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Human myotonic dystrophy protein kinase effect in *S. cerevisiae*

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Abstract

Human myotonic dystrophy protein kinase (DMPK), the product of the myotonic dystrophy (DM) locus, is a member of a novel class of multidomain serine–threonine protein kinases, which interacts with members of the Rho family of small GTPases. DMPK has been shown to affect the cell growth, size and shape in different organisms, from fission yeast to man, but its physiological role is still unclear. We examined the effect of the overexpression of two forms of human DMPK, full-length (DMFL) and a C-terminal truncated form (DMT) on the growth and cell morphology of *S. cerevisiae*, which possesses a DMPK homologous gene (CBK1) important for polarized growth and cell division. We report that the overexpression of either forms of human DMPK did not complement the CBK1 function in the haploid strain WR208-1a, deleted for CBK1. The truncated form, but not the full length one, slowed down growth rate and induced elongation of the haploid wild type strain CBK1. Similar results were obtained in the diploid wild type strain RS112 of *S. cerevisiae* where also the full-length form was effective. These effects were abolished when either DMFL or DMT were mutated in the ATP binding site (K100R mutation), suggesting that the kinase activity of DMPK is required. Interestingly, DMPK localization in yeast is similar to that of Cbk1 protein suggesting that it might affect a pathway, which regulates cell morphogenesis and progression through cell cycle, possibly involving CBK1.

Keywords: *S. cerevisiae*; DMPK; CBK; Polarized growth

1. Introduction

Myotonic dystrophy (DM) is an autosomal dominant human disorder, caused by the abnormal expansion of a CTG trinucleotide repeat in the 3′-untranslated region (3′-UTR) of the protein kinase gene (DMPK) [1–4]. It has recently been shown the structural homology between DMPK and protein kinases of the Rho family (Rho-kinase), which have an important role in the organization of the cytoskeleton, that is an essential structure for several cellular processes including cell growth, cell differentiation, intracellular transport and locomotion [5,6]. An involvement of DMPK in the cytoskeleton organization during differentiation could explain in part the pleiotropic effects associated with the decreased levels of DMPK protein in Steinert patients [7]. Moreover, in vitro studies indicate that DMPK can directly associate with the cytoskeleton linked GTPase Rac-1, and cotransfection experiments show that this association leads to the activation of DMPK, supporting the hypothesis that this protein kinase is involved in actin cytoskeletal reorganization [8]. In a previous work, we have found that DMPK in human myoblasts is localized to the terminal part of the cell during differentiation, suggesting that it might have a role in the cytoskeleton organization taking place during cell elongation (Beffy et al., submitted for publication). A role of DMPK in myoblast differentiation has been suggested by the finding that DM myoblasts differentiate to myotubes to a lesser degree than do control cells [9]. On the contrary, the overexpression of

Abbreviations: DM, Myotonic dystrophy; DMPK, myotonic dystrophy protein kinase; DMFL, full-length myotonic dystrophy; DMT, truncated myotonic dystrophy

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DMPK in murine myoblasts cells induces differentiation [10]. DMPK shows homology with a large family of serine–threonine protein kinases conserved from yeast to man, which regulate polarized growth, proliferation and division, such as Orb6 of *S. pombe* [11], Cbk1p of *S. cerevisiae* [12], Cot-1 in *N. crassa* [13] and Ndr/Warts in *Drosophila* [14,15]. It is known that many yeast proteins, involved in the signal transduction pathway for polarized growth, have functional homologous proteins in higher eukaryotes, suggesting that molecular mechanisms underlying these processes are highly conserved [16,17]. Understanding the regulation of apical growth and identifying the molecules that participate in this process in yeast could provide insights into how morphogenesis is controlled and coordinated with other cellular events in higher eukaryotes. The morphology of *S. cerevisiae* cells is normally determined during bud growth [18]. Buds growth usually occurs in two phases, the apical phase, where early growth is restricted to the bud tip and the isotropic phase, during which growth takes place over the entire bud surface [19]. In *S. cerevisiae*, the CBK1 gene encodes a protein kinase, which is supposed to have a role both in the regulation of chitinase expression, via phosphorylation of transcription factor Ace2p, and in polarized growth [12]. The deletion of CBK1 gene in strains that have a bipolar budding pattern gives rise to a random budding pattern and round rather than ellipsoidal cells, showing that Cbk1p is an important factor in the establishment of cellular morphology, although its exact role in this pathway is not completely clear.

In this paper, we investigated the effect of human DMPK on the cell growth and cellular morphology of wild type *S. cerevisiae* and a mutant lacking the DMPK homologous gene, CBK1.

Six major isoforms of DMPK are produced in transgenic mice overexpressing human DMPK, by a combination of three alternative splicing events, however it is not known whether all of these forms are expressed in human tissues [20]. In vitro experiments have shown that the full-length DMPK (DMFL) form is a membrane-associated, poorly soluble protein, with low activity; while the elimination of the C-terminal domain, due to specific proteases, confers the protein a cytoplasmic localization and induces a three-fold increase in enzymatic activity [21]. As yeast might not be able to generate the truncated form, both DMFL and a truncated form (DMT), lacking part of the COOH terminal domain [21], were expressed in *S. cerevisiae.* Our results show that neither forms of DMPK complemented Cbk1p function. However, the expression of DMPK in the wild type haploid strain YPH449 (CBK1) and diploid strain RS112 induced a reduced growth rate and elongated phenotype, suggesting that the product of CBK1 might be necessary for DMPK to exert its effect in yeast. Moreover the reduced growth rate and elongated phenotype were reversed when a point mutation in the ATP binding site (K100R) was introduced, suggesting that kinase activity was necessary to induce both effects.

