Robust yet flexible

In biological systems, resistance to change and innovation in the light of it go hand in hand

Life is a dangerous business. All living beings suffer from constant environmental wear and tear: from solar radiation and oxidative damage, to physical injuries or the onslaught of infectious agents. The robust back-up and repair mechanisms that organisms have developed to deal with these stresses, damaging mutations and infections are therefore vital to survival; the failure of these systems can cause disease or allow disease progression. At the same time, the power and robustness of protective mechanisms is a double-edged sword: tumours, for example, use repair systems to resist natural or artificial attempts to destroy them, and viruses compromise the systems for their own ends.

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Hiroaki Kitano, Director of the Japanese Systems Biology Institute in Tokyo, is one of many researchers dedicated to unravelling these mechanisms and, given the recent progress in understanding these systems on several fronts, he believes that it will be possible to develop a unified theory of biological robustness within a solid mathematical framework (Kitano, 2007). The result would be a complete model of an organism with all of its external and internal interactions and parameters, which could help researchers to understand complex diseases—first and foremost cancers—and devise new ways of fighting them.

Such a 'grand theory' might be some way off but, in the meantime, biologists already understand that organisms have

two fundamentally different responses to damage or disruption: back-up and repair, which can be used together or separately depending on the circumstances. Meanwhile, it has become clear that 'backing up' in biological systems is rarely the same as it is in engineered systems, such as planes or computers, which use redundant standby systems when the primary components fail. This is because natural selection simply does not favour the maintenance of total redundancy; with no immediate selective advantage to having back-up components, the two sets of components will gradually diverge owing to accumulating mutations.

For some time, however, molecular biologists assumed that gene duplication—which has occurred widely throughout evolution—provided a back-up to protect the organism against damaging mutations of the original, but this view has now subsided according to Maya Schuldiner from the Weizmann Institute of Science in Rehovot, Israel: "Gene duplication is a two-edged sword, since the availability of a 'back-up' copy means that there is no selective pressure for the other copy to remain intact." Without selective pressure to prevent it, the 'copy' would accumulate mutations and its encoded protein might change its function, or, in the case of multicellular organisms, could be expressed in different tissues. It has been estimated that 15% of the protein-coding genes in humans are 'duplicates' that originally encoded the same products as their 'master copy' at the time they were formed. Schuldiner, whose laboratory specializes in the quality control of protein folding, concludes that, "most gene duplications have not served to create redundancy in the function of a current biological system."

Instead, scientists have turned their thoughts to the idea of 'distributed robustness' as the main form of protection against

changes that threaten critical metabolic pathways or biological functions. "Gene duplication plays a role in such systems, but distributed robustness is by far the biggest contributor," commented Andreas Wagner, author of the seminal book on the subject, *Robustness and Evolvability in Living Systems*.

The term, distributed robustness, has been adopted from the field of information technology, in which it is used to define a network free from any single point of failure. Put another way, there must be at least two alternative paths between any two points on the network, so that removal of any one component leaves the network fully operational. This is in contrast to full redundancy, in which every node or link in the network has a direct back-up to take over in the event of failure. Distributed robustness makes more efficient use of resources, whether in computation or biology. Wagner, from the University of Zurich, Switzerland, has studied its application to metabolic networks in which groups of enzymes cooperate in the transformation of nutrients into biomass, from the synthesis of individual amino acids to complex macromolecules that comprise proteins, lipids and carbohydrates.

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"In these metabolic networks you can have this situation where every single chemical reaction is different, with no redundant reaction," Wagner explained. "Yet the network can be highly robust. You can eliminate a reaction and yet the network still synthesises all the correct biomass

compounds. It does so by rerouting the flow of matter through a different pathway that is not blocked through the reaction that you have eliminated."

