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Dissecting protein interactions during cytokinesis

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Appropriate assembly and constriction of the acto-myosin based contractile ring is essential for the final separation of the two daughter cells in mitosis. This is orchestrated by the small GTPase Rho as well as convergent signals from the prior events of mitosis. Contractile ring assembly requires the physical interaction of structural proteins like the microtubules of the central spindle, motor proteins and Rho activators. These and the interaction of newly localised proteins downstream of active Rho are essential for stability of the contractile ring and its proper constriction. Here, we discuss our recent findings that reveal a complex network of protein interactions during the early stages of cytokinesis. This includes evidence for a direct interaction between Polo Kinase and RacGAP50C as well as unpublished data suggesting other interactions of interest within the contractile ring.

Key words: polo, RacGAP50C, PavKLP, Rho, contractile ring, FRET, cytokinesis

Abbreviations: Polo, polo kinase; Plk1, polo like kinase 1; RacGAP, RacGAP50C; PavKLP, pavarotti kinesin like protein

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Rho signaling plays a major role in coordinating the final splitting of a cell at the end of cell cycle.[1] This process, termed cytokinesis, can be divided into three main events; initiation, contractile ring constriction and abscission. The initiation stage is triggered by release of the spindle checkpoint followed by the equatorial localization of Rho regulators.[2-4] This leads to localized Rho activation and the subsequent assembly and constriction of the contractile ring. Finally, the contractile ring constricts forming an intracellular bridge, which is then cleaved in a process called abscission separating the two daughter cells.

A clear understanding of how the contractile ring forms and constricts requires dissection of individual interactions that take place in a spatial manner. For this purpose, FRET is an ideal method as it allows protein interactions to be visualized in individual cells at all stages of mitosis without interfering with the normal cell cycle.[5-7]

To study the initiating events of cytokinesis, we looked at potential binding partners of Polo Kinase (Polo). Polo is required in both mitosis and cytokinesis, and has a large number of potential substrates.[3] We found that RacGAP50C (RacGAP) showed strong FRET interaction with Polo.[8] Using the yeast two-hybrid assay, we found that they interact via a region between the kinase domain and the Polo box domain of Polo. This region, which we named the intermediate domain, has no known previous function. This may be because it is not involved in the well studied mitotic events prior to cytokinesis.[3] It may now be possible to express Polo with a mutated intermediate domain in a *polo* mutant background and investigate its function specifically in cytokinesis without the mitotic abnormalities that occur in a Polo mutant.[9] Since Polo has at least two distinct protein binding domains as well as a kinase domain, it appears that it can act as a linker protein. Polo may be assembling protein complexes by binding multiple partners, while also phosphorylating targets in the complex such as Rho activators,[10] kinesins,[11] and tubulin,[12] to regulate their binding. Support for the functional importance of these interactions comes from the phenotypic similarity of Polo mutant cells,[8] to PavKLP motor dead expressing cells where PavKLP stalled at the opposite poles of the microtubules.[13] In *polo* mutant cells that had been allowed into anaphase by removing the spindle

checkpoint, we also saw PavKLP with its binding partner RacGAP stalled on the spindle.[8] Our current model is that Polo is essential for the motor activity of PavKLP through phosphorylation,[11] following the association of PavKLP with RacGAP,[14] and RacGAP with Polo,[8] so Polo is part of the mitotic signal that produces localized Rho activation at anaphase onset.

When searching for Polo binding partners, we also detected reproducible but intermittent interaction between Polo and Anillin (our unpublished data). We noticed that some cells were negative while some showed clear positive FRET signal. Interestingly, we were also able to show a strong interaction by yeast two-hybrid in independent experiments, and Anillin has previously been isolated in Plk pulldowns.[15] We speculate that an intermittent FRET signal may represent a transient association such as a kinase-substrate interaction. Therefore, Polo may be targeting Anillin and affecting its interaction with other proteins in cytokinesis. Since Anillin has been suggested to be a structural support for contractile ring components such as RacGAP,[7] and myosin,[16] it will be interesting to see if the association of Polo and Anillin is required to initiate or stabilize these structural links.

In addition, we detected an interaction between Polo and the Rho activator Pebble as well as the Rho effector Citron kinase,[17] which we then verified by yeast two-hybrid assay. This is consistent with pull-downs using the Polo box domain of Plk1, which also identified the Rho effector Rok.[15] This could be a potentially exciting lead on how Rho activity is specified towards a particular effector. Rho has many activators and effectors, and several of them are known to be specific for a particular event or tissue.[18] We hypothesize that Rho is able to choose its correct effector guided by its process/tissue specific activator when the activator and effector are linked in a complex. In this case, the cytokinesis Rho activator, Pebble, is linked by Polo to the cytokinesis Rho effector, Citron. The next question is whether Pebble is also linked with other Rho effectors, and if this model for Rho specificity can be extended into other processes.

In light of these findings, we propose that Polo is a multifunctional protein required for several cytokinetic events: Polo may act both as a trigger for initiation of cytokinesis (by regulating RacGAP/PavKLP activity) and as a regulator that allows the stable recruitment of components of the contractile ring. From our interaction data, we also propose a mechanism of Rho specificity towards its effector by an activator mediated recruitment of the correct effector, and that Polo may be playing a role in mediating this specificity in cytokinesis.

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