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 signiture 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

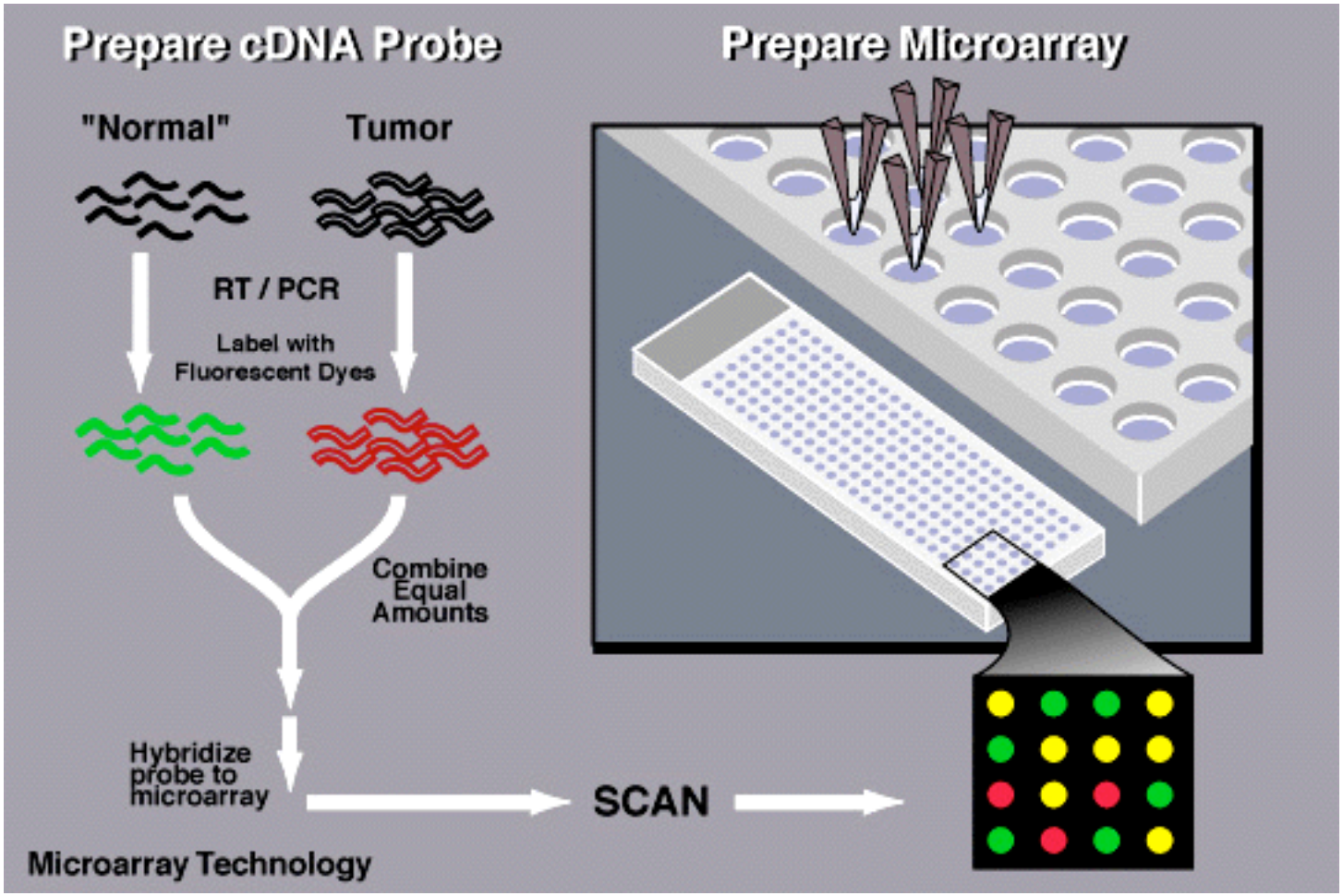
19.18; 19.25; 19.27

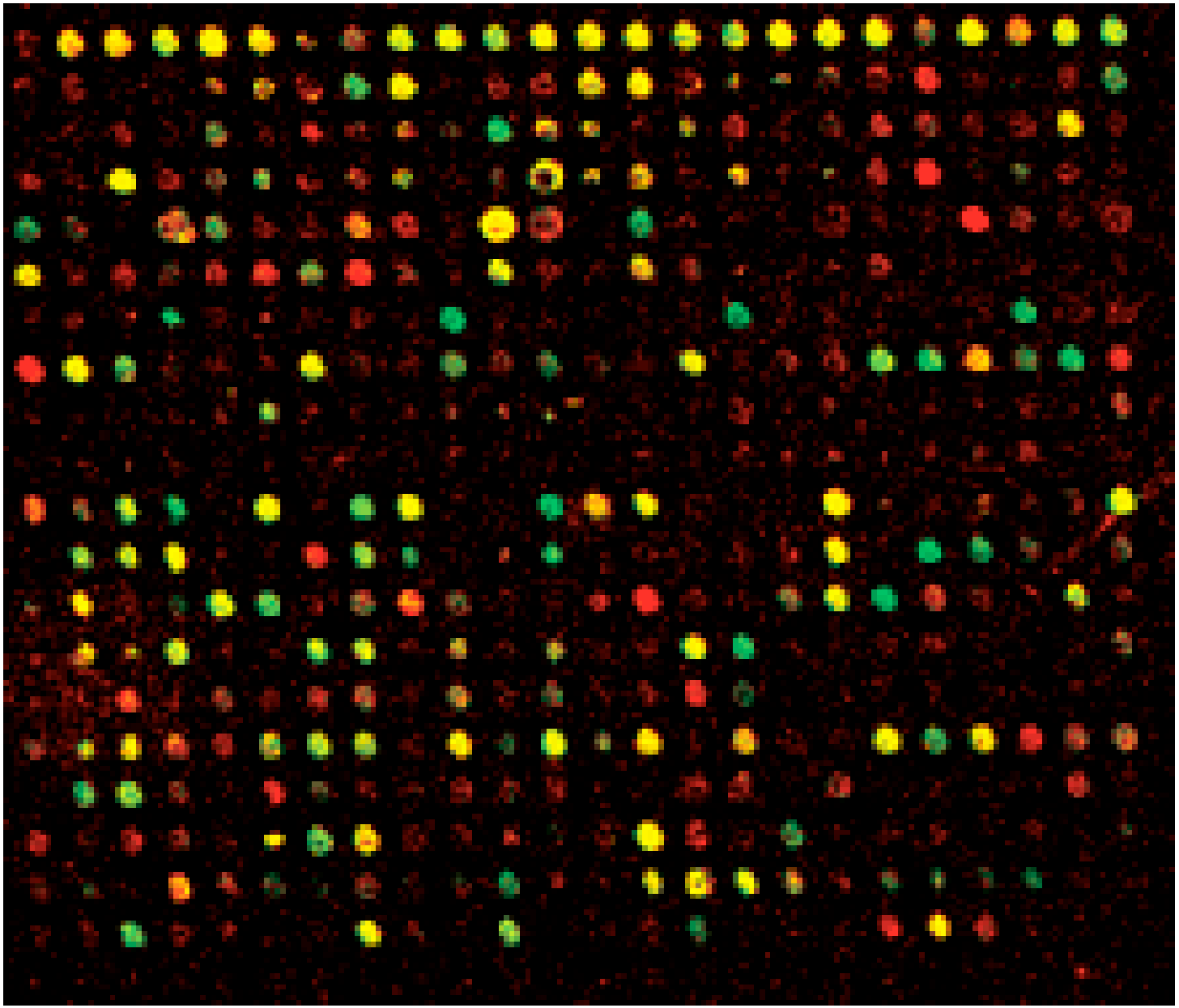
High throughput analyses of the transcriptome

