

Dynamic association of cytoplasmic dynein heavy chain 1a with the Golgi apparatus and intermediate compartment

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SUMMARY

Microtubule motors, such as the minus end-directed motor, cytoplasmic dynein, play an important role in maintaining the integrity, intracellular location, and function of the Golgi apparatus, as well as in the translocation of membrane between the endoplasmic reticulum and Golgi apparatus. We have immunolocalised conventional cytoplasmic dynein heavy chain to the Golgi apparatus in cultured vertebrate cells. In addition, we present evidence that cytoplasmic dynein heavy chain cycles constitutively between the endoplasmic reticulum and Golgi apparatus: it colocalises partially with the intermediate compartment, it is found on nocodazole-induced peripheral Golgi elements and, most strikingly, on Brefeldin A-induced tubules that are moving towards microtubule plus ends. The direction of movement of membrane between the endoplasmic reticulum and Golgi

apparatus is therefore unlikely to be regulated by controlling motor-membrane interactions: rather, the motors probably remain bound throughout the whole cycle, with their activity being modulated instead. We also report that the overexpression of p50/dynamitin results in the loss of cytoplasmic dynein heavy chain from the membrane of peripheral Golgi elements. These results explain how dynamitin overexpression causes the inhibition of endoplasmic reticulum-to-Golgi transport complex movement towards the centrosomal region, and support the general model that an intact dynactin complex is required for cytoplasmic dynein binding to all cargoes.

Key words: Brefeldin A, *Xenopus laevis*, Microtubule motor

INTRODUCTION

In most non-polarised animal cells the Golgi apparatus is a single copy dynamic organelle that consists of interconnected stacks of flattened membrane cisternae with dilated rims and associated tubules and vesicles. Functionally, the Golgi apparatus is involved in the post-translational modification of newly synthesised membrane lipids and secretory proteins, which are received from the endoplasmic reticulum (ER) and en route to the plasma membrane or the endocytic pathway.

The microtubule (MT) network plays an important role in maintaining the integrity and intracellular location of the Golgi apparatus in animal cells. Disruption of MTs using depolymerising drugs results in the fragmentation of the Golgi complex and its redistribution into multiple functional Golgi mini stacks (or stacklets) dispersed throughout the cytoplasm (reviewed in Thyberg and Moskalewski, 1999). Interestingly, when MTs are allowed to regrow, these peripheral Golgi stacklets move centripetally along newly polymerised MTs back towards the centrosome (Ho et al., 1989). Similar minus end-directed movement of entire Golgi stacks was observed using a fused-cell system (Ho et al., 1990). It is important to note, however, that in polarised epithelia, the juxta-nuclear Golgi apparatus is not localised near the minus ends of

microtubules, which in these cells lie close to the apical plasma membrane, and therefore the direction of traffic into and out of the Golgi apparatus is likely to be different in polarised versus non-polarised cells (e.g. Fath et al., 1997). Until recently, little was known about the microtubule motor involved in the reclustering of Golgi stacklets in non-polarised cells, although there was limited evidence for a role for cytoplasmic dynein in the interaction between Golgi membranes and MTs (Corthésy-Theulaz et al., 1992).

Conventional cytoplasmic dynein is a minus-end MT-based motor involved in the movement of various cargoes (reviewed in Thaler and Haimo, 1996). It is a large multisubunit complex of approximately 1400 kDa comprising two heavy chains (DHC1a, approx. 500-530 kDa); two to three intermediate chains (DIC, approx. 74 kDa); a set of light intermediate chains (approx. 51-61 kDa; reviewed in Holzbaur and Vallee, 1994) and three different light chains (approx. 8, 14 and 22 kDa; King et al., 1996). The existence of other cytoplasmic dynein complexes has been suggested following the discovery of a new cytoplasmic dynein heavy chain isoform DHC2 (Vaisberg et al., 1996), which may correspond to the DHC1b isoform described recently by Criswell and Asai (1998) and Pazour et al. (1999). Sedimentation experiments revealed that DHC2/1b is part of a 12S cytoplasmic dynein complex (Vaisberg et al.,

1996), which did not contain an anti-IC74-reactive DIC (Criswell and Asai, 1998).

A variety of studies have suggested that both conventional cytoplasmic dynein and its regulatory complex, dynactin, are involved in the central positioning of the Golgi apparatus in non-polarised cells. Firstly, overexpression of the p50/dynamitin subunit of the dynactin complex caused fragmentation of the Golgi complex (Burkhardt et al., 1997; Presley et al., 1997), similar to that seen following MT depolymerisation. Secondly, the microinjection of function-blocking antibodies directed against DIC (Burkhardt et al., 1997) resulted in the dispersal of the Golgi apparatus. Thirdly, a similar phenotype was observed in cultured blastocytes isolated from DHC1a knockout mice (Harada et al., 1998). Finally, following overexpression of centractin, a subunit of the dynactin complex, the morphology of the Golgi apparatus was altered (Holleran et al., 1996). Even though all these data suggest that the morphology and function of the Golgi apparatus is tightly linked to the function of cytoplasmic dynein or dynactin, very little is known about the association of conventional cytoplasmic dynein subunits with the Golgi apparatus. So far, Tctex-1, the 14 kDa cytoplasmic dynein light chain, has been found to colocalise with Golgi markers (Tai et al., 1998), although whether it is a subunit of both types of cytoplasmic dynein is not yet known. Interestingly, DHC2/1b was localised to the Golgi apparatus in mammalian cells, and the microinjection of anti-DHC2/1b antibodies resulted in the apparent scattering of the Golgi apparatus (Vaisberg et al., 1996). In contrast, Golgi apparatus localisation and function appeared to be normal in DHC2/1b-mutated *Chlamydomonas* cells (Pazour et al., 1999).

It is highly likely that the alteration in Golgi position that occurs following disruption of cytoplasmic dynein function is intimately linked to an inhibition of the transport of membrane material from the ER to the Golgi apparatus. This MT-dependent, minus end-directed transport has been visualised in living cells, and is inhibited by the overexpression of p50/dynamitin (Presley et al., 1997). So far, no cytoplasmic dynein components have been localised to these transport structures in non-polarised cells, although surprisingly, a member of the kinesin family of microtubule motors has, using the H1 anti-kinesin heavy chain monoclonal antibody (Lippincott-Schwartz et al., 1995).

Escaped ER resident proteins and other membrane components are recycled from the Golgi complex back to the ER, and this pathway is dramatically emphasised by treating cells with the fungal metabolite, brefeldin A (BFA). BFA induces the formation of Golgi-derived membrane tubules, which extend along MTs towards the cell periphery and fuse with the ER (reviewed in Dinter and Berger, 1998). The H1 mAb labels these tubules and inhibits their extension following micro-injection (Lippincott-Schwartz et al., 1995). The kinesin family member recognised by H1 therefore shuttles between the ER and the Golgi apparatus (Lippincott-Schwartz et al., 1995), and its activity is likely to be regulated in a way that does not involve dissociation of the motor from the membrane. There are, in fact, several candidates for the motor that drives BFA-induced tubule formation: five kinesin superfamily members, kinesin heavy chain (Feiguin et al., 1994; Marks et al., 1994; Lippincott-Schwartz et al., 1995; Minin, 1997), KIF1C (Dorner et al., 1998), Rabkinesin-6 (Echard et al.,

1998), kinesin II (Le Bot et al., 1998; Yang and Goldstein, 1998) and two KRPs of approx. 100 and 130 kDa (A. Robertson and V. J. Allan, unpublished), may be involved in some way in Golgi-to-ER traffic.

Given that cytoplasmic dynein is implicated in ER-to-Golgi transport in non-polarised cells, an obvious question is whether it also cycles in both directions between ER and Golgi, like the H1-reactive kinesin, or whether it is released from the membrane once the transport intermediates reach the Golgi apparatus. In this study we report for the first time the localisation of conventional cytoplasmic dynein heavy chain (DHC1a) by immunofluorescence on the Golgi apparatus in interphase cells. DHC1a remains associated with Golgi membranes, even following BFA treatment, suggesting that this motor shuttles constitutively between the ER and the Golgi apparatus, probably via the ER-Golgi intermediate compartment (ERGIC). We also demonstrate that conventional cytoplasmic dynein heavy chain is lost from the Golgi apparatus following overexpression of the p50/dynamitin dynactin subunit in *Xenopus* tissue culture (XTC) cells.

MATERIALS AND METHODS

All chemicals were AnalaR grade and were purchased from either BDH (Poole, UK) or Sigma Chemical Co. (Poole, UK) unless indicated otherwise.

