Chapter Six

Effect of COPI Loss on ER and Golgi Structure

6 Effect of COPI loss on ER and Golgi Structure

In this chapter the effects of loss of COPI function will be examined. Specifically, the effect of COPI loss on the structure of the ER and the Golgi. Using fluorescently tagged proteins, localised to either the Golgi or the ER to visualise these organelles, the effect of COPα loss has been examined through MO knock-down.

6.1 Introduction

Treatment with BFA and disruption of COPI function in the mutants *sly, hap* and *dop* causes a breakdown in the structure of the Golgi and the ER (Coutinho et al., 2004). The fusion proteins Cal-CFP and GalT-YFP localise to the ER and the Golgi respectively. Cal-CFP localises to the ER through the function of the Calreticulin KDEL ER retrieval sequence (Fliegel et al., 1989; Munro and Pelham, 1987). GalT-YFP localises to the Golgi through the function of the N-terminal 81 amino acids of 1, 4-galactosyltransferase (Llopis et al., 1998; Yamaguchi and Fukuda, 1995). By injecting linearised DNA vectors encoding these fusion proteins, the structure of both the Golgi and ER could be observed in individual living cells. Injection of the COPα MO or treatment with BFA in vector injected embryos enabled visualisation of ER and Golgi structure in COPI defective cells, including notochord cells.

6.2 Loss of Coatomer in Notochord and Muscle Cells

By 32 hpf, the phenotype of COPα MO injected embryos is apparent and it is therefore at this stage that the structure of the Golgi and the ER was compared to un-injected embryos. Visualisation of wild type notochord cells, in eight MO injected and ten wildtype embryos at this stage demonstrated distinct localisation of both Cal-CFP and GalT-YFP in regions resembling the ER and the Golgi respectively. This localisation is concentrated within a relatively small area of the cell, presumably due to the vacuolation of the notochord cell as it differentiates, around an area of low fluorescence that is most likely the nucleus. It is around this area that the majority of ER localised Cal-CFP is concentrated, with GalT-YFP concentrating in one large distinct body. Visualisation of ER and Golgi structure is more difficult on morphant cells, due to their small size as a result of lack of differentiation. However, close observation demonstrates that the Golgi localised GalT-YFP occupies a large body that occupies almost the entire cell, suggesting Golgi swelling, and the ER localised Cal-CFP is diffused throughout the cell, suggesting a breakdown of the ER (**Figure 6.1**).

The structure of the Golgi and ER in wild type muscle cells is comparable to the structure observed in notochord cells. Both the Golgi and the ER are localised around circular bodies resembling the multiple nuclei of the cell. Both the ER and the Golgi are smaller within muscle cells, which may be a reflection of the increased secretory demand of notochord cells. ER and Golgi are also less localised, occurring in discrete bodies spaced around the nuclei, possibly due to the lack of vacuolation. There is, as in notochord cells, no overlap of the Cal-CFP and the GalT-YFP, which befits localisation to the Golgi and ER respectively. In COPα MO injected embryos,

the affect on Golgi and ER structure is more evident in the larger muscle cells. Loss of COPI function results in a breakdown of Golgi structure and diffusion throughout the cell. Though large Golgi bodies remain, the entire cell demonstrates a much higher background level of GalT-YFP fluorescence, indicating loss of Golgi organisation. In morphant cells, Cal-CFP fluorescence is much lower and localised to several diffuse areas. Thus, there appears to be both an enlargement and a breakdown of the Golgi and a breakdown and loss of the ER (**Figure 6.1**).

