eukaryotes (organisms with nucleated cells), with microtubule-binding capacity increasing largely as a result of juxtaposing multiple copies of the core unit of the yeast kinetochore[5].

A key feature of kinetochores *in vivo* is that they can remain attached to the ends of disassembling microtubules. The kinetochores Akiyoshi and colleagues isolate can also do this. What's more, although many of the typical structural proteins are present in the isolated kinetochores, key proteins — such as the enzyme Aurora kinase[6] — that regulate chromosome attachment to the mitotic spindle are absent. These 'minimal' kinetochores therefore allow tests of how forces might regulate microtubule binding, independently of any potential regulation through protein phosphorylation.

Akiyoshi *et al.* attach the minimal kinetochores to a bead that they can manipulate with optical tweezers[7] (Fig. 1b). A bead 'trapped' by optical tweezers behaves as if it is attached to a mechanical spring, such that a force restoring its position is proportional to the change in displacement. The authors examine interactions of the kinetochores with polymerizing and depolymerizing microtubules under different forces. This *in vitro* experiment recapitulates the pulling force that a kinetochore of a bi-oriented chromosome experiences within a cell.

It is reasonable to expect that the lifetime of the attachment between any two interacting partners, such as a ligand and its receptor, decreases as an applied force increases; this is because the mechanical work helps to overcome the detachment energy barrier[8]. Remarkably, however, Akiyoshi *et al.* reveal that force — in the range relevant to physiological forces that act on chromosomes — increases the lifetime of kinetochore–microtubule attachment twofold. The authors' further analysis reveals that the kinetochore–microtubule attachment behaves like a 'catch bond' — similar to a seat belt that locks in place when pulled abruptly[9].

A catch bond can be modelled as a system with both a strongly bound state and a weakly bound state; force favours the strongly bound state. The minimal kinetochores are weakly bound to microtubules that are disassembling, and strongly bound to growing microtubules. Notably, applied force suppresses microtubule disassembly and can therefore favour the strongly bound state. On the basis of direct measurements and simple assumptions, Akiyoshi *et al.* develop a quantitative catch-bond model that accounts for the observed kinetochore–microtubule-attachment behaviour.

The catch-bond mechanism may be considered as a mechanical extension of biochemical allosteric regulation. Force can be considered to be the equivalent of a molecule binding a protein's regulatory site and inducing a conformational change that modulates activity. Evidence from other cellular components with catch-bond behaviour, such as the bacterial adhesion protein FimH, is consistent with this idea[10]. In the case of the kinetochore–microtubule interaction, it is possible that force directly induces a conformational change in microtubule tips[11]. The strongly bound state could involve kinetochore interactions with microtubule protofilaments that are relatively straight, as seen in growing microtubules *in vitro*[12]. The weakly bound state could have protofilaments splaying outwards, as seen in disassembling filaments[12].

Examining the structure of the minimal kinetochores and how they bind different microtubule-tip structures are essential next steps. Combining these structural studies with mutagenesis analysis should allow the design of experiments to test the catch-bond mechanism in dividing cells. Aurora kinases, or other proteins that correct errors in chromosome–spindle attachments, could have a role in fine-tuning the catch-bond mechanism. Experiments with purified kinetochores will also no doubt be useful in dissecting the interplay between these chemical and mechanical regulatory mechanisms.

In vitro studies of isolated kinetochores might help to settle another outstanding question regarding the regulation of chromosome segregation. If chromosomes are improperly attached to the spindle, a signalling network called the spindle-assembly checkpoint blocks mitotic cell division before its anaphase step. It is unclear whether the spindle-assembly checkpoint directly responds to force (or tension)[13]. As the purified kinetochores contain proteins required for the spindle-assembly checkpoint, these kinetochores can be used to investigate whether the recruitment of checkpoint proteins — an early step in the signalling — is sensitive to force. Keep your seat belts fastened for the next phase of this exciting journey. ■

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CANCER

The blind spot of p53

It is hoped that reactivating the tumour-suppressor protein p53 will help to combat cancer. However, fresh evidence suggests it is unlikely that all cells in a tumour will respond to such treatment. SEE LETTERS P.567 & P.572

ANTON BERNS

The tumour suppressor that is most frequently mutated in human cancers is p53. Reactivation of this protein in tumours, which induces programmed cell death or cell-cycle arrest, is therefore an appealing therapeutic strategy. In this issue, however, Feldser *et al.*[1] and Junttila *et al.*[2] report work in mouse models of cancer showing that restoring p53 activity affects only advanced tumours, leaving untouched early lesions that are likely to one day become cancerous.

