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Brain-type creatine kinase BB-CK interacts with the Golgi Matrix Protein GM130 in early prophase

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Abstract

Creatine kinase (CK) isoenzymes are essential for storing, buffering and intracellular transport of ''energy-rich'' phosphate compounds in tissues with fluctuating high energy demand such as muscle, brain and other tissues and cells where CK is expressed. In brain and many non-muscle cells, ubiquitous cytosolic ''brain-type'' BB-CK and ubiquitous mitochondrial CK (uMtCK) act as components of a phosphocreatine shuttle to maintain cellular energy pools and distribute energy flux. To date, still relatively little is known about direct coupling of functional dimeric BB-CK with other partner proteins or enzymes that are important for cell function. Using a global yeast two-hybrid (Y2H) screen with monomeric B-CK as bait and a representative brain cDNA library to search for interaction partners of B-CK with proteins of the brain, we repeatedly identified the cis-Golgi Matrix protein (GM130) as recurrent interacting partner of B-CK. Since HeLa cells also express both BB-CK and GM130, we subsequently used this cellular model system to verify and characterize the BB-CK-GM130 complex by GST-pulldown experiments, as well as by *in vivo* co-localization studies with confocal microscopy. Using dividing HeLa cells, we report here for the first time that GM130 and BB-CK co-localize specifically in a transient fashion during early prophase of mitosis, when GM130 plays an important role in Golgi fragmentation that starts also at early prophase. These data may shed new light on BB-CK function for energy provision for Golgi-fragmentation that is initiated by cell signalling cascades in the early phases of mitosis.

Key words: creatine kinase, energy metabolism, Golgi Matrix protein GM130, mitosis, yeast two-hybrid, energy shuttle, Mayven

Abbreviations: B-CK refers to either the ubiquitous brain-type CK subunit, B-CK cDNA constructs or B-CK bait constructs, whereas BB-CK refers to the functional cytosolic, ubiquitous brain-type CK dimer complex found *in vivo;* uMtCK ubiquitous mitochondrial CK; GST Glutathione-S-transferase and Y2H refers to yeast two-hybrid.

Introduction

Creatine kinase (CK) isoenzymes catalyze the reversible transfer of a high-energy phosphate group between phosphocreatine (PCr) and ADP to maintain intracellular ATP levels in cells of high and fluctuating energy demands. In vertebrates, five distinct CK isoenzymes are expressed in a tissue specific manner and localize to different intracellular compartments [1, 2]. In sarcomeric muscle, dimeric cytosolic ''muscle-type'' MM-CK and octameric sarcomeric mitochondrial sMt-CK are always co-expressed, whereas in brain and other non-muscle tissues and cells, dimeric

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cytosolic BB-CK is co-expressed with octameric ubiquitous mitochondrial uMt-CK. In heart, the cytosolic dimeric hybrid MB-CK isoform is additionally expressed in a cardiac muscle specific fashion [2].

The maintenance of a high energy state in the brain, where BB-CK and uMt-CK play a major role [3] is one of the most important aspects of brain function [4]. In the brain, ATP can be formed by mitochondrial oxidative phosphorylation and (an)aerobic glycolysis and is consumed by the activities of Ca^{2+}- and Na^{+}/K^{+} ATPases, glutamine-glutamate neurotransmitter cycling, cell signalling, and molecular synthesis and axonal transport of cell constituents [4]. The two different CK isoforms, cytosolic BB-CK and mitochondrial uMt-CK constitute the components for a functional intracellular energy shuttle in the brain [3, 5]. Octameric uMt-CK generates PCr in the peripheral mitochondrial intermembrane space, as well as in the cristae [6, 7], which is then used by the dimeric cytosolic brain-type BB-CK to generate ATP at sub-cellular sites of high ATP turnover [2, 8].

It is known that BB-CK plays an essential role in the transport of neurotransmitters in neurons [9] and ions in astrocytes [10, 11], as well as in the regeneration of ATP during thrombin receptor signalling [12]. Although it has been shown that the brain of BB-CK knockout mice can metabolically compensate to some extent for the loss of BB-CK activity, these mice revealed diminished habituation and spatial learning acquisition [13]. Moreover, BB-CK deficient mice display a larger intra-infra-pyramidal mossy fibre field size and enlarged ventricular space [13]. In the brain, BB-CK together with its substrates PCr and Cr might not only be important for energy homeostasis but can also exert a general neuroprotective role, as shown by the supplemental feeding of Cr in animal models for seizures, hypoxia, or neurodegenerative disorders [14, 15].

In order to identify novel interaction partners of BB-CK and to ascertain the role of BB-CK in the brain and other cells that express this CK isoform, we performed a yeast two-hybrid (Y2H) screen to identify B-CK-interacting proteins out of a brain cDNA library. Dimeric BB-CK is expressed as the functional complex in many cells like brain cells, fibroblasts, kidney cells, spermatozoa and last but not least also HeLa cells [3]. A brain library was chosen for the screen because the levels of BB-CK are generally very high in brain [16] and the probability to find novel interaction partners may be enhanced by using B-CK as bait in combination with a brain cDNA library. Here, we report the human Golgi Matrix protein 130 (GM130) as a novel interaction partner of human B-CK. In a LexA based Y2H screen, using a N-terminally truncated form of human B-CK as a bait (amino acids 103–381), we show that mainly a stretch of GM130 comprising the amino acid residues 433–679 of this protein are responsible for this interaction.

