Monoclonal Antibody Register

Specification of the Monoclonal Antibody PK1 Reactivity in Chinese Hamster Ovary Cells

J. HAŠEK[1], L. PEŘINKA[2], L. VALÁŠEK[1]

[1]Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[2]1st Faculty of Medicine, Charles University, Prague, Czech Republic

Background

eIF3a/Rpg1p/Tif32p is the evolutionarily conserved subunit of yeast eukaryotic translation initiation factor 3 (eIF3) that promotes multiple steps of the initiation pathway resulting in formation of the 48S pre-initiation complex at the AUG start codon of mRNA.

The monoclonal antibody PK1 (IgG_1 kappa) (Jiřincová et al., 1998) against the recombinant protein Rpg1 (Kovarik et al., 1998) has already proved itself as a useful tool for analysing the eIF3 complex in *S. cerevisiae* (Valášek et al., 1998). It binds either to native or denatured epitopes of Rpg1p and it can be used to efficiently isolate the eIF3 complex by immunoprecipitation (Valášek et al., 1999). This antibody was also used to show by immunofluorescence that yeast eIF3a is a microtubule-interacting protein (Hašek et al., 2000). In order to test whether this characteristic of yeast eIF3a is also conserved in higher eukaryotes, we analysed the immunoreactivity of the anti-Rpg1p monoclonal antibody (PK1) in Chinese hamster CHO, human HeLa and mouse 3T3 cells.

Reactivity of mAb PK1

Immunoblotting

We performed Western blot analysis of the whole cell extracts (WCE) obtained from the control *S. cerevisiae*, CHO, human HeLa and mouse 3T3 cells (Fig. 1, and not shown). As expected, mAb PK1 recognized a single 120-kD protein corresponding to yeast eIF3a (Fig. 1, lane 3) (Jiřincová et al., 1998). Remarkably, PK1 showed highly specific reaction with a single protein of molecular weight (MW) of approximately 170 kD in the WCE of CHO cells (Fig. 1, lane 4). It should be noted that the expected MW of mammalian eIF3a subunit is around 170 kD. Thus the consistency in molecular size and the specificity of the reaction indicate that the 170-kD protein might represent a Chinese hamster orthologue CH-p170 of yeast eIF3a/Rpg1/Tif32p. We observed no reaction in the WCEs from HeLa and 3T3 cells (data not shown), suggesting that the PK1 recognizable epitopes are either masked or significantly altered in both human and mouse orthologues of eIF3a. In accordance, it was previously shown that PK1 failed to recognize a recombinant human eIF3a orthologue expressed in yeast (Valášek et al., 1998).

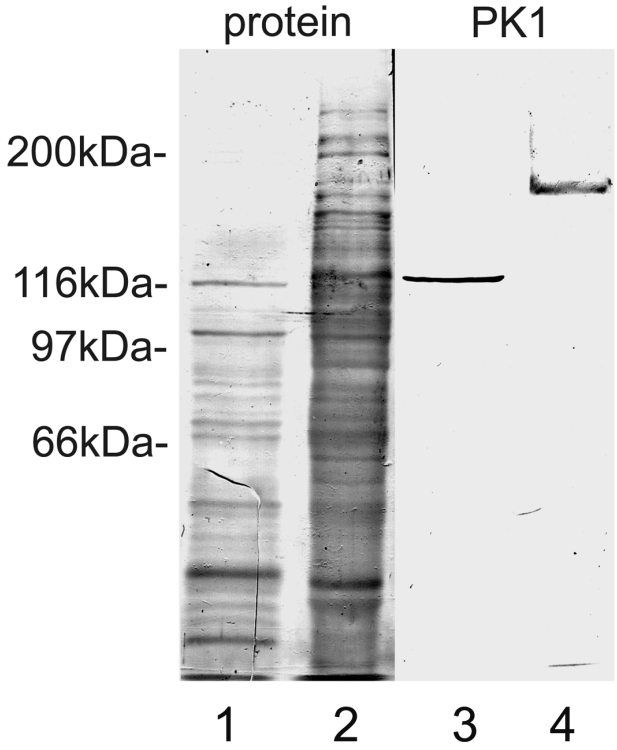

Fig. 1. Western blot analysis of whole cell lysates of *S. cerevisiae* (lanes 1 and 3) and CHO cells (lanes 2 and 4) with the monoclonal antibody PK1 against *S. cerevisiae* eIF3a/Rpg1p. Proteins were separated on 8% SDS gels.