Preparation and affinity purification of anti-DHC1a antibody

The peptide NH₂-FDPKQTDVLQQLSC-CONH₂, corresponding to amino acids 717-729 of the predicted human DHC1a sequence (GenBank accession number U53530; Vaisberg et al., 1996) followed by a single amino acid linker, was synthesised, conjugated to keyhole limpet hemocyanin, and used to immunise the rabbit 777A according to Eurogentec's immunisation protocol (Eurogentec Bel SA, Seraing, Belgium). For indirect immunofluorescence (IF) microscopy and immunoblotting (IB) experiments, antibodies were affinity-purified according to the protocol previously described by Liu et al. (1996). Bound immunoglobulins (IgGs) were eluted by 2-3 × 1 minute incubations in 100 mM glycine, pH 2.5. The pH of the eluate was neutralised immediately by adding 0.1 volume of 1 M Tris-HCl, pH 8.0. Affinity-purified IgGs were concentrated about tenfold using a Centricon 100 filter (Amicon Inc., Beverly, MA, USA) and kept at 4°C.

cDNA constructs, lectin and antibodies

Plasmid pCNG2, encoding NAGFP, a chimera between the full-length green fluorescent protein and the NH₂-terminal first 103 amino acids (containing the Golgi retention signal) of medial/trans-Golgi enzyme β 1,2N-acetylglucosaminyltransferase I, was a gift from D. Shima and G. Warren (ICRF, London, UK; Shima et al., 1997). The cDNA coding for the chicken p50/dynamitin subunit cloned in the pGW1-CMV vector was a gift from T. Schroer (Johns Hopkins University, Baltimore, USA). Both constructs are under control of the cytomegalovirus (CMV) promoter/enhancer to express high levels of exogenous proteins within *Xenopus* cells.

The following antibodies were used: anti- β -COP (coatamer protein; mAb clone maD; diluted 1/750 for IF) was a gift from T. Kreis (University of Geneva, Switzerland; Pepperkok et al., 1993). Anti- α -tubulin mAb (clone B-5-1-2; diluted 1/2000 for IF) was purchased from Sigma. Anti-DIC (diluted 1/1000 for IB) mAb was purchased from Chemicon (Harrow, UK). Anti-p150^{Glued} mAb (diluted 1/10000 for IB) was purchased from Transduction Laboratories (Lexington, KY, USA). Anti-ERGIC-53 mAb (diluted 1/50 for IF) was a gift from

H. P. Hauri (University of Basel, Switzerland; Schweizer et al., 1988). Anti-TGN46 (trans-Golgi network) sheep polyclonal antibody (diluted 1/2000 for IF) was obtained from S. Ponnambalam (University of Dundee, UK; Ponnambalam et al., 1996). Anti-cis-Golgi antibody 10E6 (diluted 1/150 for IF) was a gift from W. J. Brown (Cornell University, USA). Affinity-purified anti-DHC1a antibody was used at dilutions of 1/300 for IB and 1/60 for IF. All secondary antibodies were purchased from Jackson (West Grove, PA, USA) and used according to the manufacturer's instructions. Texas red-conjugated *Helix pomatia* lectin (diluted 1/750 for IF) was purchased from Sigma.

Cell culture and transient transfection

Xenopus XL2 (provided by C. Prigent, University of Rennes I, France), XTC and XL177 (obtained from E. Karsenti, EMBL, Heidelberg, Germany) cells were maintained in 70% Leibovitz's L-15 medium supplemented with 10% Australian foetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. *Xenopus* cell lines were grown at 20–25°C, without CO₂. Human A431 cells (a transformed keratinocyte cell line) were grown in Dulbecco's modified Eagle's medium supplemented with 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. Human, monkey and mouse cell lines were grown at 37°C in 5% CO₂. A431 cells were also cultured in CO₂-independent medium at 15°C. All cell culture reagents were purchased from Life Technologies Ltd (Paisley, UK) unless indicated.

For transient transfection, XTC cells were seeded onto sterile 13 mm round coverslips to reach 40–50% confluence after 24 hours at 25°C. Cells were then transfected according to the protocol described by Sambrook et al. (1989) with the following modifications: 3 hours before transfection, cells were washed with Dulbecco's phosphate-buffered saline (PBS; Life Technologies Ltd) and transferred to fresh culture medium. The calcium phosphate-DNA coprecipitate was prepared by mixing endotoxin-free DNA (10–25 µg), purified using Qiagen endotoxin-free kit (Qiagen, Crawley, UK), with 0.125 M CaCl₂. Then HSBP buffer (0.75 mM Na₂HPO₄, 5 mM KCl, 140 mM NaCl, 6 mM glucose, 25 mM Hepes, pH 7.0) was gently released at the bottom of the tube containing DNA and CaCl₂, followed by 10 air bubbles in order to mix the components gently. The CaPO₄-DNA precipitate was allowed to form for 30 minutes at room temperature (RT) and then mixed with 1.5 ml of fresh cell culture medium and added to the coverslips in 60 mm diameter plastic Petri dishes. After 12–14 hours at 25°C, culture medium containing DNA was removed, then the cells were washed once with Dulbecco's PBS and finally placed in fresh culture medium and incubated for 48 hours at 25°C. The cells were subsequently processed for indirect immunofluorescence as described below.

Conditions for drug treatments

XTC cells, seeded on coverslips, were incubated for various periods of time with medium containing 10 µg/ml BFA (Alexis Corp., Läufelfingen, Switzerland) from a 10 mg/ml stock in methanol. Disruption of the MT network was carried out by treating cells with 10 µM nocodazole (from a 10 mM stock in DMSO) for 3 hours at 25°C. For all drug treatment experiments, methanol and DMSO were added to a final concentration of 0.1% as a control. For recovery studies, drug-treated cells were washed three times with drug-free medium and incubated for 3.5 hours at 25°C in the absence of drug.

Indirect immunofluorescence microscopy

For indirect immunofluorescence microscopy experiments, cells were seeded on 13 mm sterile round glass coverslips for 1 or 2 days at the appropriate temperature.

In all experiments, *Xenopus* cells were rinsed in MMR buffer (100 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 5 mM Hepes, 0.1 mM EDTA, pH 7.8) whereas human (A431) cells were rinsed in PBS (0.14 M NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 8.1 mM Na₂HPO₄).

All cells were then fixed and permeabilised with methanol at –20°C for 4 minutes. After three washes in PBS, fixed cells were incubated in PBS containing primary antibodies for 30 minutes at RT. After washing in PBS (3× 5 minutes), cells were incubated with fluorescently labelled secondary antibodies for 30 minutes at RT, followed by three washes in PBS. For staining with *Helix pomatia* lectin, cells were incubated for 5 minutes at RT in the presence of lectin diluted in PBS, after staining with primary and secondary antibodies. After extensive washes in PBS, coverslips were briefly rinsed in H₂O and then mounted on glass slides in Mowiol containing 25 mg/ml 1,4-diazobicyclo-[2.2.2]-octane to reduce photo-bleaching. Pictures of fluorescently labelled cells were collected using a cooled, slow scan CCD camera CH250 (Photometrics, Tucson, AZ, USA) attached to a Leica DM RXA microscope (Leica, Wetzlar, Germany), using either a 63× Plan-Apo (NA 1.32) or a 100× PL Fluotar (NA 1.30) phase objective (Leica). Image acquisition was performed using IPLab Spectrum software (version 3.1a, Signal Analytics, Vienna, VA, USA) on a PowerMAC computer 8100/100. Images were also collected using a confocal laser scanning microscope equipped with a krypton/argon laser (Leica TCS NT). Images were transferred to Photoshop imaging software (Adobe Systems, San Jose, CA, USA) for digital processing and printed on an HP PhotoSmart colour printer (Hewlett Packard).

Xenopus egg extracts and XTC post-nuclear supernatant

Interphase extracts were prepared from freshly laid *Xenopus laevis* (Blades, Kent, UK) eggs according to Allan (1995). Ultraviolet light (UV) vanadate cleavage of *Xenopus* egg extracts was performed according to Niclas et al. (1996). XTC post-nuclear supernatant was prepared according to the protocol described by Lane and Allan (1999). Protein concentration was determined using the BCA kit (Pierce, Chester, UK) according to the manufacturer's instructions.

SDS-polyacrylamide gel electrophoresis and immunoblotting

SDS-PAGE was performed as described in Lane and Allan (1999). Proteins were electrophoretically transferred for 12 hours at 30 V onto nitrocellulose (Schleicher and Schuell, Dassel, Germany) using a mini Trans-Blot Electrophoretic Transfer Cell (Biorad, High Wycombe, UK). Immunoblotting was performed as described in Lane and Allan (1999).

Acrylamide gels were silver stained according to Heukeshoven and Dernick (1988).

