



Tumour suppression in Rome

What's All the YAPing About?

Working as a transcriptional co-activator, YAP is currently at the centre of a controversy about its role in cancer. "It's lying in one of the knots of a spider web-like signaling pathway," explains Giovanni Blandino, whose group in Rome is trying to unravel the protein's Janus-like behaviour.

"As we acquire more knowledge, things do not become more comprehensible, but more mysterious". It is a safe bet that German theologian, Albert Schweitzer (1875-1965), was not referring to cellular signaling networks as he uttered these words. But interestingly, for many cell signaling scientists, this may directly address their most recent findings in the laboratory. One protein getting some confused looks with regard to its regulation and function is the Yes-Associated Protein, or YAP.

The basics

In the early 1990s at Rockefeller University, New York, Marius Sudol was searching for proteins that could bind the SH3 domain of the protooncogene c-Yes. He cloned YAP in 1994, a relatively small protein of 65 kDa with a stretch of proline residues that facilitates its interaction with the SH3 domain of c-Yes and several other proteins. Further analysis of YAP revealed other distinct domains, most significantly a WW domain that was first identified in YAP. WW domains span ~40 amino acids and facilitate protein-protein interaction with short proline-rich regions on other proteins. The two conserved tryptophan (WW) residues, spaced 20-22 amino acid residues apart, can dictate structure and function.

YAP's function remained an enigma for some time after its discovery and a handful of ensuing studies described it as a transcriptional co-activator which required its nuclear relocalization for activity. In the mid 1990's, Giovanni Blandino began searching for proteins interacting with p73, a homolog of the p53 tumour suppressor gene, and came across YAP. His continued investigation into the relationship between p73 and YAP has since shed light on YAP's role in cancer – as a tumour suppressor or as an oncogene is highly debated.

Since the early 1990's Blandino's career has been based at the Regina Elena Cancer

Institute in Rome, Italy, where today he coordinates the Transcriptional Oncogenomics Unit. From the onset, his interests centered on p53, the gene most frequently mutated in human cancers, and he has had the opportunity to work with some of the most prominent scientists in p53 research. His first postdoctoral fellowship in 1991 was at Regina Elena Cancer Institute in the laboratory of Ada Sacchi, while the second took the native Sicilian to the laboratory of Mosche Oren at the Weizmann Institute in Rehovot, Israel. After four productive years with Oren, he returned to Rome and began searching for factors that would help discriminate between members of the p53 family.

The Italian connection

The p53 family consists of 2 members besides p53 itself: p63 and p73. Although both are structurally similar to p53, with some conserved functions, they are also functionally distinct, suggesting that each may activate a specific set of target genes. Blandino recalled the difficulty in identifying factors that discriminate between p53 and p73, but his persistence paid off. "We searched for proteins that would interact with p73 using pull-down assays and this was how we found YAP", he explains. "Of course, at the time YAP was not a well known protein, and was just cloned by Marius Sudol's lab, and this started our collaboration with Marius Sudol" a collaboration which still remains today. In 2001, their findings that YAP binds p73 and p73 isoforms via its WW domains resulting in enhanced transcriptional activity of p73 were published in the *Journal of Biological Chemistry* (vol. 276(18): 15164-73). Of equal importance was the fact that YAP did not bind p53.

Further investigations into the relationship between YAP and p73 revealed that in response to cisplatin-induced DNA damage, YAP contributed to the stabilization

and enhancement of p73-dependent apoptotic cell death. These findings, published in *Molecular Cell* in 2005 (vol. 18(4): 447-59), showed that the promyelocytic leukemia tumour suppressor gene (*PML*) was required to recruit YAP into nuclear bodies to promote p73 transcriptional activity and added support to YAP's role in tumour suppression.

The preceding results paved the way to one of the group's latest contributions, an elegant study published once again in *Molecular Cell* (vol. 32: 803-14), which demonstrates that p73/YAP directly activates *PML* transcription and YAP is a critical co-activator of p73's transactivation of *PML*. In turn, *PML* contributes to p73-dependent apoptotic response to cisplatin by stabilizing YAP via direct physical interaction between YAP and *PML*. Thus an autoregulatory feedback



Giovanni Blandino (l.) and Efrem Bertini

loop was established for the transcriptional activation of proapoptotic genes by p73 in response to DNA damage involving YAP and *PML*. According to Blandino, "some critical questions were answered in this paper but much remains untold."

