What dREAMs are made of

The retinoblastoma tumour-suppressor protein (pRb) and the E2F transcription-factor family function in cell growth and differentiation and do so by coordinating gene transcription. pRb can repress gene transcription by recruiting repressor complexes to E2F-bound gene promoters. Alexander Brehm, Nick Dyson and colleagues have now isolated from *Drosophila melanogaster* embryo extracts two such repressor complexes — which they named dREAM (*Drosophila* RBF, E2F and Myb-interacting proteins). Their results are reported in *Cell*.

D. melanogaster contains two pRb-family proteins, RBF1 and RBF2, and two E2F proteins, dE2F1 and dE2F2, which each form heterodimers with the transcription factor dDP. Chromatographic separation of *D. melanogaster* embryo nuclear extracts revealed three fractions that contained RBF1, of which one contained two separate complexes. Further purification revealed that these two complexes contained components of a dMyb complex that controls chorion gene amplification in follicle cells, as well as dE2F2, dDP and either RBF1 or RBF2.

Staining of polytene chromosomes with antibodies against various dREAM subunits showed extensive colocalization between these subunits. However, using an antibody against phosphorylated RNA polymerase II showed there was no overlap between the two staining patterns, which indicates that dREAM complexes associate with transcriptionally silent regions.

So, how does dREAM repress transcription? As dREAM complexes seem to lack chromatin-modifying enzymes, Brehm, Dyson and colleagues wanted to know whether dREAM complexes repress transcription by binding to histones directly. Indeed, several subunits bound the non-acetylated histone-H4 tail, but they failed to bind when the histone-H4 tail was acetylated. Given that transcriptionally silent chromatin is typically deacetylated, the authors propose that by binding to non-acetylated nucleosomes, dREAM complexes can protect them from modification, thereby maintaining the repressive state.

To identify the genes that are regulated by dREAM, the authors depleted dREAM subunits by treating *D. melanogaster* S2 cells with RNAi. Focusing on two sets of dE2F-regulated genes — cell-cycle-regulated A-group and E-group genes that have sex- and differentiation-specific expression patterns — they found that depletion of dREAM subunits de-repressed E-group genes, but not A-group genes.

The stresses of weight gain

Type-2 diabetes — for which weight gain is a causative factor — has become one of the most serious threats to human health. Certain cellular conditions trigger endoplasmic reticulum (ER) stress (during which unfolded or misfolded proteins accumulate in the ER). Hotamisligil and colleagues therefore proposed that obesity, by giving rise to such conditions, might stimulate ER stress in peripheral tissues, which, in turn, might be a key mechanism in triggering insulin resistance and type-2 diabetes. They report the results of their work in *Science*.

First, they studied the expression of several markers of ER stress in dietary and genetic mouse models of obesity. They found that obesity is associated with the induction of ER stress, mainly in the liver and adipose tissues. For example, the activity of Jun N-terminal kinase (JNK) — which is known to be increased by ER stress — is significantly increased in obese mice.

Next, the authors treated liver cells with agents that induce ER stress to assess whether this stress interferes with insulin function. They found that ER stress resulted in decreased insulin-stimulated Tyr phosphorylation, and increased insulin-stimulated Ser phosphorylation, of insulin-receptor substrate-1 (IRS1; Tyr phosphorylation of IRS1 propagates the insulin signal, whereas Ser phosphorylation blocks it). In addition, they found that inhibiting JNK reversed the ER-stress-induced Ser phosphorylation of IRS1. So, ER stress promotes the JNK-dependent Ser phosphorylation of IRS1, which, in turn, blocks the insulin signal.

The transcription factor X-box-binding protein-1 (XBP1) is an important regulator of ER stress, as it controls the expression of, for example, molecular chaperones. Hotamisligil and co-workers showed that the presence of XBP1 results in a suppressed ER-stress response, and vice versa, in mouse embryonic fibroblasts (MEFs). In addition, they showed that manipulating XBP1 levels alters insulin signalling — for example, overexpressing XBP1 in MEFs increased Tyr, and decreased Ser, phosphorylation of IRS1.

In the final part of this study, the authors studied *Xbp1*$^{+/-}$ mice (*Xbp1*$^{-/-}$ mice are embryonic lethal), and showed that *Xbp1*$^{+/-}$ mice that were fed a high fat diet developed continuous and progressive hyperinsulineamia, as well as high levels of blood glucose. A loss of XBP1 therefore predisposes mice to diet-induced, peripheral insulin resistance and type-2 diabetes. In addition, they showed that there was increased ER stress and disrupted insulin signalling in *Xbp1*$^{+/-}$ mice — for example, they observed increased JNK activity and increased Ser phosphorylation of IRS1 in these mice.

