Table 14.1

TABLE 14.1 Types of *lacI* mutations

Mutation	Function affected	Phenotype
lacI	Operator binding or tetramer formation	Constitutive; recessive
$lacI^{d}$	Operator binding	Constitutive; dominant
$lacI^{s}$	Inducer binding	Permanently repressed; dominant
$lacI^{rc}$	Conformational change after inducer binding	Repressed only with inducer bound; dominant

Figure 14.1

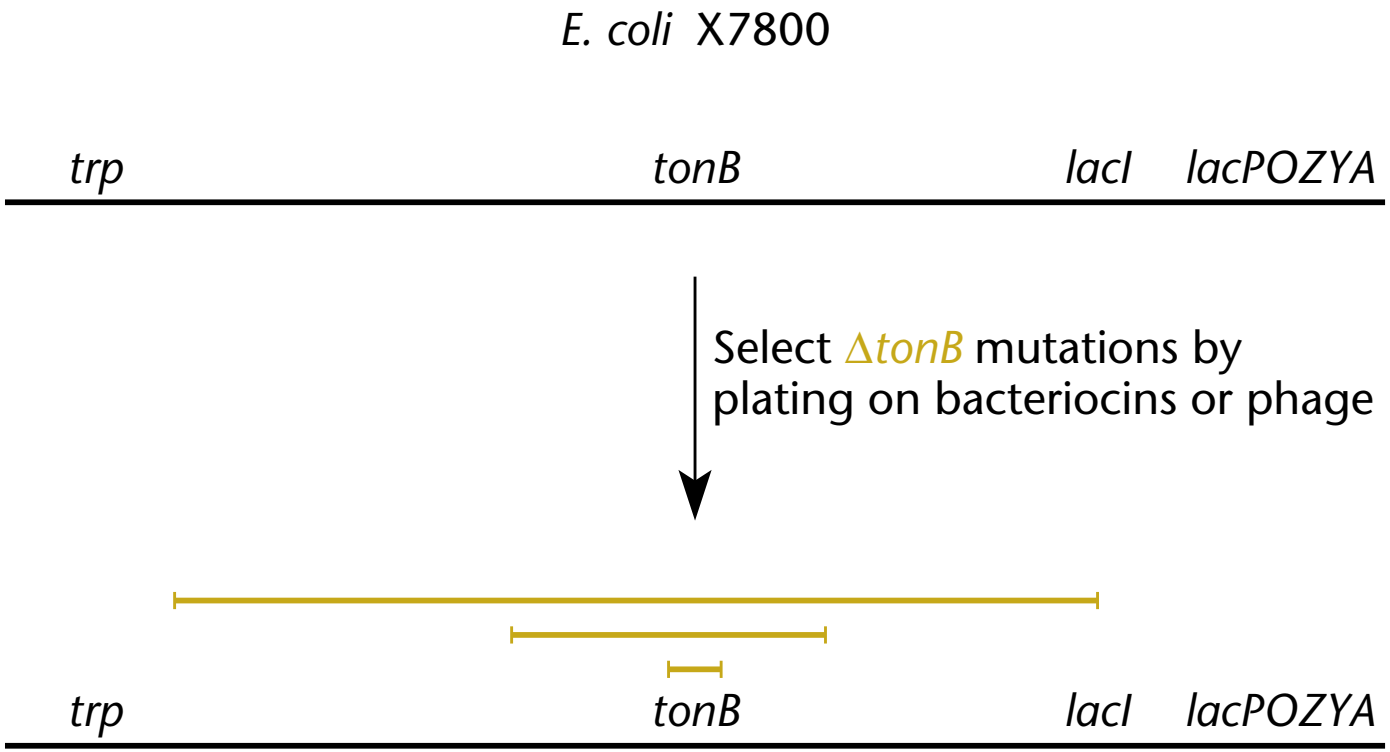

E. coli X7800
trp
tonB
lacI
lacPOZYA
Select ΔtonB mutations by plating on bacteriocins or phage
trp
tonB
lacI
lacPOZYA

Figure 14.2

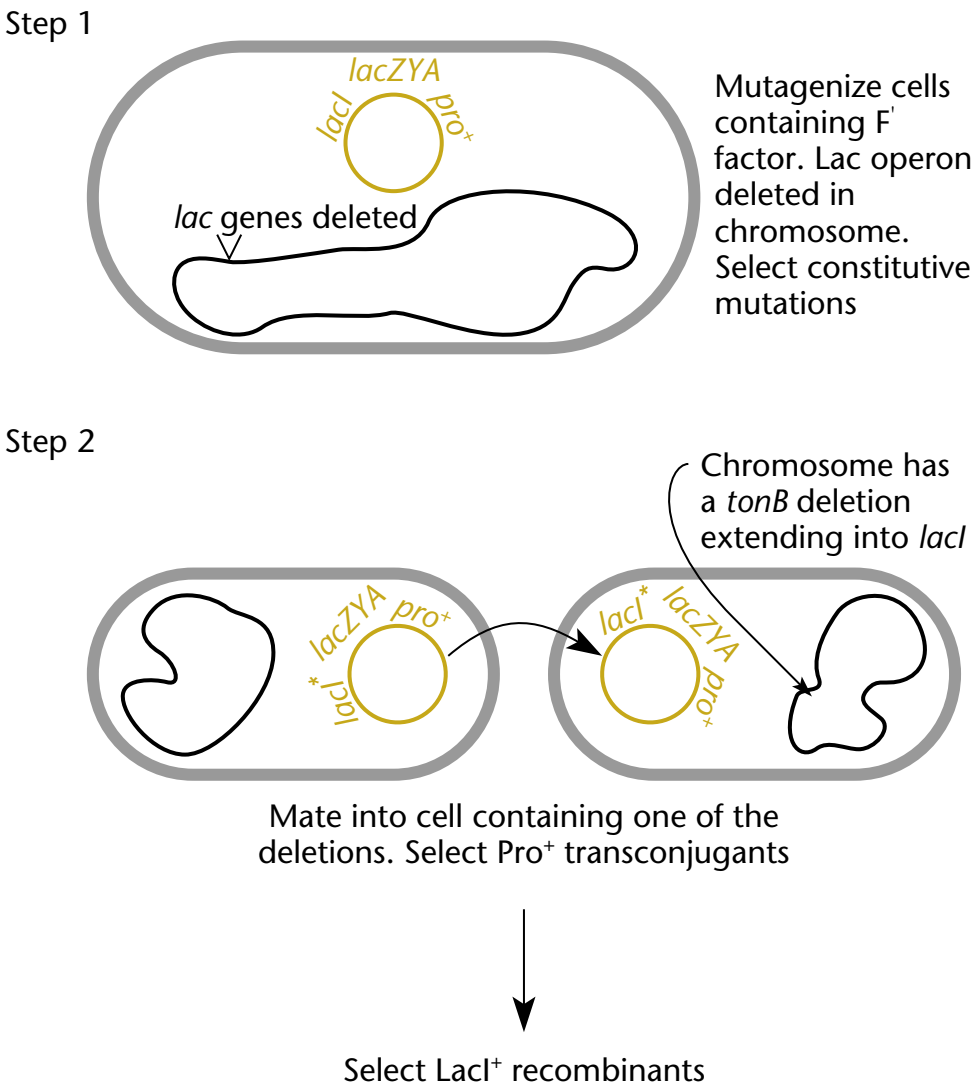

Step 1
lacZYA
lacI
pro+
lac genes deleted
Mutagenize cells containing F' factor. Lac operon deleted in chromosome. Select constitutive mutations
Step 2
Chromosome has a tonB deletion extending into lacI
lacZYA pro+
lacI*
lacI* lacZYA pro+
Mate into cell containing one of the deletions. Select Pro+ transconjugants
Select LacI+ recombinants