Immunoprecipitation

Native immunoprecipitations (IPs) were carried out as follows. *Xenopus* egg extract was diluted with 2 volumes of IP buffer (100 mM NaCl, 1% Triton X-100 (Biorad), 0.2 mM PMSF, 10 mM Tris-HCl, pH 8.3) and incubated on ice for 10 minutes. Rec-protein G sepharose A beads (15–20 µl packed beads/sample; ZYMED, San Francisco, CA, USA), blocked for 30 minutes at 4°C with 20% BSA in IP buffer, were incubated for 1 hour or overnight at 4°C with 3 µl of anti-DIC mAb or 20 µl of 777A serum per 20 µl of packed beads. Beads were then washed 3 times with a large volume of IP buffer and were added to the diluted protein extract and incubated for 2 hours at 4°C under rotation. Beads were then recovered by spinning the diluted extract for 5 seconds at 13,000 g_{max}, and washed five times in IP buffer followed by two washes in 50 mM Tris-HCl, pH 8.3. Bound proteins were eluted in gel sample buffer (10% glycerol, 3% SDS, 62.5 mM Tris-HCl, pH 6.8, 5% β-mercaptoethanol, Bromophenol blue) before being boiled and separated by SDS-PAGE on a 7% gel. For IP under denaturing conditions, the extract was diluted as above in IP buffer containing 0.4% SDS instead of Triton X-100. After incubation on ice for 10 minutes, SDS was sequestered into mixed micelles by adding Triton X-100 (Surfact-Amps® X-100 grade, Pierce) to 2% final concentration. IPs were then carried out as described above.

RESULTS

Characterisation of the anti-DHC1a antibody

We have generated an antipeptide antiserum that specifically reacts with the DHC1a isoform. A 13-amino-acid peptide sequence from the human DHC1a isotype sequence, which was not conserved in other DHC isotype protein sequences (Criswell et al., 1996; Vaisberg et al., 1996; Fig. 1A), was used for polyclonal antibody production. This peptide sequence is totally conserved in the rat DHC1a sequence (amino acids 1844-1856; Fig. 1A) and localised upstream of the proximal ATP binding P-loop motif (Zhang et al., 1993). A BLASTP sequence homology search of the Genbank database using the peptide sequence revealed no significant homology to known proteins apart from the DHC1a protein family.

Following affinity purification of the antiserum against the peptide immobilised on membranes (Liu et al., 1996), the antibody preparation was tested by immunoblotting. The affinity-purified anti-DHC1a antibody was highly specific and recognised a single high molecular mass protein in *Xenopus* egg extracts (Fig. 1B, lane 2). We also tested motor fractions separated by sucrose density centrifugation, and observed that the antibody recognised conventional cytoplasmic dynein heavy chain prepared from interphase *Xenopus* egg extracts, pig brain and HeLa cells, but did not recognise any high molecular mass protein in the 13S fractions where DHC2/1b migrates (our unpublished data). In a postnuclear supernatant prepared from *Xenopus* XTC cells, the antibody detected two

bands of similar molecular mass (Fig. 1B, lane 1). This may result from either alternative splicing or partial proteolytic degradation of DHC1a in XTC cells.

In the presence of UV and vanadate, it has been shown that cytoplasmic dynein heavy chain undergoes a partial photo-activated cleavage to generate two high molecular mass products: HUV (heavier UV cleavage product) and LUV (lighter UV cleavage product) of approximately 230 kDa and 190/200 kDa, respectively (Schnapp and Reese, 1989; Schroer et al., 1989; Pfarr et al., 1990). To confirm that the protein detected by the affinity-purified anti-DHC1a antibody was indeed a cytoplasmic dynein heavy chain, we treated *Xenopus* egg extracts and purified pig brain cytoplasmic dynein (our unpublished data) with UV and vanadate (Niclas et al., 1996) then analysed the photocleaved extract by immunoblotting using the affinity-purified anti-DHC1a antibody. UV (Fig. 1C, lane 1) or vanadate alone (our unpublished data) had no effect on the motility of DHC1a. However, in the presence of UV and vanadate, two bands were observed on the blot (Fig. 1C, lane 2) corresponding to the remaining intact DHC1a protein (upper band) and the LUV photo-cleavage product (lower band).

Next, we asked whether the antibody was able to immunoprecipitate the native cytoplasmic dynein complex, with or without the dynactin complex. An interphase *Xenopus* egg extract was incubated with rabbit 777A polyclonal serum or anti-DIC mAb antibody in the presence or absence of SDS. The immunoprecipitation reactions were then separated on a 7% polyacrylamide gel. The top of the gel was silver stained

Fig. 1. Characterisation of the antibody raised against DHC1a peptide sequence. (A) Alignment of the sequence of human DHC1a protein (Hs DHC1a, accession number: U53530) with the sequences of Rat DHC1a (Rn DHC1a; D13896), human DHC2/1b (Hs DHC2/1b; U53531) and DHC3 (Hs DHC3; U53532). The sequence of peptide 777A is underlined. (B) Protein extracts (40 µg) prepared from XTC cells (lane 1) and *Xenopus* eggs (lane 2) were subjected to electrophoresis, transferred to nitrocellulose and blotted with the anti-DHC1a antibody. Migration of the molecular mass standards is indicated on the left. (C) Protein extracts prepared from eggs were treated with UV alone (lane 1) and UV plus vanadate (lane 2), transferred to nitrocellulose and blotted with the anti-DHC1a antibody. In the presence of UV and vanadate, anti-DHC1a reacts against the intact DHC1a protein (upper band) and the LUV photo-cleavage product (lower band). (D) *Xenopus* egg extract was immunoprecipitated with beads alone (lane 1), pre-immune serum (lane 2), anti-DHC1a serum (lane 3) and anti-DIC mAb (lane 4). The top of the gel was silver-stained in order to visualise DHC1a (top panel) and the rest of the gel was transferred onto nitrocellulose and analysed by immunoblotting using anti-DIC (lower panel) and anti-p150^{Glued} (middle panel) mAbs. Anti-DHC1a serum was able to coimmunoprecipitate DHC1a along with DIC (lane 3, lower panel) and p150^{Glued} (lane 3, middle panel). None of these proteins were immunoprecipitated using beads alone (lane 1) or with preimmune serum (lane 2).

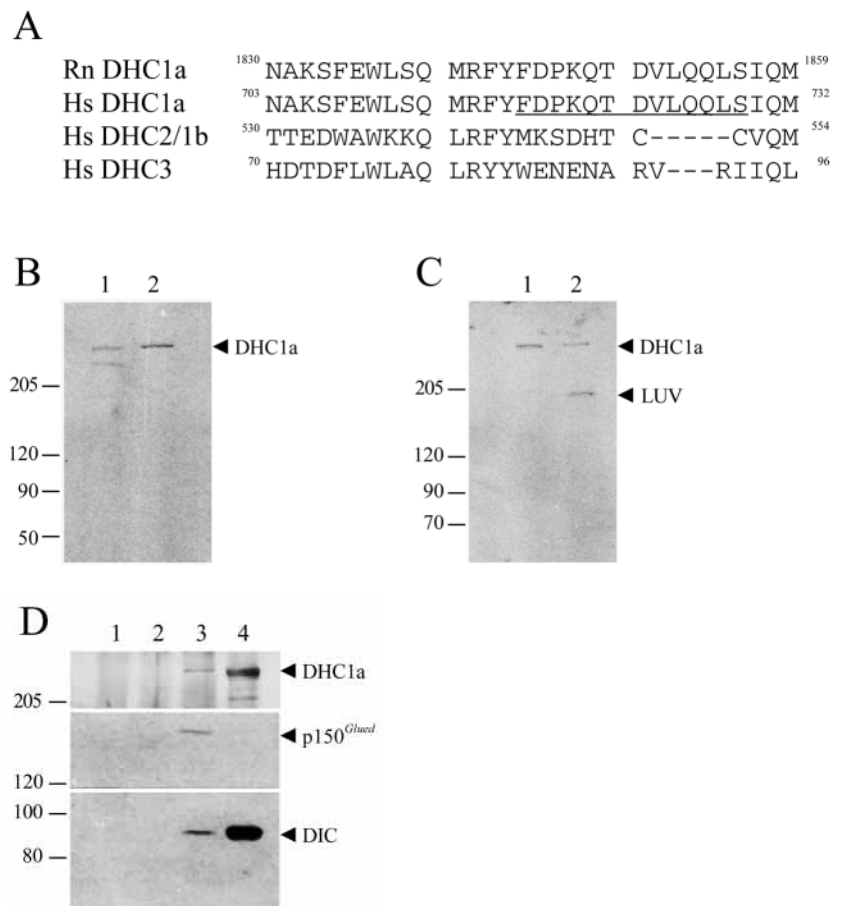
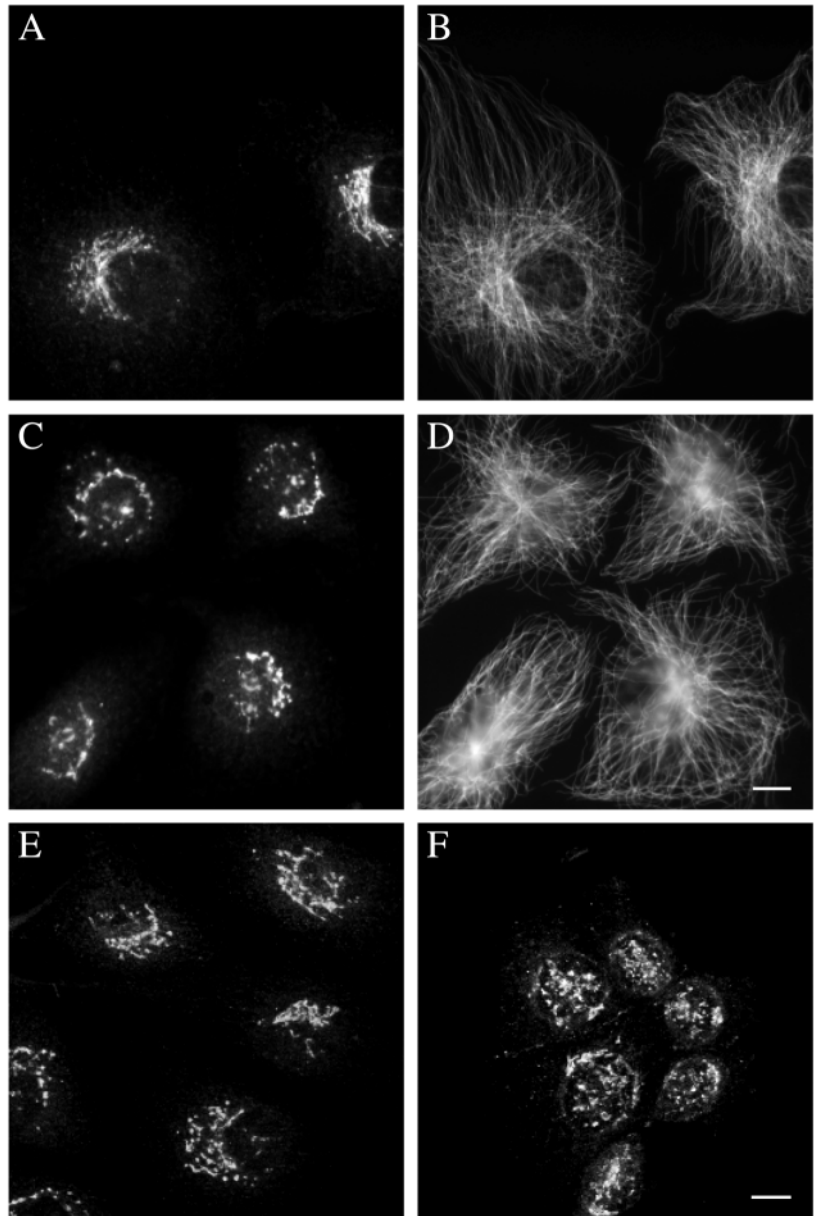


