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Minireview

Elongation factor-2 kinase and its newly discovered relatives

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Abstract Phosphorylation of elongation factor-2 (eEF-2) by the highly specific eEF-2 kinase results in eEF-2 inactivation and, therefore, may regulate the global rate of protein synthesis in animal cells. Cloning and sequencing of eEF-2 kinase led to the discovery of a new family of protein kinases, named α-kinases, whose catalytic domains display no sequence homology to conventional eukaryotic protein kinases. Several mammalian α-kinases have recently been cloned. Two of these α-kinases, named channel-kinases 1 and 2 (ChaK1 and ChaK2) represent a new type of signaling molecules that are protein kinases fused to ion channels.

Key words: Elongation factor-2; Protein kinase; Protein phosphorylation; α-Kinase; Ion channel; Channel-kinase; TRP channels

Phosphorylation of elongation factor-2 (eEF-2) was discovered in the laboratory of A.S. Spirin [1–4]. It was found that eEF-2 is one of the most prominently phosphorylated proteins in mammalian tissue extracts [1]. It was also found that phosphorylation of eEF-2 results in its inactivation, and, therefore, may represent the mechanism of global protein synthesis regulation at the elongation stage [2]. Phosphorylation of eEF-2 is catalyzed by a very specific eEF-2 kinase [3], which was identified earlier and previously called calcium/calmodulin-dependent protein kinase III [5,6]. Since eEF-2 kinase is calcium-dependent and since phosphorylation inactivates eEF-2, it was initially suggested that eEF-2 phosphorylation represents the mechanism by which changes in intracellular calcium concentration regulate the rate of protein synthesis. However, recent experiments question the role of eEF-2 phosphorylation in the regulation of protein synthesis by calcium in vivo [7]. It is now clear that the regulation of eEF-2 kinase is quite complex. It was found that several different protein kinases can phosphorylate eEF-2 kinase and that these phosphorylations can affect eEF-2 kinase activity. These protein kinases include cAMP-dependent protein kinase [8–11], stress-activated protein kinase SAPK4/p38δ [12], $p90^{RSK1}$ and p70 S6 kinase [13].

Recently we found another mechanism of regulation of eEF-2 kinase, which may provide a clue to the physiological role of eEF-2 phosphorylation. We found that eEF-2 kinase is dramatically activated by a slight decrease in pH, in the range that is observed intracellularly during hypoxia and ischemia. At normal physiological pH (7.4) eEF-2 kinase activity is very low, but increases drastically at pH 6.4–6.8 (Fig. 1). This result suggests that phosphorylation of eEF-2 may play a role in the inhibition of protein synthesis during hypoxia and ischemia. Since inhibition of protein synthesis can often protect cells from death, it is possible that the pH-dependent phosphorylation of eEF-2 is part of a protective signaling mechanism that is activated during hypoxia and ischemia.

To uncover the physiological role of eEF-2 kinase we made knockouts of eEF-2 kinase in *Caenorhabditis elegans* and

