Molecular retention mechanisms of the G1 cyclin /Cdk complex in budding yeast [

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Monografía

Budding yeast (Saccharomyces cerevisiae) cells coordinate cell growth and cell cycle progression essentially during G1, where they must reach a critical cell size to traverse Start and enter the cell cycle. The most upstream activator of Start is Cln3, a G1 cyclin that together with the cyclin-dependent kinase Cdc28 triggers a transcriptional wave that drives cell cycle entry. The Cln3 cyclin is a low abundant and very unstable protein whose levels respond very rapidly to nutritional changes. However, Cln3 expression is not sharply regulated through the cell cycle and it is already present in early G1 cells. Notably, most Cln3 is retained bound to the ER in early G1 with the assistance of Whi3, an RNA-binding protein that binds the CLN3 mRNA, and it is released in late G1 by Ydj1, a J-chaperone that might transmit growth capacity information to the cell cycle machinery. However, little is known on the molecular mechanisms that retain the Cdc28-C1n3 complex in the cytoplasm and how do these mechanisms transmit information of cell size to coordinate cell proliferation with cell growth. As Cdc28 is important for proper retention of Cln3 at the ER, we hypothesized that mutations weakening interactions to unknown ER retention factors would cause premature release of the Cdc28-C1n3 complex and, hence, a smaller cell size. This thesis describes the isolation and characterization of a CDC28 quintuple mutant, which we refer to as CDC28wee, that causes premature entry into the cell cycle and a small cell size. Next we used isobaric tags for relative and absolute quantitation (iTRAQ) to identify direct interactors with lower affinities for mutant Cdc28wee, aiming at the identification of proteins with key regulatory roles in the retention mechanism. Among the identified proteins we found Sr13, a protein of unknown function, here renamed as Whi7. Here we show that Whi7 acts as an inhibitor of Start, associates to the ER and contributes to efficient retention of the Cln3 cyclin, thus preventing its unscheduled accumulation in the nucleus. Our results demonstrate that Whi7 acts in a positive feedback loop to release the G1 Cdk¬cyclin complex and trigger Start once a critical size has been reached, thus uncovering a key nonlinear mechanism at the earliest known events of cell cycle entry. In addition to Whi7 we also identified Whi8, renamed here as Whi8, which is an RNA-binding protein present in both stress granules (SGs) and P bodies (PBs) with unknown biological function. We have found that Whi8 interacts with Cdc28 in vivo, binds and colocalizes with the CLN3 mRNA, and interacts with Whi3 in an RNA-dependent manner. Whi8-deficient cells showed a smaller budding cell size while, on the other hand, overexpression of Whi8 increased the budding volume. Cells lacking Whi8 were not capable of accumulating the CLN3 mRNA in SGs under stress conditions, and Cln3 synthesis remained high under

glucose and nitrogen starvation, two environmental stress conditions that dramatically decrease Cln3 levels in the cell. Whi8 accumulation in SGs depended on an intrinsically disordered domain (I01) identified at C-terminus of Whi8 and specific PKA phophosites. Our results suggest that Whi8 acts under stress as a safeguard that limits the influx of newly synthesized Cln3 (and likely other proteins) into the cell cycle machinery, by trapping the CLN3 mRNA in mRNA granules. Thus, we have found a unique target for signaling pathways that directly links stress response and cell cycle entry

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