Volume 255, number 1, 101–104 FEBS 07494 September 1989

A purified complex from *Xenopus* oocytes contains a p47 protein, an in vivo substrate of MPF, and a p30 protein respectively homologous to elongation factors EF-1γ and EF-1β

Robert Bellé, Jean Derancourt*, Robert Poulhe, Jean-Paul Capony*, René Ozon and Odile Mulner-Lorillon

Laboratoire de Physiologie de la Reproduction, INRA, UA CNRS 555, 4 place Jussieu, 75252 Paris Cedex 05 and *Centre de Recherches de Biochimie Macromoléculaire, UPR CNRS 41, INSERM U249, Route de Mende BP 5051, 34033 Montpellier, France*

Received 29 May 1989; revised version received 26 June 1989

A high molecular mass complex isolated from *Xenopus laevis* oocytes contains three main proteins, respectively p30, p36 and p47. The p47 protein has been reported to be an in vivo substrate of the cell division control protein kinase $p34^{cdc2}$. From polypeptide sequencing, we now show that the p30 and the p47 correspond to elongation factor EF-1β and EF-1γ. Furthermore, the p30 and p36 proteins were phosphorylated in vitro by casein kinase II.

Meiotic cell division; Elongation factor-1; Kinase, H1; Kinase, $p34^{cdc2}$; Casein kinase II

1. INTRODUCTION

Cell division is controlled by a cytoplasmic factor, MPF, which has been recently purified [1]. It is constituted in part by a protein of 34 kDa, which shares H1 kinase activity and is related to the product of the gene cdc2 controlling the cell division in the yeast, *Schizosaccharomyces pombe* [2–5].

In *Xenopus laevis* oocytes, the phosphorylation of a protein of 47 kDa (p47) is highly correlated with the appearance of MPF [6,7]. p47 is present in a complex containing three main proteins: the p47, a p30 and a p36. The complex was recovered from either prophase-arrested or matured oocytes, the p47 being phosphorylated only in matured oocytes. We further demonstrated that the kinase which is responsible in vivo for the p47 phosphorylation is the $p34^{cdc2}$ [8].

We report here using amino acid analysis of peptides, that the p30 and p47 proteins of the complex are respectively analogous to EF-1β [9] and EF-1γ [10] from *Artemia salina*.

Correspondence address: R. Bellé, Laboratoire de Physiologie de la Reproduction, INRA, UA CNRS 555, 4, place Jussieu, 75252 Paris Cedex 05, France

2. MATERIALS AND METHODS

Ovaries from *Xenopus laevis* were surgically removed from anesthetized adult females and defolliculated full-grown (stage VI) oocytes were prepared as already described [11]. The p47 complex was purified from the cytosoluble fraction by 40–50% $(NH_4)_2SO_4$ precipitation followed by three chromatographic steps, i.e. hydroxyapatite, MonoQ and heparin-Sepharose [8].

After SDS-polyacrylamide gel electrophoresis [12], proteins were electrotransferred onto either Immobilon P (Millipore) or nitrocellulose (Biorad) membranes [13]. The Immobilon P membrane was inserted into the cartridge of a model 470 A Applied Biosystems gas-phase sequencer coupled to a model 120 A PTH analyser for direct sequencing [14]. In situ digestion by *Staphylococcus aureus* protease V8 (Boehringer) (5 μg) or endoprotease Lys.C (5 μg) was performed on nitrocellulose [15]. Resulting peptides were separated by reverse-phase microbore HPLC on an Aquapore RP 300 C8 column (0.21 × 10 cm Brownlee) with a 60 min gradient of 0–60% acetonitrile. Solvent A was 0.1% TFA, solvent B 60% acetonitrile, 0.08% TFA. Flow rate was 0.2 ml/min and detection at 220 nm. Peaks of interest were further purified by reverse-phase HPLC on a C18 microbore column (0.21 × 10 cm Spheri 5 RP 18 Brownlee) us-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

ing the same acetonitrile gradient. Resulting peptides were submitted to gas-phase sequencing using the standard 03 RPTH program.