GM130 belongs to the golgin protein family [17] and is involved in the assembly and maintenance of the Golgi apparatus [18]. Golgins are coiled-coil proteins associated with the Golgi apparatus, that are believed to be involved in the tethering of vesicles and the stacking of cisternae, as well as other functions such as association with the cytoskeleton. Many golgin proteins are peripheral membrane proteins that are recruited by GTPases. Several golgins have been described in animal cells, and some in yeast, but the relationships between golgins of different species are hard to define because their protein sequences are quite divergent although they share common structural features. A subset of the golgins contains a carboxy-terminal region, called the GRIP domain that mediates binding to the Golgi membranes. The GRIP domain was named after the four proteins in which it was originally found (golgin-97, RanBP2-α, Imh1p and p230/golgin-245) [19, 20]. The N-terminal 73 amino acid residues of GM130 interact with the vesicle docking protein p115 [21], which suggests a role for GM130 in the vesicular traffic between endoplasmic reticulum (ER) and Golgi stacks. GM130 also interacts with GRASP65, a Golgi reassembly and stacking protein of 65 kDa that is involved in the reformation of the Golgi complex after mitotic cell division [22]. The interaction between p115 and GM130 is inhibited upon mitotic phosphorylation of GM130 by cyclin-dependent kinase I [23].

As a model system for dividing cells, we used HeLa cells that express BB-CK as well as GM130 endogenously. Here, we show for the first time that GM130 and BB-CK interact in cultured cells *in vivo* and that co-localization of the two endogenously expressed proteins occurs specifically in the early prophase of mitosis at the Golgi apparatus, just prior to its fragmentation for cell division. Altogether, our data reveal new aspects on the role of BB-CK for energy provision in the regulation of the cell cycle.

Materials and methods

Plasmid constructs

For the yeast two hybrid screen, human B-CK cDNA was amplified by PCR from the cDNA template (kindly provided by Dr. Jean-Paul Steghens, Lyon, France) and inserted into the pLexA-dir bait vector (Dualsystems Biotech, Zürich, Switzerland) resulting in a 480 amino acid B-CK fusion protein. The human brain cDNA library in the pACT2 vector was obtained from Clontech Laboratories, Inc, Palo Alto, USA. The full-length rat GM130 construct, as well as its deletion constructs with amino acids 433–679, 679–986, 75–271 and 272–986 in the pACT2 vector were kindly provided by Dr. Francis A. Barr, Munich, Germany [24]. For the GST construct, full-length human B-CK cDNA was

amplified by PCR and subcloned into the Glutathione S-transferase (GST) vector pET42 (+) (Novagen Inc, Madison, USA). All clones derived by PCR were verified by DNA sequencing. Protein expression was verified by Western blotting using specific antibodies for both proteins.

Bacterial recombinant fusion proteins

Glutathione S-transferase fusion protein of BB-CK was prepared according to the manufacturer's instructions. Briefly, expression of fusion protein in *E. coli* BL21 cells (Invitrogen, Carlsbad, USA) was induced by 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) for 3–5 h. Cells were pelleted at 4 °C, 4000 *g* for 20 min. The pellet was lysed in lysis buffer (0.5 M sucrose, 20 mM Tris pH 8.0, 15 mM β-Mercaptoethanol), with addition of protease inhibitor cocktail (Roche, according to the manufacturer's instruction). After incubation with lysozyme and DNase I (400 U/ml) for 30 min on ice, cells were sonicated and debris was removed by centrifugation for 15 min at 4 °C 10,000 *g*. The supernatant was affinity purified on glutathione-agarose (Sigma, Buchs, Switzerland). Expression and purification were controlled by SDS polyacrylamide gel electrophoresis.

Yeast two-hybrid assays

Yeast two-hybrid screening assays were performed as described previously [25]. Briefly, after excluding self-activation of the B-CK bait construct, the yeast reporter strain DSY-1 (Dualsystems Biotech, Zürich, Switzerland) was co-transformed with the B-CK bait and the brain cDNA library and transformants were grown on selection plates lacking histidine, leucine and tryptophan. Positive transformants were tested for β-Galactosidase activity using a filter assay [26]. Library plasmids were isolated from positive clones and assayed in a bait dependency test and with a bait encoding a LexA-laminC fusion as control, using a mating strategy [27]. The identity of positive interactors was determined by DNA sequencing.

Preparation of rat liver Golgi stacks

Rat liver Golgi stacks were prepared as described previously [28]. Briefly, rat liver was homogenized with a glass-Teflon Potter in ice-cold 0.5 M sucrose-PM buffer (0.1 M sodium phosphate, pH 6, 7.5 mM $MgCl_2$) to a final concentration of 0.6 g liver/ml. The homogenate was applied to a two-step gradient comprising 1.3 and 0.86 M sucrose-PM buffer. After centrifugation at 25,000 rpm using a SW28.Ti rotor (Beckmann) for 2 h at 4 °C, the Golgi fraction was collected from the 0.5/0.86 M sucrose interface. After dilution to 0.25 M sucrose using PM buffer, the membranes were centrifuged at 6500 *g* for 30 min at 4 °C, washed in 0.25 M PM, re-centrifuged and resuspended in the same buffer. Golgi fraction was verified by Western blotting using GM130 as Golgi marker protein.

GST-pull down assay

Pulldown of GM130 from rat liver Golgi stacks was achieved by mixing 20 μl of 50% glutathione-agarose equilibrated in binding buffer (20 mM Tris pH 7.4, 65 mM NaCl, 2.5 mM $MgCl_2$, 0.05% NP-40, 1 mM DTT) with 20 μg GST-B-CK or GST alone. After incubation at 4 °C for 1 h, 10 μg of the rat liver Golgi stacks preparation was added and incubated for 1 h at 4 °C. Glutathione beads were then recovered by centrifugation and washed three times vigorously with binding buffer. Proteins bound to the beads were eluted by SDS sample buffer and analyzed by Western blotting using mouse-anti GM130 (BD Bioscience Pharmingen, San Diego, USA) and rabbit-anti GST antibody (Sigma, Buchs, Switzerland).

