Telomeres protect the ends of linear chromosomes and by doing so safeguard genome integrity. Although this fact is well known, much remains to be learnt about the structure and elongation of telomeres, and the molecular consequences of their dysfunction. Downey et al. used a combination of genomic and genetic approaches in Saccharomyces cerevisiae to identify a new telomere-regulating protein, and describe a new but evolutionarily conserved protein complex that is involved in telomere homeostasis.

The study began with a temperature-sensitive screen of a pre-existing panel of 4,800 non-essential gene-deletion strains, into which the authors introduced a mutated allele of **cdc13-1**, which encodes a telomere-capping protein. From the recovered **cdc13-1** suppressors, the authors selected one, previously uncharacterized gene. They called it **cgi121**, after its human homologue. It turned out that **cgi121** suppresses a range of temperature-sensitive defects that are associated with loss of telomere capping and it does so by suppressing the accumulation of ssDNA at uncapped telomeres.

Guided by the fact that the human Cgi121 orthologue interacts with a putative p53 Ser15 kinase PRPK, Downey et al. showed that the yeast counterparts (**Cgi121** and **Bud32** — an orthologue of the kinase) also physically interact in vivo. **bud32Δ** suppresses **cdc31-1** and so, like **Cgi121**, **Bud32** promotes uncapping at dysfunctional telomeres. Interestingly, **Bud32** is also involved in maintaining normal telomere length, and both of these functions require its kinase activity. Double-mutant analysis revealed that **Bud32** functions mainly in telomerase-mediated pathways of telomere-length maintenance.

The fact that **Bud32** is also involved in ectopic telomere addition (at sites of double-strand breaks) indicated that its function might not depend on pre-existing telomeres. A series of elegant genetic experiments led the authors to suggest that **Bud32** might be required for the ability of telomere-bound telomerase to elongate the telomere.

Double-mutant analysis also revealed that **Bud32** and **Cgi121** function in the same pathway. In fact, using tandem affinity purification the authors showed that, together with two other proteins, they form a multiprotein complex, which they called KEOPS (kinase, putative endopeptidase and other proteins of small size). Based on their results, the authors proposed that the function of KEOPS is to facilitate access to the telomere ends. This model, which is the simplest of many possible alternatives, can explain how KEOPS can fulfil both of its reported roles — by opening up the chromatin, it could allow telomerase access to the telomere and endonuclease access to degrade chromosome ends following telomere uncapping.

The authors are well aware of the work required to test this prediction. Among their future plans is to follow up on the conservation of the components of the KEOPS complex to explore whether its function is conserved in human telomere metabolism.

Magdalena Skipper


RNAi has become a powerful research tool, but progress in developing therapeutic applications of this technology has only recently started to gather momentum. In a major advance for this field, Zimmermann, MacLachlan and colleagues report the first demonstration of gene silencing in non-human primates after systemic delivery of small interfering RNA (siRNA).

RNAi-based therapeutics harness an endogenous cellular regulatory mechanism in which small dsRNA molecules, or siRNAs, bind to and mediate the destruction of specific mRNA molecules, preventing their translation into protein. Despite its great potential, moving siRNA translation into protein. Despite mRNA molecules, preventing their mediates the destruction of specific molecules, or siRNAs, bind to and mediate the destruction of specific mRNA molecules, preventing their translation into protein. Despite its great potential, moving siRNA forward into the clinic is beset by obstacles at various stages of development. Nonetheless, this study shows that the potential of RNAi to deliver its promise is real. In this study, two collaborating groups targeted a clinically relevant gene, apolipoprotein B (APOB), in a non-human primate using systemically delivered siRNA. The gene-silencing effect was both stronger and longer-lasting than had been predicted from rodent studies — an early indication of some potentially useful therapeutic features of this agent.

