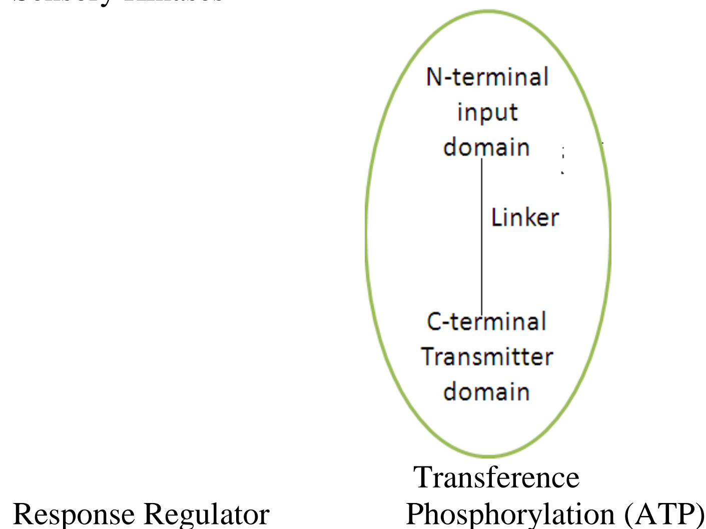
iGEM WIKI

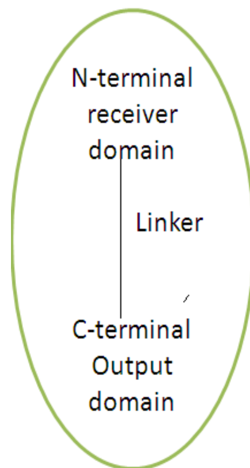
A mathematical model for the BarA/ UvrY and fluorescence of GFP two-component system is presented and its dynamics behaviour is analysed. The BarA / UvrY regulate the expression of the GFP in E-coil. The model is validated in a two steps:

(i) The signal transduction
(ii) The gene expression.

To survive, bacteria monitors their environment constantly and adapt to changing conditions immediately and bacteria have an established signal transduction systems to execute adaptive responses to changing environmental conditions.
The signal transduction systems contain of two protein components: a sensor kinase anchored in the cytoplasmic membrane, and a cytoplasmic response regulator that mediates an adaptive response, that is the gene expression.

Sensory Kinases





Sensory Kinase

Sensory kinases typically contain an N-terminal input domain which is connected via a linker to a C-terminal transmitter domain.

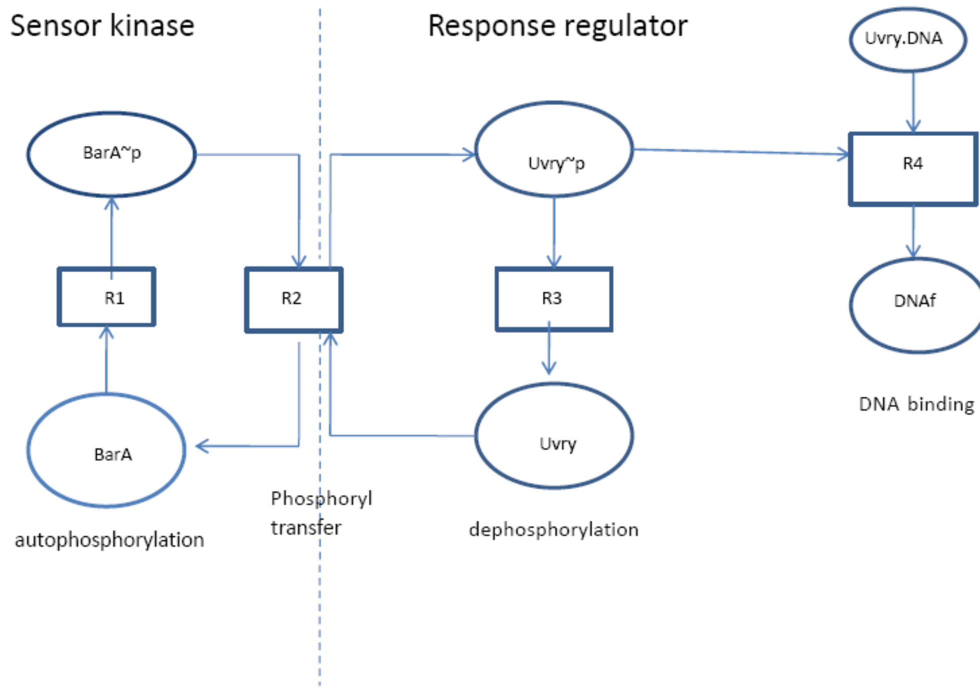
Response regulator

Response regulators typically consist of a N-terminal receiver domain coupled to C-terminal output domains.

