prostate or blood cancers — also display epigenetic changes that are crucial for tumour development and maintenance. Future experiments, including integrated genomic analyses, may provide the answer.

The underlying mechanism of alterations in gene expression by epigenetic means in retinoblastoma is still unclear. But Zhang and co-workers' observation[3] that 13% of these tumours show recurrent mutations in the *BCOR* gene offers a possible explanation. The BCOR protein is highly expressed in the fetal retina and is essential for eye development, as evidenced by a congenital eye disorder, syndromic microphthalmia, which results from a heritable mutation in *BCOR*[7]. BCOR associates with proteins that repress gene expression epigenetically[8]. This raises the possibility that loss of BCOR function, due to acquired mutations in its gene, may contribute to an altered epigenetic landscape in RB1-deficient retinal cells. Consequently, inappropriate expression of crucial genes may impair cell maturation and so facilitate the progression of retinoblastoma. It will be of interest to determine whether BCOR is part of the repression machinery that silences SYK expression in the normal retina, and whether acquired *BCOR* mutations provide at least one route to altered expression of SYK in retinoblastoma.

Nevertheless, the current work — using a comprehensive, integrated genomics approach — is notable not just for demonstrating that epigenetic alterations have a predominant role in the progression of retinoblastoma. The new possibilities it raises for therapies in this childhood malignancy, and possibly in other types of tumour, are equally noteworthy. ■

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1. Chinnam, M. & Goodrich, D. W. *Curr. Top. Dev. Biol.* **94,** 129–169 (2011).
2. Manning, A. L. & Dyson, N. J. *Trends Cell Biol.* **21,** 433–441 (2011).
3. Zhang, J. *et al. Nature* **481,** 329–334 (2012).
4. Stratton, M. R. *Science* **331,** 1553–1558 (2011).
5. Parsons, D. W. *et al. Science* **331,** 435–439 (2011).
6. Hansen, K. D. *et al. Nature Genet.* **43,** 768–775 (2011).
7. Ng, D. *et al. Nature Genet.* **36,** 411–416 (2004).
8. Fan, Z. *et al. Nature Cell Biol.* **11,** 1002–1009 (2009).

EVOLUTIONARY BIOLOGY

A ratchet for protein complexity

Molecular machines containing related protein subunits are common in cells. Reconstruction of ancient proteins suggests that this type of complexity can evolve in the absence of any initial selective advantage. SEE LETTER P.360

W. FORD DOOLITTLE

Organisms and cells are bewilderingly complicated, and the molecular machines that perform many basic cellular functions are often giant, multisubunit, multifunctional protein complexes with tangled evolutionary histories. It is generally assumed that such complexes arose by the stepwise accretion of individual proteins, each addition representing a selective advantage by adding to or refining the machine's performance. But on page 360 of this issue, Thornton and colleagues[1] argue against this standard explanation in one particular instance — that of a ring-shaped protein complex in fungi. The authors show how evolutionary processes entailing loss of function rather than gain might initially drive a system towards complexity, independently of selection*.

Many cellular molecular machines contain several proteins that self-assemble into a multi-subunit ring. In simple cases, rings are homo-oligomeric; that is, all the subunits are identical and thus probably the products of a single gene. In more complex (hetero-oligomeric) examples, the protein molecules in the ring are different, but may be related. Often, hetero-oligomeric rings seem to have arisen from homo-oligomeric complexes after a gene encoding a single subunit became duplicated, producing two or more gene copies (called paralogues), with each copy subsequently evolving to encode a slightly different protein.

The question that Thornton and colleagues address is, why does this happen? Do the structurally distinguishable subunits and/or their specific pattern of assembly confer improved or additional functions on the protein complex, with selection for their enhanced performance being the evolutionary driving force? Alternatively, might neutral processes be responsible, at least initially?