Fig. 2. DHC1a localisation in interphase cells visualised by immunofluorescence microscopy. Methanol-fixed *Xenopus* XTC (A,B), XL2 (C,D) and XL177 (E) cells, and human A431 cells (F), were labelled with the anti-DHC1a (A,C,E,F) and anti- α -tubulin (B,D) antibodies. A-D are conventional immunofluorescence micrographs. E and F were acquired by confocal microscopy and are through-focus projections of complete x/y optical section stacks. DHC1a localises to juxtannuclear reticular structures. Bars, 10 μ m.



in order to visualise DHC1a and the rest of the gel was analysed by immunoblotting using antibodies to DIC and the p150^{Glued} subunit of dynactin. Analysis of the resulting precipitates revealed that the anti-DHC1a antibody was able to coimmunoprecipitate DHC1a along with DIC (Fig. 1D, lane 3, lower panel) and cytoplasmic dynein light intermediate chain (our unpublished data) as well as p150^{Glued} (Fig. 1D, lane 3, middle panel). None of these proteins were immunoprecipitated using beads alone (Fig. 1D, lane 1) or pre-immune serum (Fig. 1D, lane 2). When SDS was added to the egg extract prior to immunoprecipitation, only DHC1a was immunoprecipitated: the coimmunoprecipitation of the other subunits of the dynein complex and p150^{Glued} with anti-DHC1a antibody was totally inhibited (our unpublished data).

Our data show that the antibody raised against a peptide sequence found only in DHC1a specifically recognises a high molecular mass protein in *Xenopus*, which is cleaved following UV/vanadate treatment. The antibody was also able to immunoprecipitate both native *Xenopus* cytoplasmic dynein complex and dynactin. We therefore conclude that our antibody recognises DHC1a in *Xenopus*.

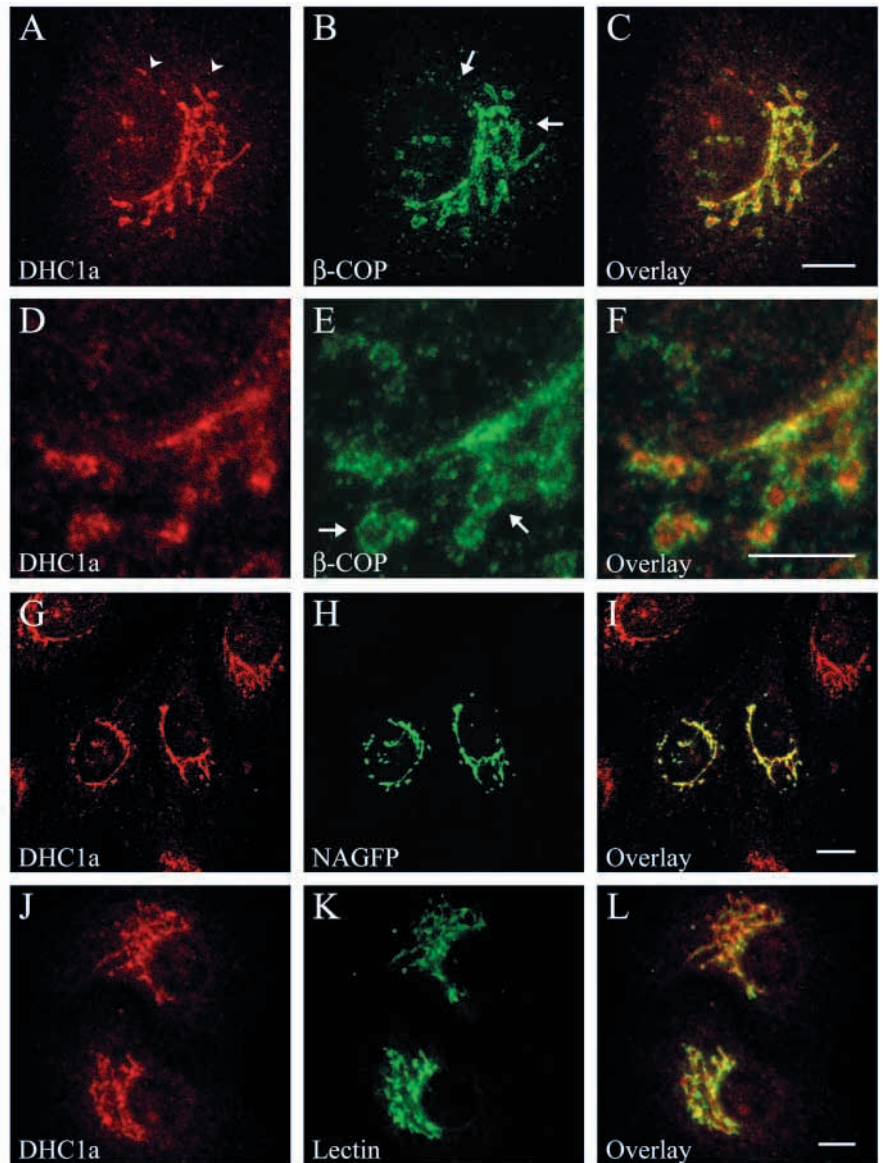
Conventional DHC is localised on the Golgi apparatus

We investigated the subcellular localisation of DHC1a by indirect immunofluorescence microscopy of cultured cells using the affinity-purified anti-DHC1a antibody in methanol-fixed XTC cells. In interphase cells, the anti-DHC1a antibody localised to a perinuclear reticular structure resembling the Golgi apparatus, with a more diffuse and punctate staining throughout the rest of the cytoplasm (Fig. 2A). In the same cell, double labelling of the MT network using an anti- α -tubulin antibody (Fig. 2B) did not reveal colocalisation of DHC1a with MTs. We detected a similar staining pattern using anti-DHC1a antibody, affinity-purified on the peptide bound to thiopropyl sepharose 6B beads (our unpublished data). Pre-absorption of the affinity purified anti-DHC1a antibody, with an excess of the peptide used for immunisation, was found to abolish the staining completely (our unpublished data). A Golgi complex-like staining pattern was also detected in a variety of other cell types, including *Xenopus* XL2 (Fig. 2C) and XL177 cells (Fig. 2E), as well as in human A431 cells (Fig. 2F). Interestingly, a dotted, vesicular

cytoplasmic dynein staining pattern, rather than a clear Golgi complex localisation, was detected in methanol-fixed human HT1080 and HeLa cells, monkey CV-1, VERO and COS cells, and rat NRK cells. This suggests a differential accessibility of the epitope recognised by the affinity-purified anti-DHC1a antibody, depending on the cell type used.