Upon perception of a stimulus, the input domain of the sensor kinase modulates the signalling activity of its transmitter domain, resulting in autophosphorylation with γ-phosphoryl group of ATP. Then, the phosphoryl group is transferred to the response regulator receiver domain, resulting in activation of the output domain(s) to trigger gene expression.

The mathematical description of a reaction mechanism representing a two component systems is presented here. The signal transduction of BarA/ Uvry expresses some reaction for auto-phosphorylation of BarA, transference of phosphoryl group of ATP between histine kinase and response regulator, and de-phosphorylation of UvrY, which is a transcription activator that initiates a response by modulating gene transcription, resulting in changes in cell physiological and metabolism to cope with the external environment.

The gene expression of CsrB and GFP is lumped together and represented by binding of the response regulator to free DNA fragments comprising the specific response regulator-binding site.



A two-component signal transduction system

The Model

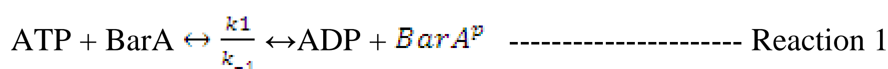
The model was set up describing autophosphorylation of BarA transfer of the phosphoryl group between sensor kinase and response regulator, dephosphorylation of Uvry~p, and binding of the response regulator to DNA fragments comprising the specific response regulator-binding site. The strategy to set-up and to analyse the mathematical model was constructed in vitro and validated by set of experiments.

Model equations for the two-component system.

A simple reaction mechanism is used to describe the two component system

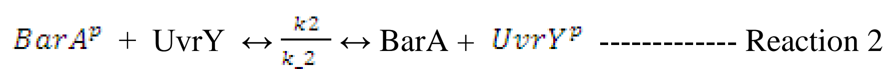
The reactions are:

Auto-phosphorylation:



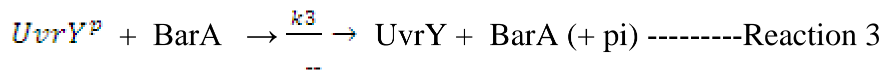
$$ATP + BarA \leftrightarrow \frac{k1}{k_{-1}} \leftrightarrow ADP + BarA^{p} \quad \text{---------------------- Reaction 1}$$

Phosphoryl group transfer :



$$BarA^{p} + UvrY \leftrightarrow \frac{k2}{k_2_} \leftrightarrow BarA + UvrY^{p} \quad \text{------------- Reaction 2}$$

Dephosphorylation :



$$UvrY^{p} + \text{BarA} \xrightarrow{k3} \text{UvrY} + \text{BarA (+ pi)} \text{ ---------Reaction 3}$$

DNA binding :

$$2UvrY^{p} + DNA_{f} \underset{k_{-b}}{\overset{kb}{\leftrightarrow}} \text{(UvrY – DNA)--------------Reaction 4}$$

In reaction R1, the stimulus enhances the kinase activity that results in auto-phosphorylation of sensor kinase (BarA, BarA~p state variable) by ATP

In reaction R2 the phosphoryl group is transferred to the response regulator (Uvry, Uvry~p state variable). Uvry~p contains the active output domain.

In reaction R3 describes the dephosphorylation of Uvry~p by cognate sensor kinase BarA. (it has been shown through reference that dephosphorylation is only dependent on BarA. Jung et.al., 1997) so that other phosphatises are not considered in the model.

In reaction R4 the activated response regulator forms a dimer and is then binds to the free DNA (DNAf, state variable) to build a transcription complex (Uvry-DNA, state variable), in presence of RNA polymerase.

Based on the analysis of the mathematical model for the two-component system a reduced model comprising O.D.Es was developed. The reduced model consist of two sub-model 1 describes the signal transduction to activate the response regulator while sub model 2 describes the interaction of the activated response regulator with DNA control sequence.
The sensor kinase and the response regulator are auto-controlled. The dynamics strongly depends on the initial conditions.

