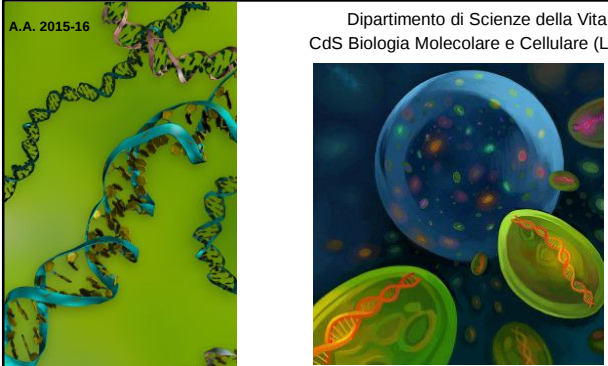


A.A. 2015-16

Dipartimento di Scienze della Vita
CdS Biologia Molecolare e Cellulare (LM-6)



Genetica dei Procarioti - 5

Prof. *Laura Marri*
ricevimento: previo appuntamento telefonico o e-mail

<http://ecocyc.org/>

LOGIN | Why Login? | Create New Account
Enter a gene, protein, metabolite or pathway: Quick Search | Gene Search | Search |
Searching *Escherichia coli* K-12 substr. MG1655 (EcoCyc) change organism database

EcoCyc *E. coli* Database

EcoCyc is a scientific database for the bacterium *Escherichia coli* K-12 MG1655. The EcoCyc project performs literature-based curation of the entire genome, and of transcriptional regulation, transporters, and metabolic pathways.

New to EcoCyc? Take the guided tour of the EcoCyc.org Web site, watch our free online instructional videos, or read our 2013 article: "EcoCyc: fusing model organism databases with systems biology"

EcoCyc User Guide >>

New version of the *E. coli* K-12 MG1655 sequence. The current release of EcoCyc uses the version of the *E. coli* genome sequence from GenBank record U00096.2. We plan to upgrade to version U00096.3 in our next release in April 2016. The nucleotide coordinates of genes and other features differ between U00096.2 and U00096.3. We will be offering a coordinate mapping service as part of the new release.

EcoCyc App is now BioCyc App
Have you ever wanted to quickly find out what is known about a gene, protein or pathway that was just mentioned in a talk or on a poster? Download the free BioCyc iPhone app to browse EcoCyc on the go! The NEW BioCyc app comes preloaded w/ EcoCyc.

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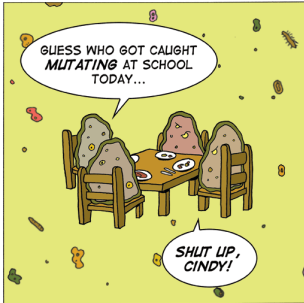
This is an alphabetical index to the individual GenePages on EcoGene. Point at a gene name for complete list. Alternatively, try EcoSearch to retrieve GenePages.

aaaD	aceE	actP	agaB	alaS	alsA	anmK	araH	argQ	aroB	artM	aspC	atpC	basR	betB	birA
cadB	cbdX	cdh	chiA	clbB	cobC	creA	csiE	cusA	cysD	cysJ	damX	denS	deoC	dicB	dmsA
dppC	dtpD	ecpD	emrB	epd	eutE	exuR	fadK	feaB	fetA	fimG	figE	flfF	flsA	frdC	frvX
ftsL	fusA	galU	gevH	gleC	glnA	glpC	gltJ	glyU	gor	gshB	gspO	hcaE	hemF	hipB	hlyE
holE	htpX	hlyB	hlyR	hnsD	ileY	insA	insC	insG	insL	intS	isrC	kdpF	laflU	lenD	llhO
lolA	lptG	lntG	macA	manX	mdtA	mdtQ	metE	nglB	nicF	nitF	moaD	mpl	mseL	murB	naaD
nanS	narW	nhaR	nobD	nrtE	nnoG	ompC	oppC	paaC	panD	penB	pfbB	pheA	phnN	pka	polA
pphB	prtB	proQ	pspD	ptaH	putA	pyrL	ralR	relB	recC	rfaA	rha	rhlA	rhlN	rhaA	rplC
rplX	rpsO	rpsP	rriD	rstS	rsuA	rutG	rzpR	sdaA	selB	sfmF	shoB	smuA	speE	sseA	stpA
tabA	tauD	tfaQ	this	tmpR	torT	trnI	trnC	tusA	ubiD	uhpC	uof	uxaB	vaaF	wcaC	wecH
xdhD	yaaW	yadS	yafS	yagN	yaiE	yajR	ybbN	ybdG	ybeY	ybgI	ybhM	ybiY	ycaI	yecF	yecJ
yegN	ygiG	ygiM	ydaQ	ydoJ	yddK	ydfD	ydgU	ydiB	ydiA	yeeH	yebG	yecR	yedZ	yegE	yehD
yehH	yfaD	yfbU	yfcZ	yfeH	yfgD	yfiN	yfiV	ygbN	ygdR	ygmM	yghA	ygiM	yhaJ	yhoB	yhdZ
yhgF	yhiL	yiaC	yihH	yicT	yieH	yihD	yiiE	yjbe	yjeZ	yjfi	yjhd	yjik	yjiv	ykgE	yliL

Frequenza di mutazione

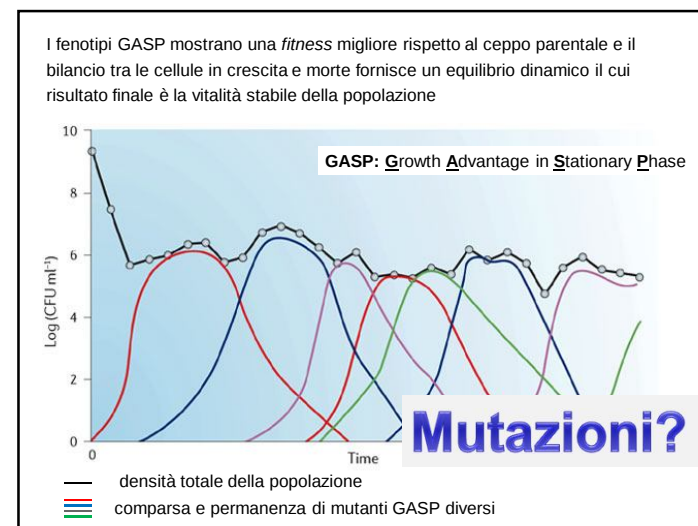
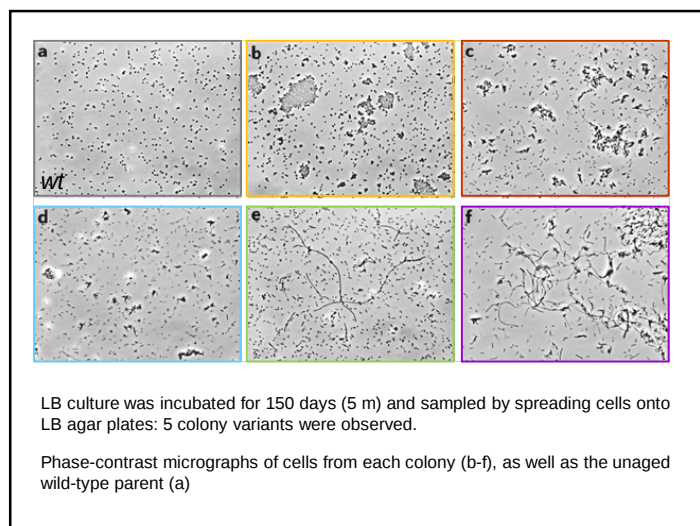
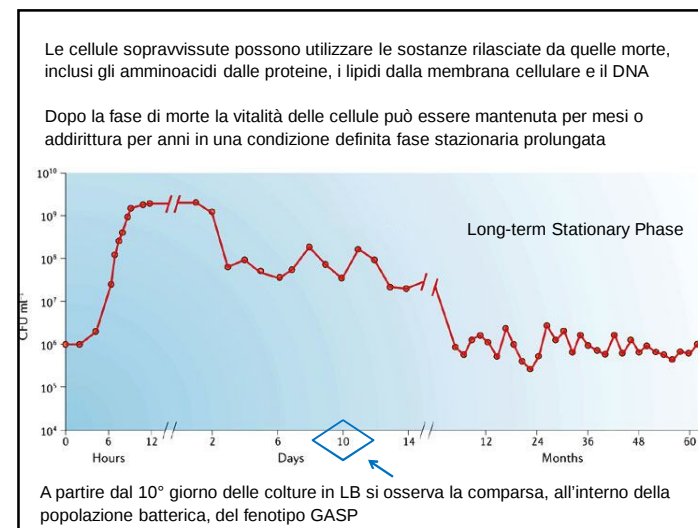
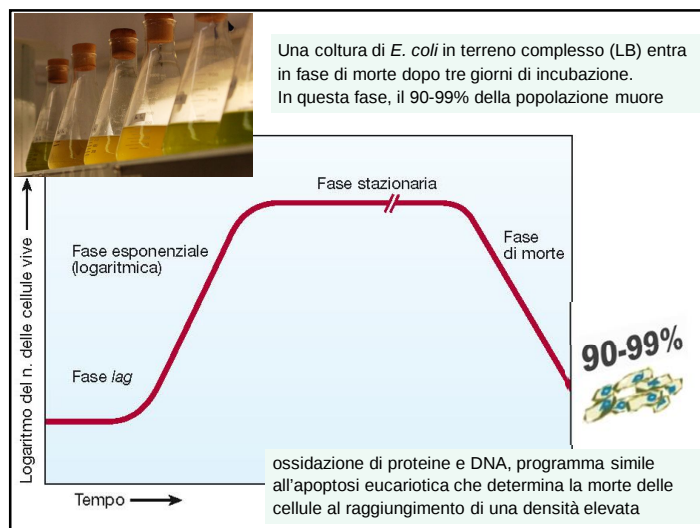
La frequenza con cui avvengono i diversi tipi di mutazioni è estremamente variabile.