Figure 14.3

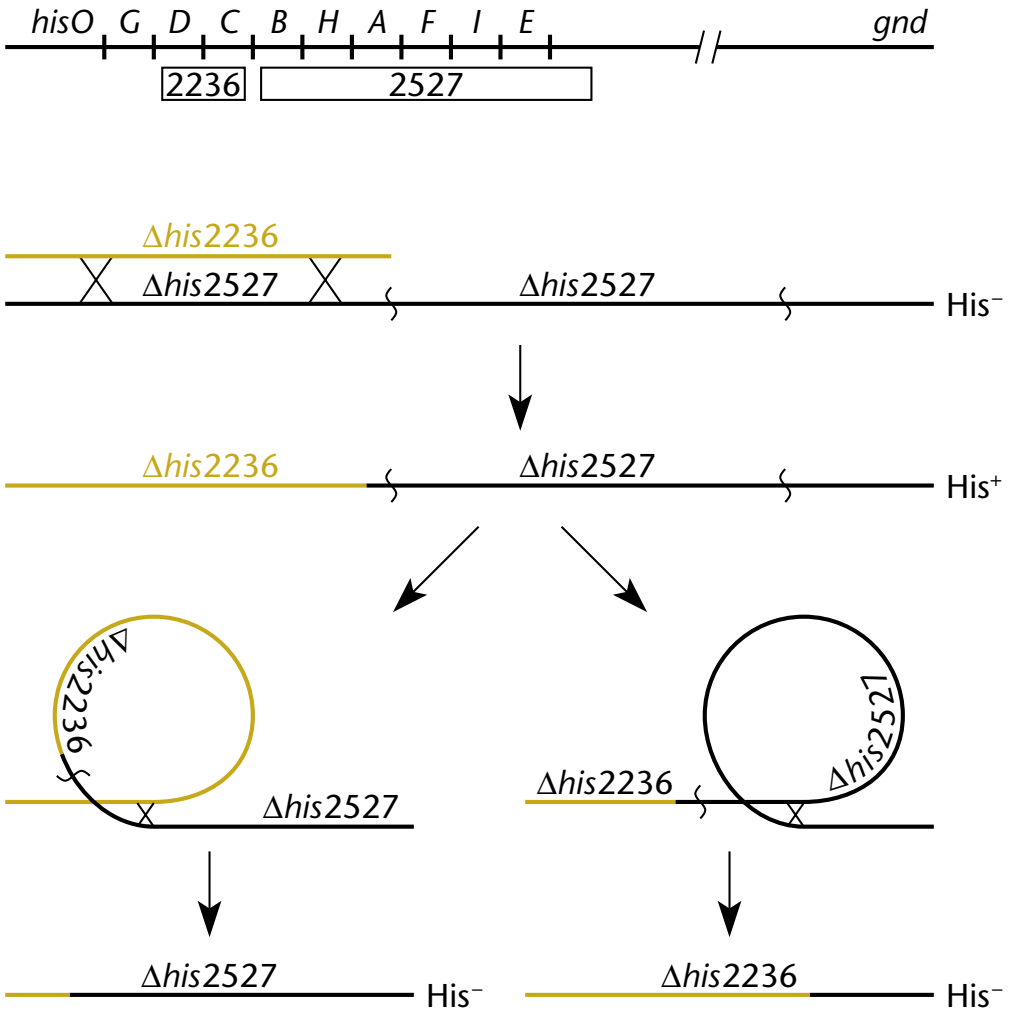

hisO G D C B H A F I E gnd
2236
2527
Δhis2236
Δhis2527
Δhis2527
His⁻
Δhis2236
Δhis2527
His⁺
Δhis2236
Δhis2527
Δhis2527
Δhis2236
Δhis2521
Δhis2527
His⁻
Δhis2236
His⁻

Figure 14.4

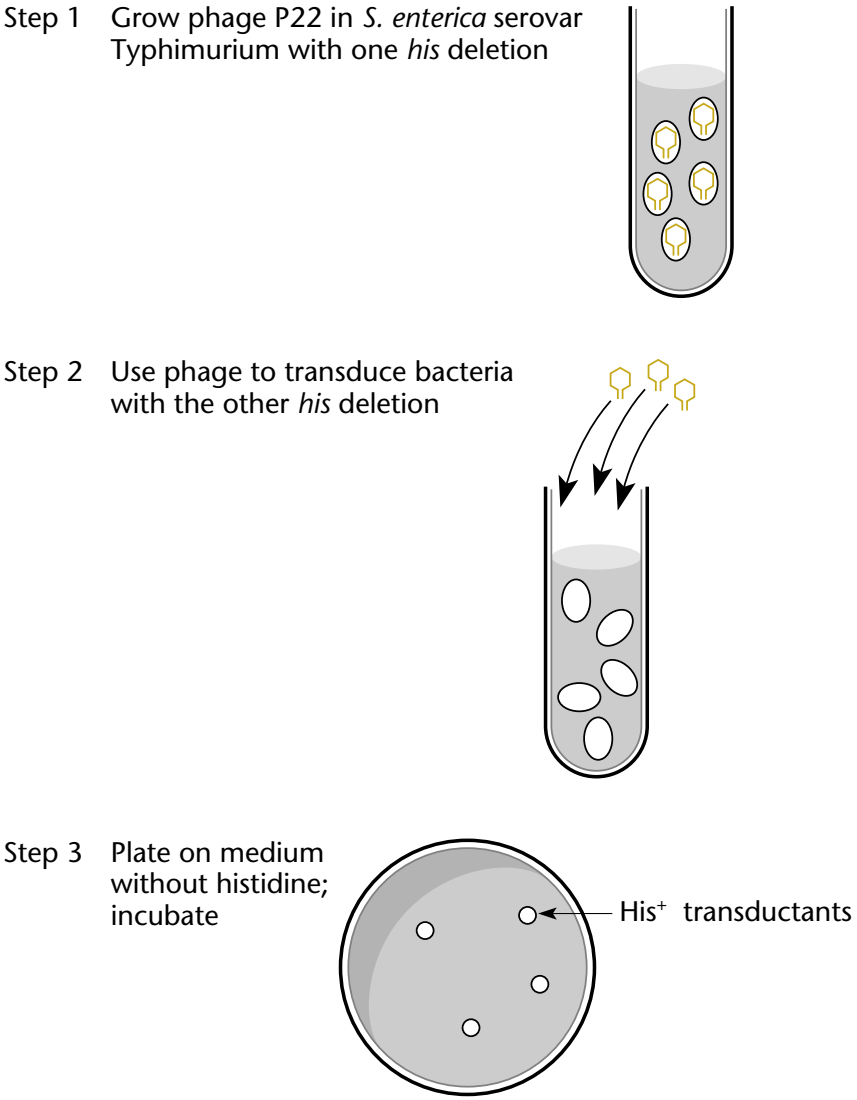

Step 1 Grow phage P22 in *S. enterica* serovar Typhimurium with one *his* deletion
Step 2 Use phage to transduce bacteria with the other *his* deletion
Step 3 Plate on medium without histidine; incubate
His+ transductants

Figure 14.5

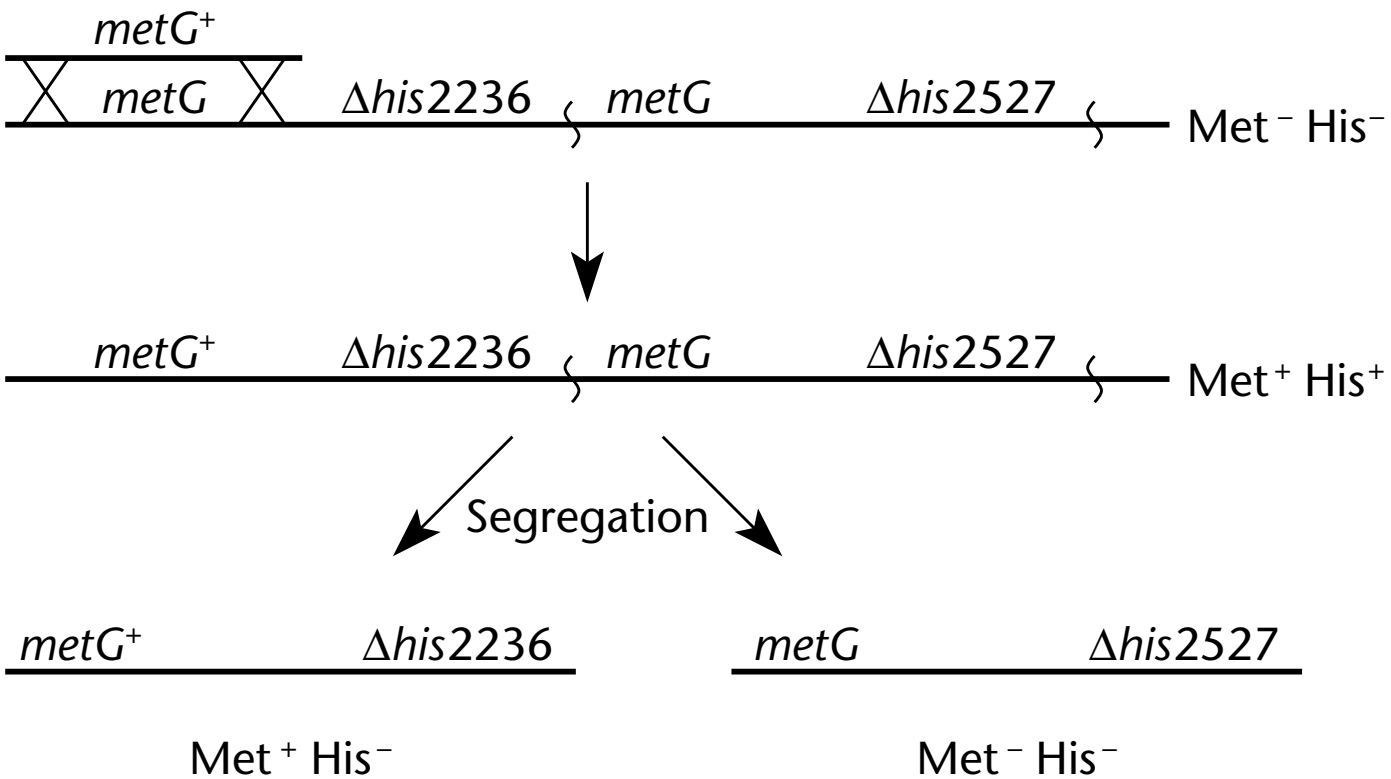

metG+
metG
Δhis2236
metG
Δhis2527
Met− His−
metG+
Δhis2236
metG
Δhis2527
Met+ His+
Segregation
metG+
Δhis2236
Met+ His−
metG
Δhis2527
Met− His−

