Advanced Engineering Journal
https://adejournal.org

Chemically Inducible Promoters in Recombinant Gene Expression and Protein Engineering. A Review

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Keywords
Chemical Indicible Promters
Recombinant Gene Expression
Protein Engieneering

ABSTRACT

In this study, one of the important topics of biotechnology, recombinant gene expression, and chemical inducers, which are becoming more and more prominent in the field of protein engineering, are explained. It has been observed that transglutaminases are an important enzyme that catalyzes acyl transfer reactions in meat products. P. pastoris is a broad expression system for the production of heterologous proteins due to its high protein expression and capacity for post-translational modification of proteins.

1. INTRODUCTION

Biotechnology, emerging as a combination of biology and engineering sciences, has taken its place in the world of science as an interdisciplinary field of study. Agricultural and medical biotechnology is one of the main research areas of biotechnology. Sub-branches include branches such as bioinformatics, blue biotechnology, industrial biotechnology, and red or biopharmaceutical biotechnology.

1.1. Recombinant Expressions

Genes, the heart of molecular genetics, can be isolated and amplified. Recombinant expression, which occurs as recombinant gene expression, recombinant DNA expression, and recombinant protein expression, are among the most important techniques used in both molecular and medical research and industrial settings. Nowadays, studies on recombinant gene expression show that 80% of the genes are with enterobacterium Escherichia coli or the methylotrophic yeast Pichia pastoris.

One method of isolating and reproducing a gene is to clone it in a living cell by inserting it into another DNA molecule that acts as a replicable vector or tool. The development of research on molecular genetics has been successfully achieved by combining molecules from different sources and the formation of new DNA molecules called recombinant DNA. Recombinant DNA molecules are formed, which do not exactly resemble the original DNA molecules, but partially carry the nucleotide sequences of DNA molecules. The technology that involves the cutting of DNA molecules obtained from different biological species by genetic engineering technology and combining the different DNA parts obtained, which cannot be formed spontaneously in nature, is called Recombinant DNA technology. Recombinant DNA technology can also be described as the sum of the techniques of studies involving the insertion and replication or expression of a gene isolated from a living thing by any means into a suitable host.

With this technology, it is possible to transfer genes and create diversity among species belonging to prokaryotic and eukaryotic groups.

Cloning a gene using recombinant DNA technology can be performed in four steps. Gene cloning can be

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Cite this article *(APA);*

Vural U S (2021). Chemically Inducible Promoters in Recombinant Gene Expression and Protein Engineering. A Review. Advanced Engineering Journal, 1(1), 01-10.

Review Article

defined as follows: It is the process of obtaining multiple copies of an important product, namely the target DNA region, in special host cells by combining it with a vector. Figure 1 shows the recombinant DNA generation schematically. rDNA is prepared in four stages (Fig. 1).

- DNA isolation
- DNA splicing
- DNA splicing
- rDNA amplification

One of the most important goals of rDNA technology is to develop organism species that have characteristics not found in natural species. Depending on the targeted results, the correct gene of interest is identified and isolated before DNA is prepared. The organism that is the source of the targeted DNA segment is called a donor, and the organism whose DNA has been changed is called a vector.

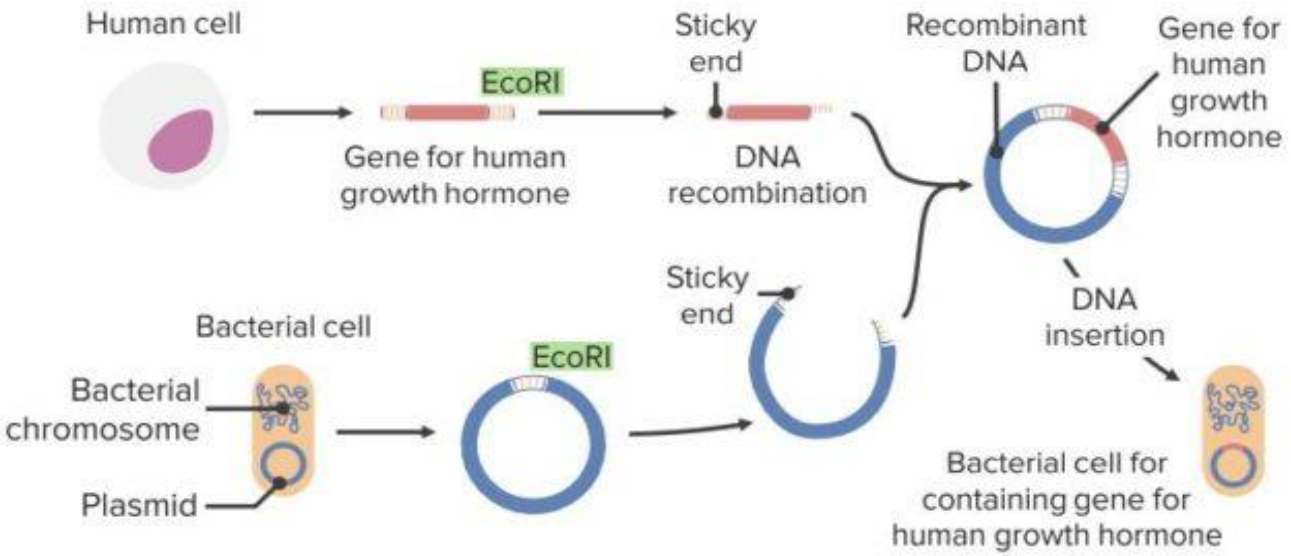

Figure. 1 The recombinant DNA generatio

The first step in preparing rDNA is to isolate both the vector and donor DNA. The most commonly used vector for prokaryotic applications is circular bacterial DNA plasmid. To isolate the plasmid from the rest of the DNA genome, differential centrifugation is performed with the addition of cesium chloride and ethidium bromide. The two DNA fragments are selectively bound to ethidium bromide and generate a gradient of cesium chloride after centrifugation. The submerged plasmid DNA is collected for the next step.