2. Materials and methods

2.1. Yeast strains and growth conditions

The diploid strain RS112 of *S. cerevisiae* (MATa/α ura3-52/ura3-52, leu2-3, 112/leu2-Δ98 trp5-27/TRP arg4-3/ARG ade2-40/ade2-101 ilv-92/ILV HIS3: pRS6/his3-Δ200 LYS/lys2-801) was provided by Dr. Robert Schiestl (UCLA Los Angels, CA, USA) [22]. The haploid strains YPH449 (MATa ade2-201 his3-200 leu2-1 ura3-52 trp1-63 lys2-801) (CBK1) and WR208-1a (MATa ade2-201 his3-200 leu2-1 ura3-52 trp1-63 lys2-801 cbk1: kanMX4) (Δcbk1) were provided by Dr. Christopher Herbert (Gif sur-Yvette, France) [12]. The complete medium (YAPD) was: yeast extract 1%, peptone 2%, glucose or galactose 2%, adenine sulfate 30 mg/l, agar 2%; the minimal selective medium (SC-URA) was: 0.67% yeast nitrogen base, 2% glucose and 1% raffinose. Cells were routinely grown in minimal selective liquid medium (SC-URA), up to the logarithmic phase (about $6-7\times10^7$ cells/ml). To induce DMPK expression, cultures were prepared in YAPD liquid medium (starting from a fresh SC-URA overnight culture) plus galactose 2% and raffinose 1% to have an OD_{600} of about 0.04–0.05, and aliquots of cells were harvested after 15 h (about $4-5\times10^7$ cells/ml), 18 h (about $6-7\times10^7$ cells/ml), 22 h (about 1.2×10^8 cells/ml) or 40 h (about 1.5×10^8 cells/ml) of growth.

2.2. Cloning of DMPK isoforms in E. coli and S. cerevisiae by Gateway technology

DMPK proteins expressed are the full-length human DMPK, DMFL (isoform I, L00727 of the published sequence) [7] and a truncated variant (DMT) lacking the C terminal membrane association domain supposed to have auto inhibitory activity [21] (Fig. 1).

For cloning and expressing DMPK in bacteria and *S. cerevisiae*, Gateway technology, a novel universal system for gene cloning and protein expression, was used (Life Technologies, Invitrogen) [23]. Both DMT or DMFL were amplified from pRSETc carrying the whole cDNA gene of human DMPK (kindly supplied by Prof. Puymirat, Canada) using the forward primer A composed by the $attB_1$ sequence: 5′-GGGG ACAAGTTTGTACAAAAAAGCAGGCT plus Shine Dalgarno-Kozak sequence and 21 bases homologous to the 1–21 5′ sequence of DMPK (ATGGGAGGGCATTTTTGGCCC). Reverse primers were composed by the 3′ $attB_2$: GGGG ACCACTTTGTACAAGAAAGCTGGGT GAC plus 21 bases homologous to 1612–1632 (GACAGCTGTGGCTCCCTCTGC) for DMT (primer B) or 18 bases homologous to 1900–1917 (GGGAGCGCGGGCGGCTCC) for DMFL (primer C) (Fig. 1).

DMPK kinase activity was abolished by introducing a point mutation, which converts lysine to arginine in the ATP-binding consensus sequence and produces the dead kinase K100R mutant described by Sasagawa et al. [28]. PCR primers used were the forward primer D 5′-

