SEQUENCING

Protein nanopores to detect DNA methylation

Two groups use nanopore sequencing through a protein pore to detect methylcytosine and hydroxymethylcytosine.

The sequence of DNA is only the first layer of information encoded in the genome. Modification of nucleotides by other functional groups is an important regulator of gene expression in both normal and disease contexts. There is intense interest in methods to distinguish these modified bases from each other and from unmodified nucleotides.

Two independent groups, that of Mark Akeson and colleagues at the University of California, Santa Cruz, and that of Jens Gundlach and colleagues at the University of Washington in Seattle, describe the use of a bacterial pore, MspA, to detect and map 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) in DNA (Laszlo *et al.,* 2013; Schreiber *et al.*, 2013). In nanopore sequencing, the current across a pore,

which varies depending on the nucleotide present at the narrowest point of its constriction, is used to read out the sequence of nucleotides in a single molecule of DNA. The approach has already shown encouraging results for detection of 5mC and 5hmC.

In the present work, both groups use a phage DNA polymerase to pull single-stranded DNA through a modified MspA pore, in single-nucleotide steps. Comparing traces of current for unmethylated, methylated and hydroxymethylated versions of short DNA molecules of known sequence, they determined that 5mC yields increased current relative to unmethylated cytosine, whereas 5hmC produces lower current.

Sequence context 5′ of the methylated cytosine, both groups observe, strongly influences the differences in current between 5mC or 5hmC and unmethylated cytosine.Both groups also report that sequence context affects the error rates of

methylation detection, as one would expect. Although performance can be very good in some contexts, Akeson and colleagues conclude that the error rate is high enough that repeated sequencing (5–19 repeats depending on the context) is likely to be needed for an error rate of less than 0.01%.

Nanopore sequencing for 5mC and 5hmC detection has several advantages: it is fast, read lengths can be long, and not much material is needed. MspA could prove useful to also detect other, nonmethyl, modifications of DNA.

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RESEARCH PAPERS
Laszlo, A.H. *et al*. Detection and mapping of 5-methylcytosine and 5-hydroxymethylcytosine with nanopore MspA. *Proc. Natl. Acad. Sci. USA* **110**, 18904–18909 (2013).
Schreiber, J. *et al*. Error rates for nanopore discrimination among cytosine, methylcytosine, and hydroxymethylcytosine along individual DNA strands. *Proc. Natl. Acad. Sci. USA* **110**, 18910–18915 (2013).