Cre recombinase fused with tamoxifen is used more often.

↳ inducible systems must regulate CRE recombinase expression or expression of a gene of interest

can tamoxifen have a side effects? (it is important to determine whether tamoxifen have side effects on the regulation of genes as it is used more often)

Using CRE recombinase : is irreversible as it removes a longere part of the sequence

Tet ON/OFF systems are reversible as it only lasts as long as the animal is fed tetracycline / doxycycline.

Tet-ON method can be used with CRE recombinase.

Tet-OFF : tetracycline switch-off transcription → two constructs are needed for this method.

↗ it is possible to have 1 construct containing all the sequences inserted in ROSA 26 chromosome

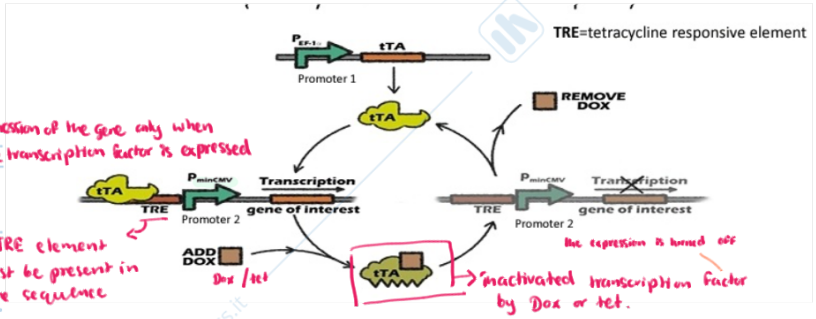
↳ tTA (transcription factor) (as it is normally not expressed in mammalian cells) – fusion / recombinant protein.

tTA binds to the promoter in normal conditions (without Dox / Tet) in this case.

↳ TRE element + promoter + gene of interest.

can have tTA ubiquitously expressed by using a promoter that is ubiquitous.

OR can have tTA expressed in tissue specific manner by using a promoter that is expressed only in a sub population of cells.



tetracyclin Transactivator factor (tTA) is a hybrid transcription factor resulting from the fusion of the prokaryotic Tet repressor with an eukaryotic transcriptional transactivation domain.

TetR confers sequence specific DNA binding and sensitivity to tetracyclines.

Binding of antibiotic (tetracycline or doxycycline) dramatically lowers the affinity of tTA to its binding sites, TRE.

Doxycycline has excellent tissue penetration and low toxicity in eukaryotes.

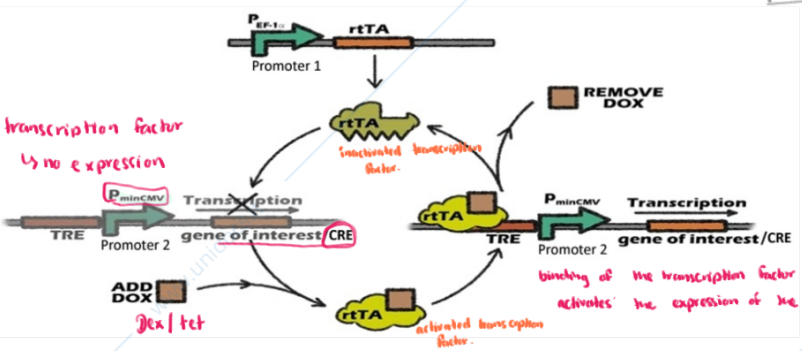
The Tet-OFF system can be used to obtain inducible repression of a specific gene.

A construct for tTA expression must be prepared and inserted into the mouse.

once Dox / Tet binds to tTA, affinity of tTA to the promoter is dramatically reduced → leading to the removal of the tTA (transcription factor) from the promoter.

Tet-ON : tetracycline switch-on transcription → two constructs.

usually this strategy is used to activated the transcription of cre (the same concept as tamoxifen).