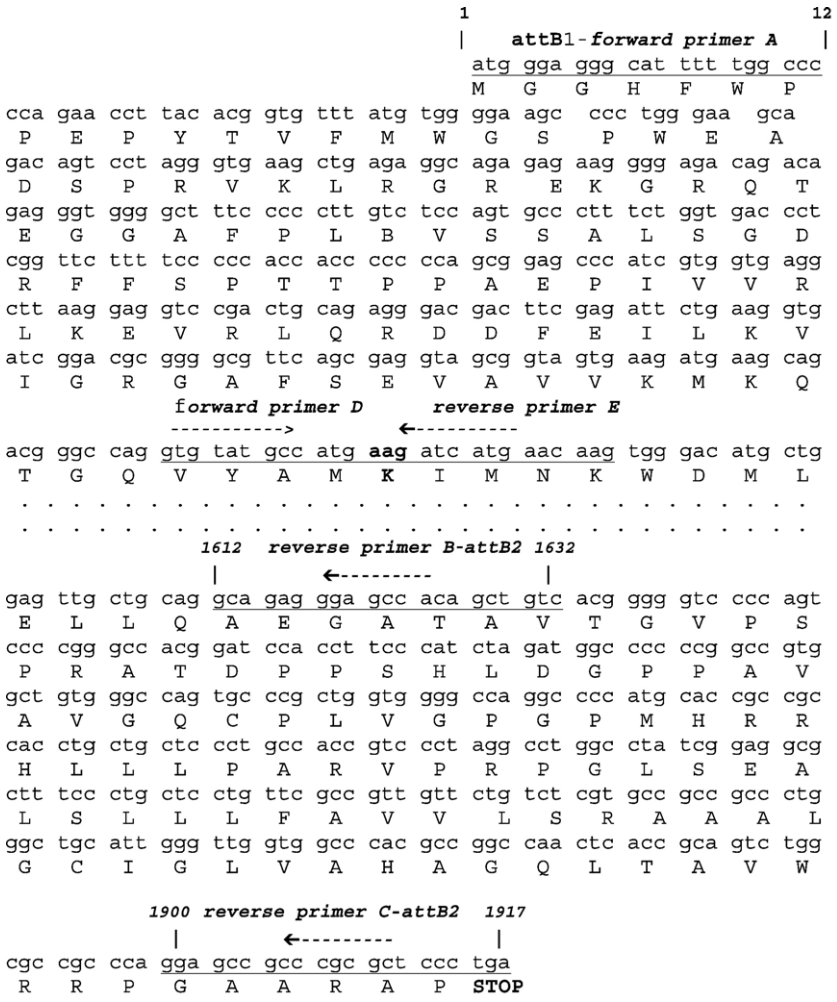

Fig. 1. Amino acid sequence of DMFL protein kinase. The sequences of primers bound to attB1–attB2 used to amplify DMFL, forward primer (A) (1–21) and reverse (C) (1900–1917), or DMT, forward primer (A) (1–21) and reverse (B) (1612–1632), are underlined. Primers forward D and reverse E, used to introduce mutation in K100R (bold), are reported.

GTGTATGCCATGcgcATCATGAACAAG-3′ and the reverse primer E 5′-CTTGTTCATGATgcgCATGGCATA-CAC-3′ (small letters represent the mutation). When the mutation is successfully introduced, a new site for the restriction enzyme *Fsp*I is produced. Primers A and E were applied to amplify the N-terminus of the mutated DMPK; primers D and B (for the truncated) or D and C (for the full length) were applied to amplify the C-terminus of the mutated DMPK. N- and C-terminus fragments were purified from the gel, mixed and reamplified with A and B (truncated) or A and C (full length) to produce the ligated K100R mutated forms.

Standard PCR conditions were used to prepare attB-cDNA products from human DMPK using 1 μl of Hot start Taq polymerase (Qiagen, Germany). Optimized cycling conditions were: starting activation at 95 °C (15′) and then 30 cycles (20 cycles for the mutated forms) of denaturation at 95 °C (30′), annealing at 68 °C (30′), extension at 75 °C (5′) followed by a final extension at 72 °C (15′). PCR products were purified by geneclean to remove the excess of unbound attB primers. To insert PCR products into pDEST17 expression vectors carrying 6× his-tail, the Gateway procedure was used. AttB-PCR-DMFL or -DMT, wild type and mutated, was cloned first in the Entry clone by BP clonase reaction using attb-PCR-DMPKs 150 ng, pDONOR vector 350 ng and 4 μl BP clonase. Entry clone was used to introduce the different forms of DMPK into bacteria pDEST17 or yeast pYES-DEST52 (p52) expression vectors by LR clonase reaction: 150 ng of entry clone, 300 ng of pDEST17 or p52, and 4 μl LR clonase were incubated at 25° C about for 2 h. All clones were sequenced and compared to the published sequence to verify authenticity.

2.3. DMPKs expression and purification from bacteria and production of polyclonal antibodies

The expression plasmid pDEST17 (100 ng) was used to transform One Shot competent *E. coli* (Invitrogen) to propagate and select the clones and BL21-SI competent *E. coli* (Invitrogen) for protein expression. The induction of DMT was obtained by the addition of 0.3 M NaCl. The recombinant human DMT was purified at the optimal

expression time (after 4 h of induction in NaCl) on nickel resin "Xpress system nickel-ProBond resin" (Invitrogen), and used to immunize rabbits to produce specific rabbit antisera anti-hDMT (Rb anti-DMT) according to described procedure (Beffy et al., submitted for publication).

The strains RS112, CBK1 and Δcbk1 were transformed with p52-DMPKs using the lithium acetate procedure as previously described [24] and transformants were selected on glucose SC-URA plates.

2.4. Protein extract preparation and Western blot

To investigate the kinetics of DMPKs induction, aliquots of *S. cerevisiae* cells were harvested during logarithmic growth (OD from 0.1 to about 1.8). For each time point, about 10^8 cells were suspended in 0.5 ml of buffer [50 mM KCl, 5 mM $MgCl_2$, 0.1 M EDTA, 25 mM HEPES, 5 mM DDT, 0.3 M $(NH_4)_2SO_4$, 10% glycerol, pH 7.4] and 10 μl of protease inhibitors solution [4.4 mg PMSF, 62 mg pepsatin, 50 mg chemostatin and 725 μl DMSO in 1 ml H_2O]. Cell lysis was performed by breaking cell walls by vortexing 30′ for 5 times with glass beads (425–600 μm). 40 μg of protein extracts was electrophoresed on 10% SDS-polyacrylamide gel and blotted onto nitrocellulose. After the transfer, membranes were blocked for 90′ in 3% BSA in PBS (137 mM NaCl, 2.7 mM KCl, 10.4 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.5) and incubated for 1 h with anti-DMT diluted 1:500 in TPBS (0.2% Tween 20) plus 1% BSA. An anti-rabbit IgG1 antibody coupled to horseradish peroxidase (Sigma, USA) was used at a dilution 1:50,000 in TPBS for 1 h. Bound antibody was detected by using the Super Signal West Dura Extended Duration Chemiluminescence Substrate detection system (Pierce, USA).

2.5. Morphological analysis and immunohistochemistry

Slides for morphological analysis and immunolocalization were prepared as follows: exponentially growing cells were collected and fixed, adding formaldehyde directly to the medium (final concentration 3.7%) and incubated for 1 h at room temperature. For cell shape analysis, cells were layered on polylysine slides, which were frozen in dry ice, and observed with a photo microscopy Zeiss (800×); images were recorded and the length (long axis relative to the birth pole) and width (maximum distance perpendicular to the length) of the cells were measured by Imagepoint Lab Spectrum software (Signal Analytics Corporation). Statistical analysis was performed on the ratio length/width by Student's *t*-test.

