Golgi Distribution of Lyn to Caveolin- and Giantin-Positive cis-Golgi Membranes and the Caveolin-Negative, TGN46-Positive trans-Golgi Network

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Src-family tyrosine kinases, classified as cytosolic enzymes, have crucial roles in regulating cell proliferation, differentiation, migration and cell-shape changes. Newly synthesized Lyn, a member of Src-family kinases, is biosynthetically accumulated at the cytoplasmic face of caveolin-containing Golgi membranes via posttranslational lipid modifications and then transported to the plasma membrane. However, the precise intra-Golgi localization of Lyn remains elusive. By means of a 19°C block-release technique and short-term brefeldin A treatment, we show here that the distribution of Lyn is not monotonously spread within the Golgi but selectively intensified in two distinct membrane compartments: giantin- and caveolin-positive membranes and trans-Golgi network protein (TGN)46-positive but caveolin-negative membranes. Furthermore, Lyn exits the Golgi from the caveolin-positive cis-Golgi cisternae or the caveolin-negative trans-Golgi network. These results suggest that Lyn moves apart from caveolin, a secretary protein, within the Golgi during Lyn’s trafficking to the plasma membrane.

Key words Lyn; Golgi; trafficking; caveolin; giantin; trans-Golgi network protein (TGN)46

Src-family non-receptor-type tyrosine kinases include at least eight highly homologous proteins: Src, Lyn, Yes, Fyn, Fgr, Hck, Lck and Blk.1,2 Src-family kinases play crucial roles in regulating cell proliferation, differentiation, migration and cell-shape changes.1,2

Src-family kinases, classified as cytosolic enzymes, are anchored to the cytoplasmic face of the plasma membrane through myristoylation and palmitoylation.3 We showed that newly synthesized Lyn, a member of Src-family kinases, in the cytoplasm is biosynthetically accumulated at the Golgi, which contains various membranous cisternae and clusters of vesicles, including cis-, medial- and trans-Golgi cisternae and the trans-Golgi network (TGN),4,5 and Golgi-associated Lyn is subsequently transported to the plasma membrane.6-10 Since the localization of proteins is linked to their functions, we also showed that Golgi-associated Lyn can serve as a signaling platform under oxidative stress.7 Although newly synthesized secretary proteins move through the secretory pathway: rough endoplasmic reticulum (ER)→cis-, medial- and trans-Golgi cisternae→secretory or transport vesicles→plasma membrane, the intra-Golgi localization of Lyn remains elusive.

In this study, we investigated the precise distribution of Lyn within the Golgi by means of temperature block and release together with brefeldin A or tannic acid treatment.

MATERIALS AND METHODS

Plasmids, Cells and Transfection cDNA encoding human Lyn11 (provided by T. Yamamoto) was subcloned into the pcDNA4/TO vector (Invitrogen, U.S.A.). cDNA encoding green fluorescent protein (GFP)-tagged neurotrophin receptor p75 (p75-GFP) was provided by E. Rodriguez-Boulan.12 COS-1 cells were cultured in Iscove’s modified Dulbecco’s medium containing 5% bovine serum. Cells were transiently transfected with polyethylenimine and cultured for 6–8 h.13

Antibodies The following antibodies were used: Lyn (H-6; Santa Cruz Biotechnology, U.S.A. and Y497; Abcam, U.K.), caveolin (BD Biosciences, U.S.A.), giantin (GI/133, ALEXIS, U.S.A.), TGN46 (Serotec, U.S.A.), GM130 (β35, BD Biosciences), and CI-MPR (Thermo Scientific Pierce, U.S.A.), and β1,4-galactosyltransferase14 (provided by M. N. Fukuda). Tetramethylrhodamine-isothiocyanate (TRITC)-, Alexa Fluor 488-, Alexa Fluor 546- or Alexa Fluor 647-labelled secondary antibodies were purchased from Sigma and Invitrogen.

Immunofluorescence Cells were fixed and stained, as described.10,15 Confocal images were obtained using a Fluoview FV500 (Olympus, Japan) laser scanning microscope with a 60× water-immersion objective. The Pearson’s R values were determined as described.15 At present, we do not have appropriate means to examine the intra-Golgi localization of endogenous Lyn because of the following reasons. In COS-1 cells, endogenous Lyn could not be visualized due to the low expression. In HeLa cells, endogenous Lyn was minimally visible in the Golgi despite the low expression, but we were unable to visualize the precise localization of Lyn within the intra-Golgi. In THP-1 monocytic cells, endogenous Lyn was sufficiently visible in the Golgi, but THP-1 cells were not appropriate for close examination of Lyn’s intra-Golgi localization because each THP-1 cell has a large nucleus and the Golgi shape is very thin in the narrow cytoplasm.