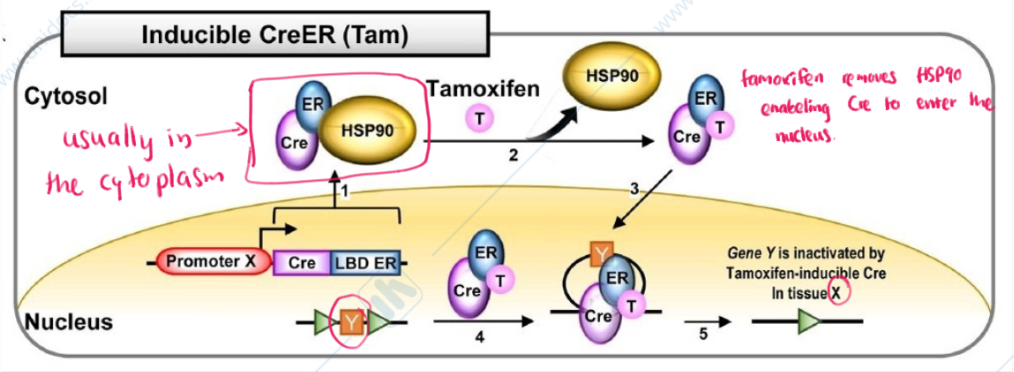
Reversal tetracycline Transactivator factor (rtTA) differs from tTA by a few point mutations within TetR which result in a complete reversal of tetracycline responsiveness of this transcription factor. ∴ rtTA will not bind to the promoter unless it is bound with Doxycycline / Tetracycline.

Binding of the antibiotic (tetracycline or doxycycline) dramatically increases the affinity of rtTA to its binding sites, TRE.

The Tet-ON system can be used to obtain inducible expression of CRE or inducible expression of a specific gene.

A construct for rtTA expression must be prepared and inserted into the mouse.

Tamoxifen (Tam) - inducible System of Estrogen receptor fused to Cre [CreER]



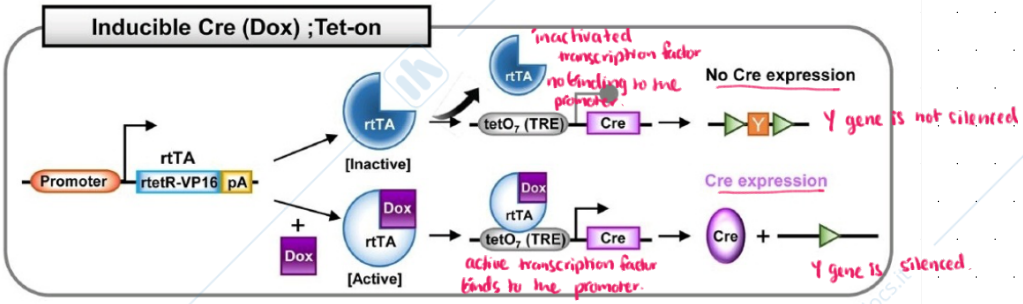
In the absence of tamoxifen, expressed fusion protein, CreER, interacts with heat shock protein 90 (HSP90) and exists in cytoplasm.

Administration of Tam disrupts the interaction of HSP90 with CreER.

Interaction of PR with Tam induces nuclear translocation of Cre.

In the nucleus, the CreER recognizes the loxP sites and inactivates the gene Y in tissue X.

Doxycycline [Dox] - induced Tetracyclin (Tet)-on system



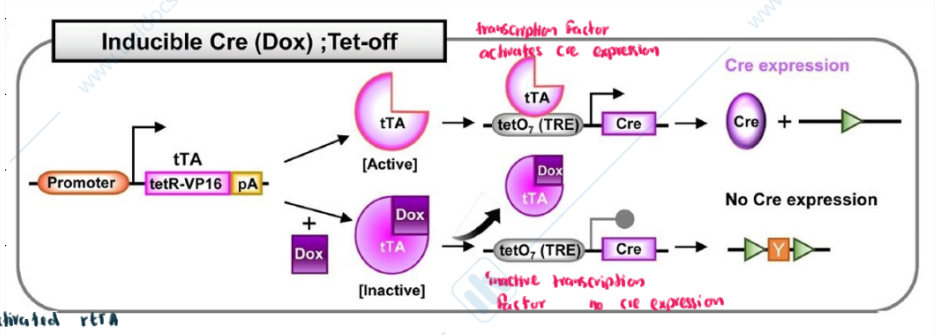
In Tet-On system, ubiquitous or tissue-specific promoter driven rtTA is expressed.

In the absence of Dox, inactivated rtTA is unable to bind tetO$_7$ (7 repeats of a 19 nucleotide tetO minimal promoter, also referred to as TRE) sequence of cre gene.

Following Dox administration, Dox interacts with the rtTA and allows to activate.

Activated rtTA binds to tetO$_7$ promoter of cre and induces the cre expression.

Doxycyline [Dox] - induced Tetracyclin (Tet) - OFF system.



Activated rtTA

In Tet-OFF system, in the absence of Dox, activated tTA is able to bind $tetO_7$ (TRE) sequence of cre and induces the Cre expression.

Upon Dox administration, tTA interacted with Dox, is inactivated.

Inactivated rTA is not able to bind to $tetO_7$ promoter and therefore Cre expression is inhibited.