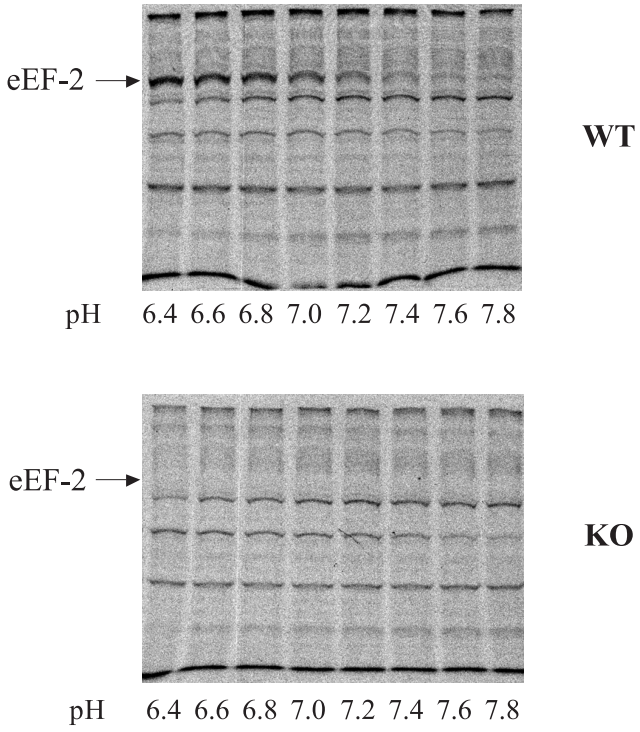

Fig. 1. pH dependence of protein phosphorylation in liver homogenates from wild-type and eEF-2 kinase knockout mice. Liver homogenates were clarified by centrifugation for 10 min at 10 000×*g* at 4°C and added in reaction mixtures consisting of 50 mM HEPES-KOH with variable pH, 10 mM magnesium acetate, 5 mM dithiothreitol, 100 μM $CaCl_2$, 60 μM ATP, and 1 μCi [γ-^{33}P]ATP. The reactions were run at 30°C for 10 min, and were terminated by incubation in an ice–water bath. Laemmli sample buffer was added, and the reaction mixtures were boiled for 5 min. Samples were analyzed by 8% SDS–PAGE and autoradiography.

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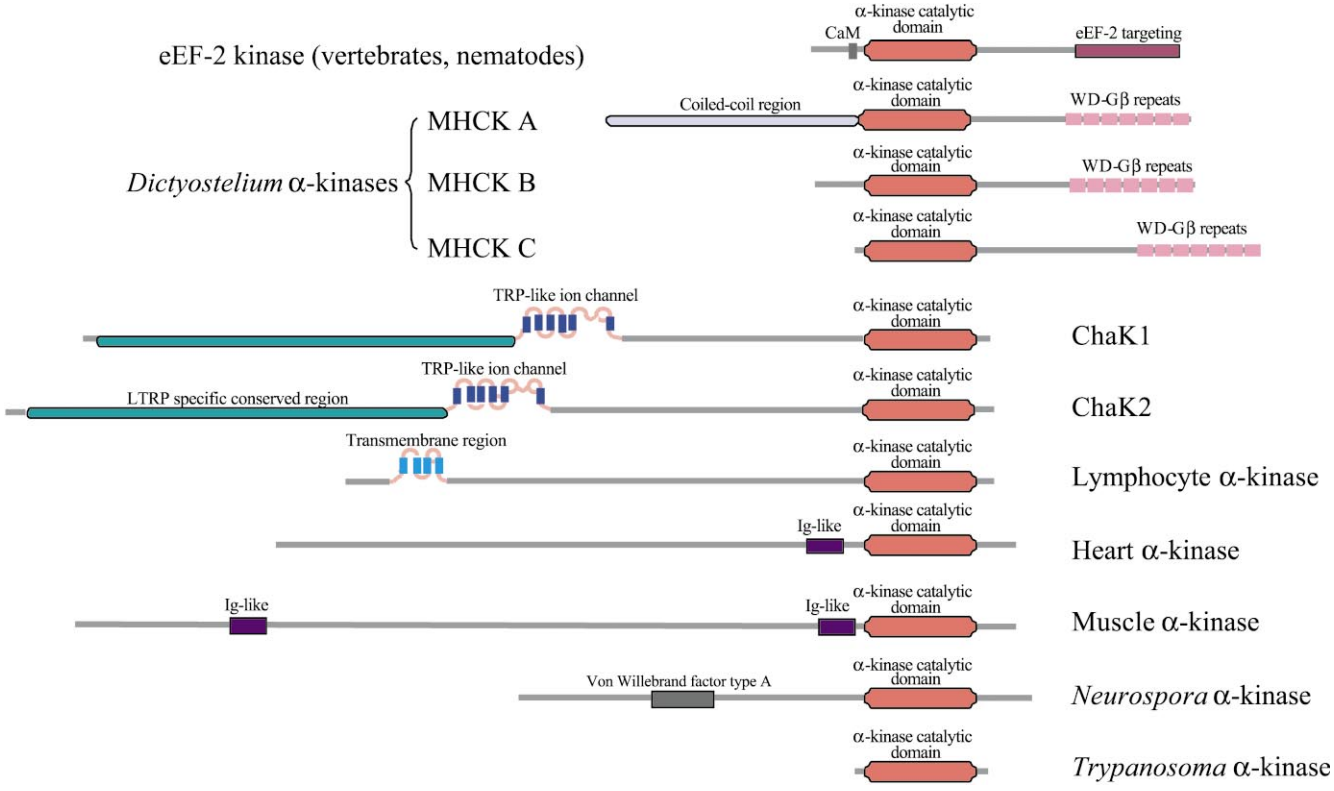