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

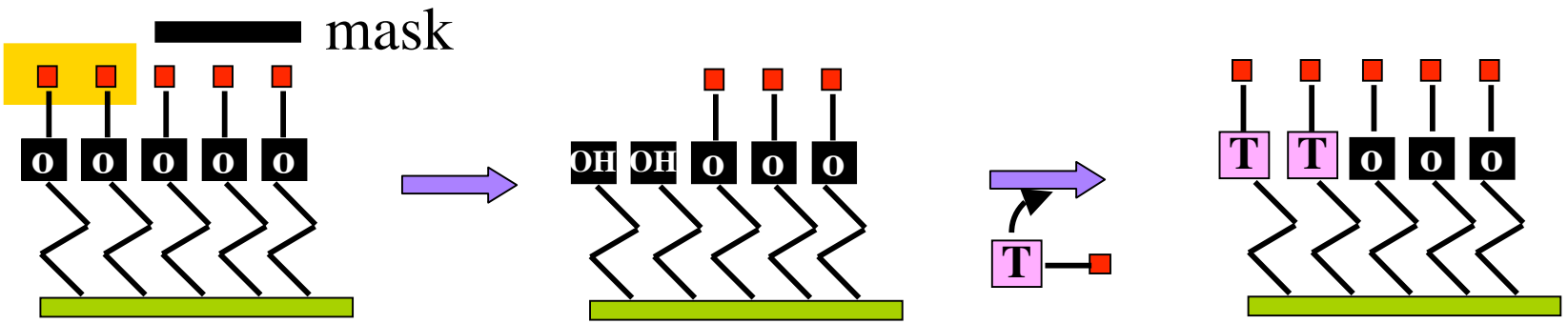
I. cDNA microarrays

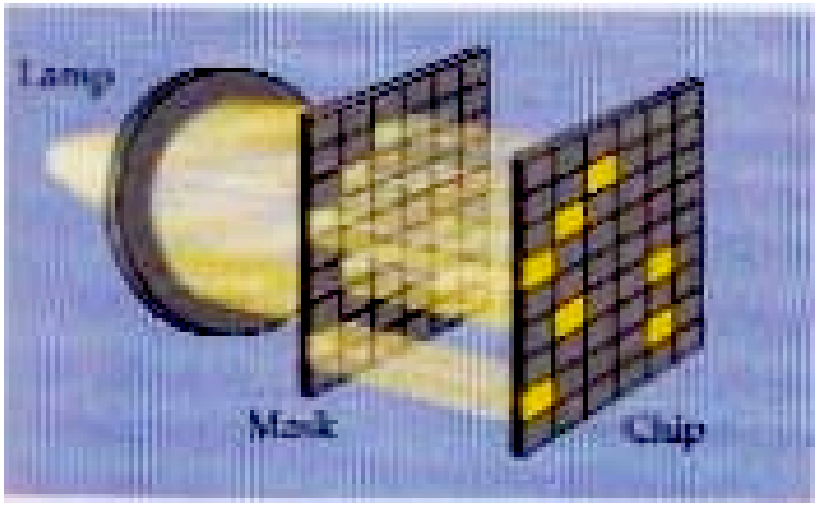




II. Oligonucleotide microarrays (Affymetrix GeneChip)

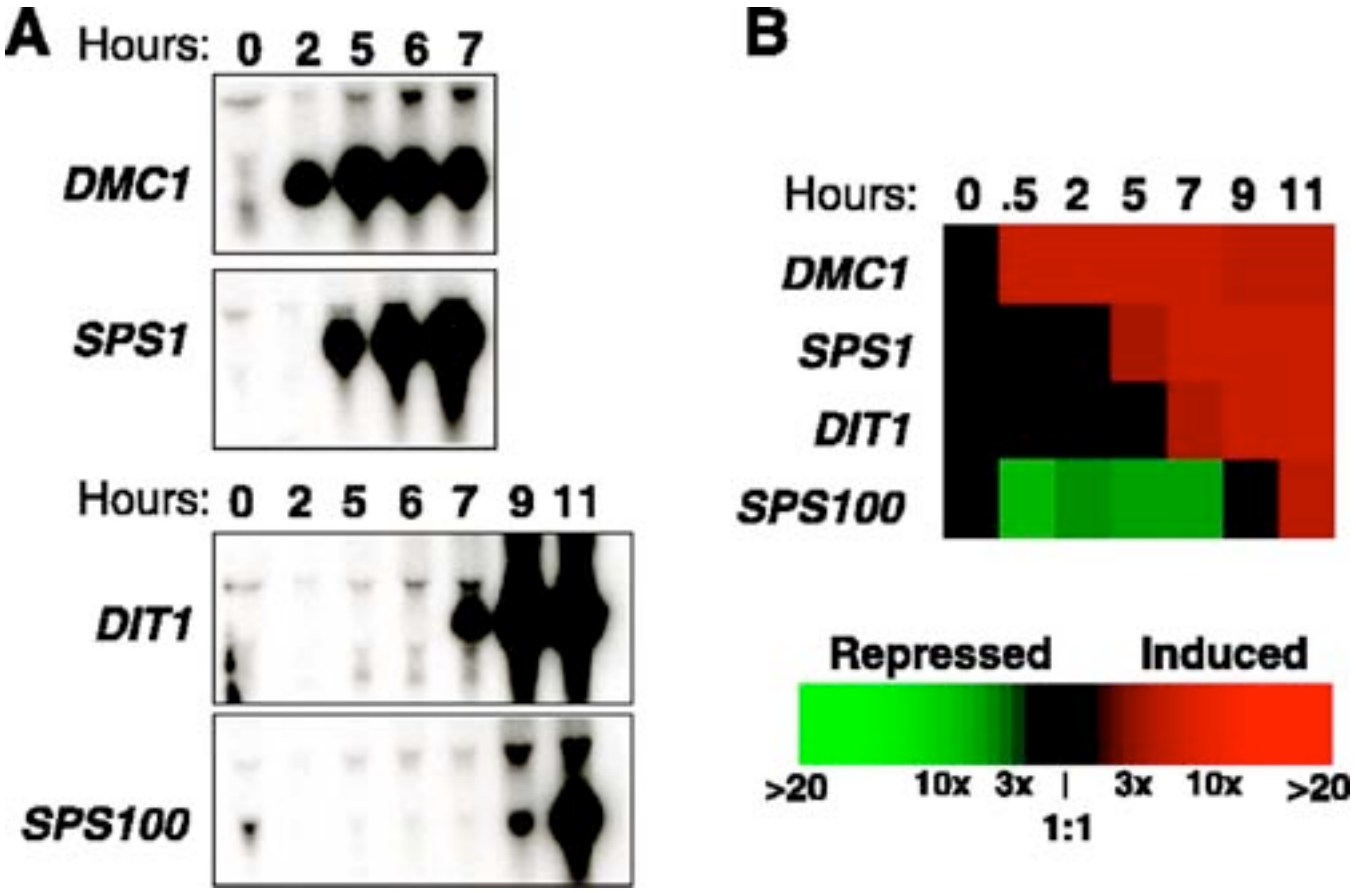
Light deprotection



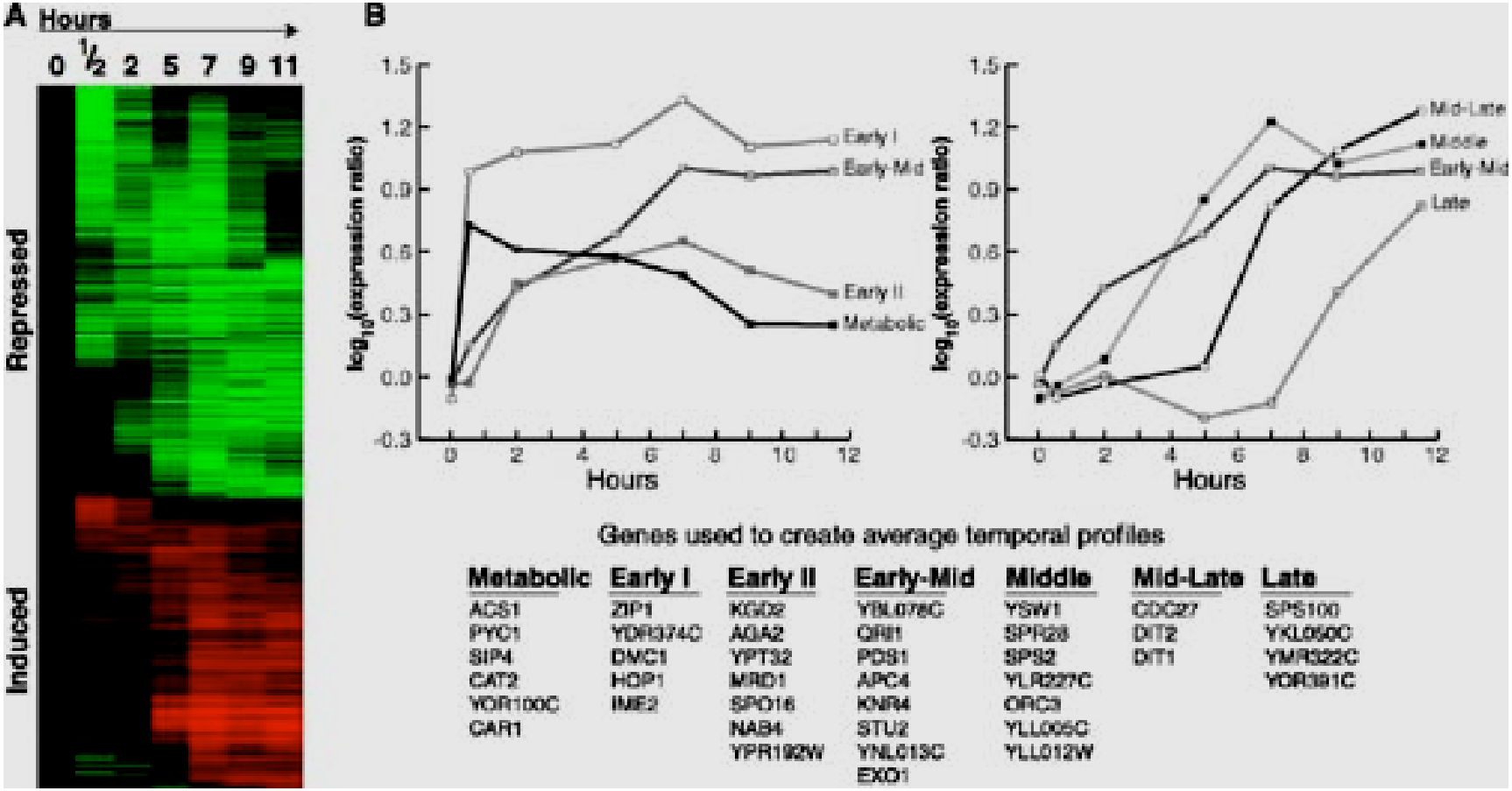


Sporulation gene expression profile in budding yeast

Chu et al., (1998) Science 282, 699-705



