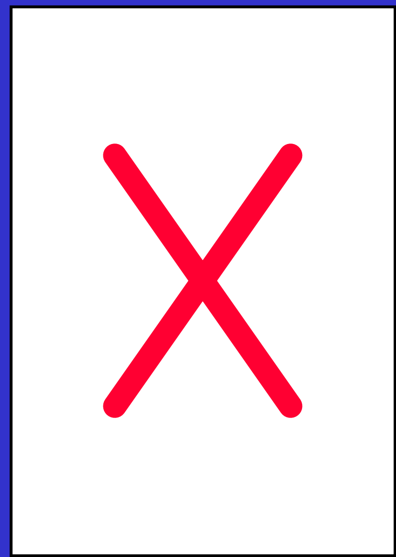
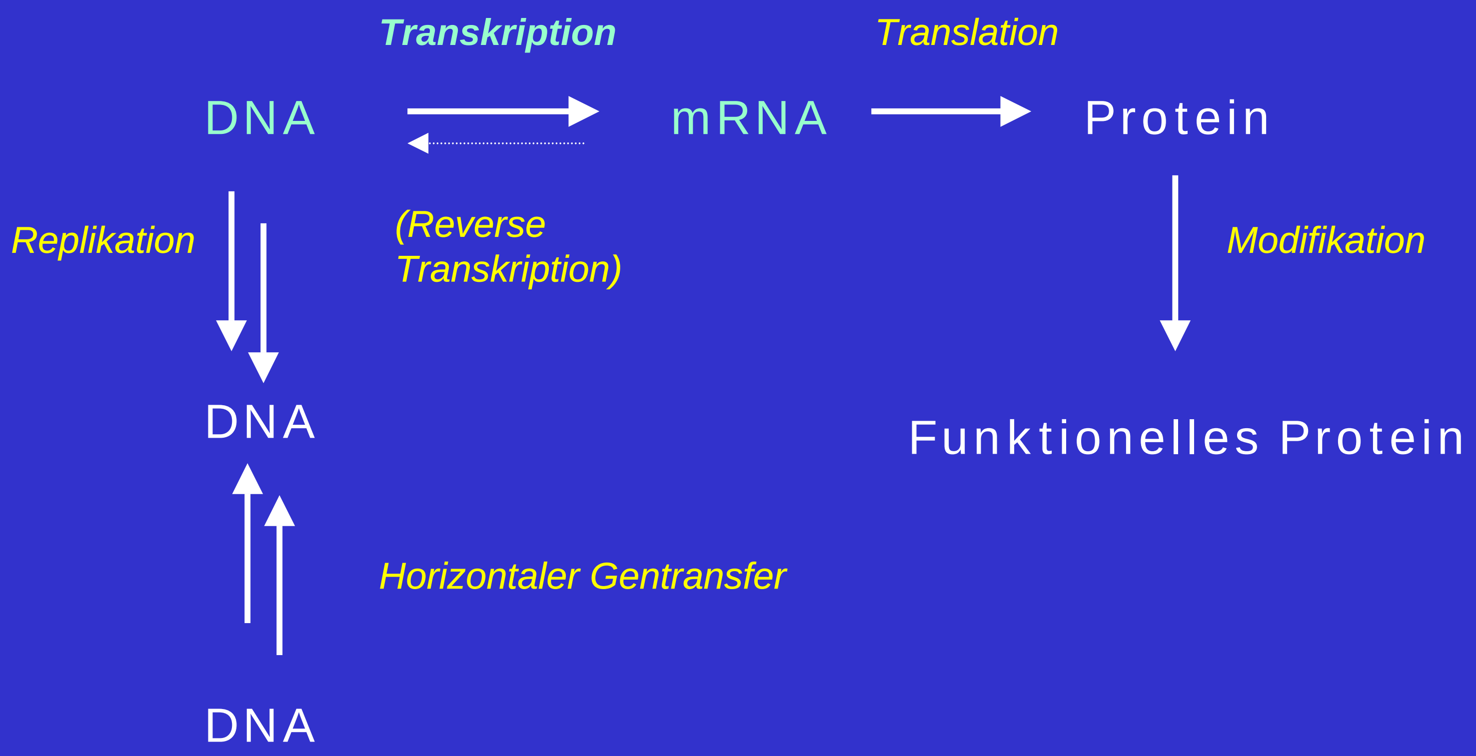
Genregulation in Bakterien