For the total concentration of the sensory kinase BarA and of the response regulator UvrY, and the entire concentration of DNA fragment. The following equations hold:

$BarA_{o}$ = BarA + $BarA^{p}$---------------------------------------equation 1

$UvrY_{o}$ = UvrY + $UvrY^{p}$ + 2(UvrY- DNA).------------------equation 2

DNA_{o} = DNA_{f} + (UvrY- DNA)--------------------------------equation 3

$BarA^{p}$ = x(1)

$UvrY^{p}$ = x(2)

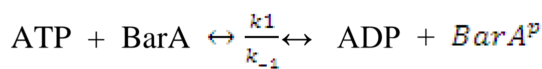
(UvrY- DNA) = x(3)

BarA = $BarA_o$ - x(1)------------------------------------- equation 4

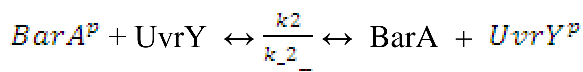
Uvry = (($UvrY_o$ - $UvrY^p$ – x(3)) /2)--------------------- equation 5

DNA_f = DNA_o - x(3)-------------------------------------- equation 6

The differential equations representing the chemical reactions are:

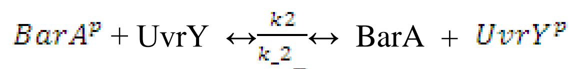


$$\text{ATP} + \text{BarA} \leftrightarrow \frac{k1}{k_{-1}} \leftrightarrow \text{ADP} + BarA^p$$

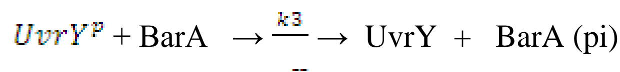


$$BarA^p + \text{UvrY} \leftrightarrow \frac{k2}{k_2_} \leftrightarrow \text{BarA} + UvrY^p$$

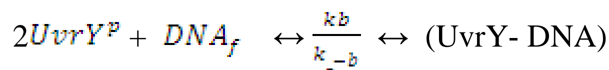
$$\frac{dBarA^p}{dt} = k_1 \text{ ATP BarA} - k_{-1} \text{ ADP } BarA^p - BarA^p \text{ UvrY} + k_{-2} \text{ BarA } UvrY^p$$



$$BarA^p + \text{UvrY} \leftrightarrow \frac{k2}{k_2_} \leftrightarrow \text{BarA} + UvrY^p$$

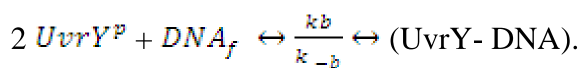


$$UvrY^p + \text{BarA} \rightarrow \frac{k3}{--} \rightarrow \text{UvrY} + \text{BarA (pi)}$$



$$2UvrY^p + DNA_f \leftrightarrow \frac{kb}{k_{-b}} \leftrightarrow \text{(UvrY- DNA)}$$

$$\frac{dUvrY^p}{dt} = k_2\, BarA^p \text{ UvrY} - k_{-2} \text{ BarA } UvrY^p - k_3\, UvrY^p \text{ BarA} - k_b DNA_f\, UvrY^{p^2} + k_{-b} \text{ (UvrY- DNA).}$$



$$2\, UvrY^p + DNA_f \leftrightarrow \frac{kb}{k_{-b}} \leftrightarrow \text{(UvrY- DNA).}$$