Western blotting

SDS-polyacrylamide gel electrophoresis was performed on 10% polyacrylamide gels. For Western blot analysis, proteins were transferred to nitrocellulose membranes (Schleicher&Schuell, Bottmingen, Switzerland). Primary antibodies were overlaid with goat anti-rabbit (Amersham Pharmacia Biotech, Sweden) or anti-mouse (Calbiochem-Novabiochem International, Inc., USA) horseradish peroxidase-coupled secondary antibodies, and chemiluminescence was detected using the Pico detection kit (Pierce Biotechnology, Rockford, USA).

Cell culture and BFA treatment

Monolayer cultures of HeLa cells were grown at 37 °C and 5% CO_2 in DMEM high glucose medium, containing 10% FCS (Invitrogen). For immunocytochemical analysis, cells were plated on gelatine coated glass coverslips 24 h before treatment with or without 200 μg/ml brefeldin A (BFA, Sigma-Aldrich). BFA incubation was performed for 60 min at 37 °C and 5% CO_2.

Immunofluorescence staining and confocal imaging

HeLa cells grown on glass coverslips were washed with pre-warmed MP buffer (65 mM PIPES pH 6.9, 25 mM

HEPES, 10 mM EGTA, 3 mM $MgCl_2$), pre-fixed for 2 min with 3% PFA in MP buffer and permeabilized for 4 min with 0.2% Triton X100 in MP. After extensive washing with MP, cells were fixed for another 6 min then blocked with blocking buffer (5% BSA in MP). Primary and secondary antibodies were diluted in blocking buffer and incubated for 60 and 40 min, respectively. Dilutions were: chicken anti-human BB-CK [29] 1:50, rat anti-alpha tubulin (Abcam) 1:100, mouse anti-GM130 (BD Pharmingen) 1:100, anti-chicken Cy3 (Molecular Probes, Leiden, the Netherlands), 1:500, anti-mouse Cy5 (rat Ig absorbed, Jackson, Bar Harbor, USA) 1:100, anti-rat Cy2 (mouse Ig absorbed; Jackson) 1:100. Pre-immune serums have been used as controls to show specificity of the antibodies under the conditions tested (data not shown).

The imaging system consisted of a Leica inverted microscope DM IRB/E, a Leica true confocal scanner TCS SP1 (Leica Microsystems AG, Glattbrugg, Switzerland) and a Fujitsu-Siemens workstation (Fujitsu Siemens computer Bv., Regensdorf, Switzerland). The images were recorded using a Leica PL APOx63/1.4 oil immersion objective. The system was equipped with Ar and He/Ne lasers. Image processing was done using Imaris 4.0 (Bitplane AG, Zurich, Switzerland). This software was used to visualize single sections from the confocal data set. Final assembly of figures was made with Adobe Photoshop CS (Adobe systems, San José, USA). For each phase of mitosis, at least three independent cells showing the same staining pattern were recorded and pictures representing one single layer of the sections were shown.

Results

Identification of GM130 as an interaction partner of B-CK using a Y2H screen

To identify putative novel interaction partners of B-CK, we screened a human brain cDNA library using a LexA based yeast two-hybrid system (Y2H), taking B-CK as bait. We fused the C-terminal 278 amino acid domain of human B-CK to lexA (Fig. 1A). This fusion protein includes the isoform specific box B260 that discriminates B-CK from M-CK, as well as the highly conserved Cys 283 near the catalytic site [30]. Expression of the fusion protein in yeast was verified by Western blot (data not shown).

Our screen with 8.6×10^6 transformants revealed 64 positive clones (Fig. 1B). Strong binders are indicated as dark grey colour in the β-Galactosidase assay at 30 min and with higher intensity at 3 h (Fig. 1B and C, grey squares). DNA sequencing identified the human Golgi Matrix protein GM130 as interaction partner for B-CK in eight of the 64 positive clones. We further identified (in three out of the 64 positive clones) the Kelch-like 2 protein Mayven [31] as interaction partner for B-CK which was also found in a former Y2H screen with human B-CK as bait of our colleagues (Dr. B. Wieringa University of Neijmegen, NL, personal communication). Here, we focus on the experiments analyzing the interaction between BB-CK and GM130 because this result suggests that BB-CK interacts with a Golgi structural protein and might be involved in the assembly, fragmentation or turnover of the Golgi apparatus.