The second and third steps involve cutting or joining the piece of DNA using enzymes. The two major classes of enzymes are important tools in recombinant DNA preparation and isolation. Restriction endonuclease: acting like scissors, they cut DNA at specific sites. Sugar phosphate in DNA cuts the backbone at both strands. Each of the restriction enzymes recognizes specific DNA sequences, cutting them at a specific place in the DNA sequence. The enzyme cuts DNA from its double-strand by breaking the covalent bonds between the phosphate group of a deoxyribonucleotide and the sugar group of the neighboring deoxyribonucleotide. The restriction endonuclease can separate the sugar-phosphate backbone to form a blunt or sticky-ended, double-stranded DNA fragment. At the blunt end; Both strands of the molecule are cut in the same place, that is, the ends are straight and each of the nucleotides is paired. At the sticky end; Each strand of the molecule is cut from different positions. A few nucleotides in the strand form protrusions, these single-stranded ends can spontaneously form base pairs with each other.

DNA ligase enzyme: It can join DNA fragments at blunt or sticky complementary ends. Ligase does not distinguish between DNAs of different origins. Thus, two DNA fragments are cut from the chromosomes of two different organisms by restriction endonuclease and joined by DNA ligase. The two fragments are now a single DNA molecule. This cut and paste technique creates the recombinant DNA molecule. This is then transferred to the host cell, where it is replicated for further studies. This process is called DNA cloning. The last step is DNA amplification. The resulting rDNA is inserted into the bacterial cell through a process called plasmid or vector transformation. The recombinant plasmid will then be replicated by natural DNA replication processes. When replicated, donor DNA is also replicated and multiple copies are produced. The continuation of cell division leads to millions of cells containing the desired piece of DNA (amplification).

The importance of gene transfer technologies is increasing day by day in studies conducted in the field of molecular biology and genetic engineering. In this technology, genes are taken from any organism and cloned. At the point reached with the researches and developing techniques in recombinant gene expression, recombinant DNA engineering, or protein engineering, it has been revealed that this technology can be used in fields such as agriculture and animal husbandry, basic sciences, environmental engineering, especially medicine. It has started to take its place in many areas such as diagnosis and treatment of diseases in medicine, smart drugs, vaccine production, increasing the yield and diversity of animal products such as meat, milk, and honey in animal husbandry, increasing the yield of oilseeds in agriculture and increasing the yield of fatty acids and lipids for the production of biofuels (biodiesel).

Interest in yeasts such as Pichia pastoris, Hansenula polymorpha (Pichia angusta), and Yarrowia lipolytica for the production of recombinant biomolecules with biotechnological applications has increased day by day. In Y. lipolytica, more than one hundred heterologous proteins have been produced with high yield and success. Y. lipolytica is a model yeast species known for its metabolic properties such as growth and accumulation of intracellular lipids at high efficiency. Y. lipolytica, which has great ability to secrete proteins and metabolites, has been used for various industrial applications such as heterologous protein synthesis, citric acid, and erythritol production, lipid production for biodiesel and bio-jet fuel, ricinoleic acid, conjugated fatty acids and derivatives of fatty acids.

Promoter selection, one of the key parameters driving recombinant gene expression, is very important when developing effective cell production. At present, few promoters have been identified and their regulations are not yet fully understood.

1.2. Chemically Inducible Promoters

Promoters act on the binding of RNA polymerase and transcription factors. As the promoter region directs the transcription of target genes, it determines the timing of gene expression and defines the amount of recombinant protein to be produced.

Inducible promoters are required for functional characterization of essential genes, as well as for the biotechnological production of heterologous protein products and fine-tuned expression, especially if the protein is toxic to the cell (Lee et al. 2010; Nevalainen et al. 2005). It was originally developed to express a gene product under uncertain conditions. An ideal inducible promoter is characterized by strong and tightly controllable regulation, cost-effective induction, and efficient expression of the gene of interest located downstream of the inducible promoter sequence. Inductive promoters are divided into chemical and physiological inducing promoters. Chemically regulated systems are induced or suppressed by the presence or absence of chemical compounds such as alcohols, antibiotics, hormones, or carbon sources (Matsuzawa et al. 2013; Meyer et al. 2011). In contrast, the regulation of physiologically controlled promoters is determined by abiotic environmental factors. In this context, systems that are regulated depending on factors such as osmotic stress, temperature, and light have been established (Fischer et al. 2016; Zhang et al. 2016). The selection of an appropriate supplement should be adapted to experimental approaches. Since not every system has optimum conditions for the selected model organism, it should be known that some inducing or suppressing substances or the protein produced have toxic effects on some organisms (Kim et al. 2016; Lee et al. 2010). Leakage of the promoter resulting in minimal structural activity leads to uncontrolled gene expression (Meyer et al. 2011). Biochemical, physiological, and genetic studies of tissue are very useful in determining the developmental processes in biochemical systems. These studies include the enhanced or ectopic expression of enzymes and regulatory proteins and the expression of antisense RNA or dominant-negative proteins that reduce the amount of a gene product. Its wide range of promoters significantly augments its research on promoters. Promoters that are activated in response to a particular chemical are particularly valuable. It is an example of a promoter in E. coli that provides the expression of a recombinant protein that will interfere with growth. An inducible promoter is required if a foreign gene product expressed in plants is to interfere with regeneration, growth, or reproduction. Other arguments than the lethality of the gene product may support the use of an inducible promoter relative to the constitutive one (List 1). Chemically inducible promoters are of interest to genetics in the improvement of crops. Examples of biotechnological applications are given in List 1.