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Examples of distributed robustness in the natural world continue to come to light. For example, Photosystem II—the light-harvesting protein complex that splits water to yield electrons and drives all of the other processes of photosynthesis—contains a major protein complex, the light-harvesting complex II (LHCII), that provides a large part of the antennae network essential for catching sunlight. However, when *Arabidopsis* plants lack the constituent proteins of LHCII, a normally minor antennae complex called chlorophyll protein complex 26 (CP26) takes over (Ruban *et al.*, 2003). The structure of the whole Photosystem II is reconfigured, with CP26 synthesized in much larger quantities than normal and organized in trimers similar to LHCII.

This raises the question of how distributed robustness operates in biological systems, and how the system recognizes when such back-up procedures need to be

invoked. According to Schuldiner, the triggers are related to stress responses and the associated changes in gene expression patterns, although much of the detail remains unclear. "There is a 'stress response' that turns on an entire barricade of proteins for nearly any given stress," she said. "Together, the upregulated proteins can help overcome the loss of any one function. For example, imagine that when you cause a mutation in one gene it causes osmotic stress in the cell—that in turn activates an entire signal transduction pathway which, in the end, leads to many cellular changes including the upregulation of gene expression."

Invoking distributed robustness is not always the appropriate response to damage or change at all levels of biological organization. In some cases, direct repair of the damage is preferable or even essential, as is the case for DNA. By virtue of its function and chemical structure, DNA is highly vulnerable to damage, whether from mis-copying, oxidative damage—caused by metabolic byproducts in the cell—mutagenic chemicals or ultraviolet radiation. Furthermore, DNA, unlike RNA and proteins, cannot be periodically degraded and rebuilt because genes need to be expressed continuously and not on a part-time basis. Not surprisingly, some of the most elaborate repair mechanisms in biology have evolved to cope with damage to DNA.

Yet, in some regards, the biological response to RNA damage is even more interesting from an evolutionary standpoint, as RNA can be repaired or replaced depending on the circumstances. For some RNA molecules, the cell has the luxury of a choice between both methods, whereas for others, only one or the other option is available. This begs two questions. First, when both methods are available, how does the cell decide whether to invoke repair or a combination of degradation and replacement? Second, if only one method is available, what is the selective advantage of giving up the other option?

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The answer to both questions lies in energy economy, according to Pål Ø. Falnes, who specializes in DNA and RNA repair at the University of Oslo in Norway. According to Falnes, the choice depends on the numbers involved: if the damage occurs frequently, it would be economical for the cell to establish a dedicated repair system to avoid wasting energy on repeated RNA synthesis; where the

damage is infrequent, the cost of a dedicated repair machinery would be higher than that of infrequent degradation and the new synthesis of a relatively small number of molecules. In those cases in which a given RNA molecule is targeted by both mechanisms simultaneously, the choice might depend on how bad the damage is. Chemistry may also have a role: some lesions are not easy to repair, which leaves the cell no choice other than to degrade and, if necessary, replace the damaged molecule.

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One of the more interesting debates about the robustness of RNA centres on the evolution of RNA itself. Robustness, some scientists argue, provides convincing evidence that an RNA genome could have existed on its own without the support of underlying DNA, as Anthony Poole, a geneticist at Stockholm University in Sweden and the University of Canterbury in New Zealand, explained. "Recognising the feasibility of repair of an RNA genome lends plausibility to the proposal that LUCA (last common universal ancestor) may have possessed an RNA genome, and eliminates an obvious criticism that there are limits to the coding capacity of RNA genomes because of the low fidelity of RNA polymerases," he commented. "If RNA polymerases are repair-competent, this concern may not be a barrier to a complex RNA genome in LUCA." The key point is that RNA itself can provide a template for its own repair, without having to refer back to the DNA template (Poole, 2006). Nevertheless, the debate over the viability of an RNA genome and whether there was once an RNA-only biosphere is clearly not yet settled.