To understand how the latter possibility might come about, imagine that a gene encoding, for example, eight subunits of a homo-oligomeric ring undergoes duplication (Fig. 1). The two paralogous gene copies that this duplication produces — and the protein subunits they encode (call them A and B) — will of necessity begin to diverge through accumulation of neutral mutations. These mutations might not at first affect any of the subunits' functions, so that functional octameric rings could continue to form by a random sampling

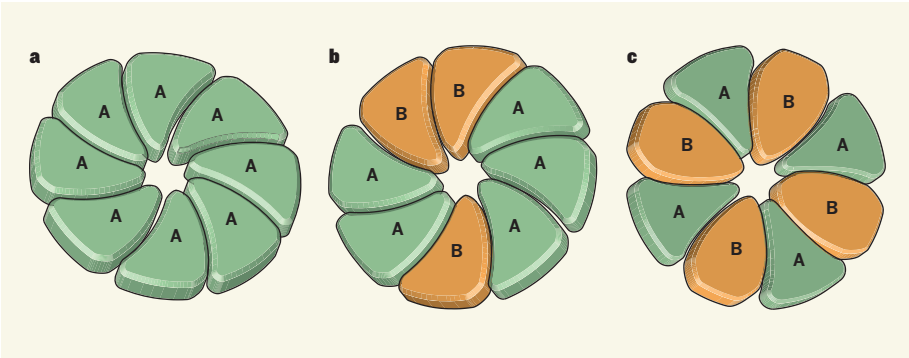

Figure 1 | Evolution of complexity in a protein ring. **a**, Homo-oligomeric protein complexes contain identical subunits (A) that are typically encoded by a single gene. **b**, If the gene is duplicated, the two gene copies can diverge through the accumulation of neutral mutations, generating structurally distinguishable but functionally unaltered subunits A and B, which can form functional hetero-oligomeric rings by random mixture. **c**, If additional mutations prevent subunits from binding to others of their own type, functional rings could still be formed by alternating subunit types. As further mutations accumulate, the probability of returning to the initial homo-oligomeric situation becomes very small. Thus, the subunit composition of a protein association may become complex in the absence of initial selective advantages. Thornton and colleagues[1] provide experimental evidence suggesting that this type of process has occurred in the evolution of a six-membered protein ring that forms part of the vacuolar H^+-ATPase enzymes in fungi.

*This article and the paper[1] under discussion were published online on 8 January 2012.

of A and B subunits. But further mutations might result in each subunit losing the ability to bind others of its own type, A to A or B to B. However, as long as A still binds B and B still binds A, functional rings can form.

As A and B continue to co-evolve through the acquisition of further neutral mutations, the likelihood of reversing back along the mutational path to the initial single-gene homo-oligomeric complex becomes vanishingly small. We can thus see the homo-oligomer to hetero-oligomer transition as an inexorable evolutionary ratchet. And even if there were some slight selective disadvantage to using a hetero-oligomeric complex (for example, complex formation could become slower), this could be overcome in small populations — in which the efficiency of selection is reduced and mildly harmful mutations can be fixed by chance[2].

Some chaperonins — molecular machines that assist other proteins to fold into functional configurations — have until recently provided the best evidence for such a scenario. Microorganisms known as archaea have one, two or three paralogous genes encoding chaperonin subunits that form rings: homo-oligomeric eight-membered rings when there is a single gene, or hetero-oligomeric eight- or nine-membered rings when there are two or three genes. Such paralogy seems to reoccur frequently in archaea, and a neutral co-evolutionary picture of the sort described above has been suggested[3] to explain it.

Comparative bioinformatic studies[4] support the idea that hetero-oligomeric archaeal chaperonins consisting of two different subunits are not functionally distinct from the homo-oligomers, and thus did arise neutrally. Adaptive changes can follow later, however. Indeed, there is direct evidence for specialized roles for the eight distinct chaperonin subunits of eukaryotes (organisms such as plants, animals and fungi)[5].

So a more realistic picture might be as follows. Rarely, gene duplication is quickly followed by functional differentiation (as proposed in early formulations of the hypothesis of evolution by gene duplication[6]). Selection against loss of either duplicate would then be ensured early on. Perhaps more often, though, the paralogous condition might first be locked in by the above-described co-evolutionary ratchet, allowing functional differences (if any) to be acquired later (if ever). That is what Thornton and colleagues[1] argue has happened in the case of a six-membered hetero-oligomeric ring that forms part of the enzymes known as vacuolar H^+-ATPases (V-ATPases) in fungi. Their argument is especially compelling because it rests not only on bioinformatics, but also on experiments, including the synthesis and testing of proteins that the authors propose are ancestral to the V-ATPase subunits.