Several classes of sporulation gene expression after transfer to sporulation media



Genes used to create average temporal profiles

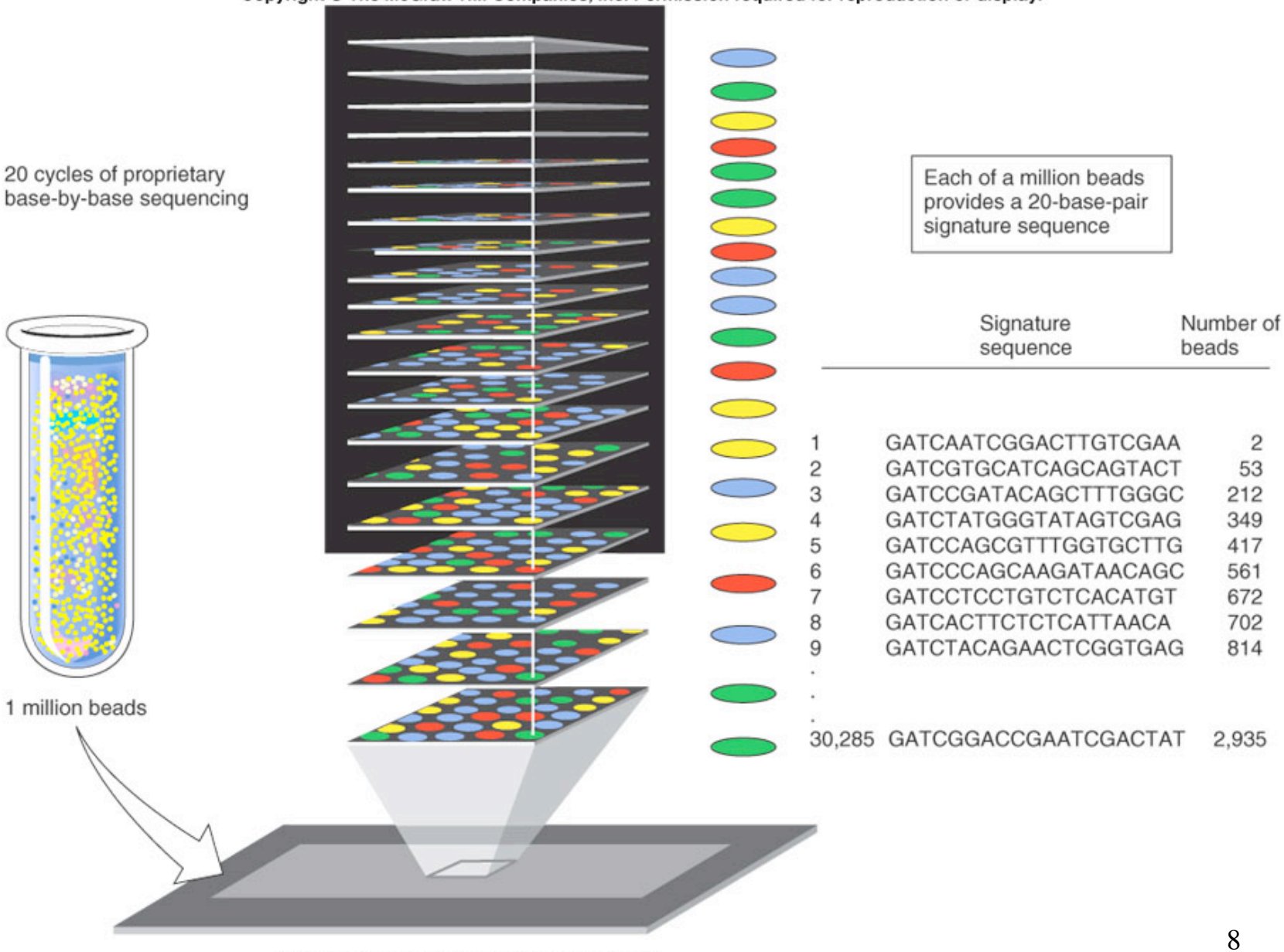
Metabolic	Early I	Early II	Early-Mid	Middle	Mid-Late	Late
ACS1	ZIP1	KGD2	YBL078C	YSW1	CDC27	SPS100
PYC1	YDR374C	AGA2	QRI1	SPR28	DIT2	YKL050C
SIP4	DMC1	YPT32	PDS1	SPS2	DIT1	YMR322C
CAT2	HOP1	MRD1	APC4	YLR227C		YOR391C
YOR100C	IME2	SPO16	KNR4	ORC3		
CAR1		NAB4	STU2	YLL005C		
		YPR192W	YNL013C	YLL012W		
			EXO1			