Immunofluorescence

eIF3a/Rpg1p/Tif32p, the largest subunit of yeast eIF3, was found to associate with yeast microtubules

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Corresponding Author: Jiří Hašek, Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague, Czech Republic. Fax: (+420) 241 062 501; e-mail: hasek@biomed.cas.cz.

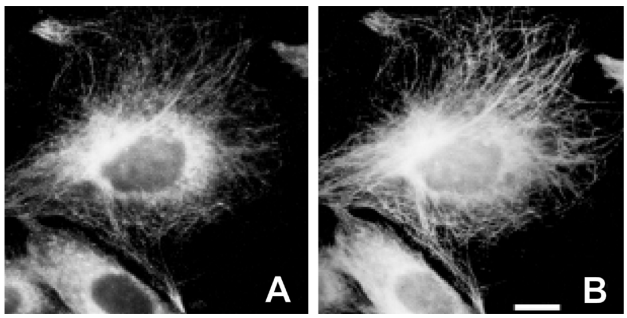

Fig. 2. Double labelling of CHO cells with the monoclonal antibody PK1 against eIF3a/Rpg1p (A) and the rabbit polyclonal anti-tubulin antibody rb4 (B) (kindly provided by Dr. Wiche, Austria). Bar 10 μm.

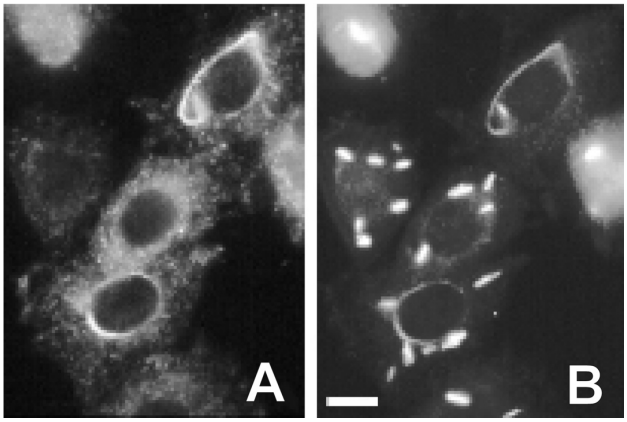

Fig. 3. CHO cells treated with vinblastine sulphate (10^{-4} M for 4 h) and double-labelled with the mAb PK1 (A) or the anti-tubulin antibody rb4 (B). Bar 10 μm.

(Hašek et al., 2000) similarly to the human p170 orthologue (Ivanov et al., 2003). We show here that CH-p170 closely co-localized with CHO microtubules, displaying a specific dotted pattern along mirotubules (Fig. 2). Control experiments showed no significant overspill of fluorescence from one optical filter system to the other (data not shown). Labelling of coiled structures instead of paracrystals in vinblastine-treated CHO cells indicates a vimentin-like behavior of the PK1-recognized protein (Fig. 3).

Properties

Despite the fact that we cannot completely rule out the possibility that the PK1 antibody just labels a single conserved epitope shared by eIF3a/Rpg1/Tif32p and CH-p170, we believe that our results provide support for our suggestion that p170 of CHO cells is a true orthologue of *S. cerevisiae* eIF3a. Thus, the availability of a highly specific antibody against CH-p170 may contribute to the investigation of the eIF3a function in CHO cells.

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