Errori nella replicazione del DNA ricorrono con una frequenza di 10^{-6} 10^{-7} per coppia di basi per singolo ciclo di replicazione.

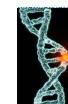


DINNER AT THE MICROBES

Aumenta in fase stazionaria: mutazione di una cellula ogni 300-400



Nome/Gene	Funzione
σ^{70} (<i>rpoD</i>)	Geni necessari durante la crescita esponenziale
σ^{54} (<i>rpoN</i>)	Geni espressi in condizioni di carenza di azoto
σ^{38} (<i>rpoS</i>)	Geni espressi durante la fase stazionaria e in condizioni di stress generale
σ^{32} (<i>rpoH</i>)	Geni espressi durante <i>heat-shock</i> (chaperoni per ristabilire il <i>folding</i> corretto delle proteine citoplasmatiche)
σ^{28} (<i>rpoF</i>)	Geni codificanti per proteine coinvolte nell'assemblaggio del flagello
σ^{24} (<i>rpoE</i>)	Geni espressi in condizioni di stress termico per ripristinare l'integrità di membrana e assicurare il <i>folding</i> corretto delle proteine di membrana
σ^{19} (<i>fecI</i>)	Geni espressi in relazione al trasporto del citrato ferrico



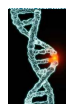
rpoS

duplicazione di 46 bp all'estremità 3' → proteina in cui i **4** aminoacidi presenti a C-terminale sono sostituiti con **39** nuovi aminoacidi

indebolimento dell'espressione del *regulon* (in *E. coli* RpoS controlla l'espressione di oltre il 10% dei geni)

sbilanciamento della competizione tra i vari fattori sigma per il core della RNA polimerasi.

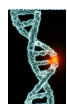
Cellule con una ridotta espressione di *rpoS* favorirebbero l'associazione dei fattori sigma **RpoD** (σ^{70}) e **RpoN** (σ^{54}) alla RNA polimerasi determinando una *fitness* migliore rispetto al ceppo *wild-type*



Irp

Lrp: regolatore trascrizionale (attivatore/repressore) che controlla l'espressione di molti geni implicati nel metabolismo e nel trasporto degli aminoacidi

maggiore capacità di recuperare gli aminoacidi rilasciati dalle cellule morte, in particolare: **serina, treonina e alanina**



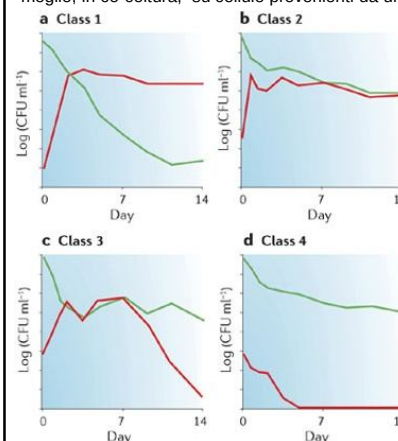
Attivazione operone (*ybeJ*)-*gltJKL*

riarrangiamento genomico prodotto da *IS5*.

Quattro geni di questo operone sono implicati nella regolazione del trasporto per l'aspartato e il glutammato

GASP: Growth Advantage in Stationary Phase

capacità delle cellule derivate da una cultura in *batch* "invecchiata" di avere la meglio, in co-cultura, su cellule provenienti da una cultura "giovane"



I grafici rappresentano il comportamento diverso che i mutanti GASP noti (linea rossa) possono assumere quando presenti, inizialmente in minoranza, in co-cultura con il ceppo *wild-type* (linea verde) [Finkel, 2006].

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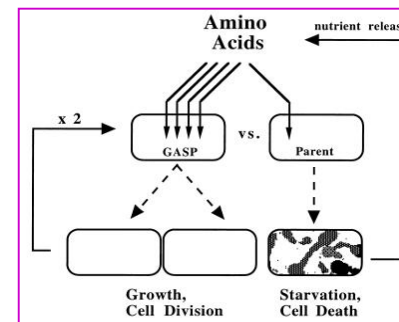
Evolutionary Cheating in *Escherichia coli* Stationary Phase Cultures

Marin Vulić and Roberto Kolter



I bari, Caravaggio (Michelangelo Merisi) 1595. Kimbell Art Museum (Fort Worth-USA)

mutanti GASP: "truffatori evolutivi", ignorano i segnali di inibizione della crescita e continuano a moltiplicarsi



Un modello delle basi fisiologiche del fenotipo GASP

(Zinser e Kolter 1999)



John F. Nash (1928) matematico ed economista statunitense.