What questions remain? Blandino referred to reviewers' comments to the initial submission to *Molecular Cell* in May 2008. "They wanted to know whether YAP, *PML* and p53 form a triple complex or if they exist as a mixture of two complexes. This is not easy to answer." Initial experiments support the latter model as p73/YAP complexes are found in mouse embryonic fibroblasts (MEFs) lacking *PML* and YAP/

PML could be detected in MEFs lacking p73. "Therefore," Blandino concludes, "the evidence suggests that there is no triple complex but a combination of the two duplexes binding together." He modestly dismisses his opinion as unimportant but philosophically adds, "We assume that transcription consists of only a target gene. Maybe there is a larger multiprotein complex that contains 5, 10 or 12 proteins. There are so many proteins that can bind together to activate or repress a gene. Who can say?"

The paradox

"When I began working on YAP three years ago, it seemed like such a quiet field", explains Efre Bertini, a doctoral student in Blandino's group and first author of a recent comprehensive review of YAP (*Cell Cycle* vol. 8(1): 49-57). "Now there are a lot of papers coming out with this YAP story." Working on the latest hot topic is always exciting but the need to publish first can cause anxiety and frustration. Bertini can surely relate to this as he was recently pipped at the post by another group which beat him to publication. Although most people would find this discouraging, Bertini excitedly discussed his opinions of YAP and its growing list of functions, including the controversy of its potential role in cancer.

"YAP: at the crossroad between transformation and tumor suppression" summarizes the current evidence, though not overwhelming, which suggests that YAP can participate in both events. YAP's role as a tumour suppressor is mainly characterized by its ability to bind p73 during DNA damage, thus enhancing p73's ability to activate pro-apoptotic genes. The evidence for oncogenic YAP centers on the observations that YAP is localized to the 11q22 amplicon which is detected in several cancers. Also contained within this amplicon is cIAP, a gene elevated in cancer. When overexpressed together with YAP, the resulting effect is synergistic. In addition, overexpressing YAP in an epithelial cell line resulted in its oncogenic transformation. YAP is also a component of the Hippo pathway, a critical pathway which co-ordinates organ size by maintaining the proliferation and apoptosis balance. YAP appears to act as a switch in this pathway as upstream regulators of YAP can influence downstream events such as cell proliferation, differentiation or death. An obvious question to this apparent duality in function is how this happens.

"It depends which partner is bound by YAP," clarifies Blandino, "but the activity of YAP does not change." His opinion is that if

an oncogene binds YAP, then YAP can promote oncogenic gene activity, whereas if the binding partner is a tumour suppressor, YAP can act accordingly. Bertini continues to explain that phosphorylation of YAP, for example by the tyrosine kinase c-Abl, can modulate the activity of YAP. Phosphorylation of YAP can also determine its localization inside the cell and alter its stability. Thinking ahead, Bertini speculates that, "the question is if this information can be useful for drugs or molecules that can target the activity of YAP; therefore you would need to know the function in the specific tissue or cells where YAP is working."

The outlook

The potential of YAP as a therapeutic target appears possible. In light of recent findings Blandino says, "One of the most important implications is that YAP can link two different tumour suppressor pathways, that of p73 and PML. If we can concomitantly activate either pathway, my opinion is that we can maximize the response of tumour cells to conventional anticancer drugs such as cisplatin." Another important implication capitalizes on the differences between p53 and p73. "Cancers with mutant p53 are more resistant to cancer therapy and thus more difficult to treat, but because the p73, YAP, PML pathway remains active in cells with mutated p53, this pathway can also be activated to induce p73-mediated apoptosis."

The search for p73 interacting factors which can distinguish p73 from p53 is ongoing, as is trying to deduce the mechanism(s) underlying YAP's regulation and function. And although Blandino would be more than happy to get his hands dirty in the laboratory again, administrative duties, including writing grant proposals to support the "young guys", keep this oncologist at his desk.

Bertini is currently in the last year of his doctoral studies, work he began at the Weizmann Institute before his move to Rome. How does he find life working in beautiful Rome? He is quick to reassure that they all work extremely hard and, Italian bureaucracy notwithstanding, the atmosphere is quite relaxed. Despite his uncertainty about what comes next, his current position in Blandino's group places him in an advantageous position as his research gains popularity and importance. As for the future of YAP research, he agrees that it is complex and though much has been deciphered with respect to regulation and function, it is only just the beginning. ROSEMARIE MARCHAN