Hotamisligil and colleagues have therefore shown "…that ER stress is a central feature of peripheral insulin resistance and type 2 diabetes at the molecular, cellular and organismal levels", although exactly how ER stress targets the insulin signalling pathway needs to be clarified. However, this work indicates that the use of therapies that regulate ER stress could be a new way to prevent and treat type-2 diabetes.

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🌐 References and links

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WEB SITE
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Intriguingly, seven out of the eight components of dREAM were found to be related to the *Caenorhabditis elegans* synMuv class-B gene products, which are important for the development of the worm's male and female reproductive systems. Brehm, Dyson and colleagues propose that synMuv class-B proteins form a complex that, like dREAM, represses sex-related gene targets and so controls the worm's sexual development. So, the evolutionary conservation of pRb-specific repressor complexes is extensive and the authors predict that 'dREAM-like' complexes might also exist in mammals.

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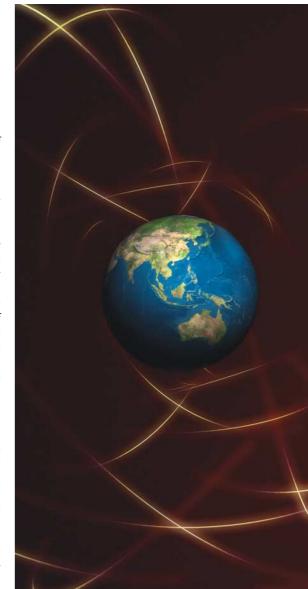
Big secrets of a small world

Micro (mi)RNAs are ~22-nt RNA molecules that inhibit the translation or induce the degradation of protein-coding mRNAs in plants and animals. Over the past decade, it has become clear that these small RNAs have important regulatory roles in fundamental cellular processes but, so far, it has been unclear how the expression of miRNA genes is controlled and which polymerase enzyme is responsible for transcribing these genes. Now, reporting in *The EMBO Journal*, V. Narry Kim and colleagues provide evidence that the miRNA genes are transcribed by RNA polymerase II (Pol II).

To find out more about miRNA transcription, the authors asked whether the primary transcripts of the miRNAs (pri-miRNAs) had 5′ methylguanosine caps and poly(A) tails — modifications that are trademarks of Pol-II transcription. First, they affinity purified cap-containing RNA from HeLa cells using glutathione *S*-transferase (GST)-immobilized cap-binding protein eIF4E and analysed the bound RNA by PCR after reverse transcription of RNA (RT-PCR). Of each tested pri-miRNA, 5–50% was bound by the GST–eIF4E column, indicating the presence of a cap modification. Similarly, oligo-dT cellulose beads were used to bind to RNA from HeLa cells that contained poly(A) tails, and the bound RNA was extracted and analysed by RT-PCR. Once again, all of the pri-miRNAs were represented in the polyadenylated-RNA fraction. So, primary transcripts of miRNAs are 'topped' and 'tailed' like their pre-mRNA counterparts, which indicates that they are transcribed by Pol II.

Another characteristic of Pol-II transcription is its inhibition by low doses of α-amantin and, when HeLa cells were treated with this peptide, the level of all the pri-miRNAs was reduced.

V. Narry Kim and co-workers then used a range of molecular techniques to define the promoter, the transcription-initiation site and the polyadenylation signal of an miRNA gene, *miR-23a~27a~24-2* (*miR-23a*). Furthermore, a reporter construct comprising the putative *miR-23a* promoter fused to a luciferase gene was transfected into a human cell line. This fusion construct was transcriptionally active and, importantly, luciferase activity was inhibited by α-amantin, which indicated that transcription of the *miR-23a* promoter was Pol-II dependent. Finally, chromatin immunoprecipitation, using antibodies that were directed against Pol II, confirmed that the enzyme did indeed bind to the promoter of an endogenous *miR-23a* gene.

This study presents evidence that miRNAs are transcribed by Pol II. But the affinity-purification experiments revealed that a large proportion of a given pri-miRNA does not contain a 5′ cap or a poly(A) tail. Also, a detailed analysis of the promoter of the *miRNA-23a* gene did not detect the DNA elements that are common to most Pol-II promoters — such as the TATA box or the TFIIB recognition elements. So, further analysis of other miRNA genes will be necessary to understand miRNA promoters and their mechanisms of transcription. It seems as if the world of small RNAs still has many secrets to reveal.

Shannon Amoils

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