EQUILIBRIUM POINTS IN N-PERSON GAMES
BY JOHN F. NASH, JR.*
PRINCETON UNIVERSITY
Communicated by S. Lefschetz, November 18, 1949

La competizione osservata all'interno di popolazioni batteriche è stata interpretata facendo riferimento alla **"teoria dei giochi"**

i "cooperatori" (WT-WT) coesistono, entrambi bloccano anticipatamente la crescita e sono premiati attraverso la redistribuzione delle risorse necessarie al mantenimento della popolazione per lungo tempo

i "cooperatori" (WT) che incontrano i "truffatori" (GASP) sono imbrogliati e le loro risorse vengono consumate prematuramente dai secondi

i "truffatori" che coesistono tra loro (GASP & GASP) sono puniti poichè continuano a crescere senza controllo mettendo a rischio la vitalità a lungo termine

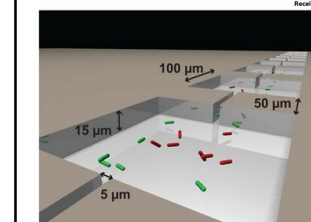
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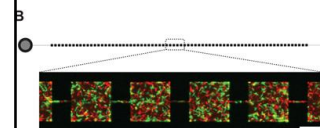
Spatial Structure Facilitates Cooperation in a Social Dilemma: Empirical Evidence from a Bacterial Community

Felix J. H. Hol^{1,2*}, Peter Galajda^{1,2,3}, Krisztina Nagy², Rutger G. Wootthuis¹, Cees Dekker¹, Juan E. Keeney^{1,2,4}

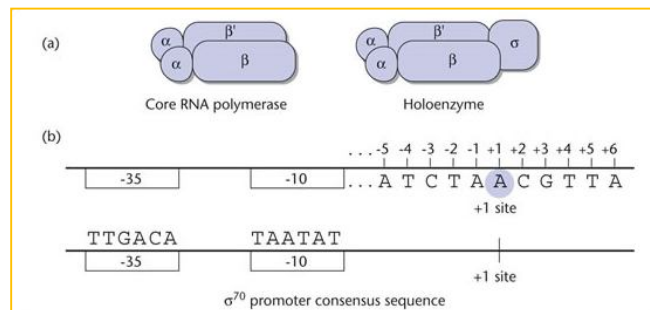
Received May 13, 2013; Accepted September 6, 2013; Published October 22, 2013



in un habitat artificiale spazialmente strutturato (micro lastre in silicone) in cui la disponibilità dei nutrienti è omogenea, WT e GASP coesistono



Most bacteria encode multiple sigma factors (some of which are specialized and only used at certain times). Each binds a different promoter consensus sequence.

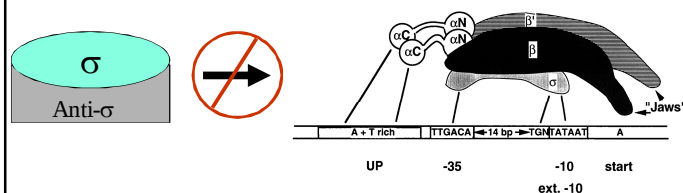


Sequenze "consenso" diverse per alcuni fattori σ .

FATTORE σ	SEQUENZA CONSENSO DEL PROMOTORE	
	Regione -35	Regione -10*
σ^{70} (<i>rpoD</i>)	TTGACA	TATAAT
σ^{32} (<i>rpoH</i>)	TCTCNCCCTTGAA	CCCCATNTA
σ^{28} (<i>rpoF</i>)	CTAAA	CCGATAT
	Regione -24	Regione -12
σ^{54} (<i>rpoN</i>)	CTGGNA	TTGCA

* La regione -10 è anche detta *Pribnow box*, dal nome di chi l'ha identificata.

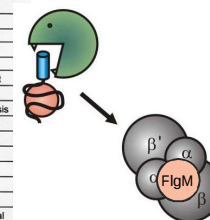
Some sigma factors are expressed but inactive due to anti-sigma factors

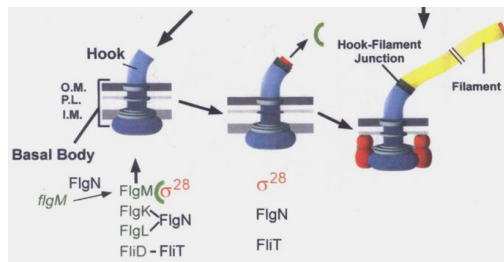


Anti-sigma factors tightly (yet reversibly) bind sigma factors and prevent their activity.

Organism	Sigma factors	Anti- σ factors	Size(aa)	Function
Bacteriophage T4	<i>E. coli</i> σ^{70}	AsiA	90	Housekeeping
<i>Bacillus subtilis</i>	σ^F	SpolIAB	147	Sporulation
	σ^G	SpolIAB	147	Sporulation
	σ^B	RabW	160	Stress response
	σ^D	FlgM	97	Flagellar biosynthesis
<i>Salmonella typhimurium</i>	σ^{28}	FlgM	97	Flagellar biosynthesis
<i>Escherichia coli</i>	σ^{24}	DnaK	638	Heat shock
<i>Streptomyces coelicolor</i>	σ^{WhiG}	?	?	Sporulation
ECF subfamily				
<i>Alcaligenes eutrophus</i>	σ^{ChrH}	Orf1	192	CorNi resistance
<i>Azotobacter vinelandii</i>	σ^{AlgU}	MucA	194	Alginate biosynthesis
<i>Bacillus subtilis</i>	σ^{SigK}	?	?	?
	σ^{SigV}	Anti-SigV	285	?
	σ^{SigZ}	?	?	?
<i>Erwinia amylovora</i>	σ^{22}	?	?	Virulence factors/
				Hypersensitivity
<i>Escherichia coli</i>	σ^E	RseA/MclA	208	Extreme temperature survival
	σ^{FedI}	FedR	317	Ferri-Citrate transport
<i>Haemophilus influenzae</i>	σ^E	RseA/MclA	195	?
<i>Mycococcus xanthus</i>	σ^{CarQ}	CarR	221	Carotenoid biosynthesis
<i>Mycobacterium tuberculosis</i>	σ^E	?	?	?
<i>Mycobacterium leprae</i>	σ^E	?	?	?
<i>Salmonella typhimurium</i>	σ^E	?	?	?
<i>Streptomyces coelicolor</i>	σ^E	?	?	Agarase synthesis
<i>Sulfolobus acidocaldarius</i>	σ^E	?	?	?
<i>Synechocystis</i> sp.	σ^E	?	?	?
<i>Photobacterium SS9</i>	σ^E	Orf2	232	High pressure survival
<i>Pseudomonas aeruginosa</i>	$\sigma^{24}(\text{AlgT/AlgU})$	MucA	194	Alginate biosynthesis
	σ^{Pds}	?	?	Pyoverdine biosynthesis
<i>Pseudomonas putida</i>	σ^{PupI}	PupR	316	Pseudobactin transport
<i>Pseudomonas syringae</i>	σ^{HPL}	?	?	Virulence factors/
				Hypersensitivity
<i>Rhodospirillum rubrum</i>	σ^E	ChrR	213	Cytochrome c_2 expression

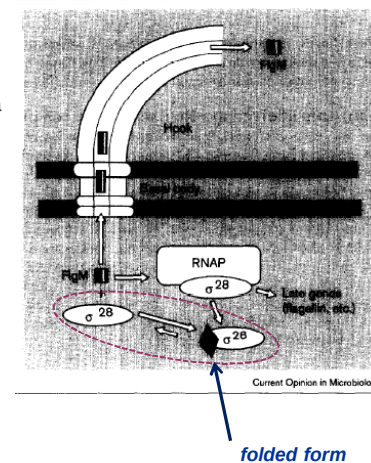
List of anti-sigma factors and their cognate sigma factors



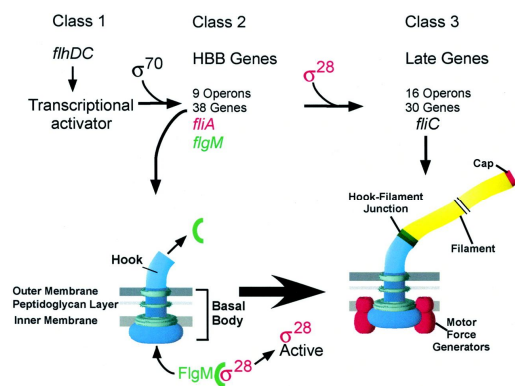


durante le fasi iniziali della biogenesi flagellare σ^{28} è mantenuto inattivo dalla stretta associazione con FigM

l'uncino ed il corpo basale sono assemblati FigM viene secreto attraverso il sistema di trasporto flagellare (T3S)

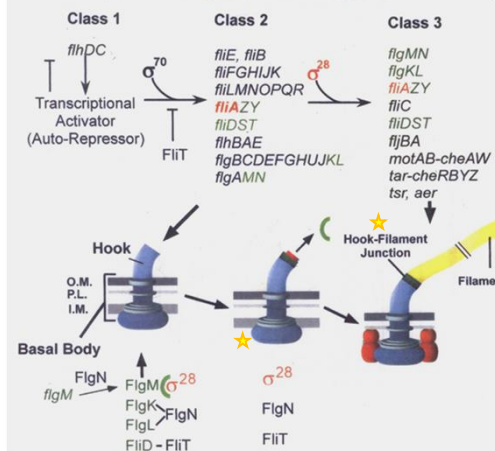


The coupling of flagellar gene regulation to flagellum assembly.