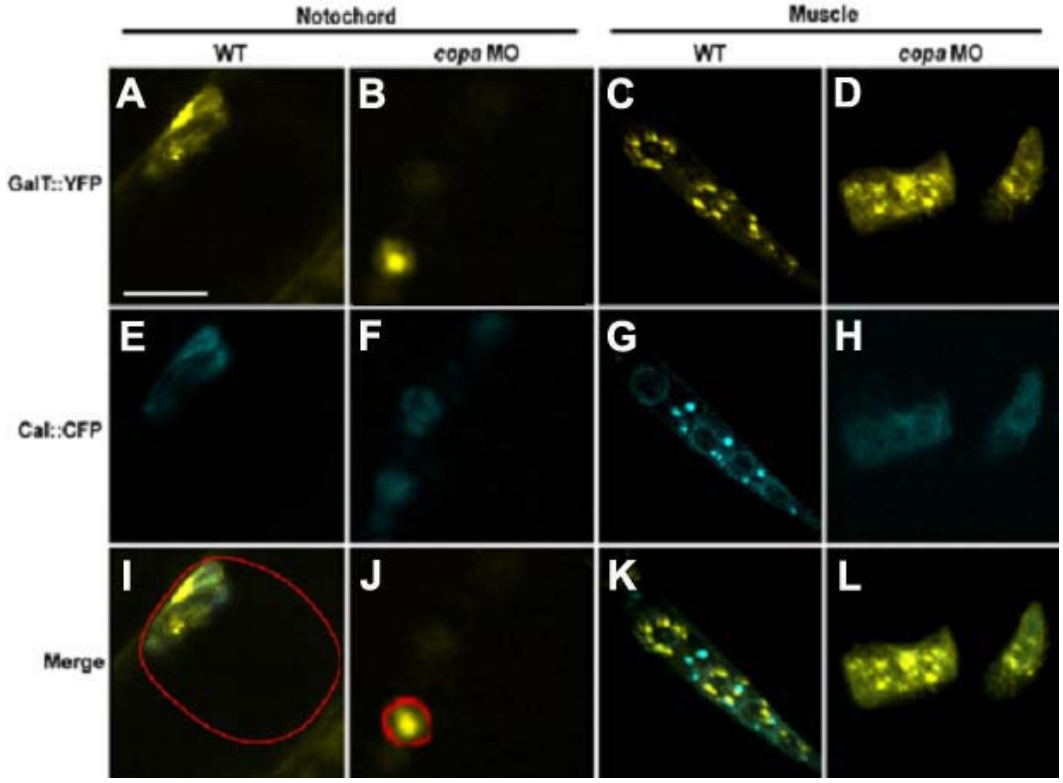

Figure 6.1 Structure of Golgi and ER in normal and COPI loss of function embryos.

Structure on the Golgi (A-C) and ER (E-H) in wild type notochord (A, E and I), COPI loss of function notochord (B, F and J), wild type muscle (C, G and K) and COPI loss of function muscle (D, H and L) cells. Red boundary in panels I and J marks the boundary of the notochord cells.
Bar (A) represents 20μM in (A, C, D, E, G, H, I, K and L) and 40μM in (B, F and J).

6.3 Effect of COPI Loss of Function Over Time

One of the benefits of using confocal techniques is that one can visualise fine structure in living cells through the use of specifically localised fluorescently tagged proteins. As such, the ER and Golgi localised fluorescent vectors were used to examine the effects of COPI loss of function over time. Embryos were injected with linearised vectors prior to 4 cell stage and raised to under normal conditions until 18 somite stage. At this point, embryos were changed to 1.8μM BFA in embryo water and were then raised under normal conditions until visualised. Thus, initial COPI activity was unaltered by COPI loss and GalT-YFP and Cal-CFP are able to localise properly in the early embryo. Addition of 1.8μM BFA at 18 somite stage has been shown to generate embryos with comparable phenotype to the COPI mutants (Coutinho et al., 2004). Addition of BFA was monitored in six embryos over a three hour period, beginning at 28 hpf, at which time the phenotype was not fully penetrant, so that Golgi and ER structure were not yet completely disrupted. A muscle cell from one embryo and three unspecified tail cells from a separate embryo are shown in **Figure 6.2**.