Purified casein kinase II was prepared according to [16]. Kinase activities were measured in 50 μl final volume containing 50 mM Hepes, 5 mM pNPP, 5 mM Mg^{2+}, 10 μM [γ-^{32}P]ATP (spec. act. ~2 μCi/nmol) and 5 μg of purified p47 fraction in the presence or in the absence of 3 μg/ml heparin. Where indicated, 5 U casein kinase II was added to the incubation medium. After 20 min incubation at 20°C, protein phosphorylation was quantified by TCA precipitation of aliquots of each assay on 3M filters, extensive washing and counting the filter by Cerenkov effect. Proteins were resolved by electrophoresis on 12% polyacrylamide gel according to [12] and radioactive proteins were analysed by autoradiography of the dried gel.

3. RESULTS AND DISCUSSION

Fig.1 shows the electrophoretic pattern of the purified complex from *Xenopus laevis* stage VI oocytes containing the three main polypeptides p47, p36 and p30 [8]. After SDS-PAGE, the proteins were transferred to Immobilon and the p30 and p47 regions submitted to gas-phase sequencing. No amino acid was detected suggesting either the presence of a naturally occurring N-terminal blocking group or that an artefactual block had arisen during electrophoresis [17]. Sequencing of internal peptides was therefore performed after in situ proteolytic cleavage of the proteins transferred onto nitrocellulose blot. Fig.2 shows the HPLC profile of peptides obtained from the p30 and p47 proteins. Four polypeptides from p30 protein were sequenced (see fig.3) and a computer search for sequence similarities in protein data bases was performed. High similitude (over 72% of strict homology) was found with elongation factor EF-1β from shrimp *Artemia salina* [9] as depicted in fig.3. Since EF-1β has been described to form a complex in association with EF-1γ [18], we have searched for homology between p47 polypeptides and EF-1γ [10].

Five out of the six polypeptides sequenced from the p47 protein could be aligned as shown in fig.3. The absence of homology of the sixth peptide suggests the presence of a non-conserved region in the proteins from *Artemia* and *Xenopus*.

Furthermore, the purified complex was occasionally contaminated by a heparin-sensitive protein kinase (see [8] and fig.4A). The endogenous kinase had more or less activity depending on the

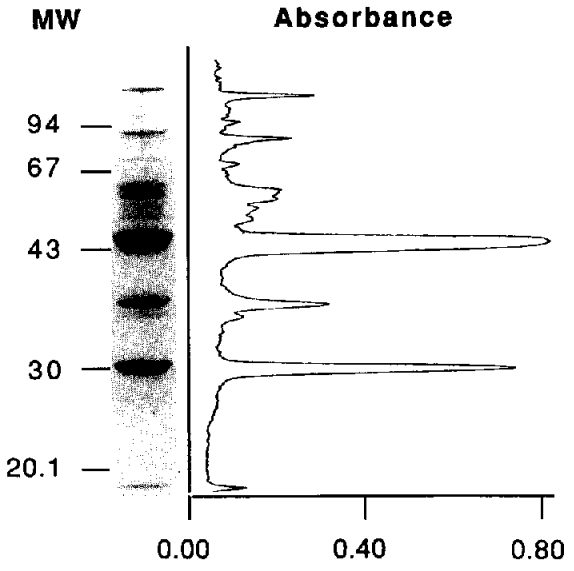

Fig.1. Electrophoretic profile of the Coomassie blue stained proteins of the purified complex from *Xenopus* oocytes, and its densitometric recording.

preparations, but when present, always phosphorylated both p30 and p36 proteins. When highly purified casein kinase II was added to the fraction, again p30 and p36 were phosphorylated whether or not the associated endogenous kinase was present (fig.4B). The phosphorylation of p30