For immunolocalization, the cells were washed once with PBS, suspended in 1 ml of PBS containing 50 μg/ml zymolyase and 2 μg/ml β-mercaptoethanol and incubated for 30′ at 30 °C. Lysed cells were washed, blocked for 1 h in 10% horse serum, and incubated with anti-DMT antibody (1:300) overnight. After washing the cells, goat anti-rabbit antibody IgG coupled to fluorescein isothiocyanate (Santa-Cruz Biotechnology, Germany) was used at a dilution 1:200 for 1 h. 50 μl of the immunostained cells was layered on polylysine slides, frozen in dry ice then treated with 100 μl of the anti-fading DABCO (diazabicilooctane) and observed by fluorescent microscopy (Zeiss).

3. Results

3.1. DMPK expression in the haploid Δcbk, the wild type CBK, and diploid RS112 strains of S. cerevisiae

The deletion of the CBK1 gene in strains that have a bipolar budding pattern gives rise to a random budding pattern and round rather than ellipsoidal cells. As Cbk1p presents a high homology with the human myotonic dystrophy protein kinase, it was interesting to investigate whether DMFL or DMT could complement Δcbk1 phenotype. DMT was chosen as it lacks the COOH domain spanning from amino acids 544 to 625 and it was shown in vitro to have higher enzymatic activity in comparison to the full-length protein. Moreover, this form has been found in human heart extracts, when protease inhibitors were not added to the extraction buffer, suggesting a physiological role of this COOH truncated protein [21]. The effect of human DMPK overexpression was studied in the haploid Δcbk1, in the parental strain CBK1 and in the diploid RS112 strain. The time course of DMT and DMFL expression in the 3 different strains of *S. cerevisiae* was studied by Western blotting analysis of cytosolic extracts of

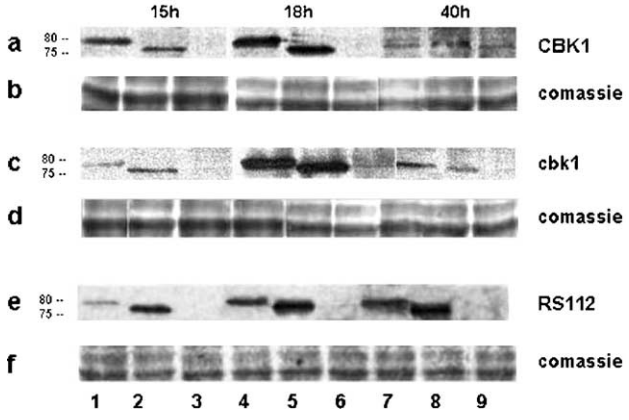

Fig. 2. Kinetic of DMT and DMFL expression in CBK1 (a) or Δcbk1 (c) or RS112 (e) strains. Western blotting of total protein extracts of exponentially growing cells transformed with the p52 plasmid carrying the DMFL (lanes 1, 4, and 7) or DMT (lanes 2, 5, and 8) probed with polyclonal anti-human DMT antibody. Protein extracts, prepared from both strains transformed with void plasmid, were used as negative control (lanes 3, 6, and 9). Cells were harvested at different times of growth: 15 h (about $4-5\times10^7$ cells/ml), lanes 1, 2, and 3; 18 h (about $6-7\times10^7$ cells/ml), lanes 4, 5, and 6; and 40 h (about 1.2×10^8 cells/ml), lanes 7, 8, and 9. 40 μg of total proteins was loaded for each sample. A protein of about ≅80 kDa is detected in extracts of cells expressing DMFL and a lower band ≅75 kDa in samples expressing DMT. Coomassie brilliant blue-stained of the same SDS-PAGE used for the Western blotting experiment is shown for comparison (b, d, f).

cells harvested at different times of growth in galactose medium. Under the culture conditions and cell density described in the experimental procedure, the optimal expression level was reached in all cases, during the logarithmic growth phase, i.e. after about 18 h of growth ($6-7\times10^7$ cells/ml) (Fig. 2a, c, e, lanes 4, 5).

3.2. Morphological alterations induced by DMPK in the haploid Δcbk1, wild type CBK1, and diploid RS112 strain of S. cerevisiae

As the structural homology between DMPK with protein kinases of the Rho family (Rho-kinase) [25] suggests that this enzyme could be involved in the organization of the cytoskeleton, we examined whether DMPK overexpression could complement Δcbk1 phenotype or alter in any way cell morphology. Although DMFL and DMT are well expressed in Δcbk1 (Fig. 2c) neither one of the two forms of DMPK rescues the Δcbk1 phenotype as far as cellular aggregates and random budding pattern (Fig. 3) characteristic of this strain. Interestingly, microscopic examination revealed that the expression of DMPK in either the wild type haploid strain CBK1 (Fig. 3) or the diploid RS112 (Fig. 4) increased the length/width ratio, while no increase in cell length was detected in Δcbk1 (Fig. 3). To quantify this effect, the length of the major axis and width of the minor axis of 100

