repeated formation (nucleation) of the tiny crystal seeds that would allow equiaxed grains to form. The challenge is to find a way of allowing this nucleation process to occur under AM conditions.

Previous attempts to solve this problem have generally focused on changing the processing parameters of AM, such as the speed, the power of the laser or electron beam used to heat the alloy feedstock, or the pattern in which the printer moves to build up an object, to disrupt the conditions that promote columnar growth (see refs 4 and 5, for example). Unfortunately, it has proved extremely difficult to exert sufficient control over such process variables to promote nucleation and hence develop the desired microstructure.

Luckily, a potential solution to this problem can be found from casting — in which additives called inoculants are commonly mixed into a liquid metal to 'seed' nuclei on which new crystals can grow, even in the presence of steep thermal gradients and high solidification velocities. The first reported instance of an addition being made to deliberately manipulate microstructure was in 1906, when ferrosilicon was added to a ladle of cast iron[6]. Since then, developments in casting have enabled the production of strong materials that lack holes or tears, and which contain equiaxed microstructures, using high-performance engineering alloys[7].

Inoculants are normally added to an alloy in its molten state. This poses a problem in AM, because the melt pool is only tens of micrometres long, and exists at any given point for just tens of microseconds[8]. Martin and colleagues' solution allows a precise quantity of inoculant to be delivered to such melt pools on this timescale.

The authors demonstrate the potential of their approach using two aluminium alloys that are well characterized and widely used: Al7075, a wrought (mechanically worked) material used in aerospace applications and which is not well suited to melt processing, and Al6061, a high-strength alloy used for casting. Crucially, both are difficult to process by AM. Martin *et al.* first modified the surface of the feedstock alloy powders by decorating them with nanoparticulate inoculants, which were tailored to the composition and crystal lattices of each alloy. These 'functionalized' powders were then used in a standard AM machine, following manufacturer-recommended processing conditions. For comparison, the authors also tested alloys that had not been surface-modified using the same processing conditions.

The difference in the microstructures obtained for the two types of sample was dramatic. The samples made using unmodified alloys contained large columnar grains and a high number density of cracks, as might be expected (Fig. 1a). By contrast, the functionalized powders produced fine, equiaxed microstructures that were free of cracks (Fig. 1b). The mechanical properties of the inoculated Al7075 were also markedly better than when it was made from the unmodified powder, and approached those of the same alloy in the wrought condition.

There is still some way to go, however, before this becomes the 'go-to' manufacturing technology for aerospace applications. In this context, the resistance of materials to fatigue — weakening caused by repeatedly applied loads — is of equal, if not greater, importance to their strength[9]. More work is needed to better understand and control the fatigue resistance of materials produced using AM. Another barrier to uptake by industry is the slow speed of current metal AM processes. Methods are emerging that deliver a step change in the speed of 3D printing of polymers[10], and the race is on to achieve the same for metals, but this presents a major technological challenge.

In the meantime, however, Martin and colleagues have identified an approach that allows alloys to be made more suitable for AM. Although they used aluminium alloys, they note that the method could be readily extended to other industrially useful alloy classes, such as non-weldable nickel alloys, superalloys and intermetallics. This might take some time to achieve, however, because inoculants for these materials remain elusive. But if inoculants can be found to functionalize the surfaces of powders of these alloys, then we really would be moving towards the 3D printing of any metal. ■

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CELL BIOLOGY

# The persistence of memory

**Live imaging reveals that whether or not a daughter cell proliferates is influenced by two molecular factors inherited from its mother, providing insight into how the behaviour of a newly born cell can be predetermined.** SEE LETTER P.404

KATARZYNA M. KEDZIORA & JEREMY E. PURVIS

A fundamental principle of cell theory is that all cells arise from pre-existing ones. Every cell, except sperm and eggs, inherits an essentially identical copy of its mother's genome, which it then passes on to two daughters when it divides. But it can also inherit a variety of other 'memories' from its mother cell, in the form of proteins, RNA and other biochemical keepsakes. Identifying these molecular memories and understanding how they influence cell behaviour has been a long-standing puzzle. On page 404, Yang *et al.*[1] tackle the question of how molecular memories acquired from the previous generation of cells influence whether daughter cells proliferate or enter a reversible resting state known as quiescence.

