seems to be extremely dynamic. During sprout lumenization, the apical compartment can break and fuse repeatedly[14], and the same phenomenon has been observed during vascular pruning[15]. It will be interesting to explore whether LROs are involved in these dynamic processes.

Luschnig and colleagues demonstrate in an impressive manner that studies elucidating the formation of tubular networks in invertebrates continue to provide fruitful ground for similar work in vertebrates. The refined toolbox available to undertake such studies in *Drosophila* will continue the cross-fertilization between different model systems. Subsequent findings might in turn propose the contribution of additional or different mechanisms that ensure that tube formation is correctly executed to meet the needs of the organism.

COMPETING FINANCIAL INTERESTS
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p21 shapes cancer evolution

Vasily S. Romanov and K. Lenhard Rudolph

Although known to induce cellular senescence, an important tumour suppressor mechanism, mutation of *CDKN1A* — the gene encoding p21 (also known as WAF1 or CIP1) — is rare in human cancers. Now, a study reports a previously unappreciated oncogenic effect of p21 overexpression that shapes cancer genome evolution through induction of replication stress.

Since its discovery more than 20 years ago, our view on p21 has changed. Rather than simply being a cell cycle inhibitor, senescence inducer and tumor suppressor, it is now appreciated as a much more complex and broader regulator of additional cellular programs such as apoptosis, DNA repair, actin cytoskeleton remodelling, and cell migration[1]. Besides the original binding partners of p21 — cyclin E/A–CDK2 complexes and the DNA polymerase δ cofactor PCNA — additional interaction partners were identified, including transcription factors (E2F1, STAT3 and c-Myc), transcription coactivators (p300 and CBP), cyclin D–CDK4/6 complexes, ASK1 and JNK stress kinases, procaspase-3, poly(ADP-ribose) polymerase 1 (PARP1) and a regulator of the actomyosin cytoskeleton, the kinase ROCK1 (Fig. 1)[2]. All these protein interactions may mediate the diverse functions of p21 in cell physiology. In addition, other as yet unknown mechanisms are likely to contribute to the regulation of cellular processes by p21.

p21-dependent suppression of cell proliferation by inhibition of cyclin–kinase complexes, PCNA, transcription factors and coactivators are regarded as mechanisms that impair the formation of tumours in response to p53 activation. However, p21 deletion does not abrogate tumour suppression mediated by p53-dependent regulation of metabolism and antioxidant function[3]. Instead, p21 contributes to leukaemia cancer growth by slowing the accumulation of DNA damage in leukaemia stem cells and thus maintaining their capacity to self-renew[4]. Moreover, p21-dependent activation of cyclin D–CDK4/6 complexes, inhibition of apoptosis and induction of cell motility contribute to p21-dependent promotion of tumorigenesis, which has been observed in several mouse models[5]. Interestingly, the tumour-suppressive activities of p21 are associated with its nuclear localization, whereas its localization in the cytoplasm associates with oncogenic effects (Fig. 1)[2]. Intriguingly, mutations or deletions of the *CDKN1A* gene are very rare in human tumours. Instead, the inactivation of p21-dependent tumour-suppressive functions and activation of its oncogenic features commonly occur by its relocation from the nucleus to the cytoplasm; overexpression or cytoplasmic localization of p21 correlates with poor prognosis in a broad range of tumours[6]. But a completely different oncogenic mechanism of nuclear p21 is now presented by Galanos *et al.*[7] in this issue of *Nature Cell Biology*. The authors demonstrate that a subset of p53-deficient cancer cells and tumours exhibit chronic overexpression of nuclear p21, which in turn leads to deregulation of replication licensing, replication stress and genomic instability.