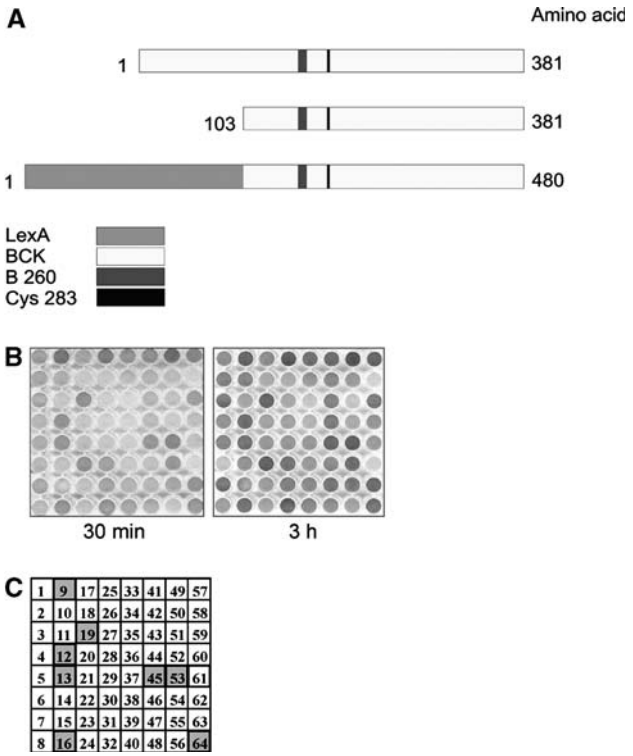

Fig. 1. Domain structure of the B-CK bait construct and identification of Golgi Matrix protein GM130 as interaction partner in the Y2H. (A) The full-length B-CK monomer (upper panel) is composed of 381 amino acids. A truncated form of 278 amino acids (103–381) was N-terminally fused to the LexA DNA binding domain and used as bait for the Y2H screen (lower panel). The isoform specific box B 260 as well as the highly conserved Cys 283 near the catalytic site is also included within this B-CK bait construct. (B, C) 64 clones rescued from the library screen were isolated and bait dependency was verified by measuring β-Galactosidase activity for 30 min up to 3 h. Grey colour indicates protein–protein interaction via X-Gal cleavage by the reporter β-Galactosidase. (B) The differences in the grey colour intensities indicate the strength of the interaction (C). DNA sequencing of strongest binders (grey squares, in C) identified human Golgi Matrix protein 130 (GM130) in eight of 64 clones.

GM130 interacts via its 433–679 amino acid domain with B-CK in the Y2H

GM130 binds via its N-terminus to the vesicle-tethering protein p115 [21] and via its C-terminus to the N-myristoylated

Golgi reassembly stacking protein (GRASP) 65 [22]. To identify the precise region in GM130 that is required for the interaction with B-CK we performed a Y2H interaction assay with various deletion constructs of rat GM130 (Fig. 2). Expression of all GM130 constructs, as well as that of BB-CK, confirmed by Western blotting (data not shown), confirmed a constant expression profile. Interaction of GM130 and B-CK, as indicated by *β*-Galactosidase activity, could be shown for the full-length GM130 construct, but also for constructs 433–679 (amino acid residues) and 272–986. The strong coloration obtained. with full length GM130 may be a hint on the quality of its interaction with B-CK. It is possible that the full-length construct of GM130 forms dimers, which then in turn would influence the quality of the interaction with B-CK. No interaction, however, could be detected for the GM130 constructs 679–896 and 75–271, indicating that the amino acids 433–679 of GM130, which include the predicted poly-proline domain (black) [24], play a role in the interaction with B-CK. This stretch in GM130 contains no coiled-coil, which argues for the specificity of the interaction with B-CK. The helical coiled-coil structures and non-helical termini of the golgin proteins are proposed to be a clue to their function [19]. These results suggest that the central part of GM130 is sufficient for the interaction with B-CK, however the increased signal in case of the full-length constructs indicates a structural stabilization that aids this interaction.

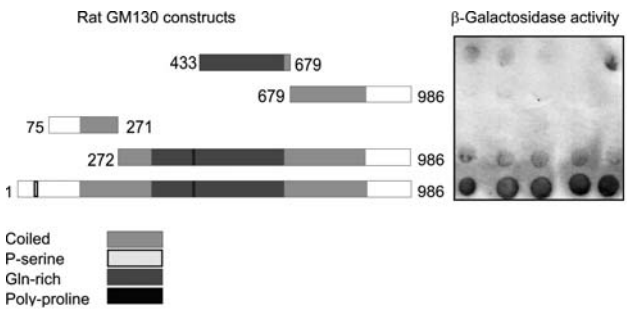

Fig. 2. Characterization of the Golgi Matrix protein GM130 binding domain that is responsible for the binding to the B-CK bait construct. Full-length GM130, as well as the indicated deletion constructs of rat GM130 with different sizes (length in amino acids), were tested against the human B-CK bait construct for *β*-Galactosidase activity. Grey colour colony indicates protein–protein interaction via X-Gal cleavage by the reporter *β*-Galactosidase. All constructs containing the poly-proline domain interact with B-CK and amino acid residues 433–679 of GM130 are responsible for this interaction.

Interaction of BB-CK with GM130 in rat liver Golgi stacks

To confirm the Y2H *in vivo* data biochemically, GST-pulldown assays were performed. For this, full-length human B-CK was N-terminally fused to GST and expressed in *E. coli*. Purification of dimeric functional BB-CK fusion protein (80 kDa) and GST alone (37 kDa) was achieved by affinity binding to glutathione agarose-beads (Fig. 3A).

To enrich GM130, rat liver Golgi stacks were prepared as described previously [28]. GM130 is a cytoplasmic protein that is tightly bound to Golgi membranes [32] and remains attached to the Golgi membrane stacks during the preparation procedure (input Fig. 3B). The proteins eluted from the pulldown complex were analyzed by Western blotting. Antibodies used were anti-GST and anti-GM130 (Fig. 3B). We demonstrate here, that GM130 from the