Figure 14.6

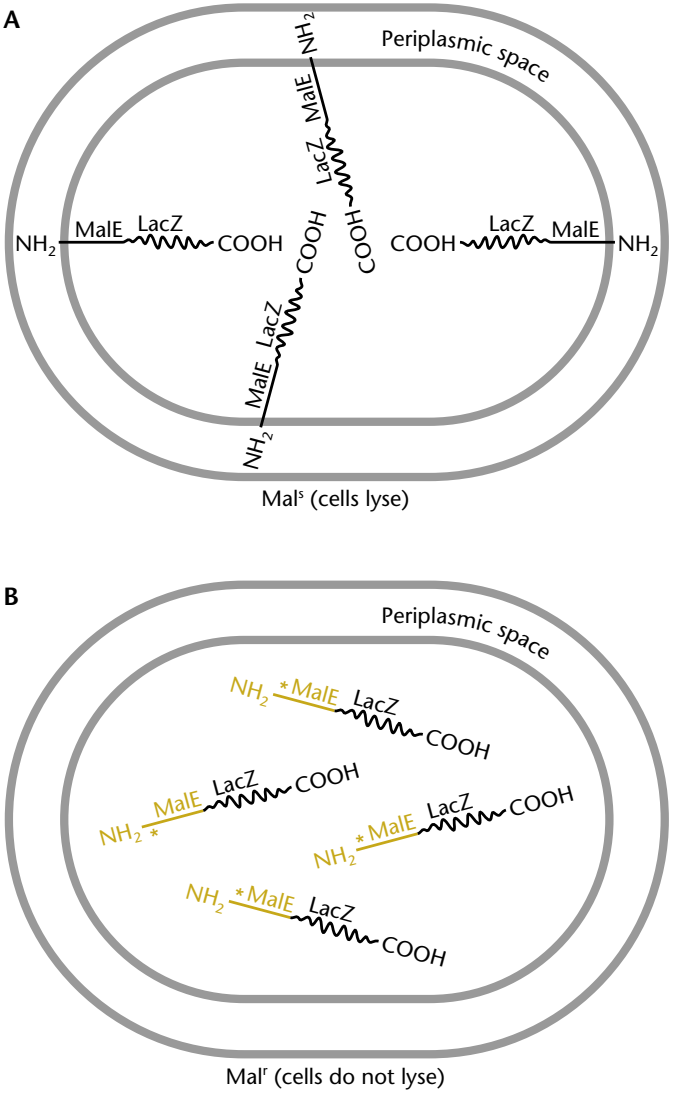

A
Periplasmic space
NH2
MalE
LacZ
COOH
NH2
MalE
LacZ
COOH
COOH
COOH
LacZ
MalE
NH2
Mal^s (cells lyse)
B
Periplasmic space
NH2 *MalE LacZ COOH
NH2 * MalE LacZ COOH
NH2 *MalE LacZ COOH
NH2 *MalE LacZ COOH
Mal^r (cells do not lyse)

Figure 14.7

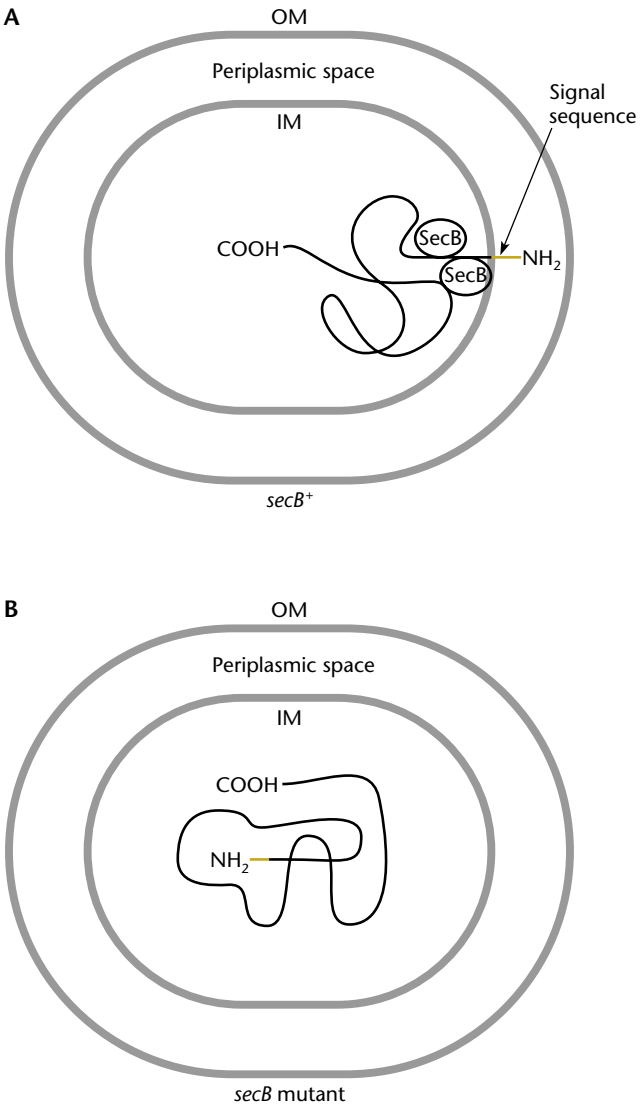

A
OM
Periplasmic space
IM
Signal sequence
COOH
SecB
SecB
NH2
secB+
B
OM
Periplasmic space
IM
COOH
NH2
secB mutant

Figure 14.8

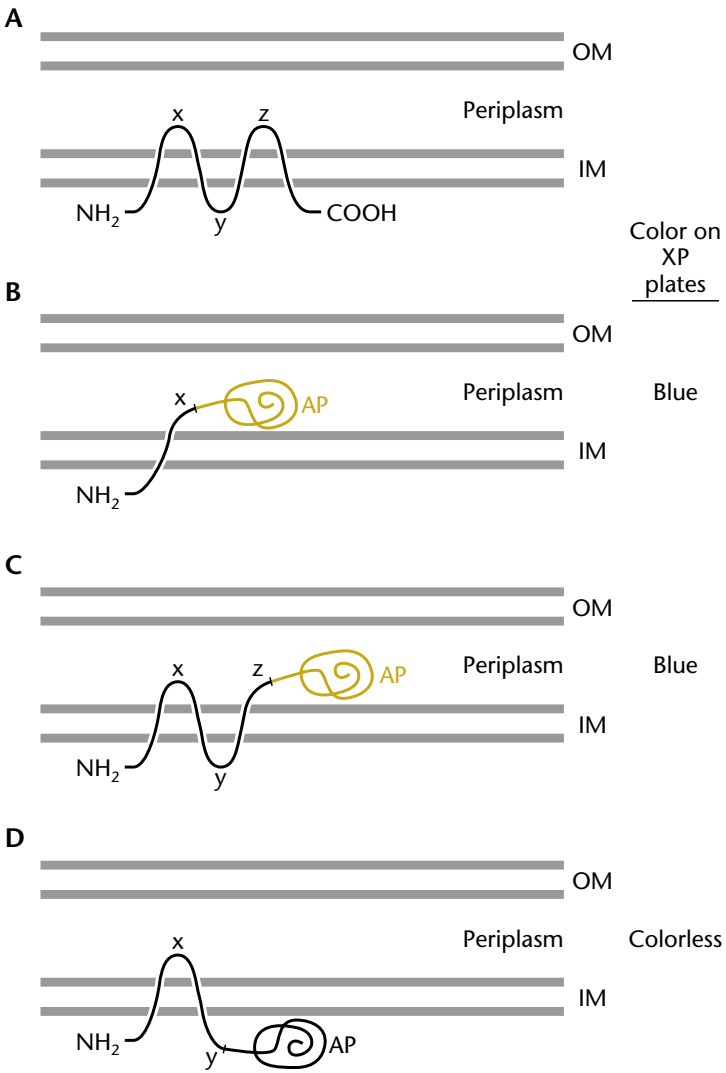

A
OM
Periplasm
x
z
IM
NH2
y
COOH
Color on XP plates
B
OM
Periplasm
Blue
x
AP
IM
NH2
C
OM
Periplasm
Blue
x
z
AP
IM
NH2
y
D
OM
Periplasm
Colorless
x
IM
NH2
y
AP