No evidence of serious toxicity was observed; however, longer-term and more detailed safety evaluations will clearly be required before this technology can be transferred to the clinic. Nevertheless, this study represents a crucial step forward in the application of RNAi-based therapeutics, demonstrating the potential for siRNA to be delivered systemically and therefore opening up the range of diseases that could be tackled using this technology. **Katherine Whalley, Locum Associate Editor, Nature Reviews Drug Discovery**

**ORIGINAL RESEARCH PAPER**

**RESEARCH HIGHLIGHTS**

**RNA INTERFERENCE**

**Breakthrough for systemic RNAi**

Following previous work, in which APOB was successfully silenced in rodents, the researchers set out to translate this strategy to a more clinically relevant model, the cynomolgus monkey. Importantly, to achieve selective and efficient delivery to the liver, where APOB RNA is synthesized, siRNA was encapsulated into small stable nucleic acid–lipid particles (SNALP), which remain stable in the bloodstream and are readily taken up by liver cells. Beginning as early as 24 hours after a single intravenous dose of siRNA, a drop in APOB protein, LDL and cholesterol levels lasting at least 11 days was observed. This was associated with a 90% reduction in APOB mRNA levels in the liver that was shown to be mediated by RNAi. Interestingly, the gene-silencing effect was both stronger and longer-lasting than had been predicted from rodent studies — an early indication of some potentially useful therapeutic features of this agent.

No evidence of serious toxicity was observed; however, longer-term and more detailed safety evaluations will clearly be required before this technology can be transferred to the clinic. Nevertheless, this study represents a crucial step forward in the application of RNAi-based therapeutics, demonstrating the potential for siRNA to be delivered systemically and therefore opening up the range of diseases that could be tackled using this technology. **Katherine Whalley, Locum Associate Editor, Nature Reviews Drug Discovery**

**TECHNOLOGY**

Cellulose synthase-like CsIF genes mediate the synthesis of cell wall (1,3;1,4)-β-D-glucans.


The tough cell walls of many cereals consist largely of the polymer β-glucan, which is a barrier to beer producers but contributes greatly to the soluble fibre content of these crops and is associated with many health benefits. This paper identifies the CsIF gene family as being responsible for the biosynthesis of β-glucan; a locus was identified as a major-effect QTL in barley, and then six genes were cloned from a syntenic region in the rice genome. The function of the genes was confirmed by the ability of three of these rice genes to produce β-glucan when expressed in Arabidopsis thaliana, which lacks the β-glucan polymer. This knowledge could be applied to improve the otherwise poor fibre content of wheat — either through traditional breeding or transgenic means — or to create improved material for biofuels.

**POPULATION GENETICS**

The distribution of fitness effects of new deleterious amino acid mutations in humans.


Describing the fitness effects of new mutations — the proportion of new mutations in our genomes that are deleterious, and to what degree — is central to many areas of biology, including understanding the genetic basis of disease and the maintenance of genetic variation. The authors have overcome the limitations of previous approaches to estimating fitness effects by analysing extensively sampled human SNP data alone. They show that the fitness effects of amino-acid changing mutations follows a gamma distribution. In addition, on average, a non-synonymous mutation reduces fitness by only ~4.3%. Any relaxation on selection that has occurred through improved medical care is therefore unlikely to cause a decline in genetic quality, although variants that underlie complex diseases might be harder to find than expected.

**RNA WORLD**

A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen.


This paper describes a new resource for high-throughput RNAi screening in mammalian cell lines. The RNAi Consortium has produced and tested a lentiviral library of short hairpin RNAs (shRNAs) directed against 22,000 human and mouse genes, with an average coverage of 5 different shRNAs per gene. The lentiviral basis of the reagents allows delivery to non-dividing cells, including primary cell lines, which overcomes an important limitation of other delivery methods. The authors have also developed a protocol that allows a high-titre stock to be generated efficiently for each shRNA. The library is available from Sigma-Aldrich (http://www.sigmaaldrich.com) and Open Biosystems (http://www.openbiosystems.com), and details can be found at the RNAi Consortium web site (http://www.broad.mit.edu/genome_bio/trc).
RESEARCH HIGHLIGHTS

IN BRIEF

LIFESPAN

Lipoprotein genotype and conserved pathway for exceptional longevity in humans.


This study took advantage of the limited genetic diversity among Ashkenazi Jews to identify a variant that might influence human lifespan. The authors genotyped offspring of 213 centenarians and an age-matched control group for variants in genes that have potential roles in cardiovascular disease. An allele of the apolipoprotein C3 (APOC3) gene, which encodes a protein that increases levels of harmful cholesterol, showed an association with parental longevity. Decreased APOC3 protein levels among carriers confirmed the variant as having a functional effect.

