Understanding the Function of Paxillin during Cell Division

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ABSTRACT

Paxillins are a family of focal adhesion associated proteins that play important roles in transducing extracellular and intracellular cues and appear to be a point of convergence of growth factor and integrin signaling. This study aims to understand the dynamics and functions of Human Paxillin during the process of Cell division in \textit{Schizosaccharomyces pombe} and Normal Rat Kidney (NRK) epithelial cells. The obtained results showed that Paxillin is diffused in the cytoplasm of \textit{S. pombe} cells during cell division, indicating that it does not play any specific role in the process of cytokinesis. The inability of Paxillin to rescue the cytokinesis defects of cells deleted for \textit{pxl1} (\textit{S. pombe} homologue of Paxillin) further justified this conclusion. On the other hand, Paxillin localized itself at the focal adhesions of NRK cells at interphase. Then it disappeared when the cell entered mitosis and reappeared mainly at the subequatorial region during cytokinesis. It also manifested, although to a slightly lesser extent, at the cell periphery. These results indicate that the down-regulation of Paxillin is essential for the cells to detach from the substratum and attain a round morphology essential for mitosis, while the reappearance of Paxillin at the end of cell division may play an important role in the restoration of the interphase morphology of the cell. In conclusion, these findings suggest that the dynamics of Paxillin, during the process of cell division, is primordial for the occurrence of changes in cellular morphology which, in turn are essential for proper division of NRK cells.

INTRODUCTION

The objective of the present study was to investigate the role of Paxillin during cell division. A series of experiments were carried out to find out firstly if (i) Paxillin localizes at the division site in \textit{S. pombe} cells in a similar manner as Pxl1p; (ii) Paxillin has the potential to rescue the cytokinesis defects of \textit{Pxl1}\Delta \textit{S. pombe} cells. Further experiments were conducted to determine the subcellular localization of Paxillin in NRK epithelial cells and their dynamics during cell division. Furthermore, it’s localization in relation to actin in interphase and dividing cells were also investigated. The mention of Paxillin in this study refers to the Human Paxillin, assuming that Human Paxillin is a good representation of all other mammalian Paxillins.

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MATERIALS AND METHODS

Plasmids containing Paxillin and GFP sequences were constructed using molecular techniques. A set of them were transformed into wild-type and Pxl1Δ S. pombe cells, while the others were transfected into NRK cells. The expression of GFP-Paxillin fusion proteins was monitored using microscopic techniques. The localization and dynamics of Paxillin in both types of cells were captured and analysed.

RESULTS AND DISCUSSION

Paxillin in Schizosaccharomyces pombe cells

Pxl1p, in S. pombe cells is known to be a component of the actomyosin ring essential for cytokinesis (Ge et al., 2008). It is important for the integrity of the ring as well as its proper constriction. Pxl1Δ cells, a Pxl1 deletion strain, display a novel phenotype, characterized by large population of septating cells, which illustrate the inability of the actomyosin ring to constrict and separate the cell into two in the absence of Pxl1p.

In this study, in order to investigate if Paxillin has a conserved function during cell division in fission yeast and mammalian cells, the capability of Paxillin to replace Pxl1p in S. pombe was examined. Therefore, Pxl1Δ cells were transformed with pREP81-HsPaxillin constructs in order to find out if the Paxillin could rescue the cell separation phenotype of the mutant S. pombe cells.

Figure 1: Fluorescence microscopy of anillin-blue-stained septa of (A) Wild-type cells carrying pREP81, (B) Wild-type cells carrying pREP81-HsPaxillin, (C) Pxl1Δ cells carrying pREP81, and (D) Pxl1Δ cells carrying pREP81-HsPaxillin. Scale bar corresponds to 5 μm.

Figure 2: The percentages of cells with no septum, a single septum, and double septa in cultures (1) Wild-type cells carrying pREP81, (2) Wild-type cells carrying pREP81-HsPaxillin, (3) Pxl1Δ cells carrying pREP81, and (4) Pxl1Δ cells carrying pREP81-HsPaxillin. No. of cells=500
These data suggests that Paxillin cannot rescue the cell separation phenotype of \(Pxl1\Delta\) cells.