Figure 14.9

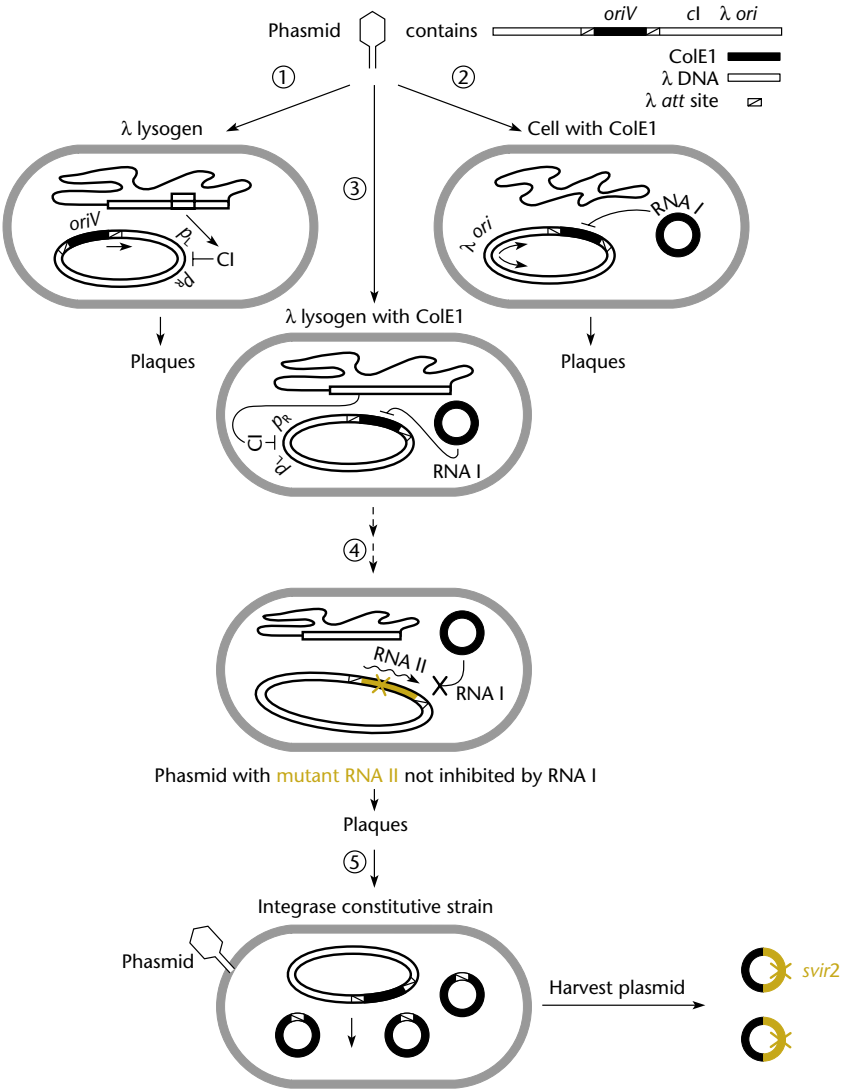
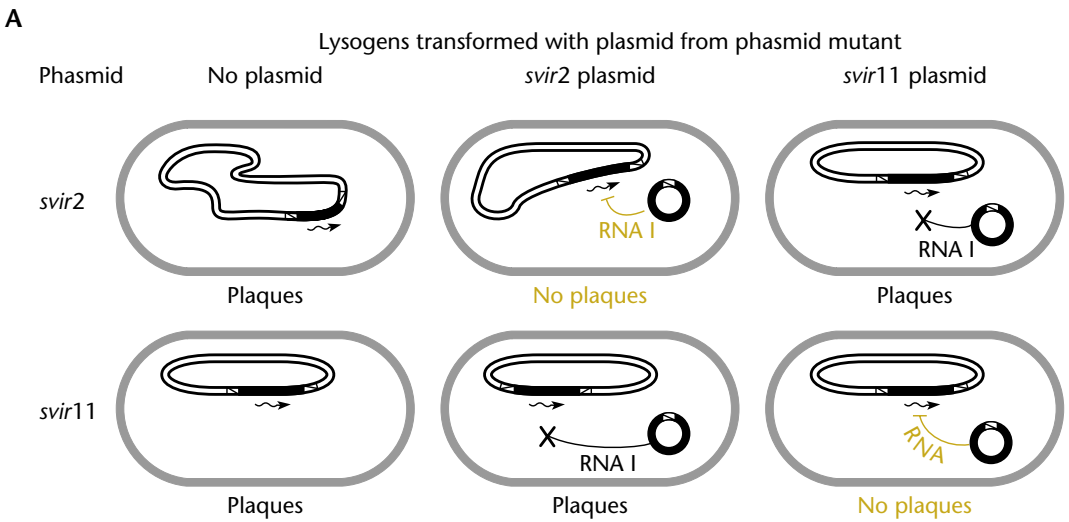

Phasmid
contains
oriV
cI
λ ori
ColE1
λ DNA
λ att site
①
②
③
λ lysogen
Cell with ColE1
oriV
CI
RNA I
λ ori
λ lysogen with ColE1
Plaques
Plaques
RNA I
④
RNA II
RNA I
Phasmid with mutant RNA II not inhibited by RNA I
Plaques
⑤
Integrase constitutive strain
Phasmid
Harvest plasmid
svir2

Figure 14.10


A
Lysogens transformed with plasmid from phasmid mutant
Phasmid
No plasmid
svir2 plasmid
svir11 plasmid
svir2
svir11
RNA I
RNA I
RNA I
RNA
Plaques
No plaques
Plaques
Plaques
Plaques
No plaques

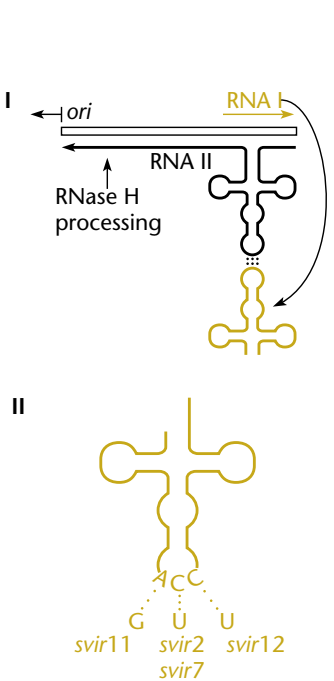

B
I
ori
RNA I
RNA II
RNase H
processing
II
A C C
G U U
svir11
svir2
svir12
svir7

Figure 14.11

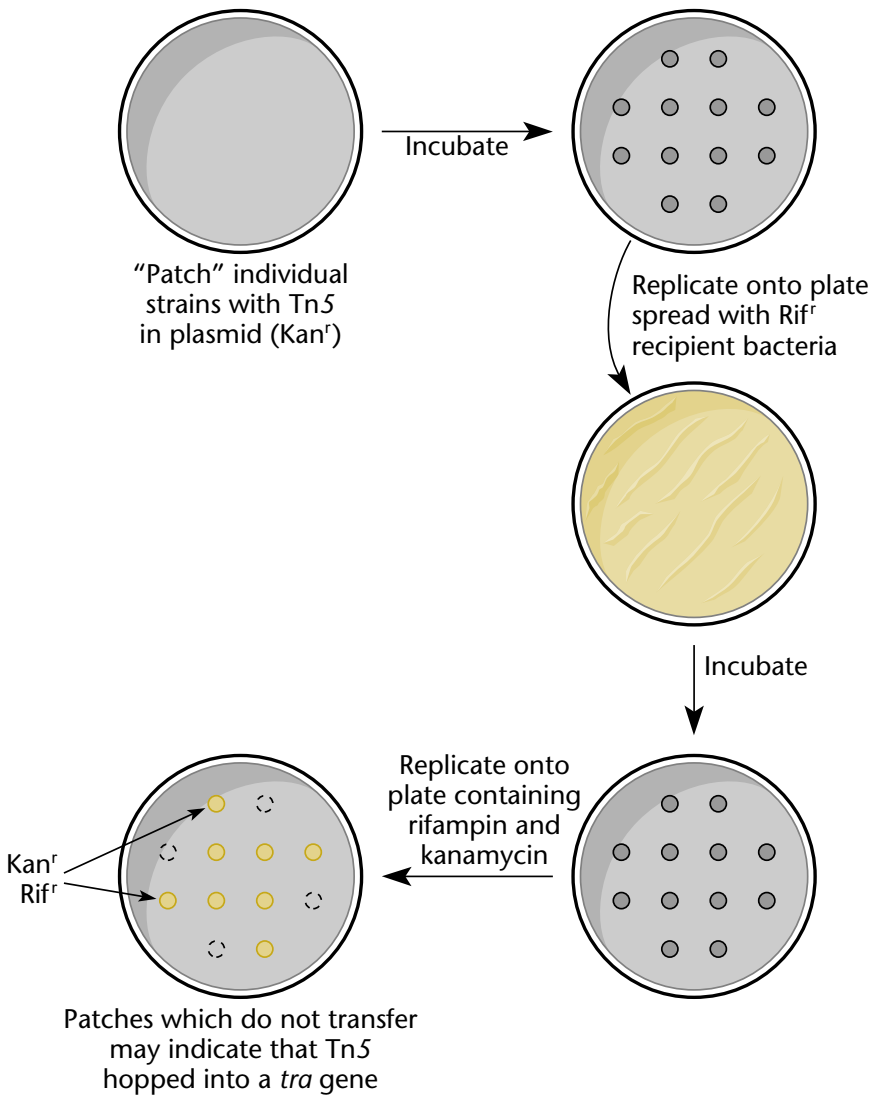
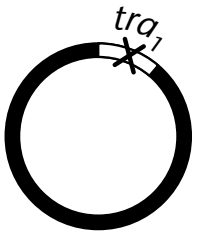
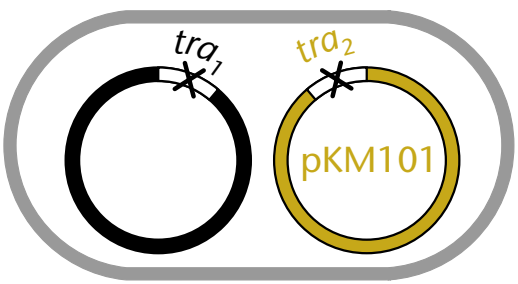