Fig. 2. Schematic representation of known proteins with an α-kinase domain. The accession numbers are as follows: human eEF-2 kinase (XM_012488), *C. elegans* eEF-2 kinase (AAB58268), *Dictyostelium* MHCK A (P42527), *Dictyostelium* MHCK B (P90648), *Dictyostelium* MHCK C (AAC31918), human ChaK1 (AAK19738), human ChaK2 (AAK31202), human lymphocyte α-kinase (AAK94675), human heart α-kinase (NP_443179), human muscle α-kinase (AAK95951), *Neurospora* α-kinase (CAC18305), *Trypanosoma* α-kinase (AC079815).

mice. Loss of eEF-2 kinase completely eliminated eEF-2 phosphorylation in the tissues of knockout animals (see Fig. 1). Surprisingly, both nematodes and mice lacking eEF-2 kinase were essentially normal; no obvious abnormalities in development, reproduction, or behavior were observed. Thus, eEF-2 kinase is not essential for viability, however it may play a role in the response to stress.

When we cloned and sequenced mammalian and *C. elegans* eEF-2 kinases, we found that they did not display sequence homology to any conventional eukaryotic protein kinase [14]. However, they displayed striking similarity to the catalytic domain of the myosin heavy chain kinase A (MHCK A) from *Dictyostelium* [15,16]. Two more protein kinases with a similar catalytic domain structure have been identified in *Dictyostelium* and were named MHCK B [17] and MHCK C [18]. Since eEF-2 kinase and *Dictyostelium* MHCKs do not display homology to conventional eukaryotic protein kinases, they clearly represent a separate family of protein kinases. We named this family α-kinases, because the existing evidence suggests that these kinases phosphorylate their substrates within α-helices [19]. Particularly striking is MHCK A, which phosphorylates three threonine residues located in the α-helical coiled-coil tail of myosin heavy chains [20,21]. This is very different from conventional protein kinases that phosphorylate their substrates within loops, turns, or regions with irregular structure [22]. α-Kinases have a catalytic domain of about 250 amino acids, which is characterized by several conserved motifs [19].

Recently, we identified, cloned, and sequenced five more human α-kinases [19,23]. All these new α-kinases are very long proteins of more than 1000 amino acids, with an α-kinase catalytic domain located at the very C-terminus. Unexpectedly, we found that two of them represent a novel type of signaling molecule, in which an α-kinase catalytic domain is fused to an ion channel. Initially we named these proteins melanoma α-kinase and kidney α-kinase [19,23] and then renamed them channel-kinases 1 and 2 (ChaK1 and ChaK2, GenBank accession numbers AAK19738 and AAK31202, respectively). During the past year several other laboratories independently cloned and characterized mouse and human ChaK1 and named it TRP-PLIK [24], LTRPC7 [25], or ChaK [26]. ChaK1, as well as ChaK2, represent the first examples of ion channels that are also enzymes and have protein kinase activity. The channel portions of ChaK1 and ChaK2 are homologous to the transient receptor potential (TRP) family of ion channels. TRP channels are homologs of the *Drosophila* TRP channel, a calcium channel that is involved in photoreception. The majority of TRP channels are Ca^{2+} permeable non-selective cation channels that can be subdivided into several subgroups (reviewed in [27–29]). ChaK1 and ChaK2 belong to one such subgroup, named long TRP channels (LTRPC) [27] or the TRPM subfamily [29]. The LTRPC (TRPM) subfamily is characterized by a very long N-terminus with a unique and highly conserved sequence. ChaK1 and ChaK2 are particularly similar to melastatin, a channel that is specifically down-regulated in metastatic melanoma [30,31]. It is likely that ChaK1 and ChaK2 are products of a recent evolutionary event – a fusion of a gene for an α-kinase catalytic domain and a gene for a melastatin-like ion channel. According to Northern analysis, ChaK1 is expressed

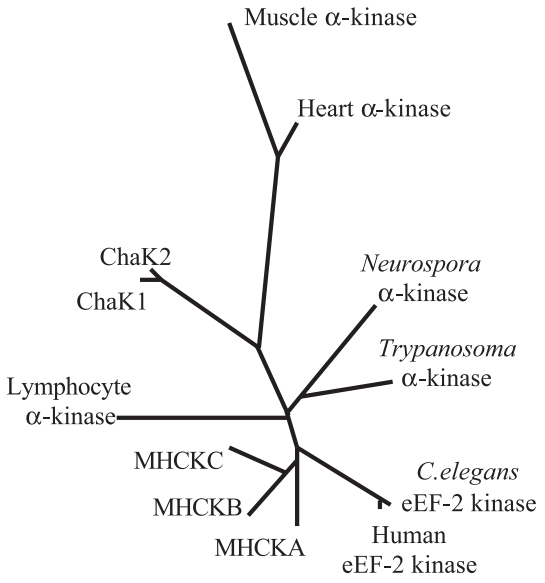

Fig. 3. Phylogenetic tree of known proteins with an α-kinase domain. Tree was created using the program 'Tree' available at 193.50.234.246./~beaudoin/anrs/Tree.html.