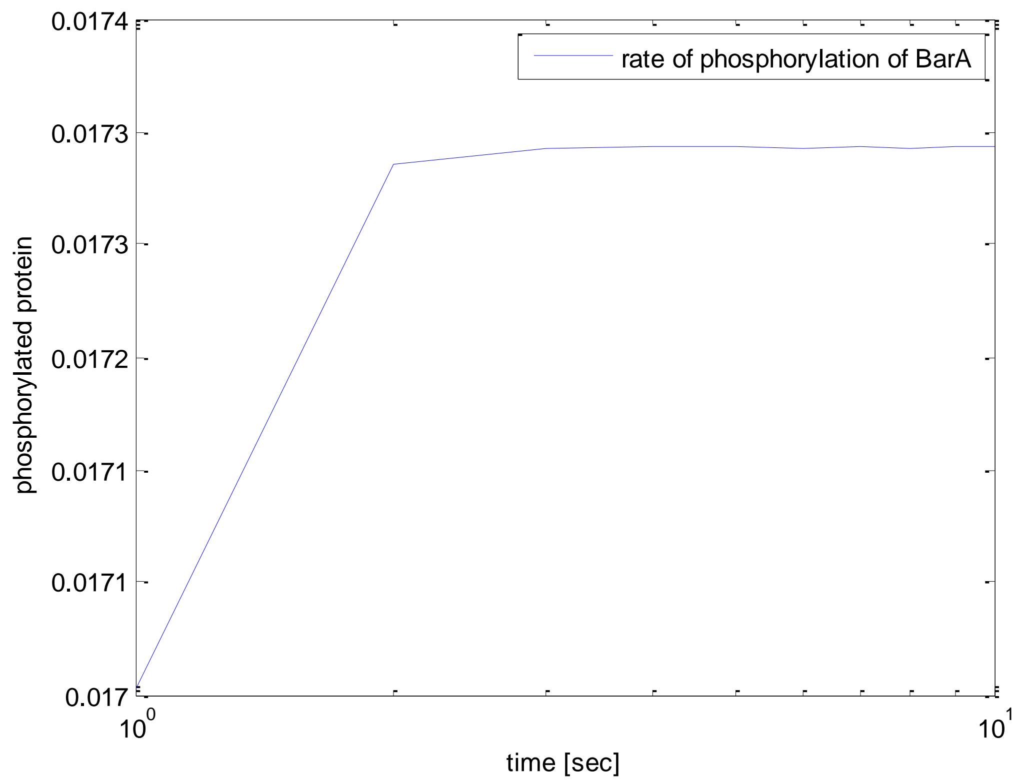
$$\frac{d(UvrY-DNA)}{dt} = k_b DNA_f UvrY^{p^2} - k_{-b} \text{ (UvrY- DNA).}$$

Substitute
x(1) = $BarA^p$,
x(2) = $UvrY^p$,
x(3)= UvrY- DNA, and equations (4) – (6)
into differential equations given above and modelling and simulating these in Matlab we have:

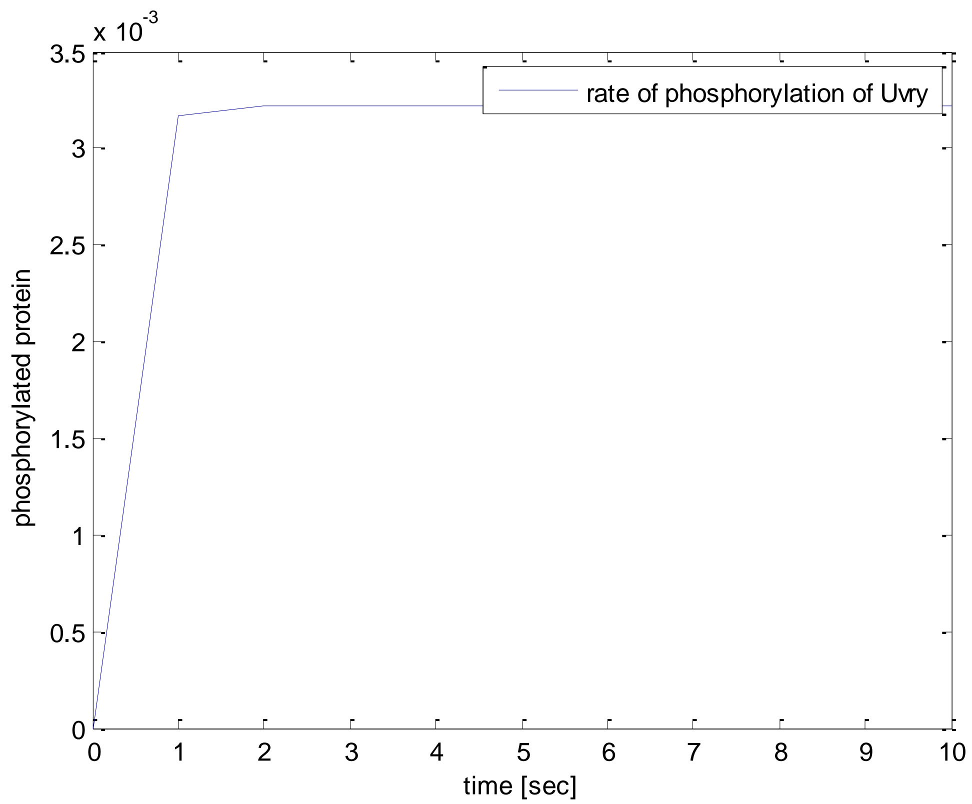
```
% the mathematical representation of the reaction mechanism for two
% component system.

function xdot=iGEM2(t,x)
k1=0.0029;
k_1=0.00088;
k2=108;
k_2=1080;
k3=90;
kb=5400;
k_b=360;
ATP=100;
ADP=8;

a = -k_1*x(1)*ADP - k2*x(1)*0.5*(4-x(3)-x(2)) + k1*(1-x(1))*ATP +
k_2*(1-x(1))*x(2);

b = -k_2*(1-x(1))*x(2) - k3*x(2)*(1-x(1)) -2*kb*x(2)*x(2)*(100-x(3))
+ k2*x(1)*0.5*(4-x(3)-x(2)) + k_b*x(3);

c = -k_b*x(3) + 2*kb*x(2)*x(2)*(100-x(3));

xdot=[a;b;c];
```

