylated when activated MKK6 is added to the complex. According to what is known about the p38α signaling pathway, activated MKK6 will phosphorylate and activate p38α and p38α will subsequently phosphorylate MK2a.

MKK6-DD is a constitutively activated form of MKK6 in which the two aspartates mimic the phosphorylated residues (Ser<sup>325</sup> and Thr<sup>211</sup>) (37) and is a very efficient activator of p38α.
When a small amount of MKK6-DD (1:100 molar ratio) was added to the p38α-MK2 complex, both p38α and MK2a became phosphorylated over time (Fig. 5). Phosphorylation of p38α was followed by Western blot analysis using an antibody that recognizes Thr180 phosphorylated p38α and phosphorylated Tyr182. Phosphorylation of MK2a was followed with two commercially available antibodies that detect phosphorylated Thr222 or Thr334.

We believe that the crystal structure represents the conformation of p38α and MK2a as it is present in the nucleus when the cell is devoid of stress. Although MK2a is a phosphorylation substrate of p38α, the conformation of the heterodimer does not represent the phosphorylation reaction but rather prevents both p38α and MK2a from phosphorylating their substrates in the absence of stress signals. Tight regulation of the p38α signal transduction pathways is important for the proper functioning of a cell, as activation of this pathway increases the levels of proinflammatory cytokines and induces cell cycle arrest. There is evidence that the conformations of unphosphorylated and phosphorylated heterodimers differ. The Kd of the unphosphorylated p38α-MK2 heterodimer is 6 nm, whereas the Kd of the phosphorylated heterodimer is 10 times weaker (16). A conformational change has also been observed in MK2a with fluorescent resonance energy transfer analysis and is triggered by the phosphorylation of Thr334 (MK2a) (19). The conformational change likely makes the NES accessible, thus allowing the p38α-MK2a heterodimer to be transported to the cytoplasm. The heterodimer structure presented here is not the translocation complex in which the two kinases migrate from the nucleus to the cytoplasm. The MK2a NES is buried between the two kinase domains of the heterodimer and not accessible to carrier proteins. The NLS is part of the p38α binding motif and is anchored in the CD domain of the docking groove and therefore not accessible to facilitate transport from the cytoplasm to the nucleus.

Cell stress activates the p38α-MK2 signaling pathway, which must have major consequences for the heterodimer. Both
kinases will be phosphorylated, p38α first by MKK3 or MKK6 and MK2a by p38α. Both kinases will in turn phosphorylate transcription factors in the nucleus, move to the cytoplasm, and phosphorylate other substrates. Eventually the kinases will be dephosphorylated by a MAP kinase phosphatase. We have shown here that MK2a binds p38α in the docking groove. All other proteins that interact with p38α also bind in this groove (27, 30, 31, 33, 36). These proteins can be p38α substrates such as MEF2c, PRAK, and MK2a, p38α activators MKK3 and MKK6, or they can be the MAP kinase phosphatase MKP7 that inactivates p38α. These substrates compete for the same binding site in p38α and consequently determine the stress response and subcellular location of p38α (Fig. 4). We have also shown that constitutively active MKK6 can phosphorylate the p38α in the heterodimer and that p38α in turn phosphorylates MK2a. We propose the following model (Fig. 6) for events that take place when the p38α signaling pathway is activated and reaches the nucleus.

In the nucleus of a normal cell, p38α and MK2a are in the heterodimeric state described here (Fig. 6A). Activated MKK3/6 displaces MK2a from p38α to phosphorylate and activate p38α (Fig. 6B). Activated p38α now has three options depending on the protein it will bind next. (i) It can bind one of its nuclear substrates such as transcription factor MEF2c (Fig. 6C) and activate gene expression in response to cell stress. The binding reaction is a typical catalytic reaction in which the kinase has much lower affinity for the product than for the substrate. Once phosphorylated, the transcription factor is released and p38α can bind a different protein. (ii) p38α encounters a MAP kinase phosphatase (Fig. 6F). This will result in dephosphorylation and inactivation of p38α. The inactivated p38α can again form a heterodimer with MK2a and this heterodimer will remain in the nucleus (Fig. 6G). (iii) Activated p38α binds and activates MK2a at its phosphorylation sites, including Thr334 (Fig. 6D). MK2a will undergo a conformational change and expose its nuclear export signal. The activated heterodimer of p38α and MK2a will then exit the nucleus and phosphorylate substrates in the cytoplasm (Fig. 6F). Most of the p38α and MK2a will eventually return to the nucleus. It is not clear whether they return as a heterodimer or as separate monomers but overexpressed p38α is mostly found in the nucleus (17), suggesting that p38α can reach the nucleus without the aid of MK2a. The bipartite NLS of MK2a is also the p38α docking motif, suggesting that binding by p38α prevents the binding of importin-α and therefore translocation to the nucleus. As a monomer, the MK2a NLS is completely exposed and importin-α could bind MK2a and transport it together with importin-β to the nucleus.