Incubate
"Patch" individual strains with Tn*5* in plasmid (Kan^r)
Replicate onto plate spread with Rif^r recipient bacteria
Incubate
Replicate onto plate containing rifampin and kanamycin
Kan^r
Rif^r
Patches which do not transfer may indicate that Tn*5* hopped into a *tra* gene

Figure 14.12

① Clone one mutant *tra* region from a *tra* mutant plasmid



② Transform into a cell with a different *tra* mutant plasmid



③ Test for transfer to a recipient. If transfer occurs, the mutations complement

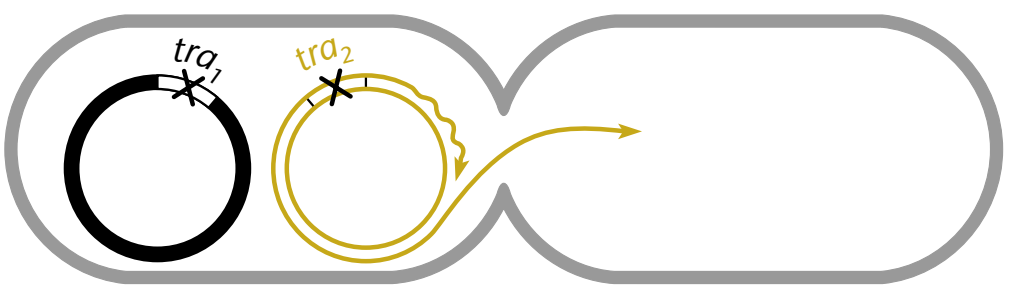


Figure 14.13

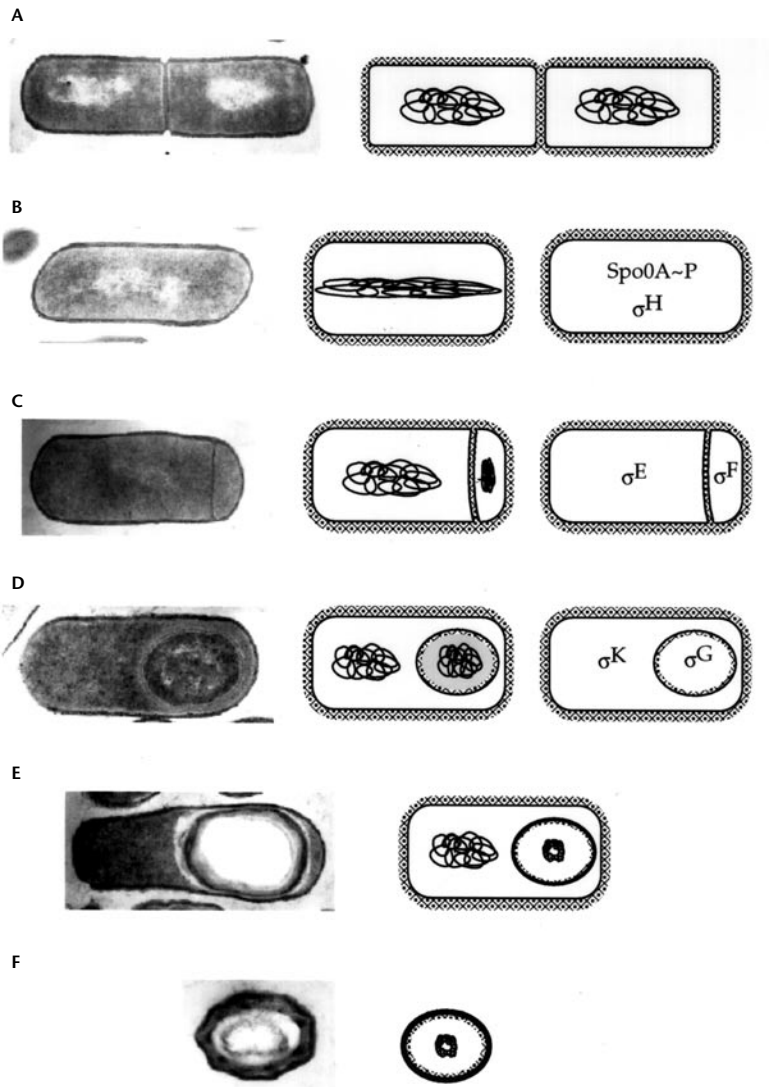


Figure 14.14

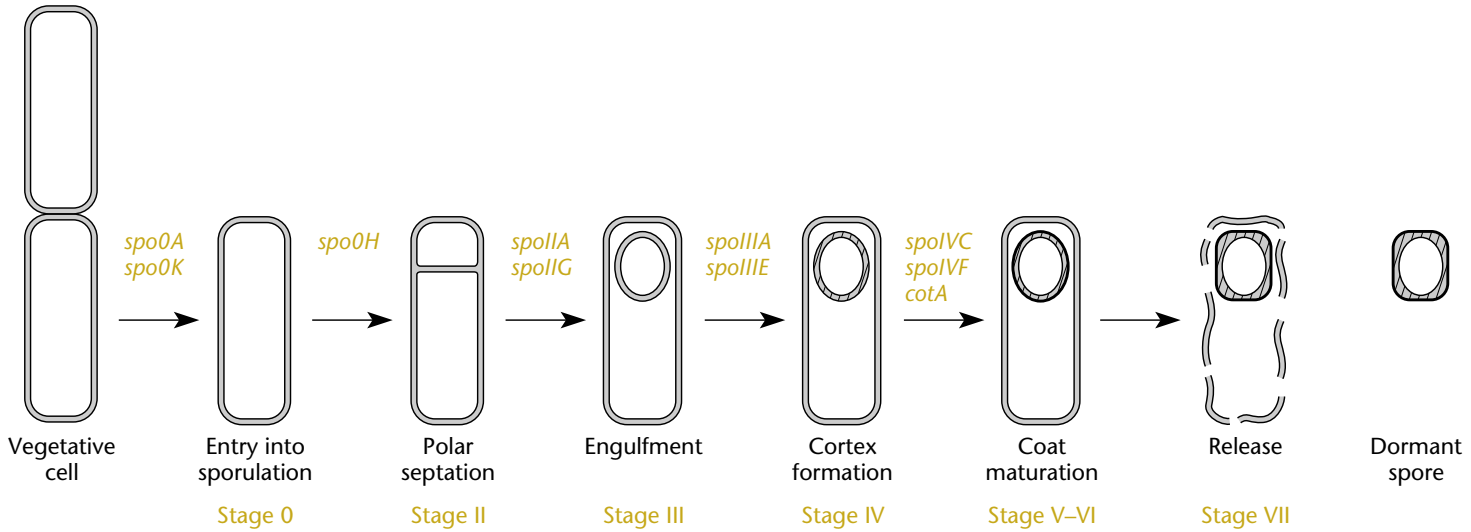

spo0A
spo0K
spo0H
spoIIA
spoIIG
spoIIIA
spoIIIE
spoIVC
spoIVF
cotA
Vegetative cell
Entry into sporulation
Polar septation
Engulfment
Cortex formation
Coat maturation
Release
Dormant spore
Stage 0
Stage II
Stage III
Stage IV
Stage V–VI
Stage VII

Table 14.2

TABLE 14.2 *Bacillus subtilis* **sporulation regulators**

Stage of mutant arrest	Gene	Function
0	*spo0A*	Transcription regulator
	spo0B	Phosphorelay component
	spo0F	Phosphorelay component
	spo0E	Phosphatase
	spo0L	Phosphatase
	spo0H	Sigma H
II	*spoIIAA*	Anti-anti-sigma
	spoIIAC	Sigma F
	spoIIE	Phosphatase
	spoIIGA	Protease
	spoIIGB	Pro-sigma E
III	*spoIIIG*	Sigma G
IV	*spoIVCB-spoIIIC*[a]	Pro-sigma K
	spoIVF	Regulator of sigma K

[a]In *B. subtilis* and some other bacilli, two gene fragments undergo recombination to produce the complete sigma K coding region.