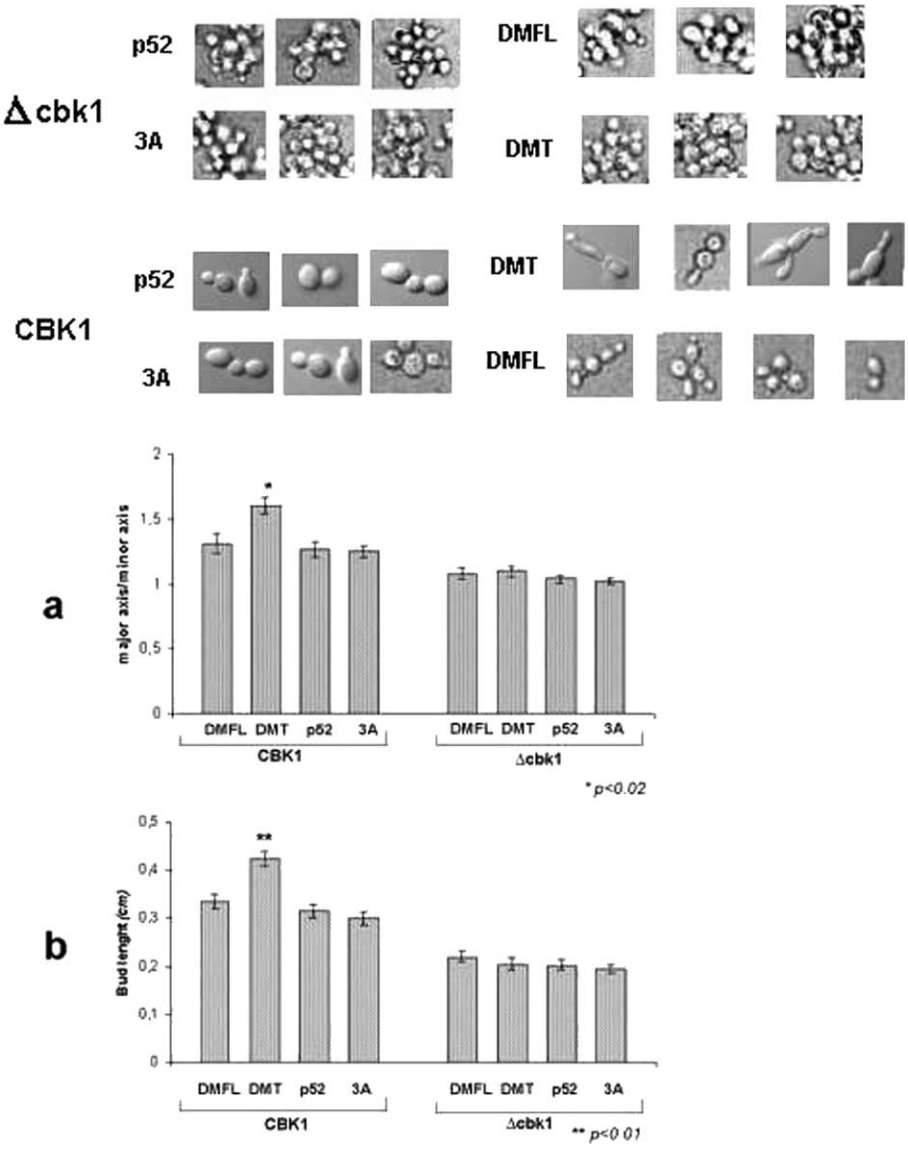

Fig. 3. Morphological analysis of CBK1 or Δcbk1 expressing DMFL or DMT. Exponentially growing cells, transformed with DMFL, DMT, void plasmid p52 or CYP3A were layered on polylysine slides, examined at 800× from images recorded by a photomicroscopy (Zeiss) and Imagepoint Lab Spectrum software (Signal Analytics Corporation). The length of the major axis and width of the minor axis of 100 randomly selected cells counted on different slides from three different cultures were measured and the distributions of the major/minor axis ratio ± standard deviation are reported in histogram (a). The effect of DMPK on bud elongation was studied by determining the length of the bud major axis of 100 randomly selected cells as reported in histogram (b). Statistical significance was determined by Student's *t*-test. $*P<0.02$; $**P<0.001$.