The authors initially observed that p21 expression correlates with the proliferation marker Ki67 in a subset of atypical cancer cells and in pre-neoplastic lesions with p53 aberrations. This led them to set up two cell-culture-based systems of inducible p21 expression in a p53-negative background. Although p21 induction results in senescence of most cells, the authors noticed the emergence of p21-positive cells that escaped senescence and re-entered the cell cycle. Proteomic and gene expression analyses of p21-positive senescence 'escapers' identified transcription-independent upregulation of replication licensing factors (RLFs) — CDT1, CDC6 and ORC2. Shutting down p21 expression conversely led to a ubiquitylation-dependent decrease in Cdt1. p21, CDT1 and CDC6 share the same E3 ubiquitin ligase, CRL4–CDT2, and, based on the fact that p21 has the strongest affinity for PCNA binding among all other PCNA-interacting proteins[8], Galanos *et al.* tested the hypothesis

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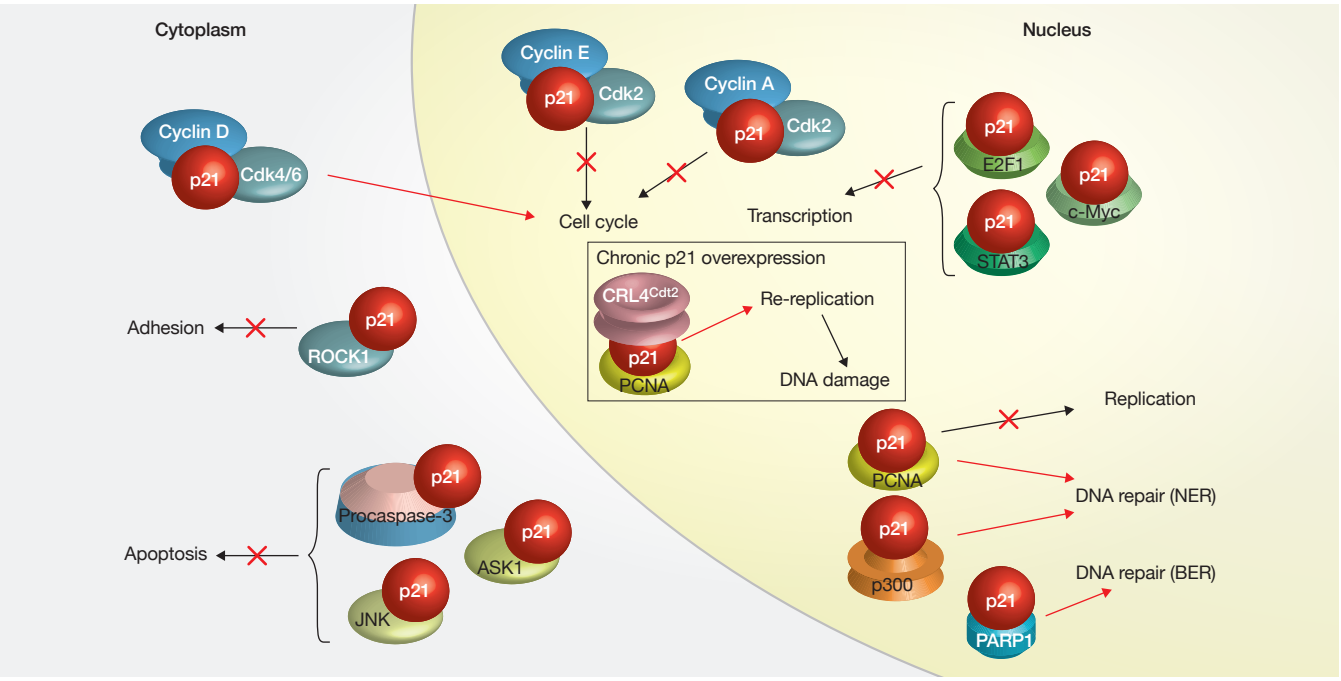

Figure 1 p21 regulates various cellular programs by different mechanisms. The subcellular localization of p21 influences its broad range of activities. According to previous studies, nuclear p21 activates tumour suppressor pathways, whereas cytoplasmic p21 induces oncogenic signals. Galanos *et al.*[7] provide experimental evidence that nuclear p21 overexpression has oncogenic effects in p53 mutant cells by inducing replication stress, DNA damage, error-prone DNA repair and cancer genome evolution. Red arrows and crosses indicate p21-mediated actions, black arrows indicate actions that are not directly regulated by p21. NER, nucleotide excision repair; BER, base excision repair.