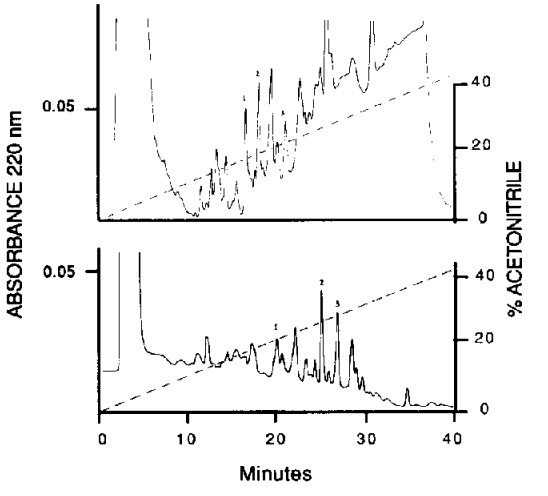

Fig.2. HPLC purification of in situ digestion by *S. aureus* on nitrocellulose blot of p30 protein (upper) and of p47 protein (lower). The numbers indicate sequenced peptides.

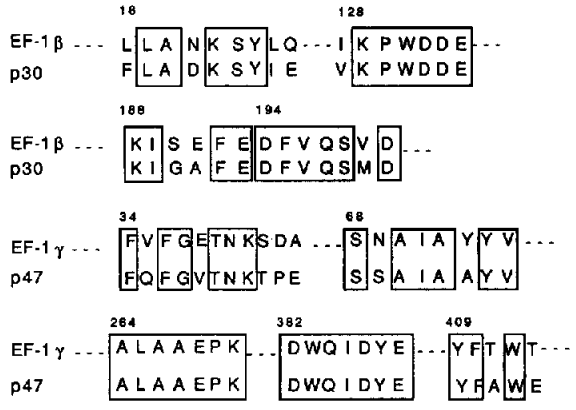

Fig.3. Alignment of p30 and p47 peptides with respectively EF-1β (upper) and EF-1γ (lower) from *Artemia salina*. The numbers indicate the amino acid position in *Artemia* proteins. The p30 peptides correspond with peaks of fig.2 (top panel): 1, VKPWDDE; 2, DFVQSMD and KIGAFE (resolved on the C18 column); 3, FLADKSYIE. The p47 peptides correspond with peaks of fig.2 (bottom panel): 1, FQFGVTNKTPE; 2, DWQIDYE; 3, YFAWE, and a minor peptide: SSAIAAYV. ALAEPK was obtained by endoprotease Lys. C digestion.

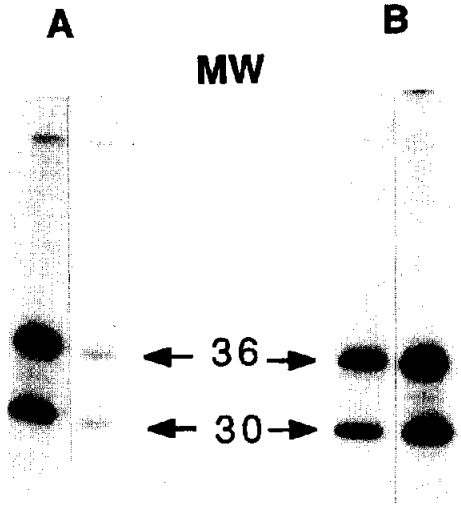

Fig.4. Autoradiography of SDS-PAGE after in vitro phosphorylation of the purified complex from *Xenopus* oocytes. (A) Autophosphorylation of the fraction in the absence (left) and presence (right) of heparin (3 μg/ml). (B) Effect of purified casein kinase II (right) compared to autophosphorylation (left) of the same fraction.

is in concordance with that demonstrated for EF-1β by casein kinase II [19]. It was shown that casein kinase II phosphorylated *Artemia* EF-1β on serine 89. The rate of guanidine nucleotide exchange on EF-1α as catalyzed by EF-1β was found to be affected reversibly by the state of phosphorylation of EF-1β; the exchange rate was higher in the presence of dephosphorylated EF-1β [19]. EF-1γ was reported to have a positive effect on the exchange rate of EF-1α in the presence of EF-1β [20], therefore it is now of interest to investigate the role of phosphorylation on the activity of EF-1γ.