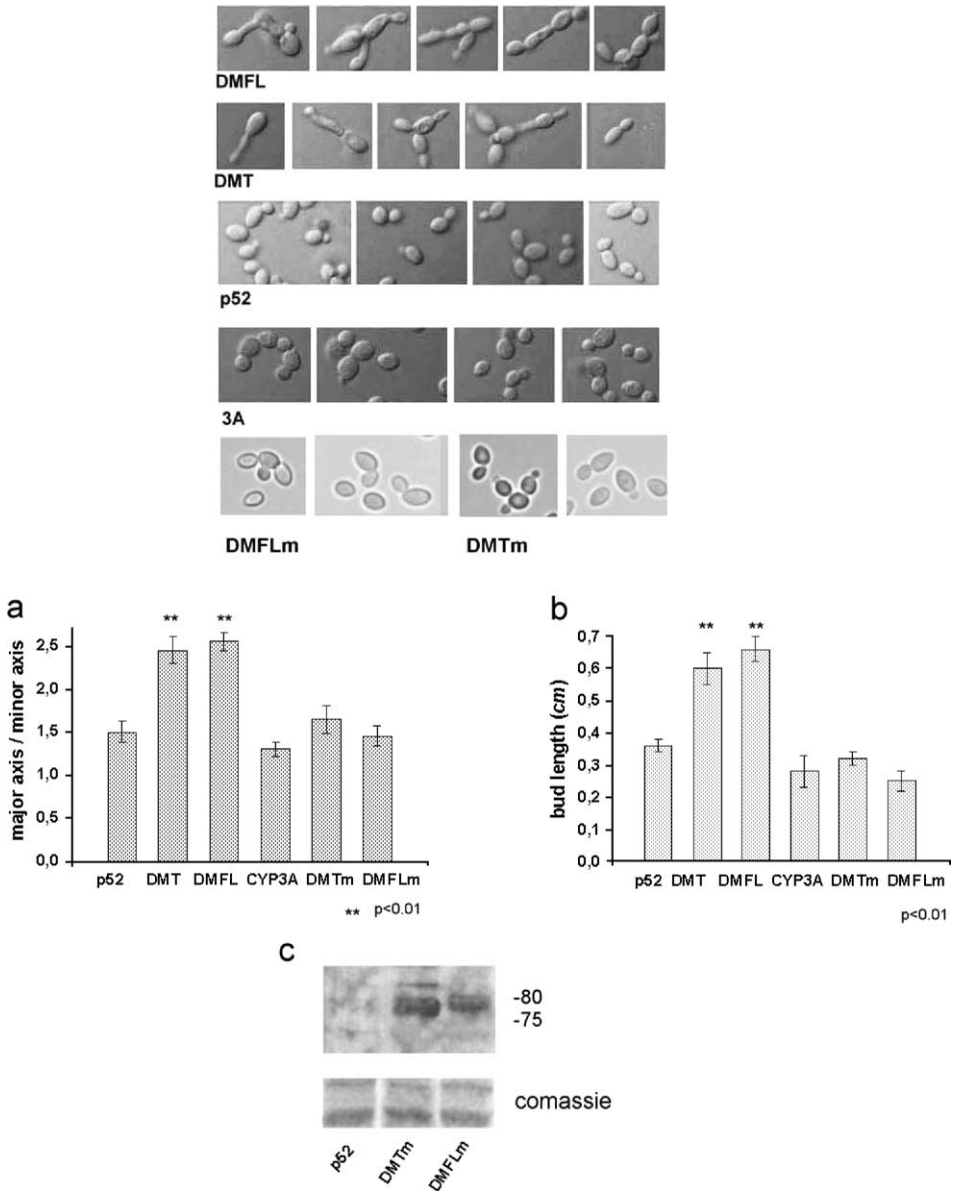

Fig. 4. Morphological analysis of RS112 cells expressing DMFL or DMT. RS112 exponentially growing cells transformed with DMFL, DMT, DMFLm, DMTm, void plasmid p52 or CYP3A were layered on polylysine slides, examined at 800× from images recorded by a photo microscopy (Zeiss) and analyzed by Imagepoint Lab Spectrum software (Signal Analytics Corporation). The length of the cells and buds was measured as described in Fig. 3. Experiments were repeated three times and different slides from three different culture were selected for each sample. The distribution of the major/minor axis ratio (a) and bud length (b) ±standard deviation are reported in the histograms. Statistical significance was determined by Student's *t*-test. $^{**}P<0.001$. The expression of DMTm and DMFLm after 18 h is reported in panel c; the Coomassie brilliant blue-stained of the same SDS-PAGE used for the Western blotting experiment is shown for comparison.

randomly selected cells from the three strains, chosen on different slides from four different cultures, were measured. As reported in the histogram (Fig. 3, histogram a) the length/width ratio of CBK1 transformed with DMT was significantly increased to 1.6 in comparison to cells transformed with the p52 void plasmid (1.25). However, no significant increase was seen in cells transformed with DMFL or with the human unrelated protein cytochrome P450 3A (CYP 3A) cloned in the same plasmid (manuscript in preparation). The effect of DMPK on cell morphology was more pronounced in the RS112 strain, where the overespression of either DMFL or DMT increased the length/width ratio to about 2.5 (Fig. 4, histogram a), while the average length/width ratio of cells transformed with the void plasmid or with CYP 3A is around 1.4. It is interesting to note that the effect on cell length was always accompanied to the induction of more than one bud per cell (Figs. 3 and 4). Our findings suggested that the expression of DMPK increased cell length probably by affecting apical growth. In *S. cerevisiae*, apical growth is restricted to the bud tip, as the bud enlarges, growth enters a second phase, the isotropic phase, during which growth involves the entire

bud surface. To further investigate and confirm the effect on apical growth, we measured the bud length, which is significantly increased in CBK cells expressing DMT (Fig. 3, histogram b) and in RS112 expressing both DMFL and DMT (Fig. 4, histogram b). The effects on cell morphology could be either caused by the kinase activity of DMPK or by an unknown structural role of this protein in yeast. To distinguish between these two possibilities, a point mutation was introduced in the ATP binding site of DMFL and DMT, which converts the amino acid lysine to arginine, resulting in the total inhibition of kinase activity [28]. The expression of DMFLm and DMTm in RS112 shows that the mutated proteins are expressed at the same levels as the wild type ones (Fig. 4c), however, no significant effect on cell morphology was detected (Fig. 4, histograms a and b).

Interestingly, the induction of cell and bud elongation correlates with a decrease of cell proliferation. In CBK1 strain only the truncated form DMT, but not DMFL, decreases growth rate (Fig. 5a) while the overexpression of either forms of DMPKs decreases significantly the cell growth of RS112 (Fig. 5b). The effect is especially evident between 10 and 30 h and it appears to be specific for DMPK, as the expression of an unrelated heterologous protein Cyp3A did not affect growth. Moreover, the overexpression of neither DMFLm nor DMTm inhibits cell growth (Fig. 5b), suggesting that the kinase activity of DMPK is required to affect both cell morphology and cell proliferation.