If very small amounts of a gene product are required for a biochemical process, the expression level of the promoter should be close to zero in the absence of the stimulus. If high amounts of a gene product are effective, activity should be tolerated in the absence of the inducer while high levels can be induced in the presence of the inducer. Both properties of very low expression levels in the absence of the stimulus and high expression levels in the presence of the stimulus must be combined in a single system as they can be applied more widely. Another advance in the use of a chemically inducible promoter is its use in combination with tissue-specific promoters. Thus gene expression can be restricted to a particular tissue at a given time.

The chemical used must be highly specific for the target promoter. It should not affect the expression of other genes and other cell functions. Additional requirements an ideal inductor must meet are listed in Schedule 2.

List 1. Applications of chemically inducible promoters

Basic research

- Expression of genes that interfere with regeneration, growth, or reproduction.
- Expression of gene products at different stages of development.
- Differentiation between primary and secondary effects.
- Analysis of primary effects before homeostatic mechanisms starts to counteract.
- Clear correlation between the induction of the transgene and occurrence of an altered phenotype.

Biotechnological applications

- Construction of a conditional male sterility system.
- Expression of transgenes that interfere with regeneration, growth, or reproduction (biofarming).
- Conditional expression of resistance genes as a means of pest management to delay adaptive processes of the pathogen.
- Simultaneous induction of processes such as flowering and leaf abscission

To provide such tools, two different strategies are being adopted. First, plant promoters that respond to a given chemical have been isolated: obviously, this

concept will provide expression systems that are not specific to the transgene alone. The second strategy involves the use of regulatory elements from other organisms that respond to chemicals that plants encounter for the first time. The second strategy involves the use of regulatory elements from other organisms that respond to chemicals that plants encounter for the first time. Both strategies have yielded useful tools that respond to a variety of unrelated chemicals (Fig. 2).

List 2 Properties required for an ideal inducer

- High specificity for the transgene, no toxicity.
- High environmental compatibility.
- Easy application by spraying or root soaking.
- High efficiency at low concentrations and low amount of use.
- Low costs.
- Derivatives with different properties: inducer chemicals, inactivating chemicals, chemicals that act systematically, chemicals with a specific application area, chemicals with different half-lives.

A prominent example of chemical treatment is the PR-1a promoter (Gatz, 1997). The PR-1a promoter, which drives the expression of a defense gene, is normally induced upon pathogen attack, forms of oxidative stress, and leaf senescence, but it also responds to benzothiadiazole (BTH, Fig. 2) (Gorlach, 1996). The PR-1a promoter has been used to drive the expression of the Bacillus thuringiensis d-endotoxin in transgenic plants. Insect damage was only inhibited in the chemically treated plants. As a permanent expression of a resistance protein favors the adaptive processes of a pathogen, the PR-1a/BTH system might constitute a means of reducing these selection pressures to ensure improved pest management. Specific steroid hormones control animal development by altering gene expression patterns, but there is no equivalent target molecule in plants. If the underlying regulatory mechanisms involve the binding of only one regulatory protein to specific sequences, they can be engineered to work in organisms, thus yielding expression systems that respond to highly specific chemicals. This strategy requires the expression of two genes in transgenic plants: the gene encoding the protein responsible for the regulation (transcriptional repressor or activator) and the gene of interest, under the control of a suitable target promoter. This strategy is more complex than the use of endogenous plant promoters, which only require the transfer of the gene of interest under the control of the inducible promoter. The use of heterologous regulatory elements includes systems that respond to the antibiotic tetracycline (Gatz et al. 1992; Weinmann et al. 1994). Plant, the steroid dexamethasone (Aoyama, 1997), the copper ion (Mett et al. 1993; 1996) or IPTG (Wilde et al. 1992), ethanol (Caddick et al. 1988). The chemically induced systems that have been tested are summarized in Table 1.

1.2.1. Types of Chemically Inducible Promoters

The Tetracycline-Inducible Promoter

In the tetracycline-inducible expression system, two types of gene constructs are followed. The first repressor protein gene (tetR) is placed under the control of Cam 35 promoter and the second gene (gene of interest) is placed under modified target promoter (Gatz, 1997; Gatz et al. 1992; Weinmann et al. 1994; Odell et al. 1985). Transposon encoded Tet repressor protein of 24 kDa is synthesized under the control of Cam35S promoter. These repressor proteins (tetR) immediately, bind to the target promoter (modified), which contains 19 bp tet operator sites. As a consequence expression of a gene of interest is blocked when antibiotic tetracycline is added and it binds to the repressor protein and forms TetR/tc complex and finally abolishing its DNA binding activity. Under these conditions, repression is relieved, leading to the synthesis of gene products (Fig. 3). This tetracycline inducer system has been known to be involved in the commercial technique of sterilized products.