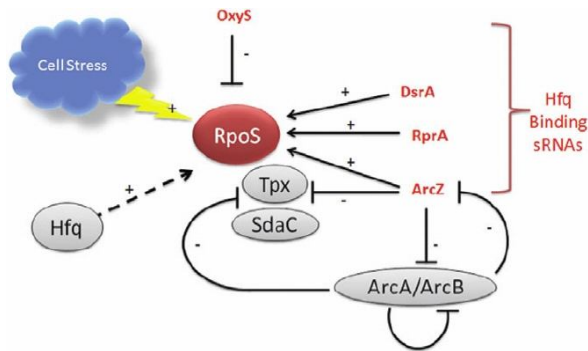


Chevance F F V et al. J. Bacteriol. 2006;188:297-304

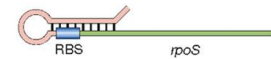
Transcriptional Regulation of Flagellar Assembly



RpoS controlla l'espressione di ~500 geni

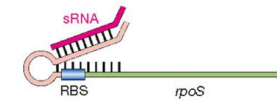


è sottoposto a un controllo post-trascrizionale



Il sito di legame al ribosoma (RBS) dell'mRNA rpoS è appaiato alla regione 5' non tradotta dell'mRNA.

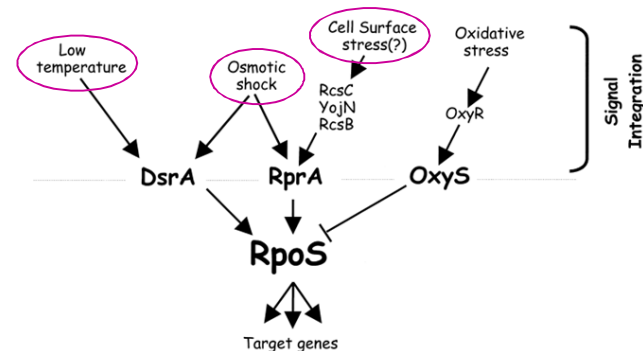
Prodotti sRNA che, legandosi al 5' dell'mRNA, impediscono la formazione della struttura secondaria e permettono la traduzione di RpoS (σ^S).



almeno 3 sRNA coinvolti: DsrA, RprA and OxyS

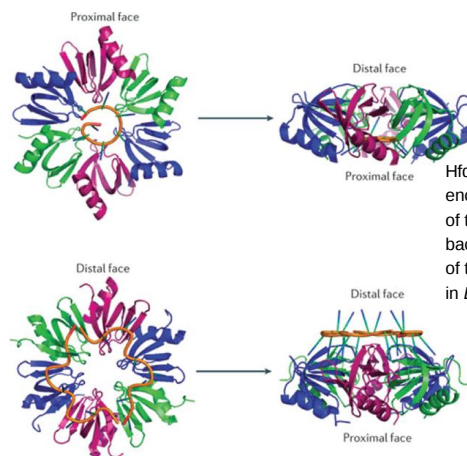
- sRNA (*small RNA*), possono svolgere la loro azione regolativa appaiandosi a regioni specifiche di un RNA bersaglio perfettamente o parzialmente complementare (sRNA antisense)
- Gli RNA antisense sono molecole piccole (da poche decine a poche centinaia di nucleotidi), diffusibili

The abundance and, therefore, the activity of each is in turn regulated by different environmental cues.



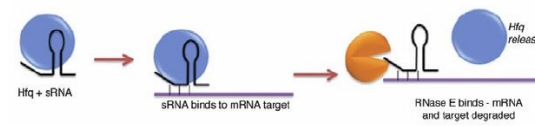
All require the protein Hfq, an RNA chaperone that facilitates RNA-RNA pairing. Hfq has a toroid structure, with a pore in the center.

Hfq consists of six identical subunits arranged in a ring shape structure



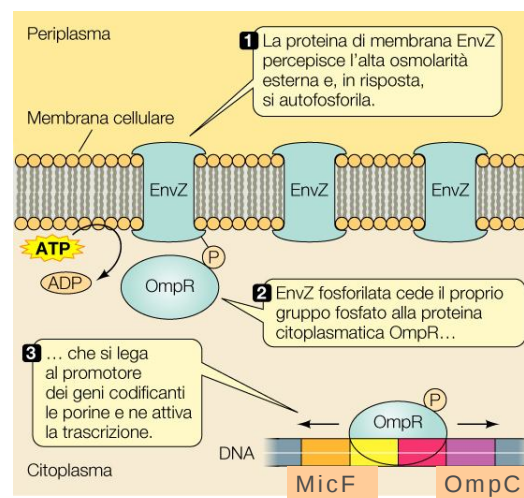
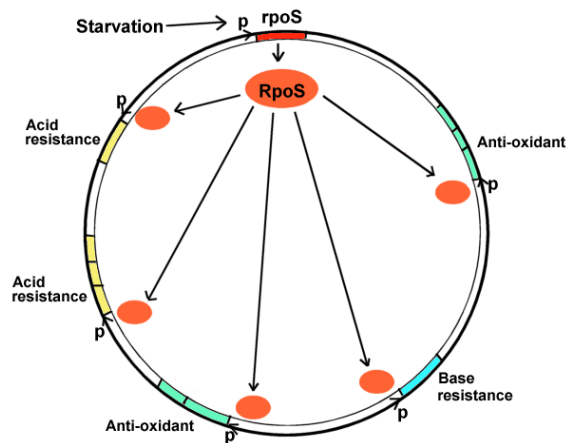
Hfq is an RNA-binding protein encoded in approximately half of the (in 2002) sequenced bacterial genomes and is one of the most abundant proteins in *E. coli*.