To confirm that DHC1a was localised on Golgi membranes, we performed double immunofluorescence labelling experiments with the anti-DHC1a antibody, along with markers for the Golgi apparatus in XTC cells. Firstly, the distribution of DHC1a was compared with that of β -COP, a subunit of the peripheral membrane coatamer complex I (COPI) that has previously been localised on rims of Golgi cisternae, Golgi stacks and the ERGIC (reviewed in Kreis et al., 1995). We observed by conventional immunofluorescence microscopy (our unpublished data) and laser confocal microscopy that DHC1a colocalised extensively with β -COP (Fig. 3A-F). Merging the signals from a single focus plane also

Fig. 3. DHC1a colocalises with different Golgi markers. Methanol-fixed XTC cells were double-labelled with the anti-DHC1a antibody (A,D,G,J) and an anti- β -COP mAb (B,E) or *H. pomatia* lectin (K). For consistency, pseudo colours have been added to black and white images such that DHC1a staining is shown in red. XTC cells were also transiently transfected with NAGFP construct (H) then stained with anti-DHC1a antibody (G). Merged images are shown in C, F, I and L. Images A-F were acquired by confocal microscopy. Other images are conventional immunofluorescence micrographs. Bars, 10 μ m (A-C, G-L); 5 μ m (D-F).



indicated colocalisation of DHC1a and β -COP (our unpublished data). Interestingly, observation at higher magnification revealed subtle differences in the staining patterns with the bulk of β -COP surrounding the DHC1a-labelled structures (Fig. 3D-F). In addition to the Golgi apparatus staining, we found that β -COP labelled some small peripheral structures, probably intermediate compartment, consisting of membrane material en route in either direction between the ER and the Golgi apparatus (Fig. 3B, arrows), which did not appear to be stained by the anti-DHC1a antibody. DHC1a, in turn, also labels a population of scattered β -COP-negative vesicles (Fig. 3A, arrowheads). A similar colocalisation between β -COP and DHC1a was observed in A431 mammalian cells as well as in XL2 and XL177 *Xenopus* cells (our unpublished data). We also observed extensive colocalisation (Fig. 3G-I) between DHC1a and β 1,2N-acetylglucosaminyltransferase I-green fluorescent protein chimera (NAGFP: see Materials and Methods, and Shima et al., 1997), which is a marker for the Golgi apparatus when expressed transiently in XTC cells. Finally, DHC1a colocalised well with *Helix pomatia* lectin (Fig. 3J-L), which has previously been shown to label the Golgi apparatus in a number of different cell types (Virtanen, 1990). However, subtle differences between DHC1a and *Helix pomatia* lectin staining patterns could be reproducibly observed (probably representing a difference in the subset of Golgi structures recognised). DHC1a also colocalised with the cis-Golgi protein recognised by the 10E6 antibody in A431 cells (our unpublished data).

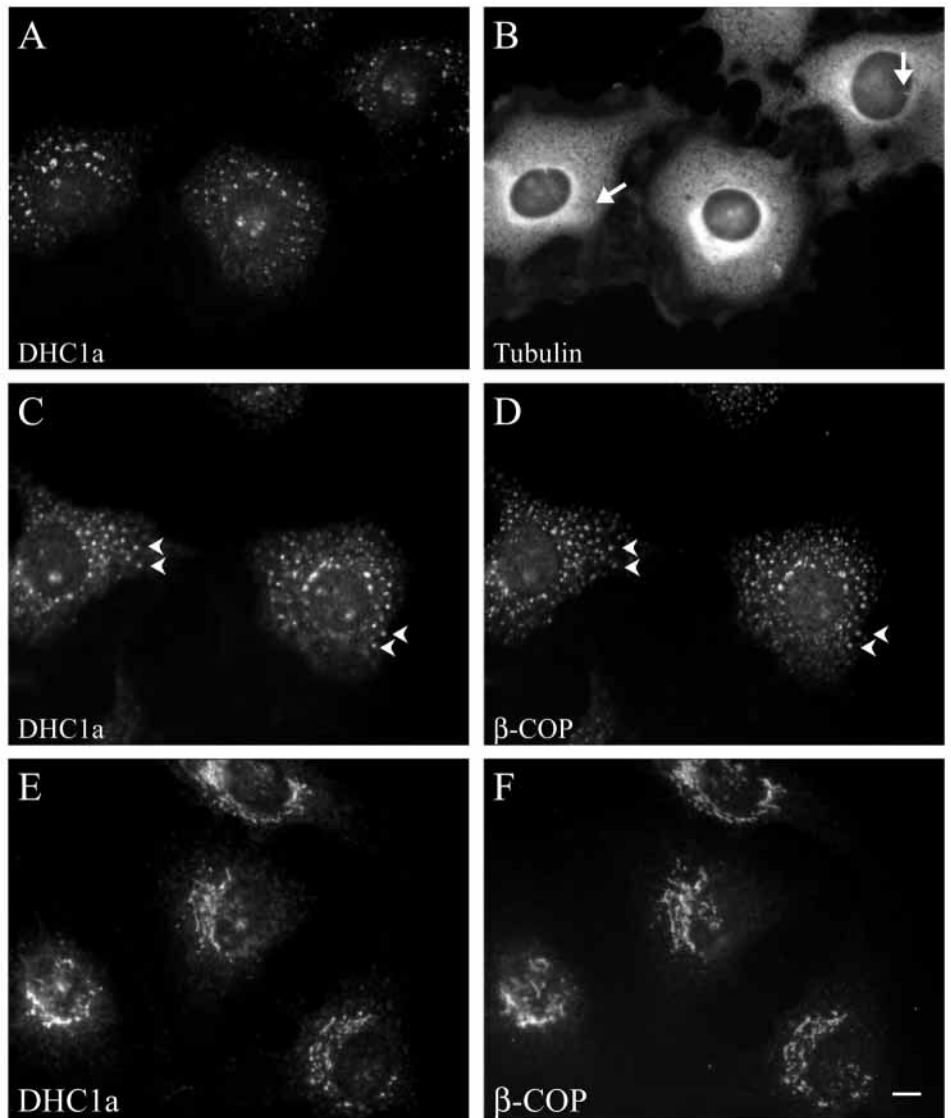
Based upon the clear colocalisation of DHC1a with Golgi markers, we conclude that the perinuclear structures stained with the anti-DHC1a antibody are Golgi membranes. However, owing to the limited resolution of fluorescence microscopy, we were unable to determine which cisternae (cis, medial, trans) is/are decorated with the anti-DHC1a antibody.

DHC1a remains associated with peripheral Golgi structures in the absence of microtubules

It is well established that drug-induced depolymerisation of the MT network results in the dispersal of the centrally located

Golgi apparatus to numerous scattered, but functional, Golgi stacklets (reviewed in Thyberg and Moskalewski, 1999). In order to assess if DHC1a is localised to peripheral Golgi stacklets, XTC cells were treated with 10 μ M nocodazole for 3 hours at 22°C to depolymerise the MTs, and were then stained with mAb anti- β -COP antibody along with the affinity-purified anti-DHC1a antibody. Microtubule depolymerisation was monitored using an anti- α -tubulin antibody. Under these conditions, most of the MTs were depolymerised and only a few nocodazole-resistant MTs were generally seen per cell (Fig. 4B, arrows). After 3 hours of nocodazole treatment, we observed the complete dispersal of the centrally located Golgi complex and the formation of peripheral DHC1a-positive structures of varying size that were distributed throughout the cytoplasm (Fig. 4A). When the distribution of DHC1a was compared with that of β -COP in double immunofluorescence labelling experiments, an extensive colocalisation of these two proteins in scattered structures was observed (Fig. 4C,D, arrows). Disruption of MTs by incubation of the cells at 4°C has little or no effect on the morphology of either the Golgi

Fig. 4. DHC1a colocalises with peripheral β -COP-positive structures following nocodazole treatment. Nocodazole-treated XTC cells were methanol-fixed and double-labelled with anti-DHC1a antibody (A,C,E) and anti- α -tubulin (B) or anti- β -COP (D,F) mAbs. After 3 hours of nocodazole treatment, microtubule depolymerisation was complete and only a few nocodazole-resistant microtubules remained in some cells (B, arrows). DHC1a (C) colocalises with β -COP-positive Golgi elements scattered throughout the cytoplasm (D, arrowheads). Treated cells were left to recover in nocodazole-free medium for 3.5 hours at 22°C, resulting in the reformation of the centrally located Golgi apparatus (E,F). All images were acquired by conventional immunofluorescence microscopy. Bar, 10 μ m.



apparatus (Moskalewski et al., 1980) or associated DHC1a (our unpublished data).

Nocodazole-induced Golgi scattering is reversible, and upon removal of the drug, the fate of these peripheral Golgi structures was followed by double labelling of fixed cells with anti- β -COP and anti-DHC1a antibodies. Regrowth of MT was very fast and a dense MT network was detected 5 minutes after nocodazole removal. We observed the continuous colocalisation of DHC1a- and β -COP-positive structures as they concentrated towards the cell centre (our unpublished data). After 3.5 hours at 25°C in drug-free medium we observed the reformation of the Golgi complex using the anti- β -COP antibody.