Earlier work[3–5] suggested that restoring p53 function in several independent oncogene-driven mouse tumours elicits a potent anti-tumour response. The outcome was either programmed tumour-cell death by the process of apoptosis, or tumour-cell senescence. In fact, in two of the three animal models[3–5], even temporary p53 reactivation led to prolonged survival. These data enhanced the appeal of p53 reactivation as a means of treating cancer.

Feldser *et al.* (page 572) and Junttila *et al.* (page 567) add a new twist to these observations. Both groups used variants of a mouse model of non-small-cell lung cancer (NSCLC) characterized by sporadic expression of a mutant *Kras* oncogene; this model closely resembles human NSCLC. Sporadic expression of physiological levels of mutant Kras in mice causes lung tumours that progress through different stages — from hyperplasia to adenoma to carcinoma. The advanced stages of the disease are marked by increased signalling flux through the RAS–MAPK pathway (the pathway in which Kras functions), probably due to additional alterations in this pathway. If sporadic tumour lesions associated with Kras mutations are also p53 deficient, they progress faster and become more malignant.

The authors[1,2] set out to determine what would happen if p53 function were restored in these tumours.

Junttila and colleagues[2] used a variant of the mouse model in which the mutant *Kras* can be switched on by inhalation of an agent called AdenoCre. The *p53* gene in these animals was replaced with a version that is inactive but can regain functionality on administration of the drug tamoxifen. The authors thus initiated tumorigenesis by AdenoCre exposure and then activated p53 functionality with tamoxifen. Feldser *et al.*[1] used a mouse model that randomly activates *Kras* at low frequency by the process of spontaneous recombination. Their animals could also be treated with tamoxifen to restore p53 functionality. The differences in the mouse models resulted in small differences in some of the measurements between the two studies, but the overall conclusions are fully congruent.

In contrast to the earlier studies[3–5], both teams found that, after induction of NSCLC by physiological levels of mutant Kras, tumour regression in response to p53 activation was hardly detectable or, at best, very modest. In both systems, in fact, only the more advanced adenocarcinoma lesions responded to induced p53 activity — by either cell-cycle arrest or a combination of cell-cycle arrest and apoptosis — whereas the less advanced lesions remained unaffected (Fig. 1).