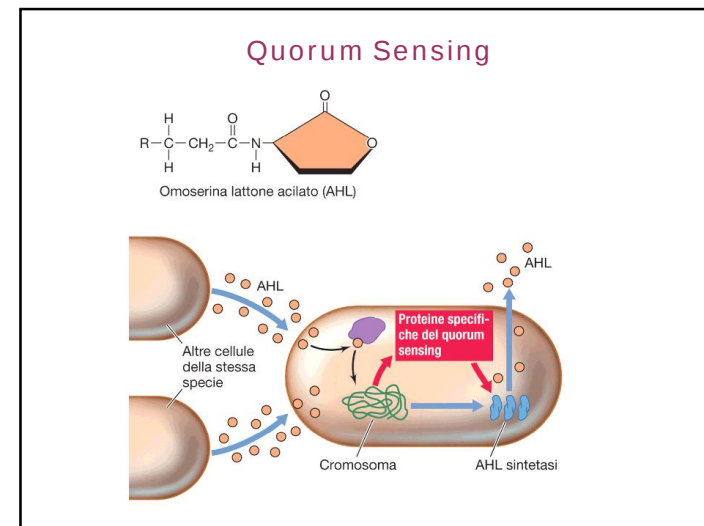
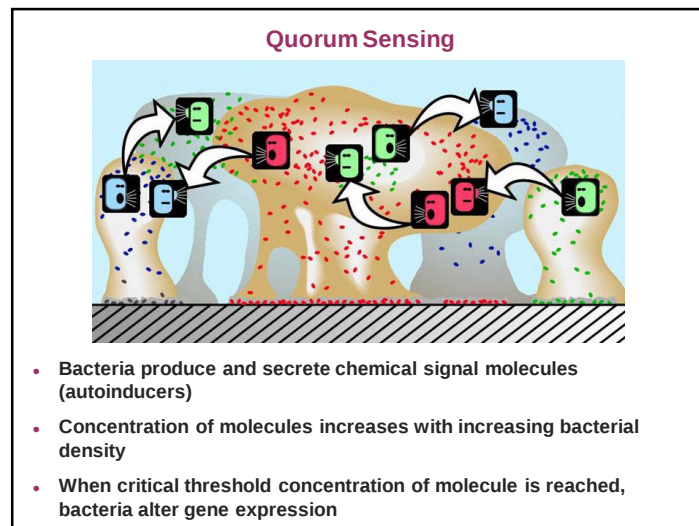
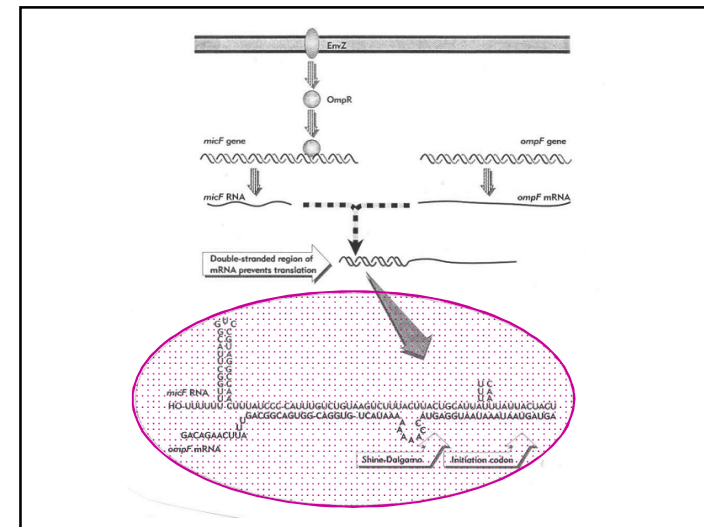
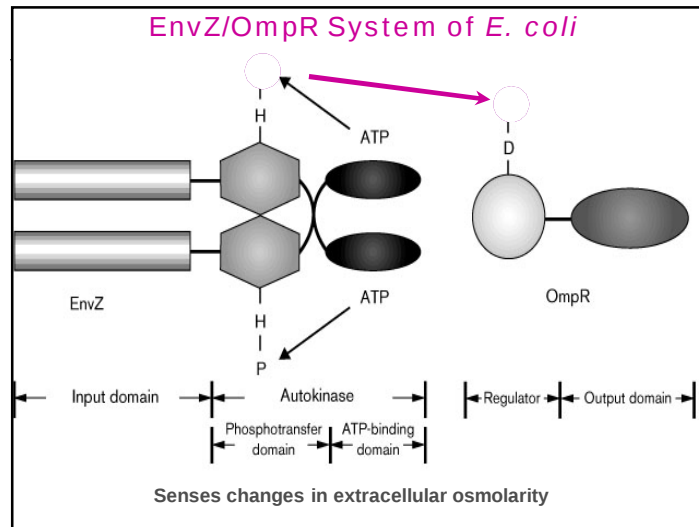
The typical action of an Hfq-associated sRNA.



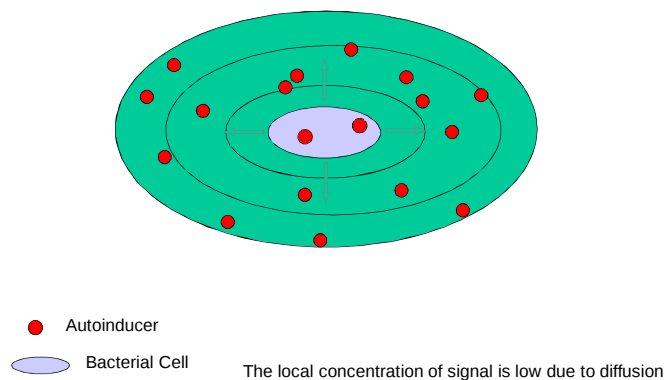
The sRNA binds to the Hfq protein and then finds its target mRNA

regolazione mediante fattori sigma alternativi

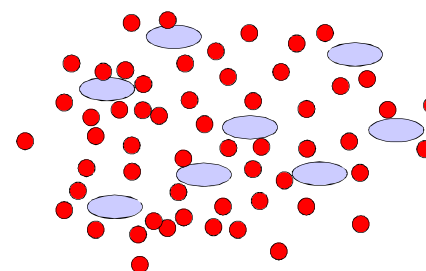




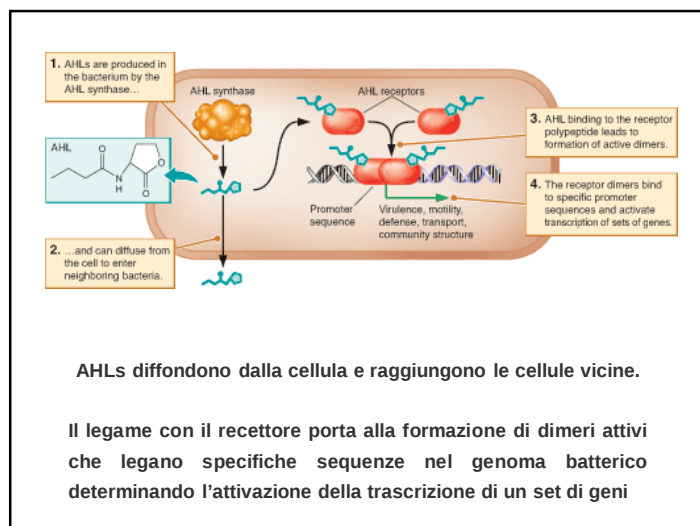
Quorum sensing



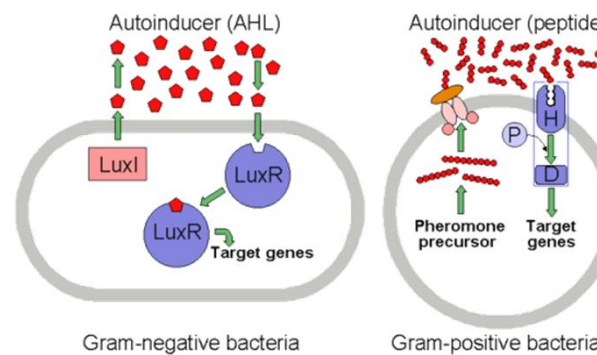
At high cell densities the local concentration of Inducer becomes high