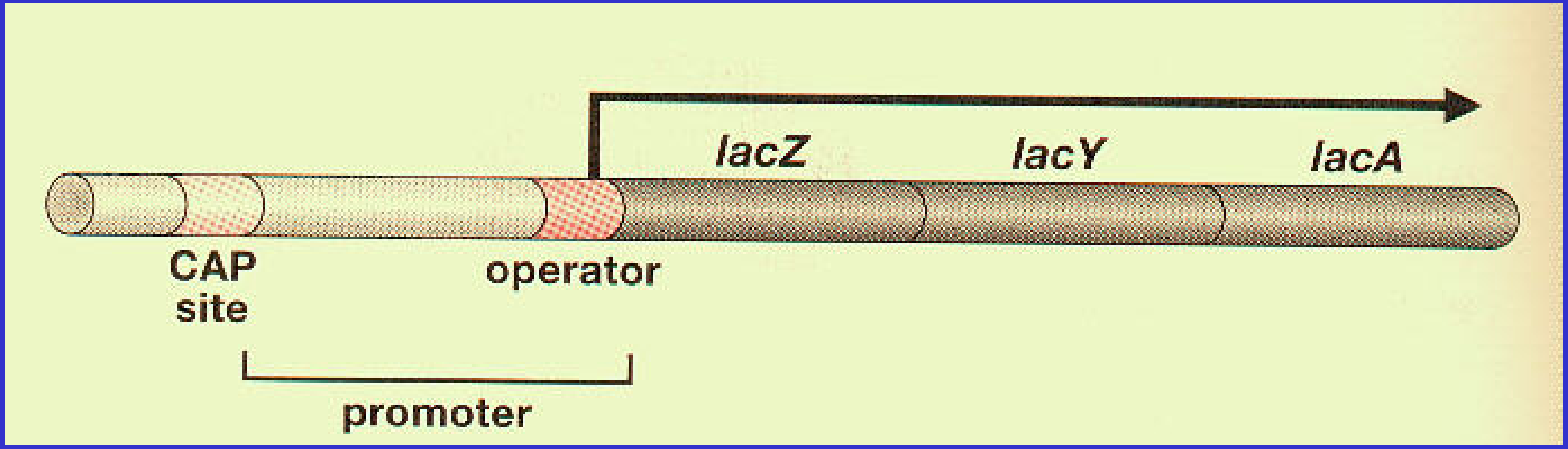
Das LAC Operon



Der Fluss der genetischen Information

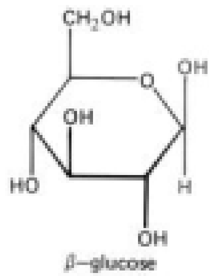


Struktur des Lac-Operons

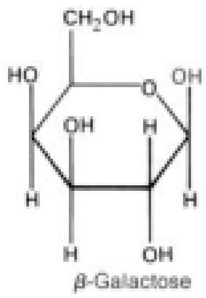


Lactose

Monosaccharides Glucose and Galactose

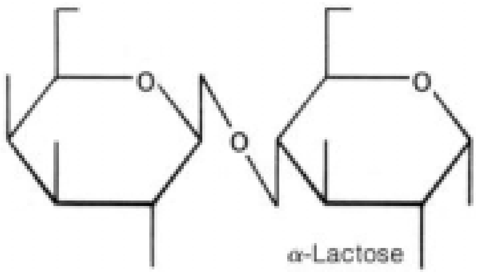


Glucose



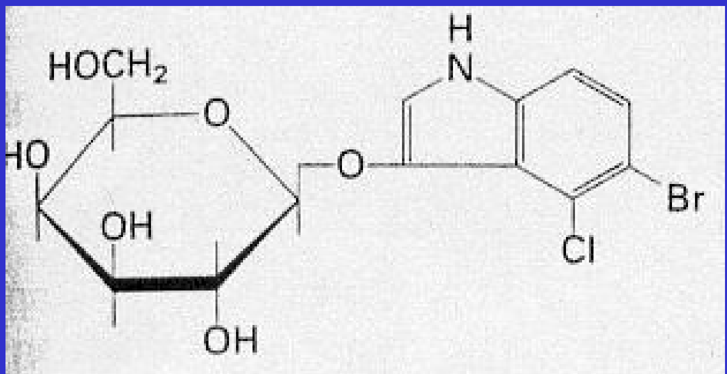
Galactose

Disaccharide Lactose consists of Galactose and Glucose

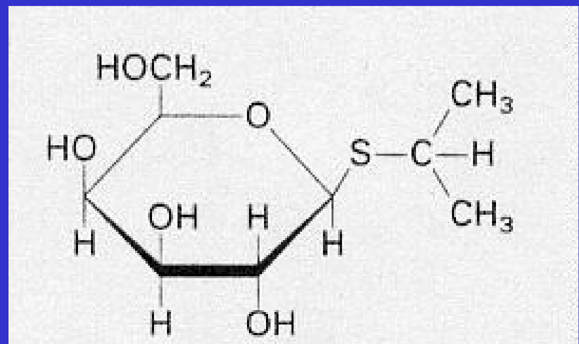


Lactose

Lactose ist gleichzeitig Substrat und Induktor...



X-gal

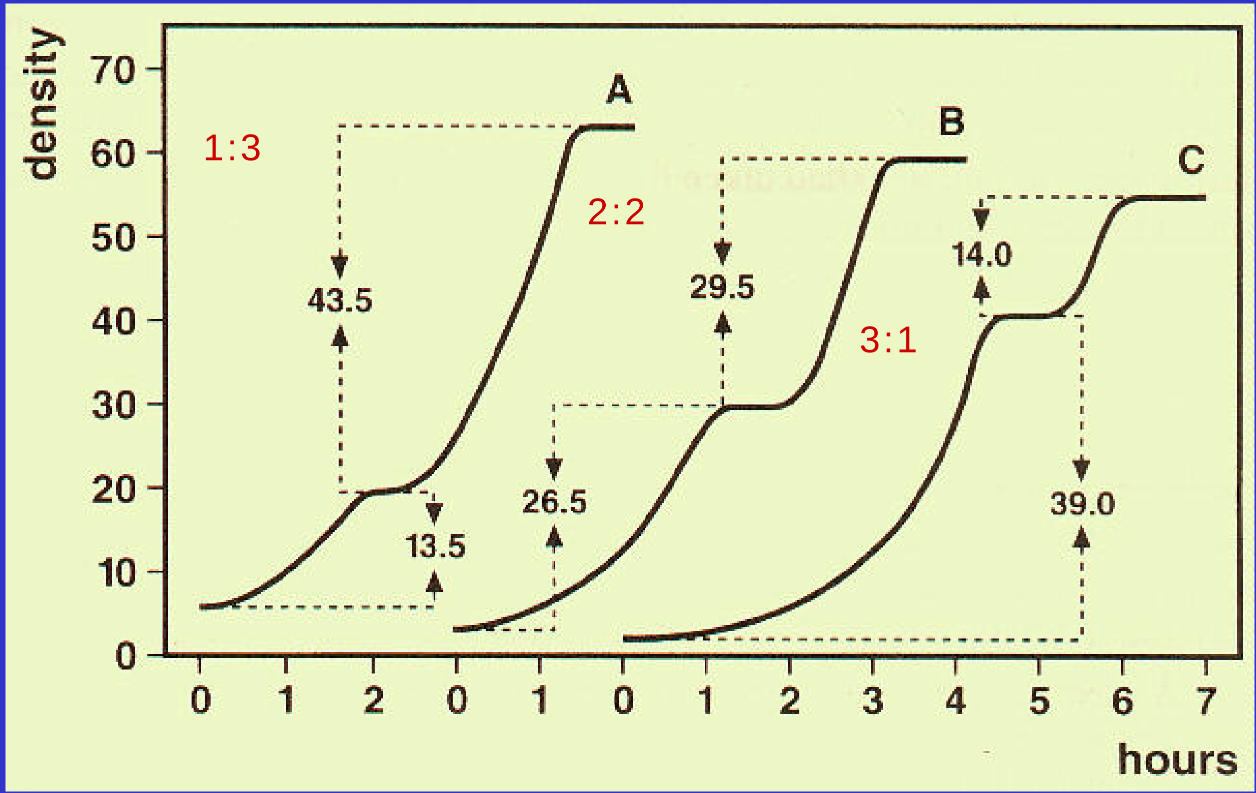


Isopropyl-β-D-thiogalactoside (IPTG)