Steroid-Inducible Promoter

Certain steroids such as glucocorticoid dexamethasone (dx) can be used as chemical inducers. Glucocorticoid mediated transcription is the process of inhibitory interaction between the heat shock protein (HSP90) and the ligand binding domain of the receptor that occurs in the absence of the ligand. In the dx-inducible expression system, the fusion protein, Tf-GR, consisting of a transcription factor as the glucocorticoid binding domains is expressed under the control of Cam35S promoter (Bruce et al. 2000, Evans, 1988). In the absence of steroid dx, the activator (TF-GR) is inactivated by the formation of a complex with heat shock protein 90 (HSP90). The binding of dx mediated dissociation from HSP90 and allows binding of the activator protein to a target promoter and transcription is induced (Fig. 4).

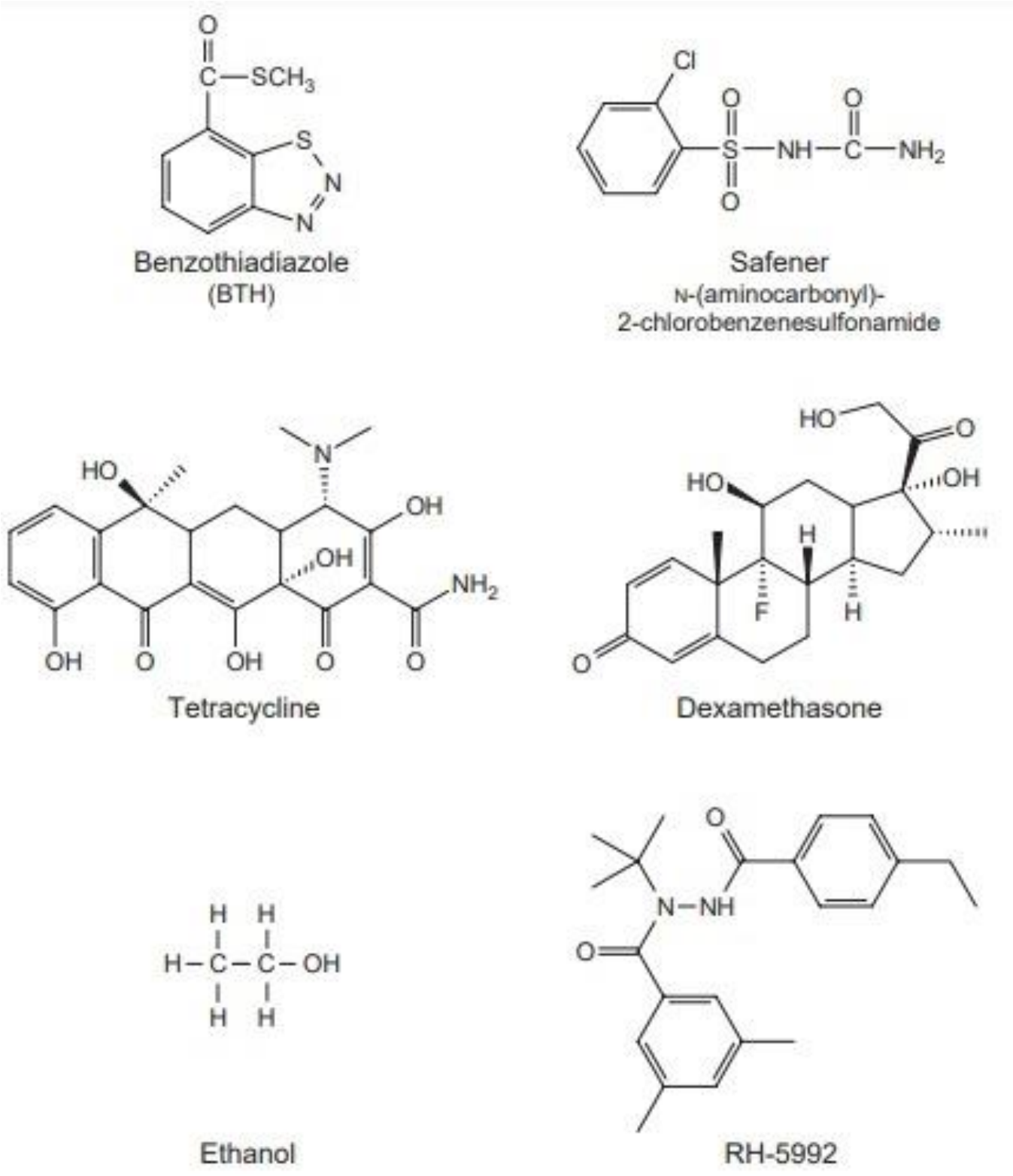

Figure 2. Molecules used for the chemical induction in gene expression

Table 1. A list of chemical-inducible systems

System	Tested	Strong	Limitations
Tetracycline-inducible TetR	tobacco, tomato, potato, BY2 cells, SR1 cells	Small amounts are sufficient for inducer activation, easily taken up by inductor cells	Leaky expression, high TetR concentrations required, the short half-life of inducer, will not work in Arabidopsis
Tetracycline can be inactivated with tTA	*Arabidopsis, tobacco, NT1 cells*	Target promoter can be efficiently closed, transgenic turnover evaluated, lower basal levels compared to TetR	The maintenance of plants with tetracycline must be negatively controlled to shut down gene expression.
Dexamethasone-inducible GR fusions	*Arabidopsis, tobacco*	GR :: transcription factor defines fusion proteins, immediate target genes, post-transcriptional activation	Suitable for transcription factors only, in some cases inductively toxic, induction of defense-related genes in Arabidopsis
Dexamethasone-inducible GVG	*Arabidopsis, tobacco*	Dexamethasone easily penetrates plant cells and can be applied in a variety of ways.	Inductor toxic in some cases, induction of defense-related genes in Arabidopsis, deceleration rate
Estradiol inducible ER - C1	Black Mexican sweet corn cells	Relatively low levels of estradiol are required, no visible toxic effects	Not tested on transgenic plants, not suitable for plants containing phytosterols
Estradiol inducible XVE	*Arabidopsis, tobacco, BY2 cells*	No visible toxic effects, low basal level, and high inducible levels	Not suitable for plants containing phytosterols (soybean), not suitable for use in the field