Repair mechanisms also exist to counter damage to some proteins, although, as with RNA, degradation is the usual response to damage. Again, the question arises as to why protein repair mechanisms evolved at all, given that it seems simpler and safer to just degrade damaged proteins that might become toxic. The answer

may again be energy efficiency: in a large three-dimensional protein complex, a small repair could make the difference between a complete, functional complex and an energy-expensive degradation and re-synthesis cycle. In other cases, no repair might be needed at all if distributed robustness can provide some margin for error in the geometry of the final tertiary structure, such that a slight distortion in shape caused by a change to one amino acid still leaves the protein fully functional.

Robustness mechanisms have evolved to respond to the problems caused by the mutations or other unanticipated changes that result from environmental wear and tear. However, in some cases, distributed robustness and other repair mechanisms have become almost too effective, leaving them open to exploitation by cancers, viruses or bacterial pathogens. According to Kitano, cancer can exploit what he calls "functional redundancy"—which is another term for distributed robustness—and feedback control systems to defend against the organism's own immune defences or even therapeutic interventions (Kitano, 2007). Functional redundancy operates at the cellular level, through cell differentiation within the tumour to produce a range of cells, some of which may be able to survive chemotherapy, radiotherapy or immune attack.

In addition, feedback mechanisms, which can be regarded as another source of robustness, allow tumour cells to survive chemical attack directly by manipulating their environment and secreting protective agents, just as some bacteria do when they occur as biofilms. Tumour cells can also escort chemotherapeutic drugs out of the cell by activating a specific ATP-dependent efflux pump, according to Kitano.

In these ways, cancer cells hijack the same mechanisms that have evolved to protect organisms against ageing, disease or mutation. Our increasing knowledge of these mechanisms will thus help to identify new and more specific targets for drugs; indeed, the role of robustness in cancer is already stimulating research in the field.

However, robustness research is also being driven by the desire to understand the role of these defensive mechanisms in evolution and, in particular, to unravel the tension between the need to protect against serious damage

while allowing the scope for genetic change and natural selection. "This is a problem Darwin wasn't able to solve," Wagner commented, "and we still don't fundamentally understand how organisms innovate." In his forthcoming book, Wagner will offer a framework for innovation and discuss how blind evolutionary pressures can lead to novelty, as indeed seems to have actually been the case. He has already presented some of his ideas in several recent papers, suggesting that molecules often have the ability to evolve neutrally through mutations that do not immediately compromise their primary function. Such 'neutral' mutations would provide robustness against change and, at the same time, would allow novel functions to slowly emerge. Indeed, the ability to innovate is itself a form of robustness against a changing environment.

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Another twist to the yin and yang of robustness and innovation is the existence of sexual recombination, which occurs in some form throughout all eukaryotic kingdoms. Sexual recombination is widely thought to have evolved as an efficient mechanism for parental organisms to combine their beneficial mutations in a single offspring. According to this theory, sexual reproduction has accelerated the generation of more competitive or better-adapted individuals. But, as Michael Knop, a group leader at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, pointed out, sexual reproduction evolved in simple unicellular organisms such as yeast that cannot move around and seek suitable partners. As such, sexual recombination in yeast does not combine beneficial mutations often enough to have been the driving force of its evolution. In fact, Knop's recent research suggests that sex evolved initially for the opposite reason: to purge harmful mutations through the process of meiosis and to ensure the propagation of some individuals without the deleterious mutation (Keller & Knop, 2009). "There is an aspect of game theory

here,” Knop said. “If beneficial mutations are not frequent enough, then it doesn’t make sense to outbreed, so if the organism concerned still does sex, it must be for another reason.”

Although sex first evolved in unicellular eukaryotes to prevent harmful change, it has been multicellular organisms—animals in particular—that have taken it to the next level by selecting partners for their desirable attributes and mutations. In this manner, sexual recombination in higher organisms has the dual role of assembling desirable alleles while protecting the population against harmful mutations.

These mechanisms—sexual recombination, distributed robustness and repair—are united by a common objective: to manage the impact of harmful mutations, while maintaining genetic flexibility within the population in order to cope with environmental or other competitive pressures. Given this common goal, a grand theory of biological robustness, as Kitano proposes, might well be possible in the future.

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