substrate

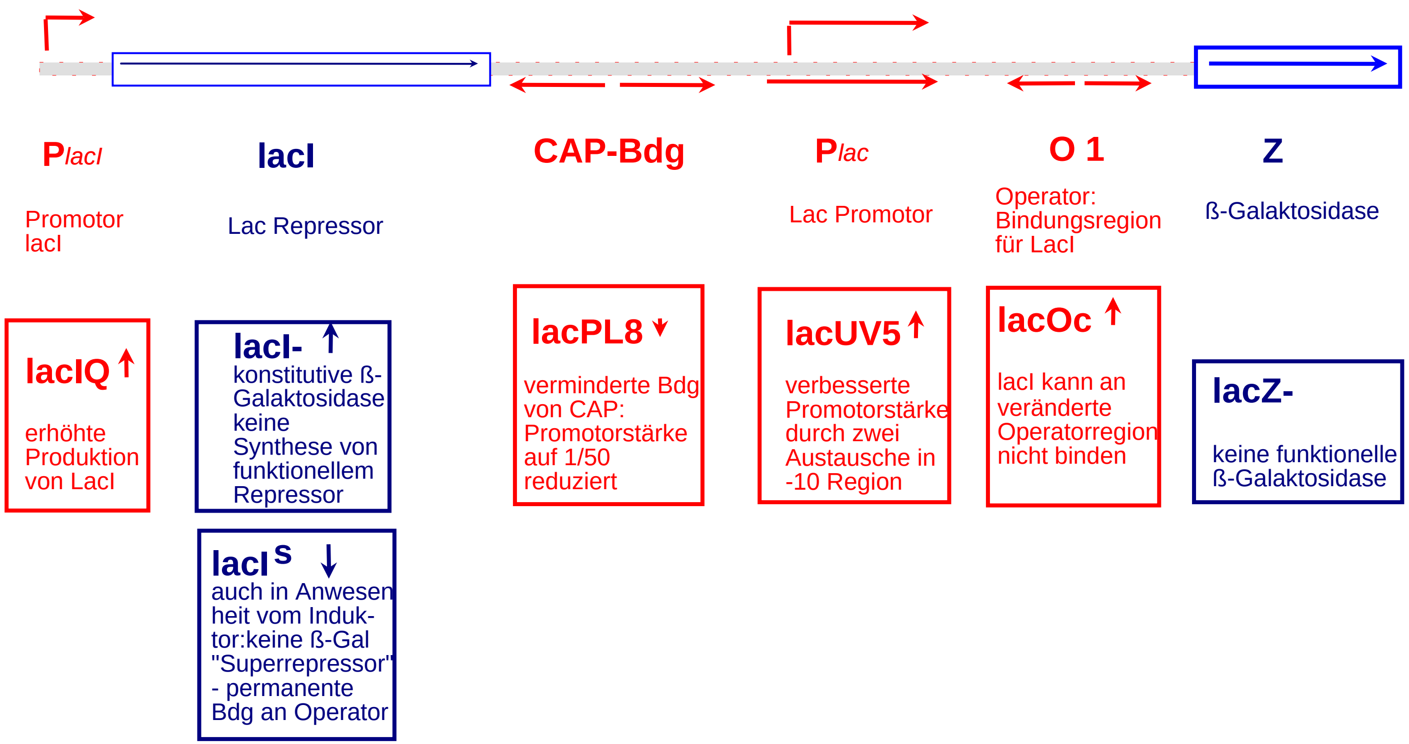
inductor

Diauxie: Wachstum von E.coli in Glucose/Sorbit



Monod 1942

System-Mutanten im Lac-Operon



Synthesis of ß-Gal and Transacetylase

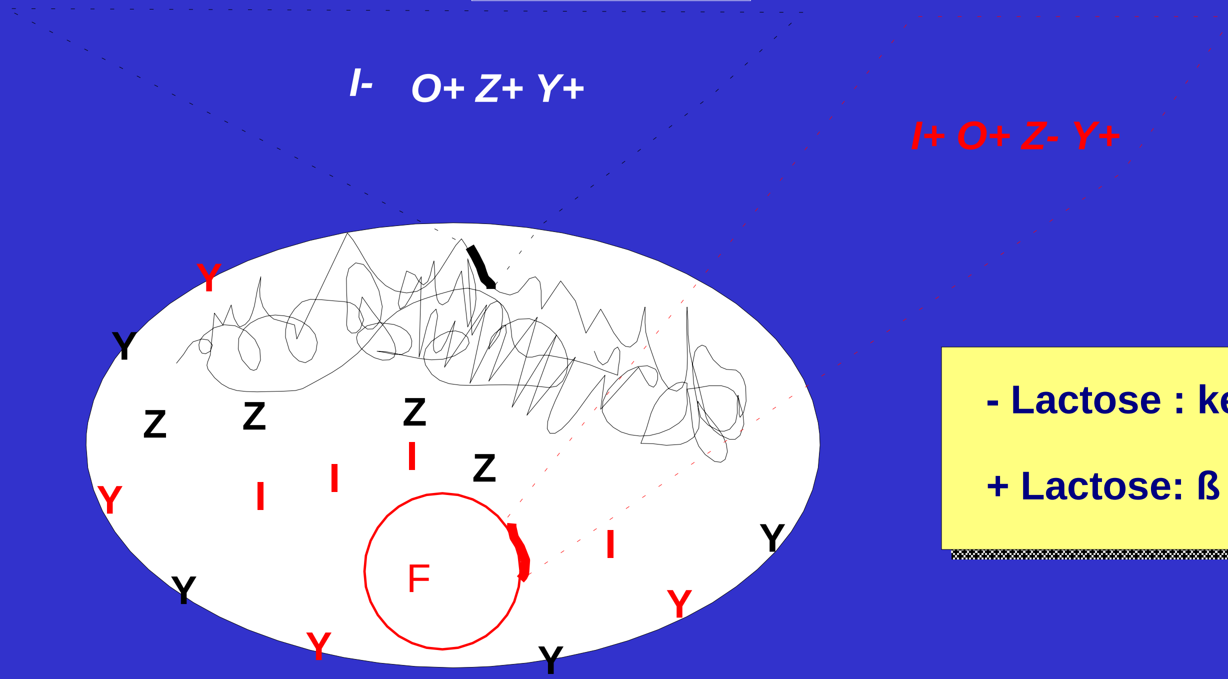
genotype	ß-Gal noninduced	ß-Gal induced	Trans-acetylase noninduced	Trans-acetylase induced
O+Z+Y+	<0.1	100	<1	100
OcZ+Y+	25	95	15	110
O+Z+Y- **F´OcZ+Y+**	70	220	50	160
O+Z-Y+ **F´OcZ+Y-**	180	440	<1	220

Monod,F.Jacob, F. 1961, J.Mol. Biol.3, 318-356

ß-Gal-Bildung in heterozygoten E.coli-Stämmen

I- O+ Z+ Y+

I+ O+ Z- Y+

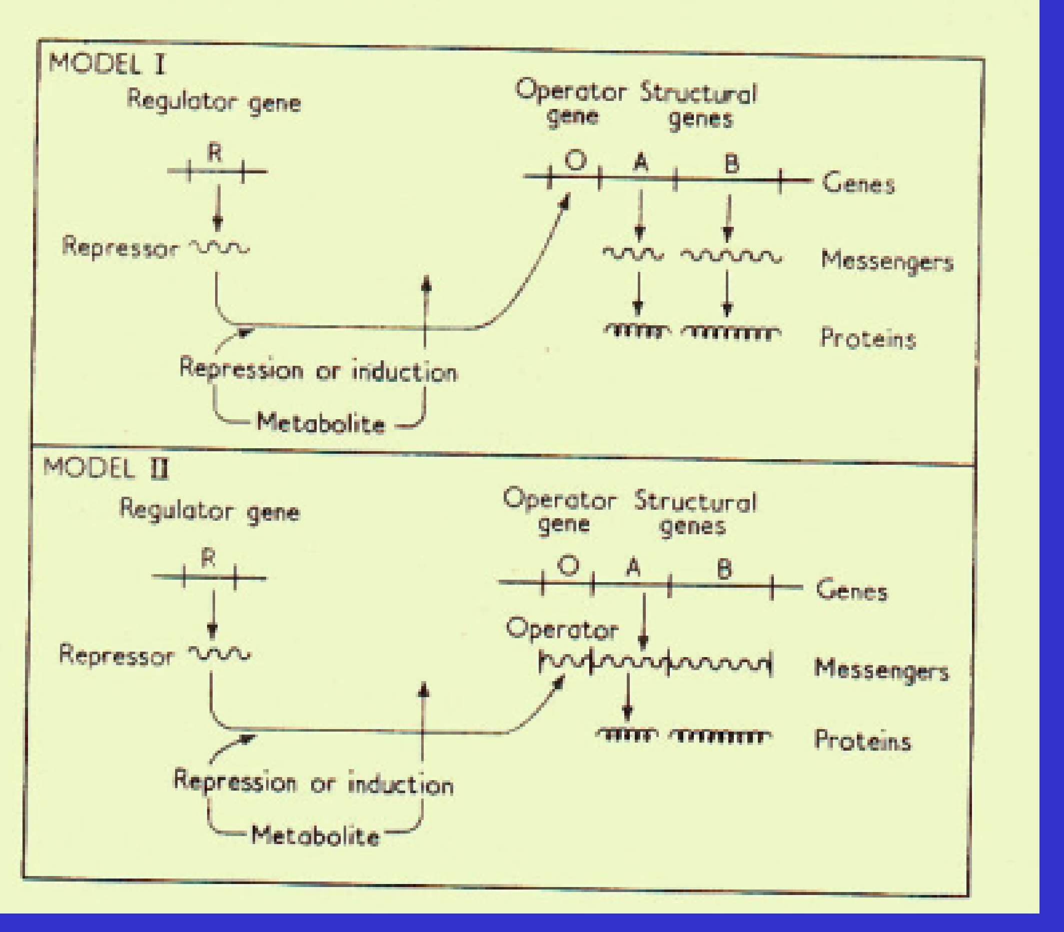


- Lactose : keine ß Gal

+ Lactose: ß Gal aktiv

Z+ ist dominant über Z-
I+ ist dominant über I-

Das Operon Modell von Jacob und Monod 1961



Monod,F.Jacob, F. 1961, J.Mol. Biol.3, 318-356

Isolierung der Lac-Operatorregion

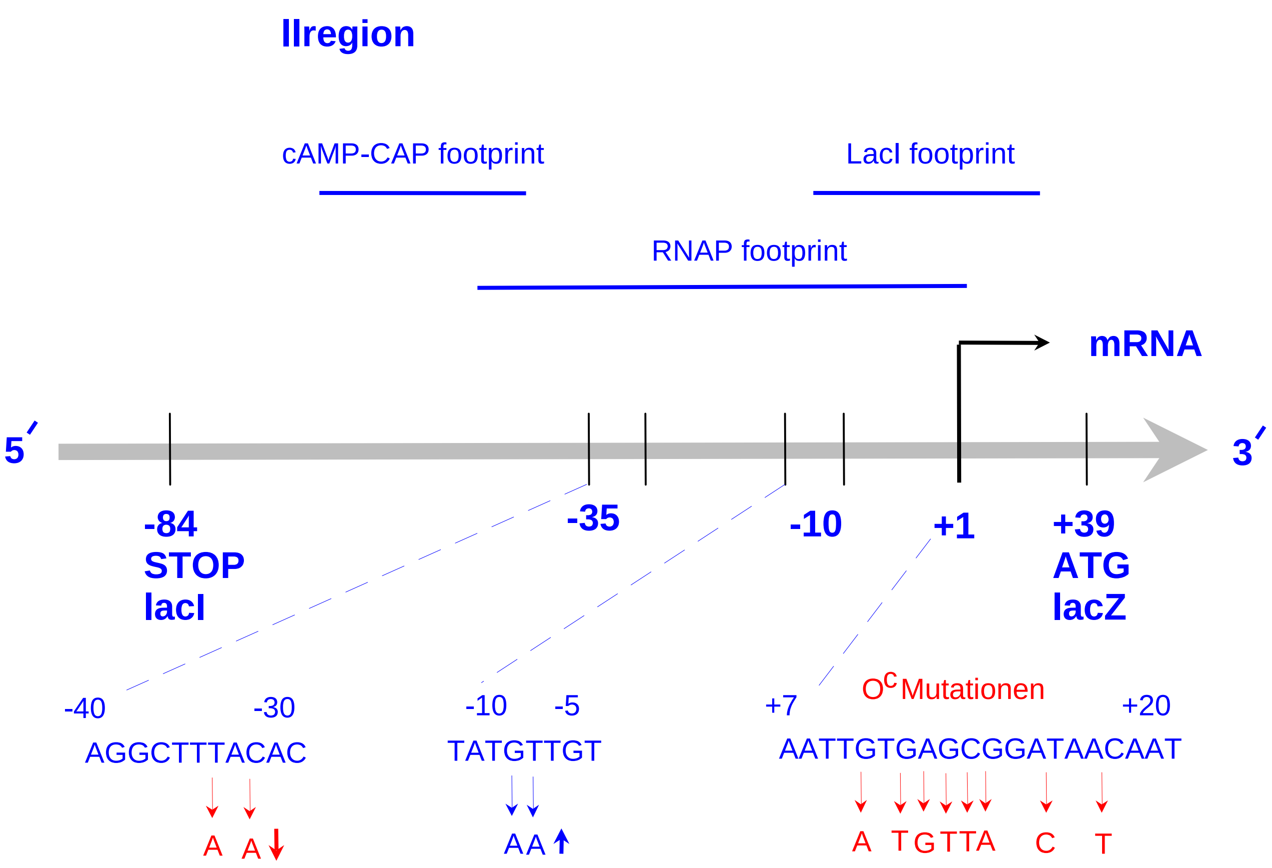
Operatorregion / LacI Bindungsregion:
Dyadische Symmetrie erlaubt Bindung des lac-Repressors als Dimer

Gilbert & Maxam, 1973; N. Maizels, 1973

Strategie:

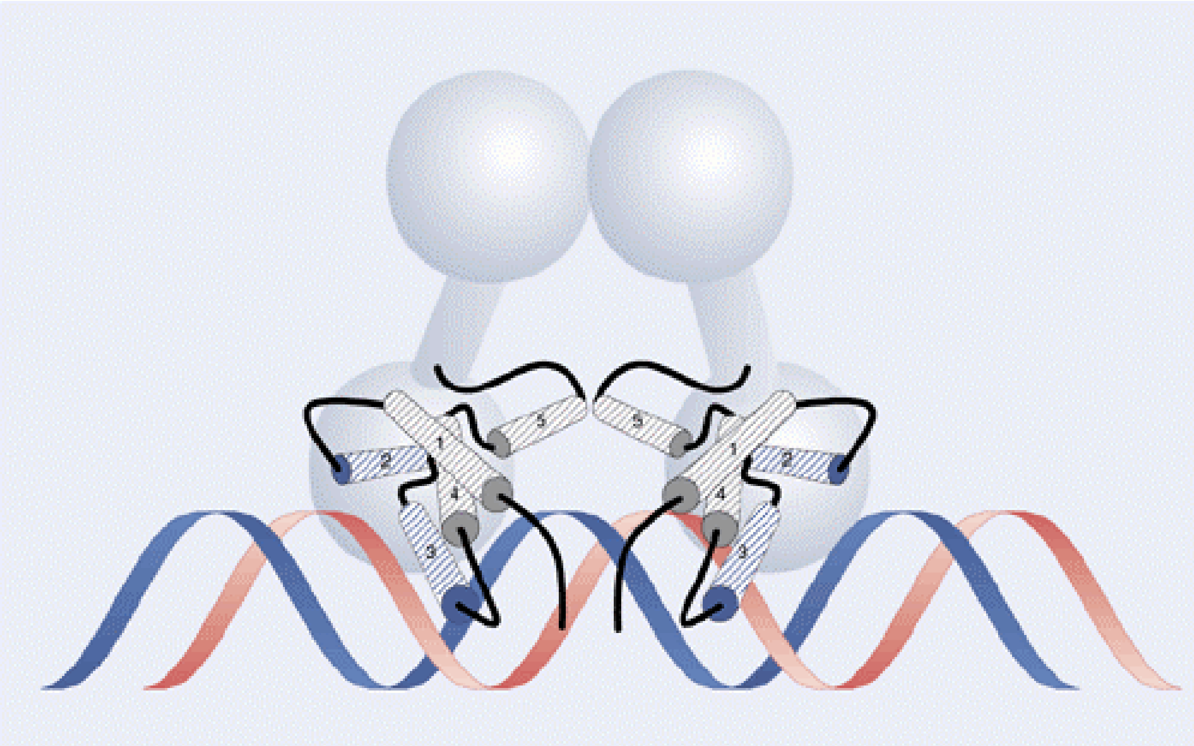
1. Spezialtransduzierende Phagen-DNAλ dlac wird in ca. 1000 bp Fragmente "zerlegt"
2. Mix DNA + Lac-Repressor und Bindung der DNA-Fragmente an Nitrocellulose
3. Verdau mit Pankreas-DNAse
4. Isolierung eines geschützten 28 bp Fragmentes
5. Sequenzbestimmung

lac-Promotorstruktur

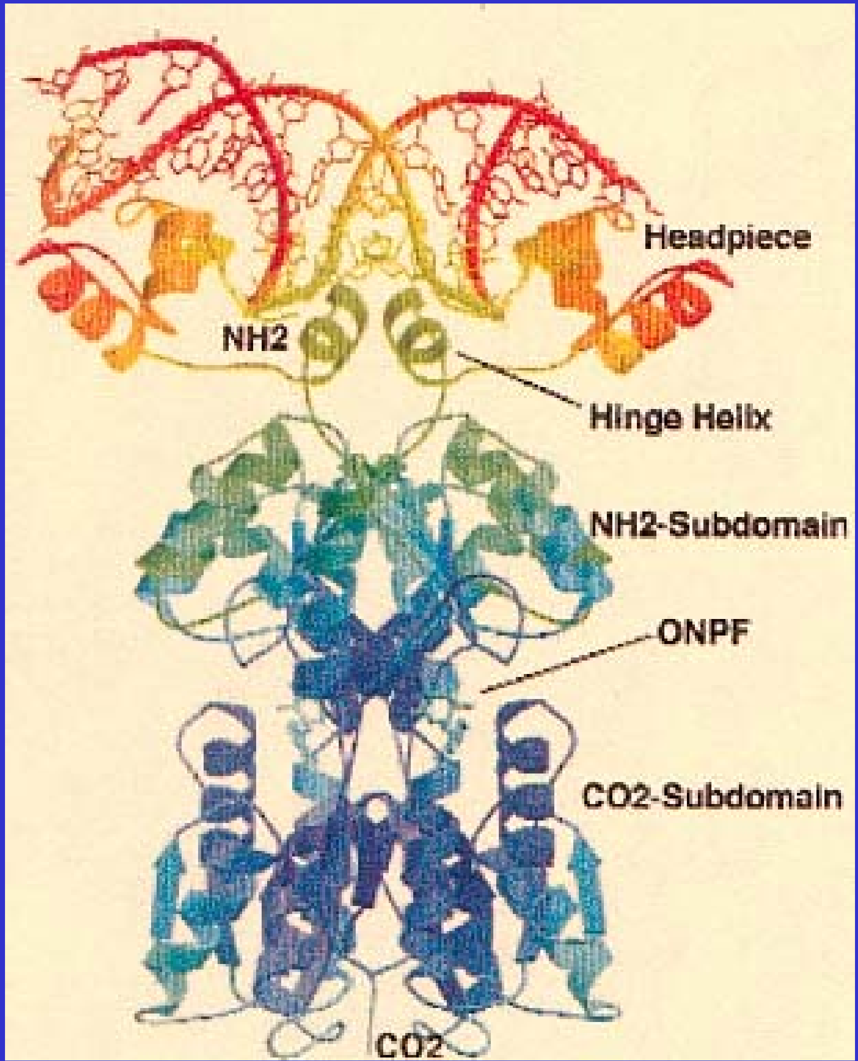


HTH Bindung an DNA

Figure 11.19 In the two-helix model for DNA binding, helix-3 of each monomer lies in the wide groove on the same face of DNA, and helix-2 lies across the groove.



Molekulare Struktur des Lac-Repressors (Dimer)



Headpiece:1-49 with HTH motif binds to the major groove of the operator

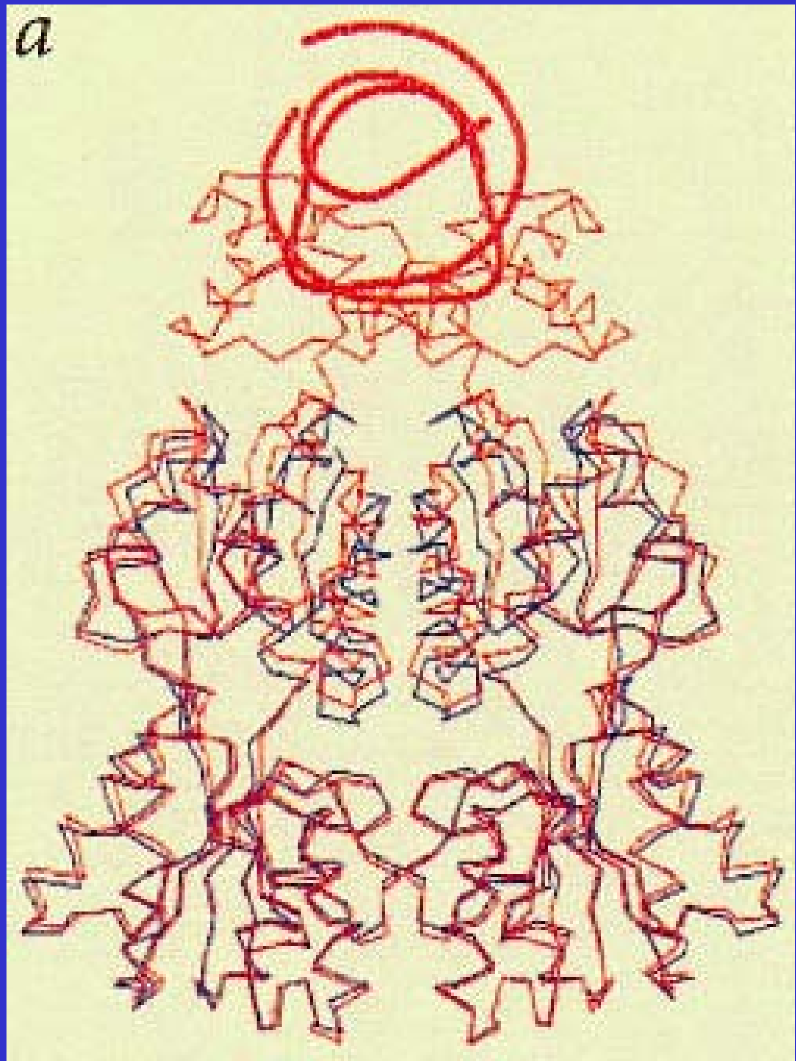
Hinge helix, 50-58 binds to the center of operator in the minor groove

Core domain,62-333 consists of two subdomains:
NH2I, 62-161 + 293-320
CO2 162-289 + 321-329

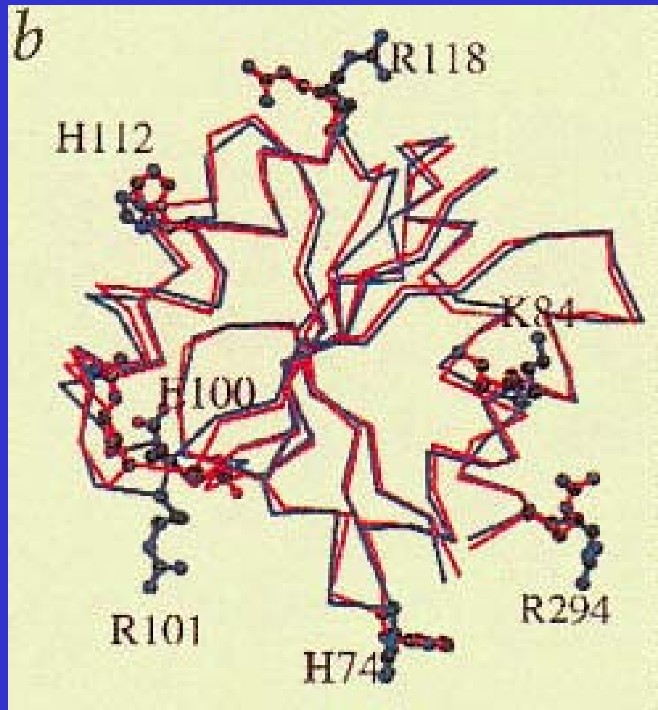
Effector IPTG binds to a pocket n their junction

Helical Tetramerization domain 340-357

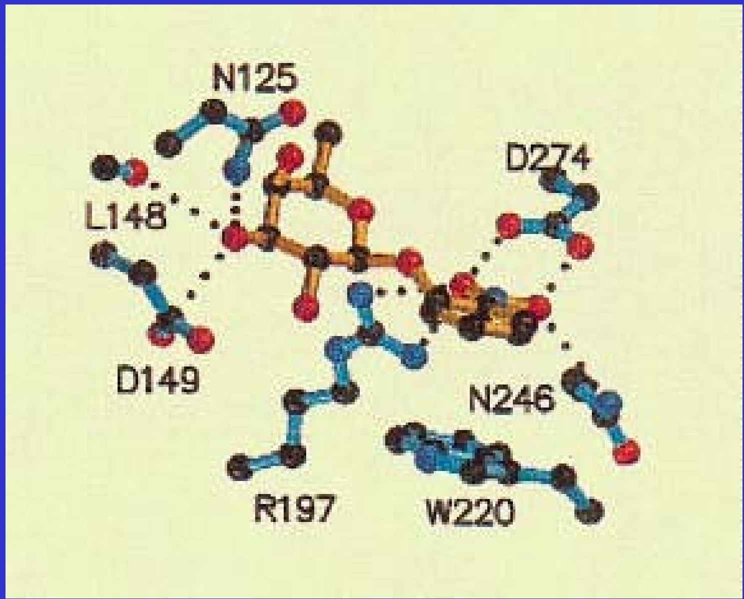
Unterschiedliche Konformationen des Lac-Repressors



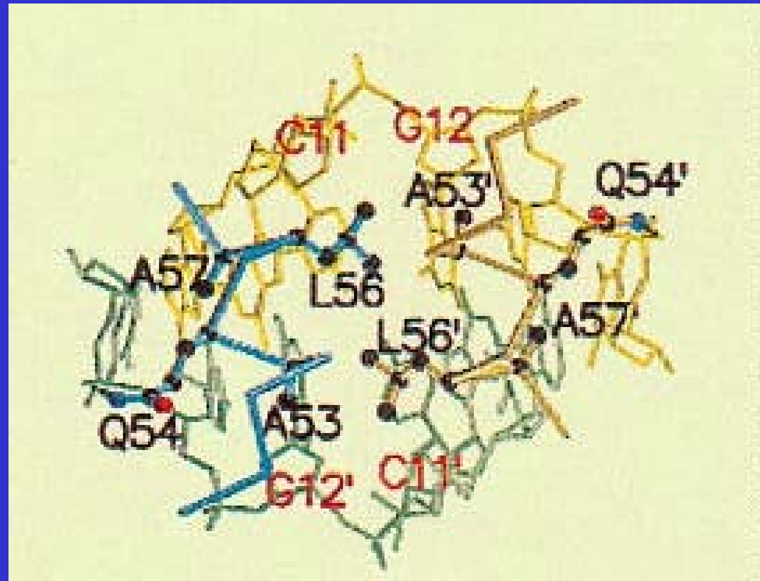
Different structure of NH2 terminal subdomain if repressor is bound to operator (red) and to IPTG (blue)