Figure 14.15

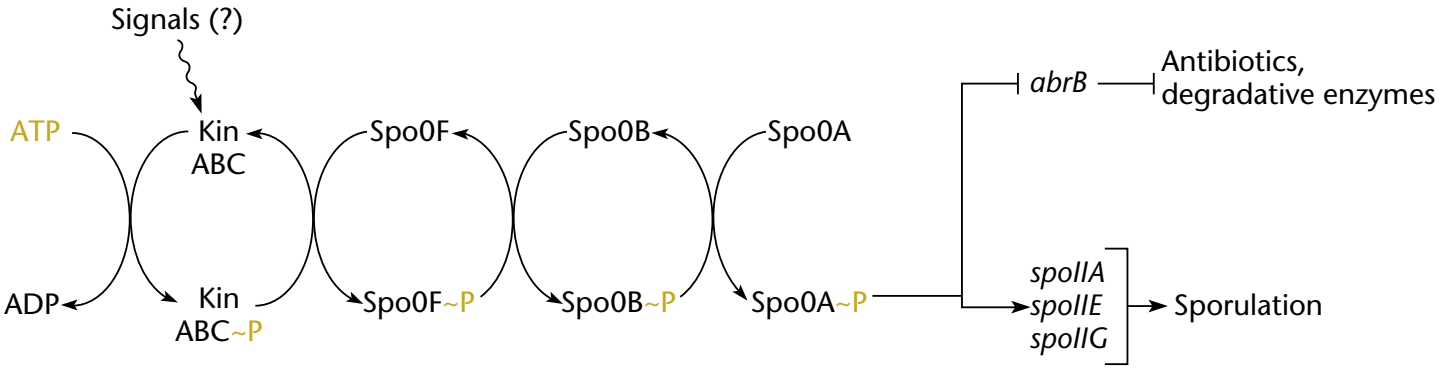

Signals (?)
ATP
Kin
ABC
ADP
Kin
ABC~P
Spo0F
Spo0F~P
Spo0B
Spo0B~P
Spo0A
Spo0A~P
abrB
Antibiotics,
degradative enzymes
spoIIA
spoIIE
spoIIG
Sporulation

Figure 14.16

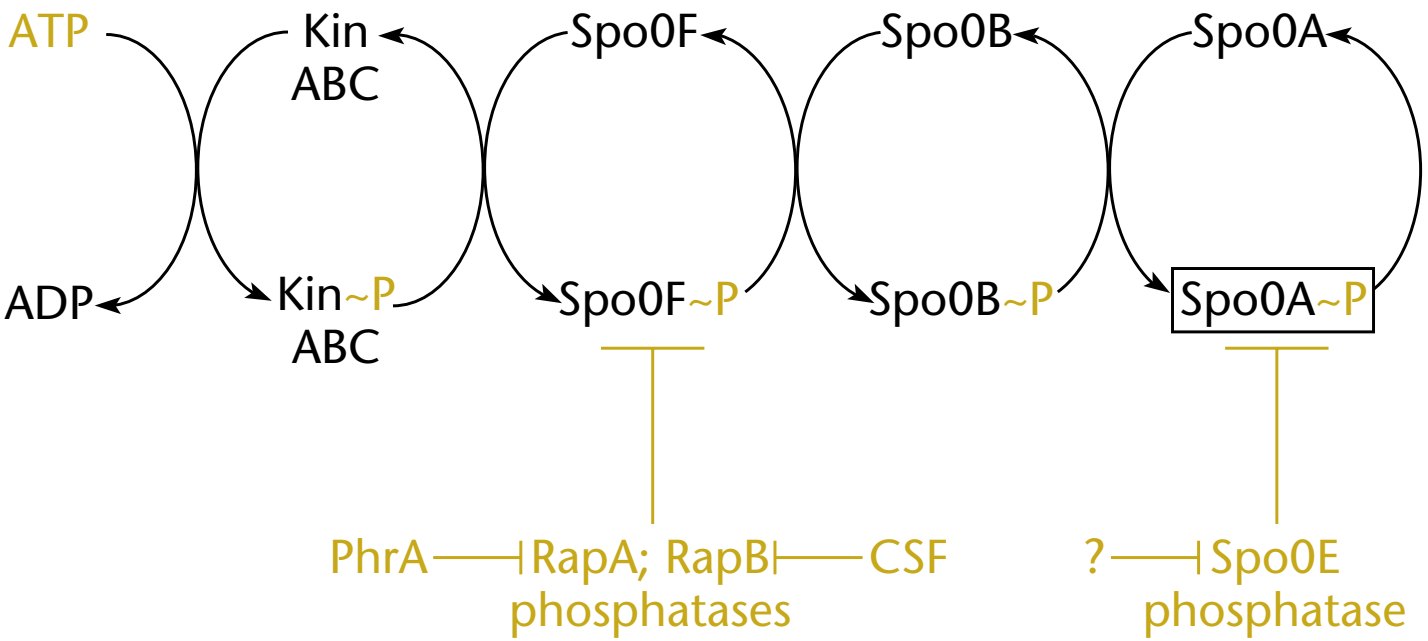

ATP
ADP
Kin
ABC
Kin~P
ABC
Spo0F
Spo0F~P
Spo0B
Spo0B~P
Spo0A
Spo0A~P
PhrA
RapA; RapB
phosphatases
CSF
?
Spo0E
phosphatase

Table 14.3

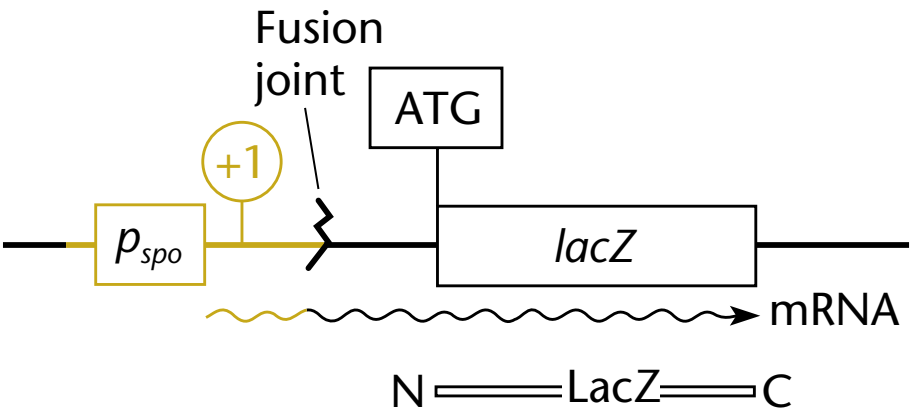
TABLE 14.3 Sporulation sigma factors and their genes

Sigma factor	*spo* gene name	*sig* gene name	Compartment of activity
σ^H	*spo0H*	*sigH*	Predivisional sporangium
σ^F	*spoIIAC*	*sigF*	Forespore
σ^E	*spoIIGB*	*sigE*	Mother cell
σ^G	*spoIIIG*	*sigG*	Forespore
σ^K	*spoIVCB-spoIIIC*	*sigK*	Mother cell

Figure 14.17

Transcriptional fusion

Promoter : *spo* gene
TIR : *spoVG* gene
Protein : LacZ



Translational fusion

Promoter : *spo* gene
TIR : *spo* gene
Protein : Spo-LacZ

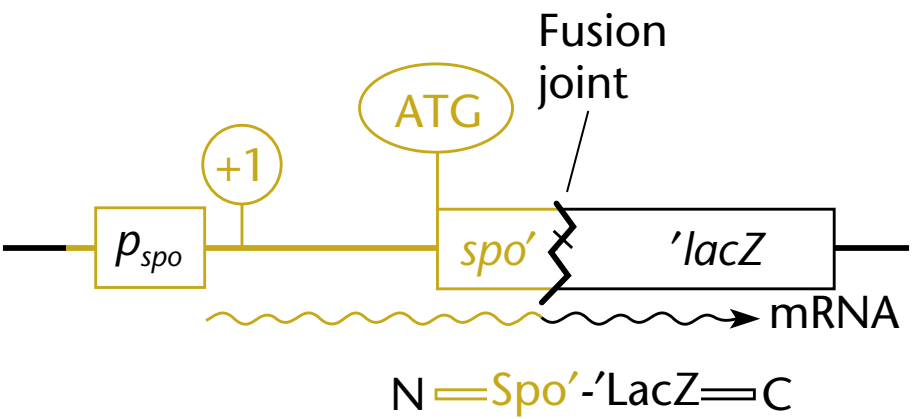
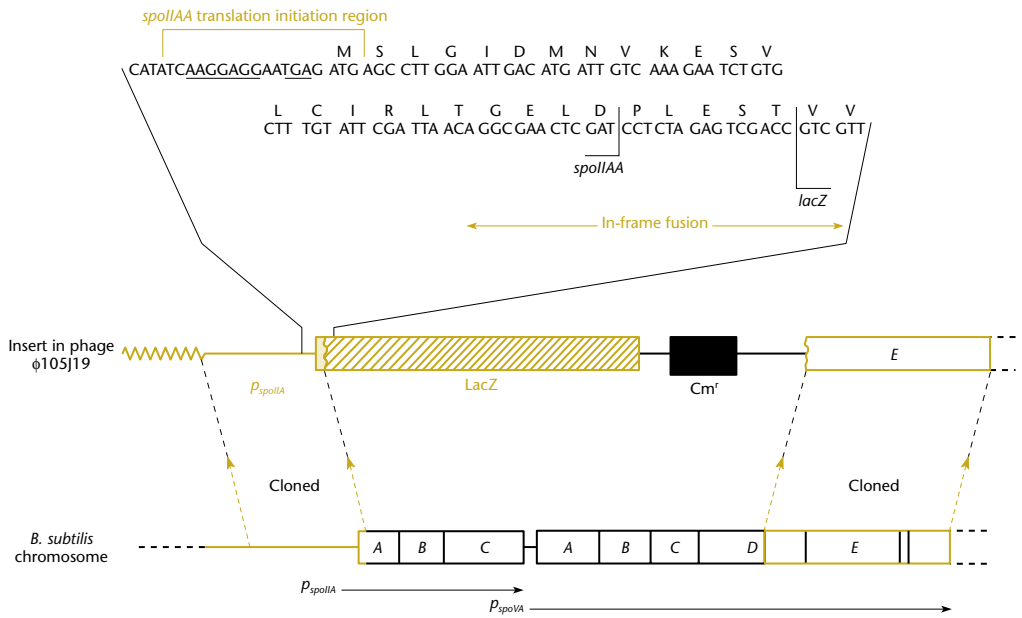