The p38α signaling pathway is a promising target for anti-inflammatory therapy. Inhibition of the p38α signaling pathway reduces the levels of proinflammatory cytokines, and p38 inhibitors have shown beneficial effects in the treatment of rheumatoid arthritis, psoriasis, and chronic obstructive pulmonary disease (7). In this study we have provided evidence that p38α forms a stable heterodimer with MK2a that is likely the preferred conformation of both kinases in the nucleus of a cell that is not receiving stress signals. The crystal structures suggest aspects of the p38α signaling pathway that could be helpful for developing novel anti-inflammatory therapies.

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REFERENCES

1. Cuenda, A., Rouse, J., Doza, Y. N., Meier, R., Cohen, P., Gallagher, T. F., Young, P. R., and Lee, J. C. (1995) FEBS Lett. 364, 229–233
2. Lasa, M., Mahtani, K. R., Finch, A., Brewer, G., Saklatvala, J., and Clark, A. R. (2000) Mol. Cell. Biol. 20, 4265–4274
3. Winzen, R., Kracht, M., Ritter, B., Wilhelm, A., Chen, C. Y., Shyu, A. B., Muller, M., Gaestel, M., Resch, K., and Holtmann, H. (1999) EMBO J. 18, 4969–4980
4. Saklatvala, J. (2004) Curr. Opin. Pharmacol. 4, 372–377
5. Shi, Y., and Gaestel, M. (2002) Biol. Chem. 383, 1519–1536
6. Salluto, F. G., Germann, U. A., Wilson, K. P., Kemper, G. W., Fox, T., and Su, M. S. (1999) Curr. Med. Chem. 6, 807–823
7. Force, T., Kuida, K., Namechuk, M., Parang, K., and Kyriakis, J. M. (2004) Circulation 109, 1196–1205
8. Stokoe, D., Campbell, D. G., Nakielny, S., Hidaka, H., Leevers, S. J., Marshall, C., and Cohen, P. (1992) EMBO J. 11, 3985–3994
9. Kumar, S., Boehm, J., and Lee, J. C. (2003) Nat. Rev. Drug Discov. 2, 717–726
10. Tan, Y., Rouse, J., Zhang, A., Cariati, S., Cohen, P., and Comb, M. J. (1996) EMBO J. 15, 4629–4642
11. Huang, C. K., Zhan, L., Ai, Y., and Jongstra, J. (1997) J. Biol. Chem. 272, 17–19
12. Stokoe, D., Engel, K., Campbell, D. G., Cohen, P., and Gaestel, M. (1992) FEBS Lett. 313, 307–313
13. Zu, Y. L., Wu, F., Gilchrist, A., Ai, Y., Labadia, M. E., and Huang, C. K. (1994) Biochem. Biophys. Res. Commun. 200, 1118–1124
14. Zu, Y. L., Ai, Y., and Huang, C. K. (1995) *J. Biol. Chem.* 270, 202–206
15. Kotlyarov, A., Yannoni, Y., Fritz, S., Laass, K., Telliez, J. B., Pitman, D., Lin, L. L., and Gaestel, M. (2002) *Mol. Cell. Biol.* 22, 4827–4835
16. Lukas, S. M., Kroev, R. R., Wildeison, J., Peet, G. W., Frego, L., Davidson, W., Ingraham, R. H., Pargellis, C. A., Labadia, M. E., and Werneburg, B. G. (2004) *Biochemistry* 43, 9950–9960
17. Ben-Levy, R., Hooper, S., Wilson, R., Paterson, H. F., and Marshall, C. J. (1998) *Curr. Biol.* 8, 1049–1057
18. Engel, K., Kotlyarov, A., and Gaestel, M. (1998) *EMBO J.* 17, 3363–3371