As previously seen in untreated cells, we observed that the distribution patterns of DHC1a and β -COP (Fig. 4F,E)

colocalised extensively on the centrally reclustered Golgi apparatus.

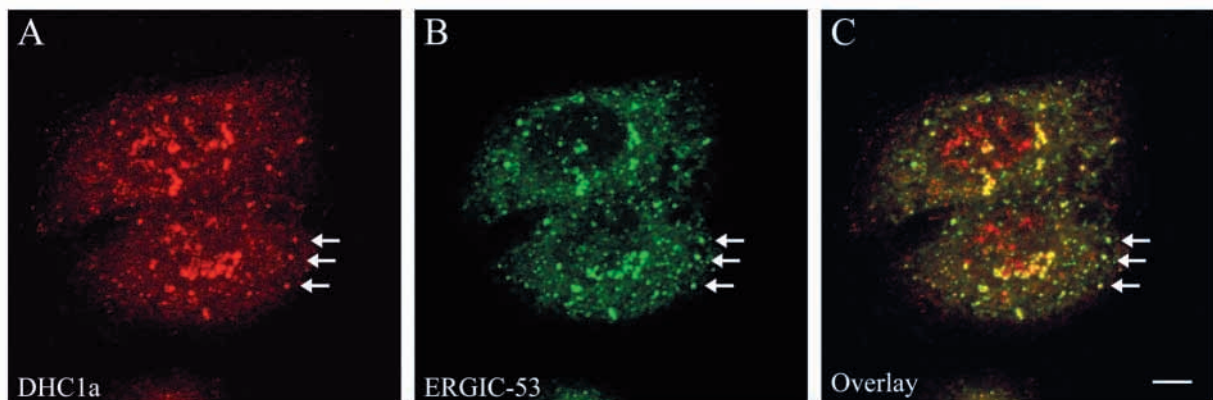


Fig. 5. Temperature-dependent redistribution of DHC1a in the intermediate compartment. A431 human fibroblasts were incubated for 3 hours at 15°C and then double-labelled with the anti-DHC1a antibody (A) and anti-ERGIC-53 mAb as an intermediate compartment marker (B). A merged image is shown in (C). DHC1a colocalises to a large number of, but not all, ERGIC-53-positive vesicular elements, both in the perinuclear region and in more peripheral locations (arrows). All images were acquired by confocal microscopy. Bar, 10 μ m.

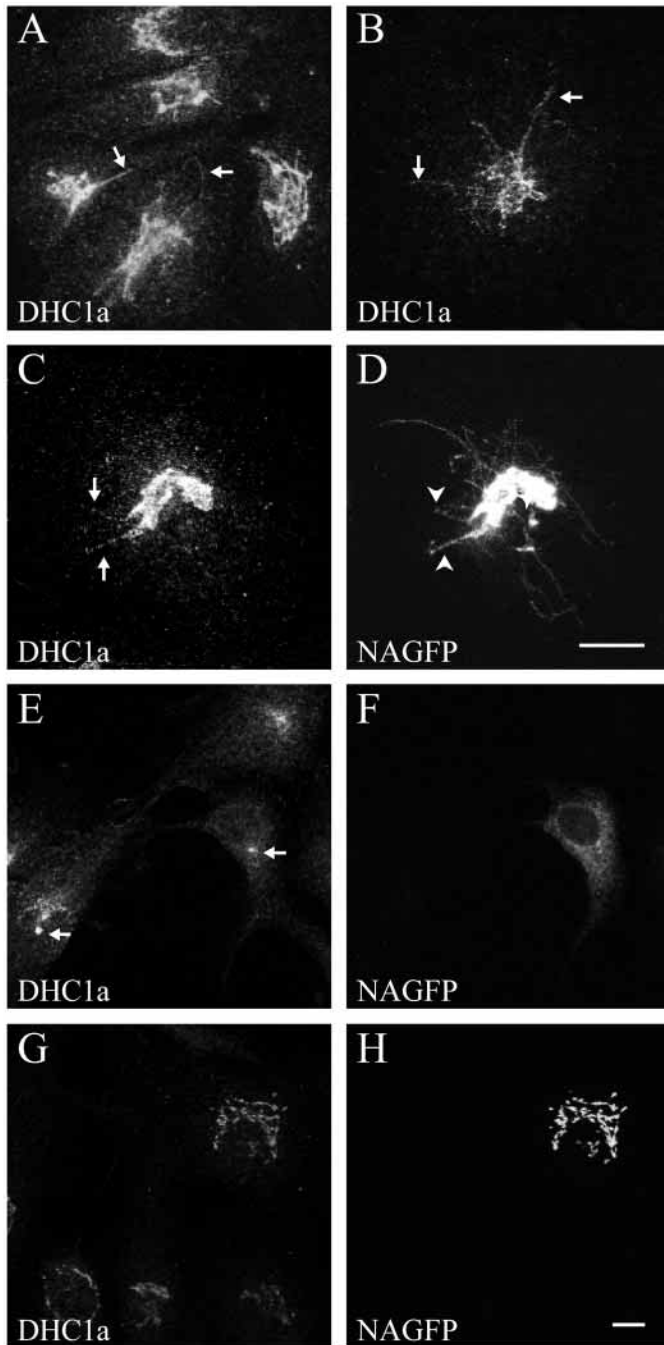


Fig. 6. DHC1a is present on BFA-induced membrane tubules. Untransfected (A,B) or NAGFP-transfected (C-H) XL177 cells were incubated with 10 $\mu\text{g/ml}$ of BFA for 7 minutes (A-D) or 25 minutes (E-H). Cells in (G,H) were incubated for an additional 3.5 hours in BFA-free medium. Cells in (A,B,E,F) were stained with the anti-DHC1a antibody. DHC1a is detected on a proportion of BFA-induced NAGFP membrane tubules that extend towards the cell periphery. After 25 minutes of treatment, DHC1a staining is largely restricted to a small bright juxtannuclear area (probably the microtubule organising centre; E, arrows), with some additional diffuse staining throughout the cytoplasm. DHC1a (G) and NAGFP (H) colocalised extensively with the centrally reclustered Golgi apparatus 3.5 hours after BFA removal. All images were acquired by confocal microscopy. Bars, 5 μm (A-D); 10 μm (E-H).

Low temperature-induced block of anterograde transport reveals localisation of DHC1a on the ERGIC

We next assessed whether DHC1a was associated with elements of the ERGIC, which consists of pleiomorphic tubulovesicular structures found in the centrosomal region and at peripheral sites (e.g. Saraste and Svensson, 1991). Since lowering the temperature of the medium to 15°C blocks the anterograde transport pathway from the ER to the Golgi apparatus, resulting in the accumulation of newly synthesised protein in ERGIC structures (Lippincott-Schwartz et al., 1990; Saraste and Svensson, 1991), we therefore examined how temperature reduction would affect the localisation of DHC1a. Human A431 fibroblasts were used because of the lack of information on the nature and structure of the ERGIC in *Xenopus* cells.

When control A431 fibroblasts were double-labelled with antibodies against DHC1a and ERGIC-53/p58 (an ERGIC marker; Schweizer et al., 1988; Lippincott-Schwartz et al., 1990; Hauri and Schweizer, 1992), we observed that none of the small, peripheral ERGIC-53-positive structures were labelled with DHC1a (our unpublished data). When the cells had been incubated for 3 hours at 15°C, ERGIC-53 underwent a characteristic redistribution, as described for other cells (e.g. Lippincott-Schwartz et al., 1990; Schweizer et al., 1990; Saraste and Svensson, 1991), and was found to be concentrated in large dots in the Golgi area, and also in smaller structures in the cell periphery (Fig. 5B). We observed that DHC1a (Fig. 5A) was localised to most, but not all, ERGIC-53-positive vesicular elements, both in the perinuclear region and in more peripheral locations (Fig. 5C, arrows). This colocalisation of ERGIC-53 and DHC1a suggests that DHC1a is present on intermediate compartment structures during the low temperature-induced transport block.

Localisation of DHC1a on BFA induced Golgi-to-ER membrane tubules

The localisation of DHC1a on centrally located Golgi membranes, as well as on peripheral Golgi stacklets and ERGIC structures that translocate inward towards the Golgi apparatus, raises the question of whether DHC1a is also cycling with membranes in the reverse direction, from the Golgi apparatus to the ER. To investigate this possibility, we tested whether DHC1a remains associated with the BFA-induced Golgi membrane tubules and cycles to the ER with these membranes.