CHROMOSOME BIOLOGY

Telomere length as a quantitative trait: genome-wide survey and genetic mapping of telomere length-control genes in yeast.


Natural variation in a subtelomeric region of Arabidopsis: implications for the genomic dynamics of a chromosome end.


Telomeric repeat number varies between and within species but excessively shortened telomeres have been linked with ageing. Gatbonton et al. carried out a deletion screen in Saccharomyces cerevisiae to identify genes that regulate telomere length. A large number of genes that encode various functions were identified. A subsequent genome-wide linkage analysis indicated that two loci accounted for 30–35% of telomere length variation between the strains under study. Combined with the observation that telomere length varies substantially among wild yeast strains, the authors concluded that polymorphisms at a large number of loci are likely to affect telomere length. Kuo et al. also studied natural variation but at the subtelomeric regions in Arabidopsis thaliana. Although expansion and deletion of blocks of repeats characterized proximal telomeres, DNA rearrangements such as inversions, deletions and transposon insertions characterized the distal subtelomeric regions. Therefore, diverse events ensure genomic variation at chromosome ends.

RECOMBINATION

Polymorphism in the activity of human crossover hotspots independent of local DNA sequence variation.


Variation of crossover activity in sperm can provide clues to how recombination hotspot activity is regulated. These authors analysed two nearby recombination hotspots on chromosome 1, one of which is considered to have only recently evolved. As well as providing the first direct evidence for rapid evolution of recombination hotspots in humans the authors show that hotspot activity might be regulated epigenetically or by distal regulators because men with active and suppressed hotspots have the same haplotypes around these sites.

TECHNOLOGY

Complexity on the nanoscale

Maps, smiley faces, triangles — these are not the sort of objects one normally associates with DNA. But nanotechnology has found many uses for DNA that are beyond this molecule’s natural calling. In the most elaborate and remarkable example of these endeavours so far, Paul Rothemund reveals a way to fold a single strand of DNA into just about any two-dimensional shape imaginable.

The method is appropriately called ‘DNA origami’, which emphasizes the main innovation of this technique — the self-assembly of a single DNA viral strand into a predetermined shape. Attempts to create nanostructures rely either on manipulating individual atoms — a laborious and expensive approach — or on directing DNA strands to their position in the final structure by exploiting the complementarity of the component strands. Sequence complementarity also lies at the heart of the new method, but some innovations allow the structures to be more complex, easier to design and faster to create.

In DNA origami, the geometrical shape and the folding path that the ssDNA must take are first designed with the help of a computer. A 7 kb ssDNA molecule is then added to a surface, which is guided to the correct position by strategically placed ‘staples’ — that is, short strands of 200 kb that hold the long DNA strand in place. Once the ssDNA and the staples have been mixed together they assemble into the desired shape in a single step, all in under 2 hours.

By using this approach Rothemund has obtained DNA shapes (such as hexagons and snowflakes) that are 100 nm in diameter, achieving a complexity that is ten times as high as for any other assembled molecular pattern. There is clearly not going to be a lucrative market for tiny art of this sort, but the author’s aim is to use these nanoscale structures for new applications in electronics and

GENE REGULATION

A closer look at conservation

In the hunt for regulatory elements that control gene expression, much emphasis has been placed on the conservation of non-coding regions at the sequence level over large evolutionary distances. A recent study demands a rethink of this approach, showing that such similarity is not enough to identify some key regulators, which might only be detectable using functional assays.

The expression pattern of the RET receptor tyrosine kinase is highly conserved in zebrafish and humans. By contrast, only part of the coding sequence of the gene shows similarity between the two species at the sequence level. So, sequence comparisons between zebrafish and human provide no clue to how patterns of RET expression have been maintained during evolution.

To identify non-coding regions that regulate RET expression, Shannon Fisher and colleagues looked for sequences in the region around the gene that are conserved at shorter evolutionary distances. They identified 38 such sequences that are conserved either between zebrafish and pufferfish, or between human and other non-primate mammals.

Using a transposon-based reporter vector, the authors looked at the patterns of expression that were driven by these sequences in zebrafish embryos. Surprisingly, almost all of the non-coding sequences — whether they were derived from zebrafish or human — directed expression in cell types
molecular biology — for example, to build smaller, faster computers, and to create larger molecular assemblies that can house a production line of enzymes.