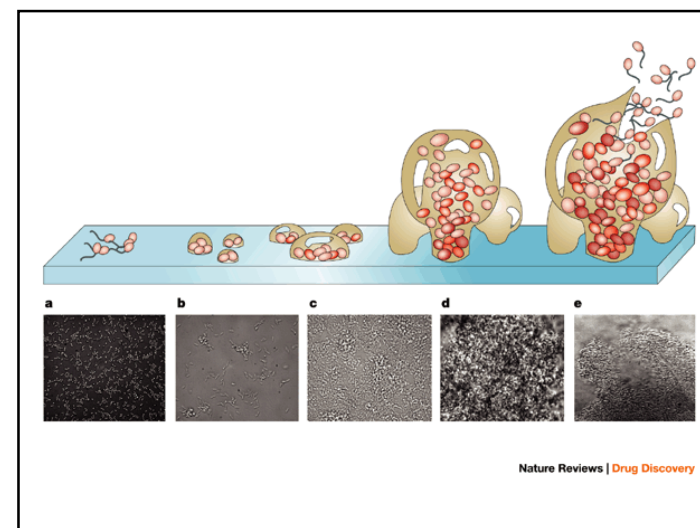
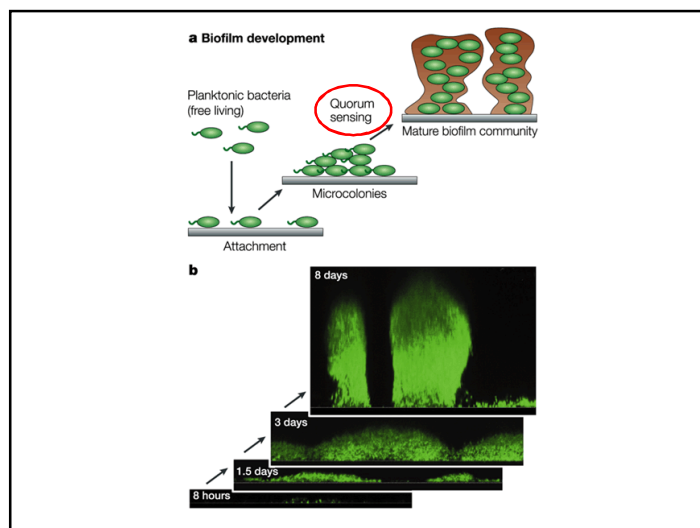
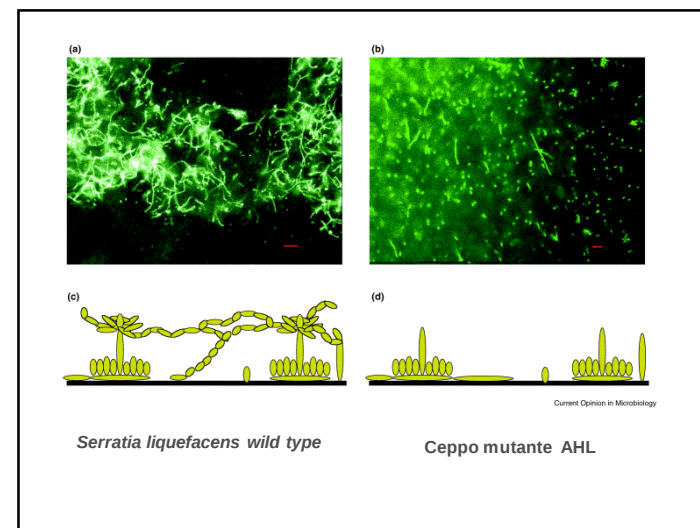
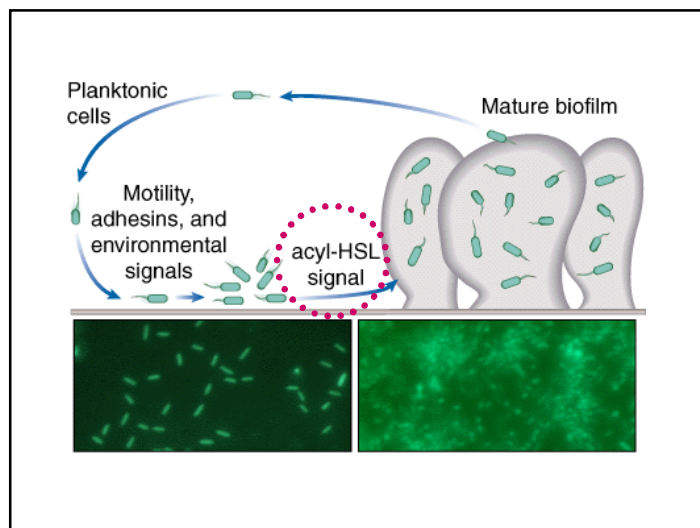


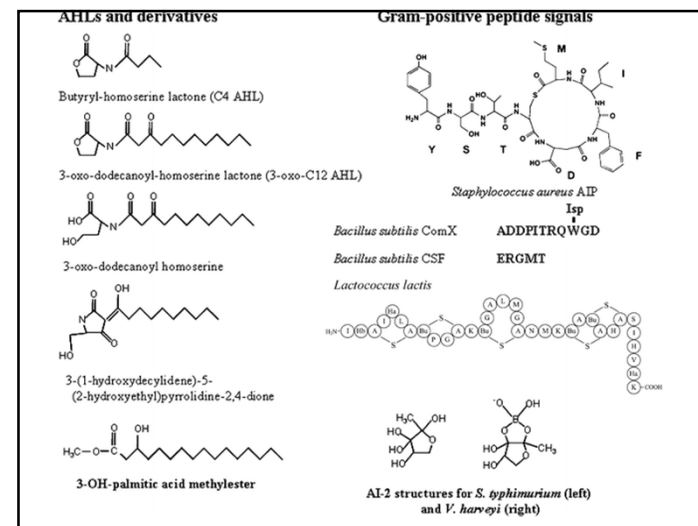
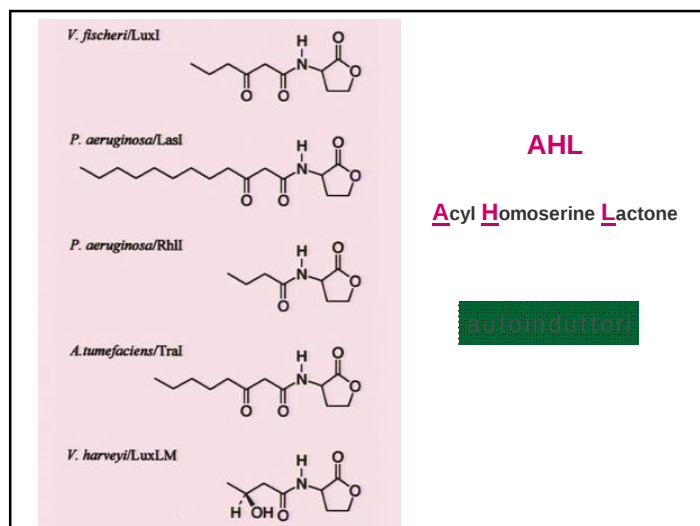
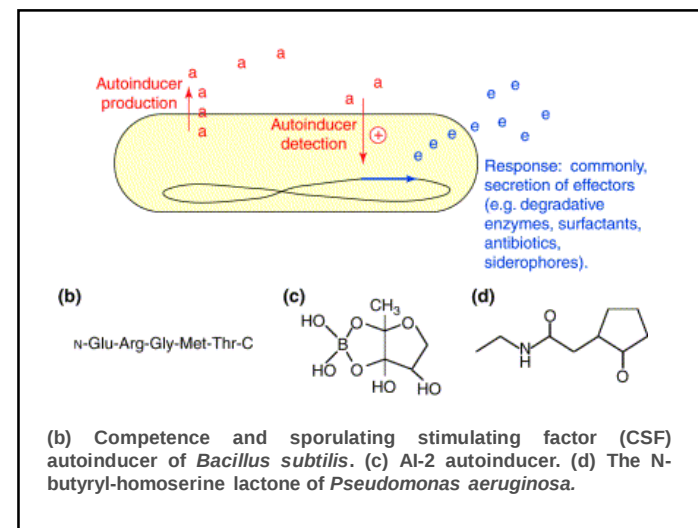
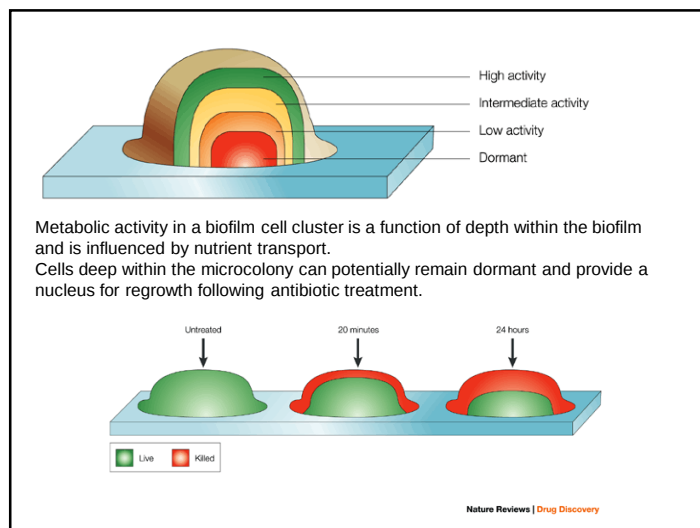
Bacteria sense this and alter gene expression