In most eukaryotes, the V-ATPase six-membered ring is made up of five copies of the protein Vma3 and one of its paralogue Vma16. In fungi such as budding yeast, by contrast, one of the five Vma3 proteins is replaced by Vma11, which is a fungus-specific paralogue of Vma3. The abilities of these subunits to bind to one another[7] are such that Vma11 must lie between Vma16 and Vma3 in the ring, and Vma3 cannot form the interface with the side of Vma16 that Vma11 can. This represents a sort of functional degeneration, because the last common ancestor of Vma3 and Vma11 must have been able to interface with either side of Vma16, as Vma3 must still do in organisms other than fungi.

Thornton and colleagues[1] synthesized proteins that matched the phylogenetically inferred ancestor of Vma3 and Vma11, as well as that of Vma16 and other intermediates in the predicted evolutionary pathway. They demonstrate that Anc.3-11, the inferred ancestor of fungal Vma3 and Vma11, does indeed interface with either side of Vma16. The authors tested the functionality of these and other inferred evolutionary ancestors and intermediates by expressing them in yeast mutants that lacked current versions of one or more of the three genes. According to this picture, when Anc.3-11 underwent duplication, one paralogue (which was to become modern fungal Vma3) lost the ability to interact with the 'anticlockwise' side of Vma16. The other paralogue (which became Vma11) lost the ability to interact with the 'clockwise' side of Vma16 and/or the anticlockwise side of Vma3.

A general theory, sometimes called constructive neutral evolution, to explain how neutral processes might drive a system towards complexity is more than a decade old[8–10]. But the study by Thornton and colleagues[1] may provide the most compelling experimental evidence to date. Of course, one can never prove that some subtle, unidentified selective advantage was not involved in the evolution of the V-ATPase protein ring, but neutrality would seem the most justifiable default hypothesis. Thus, a neutral theory of molecular evolution, normally invoked for nucleotide substitutions, may also apply to certain higher-order structures such as multi-subunit protein rings. How general such neutral mutational drives to complexity might be is one of evolutionary theory's deeper unanswered questions. ■

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1. Finnigan, G. C., Hanson-Smith, V., Stevens, T. H. & Thornton, J. W. *Nature* **481,** 360–364 (2012).
2. Lynch, M. *Proc. Natl Acad. Sci. USA* **104,** 8597–8604 (2007).
3. Archibald, J. M., Logsdon, J. M. Jr & Doolittle, W. F. *Curr. Biol.* **9,** 1053–1056 (1999).
4. Ruano-Rubio, V. & Fares, M. A. *Mol. Biol. Evol.* **24,** 1384–1396 (2007).
5. Fares, M. A. & Wolfe, K. H. *Mol. Biol. Evol.* **20,** 1588–1597 (2003).
6. Ohno, S. *Evolution by Gene Duplication* (Springer, 1970).
7. Wang, Y., Cipriano, D. J. & Forgac, M. *J. Biol. Chem.* **282,** 34058–34065 (2007).
8. Stoltzfus, A. *J. Mol. Evol.* **49,** 169–181 (1999).
9. Force, A. *et al. Genetics* **151,** 1531–1545 (1999).
10. Gray, M. W., Lukeš, J., Archibald, J. M., Keeling, P. J. & Doolittle, W. F. *Science* **330,** 920–921 (2011).

GALAXY FORMATION

Distant dwarfs

Astronomers value the smallest galaxies for the clues they can provide about how galaxies form. But only those nearest to us are easily detectable. A neat technique has allowed one such object to be found at a large distance. SEE LETTER P.341

ROBERT W. SCHMIDT

Some objects do not shine brightly enough to be seen easily. Examples of this are the small and extremely faint galaxies called dwarf or satellite galaxies. Only when they are near to our Galaxy might we be lucky enough to detect the light of their stars. Satellite galaxies are of great interest because simulations of the process of galaxy formation predict that there are many more satellites in the Local Group, which includes the Milky Way and other galaxies in its vicinity, than have yet been found[1–3]. This begs the question of whether there is something wrong with our understanding of how galaxies form. On page 341 of this issue, Vegetti *et al.*[4] report the discovery of a satellite in the vicinity of a galaxy located at a cosmological distance from Earth that allows them to test the simulation predictions for galaxies beyond those of the Local Group.

The object studied by Vegetti *et al.* is a distant elliptical galaxy that acts as a gravitational lens: light from a background source is deflected from its otherwise straight path by a massive foreground object, the lens. When compared with camera lenses, gravitational lenses have rather dismal optical properties because they can lead to large distortions and multiple images of the background object. But from an astronomer's point of view this is a good thing: the mass of the gravitational lens can be measured by reconstructing the background object from the distorted images.