Survey of 1116 genes during sporulation in budding yeast

Chu et al., (1998) Science 282, 699-705

Fig. 10.28

MPSS: Massively Parallel Signature Sequencing



	Signature sequence	Number of beads
1	GATCAATCGGACTTGTCGAA	2
2	GATCGTGCATCAGCAGTACT	53
3	GATCCGATACAGCTTTGGGC	212
4	GATCTATGGGTATAGTCGAG	349
5	GATCCAGCGTTTGGTGCTTG	417
6	GATCCCAGCAAGATAACAGC	561
7	GATCCTCCTGTCTCACATGT	672
8	GATCACTTCTCTCATTAACA	702
9	GATCTACAGAACTCGGTGAG	814
.		
.		
.		
30,285	GATCGGACCGAATCGACTAT	2,935

SAGE: serial analysis of gene expression

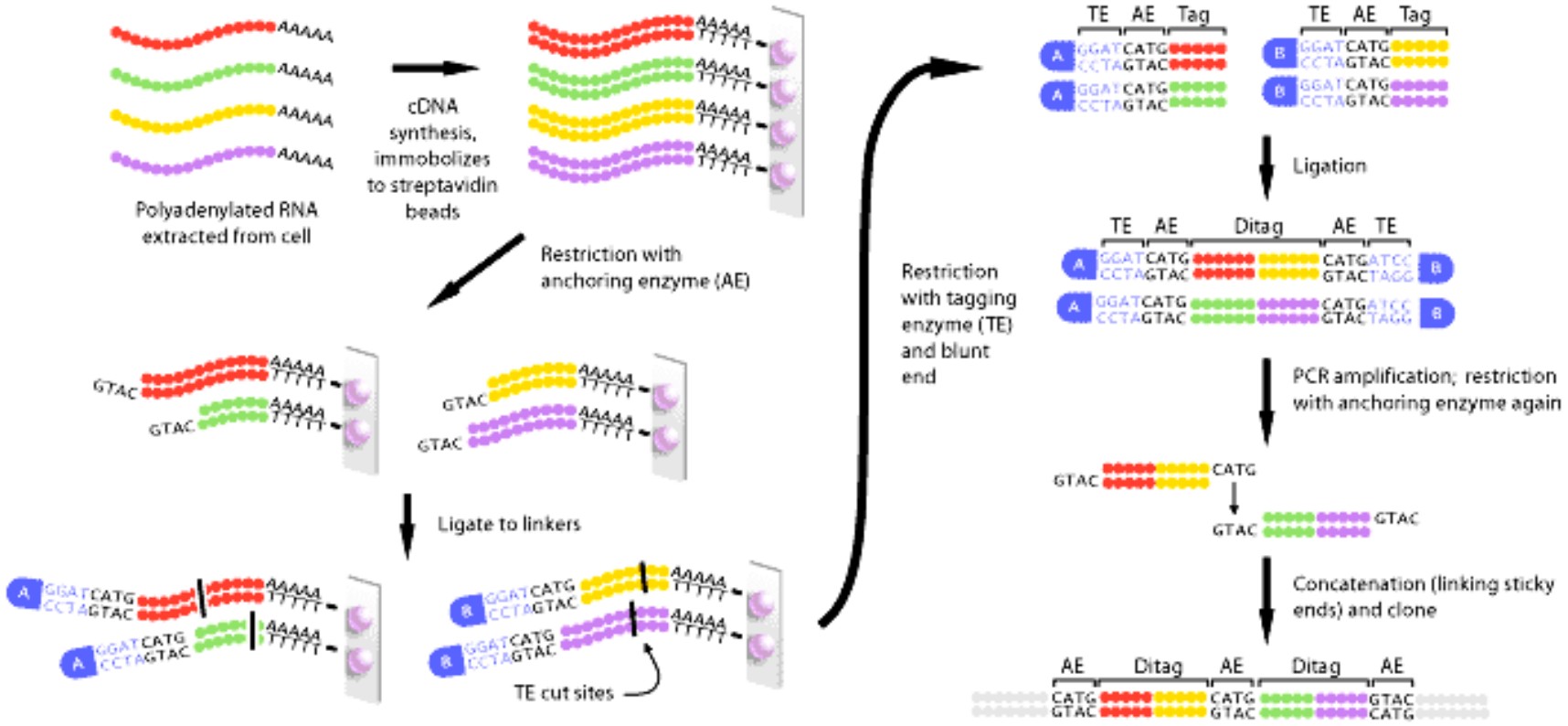


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Fig. 19.25

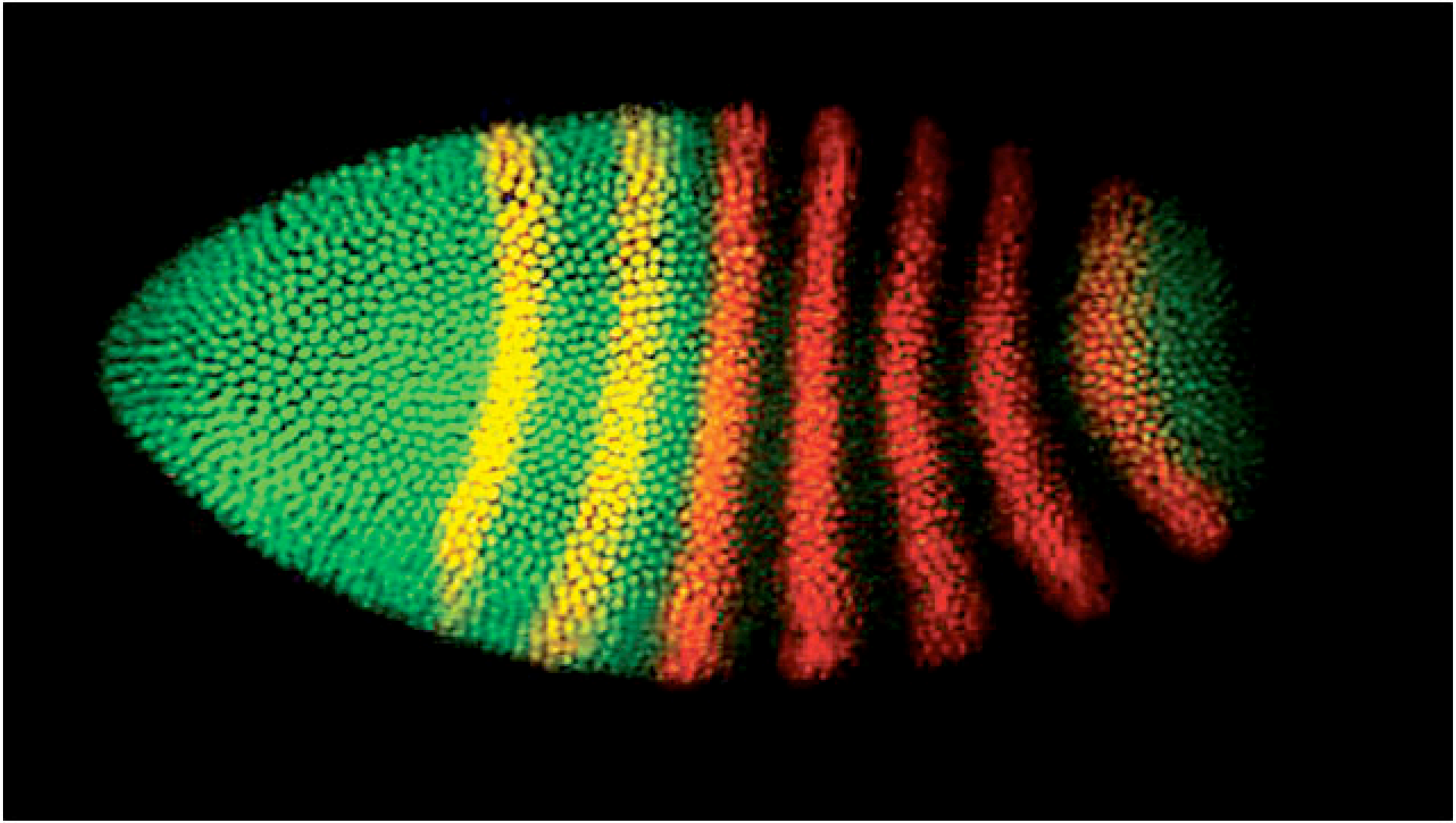
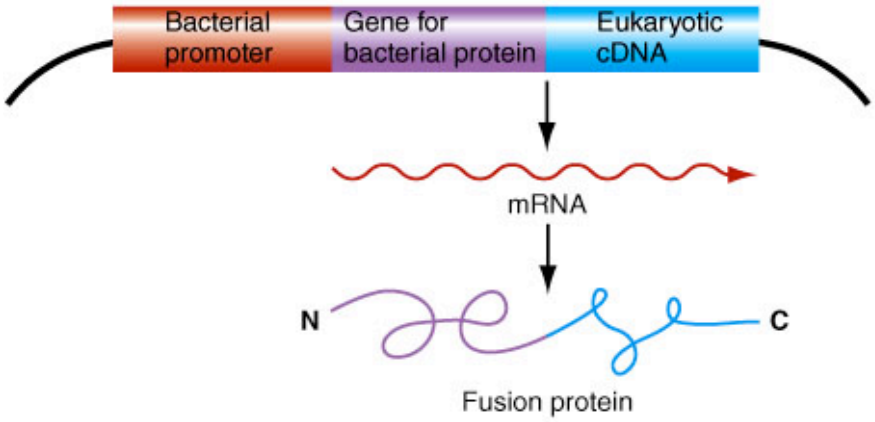
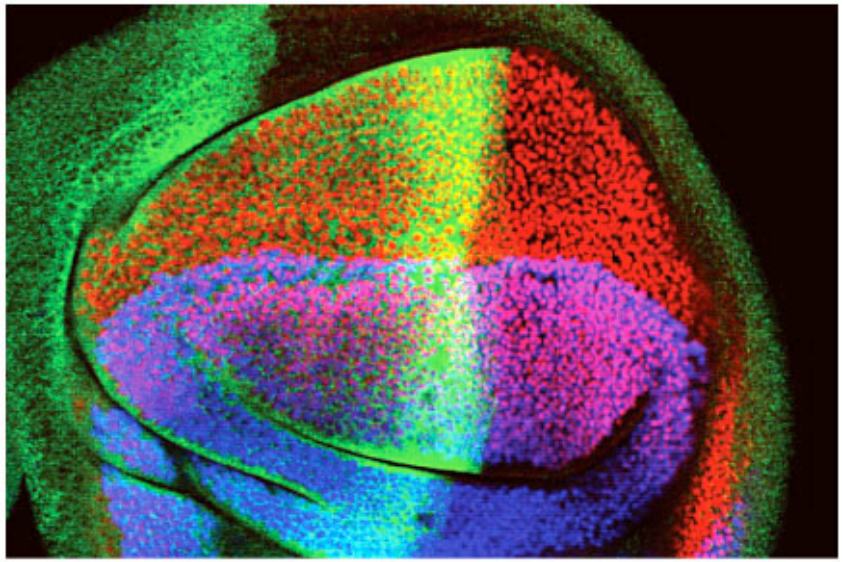