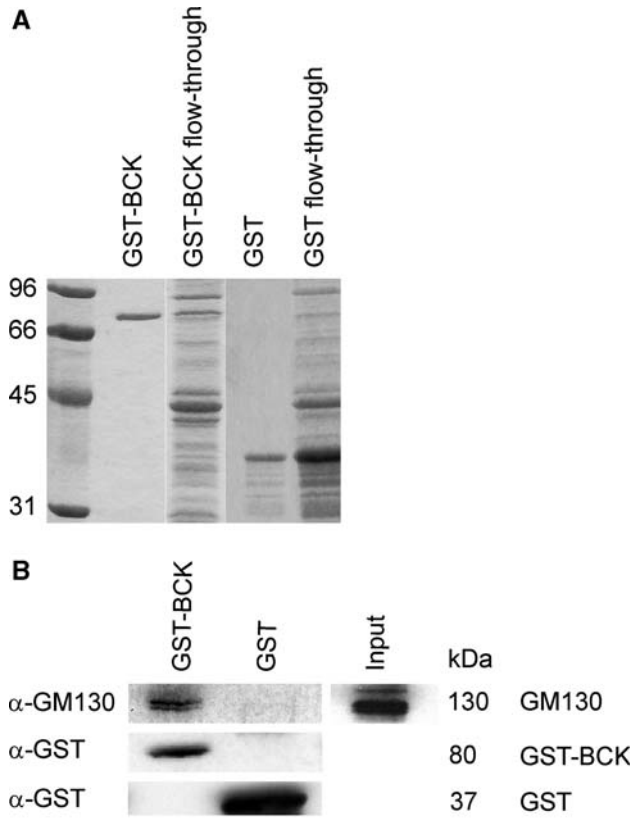

Fig. 3. GST-pulldown experiment with GST-BB-CK fusion protein and rat liver Golgi-stacks. (A) N-terminally GST-tagged BB-CK and GST alone were expressed in *E. coli* and purified on glutathione-agarose. GST-B-CK has a calculated molecular weight of 80 kDa and GST alone of 37 kDa. (B) Rat liver Golgi stacks were prepared as described [28] and incubated with GST-BB-CK and GST alone, respectively, bound to glutathione-agarose. Recovered complexes were analyzed by Western blotting. GM130 in the Golgi preparation bound specifically to GST-BB-CK but not to the GST control.

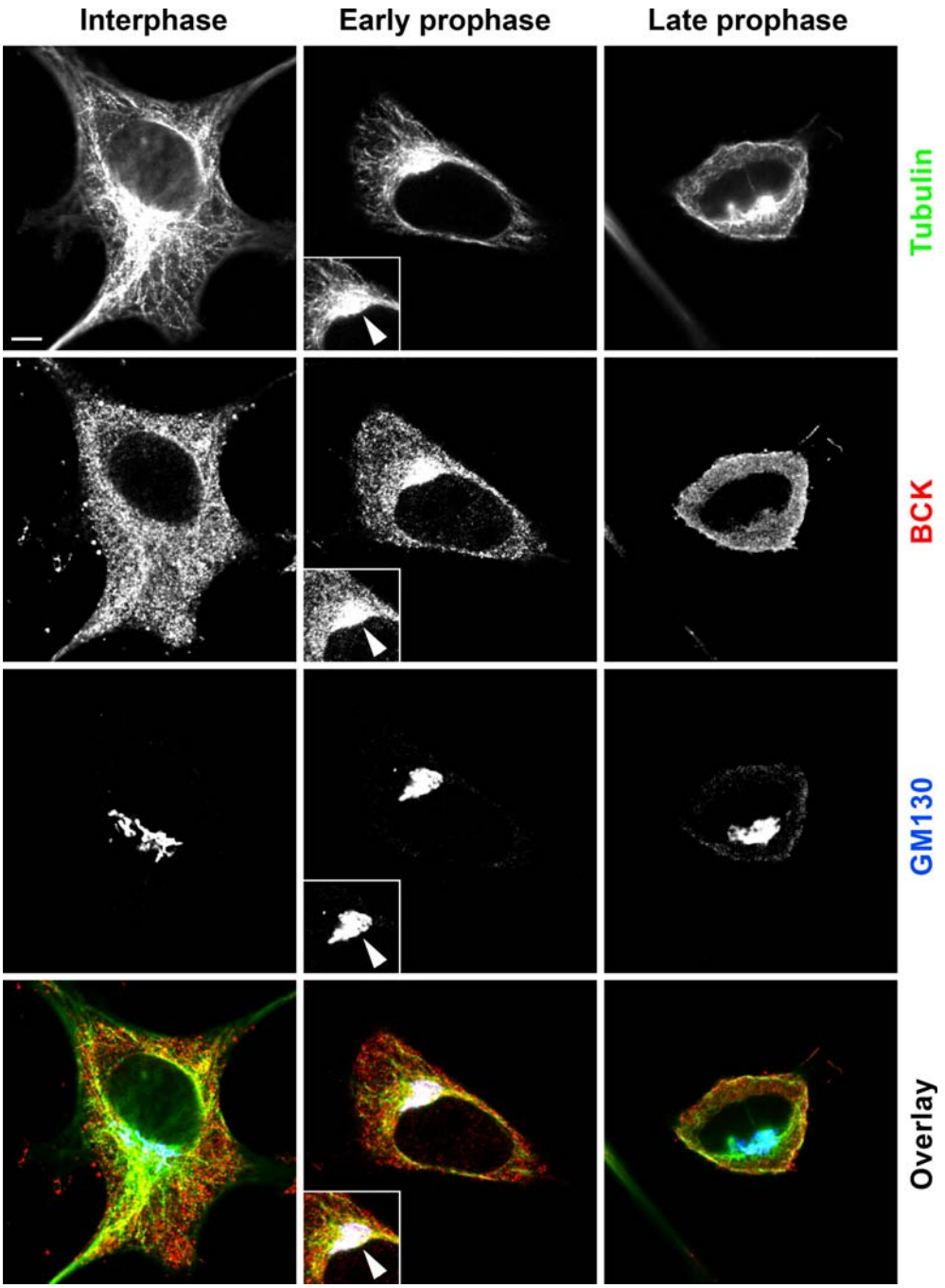

Fig. 4. Co-localization of BB-CK and GM130 is found during early prophase of mitotic HeLa cells. Cells were stained with antibodies against endogenously expressed alpha-tubulin (green) to verify the mitotic status of the cell, BB-CK (red) and GM130 (blue). Pictures shown here represent single confocal sections of the overlays and the individual channels. In the interphase, BB-CK staining shows an overall cytoplasmic distribution. In the prophase, the early and the late anaphase, BB-CK condenses to certain cellular structures, but this condensation overlaps with the GM130 staining specifically only in the early prophase, indicated by the white signal in the overlay (arrow in inset). No such overlapping was observed during the later phases of the cell cycle (see Fig. 4, continued), but a significant overlap of BB-CK staining with tubulin staining was seen during metaphase and anaphase (see Fig. 4, continued). Pictures were taken as sequential laser scans to prevent potential overlapping of fluorescence signals emitted by the secondary antibody-coupled fluorochromes of neighbouring excitation wave lengths (scale bar 5 μm).