Next, the localization of Paxillin in wild-type and \(Pxl1\Delta\) \textit{S. pombe} cells was examined.

![Image](image.png)

**Figure 3:** Fluorescence microscopy of (A) wild-type cells carrying pREP81-GFP-HsPaxillin, and (B) \(Pxl1\Delta\) cells carrying pREP81-GFP-HsPaxillin. Scale bar corresponds to 5 µm.

Paxillin does not localize at the division site during cytokinesis, in both wild-type and mutant cells (Figures 3A and B). Although some diffuse signals were found in the cytoplasm of both interphase and dividing cells, a specific localization pattern of Paxillin was not observed in most of the cells.

Sequence alignment of Pxl1p and Paxillin amino acid sequences showed that there is a 49% similarity between the two sequences, but the similarity is observed only in the C-terminal region of the two sequences. N-terminal sequence is required for the localization of Pxl1p at actomyosin ring, and the absence of this sequence in Paxillin may be the cause of its inability to localize at the actomyosin ring. These data suggest that there may be different roles of mammalian Paxillin on the regulation of actin cytoskeleton in \textit{S. pombe} and mammalian cells.

**Paxillin in Normal Rat Kidney (NRK) epithelial cells**

![Image](image.png)

**Figure 4:** Subcellular localization of GFP-Paxillin in the periphery of Live NRK cells at interphase. Cells expressing GFP-Paxillin were cultured in F12K medium and visualized using fluorescent microscopy. Red arrowheads point to the Paxillin localized at the focal adhesions.

As shown in Figure 4, GFP-Paxillin localizes efficiently at the focal adhesions. They are seen as tiny strands in the peripheral region of the cell (indicated by the red arrowheads). It was concluded, that such subcellular localization may aid in the function of Paxillin as a focal adhesion protein, thereby assisting in the communication of the extra-cellular matrix (ECM) with the interior of the cell.
Function of Paxillin during Cell division

Initially, cells appear to be rounded and the GFP-Paxillin is diffused in the cytoplasm. No Paxillin was detected at the focal adhesions as in the interphase cells. As cytokinesis takes place, Paxillin starts to reappear at the sub-equatorial region of the cell (red arrowheads). The intensity of signals continues to increase denoting a progressive accumulation of Paxillin in this region of the cell. Later, Paxillin begins to show up at the sites of focal adhesions throughout the periphery of the cell, but to a lesser extent as compared to the sub-equatorial region (yellow arrowheads). Simultaneously, the shape of the cell changes from the round morphology to its characteristic interphase morphology.

It is known that focal adhesion is disassembled during mitosis. Yamaguchi et al. (1997) has shown that during the process of mitosis, Paxillin is down-regulated in the cells. The significance of the mitosis specific down-regulation of the Paxillin protein is likely to enable the cells to round up when they enter mitosis. The first appearance and continuous accumulation of Paxillin at the sub-equatorial region during cytokinesis suggests its participation in the formation of interphase cell morphology. Their localization at the focal adhesions in the periphery of the cell, although to lesser extent as compared to the sub-equatorial region, might be for the same purpose. The higher intensity at the sub-equatorial region could be because this region of the cell has a highly rounded morphology at the end of division as compared to the rest of the cell. The observation of the simultaneous change in cell morphology with the dynamics of Paxillin further confirms the aforesaid. The proteins are not accumulated at the cleavage furrow during ingress.

**Conclusion**

In conclusion, the inability of Paxillin to localize at the division site and rescue the cell separation phenotype of *Pxl1Δ* cells indicates that it does not share a conserved role with Pxl1p in *S. pombe* cells. The dynamics of Paxillin, during the process of cell division, is primordial for the occurrence of changes in cellular morphology which, in turn are essential for proper division of NRK cells.

**REFERENCES**

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