that it may be a first-priority target of this PCNA-dependent ligase. Indeed, the authors provide experimental evidence that sustained p21 expression saturates CRL4–CDT2, thereby reducing ubiquitylation and degradation of other CRL4–CDT2 targets, including CDT1 and CDC6. Furthermore, the accumulation of RLFs results in re-replication, replication fork stalling, DNA damage and activation of the DNA damage response machinery (Fig. 2). Mechanistically, Galanos *et al.* show that DNA repair of stalled replication forks in the context of p21 overexpression involves the MUS81–EME1 resolvase complex and, importantly, the error-prone DNA recombinase, Rad52, which is engaged in low-fidelity microhomology-mediated end joining (MMEJ). Furthermore, the authors observe micronuclei as well as complex chromosomal aberrations detected by genome-wide approaches in these p21-expressing clones, suggesting that p21-dependent induction of replication stress and error-prone DNA repair contributes to the evolution of chromosomally instable clones. The induction of chromosomal instability and translocation precede transcriptome changes and the emergence of proliferative p21-positive cell clones with aggressive malignant features and an increased resistance to chemotherapy.

Taken together, these data highlight an outcome of p21 overexpression in p53 mutant cells characterized by the induction of chromosomal instability and cancer genome evolution through DNA replication stress and error-prone DNA repair. The results contrast previous findings showing that p21 can protect genome stability by regulating PCNA, p300 and PARP1, by promoting DNA repair, and by suppressing low-fidelity translesion DNA synthesis (TLS), a DNA damage tolerance pathway[9]. It will be of interest to further dissect context dependencies determining how p21 affects genome stabilization versus destabilization.

The observed mechanism of p21-induced replication stress as a driver of cancer genome evolution could be of general importance for our understanding of the role of p21 in cancer formation. Replication stress was recently identified as a driving force linking structural and numerical instability in cancer genome evolution[10]. Replication stress induced by p21 overexpression could be engaged in shaping the evolution of cancer genomes in the context of various forms of stress that lead to p21 induction. Of note, the present study shows that p21-induced cancer genome evolution only proceeds in p53 mutant cells, whereas the overexpression of p21 in cancer cells that retain functional p53 results in apoptosis. It will be of interest to investigate whether other genetic lesions that influence p53 checkpoint function cooperate with p21 induction to promote cancer genome evolution. At this stage, the findings of Galanos *et al.* appear to be most relevant for the initiation and progression of p53 mutant cancers. One important clinical example may be the evolution of liver cancer in the presence of aflatoxin or hepatitis B virus infection, both representing mechanisms of p53 inactivation. Liver cancer initiation often occurs in cirrhosis — the end stage of many chronic liver diseases — and is characterized by the activation of stress signalling pathways, for example the TGF-β and p38 pathways that are known to activate p21 independently from p53 and to promote liver cancer formation[11]. Cytotoxic cancer therapies are likely to represent another form of stress that could trigger p53-indepedent induction of p21. It is plausible that the circuit of p21-induced replication stress, error-prone DNA repair and genome instability described in the current study would contribute to the selection of resistant cancer cell clones under therapy. It is of particular interest to investigate p53-independent mechanisms of p21 induction in such scenarios, as well as to test whether p21 can be inhibited in order to minimize cancer genome evolution