Tanita Casci

It’s all in the balance

How does the 1.5-fold increase in gene dosage on chromosome 21 lead to the many phenotypes of Down syndrome? New work shows that this pleiotropy can be traced back to two genes — DSCR1 and DYRK1A — that together inhibit a family of transcription factors that have a role in many aspects of vertebrate development.

Mice in which components of NFAT signalling have been genetically inactivated have phenotypic features that resemble human Down syndrome. Prompted by these observations, Grabtree, Graef and colleagues examined the critical Down syndrome region on chromosome 21 for genes that might downregulate NFAT. One such gene is DSCR1, which is overexpressed in Down syndrome human fetuses. DSCR1 is an inhibitor of the phosphatase calcineurin, which controls the rate of nuclear import of the calcium-dependent components of NFAT transcription complexes (NFATc proteins).

Having examined the 25–30 other candidates in this region of chromosome 21, the authors came across DYRK1A. It encodes a serine/threonine kinase that primes substrates for phosphorylation by GSK3, which in the case of NFATc proteins leads to their inactivation and removal from the nucleus. Once they confirmed the DYRK1A-mediated reduction of NFAT transcriptional activity, the authors made mouse transgenic lines in which both DSCR1 and DYRK1A were overexpressed. They report that 1.5- to 3-fold increased levels of expression were sufficient to cause some of the Down syndrome developmental defects.

The effects of a modest — 1.5 fold — increase in the expression levels of DSCR1 and DYRK1A are likely to be exaggerated by the fact that members of the NFAT family autoregulate. So to better predict the outcome of NFAT signalling the authors developed a mathematical model. One of the predictions states that NFAT targets might completely fail to be expressed because activation of their promoters might require a certain NFAT threshold level. Another prediction is that the NFAT regulatory circuit might not be equally stable in different tissues, resulting in stronger phenotypic effects in some tissues than others.

This work elegantly shows that many of the developmental phenotypes of Down syndrome might be a consequence of perturbing the NFAT regulatory circuit, in response to increased dosage of DSCR1 and DYRK1A. It also has wider implications — if other diseases are also caused by specific weaknesses in genetic regulatory circuits then understanding and exploiting these weaknesses can have therapeutic potential.

Magdalena Skipper

Louisa Flintoft

...nanotechnology has found many uses for DNA that are beyond this molecule’s natural calling.
Hackable plant genes

**GENE REGULATION**

**Plastic switches**

If we omit the details, then gene expression can be defined as resulting from the way in which a gene integrates the signals that operate on its regulatory control elements. But how do we describe the effects of these regulators (the ‘input function’), and how do they evolve? A combined theoretical and experimental study now shows that mutations in a bacterial promoter do not simply abolish an input function but rather alter its nature, so that the promoter integrates signals in a qualitatively different way.

The lac operon of *Escherichia coli* is one of the most studied and best characterized of gene expression systems: this operon is controlled by cis elements that integrate signals from two molecules, the cAMP receptor protein and the Lac repressor. What is curious about this regulatory system is that the logic of the input function is intermediate between an AND and an OR gate — in practice, this means that, for example, there are two threshold levels for each input rather than just one.

What would happen if mutations disrupted this elaborate system? Avraham Mayo and colleagues generated a library of 62 point mutant combinations in the upstream control region of the lac operon: the input function of the mutant promoters was then assessed by placing each one upstream of *GFP*, and measuring expression levels in living cells. The expectation is that such mutations would ruin the input function altogether; instead, most of the 12 unique variants that were represented caused the system to respond in a different way to the input stimuli — that is, they behaved as pure AND or OR gates or as switches that responded to only one of the two inputs.

