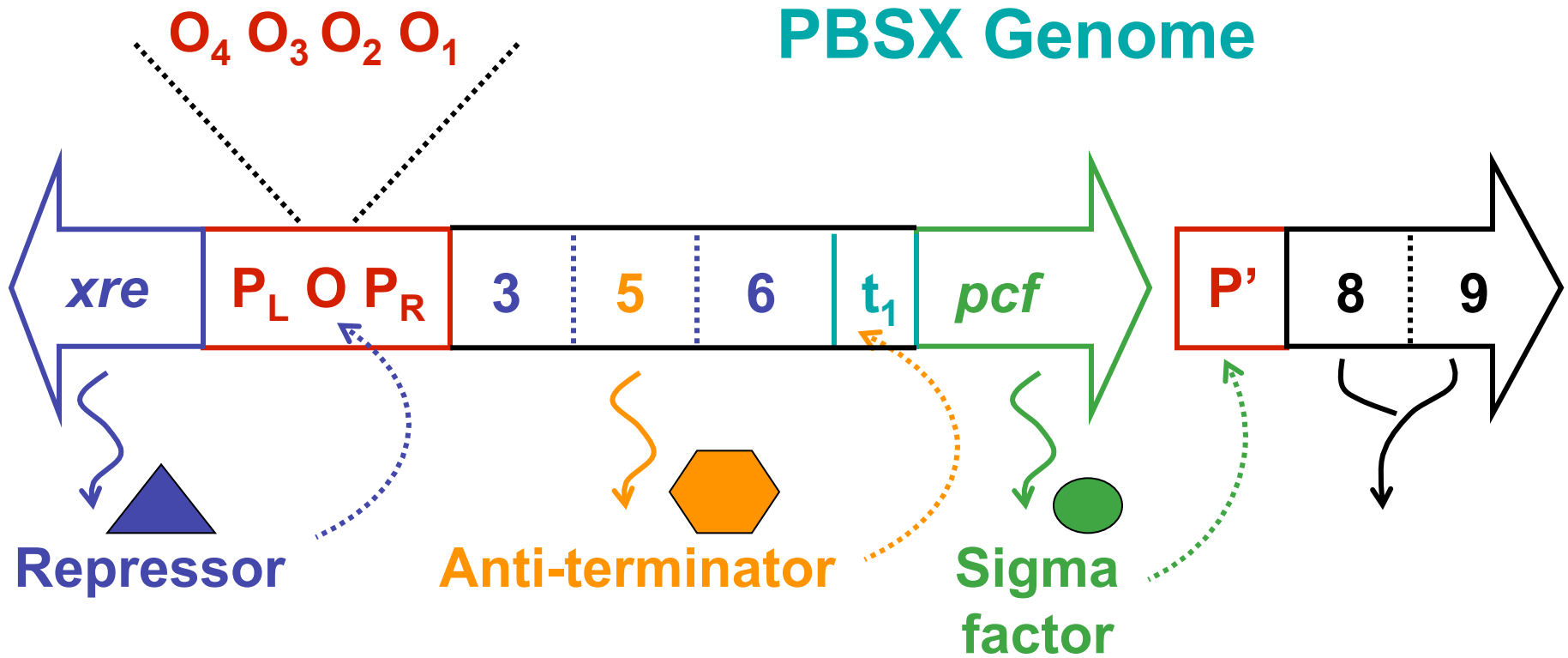


# Suicide Phage

**PBSX: defective phage of *Bacillus subtilis***



- **Temperate phage: forms lysogen**
- **Lytic phase is not reproductive**
  - 
  - **Phage particles packaged with 13 kb DNA fragments of bacterial DNA**
- **Phage particles attack and kill non-lysogens**
- **PBSX dies along with host ---> lysogen suicide**