The expression of either DMFL or DMT did not have any detectable effect on the growth rate of Δcbk1 (data not shown) in keeping with the idea that inhibition of growth rate correlates with induction of cell and bud elongation.

3.3. Subcellular localization of DMPK

To examine the subcellular localization of DMPKs in RS112 and CBK1, we carried out an immunofluorescence analysis using a fixation method that preserves the cell wall. In Fig. 6, cells representative of different stages of the cell cycle are shown. In RS112, the localization of DMT and DMFL appears to be similar: it is cell cycle dependent and distributed in a punctuate pattern around the plasma membrane during the G1 phase and in proximity of the

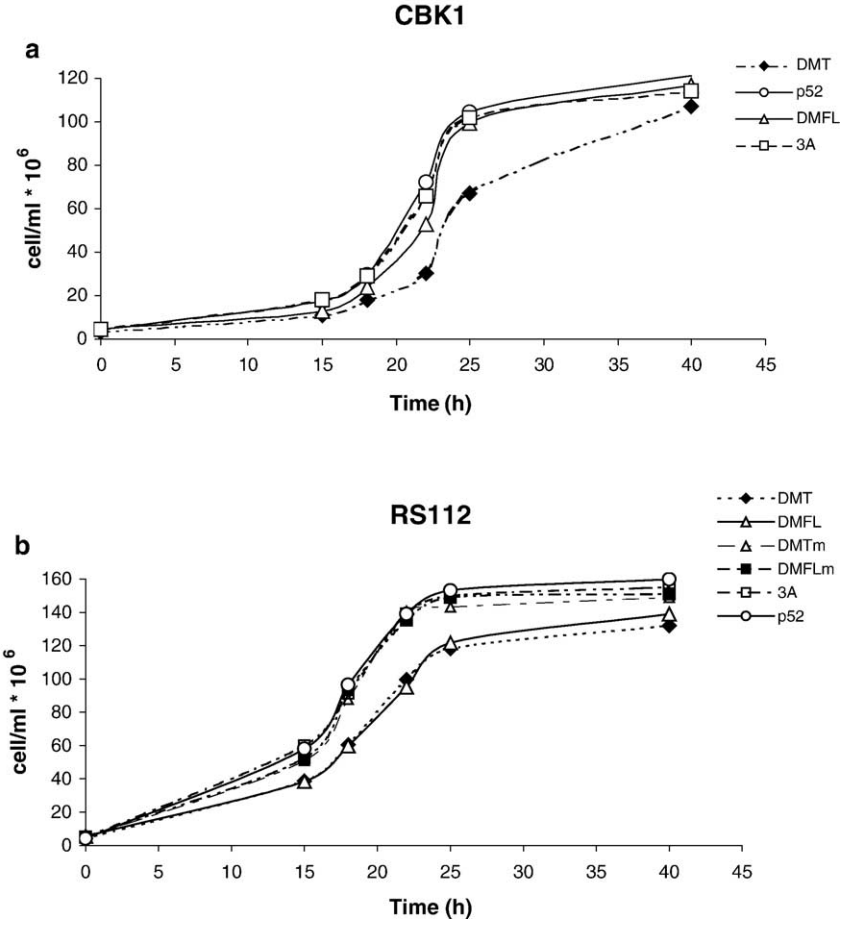

Fig. 5. Effect of DMFL and DMT expression on CBK1 or RS112 yeast cells growth. Cells were transformed with void plasmid p52 or with p52 carrying DMFL, DMT, DMFLm, DMTm or an unrelated protein CYP3A. Aliquots of cells were harvested after 15, 18, 22, 25, and 40 h. The *x* axis indicates time (h), and *y* axis the cell/ml × 10^6. Results are the mean of four independent experiments ± standard deviation.

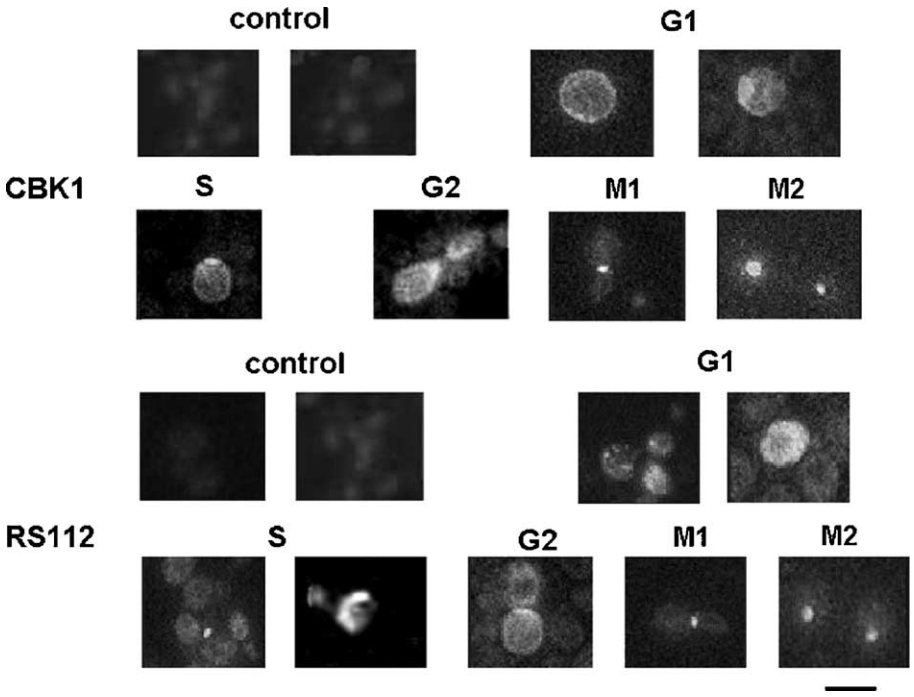

Fig. 6. Immunolocalization of human DMPK expressed in CBK1 and RS112 during the cell cycle. Cells were immunostained with anti-DMT antibody followed by goat anti-rabbit IgG FITC-conjugated. Representative cells from an exponentially growing population were fixed in different stages of the cell cycle. Representative cells from G1, S, G2, M1 and M2 phases are reported. DMPK localizes on both sides of the junction between mother and daughters, after cell division. Bar = 10 μm.