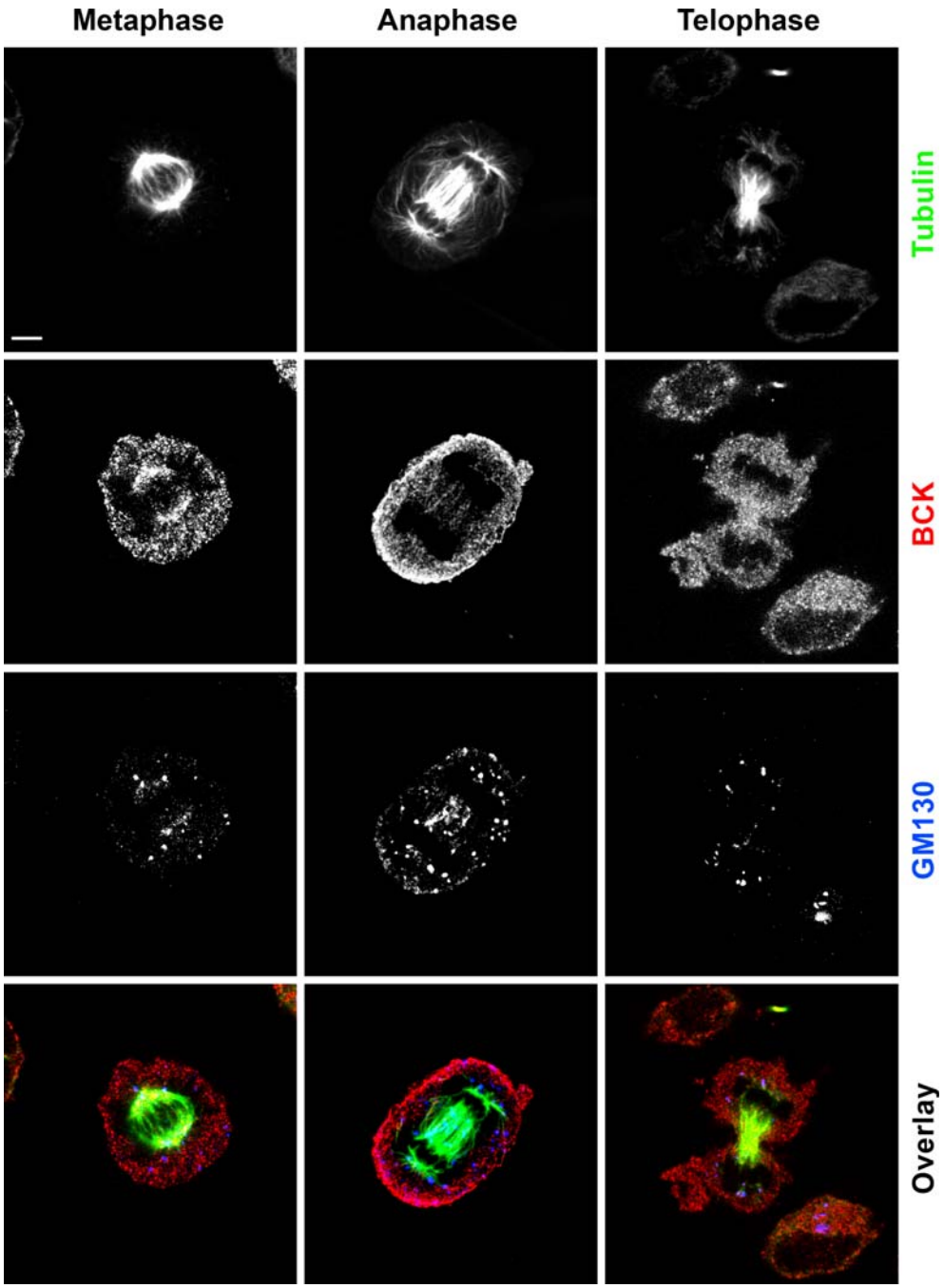

Fig. 4. (*Continued*)

Golgi membrane fraction (Fig. 3B input) binds to GST-BB-CK *in vitro* but not to the GST control. Since GM130 is firmly bound to Golgi membranes, it is likely that the latter are also pulled down via GM130 interaction with GST-BB-CK. Thus, the interaction between BB-CK and GM130 was also confirmed by a biochemical assay with functional dimeric BB-CK.

Co-localization of GM130 and BB-CK at early prophase in mitotic HeLa cells

GM130 plays an important role in the Golgi fragmentation during mitosis. Fragmentation is induced by phosphorylation of GM130 on Serine 25 by the Cdc2 kinase [21, 23]. The dephosphorylation of GM130 at the end of mitosis is

performed by the trimeric form of PP2A with the Bα regulatory subunit [33]. BB-CK catalyzes the reaction, which leads to the transfer of high energy phosphates from PCr to ADP to provide ATP and thus energy for ATP-dependent processes including cell division. To find out whether BB-CK is indeed associated with GM130 also in cells *in vivo*, we immunostained endogenously co-expressed BB-CK and GM130 (Fig. 4) in cultured HeLa cells. Further, we addressed the question of whether this association of BB-CK with this GM130 Golgi protein and thus with the Golgi apparatus were permanent or transient during certain mitotic phases. HeLa cells are used as gold-standard cell

