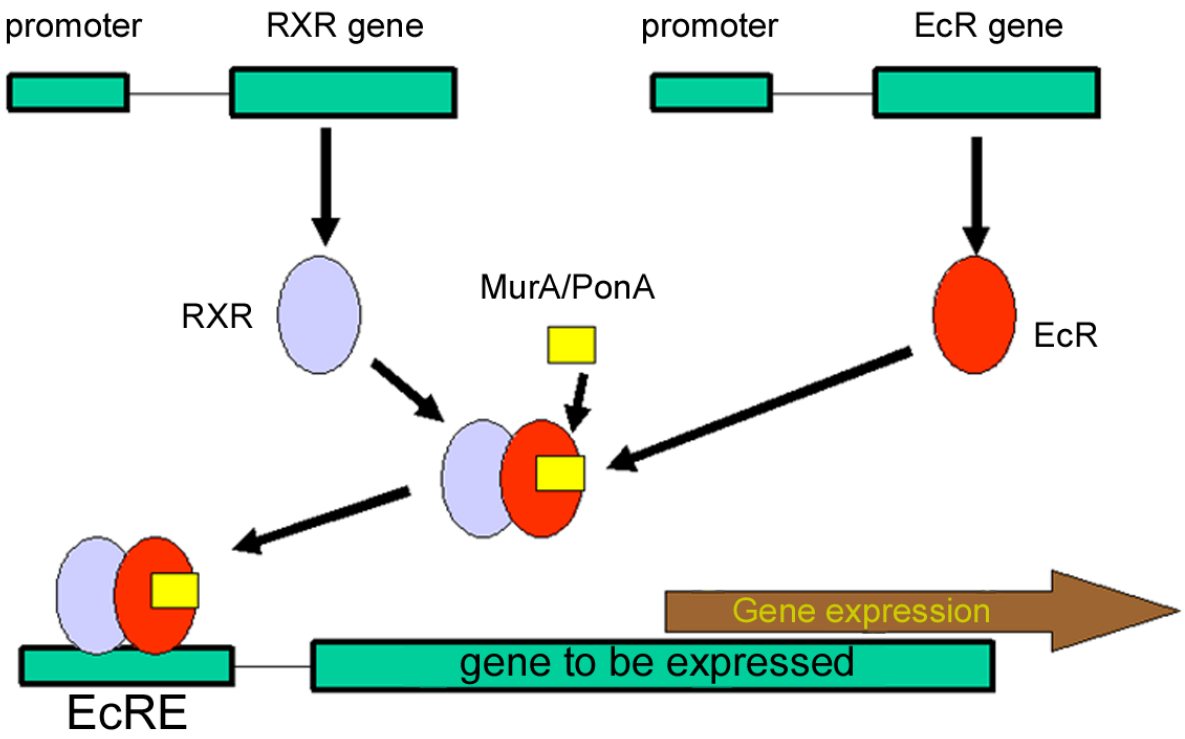
Gene expression-an overview

Regulated gene expression has recently found wide application in the laboratory and preclinical tests, including gene therapy [1,2].In clinical applications it will be beneficial to regulate the transgene expression in order to maintain concentrations within the therapeutic window and to optimize efficacy in response to the evolving nature of the disease. A regulative system would be valuable in modifying specific therapies in response to pharmacological agents that can be safely and repetitively administered.

For cancer gene therapy, therapeutic gene products would be particularly valuable, such as cytokines, prodrug activating enzymes,ribozymes antibodies, tumoricidalgenes, (or) antisense oligonucleotides [3,4]



General scheme for ecdysteroid-based gene switches.[MurA: Muristeroen A, PonA: Ponasterone A]

Gene switch systems:

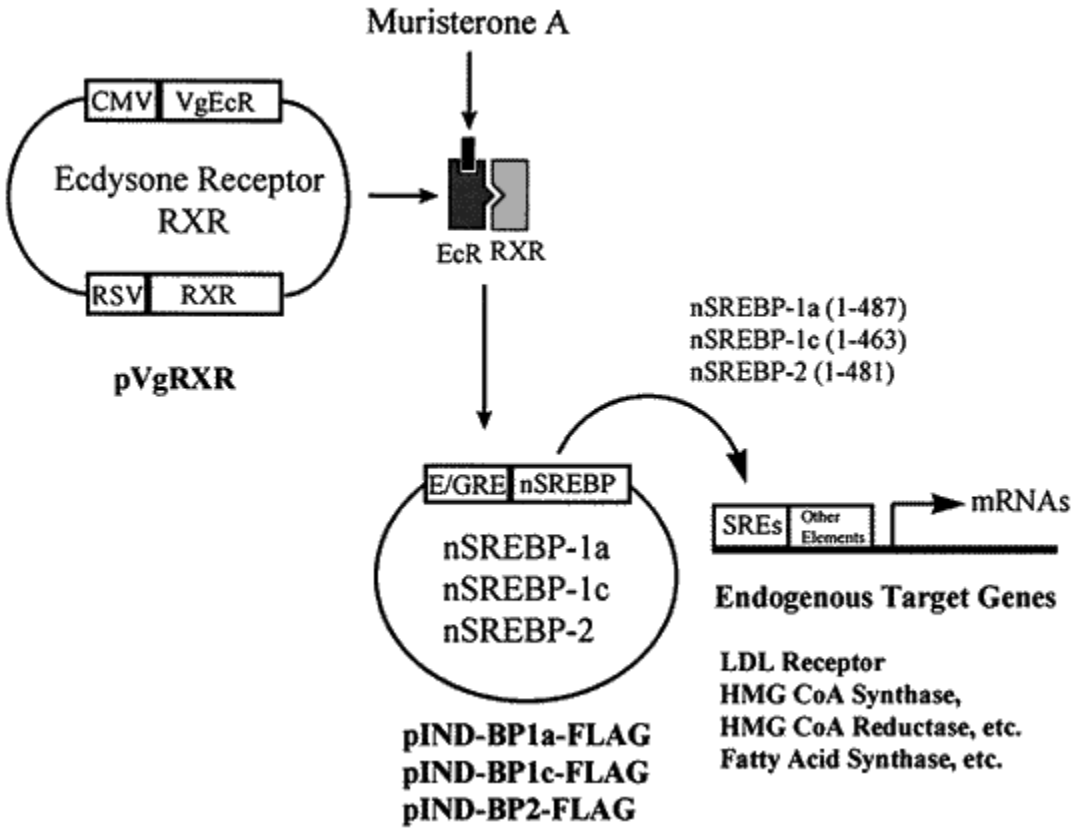
Upon completion of the human genome library sequencing it is proposed that switch genes will allow switching off cells which produce organism-destroying structures (e.g cancerous grouth) and bringing to a stop deseases, incurable by conventional treatment modes(many inherited diseases). Analogically it will be possible to implant and point wise switch on genes not present in the host cells but responsible for producing target therapeutic agents, as well as to set off the regeneration factors of damaged tissues [5, 6, 7].

Importance of the Ecdysteroids

Ecdysteroids represents natural and absolutely safe ligands in molecular systems of gene switching. Which are widely used as inducers for gene-switch systems based on insect ecdysteroid receptors and genes of interest placed under the control of ecdysteroid-response elements [8, 9, 10, 11].

Importance of the ecdysone- induced systems

The ecdysone-inducible system has proved useful for studying a multitude of processes, such as apoptosis, cancer and cell cycle regulation, embryonic development, signal transduction, lipid metabolism and neuronal function. The strength of the system appears to be its tight regulation, its dose responsiveness and the favorable uptake and clearance kinetics of the steroid inducer, which results in rapid gene switching [12, 13, 14, 15].



The ecdysone-induced gene expression system based on **Muristerone A.**

Ecdysone-inducible mammalian expression systems

Ecdysteroids are apparently not endogenously generated components of mammalian systems. However they are normal components of the diets of many animals. The low mammalian toxicity of these compounds together with the specificity of the ecdysteroid receptor complex (EcR and USP proteins) indicates that a successful gene-switching system might be developed from this system [16].

Invitrogen Corporation (Lajolla, CA, USA) claims the ecdysteroid expression system as the only truly inducible mammalian expression system. The responsive is dose dependent so the level of expression can be controlled with the concentration of Muristerone A [17].