Dex-inducible and tet-suppressible TGV	*Arabidopsis, tobacco, BY2 cells*	Double-check, fast shutdown	In some cases, the inducer is toxic, defense-related genes can be induced
GV Hv EcR inducible with tebufenozide	Tobacco	Safe inductor, suitable for field application	High basal activity, foliar uptake of inducer is poor
Methoxyfenozide-inducible GV C fEcR	*Arabidopsis, tobacco, BY2 cells*	Low basal and high inducible levels, safe inducing, suitable for field application.	Foliar inducer uptake is poor, closing is slow
EcR on methoxyfenozide inducible GV	Corn	Safe inductor, inductor act systematically, suitable for field application.	Foliar uptake of inducer is poor
Ethanol-inducible AlcR	*Arabidopsis, tobacco, potatoes*	The inductor is cheap and biodegradable, fast reversible induction, suitable for field application	Volatile inducer, induction may be accidentally triggered, induction due to anoxia, inducer cannot be used for more than 2 days
Copper-inducible ACEI Arabidopsis,	*tobacco, root nodules, BY2 cells*	Inductor application convenience, simple to use, inductive cheap Induced levels are low,	Prolonged exposure of the inducer is toxic, does not work on BY2 or protoplasts derived from tobacco leaves, expression is variable in Arabidopsis.
In2-2 inducible with Safener	*Arabidopsis, BY2 cells*	It is an inducing agrochemical suitable for field application.	Inductor causes growth abnormalities, constitutive expression in roots, promoter responsive to other chemicals.

Copper-Inducible Gene Expression

Copper is an indispensable element in plant metabolism, but accumulation to high levels leads to toxic effects. Thus, high copper levels triggered the expression of copper detoxifying genes. Regulation is mediated by the transcription of ACEI, which binds to cis-elements only in the case of copper. This simple mechanism was engineered into the plants. Any target gene can be expressed efficiently when the activator was driven by the Cam 35 promoter. While copper is absent, it cannot bind to its target promoter. The addition of copper leads to the binding of copper and induces a conformation change, facilitates the AECI activator protein to bind to a specific cis region of the target promoter, and transcription from the target promoter is induced. Using copper inducer expression system, cytokinin level was increased in plants. By placing AECI under the control of the nodule, specific nod 45 promoter antisense constructs of aspartate aminotransferase-P2 were expressed in nodule-specific fashion in transgenic lotus plants.