This tells us that the lac promoter is plastic to mutations, and indicates ways in which a gene control region can evolve — not just by destroying its regulation but by altering it in potentially useful ways. A mathematical model was then constructed to account for the effect of such mutations; the model maps the phenotypes that certain

**RNA WORLD**

**Designer plant miRNAs meet their targets**

miRNAs are produced have shown that ‘designer’ microRNAs silencing. Now, two research groups have shown that endogenous plant miRNAs are much more specific than miRNAs or siRNAs, are able to move between the RNAi machineries in plants, and are limited in their ability to silence target mRNAs — effects that are not cell-autonomous, and there plant tissues (so their effects are not normally amenable to genetic manipulation). However, although it is a tremendously useful genetic tool, it is not perfect. The agents of RNAi, small interfering (siRNAs), are able to move between plant tissues (so their effects are not cell-autonomous), and there have been suggestions that siRNAs can affect non-complementary ‘off-target’ miRNAs — effects that are hard to predict. Furthermore, siRNAs are limited in their ability to silence weakly expressed targets and in many cases lead to only a low frequency of silencing. Now, two research groups have shown that ‘designer’ microRNAs (miRNAs) that are produced in vivo overcome some of these problems, providing another powerful toolkit for gene manipulation in plants.

Detlef Weigel’s group previously showed that endogenous plant miRNAs are much more specific than miRNAs or synthetic siRNAs in animals. To confirm that this is due to genuine differences between the RNAi machineries in plants and animals, rather than selection against miRNAs that have broader specificities in plants, they designed a range of miRNAs to target various endogenous genes in *Arabidopsis thaliana*. In cases for which the effects of mutating the endogenous target genes were already known, the phenotypes that resulted from the overexpression of the corresponding designer miRNAs were remarkably similar to the outcomes of mutation. Weigel and colleagues also used microarrays to show that, like endogenous miRNAs, designer miRNAs (known alternatively as artificial or synthetic miRNAs) are highly specific — they knock down their predicted targets, but little else. This confirms the intrinsic specificity of miRNA-mediated silencing in plants and highlights designer miRNAs as potentially useful tools.

In independent work, Yuval Eshed and colleagues found that designer miRNAs work not only in *A. thaliana*, but also in other species including tomato and tobacco — a finding that is perhaps unsurprising given the considerable conservation of some miRNAs. This highlights the potential for this new tool to be applied to plant species that are not normally amenable to genetic manipulation.

Functional redundancy is common in plant genomes, so knocking down a whole gene family simultaneously could be very useful. Sure enough, both groups found that designer miRNAs can simultaneously target whole gene families, producing the same effects as combinations of multiple mutants. Designer miRNAs can also function quantitatively — Eshed and colleagues show that shifting the degree of complementarity of the miRNA to its target genes strengthens or weakens the resulting phenotypes when the miRNA is expressed. Finally, miRNAs that are expressed under the control of a tissue-specific promoter have largely cell-autonomous effects, which is a potentially large advantage over siRNAs.

Both groups discuss this and several other possible merits of designer miRNAs as genetic tools. For example, the remarkable specificity of these...
molecules raises the possibility of making the equivalent of knockouts that would be specific for particular alleles or splice variants. So, along with more established methods using siRNAs, designer miRNAs promise to make important contributions to our ability to manipulate plant genes.

Jenny Bangham

**GENE REGULATION**

Compensation culture

Dosage compensation ensures that there are equal levels of X-linked gene products in males and females. In *Drosophila melanogaster*, this is achieved by doubling the transcript levels of X-linked genes in males. The process requires the association on the X chromosomes of the male-specific lethal (MSL) complex, or dosage-compensation complex. Now, three independent reports present the results of chromosome-wide analyses of several MSL proteins. Using chromatin immunoprecipitation combined with high-throughput DNA microarray detection, the chromosome-wide binding of the *D. melanogaster* MSL complex to the single male X chromosome is detailed with unprecedented resolution, providing new insights into the dosage-compensation process.

Peter Becker’s group investigated how the MSL complex identifies and binds the X chromosome in fly embryos using high-resolution genomic tiling arrays. They found that just over half the annotated genes on the X chromosome were bound by the complex. Preferred targets were essential genes, and the greatest compensation was seen for genes that bound high levels of the MSL complex. Notably, genes, rather than intergenic regions, were favoured by the complex, which bound most strongly to the 3′ end of the transcription units. The authors proposed a model in which individual genes that are under selective pressure to equalize dosage between the sexes have evolved targeting signals to attract the MSL complex.

