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Epigenes: design and construction of new hereditary units

R.N. Tchuraev*, I.V. Stupak, T.S. Tropynina, E.E. Stupak

Institute of Biology, Ufa Research Centre, Russian Academy of Sciences, 69 Prospect Oktyabrya, Ufa 450054, Russia

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Abstract A plasmid digene construction designed before [Tchuraev, R.N. (1982) J. Gen. Biol. 43, 79–87] has been realised, including feedback by repressing proteins with given trigger regime of gene functioning. Experimental tests of the expected epigene properties of the obtained pECPI recombinant plasmid involving *lacI* and cI_{857} regulatory genes have shown a phenomenon of steady inheritance of two alternative epigenotypes $lacI^1 cI^0$ and $lacI^0 cI^1$, as well as an external toggle switch through metabolitic and temperature signals from one inherited functional state of the cyclic digene system into another. Thus, we have constructed a hereditary unit of a specific kind, namely, a two-component stationary epigene with preset properties.

Key words: Genetic network; Epigene; Epigenotype; Recombinant DNA; Functional state; Heredity

1. Introduction

In recent years a new branch of biology has been rapidly progressing. This is epigenetics, i.e. the study on hereditary variations of gene expression that occurs without any variation in the DNA sequence [2]. Epigenetics encompasses the research over a wide range of phenomena – chromosome and genome imprinting, epimutations, inheritance of acquired characters, and the possibility of non-Darwinian evolution strategies [3–8]. More than 25 years ago a hypothesis was proposed about the existence of a specific class of hereditary units: epigenes, in which part of hereditary information is stored, coded and transmitted from generation to generation regardless of the primary structure of DNA molecules in a genome [9,10]. The main feature of epigenes is the possibility for part of the hereditary information contained in them to be changed without any variation in the structure of primary nucleotide sequences. Thus, an epigene is any set of genes that implies at least two stable functional states of controlled genes and is capable of conserving each of the states from generation to generation. The occurrence of epigenes was shown by theoretical mathematical models [7,8]. A large body of experimental data supports the existence of prokaryotic and eukaryotic epigenes, which may be placed into three classes [8]. Further we shall consider one of these classes of epigenetic mechanisms – cyclic systems of genes with at least two stable steady states, each of which may be conserved from generation to generation. About 20 years ago one of the authors put forward an idea of 'epigene engineering', the essence of which was the construction, by means of gene engineering, of epigene cyclic systems with programmable inherited functional behaviours in the genes contained in them [1]. At the same time a general scheme was suggested to construct an arbitrary epigene. In an attempt to realise the idea a plasmid vector was obtained with a cascade regulation of gene expression [11], as well as recombinant plasmids with epigenetic regulation [12]. By now there are many gene engineering constructions, where gene expression is controlled by external factors (metabolites, or temperature) [13]. The present work is devoted to the construction of the simplest two-component epigene previously designed [1] that contains *lacI* and cI_{857} regulatory genes.

2. Experiments

2.1. Materials and methods

Restriction enzymes and DNA ligase were from Fermentas (Lithuania); 3-morpholinopropanesulfonic acid (MOPS), 2-morpholinoethanesulfonic acid monohydrate (MES), and isopropyl-β-D-1-thiogalactopyranoside (IPTG) were from Serva; all other reagents were of analytical grade. The host strain was *Escherichia coli* CSH36Δ (*lac pro*), *sup E, thi* (*F′ lacI pro* A^+B^+) [14]. The vectors used in this study were pCP2 [11], pET-15b (Novagene, USA), and pLACcI, pLACI constructed on the basis of pUCBM 21 (Boehringer Mannheim, Germany).

Standard methods [15,16] were used for plasmid purification, restriction digestion and ligation. As a reporter gene we used the *lacZ* gene on F′ episome. To test for the presence of β-galactosidase, bacterial colonies were plated on MacConkey plates (MacConkey Agar Base (Difco) with D(+)-lactose) containing 100 μg/ml ampicillin.