Fig. 19.18

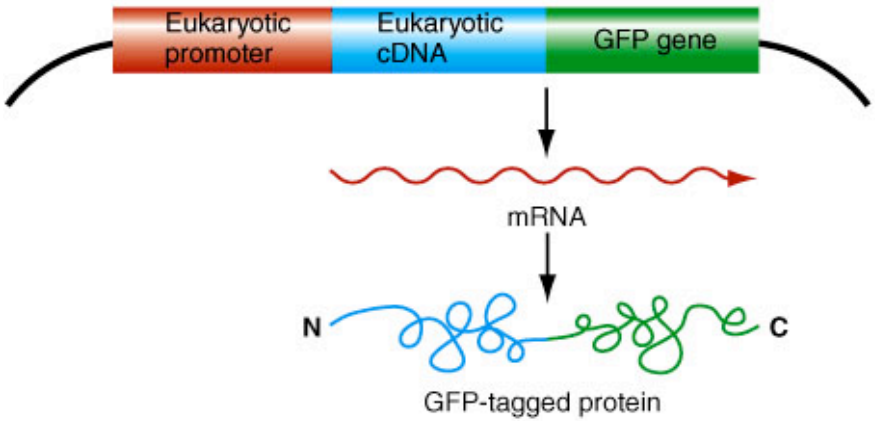
(a) Fusion protein gene in *E. coli*



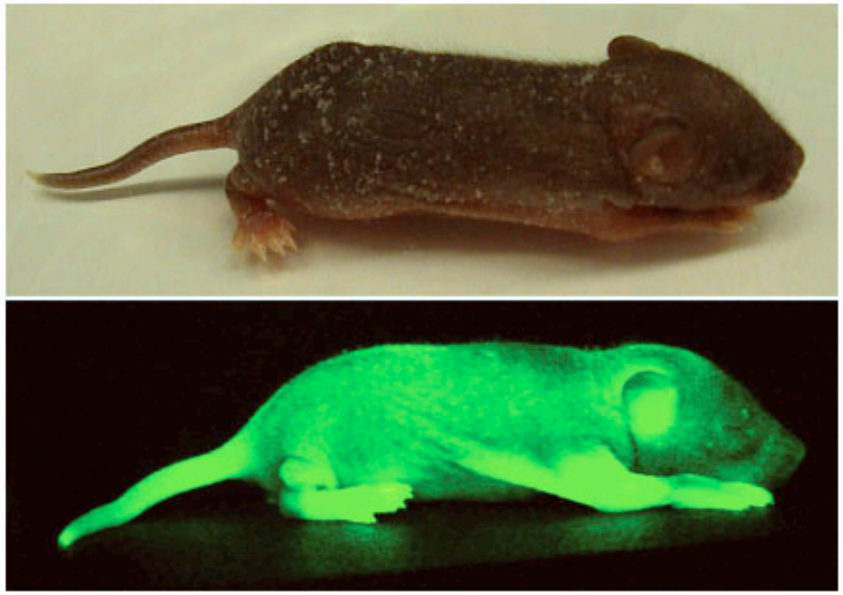
(b) A tissue stained with fluorescent antibodies



