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Hardware (DNA) circuits
Circuits génétiques

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Abstract

A scheme is presented whereby a new genetic control circuit can be introduced into an organism, permitting the experimenter to turn the expression of a given gene (or set of genes) on or off at will. The proposed scheme involves a positive feedback loop – here, a positive regulator, the CII protein of phage λ, with its structural gene engineered so as to require CII for its expression. This feedback loop creates the possibility of two stable steady states, with gene *cII* ON or OFF. Genes added downstream of *cII* and lacking a promoter will follow the same expression as *cII*. Two additional circuits allow the experimenter to switch at will between the ON and OFF states. ***To cite this article: R. D'Ari, R. Thomas, C. R. Biologies 326 (2003).***
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Résumé

Un schéma est présenté pour introduire dans un organisme un nouveau circuit génétique de contrôle, permettant à l'expérimentateur d'ouvrir ou fermer à volonté l'expression d'un ou de plusieurs gènes donnés. Le schéma proposé comprend une boucle de rétroaction positive – en l'occurrence un régulateur positif, la protéine CII du phage λ, dont le gène de structure est organisé de façon à requérir CII pour sa propre expression. Cette boucle positive crée la possibilité d'avoir deux états stationnaires stables, avec le gène *cII* exprimé (ON) ou non (OFF). Des gènes ajoutés en aval de *cII* et dépourvus de promoteur suivront la même expression que *cII*. Deux circuits supplémentaires permettent à l'expérimentateur de commuter à volonté entre les états ON et OFF. ***Pour citer cet article : R. D'Ari, R. Thomas, C. R. Biologies 326 (2003).***
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Keywords: positive feedback loop; genetic regulation; phage λ; CII protein

Mots-clés : boucle positive de rétroaction ; régulation génétique ; phage λ ; protéine CII

In this day and age of genetically modified organisms, the introduction of new genes and the inactivation or modification of existing genes in various organisms has become commonplace. These procedures, the safety of which is still hotly debated, have enormous potential for commercial and therapeutic applications. However, from the point of view of genetic circuitry they are generally rather banal. Over ten years ago we proposed a different sort of genetic engineering,

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which we called 'vicious circular DNA' and which could equally well be called 'hardware DNA circuits' [1]. We outlined a scheme that, when introduced into an organism, would permit the experimenter to turn a specific gene on or off at will, with both the ON and OFF states indefinitely stable.

Our system used genes and promoters from the bacterium *Escherichia coli* and the coliphage λ, reorganised and reintroduced into *E. coli*, and the protein whose synthesis could be turned on and off was the regulatory protein of the circuit. We present here a modified version of our scheme to illustrate the possibility of introducing into an organism not only foreign genes but an entire foreign regulatory circuit that will allow the experimenter to turn the synthesis of virtually any protein on or off at will.

The basis for our scheme is, first of all, a positive feedback circuit. This was long suspected to be a necessary condition for multistationarity, i.e., for having multiple steady states [2] and is now known to be necessary [3–6]. We chose a simple, one-element positive feedback circuit, a transcriptional activator whose structural gene is expressed only in the presence of the activator. This provides the 'vicious circle': either the activator is present and continues to stimulate the synthesis of more activator, or it is absent and therefore cannot be produced. Our suggestion was to use the CII protein of bacteriophage λ, which activates several phage promoters. We proposed cloning the *cII* gene downstream of one of these promoters, P_{int}, making CII protein synthesis dependent on the presence of CII protein.

To generalize our scheme, we propose adding, downstream of the λ*cII* gene, an additional gene, *x*, coding for a protein, X, whose presence we wish to be able to induce or turn off in our target cells. The *x* gene must not have any promoter, so that its expression will depend entirely on the activity of the upstream P_{int} promoter. This will form an operon, with coordinate expression of the CII and X proteins. If needed, several genes could be added, say *x*–*y*–*z*, provided that the cloned sequence has no internal promoters. A similar stratagem was used by Dambly-Chaudière and coworkers [7,8] in the design of their elegant epigenetic system for detection of induction of the SOS response, in which the *galETK* genes were placed downstream of the gene coding for the Cro repressor of phage λ and thus expressed co-ordinately with Cro. Since the products of the *galETK* genes confer the ability to ferment the sugar D-galactose, they provided a simple signal, visible in a bacterial colony, indicating the state of the *cro* gene, ON or OFF. This epigenetic system, which is the basis of a bacterial test for genotoxicity [9], was partly constructed in one of the authors' laboratories (R.T.).