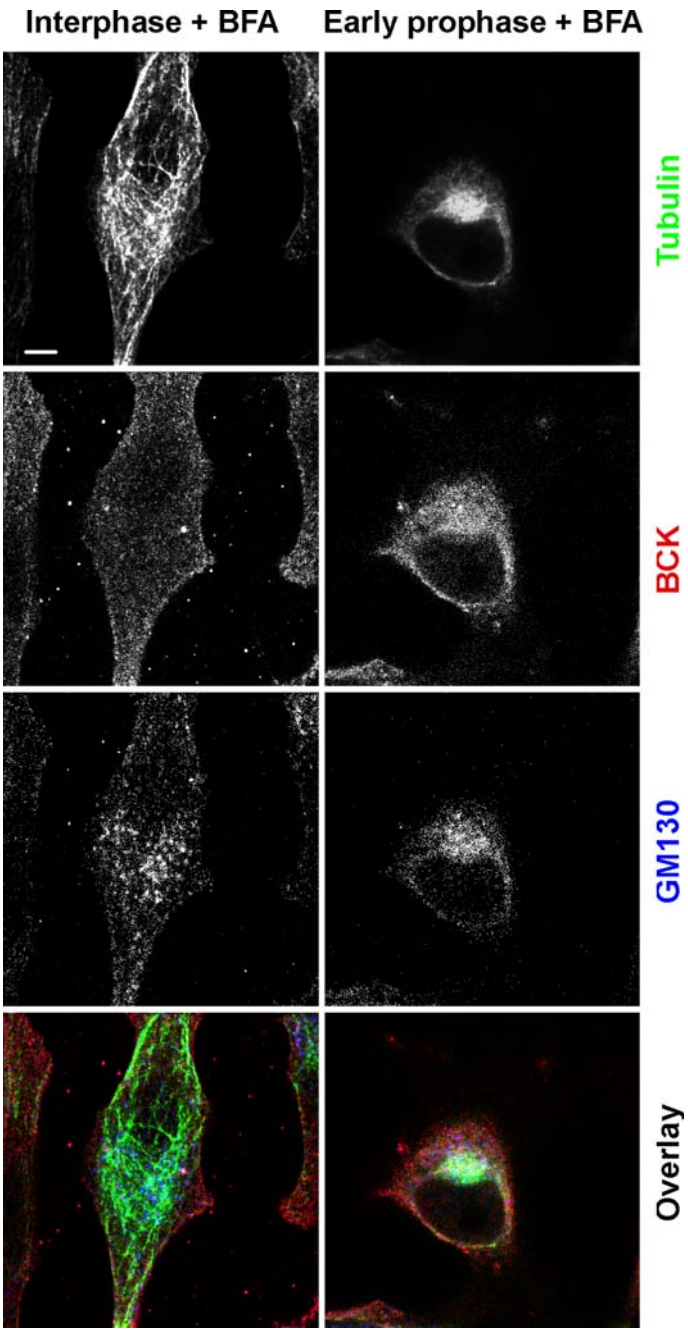

Fig. 5. BFA disrupts the Golgi apparatus and BB-CK-GM130 co-localization in early prophase is abrogated. BFA treatment alters the distribution of GM130 (blue) to be dispersed throughout the cytoplasm (interphase). At early prophase, no condensation of BB-CK (red) and GM130 was observed as it was at early prophase of control cells (Fig. 4, early prophase, in inset of BB-CK-staining). Pictures were taken as sequential laser scans (scale bar 5 μm).

culture model for studying processes of cell division since they are a well characterized cell-system to follow mitosis [33, 34]. HeLa cells are known to express both BB-CK as well as GM130 [3, 35], which is fully confirmed by our data (see Fig. 3B). Mitotically active cells were identified by staining of the microtubuli (green, alpha-tubulin), allowing the observation of all mitotic stages (Fig. 4). In interphase cells, BB-CK showed a diffuse distribution in the cytoplasm, as reported by previous studies [8, 36] and was mostly excluded from the nucleus. However, in early prophase, a clear condensation of BB-CK to one perinuclear site could be observed. This correlated with the condensation of the GM130 and the increased tubulin staining at the same site, as indicated in the inset with a white arrow in Fig. 4 (early prophase). However, during late prophase, where the separation of the centrosomes begins, the accumulation of BB-CK disappeared, indicating that the interaction between BB-CK and GM130 is only transient. Therefore, the specific co-localization of BB-CK and GM130 is clearly restricted to the onset of mitosis when disassembly of the Golgi apparatus begins (Fig. 4, compare the column labelled ‘‘early prophase’’ with the other stages of mitosis). A co-localization of BB-CK and tubulin was reported earlier [37, 38], which was also observed here during meta- and anaphase of cell division (Fig. 4, continued). As a control, the same set of experiments was carried out with Brefeldin A (BFA) (Fig. 5), which causes Golgi membranes to reversibly fuse with the ER [39]. In contrast to control cells, BFA-treated HeLa cells exhibited no condensation of BB-CK and GM130 at early prophase (Fig. 5). In normal cells, the interaction between BB-CK and GM130 at that early mitotic stage clearly was only transient. This finding may be related to the fact that provision by BB-CK of high energy phosphates provide the energy that is required to start the signalling cascade that leads to fragmentation of the Golgi apparatus during cell division [33, 40]. Subsequently, BB-CK would no longer be needed at this Golgi-related micro-compartment.

Discussion

In this study, we report that dimeric human BB-CK binds specifically to human GM130 *in vitro* and *in* cell cultures *in vivo*. Screening of a human brain cDNA library with a LexA-B-CK bait construct with the C-terminus (103–381) of monomeric B-CK, including the isoform specific box B260 and the highly conserved Cys 283 near the catalytic site of B-CK, resulted in the isolation of 64 clones of which eight were identified as GM130 (Fig. 1B). This interaction between BB-CK and GM130-golgin was confirmed by a GST pulldown assay (Fig. 3B), as well as by co-localization *in vivo* of BB-CK and GM130 in HeLa cells, which co-express both proteins endogenously, by confocal microscopy (Fig. 4). If cDNA coding for the B-CK subunit is expressed in cells or tissues, two monomers are always assembled to form functional BB-CK dimers that are the functional entities [41, 42]. This strongly suggests that *in vivo* GM130 indeed interacts with the BB-CK dimer.