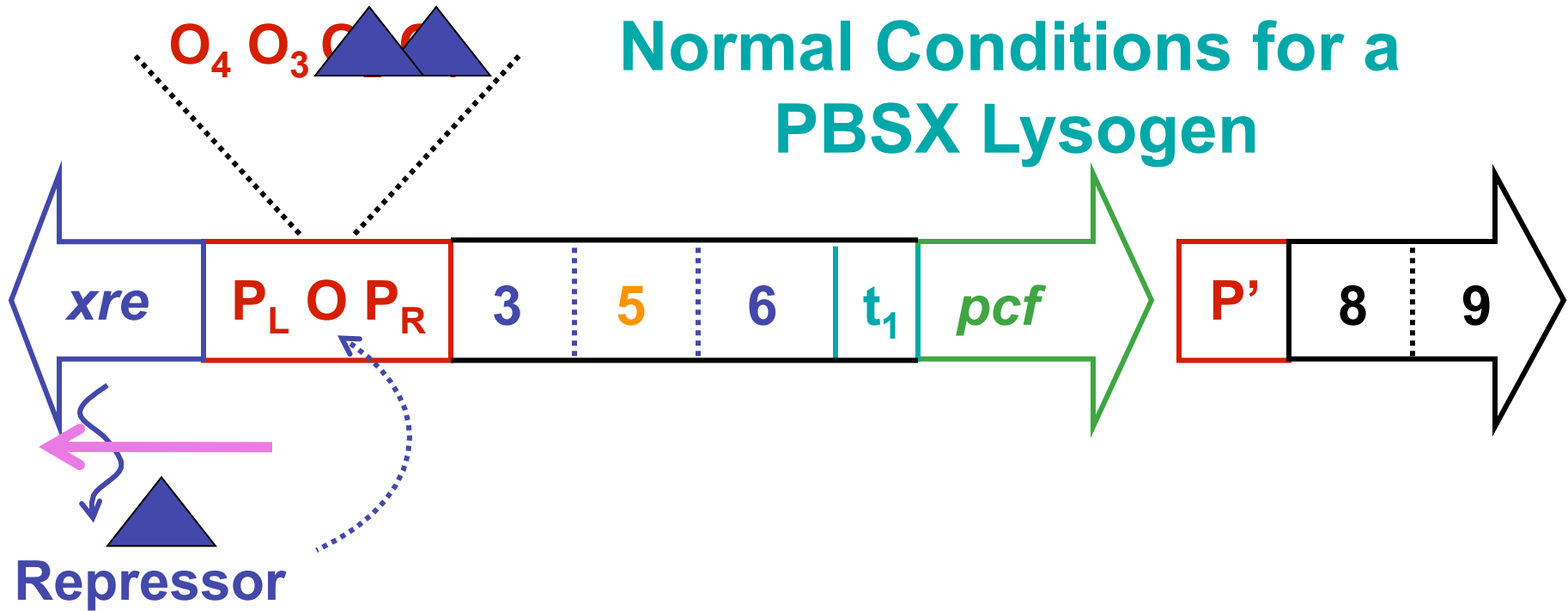


- ***xre*: encodes the phage repressor**
  - Operator affinity of Xre:
    - Low [Xre]: Xre binds to  $O_1$  and  $O_2$  repressing txn from  $P_R$
    - High [Xre]: Xre binds to  $O_3$  and  $O_4$  repressing txn from  $P_L$

## Traits of PBSX genome continued...

- **ORF5:** encodes a RNA binding protein which is an anti-terminator that allows txn through  $t_1$  to *pcf*
- *pcf*:
  - An alternate sigma factor that associates with host core RNAP to form a RNAP holoenzyme and allows for txn from  $P'$
- **ORFs 8 and 9:** Encode proteins necessary for packaging host DNA into particles that kill non-lysogens
- **3 different promoters**
  - $P_L$ - leftward promoter in which *xre* is transcribed
  - $P_R$ - rightward promoter in which *pcf* is transcribed
  - $P'$ - promoter for the lysis and packaging genes