In conclusion, the complex isolated from *Xenopus* oocytes contains p47 and p30 proteins respectively homologous to EF-1γ and EF-1β of *Artemia* which are also closely associated in an EF-1$\beta\gamma$ complex [18]. The p47 protein was demonstrated to be an in vivo substrate of the cell division control protein kinase $p34^{cdc2}$ [8] and p30 and p36 are in vitro substrates of casein kinase II. Since both kinases are activated [21–23] during meiotic cell division in correlation with changes in protein synthesis [24]. our results provide a biochemical link between these protein kinase activities and regulation of protein synthesis.

Acknowledgements: This work was supported by CNRS, INRA and MRES. We thank H. Denis and A. Mazabraud for helpful discussion in the preparation of this article.

REFERENCES

[1] Lohka, M.J., Hayes, M.K. and Maller, J.L. (1988) Proc. Natl. Acad. Sci. USA 85, 3009–3013.
[2] Gautier, J., Norbury, C., Lohka, M., Nurse, P. and Maller, J.L. (1988) Cell 54, 433–439.
[3] Dunphy, W.G., Brizuela, L., Beach, D. and Newport, J. (1988) Cell 54, 423–431.
[4] Labbe, J.C., Lee, M.G., Nurse, P., Picard, A. and Doree, M. (1988) Nature 335, 251–254.
[5] Arion, D., Meijer, L., Brizuela, L. and Beach, D. (1988) Cell 55, 371–378.
[6] Asselin, J., Bellé, R., Boyer, J., Mulner, O. and Ozon, R. (1984) CR Acad. Sci. Paris 299, 127–129.
[7] Ozon, R., Mulner, O., Boyer, J. and Bellé, R. (1987) in: Molecular Regulation of Nuclear Events in Mitosis and Meiosis (Schlegel, R.A. et al. eds) pp.111–130, Academic Press, NY.
[8] Mulner-Lorillon, O., Poulhe, R., Cormier, P., Labbe, J.C., Doree, M. and Bellé, R. (1989) FEBS Lett. 251, 219–224.
[9] Maessen, G.D.F., Amons, R., Maassen, J.A. and Moller, W. (1986) FEBS Lett. 208, 77–83.
[10] Maessen, G.D.F., Amons, R., Zeelen, J.P. and Moller, W. (1987) FEBS Lett. 223, 181–186.
[11] Bellé, R., Mulner-Lorillon, O., Marot, J. and Ozon, R. (1986) Cell Differ. 19, 253–261.

[12] Laemmli, U.K. (1970) Nature 227, 680–685.
[13] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[14] Matsudaira, P. (1987) J. Biol. Chem. 262, 10035–10038.
[15] Aebersold, R.H., Leavitt, J., Saaverdra, R.A., Hood, L.E. and Kent, S. (1987) Proc. Natl. Acad. Sci. USA 84, 6970–6974.
[16] Mulner-Lorillon, O., Marot, J., Cayla, X., Poulhe, R. and Bellé, R. (1988) Eur. J. Biochem. 171, 107–117.
[17] Moos, M., Nguyen, N.Y and Liu, T.Y. (1988) J. Biol. Chem. 263, 6005–6008.
[18] Janssen, G.M.C. and Moller, W. (1988) Eur. J. Biochem. 171, 119–129.
[19] Janssen, G.M.C., Maessen, G.D.F., Amons, R. and Moller, W. (1988) J. Biol. Chem. 263, 11063–11066.
[20] Janssen, G.M.C. and Möller, W. (1988) J. Biol. Chem. 263, 1773–1778.
[21] Meijer, L., Pelech, S.L. and Krebs, E.G. (1987) Biochemistry 26, 7968–7974.
[22] Cicirelli, M.F., Pelech, S.L. and Krebs, E.G. (1988) J. Biol. Chem. 263, 2009–2019.
[23] Labbe, J.C., Picard, A., Karsenti, E. and Doree, M. (1988) Dev. Biol. 127, 157–169.
[24] Wasserman, W.J., Richter, J.D. and Smith, L.D. (1982) Dev. Biol. 89, 152–158.