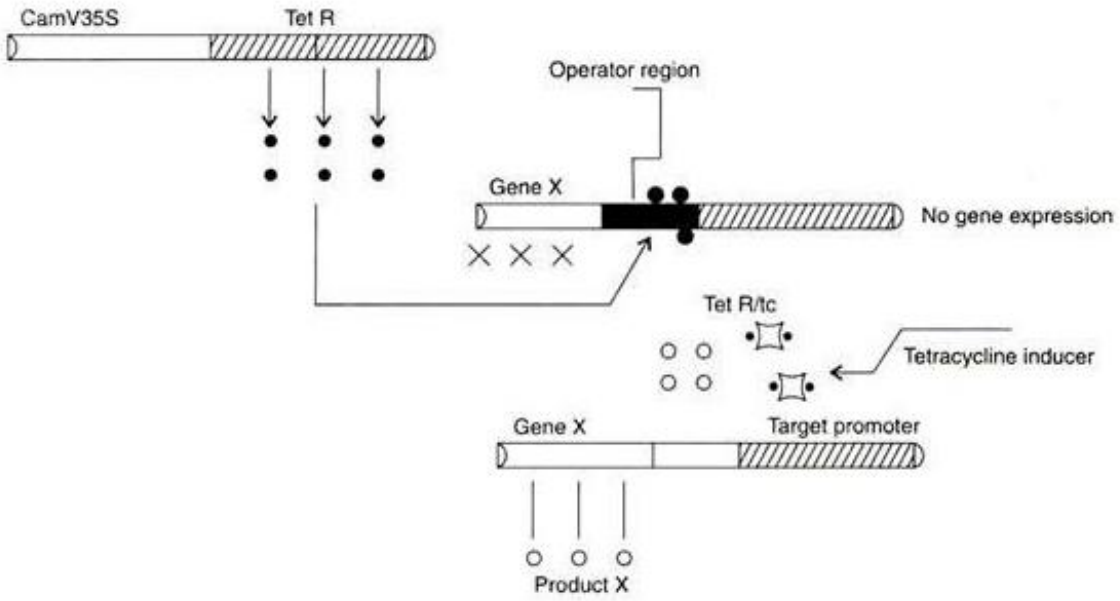

Figure 3. The tetracyline inducable promoter

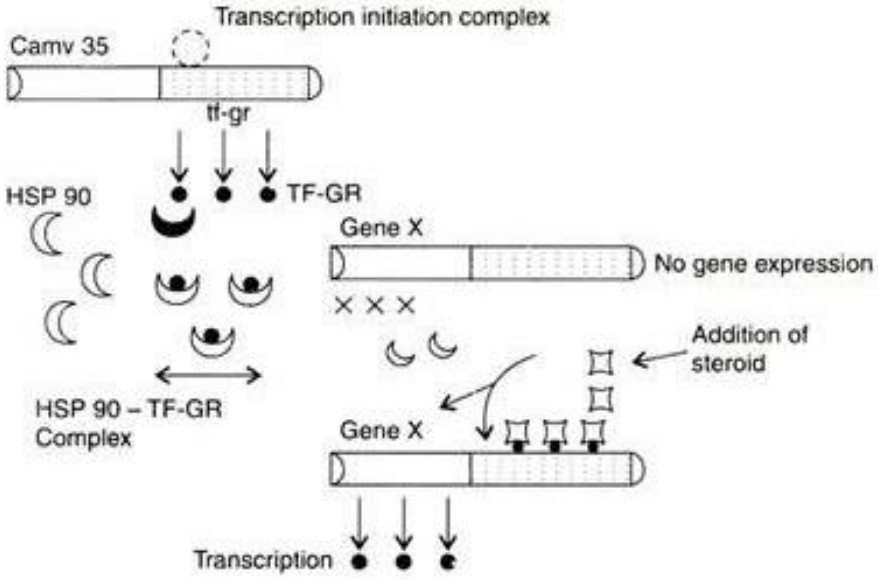

Figure 4. Sterooid inducable promoter

Ethanol-Inducible Gene Expression

Certain regulatory elements like alCA promoters are strongly inducible by ethanol. This promoter is widely used in the transgenic art to overexpress proteins. The transcriptional activator AlcR binds to the promoter when cells are grown in the presence of ethanol (Caddick et al. 1998) The system was used in vegetative organisms by placing the alcR under the control of the Cam35S promoter. The target promoter containing the TATA box was fused to the Cam35S promoter and transferred into tobacco plants. Expression of the transgene is now carried out by an ethanol-dependent process. When AlcR is expressed by the Cam35S promoter in absence of alcohol, it will not bind to the target promoter and no gene expression takes place when alcohol appeared in the cells. Transcription is induced by creating conformational changes that allow the AlcR to bind to specific cis-sequences of the target promoter.

1.3. Recombinant Transglutaminase Gene Expression in Protein Industry

Transglutaminases are a large group of enzymes that catalyze the acyl-transfer reactions. These enzymes can catalyze acyl-transfer reactions between intrachain (or interchain) glutamine residues (acyl donor) and lysine residues (acyl acceptor) to form ε-(γ-glutamyl)-lysine isopeptide bonds. The reactions catalyzed by microbial transglutaminase (MTG) can be divided into three categories. In the presence of primary amine in the acyl receptor, MTG catalyzes the acyl transfer reaction, in the presence of lysine in acyl receptor MTG catalyzes the protein crosslinking reaction and in the case of water in acyl receptor, MTG catalyzes the reaction of deamination (Kieliszek et al. 2014, Day et al. 1999). Microbial transglutaminase (MTG) is a calcium ion-independent enzyme. The ability of transglutaminases to catalyze the modifications of proteins in industrial food to improve

the functional properties was explored in several studies. Crosslinked proteins containing various amino acids improve nutritional value, and thus such proteins are valuable in food processing industries (Kieliszek et al. 2014; Salis et al. 2015). Transglutaminases (TGase) is found in microorganisms, mammals, invertebrates, vertebrates, and plants (Lorand et al. 2003; Del et al. 2005). There are several methods for generating TGase. One method is the purification of enzymes by extracting them from the tissues of plants or animals or their secreted fluids. However, this method is costly and time-consuming. The second method is the large-scale production of MTG by using traditional fermentation Technologies (Yokoyama et al. 2004). However, the traditional fermentation method for MTG production is costly and susceptible to adverse factors during fermentation and can lead to fermentation failure.

1.3.1. MTG expression systems

MTG has been successfully expressed in Escherichia coli Corynebacterium glutamicum and Streptomyces lividans. E. coli is widely applied as a host strain for protein expression owing to its rapid growth and high yield, although, as a bacterium, E. coli has endotoxin and certain pathogenicity (Javitt et al., 2017; Liu et al. 2016; Umakoshi et al. 2011; Fratamico et al. 2012). Therefore, most people cannot accept protein products expressed by E. coli. E. coli expression systems have many disadvantages that can lead to undesirable conditions such as post-translational protein modifications. MTG is expressed as inclusion bodies insoluble in E. coli, enzyme activity may not be obtained (Tzeng et al. 2005; Liu et al. 2007). P. pastoris is a broad expression system for the production of heterologous proteins because of its high protein expression and capacity for the post-translational modification of proteins. P. pastoris is a strong and tightly regulated AOX1 promoter and inexpensive medium. It has high production yields and simplified protein purification and is easy to manipulate. MTG expressed by P. pastoris is active and does not form inclusion bodies. MTG is expressed as a proenzyme in P. pastoris. In vitro activation of MTG can be achieved by enzymatic digestion of other proteases such as trypsin. (Marx et al. 2007; Eissazadeh et al. 2017).

1.4. Protein Engineering

Meat is the most important source of protein. The major problem In meat production is the reuse of by-products, such as mechanical descaling of minced meat and fat blocks attached to organs and tendons on joints, MTG is widely used for binding small pieces of meat (Sorapukdee et al. 2018).