2.2. Plasmid construction

The cyclic digene construction containing *E. coli lacI* gene under control of λ phage P_L promoter and cI_{857} gene coding for λ bacteriophage temperature-sensitive repressor (hereinafter symbolised as *cI*) under the tandem $P_{T7}P_{lac}$ promoters control has been constructed (Fig. 1). The *lacI* gene without its own promoter was excised from pLACI as a *Bam*HI–*Sal*GI fragment and ligated to *Bam*HI, *Ava*I-digested plasmid pCP2. The ligation mixture was used to transform *E. coli* CSH36 cells. In the plates with indicative medium we selected ampicillin-tolerant transformants with Lac^- phenotype at 39°C (white colonies) and Lac^+ at 30°C (red colonies). These colonies were used for plasmid pCPI preparation (Fig. 2). Then pCPI was digested with *Bgl*I, *Bgl*II and a large fragment was ligated to the *Bgl*I–*Bgl*II fragment from pET-15b, which carried the T7 promoter (yielding pEPI (Fig. 3)). Finally the plasmid pEPI cut by *Nco*I was treated with SI nuclease and ligated to the *Pbu*II fragment from the plasmid pLACcI containing *cI* gene. The ligation mixture was used to transform *E. coli* CSH36 cells; and in indicative medium we selected colonies of ampicillin-tolerant transformants with Lac^- phenotype at 39°C, Lac^+ at 30°C and Lac^+ at 39°C with IPTG. This new plasmid construction was called pCEPI (Fig. 3).

*Corresponding author. Fax: (7)-3472-356 247.
E-mail: tchuraev@anrb.ru

Abbreviations: IPTG, isopropyl-β-D-1-thiogalactopyranoside; MOPS, 3-morpholinopropanesulfonic acid; MES, 2-morpholinoethanesulfonic acid monohydrate

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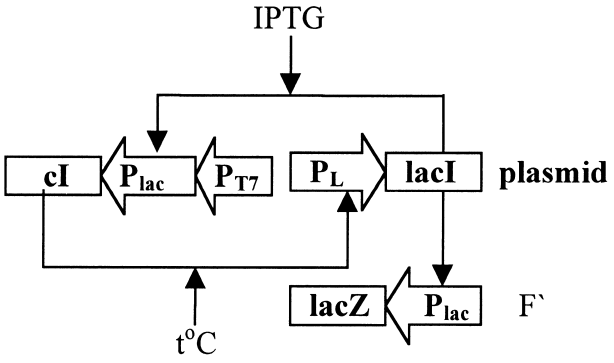

Fig. 1. Cyclic digene system with feedback via repressor proteins. Temperature-sensitive cI repressor inhibits the transcription of *lacI* gene from P_L promoter and is inactivated by thermal pulse. Lac repressor inhibits the transcription *cI* gene (on plasmid) from P_{lac} promoter and reporter gene (*lacZ*) on F′ episome and is inactivated with IPTG.

3. Results and discussion

According to the models of a cyclic digene system [9,10], one can expect, as applied to the pCEPI plasmid construction, two stable $lacI^0cI^1$ (*a*) and $lacI^1cI^0$ (*b*) epigenotypes, which reveal themselves in the expression of the *lacZ* reporter gene as alternative phenotypes – *A* (red colonies) and *B* (white colonies). Note that 'an epigenotype' is said to be a set of genes with their functional states specified [9,10]. In this case, 1 implies that the gene is switched on; 0 implies that the gene is switched off. As stated in [9,10], epigenotypes *a* and *b* must be stably inherited in the cell-division sequence. Switching from one epigenotype to another is accomplished by external metabolic (IPTG 80 μg/ml) and temperature (30 and 39°C) signals. Besides, two unstable epigenotypes – $lacI^0cI^0$ (*c*) and $lacI^1cI^1$ (*d*) – are plausible, which appear either as epigenotype *a* or *b* depending on the conditions.

To test heritability and switching of the expected epigenotypes we performed the following experiment. The CSH 36 (pECPI) liquid culture grown in 3 ml LB medium with

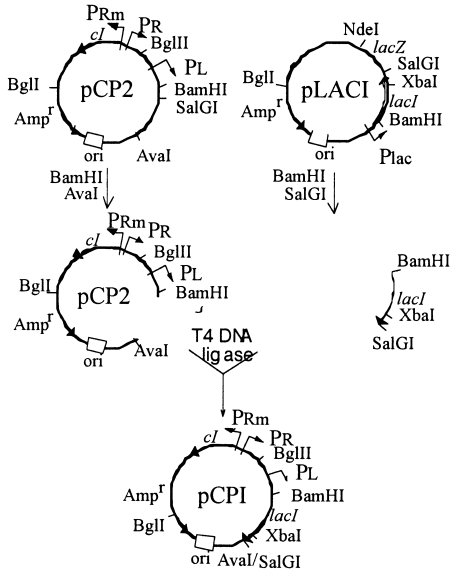

Fig. 2. Scheme of construction of pCPI recombination plasmid. See explanations in the text.

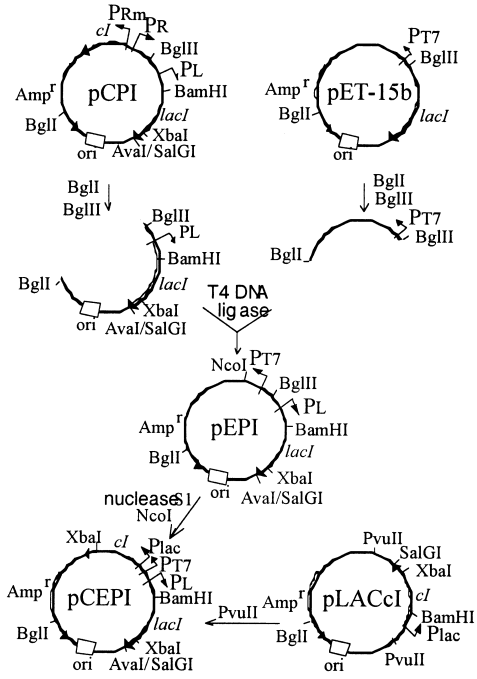

Fig. 3. Scheme of construction of pCEPI recombination plasmid. See explanations in the text.

IPTG at 30°C, plated on LB plates to individual colonies and incubated for 16 h at 30°C. New colonies (468 in number) were transferred to MacConkey plates with IPTG (by 52 colonies per plate) and incubated at 30°C. Red colonies of epigenotype *a* resulted. IPTG presence in the medium was responsible for *lacZ* gene expression on F′-episome and production of the CI repressor, which inhibited the Lac repressor synthesis. Replicas were taken from each plate, as illustrated in Fig. 4. In plate 2 incubated at 39°C all colonies produced from replicas were white; i.e. switching to epigenotype *b* took place. Switching from *a* to *b* was due to the degradation of dimers of the temperature-sensitive CI repressor, resulting in block-out of the Lac repressor synthesis, which inhibited the transcription of the *lacZ* gene (white colonies) and *cI* gene. Each of the three subsequent replicas from plate 2 to the plates incubated at 30°C (5 and others) produced white colonies; i.e. epigenotype *b* was stably inherited. The replica from plate 2 to the plate with IPTG (6) incubated at 30°C gave rise to red colonies; i.e. the reverse of epigenotype *a* was found. Each of the three subsequent replicas from plate 6 incubated at 30°C as well as from plate 1 (see plates 4 and 8) did not reveal any switch of epigenotype *a* (all colonies were red). Thus support was provided for the epigenotype stable inheritance during many cell generations on exclusion of the conditions that induced switching. In plate 3 (IPTG; 39°C) red colonies grew up. This can be explained by the fact that IPTG inactivating the Lac repressor annihilates the repression of the *lacZ* and *cI* genes, and elevated temperature leads to the degradation of the CI protein being produced. As a result, both genes – *cI* and *lacI* – were latently inactive [17], which was in line with epigenotype *d*. This epigenotype was unstable, and at subsequent replica to plate 7 (no IPTG; 39°C) white colonies were formed.