Temperature Block and Release Cells transfected with Lyn were cultured at 37°C for 6 h and then shifted to 19°C for 2 h to accumulate proteins in the Golgi.8,9 For release from a temperature block, cells were warmed to 37°C for 0, 15, 30, and 60 min in the presence of 200 µg/mL cycloheximide to inhibit protein synthesis. The addition of cycloheximide effectively creates a pulse of newly synthesized protein that can be chased synchronously from the Golgi to the plasma.
RESULTS AND DISCUSSION

To characterize the localization of Lyn within the Golgi, we visualized several endogenous Golgi proteins and evaluated the colocalization levels by determining the Pearson’s R values. First, we compared Lyn with two cis-Golgi proteins: giantin and cis-Golgi matrix protein (GM) 130. The localization of Lyn in the Golgi was appreciably similar to that of giantin, whereas the localization of Lyn was distinct from that of GM130 (Fig. 1A), suggesting that Lyn is distributed to the Golgi membranes harboring giantin rather than GM130. Next, we performed a temperature block at 19°C to prevent vesicular trafficking through the trans-Golgi network and compared the localization of Lyn with that of two trans-Golgi proteins: trans-Golgi network protein (TGN)46 and cation-independent mannose-6-phosphate receptor (CI-MPR). Note that TGN46 mediates protein trafficking between the Golgi and the plasma membrane, whereas CI-MPR mediates the transport of lysosomal enzymes. The localization and the R values showed that a large fraction of Lyn was colocalized with TGN46 but not with CI-MPR (Fig. 1B). Because TGN38/46 is closely linked to the golgin GCC88, which is required for efficient retrograde transport, TGN46-colocalized Lyn may be in-
It is known that caveolin is synthesized in the ER, transported to the Golgi and then to the plasma membrane along the secretory pathway.\textsuperscript{20} Previously, we showed that Lyn is colocalized with the Golgi pool of caveolin.\textsuperscript{6} Indeed, Lyn was nicely colocalized with caveolin in the Golgi (Fig. 1C). We further characterized Golgi membranes harboring Lyn and revealed that caveolin was not colocalized with TGN46 even at 19°C despite colocalization of caveolin with giantin (Fig. 1D), indicating that giantin-positive membranes are different from TGN46-positive membranes. Although we were unable to perform triple staining of Lyn, caveolin and giantin with the antibodies currently available because of the cross-reactivity of secondary antibodies, we showed the statistical...
data that were obtained from doubly stained cells to ensure Lyn’s intra-Golgi localization (Fig. 1E). These results suggest that Lyn is distributed to two Golgi membranes: caveolin- and giantin-positive membranes (triple colocalization of Lyn, caveolin, and giantin in the cis-Golgi) and TGN46-positive but caveolin-negative membranes (colocalization of Lyn and TGN46 in the trans-Golgi network).

We also showed that treatment with brefeldin A (BFA) for 1 h fully translocates Lyn from the Golgi to the ER, because BFA causes the disassembly of the Golgi and the redistribution of Golgi proteins to the ER. However, the trans-Golgi network is relatively insensitive to BFA compared with the Golgi cisternae. Given that Lyn was colocalized with TGN46 (Fig. 1B), we examined whether Lyn was distributed to the trans-Golgi network. After 5–10 min of BFA treatment, a fraction of Lyn still remained in the Golgi, but the trans-Golgi protein β-1,4-galactosyltransferase (GalT) disappeared from the Golgi (Fig. 2A). Furthermore, Lyn and TGN46 were found to be resistant to 5-min BFA treatment and they were partially colocalized (Fig. 2B). Despite colocalization of Lyn with caveolin and giantin (Figs. 1A, C), caveolin and giantin were dispersed by 5-min BFA treatment (Fig. 2C), suggesting that a fraction of Lyn in the Golgi is distributed to the trans-Golgi network other than the Golgi cisternae.

The trans-Golgi network consists of tubular and vesicular membranes containing secretory proteins destined to be transported to the plasma membrane. To visualize Lyn at the exit site of the Golgi, cells were cultured at 19°C for 2 h and released from the temperature block, the level of Lyn at the Golgi was gradually decreased, suggesting that Lyn is departing from the Golgi toward the plasma membrane (Fig. 3B). Moreover, a remaining fraction of Lyn was still visible in a small area 60 min after release from the temperature block, and the fraction of Lyn became apart from giantin and TGN46. These results suggest that Lyn is distributed to endomembranes other than the Golgi cisternae.

Through the Golgi is still controversial, we assume that Lyn, a cytosolic protein, exits the Golgi via a novel trafficking route other than the typical secretory pathway.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES