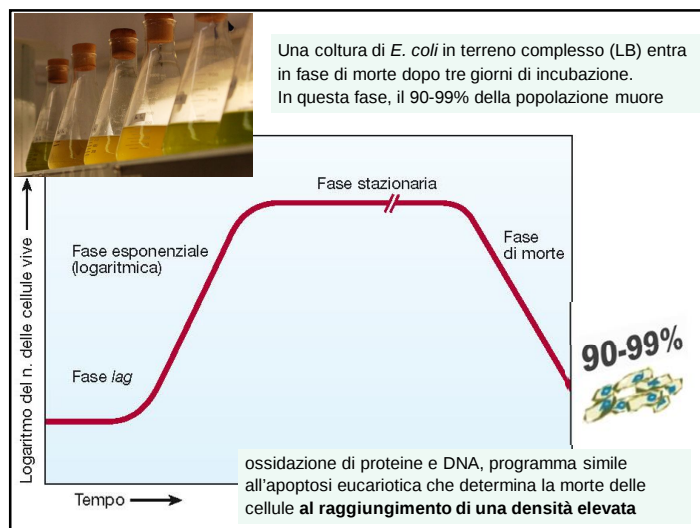


Gram negative and Gram positive bacteria use different signaling molecules (Acyl Homoserine lactones vs peptides)







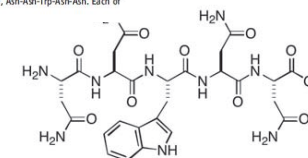


26 OCTOBER 2007 VOL 318 SCIENCE

A Linear Pentapeptide Is a Quorum-Sensing Factor Required for *mazEF*-Mediated Cell Death in *Escherichia coli*

Ilana Kolodkin-Gal,¹ Ronen Hazan,^{1*} Ariel Gaathon,² Shmuel Carmeli,² Hanna Engelberg-Kulka^{1†}

mazEF is a toxin-antitoxin module located on many bacterial chromosomes, including those of pathogens. Here, we report that *Escherichia coli mazEF*-mediated cell death is a population phenomenon requiring a quorum-sensing molecule that we call the extracellular death factor (EDF). Structural analysis revealed that EDF is a linear pentapeptide, Asn-Asn-Tyr-Asn-Asn. Each of the five amino acids of EDF is important for its activity.



EDF (extracellular death factor) attiva l'operone *mazEF* responsabile del modulo tossina-antitossina che determina la morte delle cellule al raggiungimento di una densità elevata

mazEF: a chromosomal toxin-antitoxin module that triggers programmed cell death in bacteria

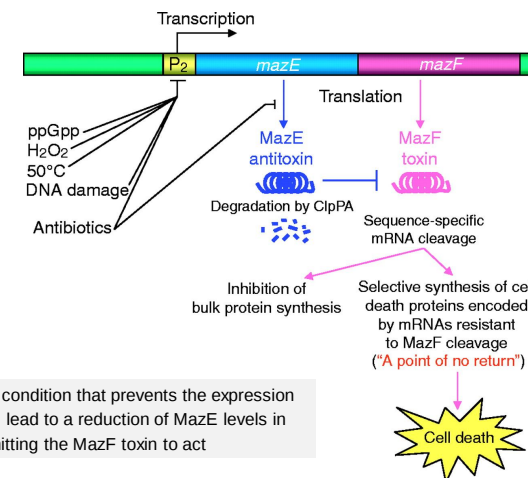
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doi:10.1242/jcs.02619

mazF encodes a stable toxin, **MazF**: a sequence-specific endoribonuclease that preferentially cleaves single-stranded mRNAs at ACA sequences

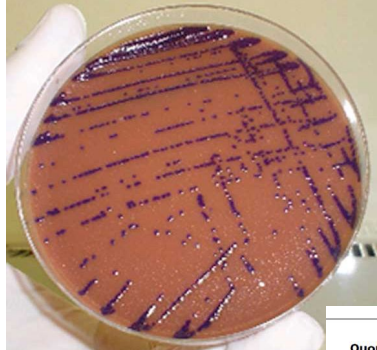


mazE encodes a labile antitoxin, **MazE**, that counteracts the action of **MazF**



Any stressful condition that prevents the expression of *mazEF* will lead to a reduction of MazE levels in the cell, permitting the MazF toxin to act

Chromobacterium violaceum



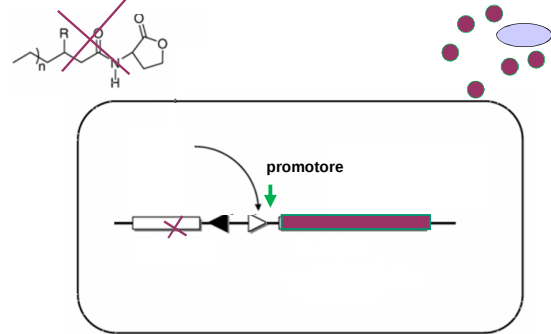
A mutant of *C. violaceum* ATCC 31532, designed strain CV026, does not produce C6-HSL but does produce violacein in response to the presence of exogenous C6-HSL (McClellan *et al.*, 1997)

Microbiology (1997), 142, 3709-3711

Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of *N*-acylhomoserine lactones

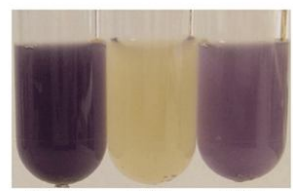
Kay H. McClellan,^{1,2} Michael K. Wilson,^{1,2} Leigh Fish,^{1,2} Adrian Taylor,^{1,2} Siri Ram Chhabra,² Miguel Camara,² Mavis Daykin,^{1,2} John H. Lamb,¹ Simon Swift,^{1,2} Barrie W. Bycroft,² Gordon S. A. B. Stewart¹ and Paul Williams²

Biosensore per la rivelazione di AHLs

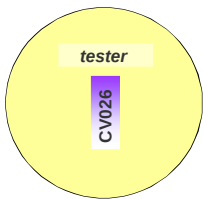


production of AHL molecules has been blocked by mutation of the AHL synthase but the gene encoding the production of the purple pigment violacein remains AHL-responsive.