## Normal Conditions for a PBSX Lysogen



- Xre binds to  $O_2$  and  $O_1$  repressing transcription from  $P_R$

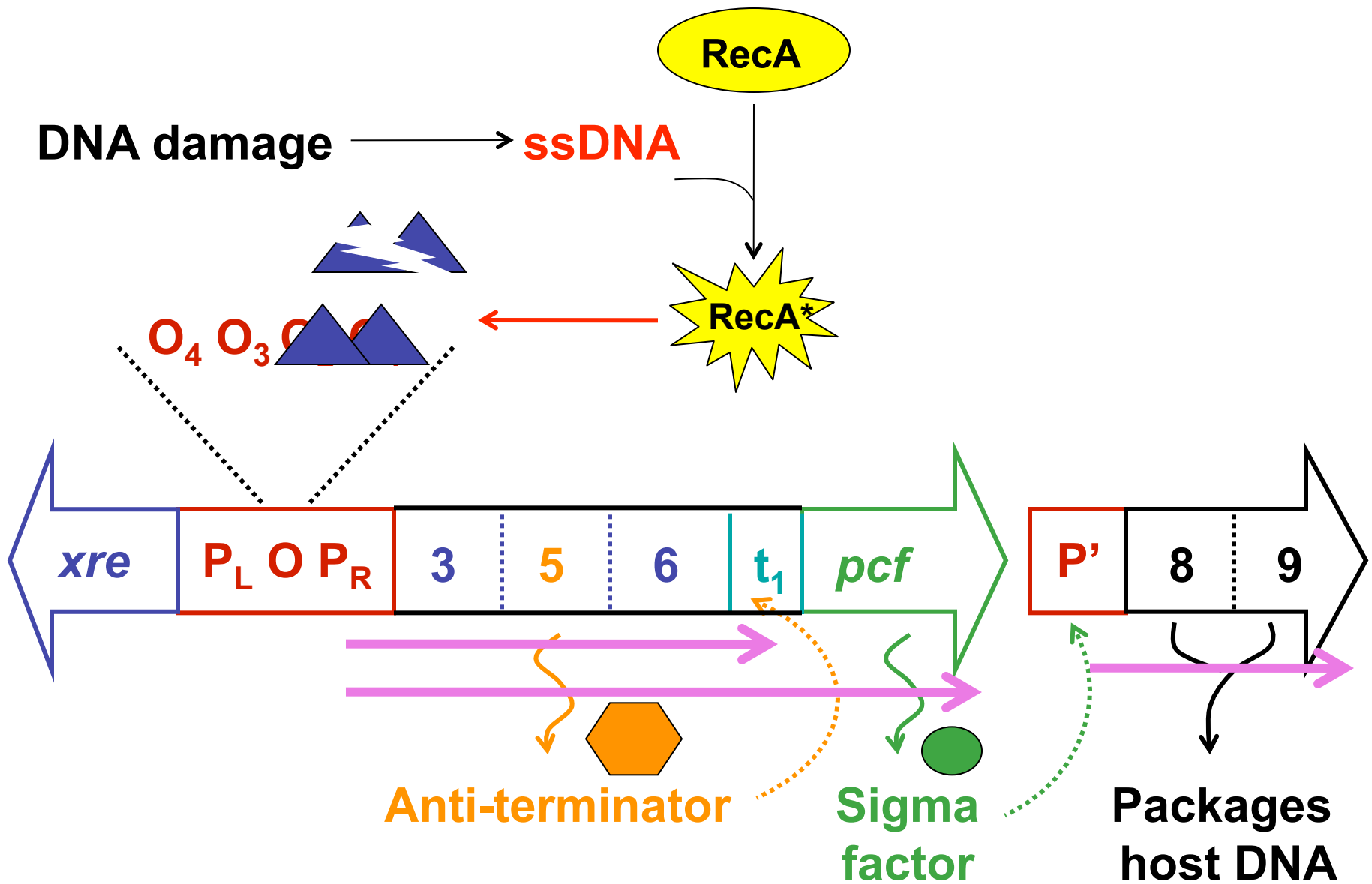


- Xre negatively autoregulates itself by binding to  $O_3$  and  $O_4$  repressing transcription from  $P_L$



# Induction of PBSX

- Under damaging conditions, PBSX lysogens will kill themselves to benefit other cells of its type (other PBSX lysogens)
  - 
  - Reduces competition for nutrients
  - Releases new nutrients from dead non-lysogens
  - 
  - Phage particles produced from induction adsorb to a new cell and kill the host before any DNA is injected
    - ↳ Only nonlysogenic *B. subtilis* cells



# Sequence of Events During Induction

1) Lethal damage occurs to the lysogen triggering the SOS response

2) RecA\* cleaves Xre → no longer bound to operator region

3

➤ ORF 5 is transcribed and translated: antiterminator

4) Antiterminator allows transcription from  $P_R$  thru  $t_1$  to *pcf*

➤

5) Pcf recognizes P' and allows transcription of genes for packaging and lysis

6) Released particles attach to non-lysogen cells and kill them by disrupting their cell walls

# How was the PBSX model determined?

- Cloned the DNA fragment containing P' upstream of a *lacZ* gene
- Integrated this cassette via homologous recombination into the genome of both a non-lysogen and a lysogen *B. subtilis* cell
- - Only the PBSX lysogen expressed  $\beta$ -galactosidase after treatment with mitomycin C
    - ↳ Results suggest that the cloned region contains a promoter sequence and that something encoded by the PBSX DNA activates TXN of the promoter

## Discovery of Pcf

- Created a library of plasmids containing ORFs from PBSX under the control of a constitutive promoter
- Introduced the library into the non-lysogenic strain containing the P' – *lacZ* fusion
- Isolated colonies with  $\beta$ -galactosidase activity and looked at the PBSX ORF from the plasmid



- Found that Pcf associates with RNAP core enzyme from host, thus hypothesized that Pcf is a sigma factor

# Overview of PBSX

- **Phage is defective in that it can no longer infect other cells and produce progeny or produce a lysogen**
- **PBSX lysogens were formed before the phage became defective, but can be induced under damaging conditions**
- **While phage particles are produced, they immediately kill the cell they attach to by cell wall interruption**
- **The mechanism is unknown, but the PBSX particles only kill the original lysogen and other non-lysogenic cells**
- **PBSX lysogens kill themselves to provide more nutrients to other PBSX lysogens that have not yet had the SOS response triggered**