Lecture 14: Gene expression --continued

B. Analyses of transcriptome
I. cDNA microarrays
II. Oligonucleotide arrays
III SAGE (serial analysis of gene expression)
VI MPSS (massively parallel signature sequence)

C. Protein expression pattern
Immunohistochemistry (p661-662)
Reporter (p798; 661-662)
Western blot
Mass spectrometry

Read 348-354
Fig. 10.6; 10.24; 10.25; 10.26; 10.28; 10.29
19.18; 19.25; 19.27
High throughput analyses of the transcriptome

Documenting gene expression on a genome wide scale

Transcriptome: complete set of transcripts and their relative expression levels in a particular cell or tissue under defined conditions
I. cDNA microarrays
II. Oligonucleotide microarrays (Affymetrix GeneChip)

Light deprotection

![Diagram of light deprotection process involving oligonucleotides and a mask.]
Sporulation gene expression profile in budding yeast


Several classes of sporulation gene expression after transfer to sporulation media
Survey of 1116 genes during sporulation in budding yeast
Fig. 10.28

MPSS: Massively Parallel Signature Sequencing

20 cycles of proprietary base-by-base sequencing

Each of a million beads provides a 20-base-pair signature sequence

<table>
<thead>
<tr>
<th>Signature sequence</th>
<th>Number of beads</th>
</tr>
</thead>
<tbody>
<tr>
<td>GATCAATCGGACTTGTCGAA</td>
<td>2</td>
</tr>
<tr>
<td>GATCGTGCATCAGCAGTACT</td>
<td>53</td>
</tr>
<tr>
<td>GATCCGATACAGCTTTGGGC</td>
<td>212</td>
</tr>
<tr>
<td>GATCTATGGGTATAGTCGAG</td>
<td>349</td>
</tr>
<tr>
<td>GATCCAGCGTTTGGTGCTTG</td>
<td>417</td>
</tr>
<tr>
<td>GATCCCAGCAAGATAACAGC</td>
<td>561</td>
</tr>
<tr>
<td>GATCTCTCTGTCACATGT</td>
<td>672</td>
</tr>
<tr>
<td>GATCCTTCTTCATTAACA</td>
<td>702</td>
</tr>
<tr>
<td>GATCTACAGAACTCGTGAG</td>
<td>814</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>GATCGGACCAGAATCGACTAT</td>
<td>2,935</td>
</tr>
</tbody>
</table>

1 million beads arranged in a monolayer
SAGE: serial analysis of gene expression
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Fig. 19.25
Fig. 19.18

(a) Fusion protein gene in *E. coli*

(b) A tissue stained with fluorescent antibodies

(c) Tagging a protein with GFP

(d) A mouse with a GFP-tagged transgene
Fig. 19.27

(a) Asymmetric neuroblast stem cell divisions

(b) Asymmetric distribution of Prospero protein

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RNA interference

post-transcriptional gene suppression (PTGS)

RNAi movie [www.nature.com/focus/rnai/animations/index.html]

Fig. 19.8
siRNAs have a defined structure

19 nt duplex

2 nt 3' overhangs
RNAi mechanism

- Cellular RNase recognizes dsRNA
- Cleaves to small (23 bp) fragments
- Fragments hybridize to transcripts
- RNA-dependent RNA polymerase forms dsRNA
- RISC nuclease chews up dsRNA

Good RNAi resource: http://www.ambion.com/techlib/resources/RNAi/
RNAi mechanism

- **Function of RNAi likely used to detect:**
  - genome-invading transposable genetic elements and double-stranded (ds) RNA viruses
  - Other abnormal gene expression

- **Initially characterized in the following:**
  - *C. elegans*
    - Antisense injection resulted in predicted phenotype
  - Plants
    - Resistance to spread of virus