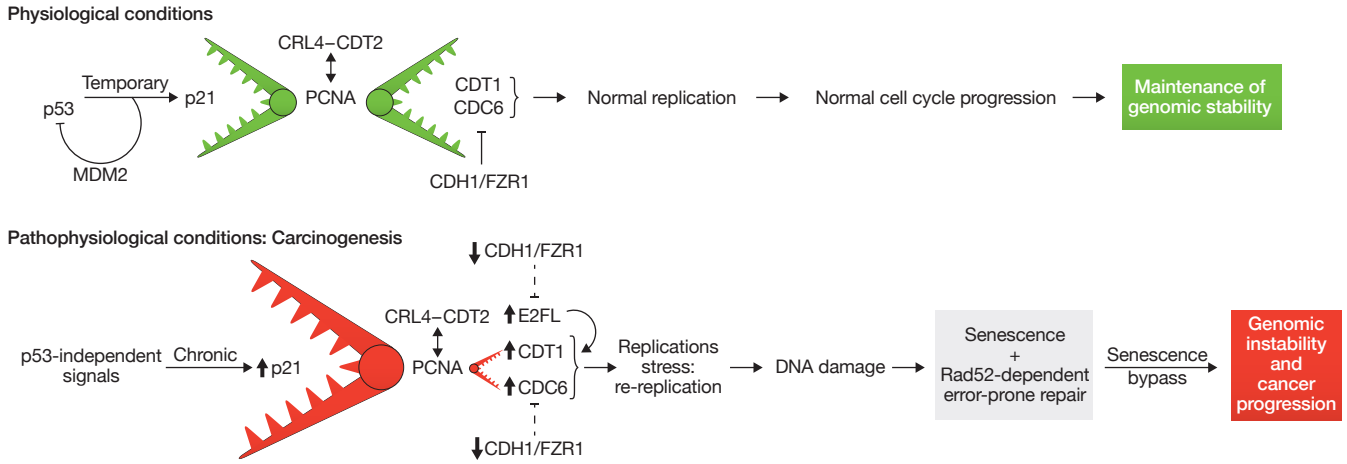

Figure 2 The model proposed by Galanos *et al.* Whereas transient induction pf p21 contributes to the maintenance of genomic stability (top), chronic p53-independent expression of p21 leads to re-replication, DNA damage, error-prone repair, genomic alterations and eventually cancerous evolution (bottom). Figure adapted from ref. 7, Nature Publishing Group.

and resistance in response to therapies that may induce p21. Intriguingly, the inhibition of p38 was recently shown to enhance the sensitivity and impair the emergence of resistant tumours in mouse models of hepatocellular carcinoma treated by sorafenib, a multikinase inhibitor[12]. It is tempting to speculate that abrogation of p38-dependent p21 induction[11] contributes to the mode of action.

In conclusion, the current study of Galanos *et al.* enhances our understanding of the 'dark side' of p21 that can be exploited by cancer cells by triggering replication stress and cancer genome evolution. This mechanism may also be relevant for cancer initiation in ageing tissues. p21 is upregulated in senescent cells and tissues during ageing, and this can impair regenerative capacity and organ homeostasis in response to telomere shortening[13]. p21-induced replication stress may contribute to regenerative impairments in ageing cells but could also lead to the transformation of p53 mutant cells that accumulate in somatic tissues and stem cell populations during ageing[14,15]. The findings support the concept that p21 inhibition could be explored as an anti-cancer approach in addition to its possible use in improving regeneration in aged tissues[13].

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New MAPS for misfolded proteins

Norbert Volkmar, Emma Fenech and John C. Christianson

Clearing misfolded proteins from the cytoplasm is essential to maintain cellular homeostasis. Now, a parallel clearance system is described that uses the deubiquitylase USP19 to enable secretion of misfolded cytoplasmic proteins when conventional proteasomal degradation is compromised. Misfolding-associated protein secretion (MAPS) has important implications for protein quality control and prion-like transmission.

Eukaryotic cells have evolved a diverse range of protein quality control (PQC) mechanisms to prevent, respond to and resolve proteotoxic stress elicited by proteins misfolding and accumulating in the cytoplasm[1]. Aberrant proteins may aggregate, undergo sequestration, be remediated by chaperone-assisted refolding, or be degraded by pathways including the ubiquitin–proteasome system, autophagy and breakdown by lysosomes. Proteins that are misfolded in the cytoplasm may also be released into the extracellular environment independently of conventional secretion via the endoplasmic reticulum (ER)–Golgi network, notably in the cases of aggregation-prone α-synuclein and tau proteins[2], but the mechanisms remain under debate. It has been proposed that forms

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