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p38 MAPK regulates COPII recruitment

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Abstract

Here, we investigate regulation of coat protein complex II (COPII) recruitment onto ER export sites in permeabilized cells. In cytosols from nocodazole treated HeLa cells we find COPII loading is inhibited. The stress kinase p38 MAPK is activated in these cytosols and COPII loading can be rescued by depletion of p38 MAPK α or by the p38 MAPK inhibitor (SB203580) but not by inhibition/depletion of cdc2. These observations indicate regulation of the early secretory pathway by p38 MAPK.

Keywords: Mitosis; Endoplasmic reticulum; COPII; Budding; p38 MAP kinase; ER export; Permeabilised cells

The first step in secretory protein trafficking is the packaging of secretory cargo into vesicular carriers that arise from specialized ER exit sites (ERES) on the endoplasmic reticulum. At the ERES, vesicle budding is mediated via action of the multimeric COPII coat, assembled by recruitment of Sec23/24p and Sec13/31p from the cytosol. Assembly is triggered by a transmembrane guanine nucleotide exchange factor (GEF) Sec12p, which promotes exchange of GDP for GTP on the small GTP-ase sar1p. This protein recruits Sec23/24p for cargo binding followed by Sec13/31p to complete coat formation and the budding process (for details, see [1,2]).

Transport in the early secretory pathway of mammalian cells is considered to be a largely constitutive process but some data indicate regulation of this transport step by intracellular signals. For example, in mitosis there appears to be a profound inhibition of ER-Golgi traffic [3–6], with a restriction in the assembly and production of ER export structures [3,7]. During interphase there is also regulation of ER-Golgi trafficking by signalling pathways, protein phosphatases and protein kinases [8–11].

Here, we use semi-intact cells to study loading of COPII coat components from cytosols derived from untreated and nocodazole-arrested suspension HeLa cells. Cytosols from nocodazole arrested cells exhibit strong activation of p38 MAPK and also inhibition of COPII loading onto ERES that requires p38 MAPK α.

Materials and methods

Cell lines, antibodies, and reagents. HeLa cells were grown in modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Spinner HeLa (sHeLa) cells were grown in RPMI 1640 medium containing 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin. The following antibodies were used: affinity-purified sheep antiserum against human Sar1; affinity-purified rabbit antisera against human Sec13 and Sec31 (provided by Wanjin Hong, NUS, Singapore); anti-p38 MAP kinase α (provided by Dr. Ana Cuenda, University of Dundee); anti-phospho-cdc2 and anti-phospho-MAPKAP kinase 2 (MK2; Cell Signalling Technology); anti-total cdc2, anti-total MK2, and $p13^{SUC1}$-agarose (Upstate Biotechnology); secondary antibodies conjugated to horseradish peroxidase (Scottish Antibody Production Unit); antibodies for immunofluorescence (IF) were from Stratech Scientific. Recombinant Sar1 was prepared as described [7]. FBS and other cell-culture reagents were from Life Technologies, Complete Protease Inhibitor Cocktail (PIC) tablets from Roche, ^{3}H-GDP, ECL reagents and protein G–Sepharose from Amersham Pharmacia Biotech. PD184352 was provided by the DSTT (University of Dundee), and other signalling inhibitors from Calbiochem. Chemicals were from Sigma unless otherwise stated.

Nocodazole treatment and preparation of cytosols. sHeLa cells were grown in spinner flasks to between 4 and 7×10^5 cells/ml. For interphase cells, ½ volume fresh medium was added and cells cultured for 24 h. For

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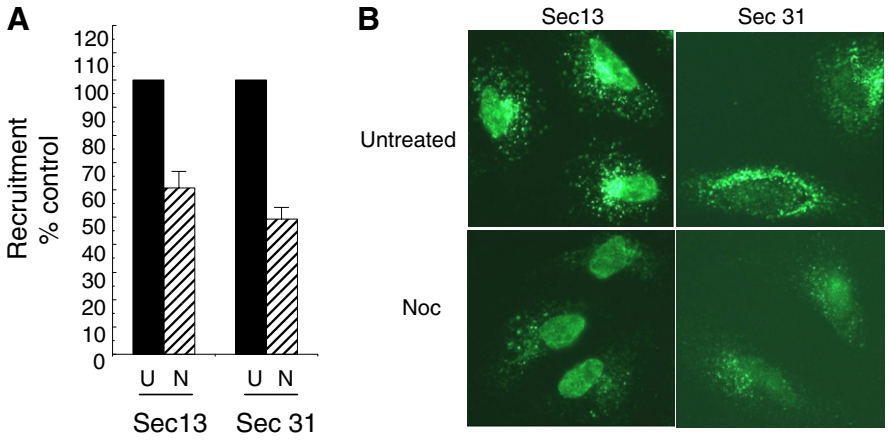

Fig. 1. Restricted recruitment of COPII components in cytosols from nocodazole-arrested cells. Hela cells were permeabilized and Sec13/Sec31 recruitment assays performed using cytosols from untreated (U) or nocodazole-treated (N) cells as described in Materials and methods. Cells were fixed and processed for IF localisation of Sec13 or Sec31. (A) The data are percentages of Sec13- or Sec31-positive ERES/cell in untreated cytosol control, counted using NIH Image program 1.63 (±SEM; $n = 5$. (B) Qualitative examples of Sec13 and Sec31 localization.