In order to assess if *Xenopus* XL177 and XTC cells were sensitive to BFA, cells were treated with 10 μM BFA for various times and the behaviour of β -COP observed. Within 2 minutes after addition of the drug, β -COP was found to redistribute to a diffuse pattern (our unpublished data) in either cell-line, suggesting that both were sensitive to BFA. DHC1a distribution was analysed in *Xenopus* XL177 cells treated with 10 $\mu\text{g/ml}$ of BFA at 20°C. After 7 minutes incubation, DHC1a was detected on fine tubule processes emanating from the Golgi apparatus (Fig. 6A-C, arrows). DHC1a-positive BFA-induced membrane tubules were also observed in XTC cells (our unpublished data). In order to confirm that the DHC1a labelling corresponded to BFA-induced Golgi membrane tubules, we treated XL177 cells that had been transiently transfected with the NAGFP fusion

construct, with 10 $\mu\text{g/ml}$ BFA. In cells overexpressing NAGFP we observed that, 7 minutes following drug

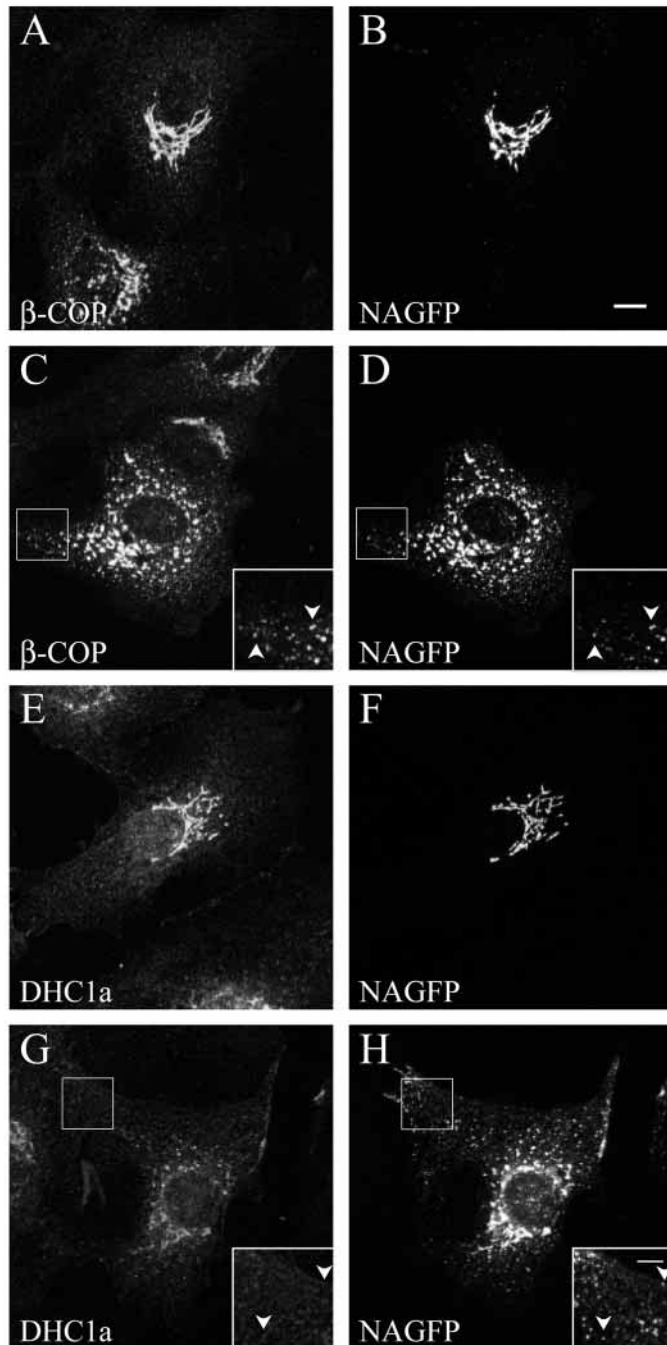


Fig. 7. DHC1a is lost from peripheral Golgi stacklets following overexpression of p50/dynamitin. XTC cells transiently transfected with NAGFP construct alone (A,B,E,F), or with NAGFP and p50/dynamitin (C,D,G,H), were stained with anti- β -COP antibody (A,C) or with the anti-DHC1a antibody (E,G). Overexpression of p50/dynamitin induced Golgi scattering and a reduction in the intensity of DHC1a labelling of NAGFP-positive structures. This is particularly clear in the cell periphery, where NAGFP-positive structures possessed β -COP (compare inserts in C,D, arrowheads), but not DHC1a (compare inserts in G,H, arrowheads). All images were acquired by confocal microscopy using identical confocal and printer settings for all images. Bars, 10 μm (5 μm in enlarged regions).

exposure, NAGFP was found in tubular processes that extended toward the cell periphery (Fig. 6D, arrowheads). A proportion of these NAGFP tubules were also positive for DHC1a (Fig. 6C, arrows). In contrast, DHC1a was not associated with any of the BFA-induced TGN membrane tubules emanating from the Golgi area of A431 cells treated with BFA (our unpublished data).

Immunofluorescence of cells incubated for more than 25 minutes with BFA revealed a significant alteration of the DHC1a (Fig. 6E) and NAGFP (Fig. 6F) fluorescence-staining patterns. DHC1a was largely restricted to a small bright juxtannuclear area (possibly the centrosome; Fig. 6E, arrows), with some additional diffuse staining throughout the cytoplasm (probably ER staining; Fig. 6E). Removal of BFA after 30 minutes and incubation of the cells for 3.5 hours at 25°C resulted in the reassembly of the Golgi apparatus as visualised in NAGFP-transfected XL177 cells (Fig. 6H). As previously observed in untreated cells, the distribution patterns of DHC1a and NAGFP (Fig. 6G,H) localised extensively on the centrally re-clustered Golgi apparatus.

Although redistribution of the Golgi membranes into the ER during BFA treatment is now well characterised, the presence of DHC1a on BFA-induced tubules was unexpected. This result supports the hypothesis that DHC1a constitutively cycles between Golgi complex and ER. Such a cycling pathway has already been suggested for another microtubule motor, kinesin (Lippincott-Schwartz et al., 1995), as well as for the membrane proteins p58 (Saraste and Svensson, 1991) and ERGIC-53 (Hauri and Schweizer, 1992).

Overexpression of p50/dynamitin causes release of DHC1a from peripheral Golgi stacklets

In the light of recent studies implicating dynactin in ER-to-Golgi membrane traffic, we hypothesised that the binding of DHC1a to the Golgi apparatus (and probably ERGIC membranes) might be disrupted following p50/dynamitin overexpression, thus inducing dispersal of the Golgi apparatus. XTC cells were transiently cotransfected with the NAGFP Golgi marker to label the Golgi complex fluorescently, together with chicken p50/dynamitin. In cells transfected only with NAGFP, we observed fluorescent labelling of the Golgi apparatus (Fig. 7B,F) and its colocalisation with β -COP (Fig. 7A) and DHC1a (Fig. 7E). In XTC cells cotransfected with NAGFP and p50/dynamitin, we observed that overexpression of p50/dynamitin induced a dramatic redistribution of NAGFP staining into numerous punctate structures scattered throughout the cytoplasm (Fig. 7D,H), and that these structures also possessed β -COP (compare inserts, Fig. 7C,D, arrowheads). When we compared the DHC1a labelling seen in cells transfected with NAGFP alone versus those coexpressing p50/dynamitin and NAGFP, we observed a general reduction in the intensity of DHC1a labelling of all NAGFP-positive structures (identical confocal microscope and printer settings were used in all cases). This was clearest in the cell periphery (compare inserts, Fig. 7G,H, arrowheads), where almost no DHC1a labelling was detected, even though NAGFP- and β -COP-positive structures were present (inserts, Fig. 7C,D,H). These results suggested that overexpression of p50/dynamitin induced a dissociation of DHC1a (and probably the entire conventional dynein complex) from the Golgi apparatus.

DISCUSSION

Two forms of cytoplasmic dynein (DHC1a and DHC2/1b) are believed to be involved in Golgi function in interphase cells (Corthésy-Theulaz et al., 1992; Holleran et al., 1996; Vaisberg et al., 1996; Burkhardt et al., 1997; Presley et al., 1997; Harada et al., 1998; Tai et al., 1998). The DHC2/1b isoform partially colocalises with Golgi markers (Vaisberg et al., 1996), and microinjection of an anti-DHC2/1b antibody induces apparent fragmentation of the Golgi complex into multiple cytoplasmic structures scattered throughout the cytoplasm, in 40–45% of cells, which has been interpreted as indicating a role for DHC2/1b in Golgi positioning and/or the centripetal transport of Golgi membrane along MTs (Vaisberg et al., 1996). A similar function has also been proposed for DHC1a because of the observed dispersal of the Golgi apparatus in blastocytes cultured from DHC1a knockout mice (Harada et al., 1998). However, without the direct visualisation of DHC1a on Golgi membranes it is impossible to conclude whether dispersal of the Golgi complex results directly or indirectly from the lack of DHC1a protein on the Golgi apparatus itself or whether it reflects the consequences of altered membrane delivery to the Golgi apparatus.

