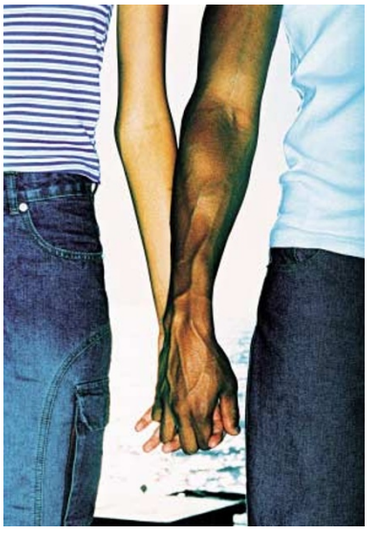
EVOLUTION

A tale of courtship



Far from being a prurient pastime, scrutinizing the interaction between the sexes is the only means of studying the most fundamental problem there is — the propagation of life. Two recent papers on *Drosophila* now highlight two of the topics tackled by this rich area of research: in one, Dauwalder *et al.* describe a gene that is expressed specifically in males and is required for courting behaviour; in the other, Miller and Pitnick reveal, for the first time, how post-copulatory interactions between the sexes drives the evolution of a reproductive trait, in this case longer sperm tails.

It is the male fly that takes the initiative to mate through an elaborate courtship ritual. Like other somatic sexual features, sex-specific behaviours, such as courtship, are controlled by the combined action of the sex-specific forms of Doublesex (Dsx) and Fruitless (Fru) proteins. But how? Dauwalder and colleagues provide part of the answer to this question in their study of the *takeout* (*to*) gene, which they found — in an RNA subtractive hybridization screen — is specifically expressed in male heads. Subsequent mutant studies showed that *to* is required for male courting behaviour: *to* mutant male flies could distinguish between males and females, but courted less often. The expression of *to*, which encodes one in a family of 20 secreted proteins, depends on the male-specific forms of Dsx and Fru; this study therefore identifies the first target of Dsx and Fru that is involved in sex-specific behaviour, as well as providing the curious puzzle of how fat cells (the cells in the head in which *to* is expressed) might control courtship.

One of the driving forces behind the evolution of many male traits is female-driven sexual selection, the peacock's tail being the most famous example. Miller and Pitnick now show experimentally how female choice drives the evolution of one such sexually selected trait: longer sperm tails. Sperm tail length is highly heritable, so populations of flies could be bred that had either giant or very short sperm tails. It was clear from mating these males to females that were bred to have either very long or very short sperm storage organs (seminal receptacles, SRs) that longer sperm were more successful at fertilizing females — especially those with long SRs — when competing with shorter sperm. As well as showing that

GENE EXPRESSION

Making long-distance contact

Enhancer elements are a somewhat mysterious feature of higher eukaryotic genomes, predominantly because how they enhance the expression of genes that lie far away from them remains unknown. Do they, for example, make direct contact with their target gene by looping out the intervening DNA or do they act indirectly by producing a transcriptionally favourable environment?

Answers to these questions now come from a recent paper by David Carter and colleagues who have developed a new technique — RNA TRAP — to investigate enhancer elements. They show, for the first time, that long-range enhancer elements very likely come into physical contact with the genes they regulate — results that shed doubt over non-contact models of enhancer function and demonstrate the usefulness of this technique for exploring transcription-regulating elements.

Step one of RNA TRAP involves localizing horseradish peroxidase (HRP) to oligos that are targeted to an RNA as it is being transcribed. In this study, oligos were directed against two genes that lie in the mouse β-globin cluster, downstream of a locus control region (LCR). This LCR contains six DNase-I hypersensitive sites (HS1–6) and is required for the high-level expression of β-globin locus genes in erythroid cells. In step two, the localized HRP catalyses the covalent deposition of biotin onto chromatin proteins in close proximity to the transcribed gene. The labelled chromatin is then purified by affinity chromatography and the DNA sequences bound to it are identified by PCR.

In their study, Carter *et al.* used mouse E14.5 fetal liver cells, which express only two of the four genes at the *Hbb* locus, *Hbb-b1* and *Hbb-b2*. In their first RNA TRAP experiment, probes were targeted to the 3′ intron of *Hbb-b1*, and the enrichment of sequences across the *Hbb* locus was measured. The sequence around the targeted region was most greatly enriched, as expected, with enrichment dropping off sharply over the silenced regions of the locus. The enrichment picked up again around the LCR, especially at HS2, and to a lesser extent at HS1 and HS3. A similar enrichment pattern was detected when a 3′ intron of *Hbb-b2* was targeted with oligos. Again, HS2 was highly enriched; as was HS4, but to a lesser extent. These findings indicate that certain regions of the LCR, especially HS2, come into close physical proximity to the active *Hbb-b1* and *Hbb-b2* genes. Moreover, these results tie in nicely with previous *Hbb*-locus deletion studies in mice that have shown that gene expression in this region is most drastically reduced by the deletion of HS2.