Proliferation drives both the development of an organism and the maintenance of its tissues. In response to growth signals, proliferating cells proceed through an initial phase of growth (known as G1), after which they begin DNA synthesis (S phase). Following a second growth phase (G2), the mother cell divides its contents into two daughter cells through a process called mitosis. Not all cells proceed swiftly through these phases, however. Instead, some temporarily withdraw from the cell cycle before S phase, entering quiescence[2].

How does a cell 'decide' between proliferation and quiescence? A study in the 1970s suggested that this decision is made during G1, before a cell commits to DNA synthesis[3]. According to this model, each cell is a clean slate, able to make an independent decision on the basis of the signalling molecules to which it is exposed. However, this idea was challenged in 2013 by the discovery that some cells are born predisposed to rapidly enter S phase[4]. For these cells, the decision is influenced by the experience of the mother during its G2. Precisely

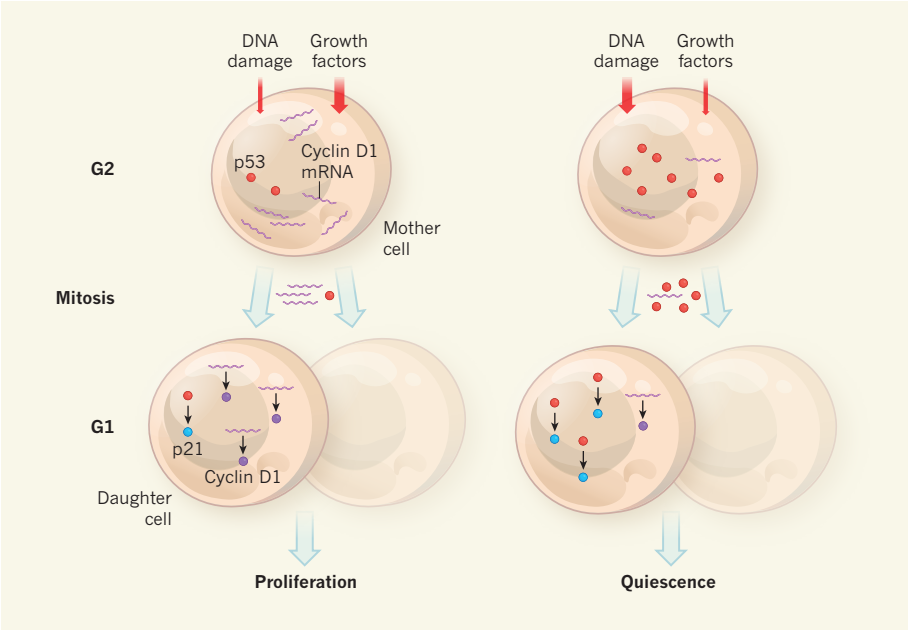


**Figure 1 | Making cellular memories.** Dividing cells progress through a growth phase called G2, and then undergo mitotic cell division. Daughters either undergo another growth phase, G1, before committing to proliferation, or become quiescent. The addition of growth factors to the mother cell leads to the accumulation of cyclin D1 messenger RNA, whereas DNA damage leads to activation of the protein p53; the sizes of the red arrows indicate the amounts of DNA damage and of added growth factors to which the mother cells were exposed. Yang *et al.*[1] report that cyclin D1 mRNA and p53 persist through mitosis into daughters, where cyclin D1 mRNA is translated into protein and p53 promotes the production of the p21 protein. The authors show that the ratio of cyclin D1 to p21 is an accurate predictor of whether a daughter will proliferate or enter quiescence.

how this memory is transmitted from mother to daughter has remained elusive.