In both groups of cells, the Golgi and the ER structure initially resembled wild type embryos. GalT-YFP is localised in discrete bodies surrounding the nuclei. Cal-CFP is present throughout the cell, but concentrated in distinct, regular bodies around the nucleus. After one hour (29 hpf), there is an obvious breakdown and fragmentation of the ER and GalT-YFP localisation becomes more diffuse, with the Golgi bodies reducing in number. At two hours, Cal-CFP is more diffuse and remains localised in only a small region surrounding the nucleus. At this point the level of GalT-YFP fluorescence within the cell is higher and the Golgi bodies have

become fewer. By three hours, the ER is a thin body surrounding the nucleus, with Cal-CFP diffused throughout the cell. CFP fluorescence is much lower and the ER is reduced is size and highly fragmented and fractured. GalT-YFP has become diffused highly throughout the cell and there are few remaining Golgi bodies. Localised fluorescence of GalT-YFP within the Golgi bodies is less than at 28 hpf (**Figure 6.2**).

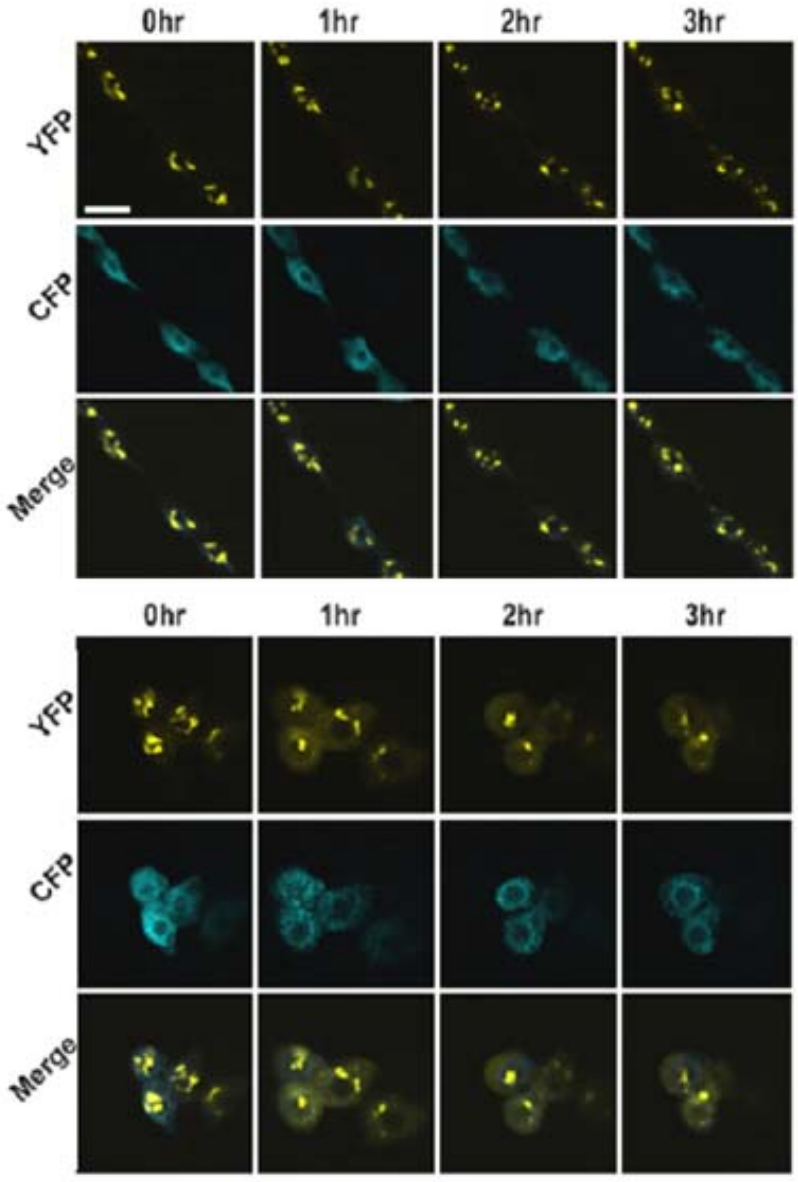

Figure 6.2 Effect of COPI loss of function on the localisation of ER-CFP and Golgi-YFP.