This process increases the economic value of the meat products and reduces waste. MTG can also improve the quality and properties of the gels from the minced mixtures of meat and fish proteins through crosslinking (Li et al. 2017). Excessive meat consumption increases the risk of obesity, hyperlipidemia and high blood pressure. Such health problems, as in meat consumption, have led scientists to improve the nutritional content of meat products and produce food products with low fat and cholesterol content.. Plant proteins (such as soybean) and animal proteins (such as meat) are the most known two sources of proteins. Meat has good taste but has high-fat content, whereas soy protein contains amino acid composition but has a poor taste. Mixing meat and soy protein and using their different protein functionalities may improve the fat contents and gel properties of meat products. The addition of soy protein can favorably affect the hydration properties of meat gels, as nonmuscle proteins interact with MTG during gel formation. (Pietrasik et al. 2007). Myofibrillar proteins (MP) play an important role in the functional properties of meat. These proteins are the largest part of proteins in muscle tissue (Yu et al. 2016). Myofibrils are composed of actin, myosin, and troponin, and other proteins that hold them together. Myofibrillar proteins are usually able to form the gel network and increase the water holding capacity due to the entrapment of moisture (Amiri et al. 2018). MTG improves the gel properties of heat-induced myofibrillar protein and pea protein isolate gel (Sun et al. 2012). However, MTG expressed by engineered bacteria has been not applied to the reconstruction of meat products. Researchers also investigated the effect of MTG on the quality of restructured meat through texture profile analysis and on the crosslink of soy protein isolate (SPI) and MP. This method will make a significant contribution to the more efficient use of meat and plant proteins and to the development of new restructured food products.

2. CONCLUSSION

Proteins are one of the essential foods that improve the quality of life. As the quality of life increases in the developing world, protein consumption also increases. Excessive protein consumption brings along various health problems such as hyperlipidemia, high blood pressure and cholesterol in humans. New technologies and techniques developed in protein engineering, along with innovative studies in genetic science, have led to many steps in the production of recombinant proteins. In studies on gene recombination, it is still unclear to solve many problems such as the occurrence of microbiological events that are difficult to understand due to parameters such as inducers, temperature and light, and so on. Therefore, much more studies are needed on recombinant genes, recombinant DNA or recombinant proteins. Studies on recombinant genes are not limited to protein engineering such as the taste, smell and production efficiency of foods, but will also provide significant improvements in vaccine and smart drug production, especially in the health sector. Although recombinant gene or recombinant DNA studies are to improve the quality of life of living things, scientists are also risky enough to be a biological weapon that threatens the lives of living things. Therefore, national and international ethical principles regarding recombinant genes or recombinant DNA studies should be clearly determined and restrictive regulations related to this should be set forth. While recombinant gene technology promises great hopes for human beings, it should be understood that it can bring irreversible problems that threaten humans.

REFERENCES

Amiri A, Sharifian P & Soltanizadeh N (2018). Application of ultrasound treatment for improving the physicochemical, functional, and rheological properties of myofibrillar proteins. International Journal of Biological Macromolecules, 111, 139–147.

Aoyama T & Chua N-H (1997) A glucocorticoid-mediated transcriptional induction system in transgenic plants, Plant J. 11, 605–612

Bruce W, Folkerts O, Garnaat C, Crasta O, Roth B & Bowen B (2000). Expression profiling of the maize flavonoid pathway genes controlled by estradiol-inducible transcription factors CRC and P. Plant Cell. 12, 65-80.

Caddick M X, Greenland A J, Jepson I, Krause K P, Qu N, Riddell K V, Salter M G, Schuch W, Sonnewald U & Tomsett A B (1988). An ethanol inducible gene switch for plants used to manipulate carbon metabolism. Nat Biotechnol, 16, 177-180.

Day N & Keillor J W (1999). A continuous spectrophotometric linked enzyme assay for transglutaminase activity. Analytical Biochemistry, 274(1), 141–144.

Del Duca S & Serafini-Fracassini D (2005). Transglutaminases of higher, lower plants and fungi. Progress in Experimental Tumor Research, 38, 223–247.

Eissazadeh S, Moeini H, Dezfouli M G, Heidary S, Nelofer R & Abdullah M P (2017). Production of recombinant human epidermal growth factor in Pichia pastoris. Brazilian Journal of Microbiology, 48(2), 286–293.

Evans R M (1988) The steroid and thyroid hormone receptor superfamily, Science 240, 889–895

Fischer S, Engstler C, Procopio S & Becker T (2016) Induced gene expression in industrial Saccharomyces pastorianus var carlsbergensis TUM 34/70: evaluation of temperature and ethanol inducible native promoters. FEMS Yeast Res 16(3), 014.

Fratamico P M & Bagi L K (2012). Detection of Shiga toxin-producing Escherichia coli in ground beef using the GeneDisc real-time PCR system. Frontiers in Cellular and Infection Microbiology, 2, 152.

Gatz C (1997) Chemical control of gene expression, Annu. Rev. Plant Physiol. Plant Mol. Biol. 48, 89–108

Gatz C, Frohberg C & Wendenburg R (1992). Stringent repression and homogeneous de-repression by tetracycline of a modified CaMV35S promoter in intact transgenic tobacco plants. Plant J, 2, 397-404.

Görlach J, Volrath S, Knauf-Beiter G, Hengy G, Beckhove U, Kogel K H & Ryals J (1996). Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. The Plant Cell, 8(4), 629-643.

Javitt G, Ben-Barak-Zelas Z, Jerabek-Willemsen M & Fishman A (2017). Constitutive expression of active microbial transglutaminase in Escherichia coli and comparative characterization to a known variant. BMC Biotechnology, 17(1), 23.

Kieliszek M & Misiewicz A (2014). Microbial transglutaminase and its application in the food industry. A review. Folia Microbiol, 59, 241–250 https://doi.org/10.1007/s12223-013-0287-x

Kim S I, Ha B S, Kim M S, Park M & Ro H S (2016) Evaluation of copper-inducible fungal laccase promoter in foreign gene expression in Pichia pastoris. Biotechnol Bioprocess Eng 21(1), 53–59, https://doi.org/10.1007/s12257-015-0567-1

Lee J, Son H, Lee S, Park A R & Lee Y W (2010). Development of a conditional gene expression system using a zearalenone-inducible promoter for the ascomycete fungus Gibberella zeae. Appl Environ Microbiol 76(10), 3089–3096. https://doi.org/10.1128/AEM.02999-09

Li Q, Gui P, Huang Z, Feng L & Luo Y (2017). Effect of transglutaminase on quality and gel properties of pork and fish mince mixtures. Journal of Texture Studies, 2017, 1–9

Liu S, Wang M, Du G & Chen J (2016). Improving the active expression of transglutaminase in Streptomyces lividans by promoter engineering and codon optimization. BMC Biotechnology, 16(1), 75.

Liu X Q, Yang X Q, Xie F H, Song L Y, Zhang G Q & Qian S J (2007). Oncolumn refolding and purification of transglutaminase from Streptomyces fradiae expressed as inclusion bodies in Escherichia coli. Protein Expression and Purification, 51(2), 179–186.

Lorand L & Graham R M (2003). Transglutaminases: Crosslinking enzymes with pleiotropic functions. Nature Reviews Molecular Cell Biology, 4(2), 140–156.

Marx C K, Hertel T C & Pietzsch M (2007). Soluble expression of a pro-transglutaminase from Streptomyces mobaraensis in Escherichia coli. Enzyme and Microbial Technology, 40(6), 1543–1550.

Matsuzawa T, Tohda H & Takegawa K (2013) Ethanol-inducible gene expression using gld1 (+) promoter in the fission yeast Schizosaccharomyces pombe. Appl Microbiol Biotechnol 97(15):6835–6843. https://doi.org/10.1007/s00253-013-4812-2

Mett V L, Podivinsky E, Tennant A M, Lochhead L P, Jones W T & Reynolds P H (1996). A system for tissue-specific copper-controllable gene expression in transgenic plants: nodule-specific antisense of asparate aminotransferase-P 2. Transgenic research, 5(2), 105-113.

Mett V L, Lochhead L B & Reynolds P H S (1993) Copper controllable gene expression system for whole plants, Proc. Natl. Acad. Sci. U. S. A. 90, 4567–4571

Meyer V, Wanka F, van Gent J, Arentshorst M, van den Hondel C A & Ram A F (2011). Fungal gene expression on demand: an inducible, tunable, and metabolism-independent expression system for Aspergillus niger. Appl Environ Microbiol 77(9):2975–2983. https://doi.org/10.1128/AEM.02740-10

Nevalainen KM, Te'o VS & Bergquist PL (2005) Heterologous protein expression in filamentous fungi. Trends Biotechnol 23(9):468–474. https://doi.org/10.1016/j.tibtech.2005.06.002

Odell J T, Nagy F & Chua N H (1985). Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature, 313, 810-812.

Pietrasik Z, Jarmoluk A & Shand P J (2007). Effect of non-meat proteins on hydration and textural properties of pork meat gels enhanced with microbial transglutaminase. Lwt-Food Science and Technology, 40(5), 915–920.

Salis B, Spinetti G, Scaramuzza S, Bossi M, Saccani Jotti G, Tonon G & Schrepfer R (2015). High-level expression of a recombinant active microbial transglutaminase in Escherichia coli. BMC Biotechnology, 15, 84.

Sorapukdee S & Tangwatcharin P (2018). Quality of steak restructured from beef trimmings containing microbial transglutaminase and impacted by freezing and grading by fat level. Asian-Australasian Journal of Animal Sciences, 31(1), 129–137

Sun X D & Arntfield S D (2012). Gelation properties of myofibrillar/pea protein mixtures induced by transglutaminase crosslinking. Food Hydrocolloids, 27(2), 394–400.

Tzeng S S, Yin L J, Chen G H & Jiang S T (2005). Molecular cloning of microbial transglutaminase from Streptoverticillium ladakanum BCRC 12422. Journal of the Fisheries Society, 32, 53–67.

Umakoshi M, Hirasawa T, Furusawa C, Takenaka Y, Kikuchi Y & Shimizu H (2011). Improving protein secretion of a transglutaminase-secreting Corynebacterium glutamicum recombinant strain on the basis of 13C metabolic flux analysis. Journal of Bioscience and Bioengineering, 112(6), 595–601.

Weinmann P, Gossen M, Hillen W, Bujard H & Gatz C (1994). A chimeric transactivator allows tetracycline-responsive gene expression in whole plants. Plant J, 5, 559-569.

Wilde R J, Shufflebottom D, Cooke S, Jasinska I, Merryweather A, Beri R & Schuch W (1992). Control of gene expression in tobacco cells using a bacterial operator-repressor system. The EMBO journal, 11(4), 1251-1259.

Yokoyama K, Nio N & Kikuchi Y (2004). Properties and applications of microbial transglutaminase. Applied Microbiology and Biotechnology, 64(4), 447–454

Yu T Y, Morton J D, Clerens S & Dyer J M (2016). Cooking-induced protein modifications in meat. Comprehensive Reviews in Food Science and Food Safety, 16(1), 141–159.

Zhang G, Liu P, Wei W, Wang X, Wei D & Wang W (2016). A light-switchable bidirectional expression system in filamentous fungus Trichoderma reesei. J Biotechnol 240:85–93. https://doi.org/10.1016/j.jbiotec.2016.11.003