The positive feedback loop of the construct P_{int}–*cII*–*x*, introduced into a target cell, would already be expected to give rise to two possible stable steady states: CII and X present and actively synthesized (the ON state), or CII and X absent and not synthesized (the OFF state). We now must add devices to switch between these steady states.

To go from the OFF state to the ON state, a push-button device is needed, something which, in response to a transient signal controlled by the experimenter, turns the system on. A very simple possibility is the introduction of a second *cII* gene, whose expression is controlled, not by CII but by a negative regulator the activity of which can be manipulated. In our initial scheme we suggested placing this second *cII* gene under the control of P_{lac}, promoter of the *lac* operon and subject to repression by LacI, the *lac* repressor; the repressive activity of LacI can be neutralized by adding an appropriate inducer to the medium. For practical reasons, we now propose using a different promoter, P_{araBAD}. This promoter is activated in the presence of the sugar D-arabinose and repressed in its absence, making for essentially negligible expression in the absence of inducer (D-arabinose). The regulatory element that responds to D-arabinose is the AraC protein. To use our new circuit in organisms other than *E. coli*, the structural gene *araC* will have to be included in the construct, since most cells in nature do not make this regulator.

Our scheme now includes the P_{int}–*cII*–*x* construct, the P_{araBAD}–*cII* construct and the *araC* gene. If the initial state is OFF (no CII present), the transient addition of the inducer D-arabinose will turn it ON. This is because the inducer will activate expression of the second *cII* gene (the push-button device), which will synthesize CII protein, and this CII will activate the P_{int}–*cII*–*x* construct. When D-arabinose is removed from the culture, the second *cII* gene (the P_{araBAD}–*cII* construct) will be turned off, but since CII is present, it will continue to be synthesized from the

P_{int}–*cII*–*x* construct; the system will be stably in the ON state.

To switch from the ON state to the OFF state, we have to introduce a means of transiently inactivating CII activity. A simple way to do this, as in our original scheme, is to use a modified *cII* gene in the P_{int}–*cII*–*x* construct, such that its product, CII′, is temperature sensitive; a brief passage to a higher, 'non-permissive' temperature (but still permissive for the growth of the host organism) will denature the CII′ protein. Assuming the second *cII* gene is off, i.e., that the cell has not seen any D-arabinose for some time, there will be no active CII protein at high temperature, so the P_{int}–*cII′*–*x* construct will be turned off. On return to the lower temperature, the cell will still have no active CII protein to express the P_{int}–*cII′*–*x* construct, so it will remain OFF. Thus to put the system in the OFF state, it suffices to cultivate the cells briefly at non-permissive temperature (in the absence of D-arabinose).

Our complete circuit will thus be composed of the following elements: the P_{int}–*cII′*–*x* construct, the P_{araBAD}–*cII* construct and the *araC* gene. Introduced into a target cell, this system will permit the experimenter to turn the synthesis of protein X on or off at will. It can be turned on by brief exposure to D-arabinose and turned off again by brief exposure to a non-permissive temperature.

The introduction of new genetic circuits into an organism can thus be used to create epigenetic states with varying gene expressions. It should also be possible to construct oscillating circuits, in which the expression of a foreign gene oscillates with time, with the period and amplitude of the oscillation determined by the details of the circuit, fixed by the experimenter. In this case, a negative feedback loop would be required (2). An oscillating circuit of this type in *E. coli* was able to maintain the oscillations over a few generations [10]. On the other hand, several examples of multiple steady states have been introduced successfully introduced into *E. coli* [7–9,11].

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