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A model for NF-κB regulation by GSK-3β

Glycogen synthase kinase-3β (GSK-3β) was identified some 20 years ago as one of several protein kinases that can phosphorylate glycogen synthase and thus regulate glucose metabolism [1]. It has since been recognized as participating in a multitude of cellular processes, including the control of gene expression, and has recently received considerable attention within the pharmaceutical industry as a drug target.

Three years ago, James Woodgett's laboratory published [2] the intriguing observation that GSK-3β knockout mice show a phenotype that is similar to that produced by gene targeting of IκB kinase-β (IKKβ), a protein kinase involved in NF-κB activation, or of p65, a component of NF-κB itself, strongly implicating GSK-3β in the activation of this transcription factor. However, the mechanism by which the kinase stimulates the activity of NF-κB has thus far remained elusive.

In a recent publication [3], the group of Claudio Schneider has shed some light on how GSK-3β can modulate NF-κB. The authors report that the kinase regulates the stability of p105, precursor of p50, which is a component of NF-κB, typically consisting of a dimer of p50 and p65 (p65 provides the transactivation function). This group proposes a model in which GSK-3β, which is active in cells in the absence of stimuli, stabilizes p105 by phosphorylation. Once a signal that activates NF-κB – such as tumour necrosis factor-α (TNF-α) – is received by the cell, IKK (activated by the TNF receptor via the PI3-kinase/Akt pathway) phosphorylates p105 primed by GSK-3β. At the same time, GSK-3β is phosphorylated by Akt and is thus inactivated. Under these conditions, newly synthesized p105 is not phosphorylated by GSK-3β; consequently it is also not phosphorylated by IKK and thus not degraded. Instead, it is processed to produce p50.

So, how do the results of Schneider's group fit in with the observations of Woodgett's laboratory? According to Schneider and co-workers, increased production of p50 in the absence of GSK-3β activity will result in increased p50-homodimer formation, which will 'outcompete' p50–p65 heterodimers at NF-κB binding sites in promoters of genes regulated by the transcription factor. Because p50 does not have a transactivation domain, transcription from such promoters will be much reduced, resulting in inhibition of NF-κB activity in the absence of functional GSK-3β as observed by Woodgett's team. Moreover, because not all undegraded p105 is immediately converted to p50, an additional mechanism of NF-κB inhibition proposed by Schneider's group is increased retention of the transcription factor in the cytoplasm by p105, which can act as an IκB protein and can prevent nuclear translocation of NF-κB.

In their paper, Schneider and co-workers not only present results obtained with GSK-3β$^{-/-}$ cells but also data from experiments with wildtype cells in which the p105 gene was silenced by siRNA. Using this technique, they observed a reduction in the p105:p50 ratio similar to that found in the cells from GSK-3β knockout mice. They attribute this to p50 being degraded less rapidly than p105 after cessation of p105 synthesis. When comparing the sensitivity to TNFα-induced apoptosis of wildtype cells after p105 gene silencing to that of GSK-3β knockout cells, they found the two to be similar.

To my mind at least, Schneider's model leaves several questions open. Would increased generation of p50 not be compensated for by enhanced synthesis of p65? After all, p50–p65 heterodimers are the most common form of NF-κB found in cells, implying coordinated production of their two components.

A second question is: do the cells in which p105 expression has been silenced actually have the increased amounts of p50 required for the generation of enhanced levels of p50 homodimers needed to inhibit NF-κB-dependent gene expression. Upon silencing, the ratio of p50:p105 should indeed increase if preexisting p50 has a longer half life than p105 present in the cells before silencing but the absolute amount of p50 should not increase. Instead, it should eventually decrease, albeit more slowly than that of p105, which is exactly what the results of Schneider's team show. Moreover, I

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believe that the loss of p105 should if anything result in reduced inhibition of NF-κB and consequently in diminished sensitivity to apoptosis induced by TNFα.

In my opinion, the increased sensitivity to TNFα-induced apoptosis that is observed in cells with silenced p105-expression is more likely to be due to a lack of p50-p65 heterodimers, which might not form in sufficient numbers if p50 levels are much reduced.

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Probiotics: time to move beyond Metchnikoff?

It is almost a century ago since Eli Metchnikoff proposed the, then revolutionary, idea to consume viable bacteria to promote health. Since that time, the area of what is now know as 'probiotics' has made dramatic progress, particularly during the past two decades. These two decades have also seen the emergence of a new, related, area of study – 'prebiotics'. The review by Touhy and co-workers in a recent issue of *Drug Discovery Today* [1] nicely summarizes the main achievements in these two fields and also indicates the future challenges.

The authors of the review restricted themselves to the effects of pre- and probiotics in the gut. This is understandable because most of the work has been done on gastrointestinal tract-related disorders. However, the basic idea with pre- and probiotics is modulation of the activity and/or composition of the endogenous microbiota. Many other parts of the human body have an endogenous microbiota too. The potential health benefits that could be provided by probiotics in non-intestinal applications have to date received little attention [2].

The non-intestinal application of probiotics that has received most attention, albeit much less than intestinal probiotics, is that of use in urogenital tract-related disorders. The challenges for this kind of application of probiotics are to some extent similar to those for intestinal applications. Some studies have also used the oral administration of the preparation. Selected probiotics have been observed to be able to reduce the risk of urinary tract infections [3] indicating that this is a feasible approach.

A different application is to use oxalate metabolizing bacteria in the intestine to reduce the urinal load of oxalate, thereby diminishing the risk for the formation of calcium oxalate renal stones [4]. Although the application is still intestinal, the target is novel and outside of the intestine.

Modulation of the nasopharyngeal microbiota has also been found to be a potentially successful approach. Children who have experienced recurrent otitis media have been found to have reduced levels of α-haemolytic streptococci. Application of a nasal spray with six strains of α-haemolytic streptococci reduced the recurrence rate of otitis media [5].

Probiotics for the skin have been proposed but not yet investigated, either in experimental animals or in a clinical setting [6].

Metchnikoff founded the research field of probiotics, aimed at modulating the intestinal microbiota. Have his ideas held us back to explore additional applications for probiotics? Although much work remains to be done on the intestinal applications of pre- and probiotics, the time has come to move on and extend Metchnikoffs ideas to other parts of the body with an endogenous microbiota. The above examples show that non-intestinal applications of probiotics can be successful and warrant further investigations.

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