Yang *et al.* exposed mother cells to different combinations of growth signals and DNA damage. They then withdrew the signals and used live imaging to chart the proliferation–quiescence status of the daughters. They found that newly born cells 'remembered' the signalling history of their mothers. Specifically, cells from mothers exposed to growth signals had high levels of the protein cyclin D1, which promotes progression from G1 to S phase[5]. By contrast, cells from mothers exposed to DNA damage had high levels of the p21 protein, a potent inhibitor of G1 progression[6]. In fact, the balance between these two factors was highly predictive of whether a cell would undergo proliferation or quiescence. At the molecular level, cyclin D1 and p21 compete to control phosphorylation of the retinoblastoma protein, which acts like a switch that determines whether cells enter S phase.

However, p21 and cyclin D1 have short lifetimes, making it unlikely that inheritance of these factors is the basis of cellular memory. The authors therefore reasoned that cells needed a more persistent form of memory that would last from the previous G2, through mitosis and well into the daughter cell's G1. The messenger RNA molecule that encodes cyclin D1 is much longer-lived than its protein product. Similarly, the stress-response protein p53, which is an activator of p21, becomes stabilized when it is activated by DNA damage. The researchers found that, in this activated form, it can last roughly ten times as long as when it is in its inactivated form.

Directly visualizing activated p53 and cyclin D mRNA as they are passed from mother to daughter is technically challenging. To work around this difficulty, Yang and colleagues demonstrated that both long-lived factors are generated in the mother cell and detectable soon after daughter-cell birth. Moreover, changes in the levels of these factors in the mother influenced daughter-cell fate. Cyclin D1 mRNA and p53 protein therefore represent opposing molecular memories that alter the proliferation–quiescence decision of daughter cells (Fig. 1). To our knowledge, this is the first identification of molecular factors that directly compete for influence over daughter-cell fate.

An unexpected secondary finding of this study is that levels of just two molecules can predict a single cell's behaviour with exceptional accuracy. The proliferation–quiescence decision shows an ultrasensitive response to changes in the ratio of cyclin D1 to p21 — tiny changes in this ratio could dramatically switch the fate decisions of daughter cells. This finding might indicate that cyclin D1 and p21 represent the end of a complex molecular funnel that compresses multiple proliferation-promoting and -inhibiting signals carried by upstream factors into a single output. It also opens up the possibility that other binary cell-fate choices (such as a stem cell's decision to self-renew or differentiate) is predetermined by a relatively small set of inherited, competitive memory signals.

Another interesting aspect of Yang and colleagues' work is that, in the case of DNA damage, only the memory of damage — and not the damage itself — is passed from mother to daughter. This finding contrasts with recent reports showing that replication stress in mother cells leads to DNA damage that persists through mitosis, causing quiescence in daughter cells[7,8]. A possible explanation for this discrepancy is that Yang *et al.* induced high levels of DNA damage, instead of looking at the less abundant breaks that occur naturally. Greater DNA damage can more efficiently trigger a response[9] that temporarily halts the cell cycle at G2, forcing cells to stop and repair the damage before entering mitosis. In either case, a history of DNA damage seems to be an important factor in a cell's proliferation–quiescence decision. Both DNA damage and the memory of such damage, by inducing quiescence, reduce the accumulation of potentially cancer-causing mutations in growing tissues.

The concept of competing molecular memories is attractive, but raises questions about the behaviour of individual cells. For example, why do some pairs of daughter cells make different decisions from one another? One possibility is that p53 and cyclin D1 mRNA are not equally distributed between daughter cells during division. This hypothesis could be tested by comparing the relative levels of these factors between sister cells immediately after division. Another question is how molecular memories cooperate with external signals — extra DNA damage during G1, signals from neighbouring cells and mechanical forces[10], for instance. These external factors probably have a role *in vivo*, where the heterogeneous make-up of complex tissues could act either to strengthen or repress the memories of individual cells. ■

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