We used an affinity-purified anti-DHC1a antibody to study the localisation of DHC1a in a number of non-polarised cell lines. A striking finding, which differs from other reports (Pfarr et al., 1990; Criswell et al., 1996; Vaisberg et al., 1996), is that DHC1a displays a typical Golgi localisation at the light microscope level. Since a different, function-blocking anti-DHC1a antibody, previously used to inhibit cytoplasmic dynein-dependent mitotic spindle formation (Vaisberg et al., 1993), was unable to recognise DHC1a on the Golgi apparatus by immunofluorescence, this may reflect the differential accessibility of epitopes within a fixed protein. Our visualisation of DHC1a on the Golgi apparatus indicates that conventional cytoplasmic dynein is likely to play a role in the structure and function of the Golgi apparatus, and therefore suggests that DHC2/1b is unlikely to be solely responsible for Golgi apparatus positioning.

Why should two different types of heavy chains be present on the Golgi complex? Emerging evidence suggests that numerous biochemically and functionally distinct forms of cytoplasmic dynein exist, probably performing specialised cellular tasks (Vaisberg et al., 1996; Criswell and Asai, 1998; King et al., 1998; Nurminsky et al., 1998). It seems reasonable that conventional cytoplasmic dynein and DHC2/1b-containing complexes may each perform different functions on the same organelle. For instance, given that the Golgi apparatus receives membrane material from both the ER and the endocytic pathway, and that the organelle as a whole is motile (Cooper et al., 1990), the two complexes may be found on different membrane domains within the Golgi apparatus. Indeed, the overlap between DHC1a and either *Helix pomatia* lectin, β -COP or NAGFP is close, but not absolute. In addition, we observed that BFA-induced tubules containing NAGFP fell into two classes: those with, and those without DHC1a. These data, coupled with the absence of DHC1a from BFA-induced TGN tubules, certainly suggest that DHC1a is not found in all regions of the Golgi apparatus. It will be very interesting to observe the localisation of both dynein complexes by immunoelectron microscopy in order to compare their distributions within the Golgi apparatus.

Inhibition of cytoplasmic dynein function, and the depolymerisation of MTs, both result in the formation of peripheral Golgi stacklets (Cole et al., 1996; Vaisberg et al., 1996; Burkhardt et al., 1997; Presley et al., 1997; Harada et al., 1998; Shima et al., 1998; Storrie et al., 1998; Thyberg and Moskalewski, 1999). How, and why, these stacklets form is currently the subject of controversy, since it is unclear whether it is due to an imbalance between plus end-directed motors and cytoplasmic dynein, resulting in the active movement of Golgi stacks towards the periphery (Minin, 1997), or whether they form *de novo* at ER exit sites from Golgi membrane components that have recycled through the ER (Cole et al., 1996; Storrie et al., 1998; but see Shima et al., 1998 for a conflicting view). In addition, although the Golgi stacklets formed following MT depolymerisation can subsequently move towards the cell centre if MTs are allowed to regrow (Ho et al., 1989), it is not known whether this is achieved using cytoplasmic dynein on the stacks themselves, or whether the motor is attached to moving cis-Golgi/ERGIC components that drag the associated stacks along with them. Our observations suggest that DHC1a is present not only on the Golgi apparatus, but also on elements of the ERGIC, since we see considerable colocalisation of DHC1a with β -COP under control conditions, and with both β -COP and ERGIC-53 in cells incubated at 15°C. This provides further indirect support for the hypothesis, based on observations in cells overexpressing p50/dynamitin (Presley et al., 1997), that conventional cytoplasmic dynein is responsible for the movement of ER-to-Golgi transport complexes.

The presence of excess p50/dynamitin, both *in vivo* and *in vitro*, has been shown to cause dissociation of the dynactin complex, separating p150^{Glued} from the Arp1 filament (Echeverri et al., 1996; Wittmann and Hyman, 1999). It has been proposed that this, in turn, could cause the loss of conventional cytoplasmic dynein from its cargoes, and indeed, overexpression of p50/dynamitin in mitotic cells induced the release of cytoplasmic dynein from the prometaphase kinetochore (Echeverri et al., 1996). We provide further evidence for this model by showing for the first time that when p50/dynamitin is over-expressed, DHC1a is progressively lost from the Golgi apparatus, which would explain why the Golgi apparatus becomes scattered (our results; Burkhardt et al., 1997; Presley et al., 1997) and why ER-to-Golgi traffic is inhibited (Presley et al., 1997).

It has been suggested that cytoplasmic dynein and dynactin bind to the Golgi apparatus via an organelle membrane cytoskeleton containing Golgi spectrin and ankyrin (reviewed by Beck and Nelson, 1998). In BFA-treated cells, where the Golgi-to-ER retrograde pathway is thought to be exaggerated (Klausner et al., 1992), the Golgi apparatus is transformed into membrane tubules that fuse with the ER (e.g. Lippincott-Schwartz et al., 1989; reviewed in Dinter and Berger, 1998). Golgi-associated spectrin and ankyrin have been reported to dissociate from BFA-induced Golgi tubules (Beck et al., 1994, 1997), whereas we have shown that DHC1a remains membrane-associated under these conditions. In addition, Fath and coworkers presented evidence that cytoplasmic dynein associates with Golgi membranes using peripheral membrane proteins other than the spectrin/ankyrin membrane lattice (Fath et al., 1997), although the identity of the proteins involved remains to be determined. Taken together, these data raise the

question of how conventional cytoplasmic dynein associates with the Golgi apparatus, if not through spectrin and ankyrin. Conventional cytoplasmic dynein might bind directly to membrane lipid, since purified bovine brain cytoplasmic dynein (perhaps containing some dynactin) and squid axoplasmic cytoplasmic dynein have both been reported to bind to unilamellar liposomes (Lacey and Haimo, 1994; Muresan et al., 1996). Whether an interaction with lipid could account for the specificity of dynein and/or dynactin binding within the cell remains to be seen. It would seem more likely, however, that organelle-specific membrane proteins, as yet unidentified, are involved in targeting these complexes.

Observations of the bidirectional movement of organelles such as mitochondria in axons (Morris and Hollenbeck, 1993), pigment granules in melanophores (Rozdzial and Haimo, 1986), ER (Lane and Allan, 1999) and phagosomes (Blocker et al., 1997), suggests that both plus and minus end motors may be bound to the same structure and that the direction of movement is probably controlled by regulating the activity of the motors (reviewed in Thaler and Haimo, 1996; Lane and Allan, 1998). Our BFA experiments imply that conventional cytoplasmic dynein is present, though enzymatically inactive, on membranes moving from the Golgi apparatus to the ER, but is fully functional for the reverse transport step. Since both plus and minus end-directed motors cycle constitutively between ER and Golgi apparatus (our data and Lippincott-Schwartz et al., 1995), the activity of the motors must be coordinately regulated by a mechanism which does not involve motor dissociation from the membrane. Allan (1995) has previously shown that cytoplasmic dynein-driven ER movement in interphase *Xenopus* egg extracts can be activated up to 30-fold from a basal level, without the recruitment of an additional motor (or dynactin: S. Addinall and V. Allan, unpublished data). This supports the proposal that the activity of membrane-associated motors can be modulated. In addition, a stably bound and potentially inactive cytoplasmic dynein has been reported in cells with dispersed lysosomes (Lin and Collins, 1992, 1993). We should point out that a different regulatory mechanism may be involved on entry into metaphase, when conventional cytoplasmic dynein is released from *Xenopus* egg ER membranes co-ordinately with an inhibition of organelle movement (Allan and Vale, 1991; Niclas et al., 1996). We are currently investigating whether there is a similar release of DHC1a from the Golgi apparatus in mitotic cultured cells.

We do not yet know how the activity of DHC1a on Golgi membranes is regulated during interphase, but we can speculate on the possible mechanisms. Post-translational modification of conventional DHC1a or other subunits of the complex could affect conventional cytoplasmic dynein motor activity. Indeed, it has been suggested that in rat optic nerve, cytoplasmic dynein heavy chain is less phosphorylated on vesicles moving towards MT plus ends as compared to the total cytoplasmic dynein pool. This implies that dephosphorylation of the heavy chain may inactivate the dynein motor activity, allowing the vesicles to be carried towards the nerve terminal by a plus end-directed motor (Dillman and Pfister, 1994). The effect of phosphorylating other proteins, such as subunits of the dynactin complex, must also be considered. A further possibility is that motor activity could be regulated by components of the membrane budding or fusion machinery, such as the COPI and COPII coats. However, any such link is

likely to be indirect, since we have not observed any correlation between the membrane association of cytoplasmic dynein and the presence of β COP. The possibility that Rabs may be involved in motor regulation has been made very attractive by the identification of Rabkinesin6, a kinesin-related protein which interacts with Rab6 in its GTP- but not GDP-bound form (Echard et al., 1998), and which is involved in some aspect of traffic from the Golgi apparatus to ER. Whatever the mechanism, it seems highly likely that the activity of the motors that cycle between these two organelles will be tightly coupled in some way with the process of membrane traffic.

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