Figure 14.18

A



B

	Mutation	β-Galactosidase activity (units ml^{-1}) $t_{1.5}$	t_4	No. of determinations
spo0	spo0A43	0.016	0.037	2
	spo0B136	0.023	0.054	2
	spo0E11	0.018	0.015	2
	spo0F221	0.021	0.050	2
	spo0H17	<0.01	0.018	2
	spo0J93	0.048	0.25	2
	spo0K141	0.013	0.050	2
spoII	spoIIAA562	0.41	0.38	2
	spoIIAC1	0.44	0.51	2
	spoIIB131	0.36	0.17	3
	spoIID298	0.43	0.11	2
	spoIIE48	0.67	0.77	3
	spoIIG55	0.69	0.58	3
spoIII	spoIIIA65	0.18	0.22	4
	spoIIIB2	0.65	0.13	2
	spoIIIC94	0.24	0.23	4
spoIV	spoIVA67	0.29	0.080	3
	spoIVB165	0.12	0.19	3
	spoIVC23	0.29	0.15	3
spoV	spoVA89	0.44	0.34	2
	spoVB91	0.49	0.21	2
	spoVC134	0.61	0.46	2

Table 14.4

TABLE 14.4 Timing and dependence patterns of gene expression

Gene or operon fusion[a]	Time of expression (min)	Expression in mutants of genes or operons:					
		spo0	*spoIIA* (σ^F)	*spoIIE*	*spoIIG* (σ^E)	*spoIIIG* (σ^G)	*spoIVC* (σ^K)
spoIIA	40	–	+	+	+	+	+
spoIIE	30–60	–	+	+	+	+	+
spoIIG	0–60	–	+	+	+	+	+
gpr	80–120	–	–	–	+	+	+
spoIIIG	120	–	–	–	–	+	+
ssp	>120	–	–	–	–	–	+
spoIVC	150	–	–	–	–	+	+
cotA	240	–	–	–	–	–	–

[a]The functions of these are described in the following sections.

Figure 14.19

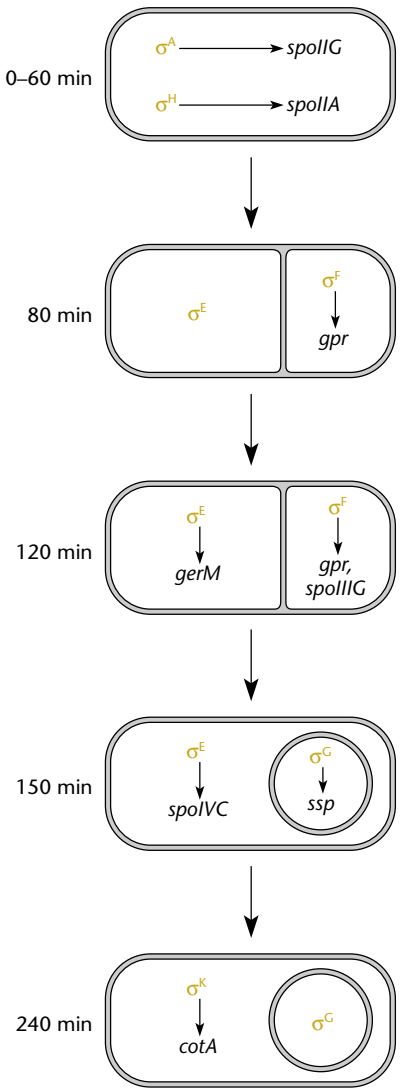

0–60 min
σA
spoIIG
σH
spoIIA
80 min
σE
σF
gpr
120 min
σE
gerM
σF
gpr,
spoIIIG
150 min
σE
spoIVC
σG
ssp
240 min
σK
cotA
σG

Figure 14.20

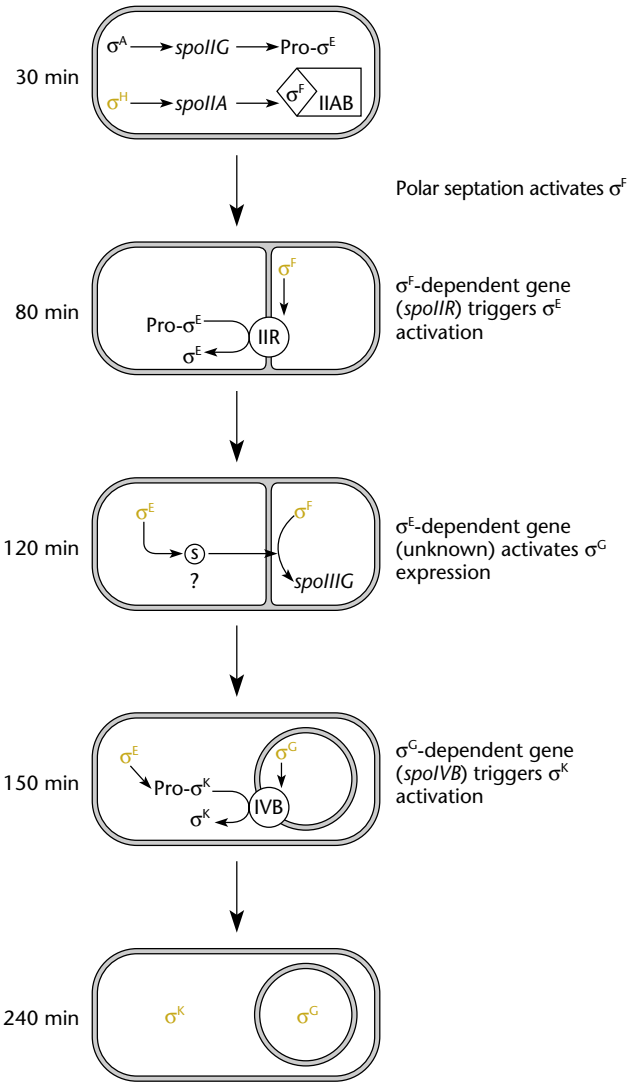

30 min
σA → spoIIG → Pro-σE
σH → spoIIA → σF IIAB
Polar septation activates σF
80 min
σF
Pro-σE
IIR
σE
σF-dependent gene (spoIIR) triggers σE activation
120 min
σE
S
?
σF
spoIIIG
σE-dependent gene (unknown) activates σG expression
150 min
σE
Pro-σK
σK
IVB
σG
σG-dependent gene (spoIVB) triggers σK activation
240 min
σK
σG

Figure 14.21

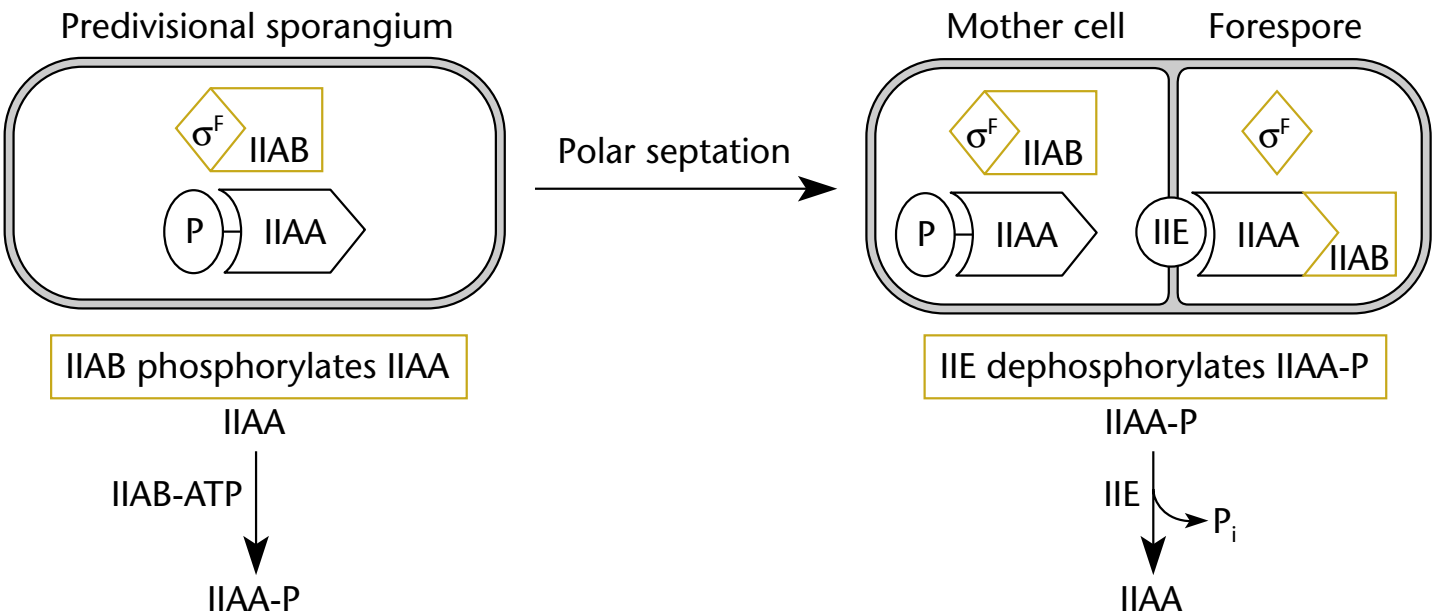
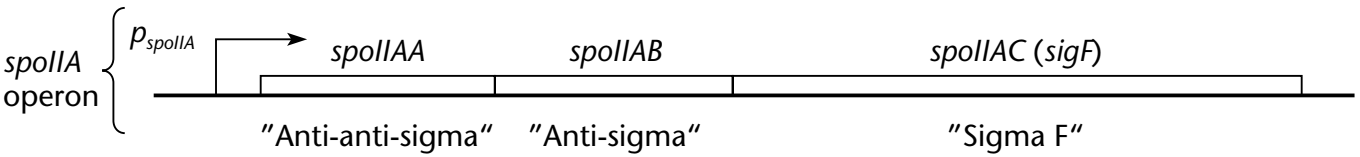