To rule out the possibility that these results might be caused by the preferential deposition of biotin in certain chromatin regions, Carter *et al.* also ran control experiments in which they omitted the intronic probes at step one, causing biotin to be randomly deposited across the genome. No preferential labelling of *Hbb*-locus sequences occurred as a result, lending further weight to their findings. The authors' planned improvements to this assay should shed more light on the exact nature of the interaction that occurs between enhancers and the genes they regulate.

Jane Alfred

References and links

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WEB SITE

Peter Fraser's lab:
http://www.babraham.ac.uk/research/developmental_genetics/chromatin.htm#fraser

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sperm length is adaptive, these results, and others, show that physical selection by females influences sperm morphology — presumably because longer sperm preferentially occupy a favourable position in longer SRs.

The biological implications of both studies go well beyond flies. For example, given that the *takeout* gene was previously described for promoting starvation tolerance in flies, it would be interesting to find out any existing link between the appetite for food and for sex.

Tanita Casci

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WEB SITE

Scott Pitnick's lab: http://www-hl.syr.edu/depts/biograd/FACULTY/Pitnick.html

GENE REGULATION

Small but perfectly … regulating

One way for bacteria to modulate their gene expression is by attenuation — by which structural changes in the 5′ leader of a transcript bring about transcription termination of downstream genes in the same operon. In most cases, a ribosome, an RNA binding protein or an uncharged tRNA can alter RNA structure so that the so-called terminator loop doesn't form; instead, the alternative structure (referred to as the antiterminator) allows transcription to proceed. Now, Mironov and colleagues uncover a new mechanism of attenuation. They show that, in *Bacillus*, some small molecules can regulate their own transcription by interacting directly with specific sequences on RNAs that are transcribed from the operon to which they belong.

In *Bacillus*, genes that are involved in thiamin and riboflavin synthesis are organized in operons. Although much is known about these synthetic pathways, how the genes involved are regulated is unclear. Recent studies found that the 5′ leaders of RNAs from both operons contain evolutionarily conserved sequences, the structure of which indicated that they might fold into secondary structures — the *rfn* box (for the riboflavin (*rib*) operon) and the *thi* box (for the thiamin (*thi*) operon) — that could regulate the expression of genes in the two operons. To achieve this they would need to bind a cofactor. As no protein or tRNA — the usual suspects — could be found, it was suggested that riboflavin and thiamin or their derivatives, such as FMN and TPP, respectively, could have this role. Mironov *et al.* showed that this was indeed the case.

First, the sequence of both RNAs suggested that the binding of FMN or TPP could mask the antiterminator, allowing the formation of a termination loop and preventing downstream transcription. Conversely, in the absence of the ligand, the antiterminator loop would allow transcription to proceed.

To test whether this regulation involves antitermination, Mironov and colleagues made several RNA leader–*lacZ* fusions that included specific point mutations in the leader sequence of the *rib* operon. Monitoring the levels of β-gal expression, a marker of operon expression, in the different transgenic strains showed that FMN suppressed the *rib* operon through *rfn*-box-mediated termination. The same results were obtained when they monitored transcription directly *in vitro*.

The idea that FMN promotes the formation of an anti-antitermination loop was confirmed when the authors looked at the structure of the RNA leader in the presence or absence of FMN — by looking at which of the oligonucleotides that were homologous to different parts of the leader could base pair with the leader as transcription elongation proceeded. The results were clear — FMN triggers the formation of an anti-antitermination loop even before the elongation complex has reached the terminator.

So, Mironov *et al.* found a new way in which bacterial genes can be acutely and specifically responsive to changes in their environment. RNA sequences similar to *rfn* and *thi* boxes have been found in other bacteria, so this method of gene regulation might be widespread. Because some of these sequences overlap with the translation initiation sites and the start sites, the authors speculate that similar mechanisms might also operate at the level of translation.

Magdalena Skipper

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WEB SITE

Evgeny Nudler's Lab: http://www.med.nyu.edu/research/nudlee01.html

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