Interaction of the Lac Repressor with operator and inducer

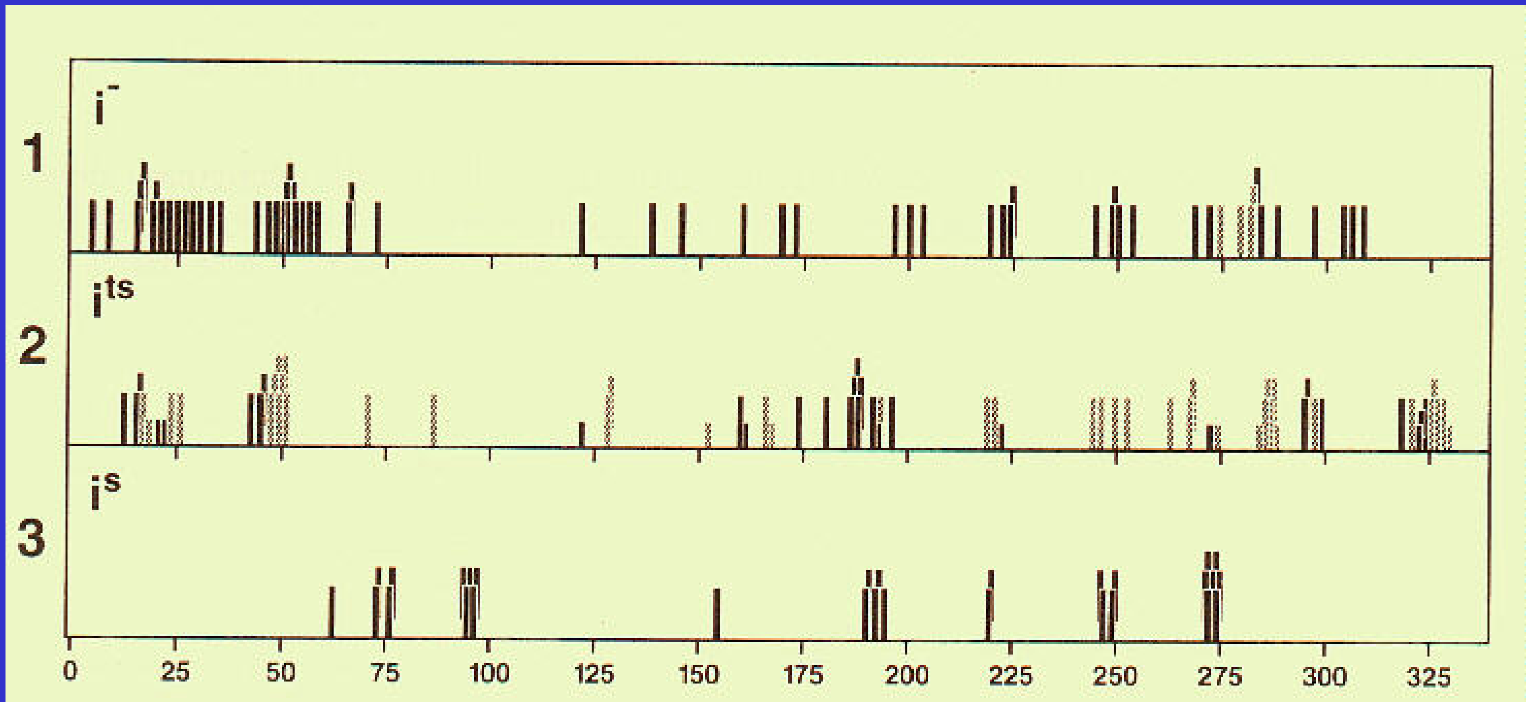


Inducer binding pocket. Residues contacting ONPF are shown



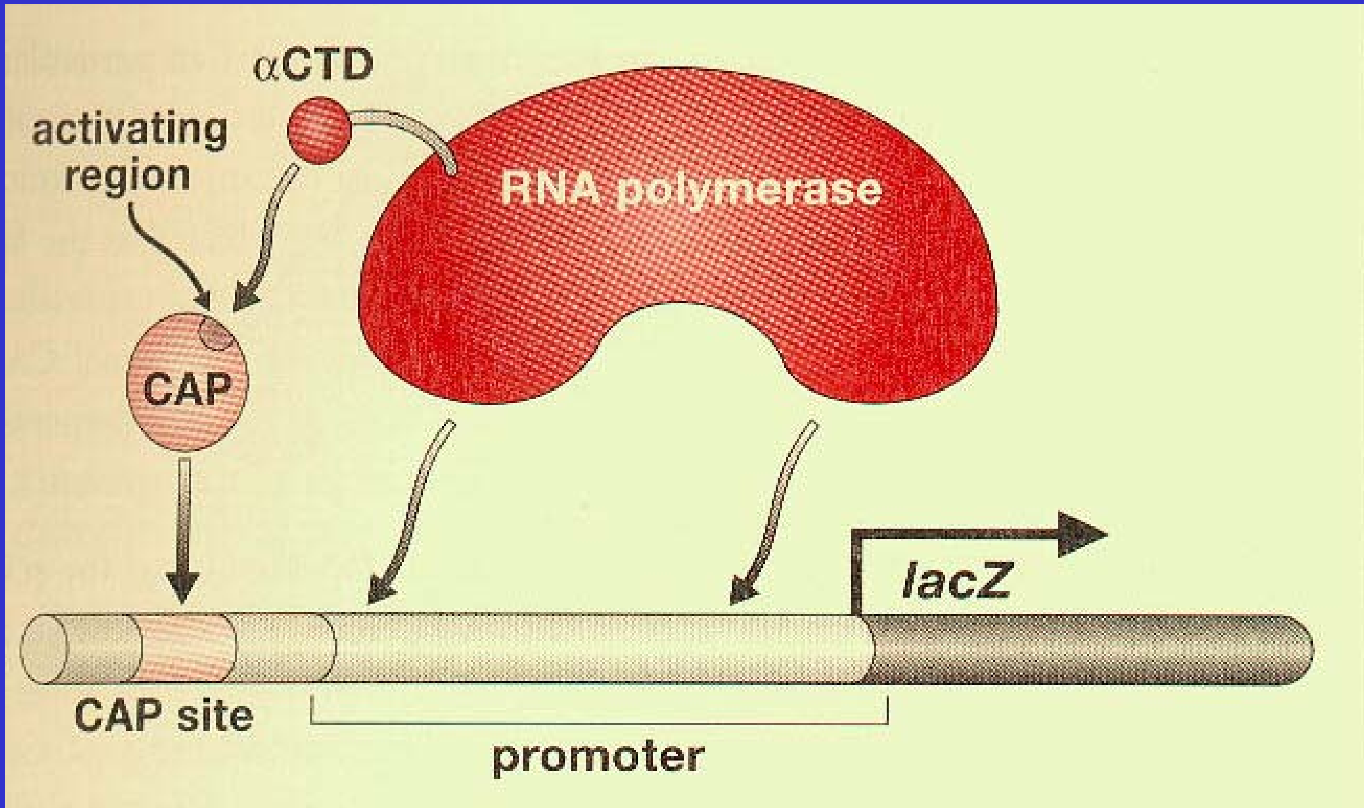
Interaction between the hinge helices of the repressor (blue and brown) and the minor groove of the operator (green and yellow)

Mapping of mutations in lacI by J. Miller,1977



2000 lacI- missense mutations were mapped by selection of I+ recombinants.
I^- constitutive, I^{ts} heat sensitive, I^s noninducible