GM130 is a cytoplasmic protein that is tightly bound to Golgi membranes and represents a part of a larger oligomeric complex. This complex consists of Golgi reassembly stacking protein 65 (GRASP65), p115 and giantin, which are referred to as tethering proteins [43]. GM130 belongs to the golgin protein family and predictions from the protein sequence suggest that GM130 is an extended rod-like protein with coiled-coil domains. The so called “string theory” [44] proposes a predominant role for the GM130 protein in holding transport vesicles in place [45, 46]. Our deletion analysis showed that a region around the predicted polyproline domain of GM130 in Fig. 2 (amino acids 433–679) is involved in the interaction with B-CK. As this domain is not part of the helical coiled-coil structure that may be sticky by nature, it is less likely that BB-CK is just simply non-specifically sticking or adventitiously bound to GM130. The association between the two proteins is transient and limited to a very narrow time window during mitosis, which strongly argues against a non-specific interaction between BB-CK and GM130. To determine whether the above domain of GM130 causes the interaction, deletions of different residues, e.g., the prolines within this region of GM130 may give further insights.

The Golgi apparatus occupies a central position in the classical secretory pathway, where it receives *de novo* synthesized proteins from the ER, and functions to distill posttranslational processes and to sort cargo to their final destinations [47]. Its mode of inheritance after cell division is still under debate and several models have been proposed: *de novo* formation [48], fission [49], and disassembly–reassembly [50]. The latter process is regulated by Cdc2-cyclin B kinase [51]. GM130 is a substrate of Cdc2 [21, 52] and upon phosphorylation on serine 25, the vesicle tethering factor p115 can no longer bind to GM130 [21]. Mitotic Golgi fragmentation *in vitro* depends on Cdc2-cyclin B activity. Phosphorylation of GM130 and dissociation of p115 are synchronized with the conversion of Golgi cisternae into small vesicles. Since cyclin B2 has been localized to the Golgi complex in interphase cells [53], it is possible that Cdc2 phosphorylates GM130 during prophase to initiate the disassembly of the Golgi complex [33]. Interestingly, many different ATP-requiring processes are taking place in late prophase at the Golgi: mitogen-activated protein kinase 1 (MEK1) and Polo-like kinase 1 are required for Golgi complex fragmentation [40], while RAF1 is necessary to activate MEK1 in the first place. Moreover, MEK1 is found on the Golgi membranes in late prophase [54].

As high energy phosphates are needed for the various phosphorylation processes, it is likely that the interaction between GM130 and BB-CK would facilitate GM130 phosphorylation by ATP-requiring protein kinases. Indeed, the interaction between BB-CK and GM130 seems to be transient and restricted to the prophase of the cell cycle. This is exactly the time point when energy is needed to initiate the fragmentation of the Golgi apparatus by signalling pathways. ATP needed for these processes could thus be provided by BB-CK associated with GM130 on the Golgi membrane. The transient co-localization of BB-CK and GM130 was demonstrated with immunostaining of HeLa cells at different phases of mitosis for endogenously co-expressed GM130 and BB-CK. During interphase, BB-CK was always distributed diffusely throughout the entire cytoplasm, however, in early prophase, a typical condensation of BB-CK that clustered together with GM130 on the Golgi apparatus (Fig. 4) could be demonstrated very clearly and reproducibly. Co-staining experiments in BFA treated HeLa cells (Fig. 5) did not show a clear association between the two proteins. Nevertheless, we suggest that the interaction of BB-CK with GM130, due to its transient appearance during early prophase only, is specific and cannot be explained by adventitious binding of BB-CK to GM130 due to a potentially stickiness between the two proteins. Second, since the interaction is only transient, this indicates that BB-CK associates with GM130 and thus with the Golgi apparatus at times when energy provision to the Golgi must be high, e.g., for Golgi fragmentation that is initiated by energy-requiring signalling pathways [23, 40, 54]. Thus, it is tempting to speculate that BB-CK may facilitate the provision of energy to kinases that are recruited to signalling steps upon initiation of the prophase. The action of BB-CK would be to maintain locally high ATP levels and a high ATP/ADP ratio that would favour kinase reactions. Since the interaction between BB-CK and GM130 obviously does not seem to be permanent in HeLa cells *in vivo*, albeit we found it in the Y2H screen, it is likely to be a regulated phenomenon itself, dependent on the cell cycle. Such transient, possibly weak, but specific CK micro-compartments have been found earlier in another context [55].

Interestingly, BB-CK was recently shown to be in a complex together with the chloride intracellular channel (CLIC), dynamin I, α-tubulin, β-actin and 14-3-3 [37]. In line with the idea that GM130 may recruit BB-CK to form specific micro-compartments, GM130 was recently shown to target the protein kinase YSK1 to the Golgi, which has protein 14-3-3 as a substrate [24]. 14-3-3 is a protein enriched in brain and modulates ion channel functions that regulates diverse cell signalling pathways, including activation of protein kinases, cell cycle control, neural development, cell migration and polarization and pathogenesis of bacteria and viruses [56]. In full support of the data reported herein, recent results revealed a co-purification of BB-CK and GM130 in a proteomic approach with Golgi fraction isolated from lactating mammary gland [57].

Taken together, these data confirm that BB-CK is structurally and functionally coupled in a dynamic and cell cycle dependent manner to the Golgi apparatus. There, BB-CK is most likely a member of a large protein complex that also comprises several protein kinases. This raises a novel aspect for a new function of members of the CK family and represents a first indication that CKs are linked to signalling cascades regulating the integrity of the Golgi apparatus and thus may be involved in the control of the cell cycle.

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