Peter Park, Mitzi Kuroda and colleagues also determined the X-chromosome-specific binding pattern of the MSL complex in two male cell lines and in embryos, again using high-resolution genomic tiling arrays. Similarly to Becker’s group, the authors found that the MSL complex recognizes and preferentially binds to genes rather than intergenic regions; the binding is also biased towards the 3′ end of the transcription units. The same gene sequences can be clearly bound or unbound by the MSL complex, depending on cell type, indicating that MSL targeting cannot be explained by sequence alone. The authors proposed that the MSL complex selectively targets most X-linked genes, but only within the context of active transcription.

Asifa Akhtar and colleagues mapped the distribution of MSL1, a component of the MSL complex, in *D. melanogaster* embryos and larvae using an expression array that represented more than 10,000 annotated fly genes. They found that most MSL1-bound genes were expressed early in embryogenesis. In larvae, the recruitment of MSL1 did not rely solely on transcription on the X chromosome. Moreover, many genes on the X chromosome seemed to be dosage compensated without direct MSL1 binding. The authors therefore suggested that the MSL complex might also regulate transcription of X-linked genes at a distance in larvae.

Taken together, these papers raise new questions about dosage-compensation selectivity and highlight the need for further investigation into selectivity determinants, while helping to redefine current models of dosage compensation. Given that the vertebrate X chromosome has also been shown to be subject to activating dosage compensation, lessons that have been learned from studying fruitflies could also help to understand dosage compensation in other species.

Sharon Ahmad, Assistant Editor, Nature Reviews Molecular Cell Biology

**ORIGINAL RESEARCH PAPERS**


**FURTHER READING**


**WEB SITE**

miRnGro | http://wmd.weigelworld.org/bin/mirnGro.pl
**Unscrambling the egg–sperm divide**

An important breakthrough has been made in understanding the process that produces eggs or sperm in mammals. Contrary to previous theories that the two types of gamete are intrinsically programmed to enter meiosis, recent papers have shown that levels of an external signal — retinoic acid (RA) — controls the timing of this event, and therefore the type of gamete that is produced.

Key to the production of gametes is the time at which the germ cells are derived from the primordial germ cells, which are present during embryonic development (at E13.5), whereas in males this occurs only after birth. This timing is crucial, as the later meiosis of male germ cells allows sperm rather than eggs to be made.

David Page and colleagues showed that an RA receptor (RAR) antagonist suppresses the expression of a pre-meiotic marker (Stra8) in cultured mouse embryonic ovaries. Furthermore, although Stra8 is not normally expressed in the male gonad until after birth, addition of RA to testes in culture stimulates expression of the marker. This led the authors to propose that RA is required for germ cells to enter meiosis, and indicates that regulated RA degradation might control the timing of this event.

In support of this, Page and colleagues showed that an inhibitor of the RA-degrading enzyme CYP26B1 induces embryonic testes to express Stra8. Their findings — in line with previous studies — also indicated that the Cyp26b1 gene is expressed in embryonic testes, but not in embryonic ovaries, suggesting how the difference between the time of egg and sperm production might arise.

In a separate study, Peter Koopman and colleagues extend these findings, adding some important refinements to how the timing of meiosis induction is controlled. They carried out a screen for genes that are expressed sex-specifically during gonad development. This picked up Cyp26b1, which they found to be expressed initially in the developing gonads of both sexes — in contrast to the findings of Page and colleagues — but which became male-specific by E12.5.

The authors then tested for the presence of RA in the developing urogenital system. In both sexes, RA expression was detected in the mesonephros (a structure that is physically attached to the gonad). Furthermore, RA activity was found to be much higher in the developing ovary than in the developing testes. Similar to the study by Page and colleagues, but using a wider range of meiotic markers, this study also showed that RA can induce germ-cell meiosis in cultured organs, and that this induction is inhibited by an RAR antagonist.

Koopman and colleagues also provide strong genetic evidence that CYP26B1 is responsible for controlling the timing of germ-cell meiosis in both sexes. Male Cyp26b1-null mice showed increased expression of meiotic markers in embryonic gonads, and germ-cell meiosis also took place earlier in female mice with the same genetic defect.

As well as revising our understanding of how sperm and eggs are produced, these results have potential practical applications. The timing mechanism provides a target for treatments to increase or suppress fertility, and understanding how it works could prove crucial in attempts to produce gametes from cultured germ stem cells.

Louisa Flintoft

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**FURTHER READING**


**ORIGINAL RESEARCH PAPERS**