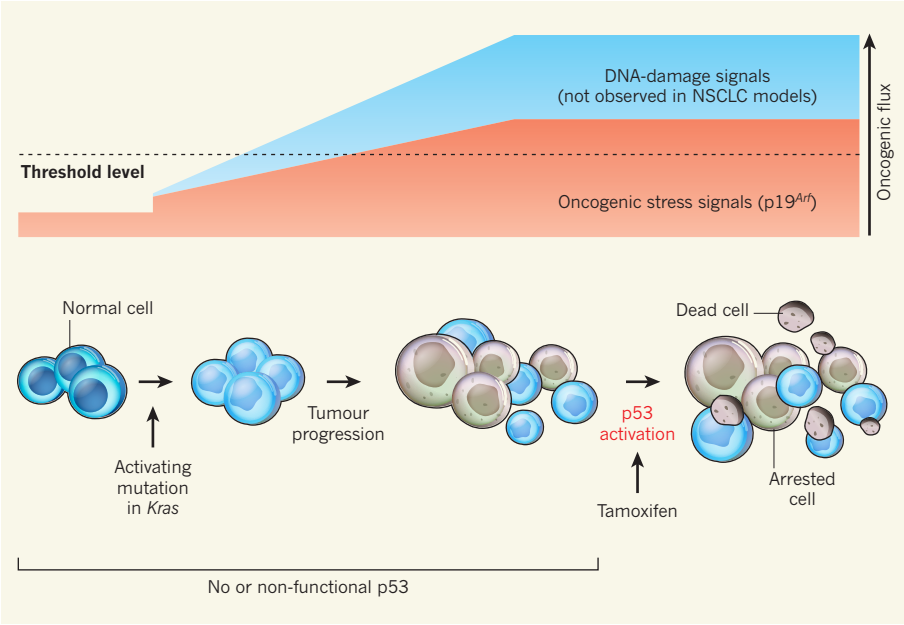

Figure 1 | Prerequisites for p53 activation. It is thought that a minimal level (threshold level) of oncogenic stress and/or DNA damage activates p53. Feldser *et al.*[1] and Junttila *et al.*[2] studied mouse models of non-small-cell lung cancer (NSCLC) characterized by oncogenic mutation of *Kras* to investigate what happens in the absence of p53. They find that, without p53, tumours could progress even if the oncogenic stress level increased above threshold level and the tumour-suppressor protein $p19^{Arf}$ was activated. When the authors restored p53 function with tamoxifen, tumour cells with an increased oncogenic flux were either arrested or killed. Less advanced lesions were unaffected, however, probably because their oncogenic flux remained below the threshold level. The authors did not detect DNA-damage response, indicating that, at least in their models, it does not contribute to p53 activation.

At a molecular level, cells in the more malignant lesions showed enhanced signalling flux through the RAS–MAPK pathway, owing to amplification of the mutant *Kras*, loss of the normal *Kras* allele (gene copy) or other alterations affecting this pathway. Intriguingly, the high signalling flux was associated with high levels of another tumour-suppressor protein, $p19^{Arf}$, which acts upstream of p53. The less advanced lesions did not have increased $p19^{Arf}$ levels, suggesting that enhanced activity of this protein is required to trigger the tumour-suppressive function of p53. Neither paper reports evidence of DNA damage in either the early or the advanced lesions: in this NSCLC model, therefore, DNA-damage response does not seem to play a significant part in activating p53.

These observations have important implications for understanding not just the 'surveillance' function of p53, but also the usefulness of restoring this tumour suppressor's function as a therapeutic strategy. p53 does not affect early cancerous lesions that have a low oncogenic flux and retain low levels of $p19^{Arf}$; indeed, only after $p19^{Arf}$ levels increase does p53 spring into action. This could be because organisms do not distinguish between normal pathway activation and moderate oncogenic signals: reacting to the latter would also compromise normal cell proliferation, which is essential for tissue maintenance, as well as tissue restoration after injury.

What do these findings[1,2] mean for human cancers and their treatment? There is no reason to be discouraged by them. By the time they are diagnosed, human tumours are usually much more advanced and so will more resemble the tumours described in the earlier papers[3–5] — those with a high oncogenic flux. Although advanced tumours might still contain cells with a low oncogenic flux from the earlier lesions, such cells probably constitute only a small fraction. Restoring p53 activity should, therefore, have a considerable effect on human tumours.

Nevertheless, Feldser and colleagues and Junttila and co-workers observe that cells with 'early-lesion' features are still present in the animals, even after p53 reactivation. Lesions containing such cells are obviously prone to progress to more advanced stages of cancer. Moreover, the two teams show that, at least in their NSCLC models, the DNA-damage-response pathway does not have a sizeable role in inducing p53's anti-tumour activity — an observation that was also highlighted in a previous investigation of another cancer model[6]. The idea that the DNA-damage-response pathway does not contribute to p53's tumour surveillance function is counterintuitive and warrants further research.

The studies[1,2] do demonstrate that oncogenic flux is the main trigger for effective p53 action. In view of the crucial role of $p19^{Arf}$ in this response — which might be more prominent than the role of its related human protein $p14^{ARF}$ — the NSCLC models seem particularly suitable for addressing the question of whether p53 responds to signals other than those from either the oncogene stress pathway, as governed by $p19^{Arf}$, or DNA damage.

Previous studies[7–9] have pointed to p53 and $p19^{Arf}$ having independent functions in tumour surveillance. A careful comparison of the loss of function of either $p19^{Arf}$ or both $p19^{Arf}$ and p53, with subsequent reactivation of p53, in these NSCLC models might help to further clarify the $p19^{Arf}$-independent tumour-suppressor roles of p53. This might also provide clues about how to selectively trigger p53 activity in the many human tumours in which the *INK4AB/*$p14^{ARF}$ tumour-suppressor genes are either deleted or silenced. ■

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