Thus, the pCEPI cyclic digene construction is capable under given conditions (30°C) to inherit stably either of the two

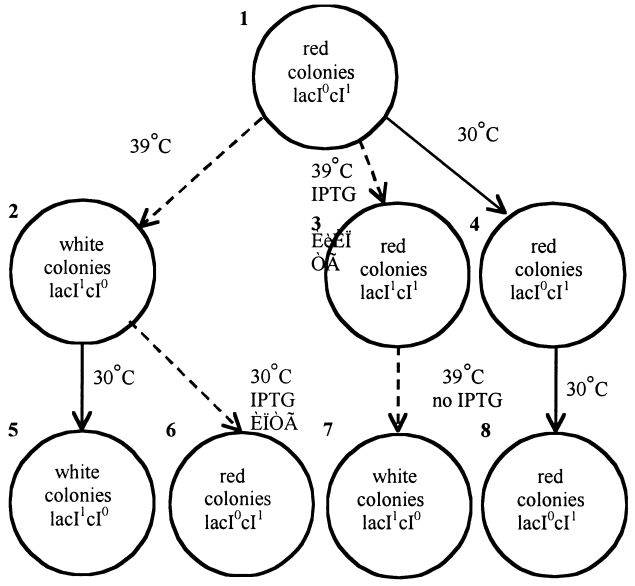

Fig. 4. Tests for epigenotype heredity and switching in the cyclic system of genes *lacIcI*. Switching (---→) from $lacI^0cI^1$ (*a*) to $lacI^1cI^0$ (*b*) occurs at 39°C; switching from *b* to *a* is found when cells are plated with IPTG. Stable epigenotypes *a* and *b* are inherited (→) over 72 h during three replicas even in the absence of factors responsible for switching, i.e. at 30°C and without IPTG.

alternative states – *a* or *b*, in a sequence of *E. coli* cell generations, i.e. as in [1], it is a two-component stationary epigene.

Such synthetic networks can be applied in gene therapy to have a particular effect at any desired moment, and also to control the synthesis of biologically active substances.

Upon completion of our experimental research we became aware of a similar pTAK construction created not long ago quite independently of us [18]. In a series of pTAK plasmids genes and promoters were obtained through PCR amplification; in that case the following sources of genes were used - Ptrc-2 (pTrc99a), P_L (pXC46), *cIts*(pGW7), and *lacI*(pTrc-99a). Gardner et al. [18] used quite another reporter system. As a reporter gene they took the *gfpmut3* gene arranged as the second cistron downstream of the Ptrc-2 promoter. Transcription of the *cI* gene from Ptrc-2, and hence, repression of the P_L promoter resulted in the synthesis of green fluorescent protein (GFP)mut3. In our construction the *lacZ* reporter gene is arranged downstream of its own promoter on the F′ episome. Expression of the *lacI* gene results in the white colouring of the colonies, and the absence of the Lac-repressor makes the colonies red. The pTAK construction like our pECPI plasmid has two stable steady functional states, and the toggle switch from one state to another is performed by chemical and temperature factors. Thus, genetic toggle switch constructed in the cited paper is also a two-component epigene. It should be noted that contrary to this paper our main goal, alongside with the construction of a cyclic digene system, was to show its being a hereditary unit. That is why in our work we have emphasised the experimental aspect of the heredity of epigenotypes in the sequence of generations.

4. Conclusion

It is well known that information can be stored either by conservation of a specific distribution of spatial structure elements ('structural' way) or by circulation of signals in a cyclic system of elements ('dynamical' way). DNA molecules of which genes form parts store information in the structural way. Thus, information inherited in genes is stored in the structural way within the linearly ordered DNA structure, encoded by four-letter (A,G,C,T) words, that are the elements of this structure and transmitted from generation to generation by means of covariant reduplication. By contrast, epigenes as cyclic sets of genes store part of the inherited information they contain in the dynamical way, by circulation of molecular signals (repressors, or activators) in cyclic gene subnetworks. Information in them may be coded with a binary code (i.e. by either presence or absence of threshold doses of specific regulatory molecules) and transmitted in the cell-division cycle by distributing extragenomic regulatory proteins among daughter cells. Consequently, epigenes differ from genes by three principal criteria: the way of storing, coding and transmitting part of hereditary information. That is why it can be asserted that we have got a new hereditary unit of the complexity level higher than the gene.

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