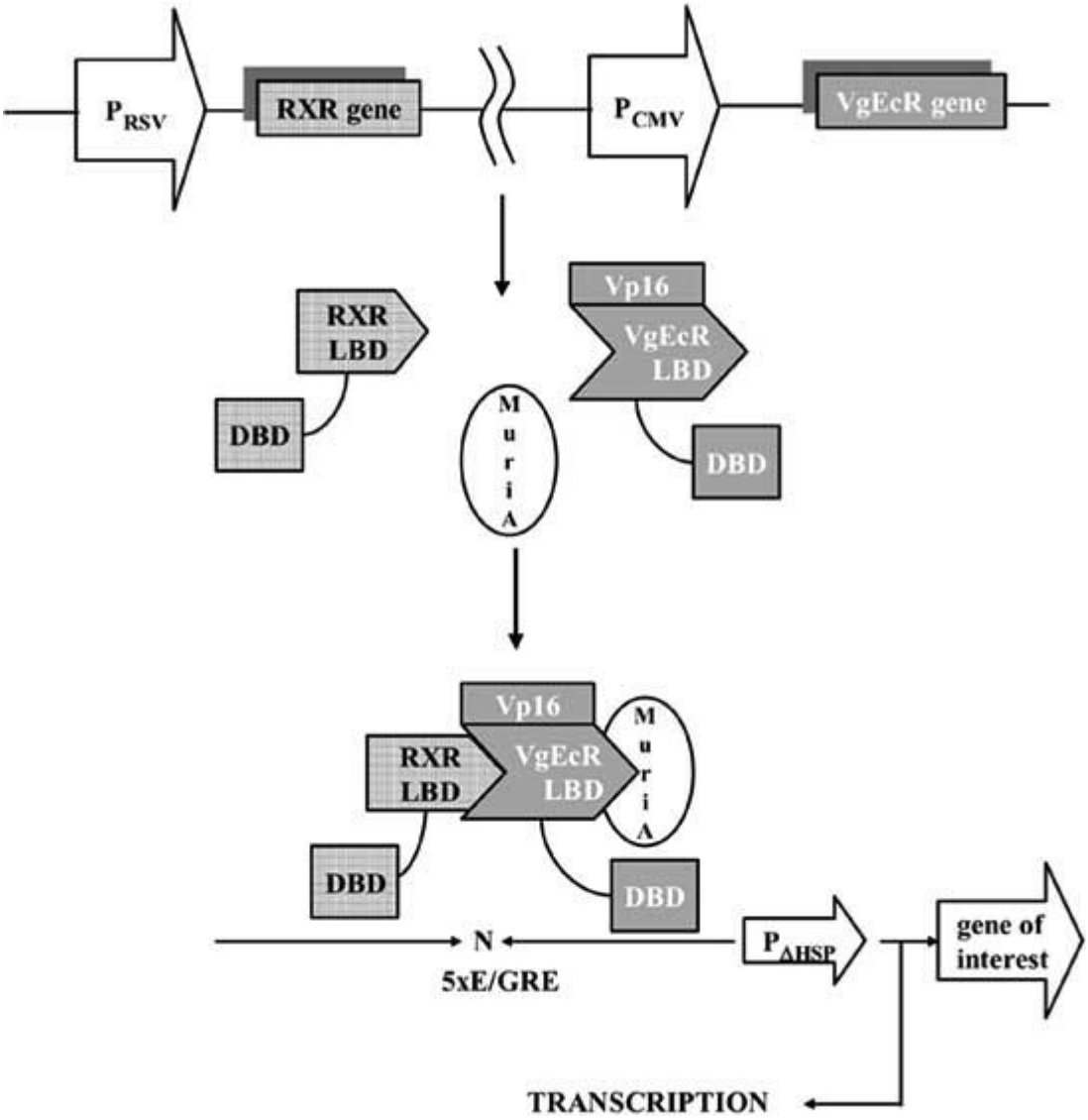
The ecdysone- inducible mammalian expression system was developed at The salk institute for biological studies (sandiego, CA, USA)[18].

Inducible expression systems are already showing great potential for clinical use in humans [19]. For example, applications for regulated gene expression include inducible gene targeting, over expression of toxic and teratogenic genes, block of the antisense RNA expression, studying development and other physiological processes and gene therapy [20]. Examples might include the controlled expression of anti-inflammatory cytokines to treat autoimmune diseases and graft versus host pathology, (or) the regulated production of recombinant insulin to treat type 1 diabetes [21]. The benefits of regulated transgene expression in gene therapy have been reviewed [22].

The ecdysteroid-controlled system is one of the promising candidates for the future of gene therapy applications with the prospect of clinical human gene therapy on the horizon [23].

A possible realistic procedure for germ line improvement has already been written [24]. If prostate cancer were ever diagnosed (or) even suspected, a man would get an injection of ecdysteroid. The hormone would activate the ecdysteroid receptor in his prostate glandular cells, which would turn on the diphtheria gene. Diphtheria toxin would kill the cells. One shot and the cancer would be gone [25, 26].

Inducible gene expression systems in mammalian cells have been shown to be valuable process to study the specific function of a protein in differentiation, proliferation or survival/apoptosis. Ecdysone analogues potentiated the IL-3 dependent activation of the signaling pathway of the pro-B cell-line, which could ultimately interfere with the growth, and / or survival of these cells [27].



Ecdysone-Inducible Mammalian Expression System: Expression of the mammalian protein RXR and the modified ecdysonereceptor VgEcR (a fusion of Vp16 transactivation domain and the NH2-terminal truncation of a mutant ecdysteroid receptor and theDNA-binding domain of the glucocorticoid receptor) is under control of the constitutive promoters, such as PRSV and PCMV, respectively. Co-expression of receptor proteins results in the formation of VgEcR/RXR heterodimer which binds by interaction of their DNA-binding domains (DBD) specifically to the response elements (5xE/GRE) that are present in the promoter of the transgene. Addition of an EcR agonist (either **muristerone A** or ponasterone A) causes a conformational change in theVgEcR ligand binding domain (VgEcR LBD), which results in activation of a core promoter, the minimal heat shock promoter (PΔHSP).The resulting complex is active to cause transcription of the gene of interest.

Importance of the Muristerone A

The activity rate of isolated ecdysteroids is evaluated by biotesting with insect cells containing natural ecdysteroid receptors (EcR). Muristerone A, Ponasterone A and 20-Hydroxy ecdysone (20-E) are regarded to be most active ecdysteroids with large practical use possibilities. Each of them can show different results with different receptors.

Muristerone A isolated from kaladana seeds, is a member of the ecdysteroid family and is used to induce expression of the gene of interest from any of the ecdysone inducible system expression vectors. Since the discovery of Muristerone A in 1972, more than 1500 articles have been published on various aspects of its research. Scientists give more preference to Muristerone, despite it is a rare and expensive substance. The Ponasterone A use is complicated because of its instability: after 3 hours the receptor complex decays by 50 % in buffer solutions, where as for Muristerone the figure lie by 5% [28].

The expression of the reporter gene was markedly increased in cells treated with Muristerone A and lesser extent in cells treated with Ponasterone A. The addition of Muristerone A causes a greater than 200 fold induction of expression of the target gene over basal levels in mammalian cells. The strong agonist Muri A differs from Pon A solely by the addition of hydroxyl groups at position 5 and 11 [29, 30].

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S.No	Reason	Present Muristerone Supplying Companies	AltaVista Phytochemicals
1	Price	$ 140-289/1 mg	**10-999 mg---------- $ 10/1 mg 1-100 grams --------$ 5000/1 gram >100 grams----------$ 3000/ 1gram**
2	Packing	250 ug to 1 mg	**10 mg to 1 gram**
3	Quantity Available	500 mg to 1 gram	**1000 to 3000 grams/year**
4	Regular Supply	Not guaranteed	**Guaranteed supply**
5	Period of Supply	--------	**Minimum 5-10 Years**
6	Scheduled Delivery	Some times back ordered	**No back orders at any time**
7	Purity	90-99%	**>97 %, Same purity will be supplied regularly**
8	Supporting Spectral Data	----	**NMR, HPLC, LC-MS, UV-Vis. SOR.**