P53 REGULATION

Hitting the G spot

Cyclin G — one of the first transcriptional targets of p53 to be discovered — is a bit of an oddball. It looks like a common-or-garden cyclin but, unlike its relatives, doesn't seem to activate a kinase. Nevertheless, most of the evidence points towards it promoting cell proliferation. So how does it do it? Koji Okamoto *et al.* have found that cyclin G activates not a kinase, but a phosphatase, providing a new way to regulate p53.

In a fishing expedition for cyclin-G-binding proteins, the authors had previously found that cyclin G binds to a subset of protein phosphatase 2A (PP2A) subunits. PP2A is a trimeric serine/threonine phosphatase with a catalytic A-subunit, a C-subunit that seems to have a scaffolding function, and one of three subtypes of regulatory B-subunit: B, B′ or B″. Cyclin G binds specifically to B′ subunits, but can it bind the functional PP2A enzyme? By co-expressing epitope-tagged constucts of cyclin G and the PP2A B′ subunit, followed by density-gradient separation of cell extracts, the authors found cyclin G exclusively in a fraction that contained all three subunits of PP2A. Phosphatase activity could be immunoprecipitated from these extracts using an antibody against cyclin G. Furthermore, endogenously expressed cyclin G was associated with protein phosphatase activity.

Under some circumstances, cyclin G also colocalized with another of p53's targets, Mdm2, and about 10% of transfected cyclin G could be co-immunoprecipitated with Mdm2. This interaction could be reproduced *in vitro* using purified or *in-vitro*-translated cyclin G and Mdm2. Might cyclin G facilitate dephosphorylation of Mdm2 by PP2A? The authors took advantage of the fact that phosphorylation of Mdm2 on threonine 216 (T216) prevents Mdm2 from being recognized by an Mdm2-specific antibody known as SMP14. Mdm2, phosphorylated on T216 by cyclin-A–CDK2, was readily dephosphorylated (as measured by its increased affinity for SMP14) in the presence of both cyclin G and the PP2A holoenzyme, but not in the presence of either alone, and not in the presence of PP2A that contained B subunits instead of B′ subunits.

So, cyclin G seems to target PP2A to Mdm2. But does dephosphorylation of Mdm2 by PP2A alter Mdm2's ability to inhibit p53 and target it for proteasomal destruction? In cyclin-G-null cells, p53 levels were markedly elevated and Mdm2 was relatively highly phosphorylated. Re-expression of low levels of cyclin G in these cells increased the amount of p53-associated Mdm2, although, paradoxically, higher levels of cyclin-G expression had the opposite effect.

By activating the transcription of both Mdm2 and cyclin G, p53 therefore drives a two-level negative-feedback loop: increasing the level of Mdm2 switches off the p53 response, but only if Mdm2 remains in a hypophosphorylated state (see figure). Tantalizingly, Michael Jensen in Snorri Thorgeirsson's lab (National Cancer Institute, Bethesda, Maryland, USA) has found that cyclin-G1-null mice get fewer, smaller and less aggressive tumours than wild-type mice after treatment with a potent hepatocarcinogen (unpublished results), so cyclin G and PP2A might prove to be useful therapeutic targets, especially in cancers that are driven by *Mdm2* overexpression.

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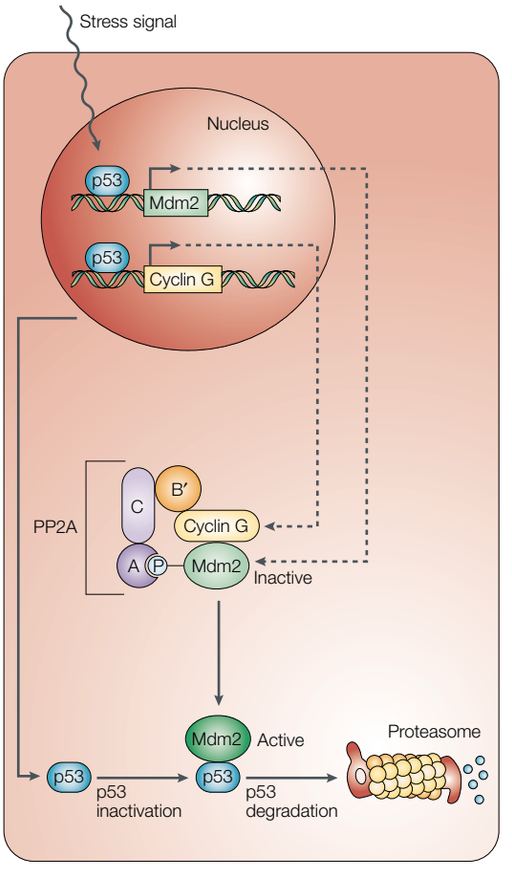
References and links

ORIGINAL RESEARCH PAPER Okamoto, K. *et al.* Cyclin G recruits PP2A to dephosphorylate Mdm2. *Mol. Cell* **9**, 761–771 (2002)
FURTHER READING Goldberg, Y. Protein phosphatase 2A: who shall regulate the regulator? *Biochem. Pharmacol.* **57**, 321–328 (2002)
WEB SITE
Carol Prives' lab: http://www.columbia.edu/cu/biology/faculty/prives/



WEB WATCH

Microarray mania

- http://www.mged.org/
- http://www.ebi.ac.uk/array-express
- http://www.frontiersinge-nomics.com

Gene-expression microarrays are revolutionizing the way we categorize tumours. The problem is that everyone is using different platforms and analysis tools, and storing different types of data. Will we ever be able to compare data from different experiments?

The Microarray Gene Expression Data Group (MGED) was set up to create order from the chaos, by introducing standards for the design of microarray experiments and the annotation of results. If you want to make sure that your microarray experiments will be interpretable by the wider community, check out their minimal information about a microarray experiment (MIAME).

You can also deposit your data at ArrayExpress — a public repository of microarray data. ArrayExpress accepts data in MAGE-ML, the standard format developed by MGED, but don't worry if you're not a bioinformatics whizz because there's also a simple web-based submission tool called MIAMExpress that takes you through the whole process in plain English.

And if you need a good introduction to the use of microarrays in biomedical research, take a look at Frontiersingenomics.com. This site, developed by the Johns Hopkins Program in Applied Genomics and the United States Children's National Medical Centre, uses streaming media technology to introduce novices to microarrays, and take them through the intricacies of experimental design.

With these tools in hand, cancer researchers should be able to make optimal use of the vast amount of microarray data that is accumulating, and eventually apply it to the diagnosis and treatment of patients.

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