Jewels among the junk

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Regions of the genome that have long been thought of as junk are now being resurrected as potential links to behavioural disease.

For over 50 years, DNA and proteins were at the centre of attention of molecular biologists. However, in 2002, tiny RNA molecules that have nothing to do with the protein synthesis machinery stole the show [1]. A series of discoveries showed that these small non-messenger (snm; non-coding) RNAs have a crucial role in gene regulation: They are the main players in poorly understood genetic phenomena such as RNA interference, transgene silencing, X-chromosome dosage compensation and imprinting [1,2].

Hunting for novel RNA genes

The total number of these snmRNAs remains a mystery, partly because they cannot be detected with conventional methods used to search for novel genes. However, the field of RNomics (the identification of novel snmRNAs and their genes; a term coined by Alexander Hüttenhofer at the University of Münster, Germany; http://www.uni-muenster.de) is gaining momentum. While some scientists are busy writing computer programmes that can detect snmRNAs [3], Hüttenhofer works with cDNA libraries of expressed sequences. 'We are the dustmen of the human genome project,' he jokes. When constructing cDNA libraries to find novel genes, scientists usually throw the non-polyadenylated RNAs in their RNA preparation away to get rid of anything that is not mRNA and thus coding for protein. Hüttenhofer shifted the protocol: he studies what others used to regard as junk and has already found hundreds of novel snmRNAs. More and more research groups are now adopting his approach.

One of Hüttenhofer's current projects is to mine snmRNAs that are specifically expressed in the mouse brain. Among his trophies are several snmRNAs that are located in the genome region associated with Prader-Willi-Syndrome, a chromosomal disorder that results in an obsession with food and other difficult behaviours [4]. Previously, scientists had failed to come up with a protein-coding candidate gene.

Overlooked culprits?

Could this be just the first example where a non-coding gene is the culprit for an inherited behavioural disorder? Alison McInnes, a psychiatrist at Mount Sinai Medical School (http://www.mssm.edu), believes so. Speaking at the annual meeting of the American College of Neuropsychopharmacology, held in December 2002 in San Juan (Puerto Rico), she suggested that scientists could have looked in the wrong places in the genome when searching for a genetic link to psychiatric disease. 'It appears that smnRNA may be especially relevant for understanding behavioural differences,' she says, 'because they appear to be particularly enriched in the brain. They represent a swift and energy efficient means of regulating gene expression and may be especially important for rapid regulatory events.'

McInnes launched an ambitious programme, using computational and experimental methods, to hunt for snmRNA genes that are lurking in regions of the genome that have been linked to behavioural disorders. Eventually, she hopes, this will lead to novel RNA-based therapies that target genetic disorders.

Her initial screen has already revealed an snmRNA in the intron of the human corticotrophin-releasing hormone (CRH) gene, she reported at the meeting in San Juan [5]. Altered levels of CRH could contribute to depression, anxiety disorders and anorexia nervosa.

But it could take many years to nail down details of the link between snmRNAs and disease. 'We have so few tools for working on RNAs,' says Sean Eddy at Washington University School of Medicine in Saint Louis (http://medicine.wustl.edu). 'You cannot frameshift a non-coding RNA,

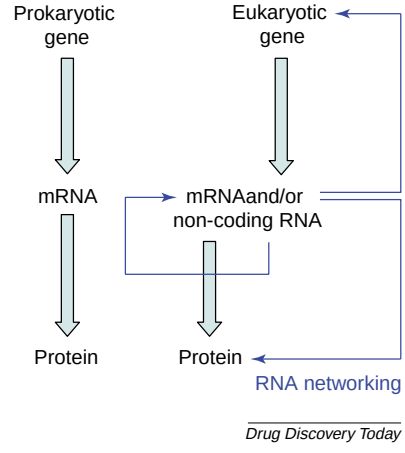

Figure 1. According to the central dogma of biology, DNA encodes proteins via mRNA intermediates; proteins perform the majority of structural, catalytic and regulatory tasks in the cell. This might hold true for prokaryotes. However, complex organisms might have evolved another level of regulation: their genomes contain information for proteins – the functional components of a cell – and for non-coding RNAs – the control architecture that regulates a cell's overall activity. Adapted from a schematic in Ref. [2].

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you cannot put a stop-codon in – a lot of the standard ways to try to verify that you are dealing with the right locus are just not available, so you have to invent new techniques.'

Hüttenhofer adds that it will be tricky to find a phenotype: 'Mutations in snmRNAs usually do not have the same dramatic effect as in proteins, since the nucleotide sequence is not translated into an amino acid sequence.' And, he continues, with behavioural diseases, it is a problem to find valid mouse models in the first place.

Damage to the control architecture

Despite all these hurdles, John Mattick, a geneticist at the University of Queensland (Brisbane, Australia; http://www.imb.uq.edu.au), believes that McInnes is heading in the right direction. Mattick has argued for years that RNAs have a crucial role in regulating gene expression. In fact, he thinks they might be more important than most people realize even today: 'While the genomes of the higher organisms have increased their protein-coding capacity substantially, the major increase has been a massive expansion of non-coding RNA sequences. I think that the shift from simple organisms to complex organisms required a radical change in the genetic operating system, so that gene expression could be co-ordinated and networked far more densely.'

Mattick claims that the regions in our genome that had been thought to be largely junk are in fact a sophisticated control architecture for eukaryotic systems (see Fig. 1, [2]). He is therefore convinced that snmRNAs have a crucial role in the development of disease. 'I think we have got past the phase now of identifying mutations that affect proteins and give you component damage (even though we have not identified all of them, by any means). Now, we are in the much more difficult phase of trying to identify mutations in this much larger amount of control architecture, which affect growth, development and physiology in much more subtle ways. But my bias is to think that the majority of the genetic components of common diseases lie in this architecture, rather than in changes or damage to the components.'

References

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Encapsulated cell technology could prevent blindness

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Inevitable blindness caused by retinitis pigmentosa could be a thing of the past. A team of researchers led by Weng Tao of Neurotech USA (http://www.neurotech.fr) have demonstrated a therapeutic technique in dogs – encapsulated cell technology – which holds promise for preventing the retinal degeneration of human retinitis pigmentosa [1].

Retinitis pigmentosa is a group of hereditary retinal diseases characterized by degeneration of the photoreceptor cells. Beginning in childhood, night blindness occurs first, then a loss of the peripheral visual fields and finally loss of the central visual fields. Over the course of several decades, the usual endpoint is total blindness. Seven of the 20+ mutations that cause the disease have been identified and characterized [2] with most of the described mutations affecting the phototransduction mechanism in the photoreceptor cells.

Preserving function

Several growth factors, neurotropic factors and cytokines have been shown to preserve photoreceptor cell function in animals [3]. Unfortunately, in most of these early studies the therapeutic agent was introduced into the eyeball by repeated injections. This is not, of course, a desirable technique for treating a long-term human condition.

Based on earlier work by her group at Neurotech USA, and by others, Tao engineered cells to produce human ciliary neurotrophic factor (CNTF), a factor that is protective of photoreceptor cells. Tao's group encapsulated the cells in a proprietary

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