emerging bud sites during the S phase. As the bud grows during the G2 phase, DMPK is present in the whole bud. During and after division (M1, M2), it appears on both sides of the junction between mother and daughter cells (Fig. 6, upper panel). This subcellular localization pattern is similar to the one described for cbk1 protein [12]. In CBK1, the DMT form has a cell cycle-dependent localization essentially similar to that observed for RS112 strain (Fig. 6, lower panel), while no specific localization was observed for DMFL (data not shown).

4. Discussion

Cellular morphology, polarity and shape in eukaryotic organisms are complex processes controlled by intricate signaling pathways and are fundamental for cell growth and other cellular function such as cell locomotion and differentiation [18,26]. Since it has been suggested that DMPK exerts a role on cellular growth and differentiation [3,27], we studied the effect of overexpressing two isoforms of human DMPK, DMT and DMFL, on the cell growth and morphology of *S. cerevisiae*. Overall, our results indicate that the expression of DMPK in two strains of *S. cerevisiae*, RS112 (diploid) and CBK1 (haploid), induces a significant increase in bud and cell apical growth, suggesting an involvement of DMPK in pathways that control cytoskeletal organization. A further observation is that the induction of cell elongation is accompanied by cell growth inhibition; this effect was not due to cell death and it was specific for DMPK, as the overexpression of an unrelated protein Cyp3A did not inhibit growth. Moreover, the overexpression of DMFLm and DMTm, mutated in the ATP binding site, completely reverts both growth inhibition and cell elongation induced by the wild type proteins. As the two dead kinase mutants are expressed at the same levels as the wild type DMPKs, the effects induced by DMPK are probably due to the kinase activity. Although DMPK is homologous to CBK (44% sequence identity), it did not complement the function of Δcbk1, suggesting that these two forms of human DMPK do not replace the CBK1 protein kinase activity in *S. cerevisiae*. It has to be pointed out that in general, the haploid strain CBK1 appears to be less responsive to DMPK effects than the diploid strain RS112, and that only the truncated form of DMPK is effective, while DMFL is inactive. As the C-terminally deleted forms of DMPK are supposed to be more active than the full-length [21], a possible explanation is that, in this less responsive strain, only the truncated form DMT is effective because of a higher enzymatic activity.

The effects on cell growth and morphology similar to those described in this paper were observed in *S. pombe* by overexpressing human DMPK. The authors show that the C-terminal domain was necessary to induce polarized growth and intracellular localization [28]. As DMT used in our experiments lacks only part of the C-terminus (544–625 amino acids), while the truncated inactive form expressed in *S. pombe* lacks all the C-terminal domain (401–625), it is

tempting to speculate that peptides 401–544 might be important for this function. Our findings are somewhat different from those seen in *S. pombe* [28], inasmuch as the point mutation K100R completely reverted cell growth inhibition, as well as cell elongation and polarization induced by the wild type forms. Moreover, our results imply that in *S. cerevisiae*, DMPK requires CBK1 to exert its effects, as in the deleted strain, no effect was detectable although DMPK itself was well expressed. It is possible that the overexpressed DMPK might act in the morphogenetic pathway involving Cbk1p, which regulates polarized morphogenesis (RAM) [29,30]. In agreement with this hypothesis is the finding that DMPK localization is cell cycle dependent, and similar to that observed for Cbk1p [12]. This aspect is especially evident at the beginning of bud formation, when DMPK localizes at the site of bud emergence, where Cbk1p is also localized [31].

Finally, the increase in cell length induced by DMPK appears to be tightly associated with the inhibition of cell proliferation in *S. cerevisiae.* It is intriguing that an elongated phenotype, with multiple buds, is observed in mutants deleted for the cell cycle regulating gene CDC34. In these cells, CDC28 activity is inhibited, because the CDK inhibitory kinase Swe1 is not properly degraded [32] and cells are blocked in a state of apical growth, so it is possible that the decreased cell proliferation and elongated phenotype induced by DMPK is due to the inhibition of cdc28 mitotic kinase [18]. Interestingly, the *S. pombe* homologous protein, ORB6, has been shown to inhibit mitotic entry by blocking p34cdc2 activation [11]. The findings that DM patients suffer from certain types of tumors [33] and have an increased rate of chromosome loss [34] further support the hypothesis that DMPK might participate in cell-cycle checkpoint.

In summary this is, to our knowledge, the first report, which shows that overexpression of human DMPK in *S. cerevisiae* induces an elongated phenotype and inhibits cell growth, suggesting a role of DMPK as modulator of the cytoskeleton and cell cycle. Both effects appear to require the kinase activity of DMPK and the product of CBK1 gene, a protein kinase homologous to the myotonic dystrophy protein kinases family. These findings, together with the observation that DMPK localization is similar to that described for cbk1, suggest that in *S. cerevisiae*, DMPK might act in a morphogenetic pathway involving CBK1.

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