Predivisional sporangium
σF
IIAB
P
IIAA
Polar septation
Mother cell
Forespore
σF
IIAB
P
IIAA
IIE
IIAA
IIAB
σF
IIAB phosphorylates IIAA
IIAA
IIAB-ATP
IIAA-P
IIE dephosphorylates IIAA-P
IIAA-P
IIE
Pi
IIAA

Figure 14.22

A



B

Figure 14.23

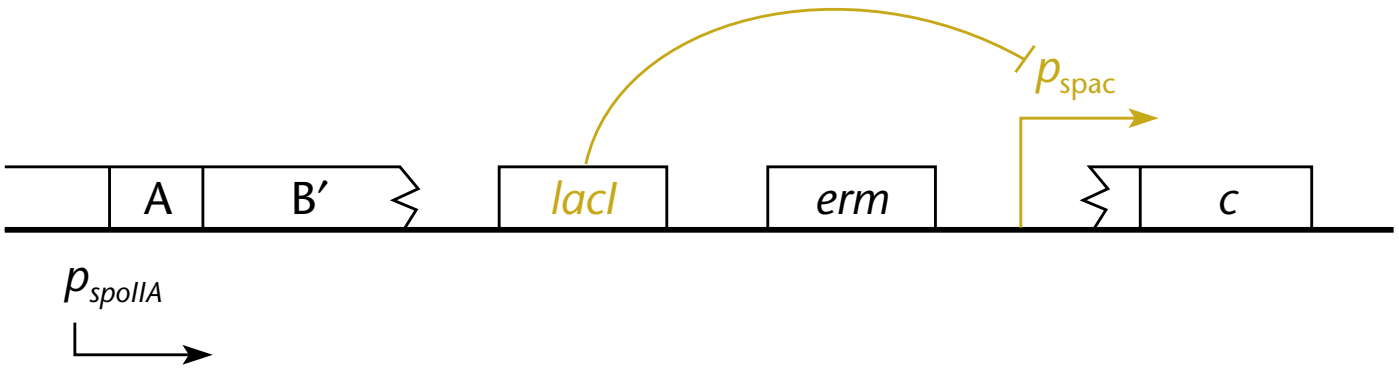


Figure 14.24

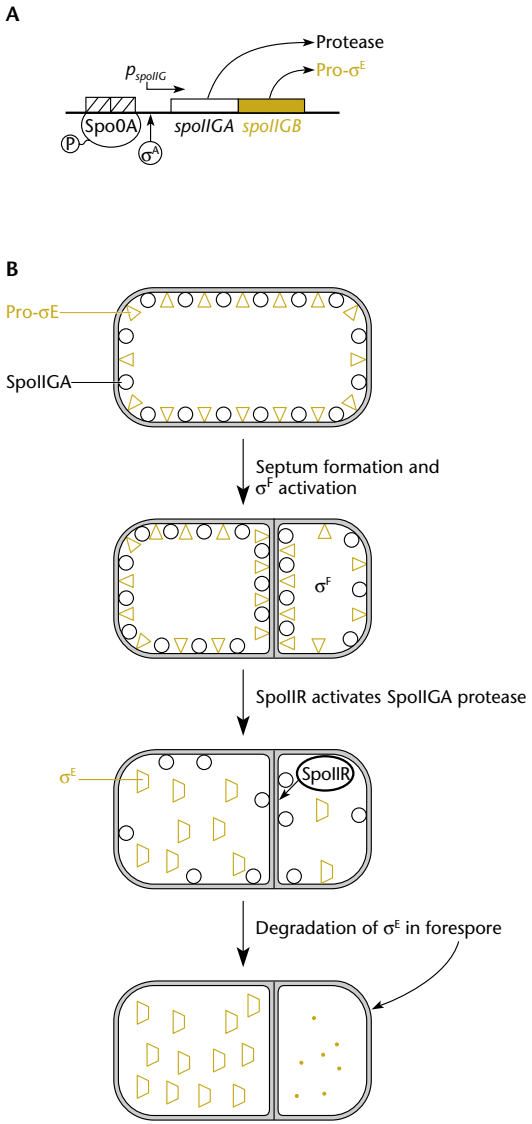
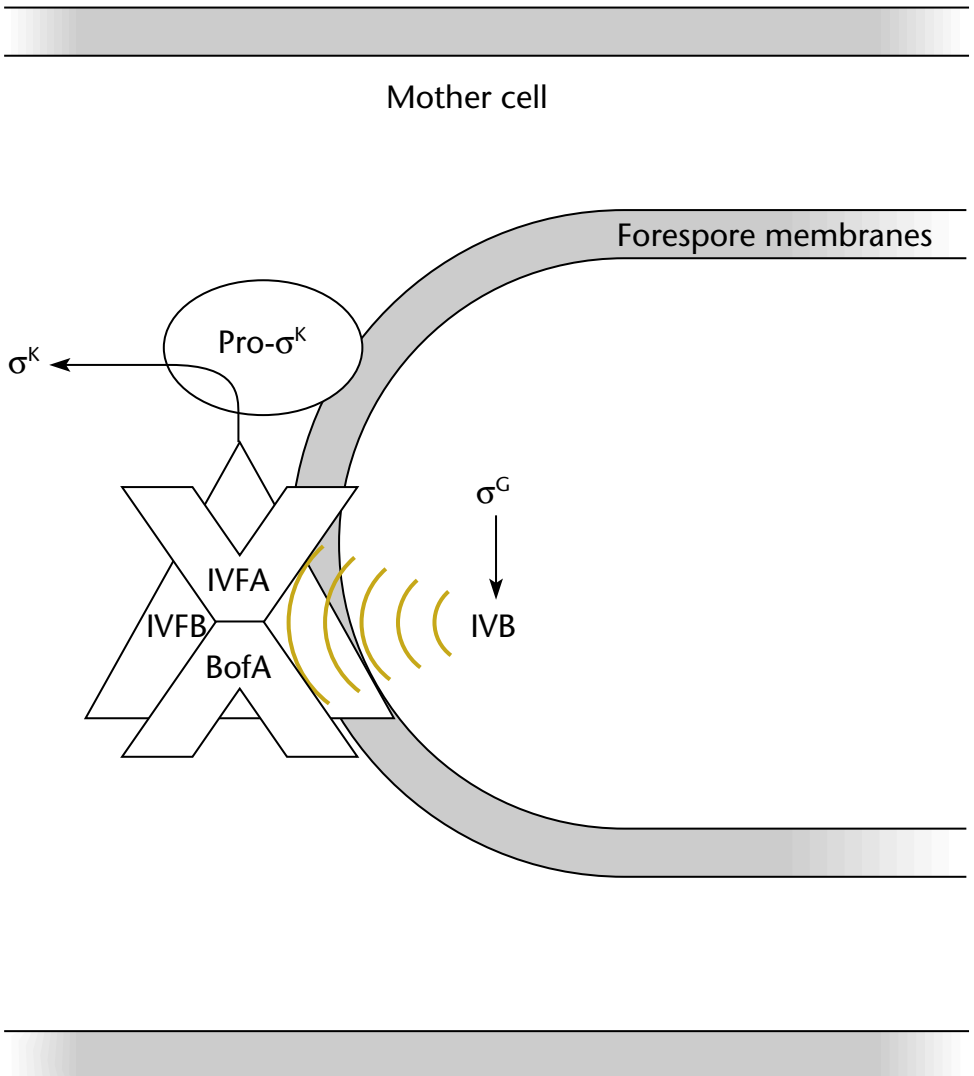

A
Protease
Pro-σE
p_{spoIIG}
Spo0A
P
σA
spoIIGA spoIIGB
B
Pro-σE
SpoIIGA
Septum formation and
σF activation
σF
SpoIIR activates SpoIIGA protease
σE
SpoIIR
Degradation of σE in forespore

Figure 14.25


Mother cell
Forespore membranes
Pro-σK
σK
σG
IVFA
IVFB
BofA
IVB