19. Neininger, A., Thielemann, H., and Gaestel, M. (2001) *EMBO Rep.* 2, 703–708
20. Wilson, K. P., Fitzgibbon, M. J., Caron, P. R., Griffith, J. P., Chen, W., McAffrey, P. G., Chambers, S. P., and Su, M. S. (1996) *J. Biol. Chem.* 271, 27696–27700
21. Meng, W., Swenson, L. L., Fitzgibbon, M. J., Hayakawa, K., Ter Haar, E., Behrens, A. E., Fulghum, J. R., and Lippke, J. A. (2002) *J. Biol. Chem.* 277, 37401–37405
22. Vagin, A., and Teplyakov, A. (2000) *Acta Crystallogr. Sect. D Biol. Crystallogr.* 56, Pt. 12, 1622–1624
23. Chang, C. I., Xu, B. E., Akella, R., Cobb, M. H., and Goldsmith, E. J. (2002) *Mol. Cell.* 9, 1241–1249
24. Blanc, E., Roversi, P., Vonrhein, C., Flensburg, C., Lea, S. M., and Bricogne, G. (2004) *Acta Crystallogr. Sect. D Biol. Crystallogr.* 60, Pt. 12, 2210–2221
25. Underwood, K. W., Parris, K. D., Federico, E., Mosyak, L., Czerwinski, R. M., Shane, T., Taylor, M., Svenson, K., Liu, Y., Hsiao, C. L., Wolfrom, S., Maguire, M., Malakian, K., Telliez, J. B., Lin, L. L., Kriz, R. W., Seehra, J., Somers, W. S., and Stahl, M. L. (2003) *Structure (Camb.)* 11, 627–636
26. Stokoe, D., Caudwell, B., Cohen, P. T., and Cohen, P. (1993) *Biochem. J.* 296, Pt. 3, 843–849
27. Tanoue, T., Adachi, M., Moriguchi, T., and Nishida, E. (2000) *Nat. Cell. Biol.* 2, 110–116
28. Bardwell, A. J., Flatauer, L. J., Matsukuma, K., Thorner, J., and Bardwell, L. (2001) *J. Biol. Chem.* 276, 10374–10386
29. Fantz, D. A., Jacobs, D., Glossip, D., and Kornfeld, K. (2001) *J. Biol. Chem.* 276, 27256–27265
30. Tanoue, T., Maeda, R., Adachi, M., and Nishida, E. (2001) *EMBO J.* 20, 466–479
31. Tanoue, T., Yamamoto, T., and Nishida, E. (2002) *J. Biol. Chem.* 277, 22942–22949
32. Tanoue, T., and Nishida, E. (2002) *Pharmacol. Ther.* 93, 193–202
33. Bardwell, A. J., Abdollahi, M., and Bardwell, L. (2003) *Biochem. J.* 370, Pt. 3, 1077–1085
34. Ho, D. T., Bardwell, A. J., Abdollahi, M., and Bardwell, L. (2003) *J. Biol. Chem.* 278, 32662–32672
35. Tanoue, T., and Nishida, E. (2003) *Cell. Signal.* 15, 455–462
36. Tanoue, T., Yamamoto, T., Maeda, R., and Nishida, E. (2001) *J. Biol. Chem.* 276, 26629–26639
37. Takekawa, M., Maeda, T., and Saito, H. (1998) *EMBO J.* 17, 4744–4752
38. Lee, J. C., Laydon, J. T., McDonnell, P. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Heys, J. R., Landvatter, S. W., Strickler, J. E., McLaughlin, M. M., Siemens, I. R., Fisher, S. M., Livi, G. P., White, J. R., Adams, J. L., and Young, P. R. (1994) *Nature* 372, 739–746
39. Kumar, S., McDonnell, P. C., Gum, R. J., Hand, A. T., Lee, J. C., and Young, P. R. (1997) *Biochem. Biophys. Res. Commun.* 235, 533–538
40. Cuenda, A., Cohen, P., Buee-Scherrer, V., and Goedert, M. (1997) *EMBO J.* 16, 295–305

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