bases in the template strand. By directly comparing the interpulse duration using templates of identical nucleotide sequence but containing methylated or unmethylated bases at specific locations, an interpulse duration ratio can be derived for each base. This approach can be used to detect mA, hmC and mC (in order of sensitivity and specificity) over a few hundred nucleotides with good discrimination from unmodified bases using both synthetic templates as well as genomic DNA (an mA-containing *Caenorhabditis elegans* fosmid prepared from *Escherichia coli*) over a wide range of G-C contexts.

Using circular consensus sequencing, the repeated resequencing of a circularized template, 'a SMRTbell', the accuracy of mA base calls can be increased to >85% with ~5% false positive calls after five subreads. Unlike for mA, however, the kinetic signatures for mC (and hmC)—base modifications critically important in eukaryotic genomes—are complex, and base-pair resolution accuracy is not yet possible. Additionally, improvement in experimental and/or computation methods will be required to match the precision of the gold standard, bisulfite treatment–based sequencing, for mC detection. Another limitation is that the current method[3] does not allow for *de novo* identification of methylated bases, that is, the detection of kinetic signatures without comparison of unmethylated and methylated template sequences.

But as SMRT sequencing technology is applied to more and more genomes, a database of kinetic profiles in many sequence contexts will emerge that can be used for these comparisons. Alternatively, with such rich kinetic signatures, one can envision that machine-learning approaches might be developed that allow for direct, *de novo* methylated-base calling. Either way, further pioneering of single-molecule approaches, including nanopore-based methods[13], with the capacity to discriminate native from modified bases may enable methylome profiling for hundreds of thousands of contiguous bases, or even entire chromosomes, as single long reads in which both primary sequence and methylome are simultaneously determined—with no BS.

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Do mice have a pain face?

Paul A Flecknell

Assessing changes in facial expression may enable us to assess pain in animals more accurately and more effectively.

Pain is a highly individual, subjective experience, which we communicate to each other primarily through language, and the clinical assessment of pain relies upon this ability to communicate. As this ability is very limited in infants, in comparison to adults, assessing pain in human infants is both controversial and problematic, but can be achieved by observing behavior and facial expression[1]. A report in this issue of *Nature Methods* now applies a similar measure of facial expressions to evaluate pain in animals, namely in mice[2].

Attempting to assess pain in animals is problematic[3] but is of fundamental importance for veterinarians who need to assess pain to treat it in their patients and for pain researchers who need to assess pain to develop new therapies in laboratory animal models.

Although there is evidence for face recognition and detection of facial expressions that could indicate emotional state in animals[4], there has been no systematic evaluation of facial expressions that may indicate pain. This may be due to assumptions that animals cannot display a range of facial expressions comparable to those shown by humans. Although various behavior-based approaches to animal pain assessment have been proposed, these can be difficult and time-consuming to apply[3]. In laboratory animals, simpler behavioral assessments, or even reflex responses, such as the tail-flick test and abdominal constriction test, have been widely used to quantify pain and evaluate analgesics[5]. In models of chronic pain, although changes in sensitivity to evoked pain can be measured, ongoing pain may not be assessed effectively. Although broadly successful, both the models themselves and the methods of pain assessment may have considerable limitations[6].

Langford *et al.*[2] have adopted a new approach. Their data indicate that changes in facial expression, coded using a method analogous to facial action coding systems in humans, can be used to evaluate pain in mice. Several specific changes in facial expression occurred in mice that were in pain, enabling the authors to develop a 'mouse grimace scale' for each of these expressions, and to use this to score the animals' pain. Administration of analgesics attenuated these changes in expression. Furthermore, morphine not only reduced the frequency of 'pain faces' but had no effect on facial expression in pain-free mice. This is in contrast to this analgesic's effects on other aspects of behavior in pain-free animals—for example, producing either excitement and increased activity or sedation—which can hamper assessment of its action on pain.