(c) Tagging a protein with GFP



(d) A mouse with a GFP-tagged transgene



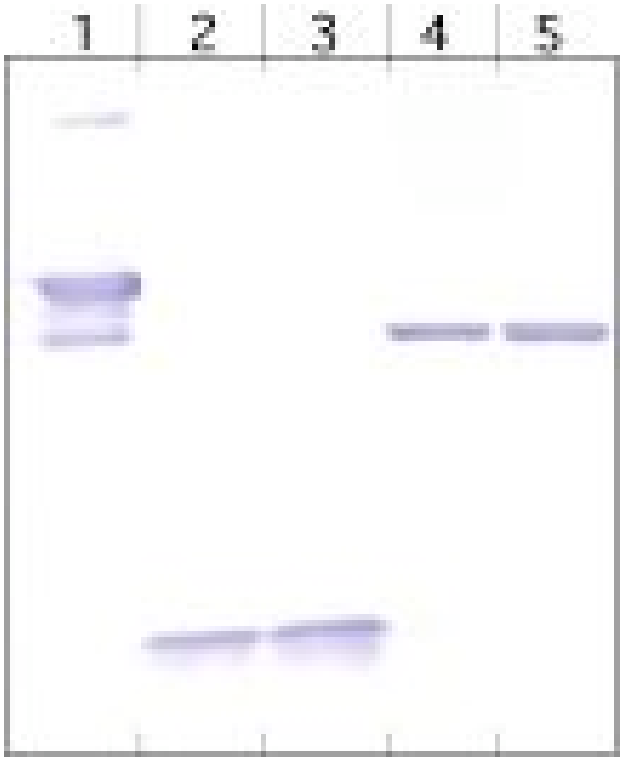

1
2
3
4
5

Fig. 19.27

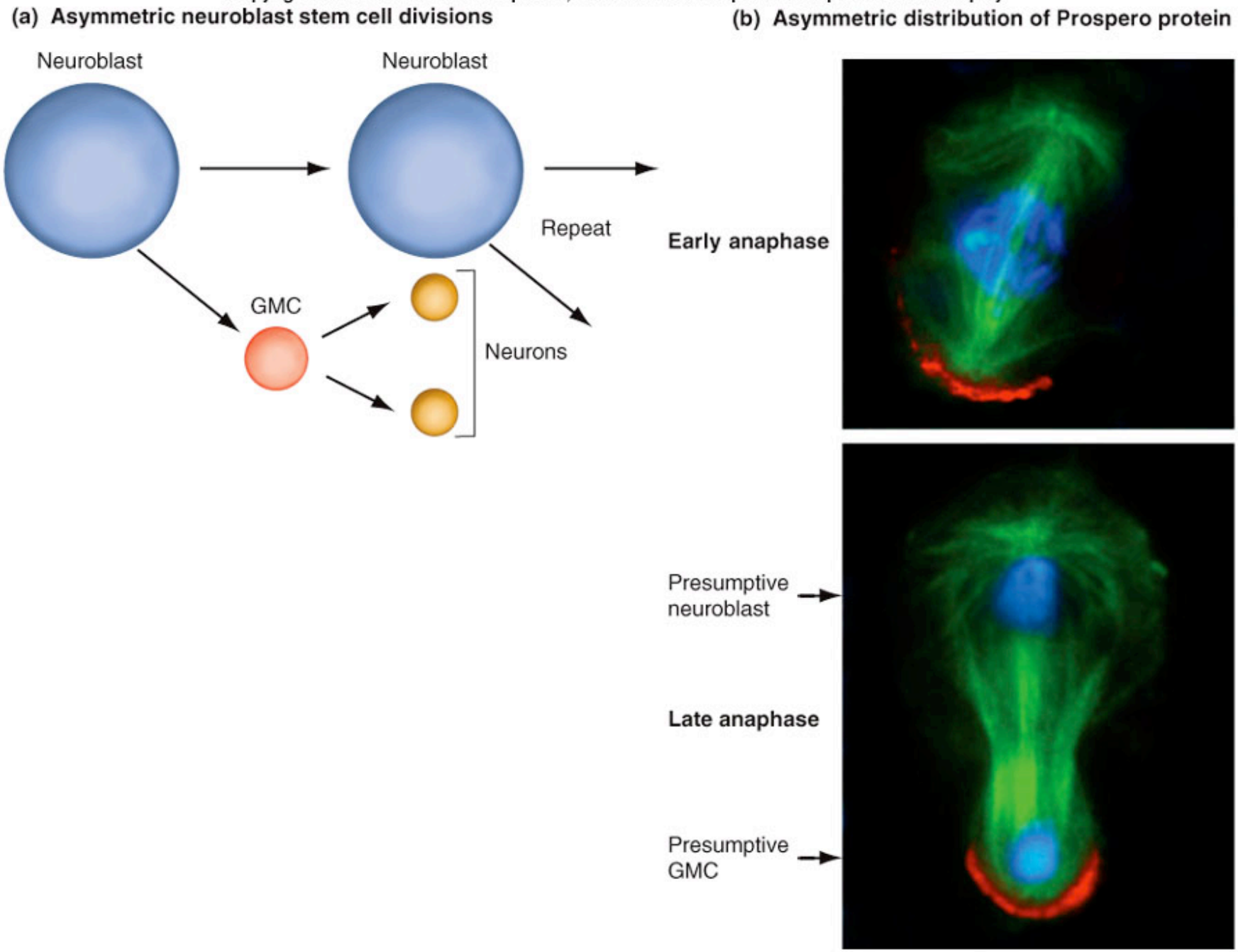
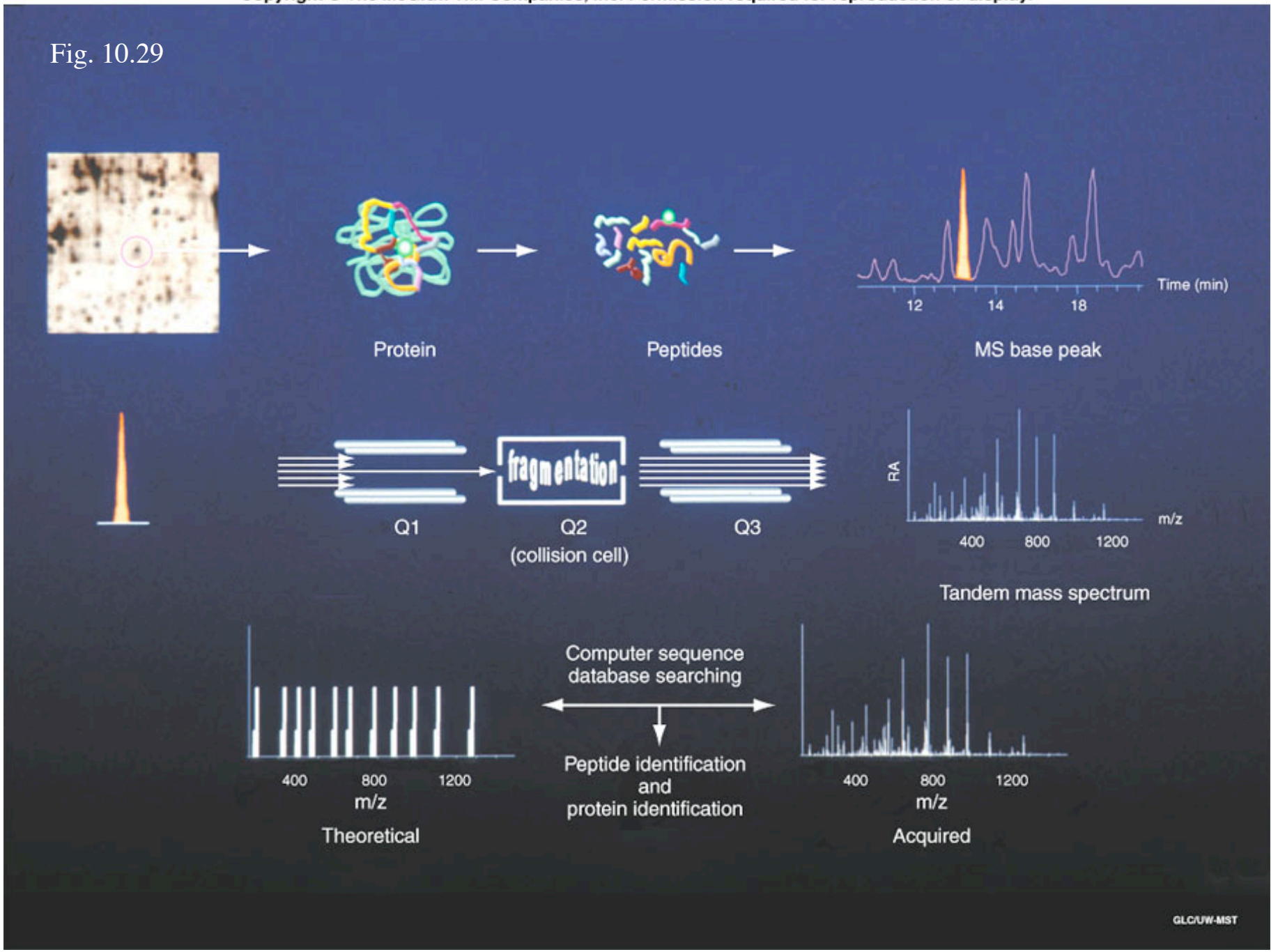


Fig. 10.29



RNA interference

post-transcriptional gene suppression (PTGS)