In the presence of specific AHLs with acyl chain lengths shorter than C₁₀, this strain will therefore produce purple pigment due to violacein production

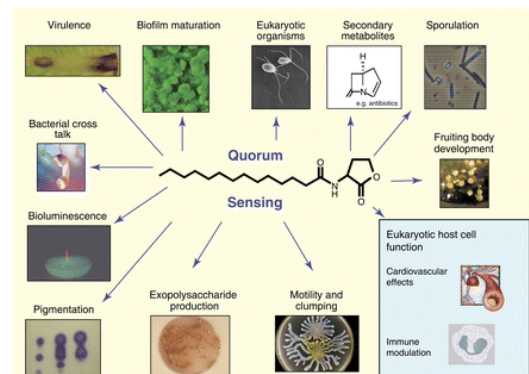


CV wild type CV AHL⁻ CV AHL⁻ + AHLs



Quorum Sensing

- When critical threshold concentration of molecule is reached, bacteria alter gene expression



Current Biology

Controllo post-traduzionale

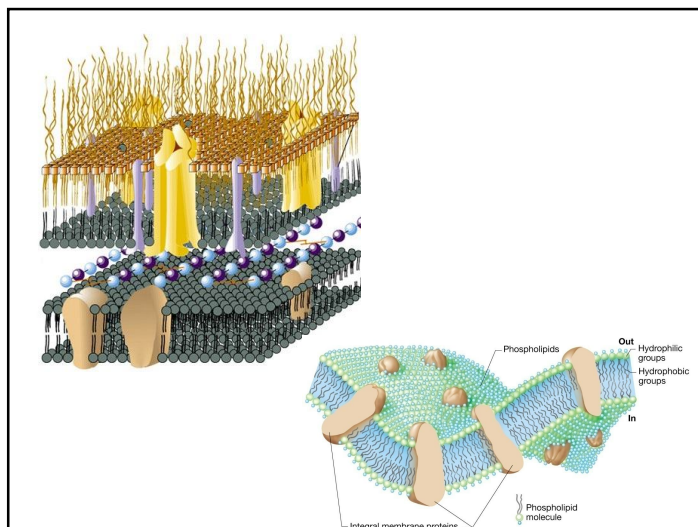
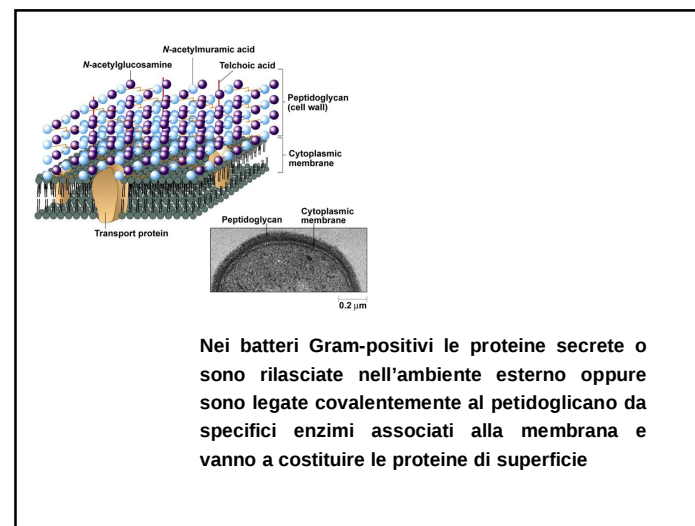
SECREZIONE PROTEINE

Proteine specifiche si trovano:

associate alla IM
nello spazio periplasmatico
OM
in alcuni casi secrete completamente dalla cellula

Le proteine legate alle membrane comprendono:

Citocromi
F1/Fo ATP sintasi
Proteine di trasporto
Porine



Molte proteine sono secrete attraverso entrambe le membrane cellulari (IM, OM) dei batteri GRAM-negativi

Alcune usano la via Sec per attraversare la IM ed un'altra via per la OM

Altre dipendono da altri sistemi per attraversare entrambe le membrane



Sec-pathway

Il trasporto è realizzato attraverso un meccanismo dipendente da una

SEQUENZA SEGNALE

Met-X - Lys/Arg - Z(10-12x) - Gly/Ala -

Regione basica core idrofobico N-terminus proteina matura
5-6 residui

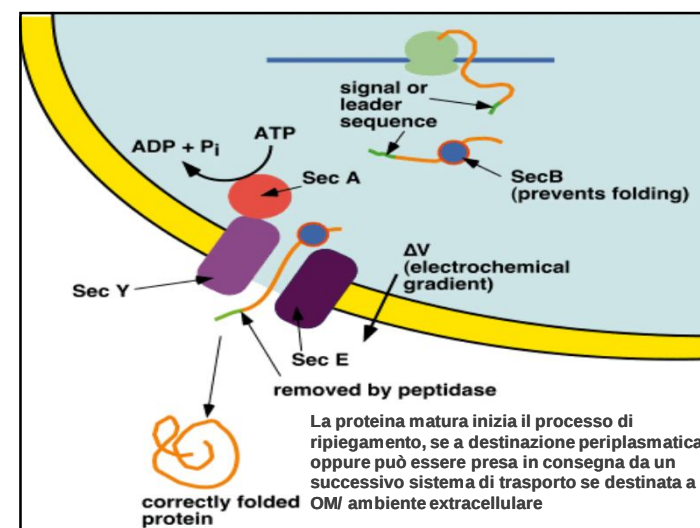
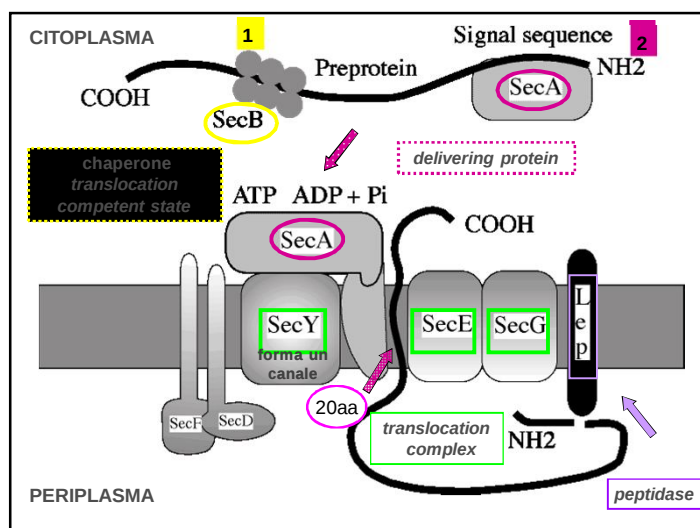
X = residuo idrofilico o idrofobico

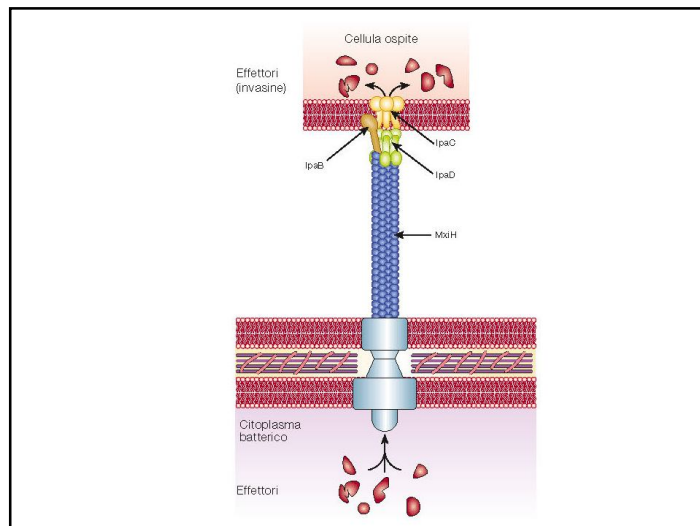
Z= residuo prevalentemente idrofobico

CITOPLASMA



chaperone
translocation
competent state





Type IV – T4S

ancestralmente correlato all'apparato della coniugazione che transloca DNA o substrati proteici attraverso la parete (spesso in relazione alla patogenesi)