This novel technique[2], of using the mouse grimace scale, also offers some tantalizing possibilities. Chemical destruction of a small area of the mouse brain that, in humans, is associated with the emotional component of pain, prevented expression of the 'pain face' but did not block the abdominal contractions previously considered to

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be markers of pain in this model. One long-standing debate has been the nature of pain in animals. In people, pain has both sensory (what type of pain, where it is and how intense it is) and emotional (how it feels) components. It is the emotional component that makes pain unpleasant and distressing. In animals, it has been suggested that this emotional component is either greatly reduced or completely absent[7]. However, a recent consensus view was that animal pain does have an emotional component, but it was acknowledged that measuring this component would prove difficult[8]. It is possible that assessment of facial expressions, which may measure the emotional component, could be of value in this context. Assessing 'pain faces' in animals may also be easier for humans to learn than other behavioral measures because we have specialized neural apparatus for attending to and processing faces[9].

The methodology[2] is at an early stage of development, but Mogil and colleagues have already completed a broad range of investigations using different pain assays to substantiate their initial studies. It remains to be seen whether the technique proves more sensitive and better able to detect changes in pain state than other assays used in pain research. It may prove to be an adjunct rather than a replacement for current approaches, or it may primarily be measuring a different dimension of pain. The authors[2] point out that other behavioral states—for example, sleep—have in common some, but not all, facial expressions with pain faces, and other measures may be needed to differentiate between other situations that could produce some of these changes in expression.

Despite these caveats, this study could lead to a radical change in approach to assessing pain, not only in animal models in pain research but more generally in animal husbandry. Changes in legislation in Europe will require more detailed evaluation of the welfare of laboratory animals, and assessment and alleviation of pain will be of central importance. Similarly, the last decade has seen an upsurge in interest in the welfare of other animals, notably farm and companion animals. If mice and human infants have a pain face, do rats, rabbits, dogs, cattle and the other species that are used by society? If they do, can we use this not only as a research tool to develop better pain therapies for ourselves but also as a means of improving the welfare of the animals we use?

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Knock it down, switch it on

Jean-Louis Bessereau

The arsenal of methods to investigate gene function in *Caenorhabditis elegans* continues to grow—with new approaches to generate targeted deletion mutants and to control gene expression.

The genetic toolbox for a model organism (**Figure 1**) should contain efficient methods for targeted gene inactivation and conditional gene expression. Surprisingly, these two major tools remain relatively unsatisfactory for the nematode *Caenorhabditis elegans*. In recent papers in *Nature Methods*[1,2], researchers in the laboratories of Erik Jorgensen and Martin Chalfie independently report new strategies that should expand the ability to control gene expression in the worm and could even be a source of inspiration for developing techniques in other systems.

A standard and powerful strategy to get at the function of a gene is to inactivate its expression and analyze the resulting phenotype, a so-called 'reverse genetics' approach. The sequencing of the *C. elegans* genome and the discovery of RNA interference (RNAi) opened up the exciting perspective of being able to inactivate every worm gene[3]. RNAi is easily achieved in the worm and has thus become widely used for *C. elegans* research, but it has a few intrinsic limitations. First, RNAi efficiency is very sensitive to the experimental conditions, and output can be variable. Second, residual gene expression persists to an extent that is difficult to predict for a given gene. Third, some tissue types such as neurons are partially resistant to RNAi. Fourth, genes sharing sequence similarity with the primary target can sometimes be undesirably downregulated. Obtaining strains containing heritable null mutations in every gene therefore remains complementary to RNAi-based analysis.

In *C. elegans*, most strategies used so far to isolate gene knockouts relied on randomly mutagenizing large worm populations and then screening by PCR for the presence of a specific deletion. Thanks to the efforts of the *C. elegans* Gene Knockout Consortium in the United States and Canada and the National BioResource Project in Japan, deletion alleles have been obtained for about 5,500 out of 20,000 predicted genes[4]. These strains are extremely useful but also have a few limitations. First, deletions are usually small and are sometimes not molecularly null mutations. Second, strains have been heavily mutagenized, and mutations are ineluctably present in the background, sometimes tightly linked to the deletion allele. Third, deletions can be associated with complex chromosomal rearrangements. Fourth, some genes are difficult to target because they are small or because null alleles cause lethality and sterility, and are therefore difficult to recover from the

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