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

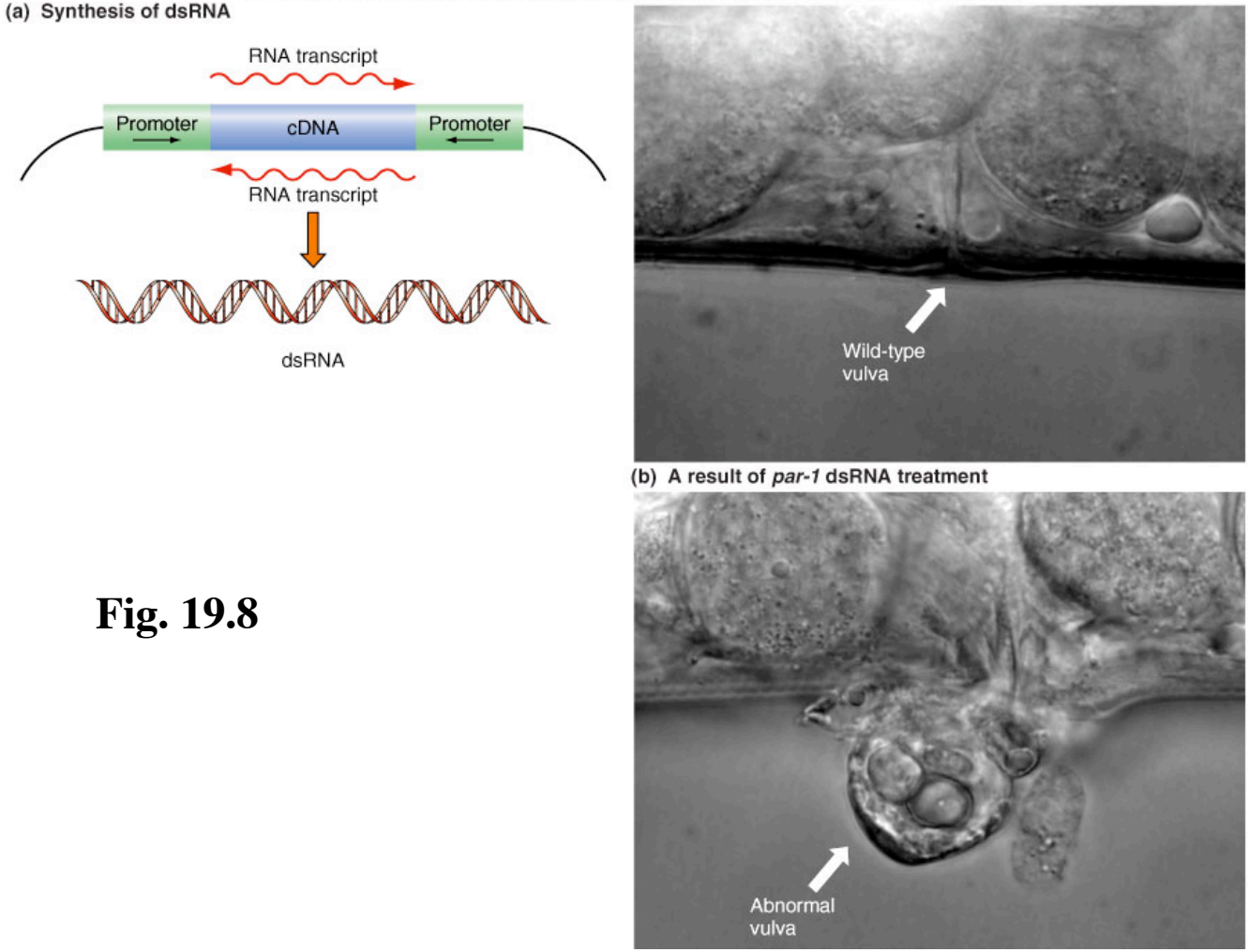
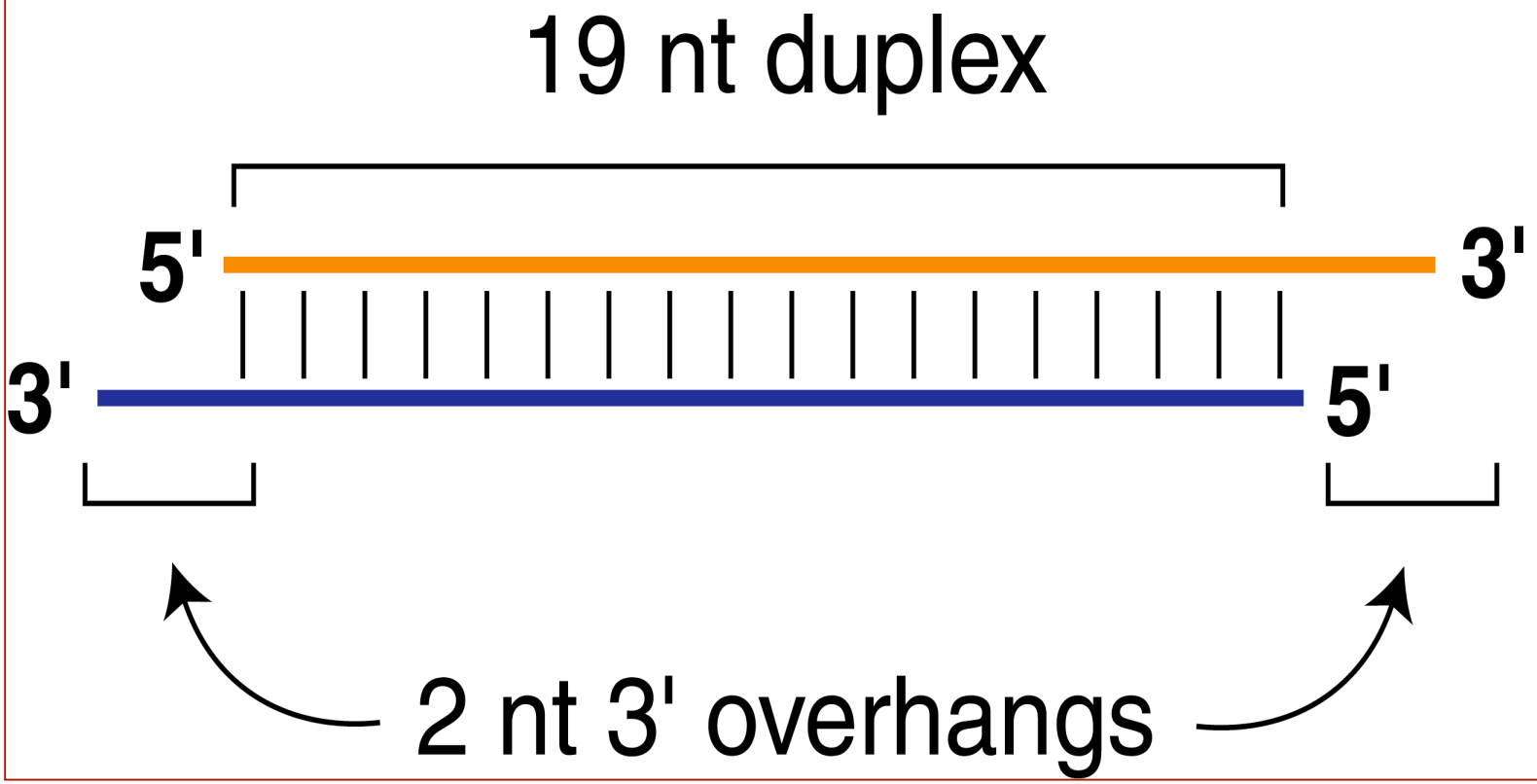


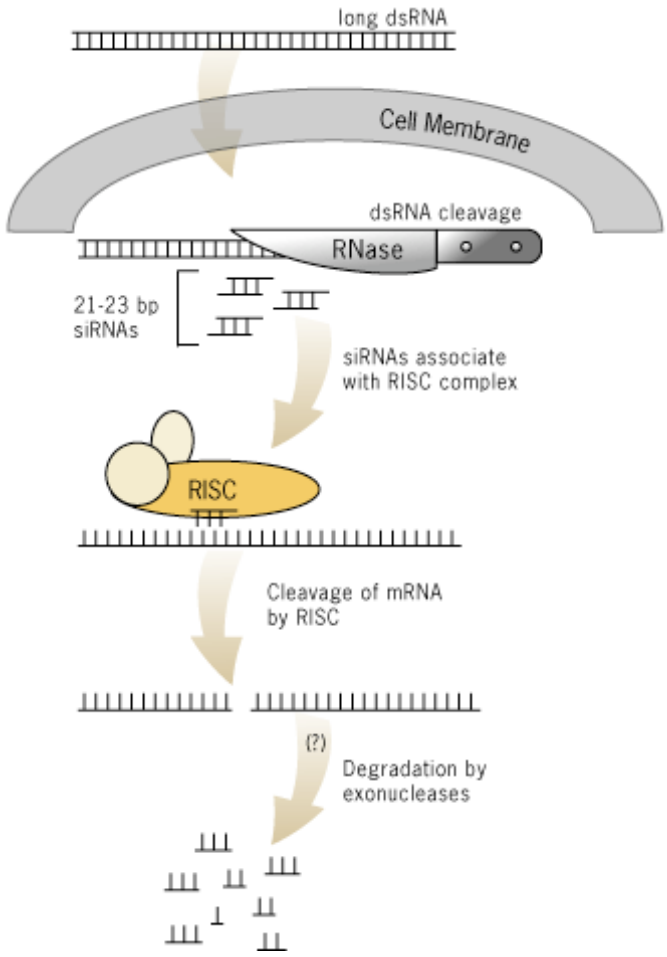
Fig. 19.8

siRNAs have a defined structure



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