CAP acts as positive regulator



Kooperative Bindung von CAP und Polymerase an RNA

CAP Bindungsprotein – Positiver Regulator des C-Katabolisms

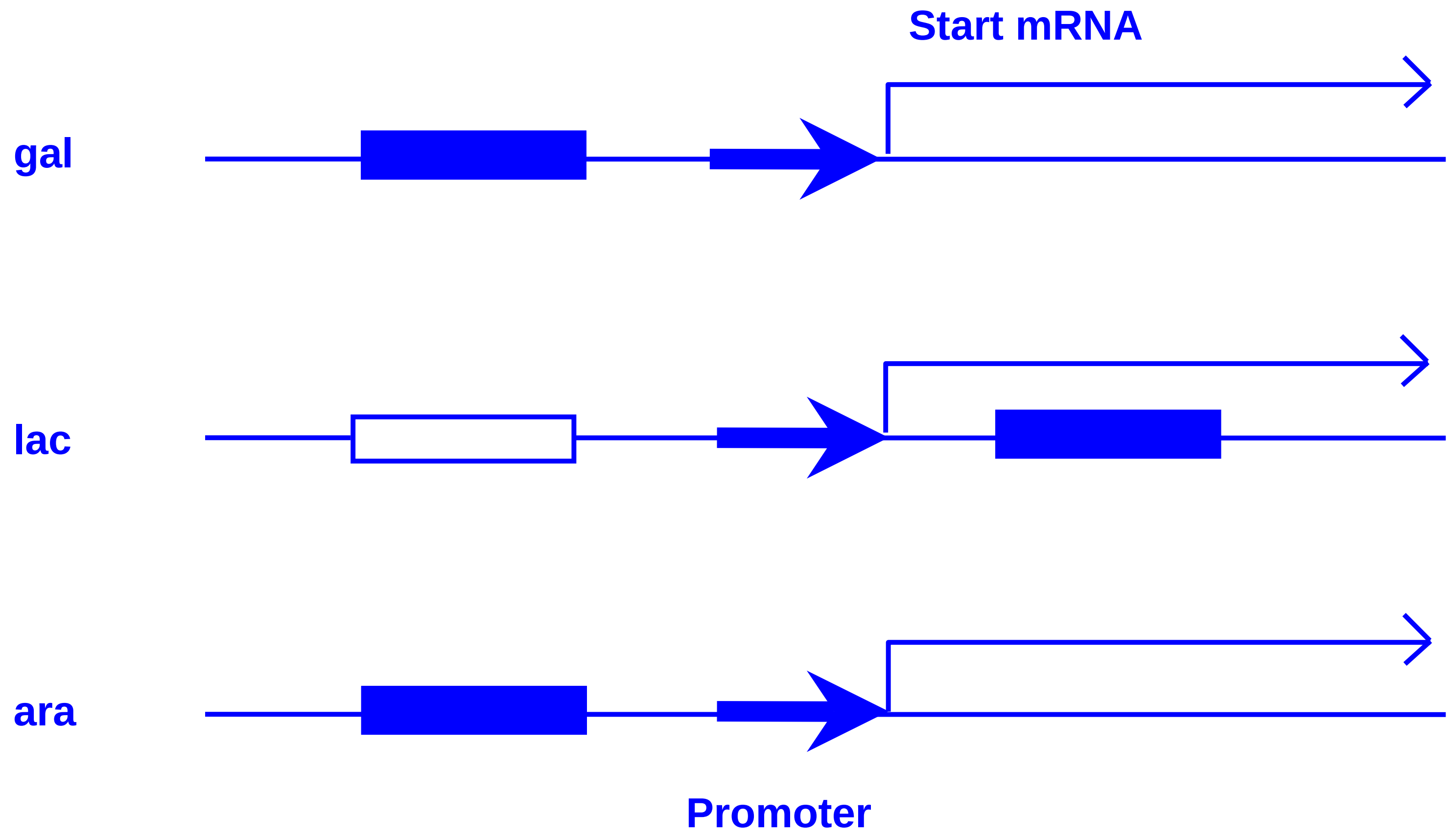
1982: Klonierung und Sequenzierung von CAP (209 AS)
Dimer: jedes Monomer hat eine DNA-Bindungsstelle (nur in Gegen-wart von c-AMP)
Consensus-DNA-Bindungssequenz (CAP-Bindungsstelle):

```
         <-------------
5   CGAAAAGTGTGACAT  ATGTCACACTTTTCG
    GCTTTTCACACTGTA  TACAGTGTGAAAAGC   5
                      ------------->
```

Positiver Regulator durch DNA "bending"
Abhängigkeit von cAMP-Konzentration: Niedrige Glukosekonz.

Glucose ↕ → **PTS:Enzym IIGlu** → **Adenylatcyclase inaktiv**
→ **PTS:Enzym IIGlu-P** → **Adenylatcyclase aktiv** → **ATP -> cAMP**

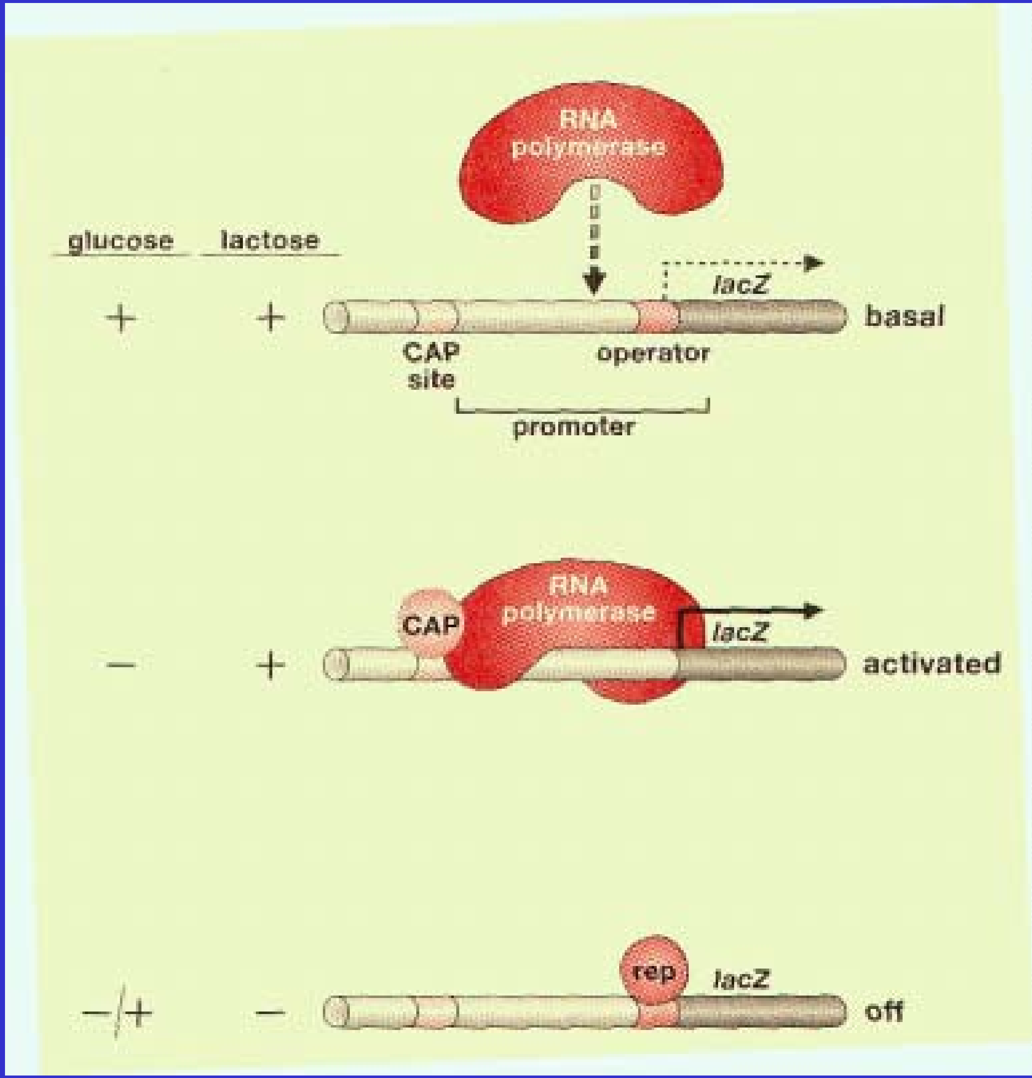
Das CAP Protein kann in verschiedenen Bereichen des Promotors binden



Die drei Stadien der lac Gene

Positive control

Negative control



basal

active

off

LAC-Promotor Kontrolle