Top panel: Effect of loss of COPI function in muscle cells on Golgi structure (top row) and ER structure (middle row). Merged displayed on bottom row.
Bottom panel: Effect of loss of COPI function in undefined trunk cells on Golgi structure (top row) and ER structure (middle row). Merged displayed on bottom row.
Time 0 represents 28 hpf through to 3 hr, which represents 31 hpf.
Bar (top left micrograph) represents 20 μm.

6.4 Discussion

Examination of both Golgi and ER structure through the use of ER localised, CFP tagged, Calreticulin and Golgi localised, YFP tagged 1,4-galactosyltransferase has demonstrated that loss of COPI function, through MO knock down of COPα, results in disruptions to the Golgi and ER structure. Loss of COPI function, through either MO knock-down of COPα or treatment with BFA, results in a breakdown of the ER and a dispersal of Cal-CFP throughout the cell. GalT-YFP fluorescence becomes highly diffuse under conditions of COPI loss and Golgi bodies decrease in number and increase in size. These observations fit with a model of loss of transport between the Golgi and the ER, as fits with a loss of COPI function. The major role of COPI is in retrograde transport, in the Golgi and from the Golgi to the ER. Under normal conditions, ER resident proteins are captured in forming vesicles and transported to the Golgi, to then be returned to the ER through identification of target sequences, such as those within calreticulin, through the action of COPI vesicles. These vesicles also function in maintaining Golgi components in their proper localisation. Under conditions of COPI loss, retrograde transport is lost, ER resident proteins are transported to the Golgi and lost from the ER and Golgi components are lost from their proper Golgi location, again, due to a lack of retrograde transport functioning within the Golgi network.

Observations of Cal-CFP localisation support this, where initial fluorescence is localised to small bodies around the nucleus, only to fragment and diffuse throughout the cell as loss of COPI function takes effect. Such fragmentation and diffusion is likely to be due to the dispersal of Cal-CFP through the Golgi bodies and beyond, being lost to the cytosol of the cell as the ER breaks down. The localisation of GalT-

YFP also fits with the role of COPI and predictions on the effect of COPI loss on secretory network structure. A loss of retrograde transport within the Golgi results in a loss of Golgi components to the cytosol as well as the incorporation of ER resident proteins to the Golgi. Observations of GalT-YFP localisation demonstrate both a fusion of individual bodies of GalT-YFP fluorescence as well as a diffusion of fluorescence throughout the cell. Such observations can be explained through the fusion of individual Golgi bodies, due to an increase in the influx of material from the ER which is incapable of undergoing retrograde transport as well as the loss of retrograde transport within the Golgi, so that individual compartments are lost and incorporated into one large abnormal Golgi body. The diffusion of GalT-YFP throughout the cell is likely due to the loss of Golgi components to the cytosol due to both a loss of retrograde transport, so that Golgi components are secreted from the network, and the breakdown of Golgi bodies under the abnormal stress, releasing their contents into the cytosol. Hence, loss of COPI function results in a breakdown and loss of the ER, with dispersal of ER components throughout the Golgi and cytosol. Golgi bodies become fused and Golgi contents becomes dispersed throughout the cell under conditions of COPI loss.

6.5 Summary

- In wild type cells Cal-CFP and GalT-YFP localise to organelles that closely resemble the ER and the Golgi respectively.
- Notochord cells lacking COPI function are much smaller than wild type notochord cells due to a lack of vacuolation.

- In cells lacking COPI function, at 32 hpf, there is a dispersal of GalT-YFP throughout the cell and a reduction in the number of highly fluorescent bodies.
- At 32 hpf, COPI deficient cells show a loss of Cal-CFP fluorescent bodies and low levels of Cal-CFP throughout the cell.
- COPI loss of function causes a breakdown of the ER and dispersal of its contents throughout the cell combined with a reduction in the number of Golgi bodies and dispersal of Golgi contents into the cytosol.
- COPI deficient cells have ER and Golgi structure resembling wild type cells at 28 hpf, but by 31 hpf show breakdown of ER and Golgi structure.