

## Gene dosis and the timing of mitosis

Victor A. Tallada<sup>1,\*</sup> and Jürg Bähler<sup>2</sup>

<sup>1</sup>Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide/CSIC, 41013 Sevilla, Spain.

<sup>2</sup>Research Department of Genetics, Evolution & Environment and UCL Cancer Institute, University College London, London WC1E 6BT, United Kingdom.

\*Correspondence to Victor A. Tallada; Email: valvtal@upo.es

News and Views: Moris N. et al. A genome-wide screen to identify genes controlling the rate of entry into mitosis in fission yeast. *Cell Cycle*. 2016 Oct 13:0. [Epub ahead of print]. DOI: 10.1080/15384101.2016.1242535.

### Keywords

mitotic entry, cell size, haploinsufficiency, *S. pombe*, rate-limiting genes, genetic screen, G2-M transition, nuclear transport, nucleotide metabolism.

In all organisms, cell size is crucial for biological function and is tightly controlled by genetic, environmental and physiological factors. Cell-size homeostasis is mediated by a finely balanced coordination between cell growth and division. At a critical point in the cell cycle, the G2-M transition, cells stop growth and enter mitosis; advancing or delaying this transition leads to smaller or larger cells, respectively<sup>1</sup>. Uncoordinated cell growth and mitotic entry can lead to diseases. The fission yeast, *Schizosaccharomyces pombe*, has long served as a potent genetic model system to study mitotic entry and cell-size regulation. The rod-shaped *S. pombe* cells grow by length extension until they reach the size requirement for the next cell division<sup>2</sup>; thus, longer or shorter cells at division directly point to a delayed or accelerated mitotic entry. In this way, conserved core regulators and modulators of cell-cycle transitions have been identified a while ago<sup>3</sup>. More recently, a systematic study of nearly all *S. pombe* genes has uncovered 538 mutants showing aberrant cell-cycle progression, leading to longer or shorter cells<sup>4</sup>.

In order to identify rate-limiting steps for mitotic entry, Moris, Shrivastava et al.<sup>5</sup> have now analyzed which of these genes act in a dose-dependent manner by using diploid cells with heterozygous deletion mutants, where one of two gene copies is deleted and the expression level of the corresponding gene is typically halved. They screened for so-called haploinsufficient mutants in which the single gene copy is not sufficient for normal control of mitotic entry, leading to longer or shorter cells at division. Notably, only 13 such haploinsufficient mutants were more than 10% longer or shorter than control cells. This surprisingly low number points to a remarkable cellular resilience to gene copy decreases, which suggests that the expression levels of other cell-cycle regulators are not rate limiting even when reducing expression levels by 50% or that cells can adjust the expression of these genes by compensatory mechanisms. Moreover, all 13 rate-limiting factors appear to act during the G2-M transition, suggesting that regulators functioning during the G1-S transition, another key control point for cell size which is cryptic in *S. pombe*, are present in excess or that the screen is not sufficiently sensitive to uncover such factors<sup>5</sup>. These findings highlight the distinct and complementary insights that can be obtained from haploinsufficiency screens.

The 13 rate-limiting proteins identified in this screen can be grouped into three functional categories<sup>5</sup>: 1) regulation of G2-M transition (7 proteins), including proteins well-known to control the timing of mitotic entry via the CDK network, such as Cdc2, Cdc25, Pom1 and Wee1, which validate the screen; 2) nucleocytoplasmic transport (5 proteins), including four nucleoporins and an importin; and 3) nucleotide metabolism (1 protein, Dea2). These results indicate that the CDK network is regulated by multiple rate-limiting steps, each contributing to the timing of mitotic entry. The proteins functioning in nucleocytoplasmic transport could be indirectly involved in controlling G2-M progression by influencing the localization of rate-limiting regulators. However, a more direct role in cell-cycle progression for certain nuclear pore proteins is plausible, because their cell cycle-related functions besides transport are well documented<sup>6,7</sup> and the haploinsufficient nuclear-pore mutants showed remarkably specific phenotypes<sup>5</sup>. It may be informative to assess epistasis relationships in diploid cells containing different combinations of haploinsufficient mutants.

The nucleotide-metabolism protein Dea2 is the most unexpected rate-limiting factor emerging from this screen. Dea2 is an adenine deaminase involved in the adenine salvage pathway and catabolism. The heterozygous *dea2* mutant actually leads to cells being 32% longer than the control, by far the strongest cell elongation phenotype emerging from the screen<sup>5</sup>. This substantial delay in G2-M progression probably reflects activation of DNA damage or replication checkpoints caused by aberrant nucleotide metabolism<sup>5</sup>, which suggests that Dea2 is rate-limiting for nucleotide metabolism. This idea could be tested by analysing the phenotypes of *dea2* haploinsufficient mutants upon DNA damage or replication interference and in combination with checkpoint mutants. The remarkably specific cell-cycle phenotype of *dea2* haploinsufficient mutants, unlike other nucleotide-metabolism mutants, raises the intriguing possibility that Dea2 plays a key role at the nexus of nucleotide metabolism 'health' and cell-cycle progression.

## References

1. Fantes PA, Grant WD, Pritchard RH, Sudbery PE, Wheals AE. The regulation of cell size and the control of mitosis. *Journal of Theoretical Biology* 1975; 50:213-44.
2. Mitchison JM. The growth of single cells. I. *Schizosaccharomyces pombe*. *Exp Cell Res* 1957; 13:244-62.
3. Nurse P. Universal control mechanism regulating onset of M-phase. *Nature* 1990; 344:503-8.
4. Hayles J, Wood V, Jeffery L, Hoe KL, Kim DU, Park HO, Salas-Pino S, Heichinger C, Nurse P. A genome-wide resource of cell cycle and cell shape genes of fission yeast. *Open Biol* 2013; 3:130053.
5. Moris N, Shrivastava J, Jeffery L, Li JJ, Hayles J, Nurse P. A genome-wide screen to identify genes controlling the rate of entry into mitosis in fission yeast. *Cell cycle* 2016:0.
6. Ibarra A, Hetzer MW. Nuclear pore proteins and the control of genome functions. *Genes Dev* 2015; 29:337-49.
7. Rodriguez-Bravo V, Maciejowski J, Corona J, Buch Håkon K, Collin P, Kanemaki Masato T, Shah Jagesh V, Jallepalli Prasad V. Nuclear pores protect genome integrity by assembling a premitotic and Mad1-dependent anaphase inhibitor. *Cell* 2014; 156:1017-31.