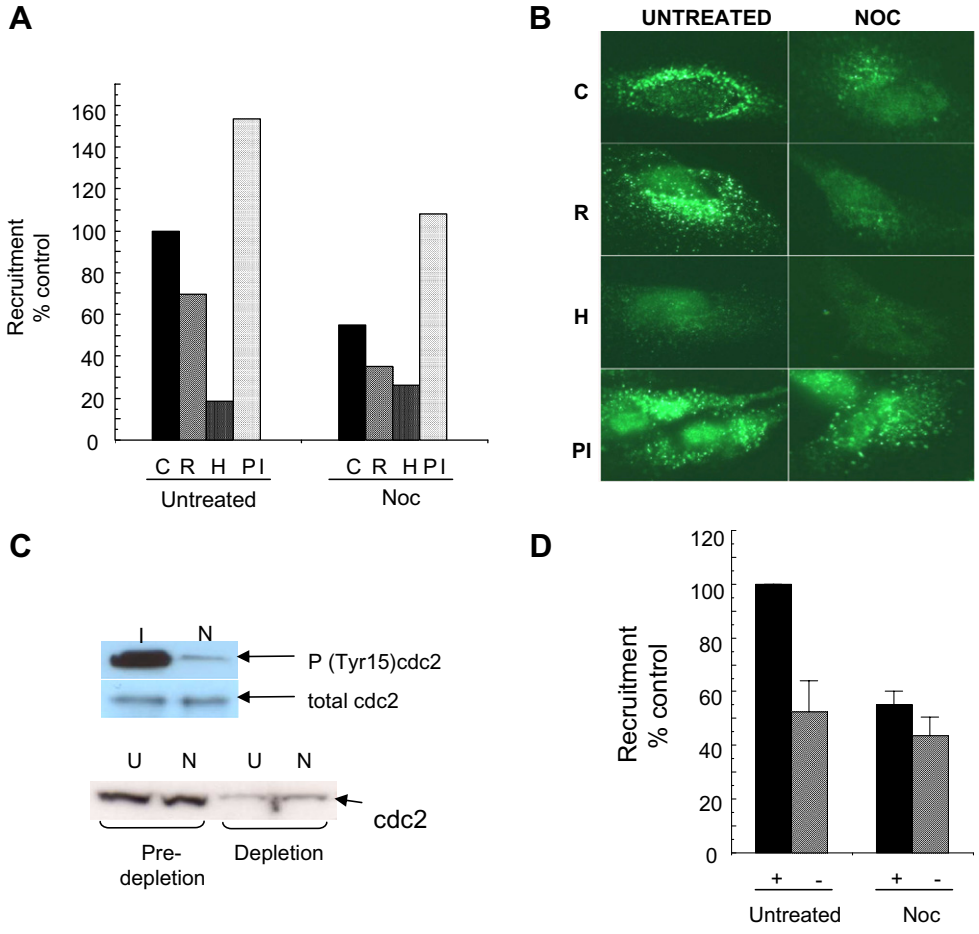

Fig. 2. cdc2 inhibition/depletion does not recover COPII loading from nocodazole treated cytosols. (A,B, and D) COPII recruitment assays were carried out with control (untreated) and Nocodazole treated (Noc) cytosols. In some cases, roscovotine (R, 10 μM) H89 (H, 1 μM), phosphatase inhibitors (PI) were present or absent (control, C) in the assay, and where appropriate, assays were done in presence of non-depleted (+) or cdc2-depleted cytosols (−). (A) Data are compared to untreated control ($n = 2$) and representative examples presented in (B). (C) Immunoblot of an antibody that recognizes only cdc2 phosphorylated at Tyr15. Cdc2 is phosphorylated in interphase cells and dephosphorylated (activated) in nocodazole-arrested cells (upper panel). Total-cdc2 was also analysed using antibody against total cdc2 irrespective of its phosphorylation state to confirm equal loading (upper panel). Results are representative of three experiments. Cytosols were immunoblotted for cdc2 to confirm the extent of depletion of this protein, which is a typical of four experiments (lower panel). (D) Data are expressed as a percentage of mean value in untreated cytosol (±SEM; $n = 4$).