in all mammalian tissues and cell lines tested, while ChaK2 is expressed only in some tissues, being most abundant in kidneys [23]. The exact function of channel kinases is unknown, although experiments with targeted deletion of ChaK1 (LTRPC7) in DT-40 B cells demonstrated that this gene is essential for viability [25]. It is also unclear whether the main purpose of having a protein kinase covalently linked to an ion channel is to regulate the channel by the kinase or to regulate the kinase by the channel. Runnels et al. [24] provided evidence that kinase activity is required for ChaK1 (TRP-PLIK) ion channel activity; however, this conclusion was challenged by another group [25]. This group also showed that the ion channel activity of ChaK1 (LTRPC7) is modulated by Mg^{2+} and ATP [25].

Another protein with the α-kinase catalytic domain, which we cloned from a lymphocyte cDNA library and called lymphocyte α-kinase, has four predicted transmembrane segments close to its N-terminus [23]. However, this region is not homologous to any ion channel. Interestingly, the pattern of expression of lymphocyte α-kinase is very similar to ChaK1 [23], suggesting that these two proteins have a common mode of regulation and perhaps related functions.

The two other mammalian proteins with the α-kinase catalytic domain, named heart α-kinase and muscle α-kinase, are expressed predominantly in heart and skeletal muscle [23]. Both the heart and muscle α-kinases have an Ig-like domain preceding the catalytic domain. Recently, a mouse homolog of muscle α-kinase has been cloned and named Midori [32]. Midori is a nuclear protein that is expressed in the heart early during development and can regulate cardiomyocyte differentiation [32].

Thus in humans, and perhaps in other mammals, there are six α-kinases. Analysis of the completed human genome sequence revealed the location of all six α-kinases on various chromosomes and did not reveal any other genes whose products contain an α-kinase domain. Analysis of various genome sequencing data revealed that *C. elegans* has only one α-kinase, eEF-2 kinase. There are no α-kinases in *Drosophila*, *Arabidopsis*, or yeast genomes; however, there is one protein encoded in the *Neurospora* genome with a typical α-kinase domain (GenBank accession number CAC18305). This protein also contains a von Willebrand factor type A domain, a module that is present in many proteins (mostly extracellular matrix proteins) and that can interact with various ligands. There are probably many α-kinases in protozoan genomes, since the genomes of *Trypanosoma*, *Leishmania* and *Entamoeba* each contain several DNA sequences that encode hypothetical proteins with α-kinase domains. A schematic representation of all currently known proteins having α-kinase domains is shown in Fig. 2. The phylogenetic relationship of these proteins is shown in Fig. 3.

Recently α-kinases gave another surprise. When the three-dimensional structure of mouse ChaK1 was determined [26], it appeared that the fold of the α-kinase catalytic domain is similar to conventional eukaryotic protein kinases, despite the absence of similarity at the amino acid sequence level. The major structural difference was in the putative substrate-binding region [26]. This suggests that α-kinases are evolutionarily related to conventional eukaryotic protein kinases and utilize a similar catalytic mechanism, but that they may have a different mechanism of substrate recognition.

