PERGAMON

European Journal of Cancer 39 (2003) 1337–1338

European Journal of Cancer

www.ejconline.com

Editorial

Sometimes size does matter

M. Muzi-Falconi[a,*], S. Sabbioneda[a], Paolo Plevani[a], Marco Foiani[a,b,*]

[a] *Dipartimento di Genetica e Biologia dei Microrganismi, Università degli Studi di Milano. Via Celoria 26, 20133 Milano, Italy*
[b] *FIRC Institute of Molecular Oncology (IFOM). Via Adamello 16, 20139 Milan, Italy*

Wider and faster, those two simple words have assumed a totally new importance in the so-called post-genomic era. After the complete sequencing of various organisms genomes (*Saccharomyces cerevisiae, Arabidopsis thaliana, Homo sapiens* just to mention a few), the huge amount of data that has been newly accessible has changed our view to a wider angle. Where once it was necessary to work on a gene by gene basis, today it is possible to approach any problem in a more systematic, comprehensive way.

A great example of this is proposed by a recent work of Jorgensen and colleagues that try to shed some light on the connection between cell growth and division in the budding yeast *S. cerevisiae* [1].

In eukaryotic cells, the commitment to a round of replication is decided in the late G1 phase after passing a restriction point that in yeast is called Start. Once the decision to go through this point is taken, the cell is committed to another round of the cell cycle, DNA replication occurs in S phase and the cell undergoes mitosis. The passage through Start requires that the cell has reached a critical size, which seems to be dependent upon ploidy and growth conditions. During G1 and before Start, the cell increases in size in order to obtain an adequate mass before dividing. Having such importance in a cell's life cycle, the restriction point is accurately regulated through a series of promoting and repressing factors, so that a faster cell division cycle leads to a smaller cell size (*wee* or *whi* phenotype). The most noticeable result of Tyers' colleagues is the identification of a series of proteins that actively, and not in a passive form as generally thought, monitor the cell size and only upon reaching a certain condition allow the cell to begin the pre-Start transcriptional programme (the *CLN2* cluster). The first step of this elaborate work was the isolation of a series of mutants having alterations in cell size distributions, starting from a series of 4812 viable haploid and 1142 diploid deletion strains. The data obtained from the analysis allowed the strains to be grouped in clusters according to their mean cell size and the mutants showing the most significant variation from the wild-type size were further investigated. The authors identified 249 large (*lge*) and 61 small (*whi*) mutants. It has to be mentioned that factors already known to play a major role in passage through Start (*CLN3, SWI4, SWI6, BCK2* and *WHI3*) were isolated either with a large or small phenotype, confirming the validity of the approach. Deletion of the first four genes produces an increase in cell size, as a consequence of the absence of key factors activating the cell-cycle transcriptional programme, and thus leads to a delay in cell division. From this first screen, the authors isolated genes in functional groups ranging from actin organisation to ribosome biogenesis, to cell cycle control. In order to identify those mutants affected in Start control, the authors decided to look for genetic interactions with known regulators of Start. Having to deal with a great number of genes a classical screening for synthetic interaction was out of question, and for that reason the Synthetic Genetic Array developed by Charlie Boone was used [2]. This is a very elegant genetic technology that allows large numbers of genetic interactions to be tested rapidly using an automated system. Briefly, an array of strains, in this case the *lge* mutants, is created and these strains are then mated with the testing strains, carrying the deletions to be analysed (in this particular case *cln3Δ, bck2Δ* and *swi4Δ*). After mating, all diploids are sporulated and the spores carrying the double mutations are selected. These strains are then tested for a specific phenotype, in this case the colony growth was checked. It is important to underline the power of this approach that avoids the limiting step of a traditional screening, opening a wider range of possibilities while dealing with genetic interactions. A very interesting result was the creation of a first genetic interaction map

* Corresponding author.
E-mail address: foiani@ifom-firc.it (M. Foiani).

0959-8049/03/$ - see front matter
doi:10.1016/S0959-8049(03)00127-8

between the genes responsible for the large or small phenotype and regulators of Start. To isolate a putative Start repressor, a group of 61 *whi* mutants was analysed, looking for strains that were smaller than mutants affected in ribosomal function. The assumption being that an acceleration of Start progression would lead to a smaller cell then a metabolic defect. In fact, 25 *whi* mutants turned out to be smaller then the controls, when doubling time was taken into account. The genes belonging to this group included known cell cycle regulators such as *WHI3* and *CDH1*, Sfp1 a transcription factor involved in the DNA damage response and Sch9, which is highly similar to a metazoan kinase, Akt1, implicated in longevity and stress response. *SFP1* is extremely interesting, not only because its deletion strain was the one with the smallest cell size, but also because of its possible role as a transcriptional regulator; moreover, increasing its expression levels causes a *lge* phenotype. By microarray analysis of a conditional *GAL-SFP1* strain, the authors identified several SFP1-controlled genes that may be involved in cell size regulation. Some of them code for ribosomal biogenesis factors, suggesting a more direct connection between cell cycle progression and ribosomal activity and supporting what had been formerly hypothesised in two recent papers, which related ribosome biogenesis to the initiation of DNA replication [4,5]. This may well be the next frontier to pass in order to understand the molecular mechanisms controlling cell-cycle progression and cell division.

Jorgensen's data [1] suggest that the control of the restriction point is more complex than was previously thought and that the regulation is exercised involving many different factors. How can these results be extended to other eukaryotic organisms? A couple of recent papers have discovered a gene, which seems to be involved in cell size control and is associated with various pathologies of the Central Nervous System. This gene is called *PTEN* and had been initially discovered as a tumour suppressor that was mutated in a series of cancers (glioblastomas, breast, prostate and kidney). This gene is also associated to Lhermitte–Duclos disease, where the cerebellum cells show an increase in size. *PTEN* is a known negative regulator of the Protein Kinase B/Akt activity, which is a key mediator of cell survival after stimulation by growth factors. Mutations in *PTEN* in both *Drosophila melanogaster* and the mouse cause an increased cell size and proliferation in undifferentiated cells, whereas overexpression of loss-function-mutants of *PTEN* causes the opposite phenotype [3]. It is worth remembering that Akt1 shares at its C-terminus a good degree of identity with yeast Sch9, which was also isolated by Jorgensen's screening procedure; the analogies are just the beginning of a new road to be walked in the future.

References

1. Jorgensen P, Nishikawa JL, Breitkreutz B-J, Tyers M. Systematic identification of pathways that couple cell growth and division in yeast. *Science* 2002, **297**, 395–400.
2. Tong AH, Evangelista M, Parsons AB, *et al*. Systematic genetic analysis with ordered arrays of yeast deletion mutants. *Science* 2001, **294**, 2364–2368.
3. Backman SA, Stambolic V, Mak TW. PTEN function in mammalian cell size regulation. *Curr Opin Neurobiol* 2002, **12**, 516–522.
4. Du YC, Stillman B. Yph1, an ORC-interacting protein: potential links between cell proliferation, DNA replication, and ribosome biogenesis. *Cell* 2002, **109**, 835–848.
5. Yuexuan Zhang Zhiling Yu, Xinrong Fu Chun Liang. Noc3p, a bHLH protein, plays an integral role in the initiation of DNA replication in budding yeast. *Cell* 2002, **109**, 849–860.