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References

[1] Ryazanov, A.G. (1987) FEBS Lett. 214, 331–334.
[2] Ryazanov, A.G., Shestakova, E.A. and Natapov, P.G. (1988) Nature 334, 170–173.
[3] Ryazanov, A.G., Natapov, P.G., Shestakova, E.A., Severin, F.F. and Spirin, A.S. (1988) Biochimie 70, 619–626.
[4] Ryazanov, A.G. and Spirin, A.S. (1993) in: Translational Regulation of Gene Expression II, pp. 433–455, Plenum Publishers, New York.
[5] Nairn, A.C., Bhagat, B. and Palfrey, H.C. (1985) Proc. Natl. Acad. Sci. USA 82, 7939–7943.
[6] Nairn, A.C. and Palfrey, H.C. (1987) J. Biol. Chem. 262, 17299–17303.
[7] Laitusis, A.L., Brostrom, C.O., Ryazanov, A.G. and Brostrom, M.A. (1998) Arch. Biochem. Biophys. 354, 270–280.
[8] Redpath, N.T. and Proud, C.G. (1993) Biochem. J. 293, 31–34.
[9] Diggle, T.A., Redpath, N.T., Heesom, K.J. and Denton, R.M. (1998) Biochem. J. 336, 525–529.
[10] Hovland, R., Eikhom, T.S., Proud, C.G., Cressey, L.I., Lanotte, M., Doskeland, S.O. and Houge, G. (1999) FEBS Lett. 444, 97–101.
[11] Diggle, T.A., Subkhankulova, T., Lilley, K.S., Shikotra, N., Willis, A.E. and Redpath, N.T. (2001) Biochem. J. 353, 621–626.
[12] Knebel, A., Morrice, N. and Cohen, P. (2001) EMBO J. 20, 4360–4369.
[13] Wang, X., Li, W., Williams, M., Terada, N., Alessi, D.R. and Proud, C.G. (2001) EMBO J. 20, 4370–4379.
[14] Ryazanov, A.G., Ward, M.D., Mendola, C.E., Pavur, K.S., Dorovkov, M.V., Wieldmann, M., Erdjument-Bromage, H., Tempst, P., Parmer, T.G., Prostko, C.R., Germino, F.J. and Hait, W.N. (1997) Proc. Natl. Acad. Sci. USA 94, 4884–4889.
[15] Futey, L.M., Medley, Q.G., Côté, G.P. and Egelhoff, T.T. (1995) J. Biol. Chem. 270, 523–529.
[16] Côté, G.P., Luo, X., Murphy, M.B. and Egelhoff, T.T. (1997) J. Biol. Chem. 272, 6846–6849.
[17] Clancy, C.E., Mendoza, M.G., Naismith, T.V., Kolman, M.F. and Egelhoff, T.T. (1997) J. Biol. Chem. 272, 11812–11815.

[18] Luo, X., Crawley, S.W., Steimle, P.A., Egelhoff, T.T. and Cote, G.P. (2001) J. Biol. Chem. 276, 17836–17843.
[19] Ryazanov, A.G., Pavur, K.S. and Dorovkov, M.V. (1999) Curr. Biol. 9, R43–R45.
[20] Vaillancourt, J.P., Lyons, C. and Cote, G.P. (1988) J. Biol. Chem. 263, 10082–10087.
[21] Luck-Vielmetter, D., Schleicher, M., Grabatin, B., Wippler, J. and Gerisch, G. (1990) FEBS Lett. 269, 239–243.
[22] Pinna, L.A. and Ruzzene, M. (1996) Biochim. Biophys. Acta 1314, 191–225.
[23] Ryazanova, L.V., Pavur, K.S., Petrov, A.N., Dorovkov, M.V. and Ryazanov, A.G. (2001) Mol. Biol. (Moscow) 35, 271–283.
[24] Runnels, L.W., Yue, L. and Clapham, D.E. (2001) Science 291, 1043–1047.
[25] Nadler, M.J.S., Hermosura, M.C., Inabe, K., Perraud, A.L., Zhu, Q., Stokes, A.J., Kurosaki, T., Kinet, J.P., Penner, R., Scharenberg, A.M. and Fleig, A. (2001) Nature 411, 590–595.
[26] Yamaguchi, H., Matsushita, M., Nairn, A.C. and Kuriyan, J. (2001) Mol. Cell 7, 1047–1057.
[27] Harteneck, C., Plant, T.D. and Schultz, G. (2000) Trends Neurosci. 23, 159–166.
[28] Clapham, D.E., Runnels, L.W. and Strubing, C. (2001) Nat. Rev. Neurosci. 2, 387–396.
[29] Montell, C. (2001) Science's STKE, http://stke.sciencemag.org/cgi/content/full/OC_sigtrans;2001/90/re1.
[30] Duncan, L.M., Deeds, J., Hunter, J., Shao, J., Holmgren, L.M., Woolf, E.A., Tepper, R.I. and Shyjan, A.W. (1998) Cancer Res. 58, 1515–1520.
[31] Hunter, J.J., Shao, J., Smutko, J.S., Dussault, B.J., Nagle, D.L., Woolf, E.A., Holmgren, L.M., Moore, K.J. and Shyjan, A.W. (1998) Genomics 54, 116–123.
[32] Hosoda, T., Monzen, K., Hiroi, Y., Oka, T., Takimoto, E., Yazaki, Y., Nagai, R. and Komuro, I. (2001) J. Biol. Chem. 276, 35978–35989.