All units in μM

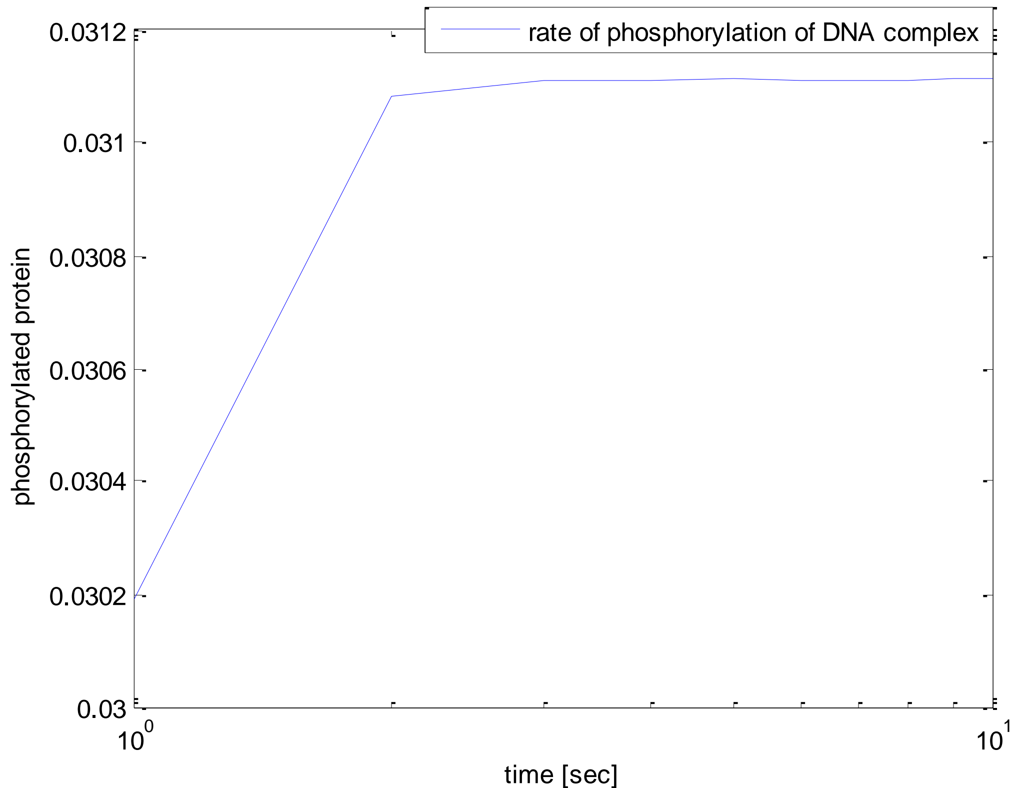
```
t0=0;
tf=5;
tspan=[t0:tf];
x_initial=[0;0;0];
[t,x]=ode45(@iGEM2,tspan,x_initial);

figure(1);
semilogx(t,x(:,1));
xlabel('time [sec]');
ylabel('phosphorylated protein ');
legend('rate of phosphorylation of BarA');
```




```
figure(2);
plot(t,x(:,2));
xlabel('time [sec]');
ylabel('phosphorylated protein ');
legend('rate of phosphorylation of Uvry');
```




```
figure(3);
semilogx(t,x(:,3));
xlabel('time [sec]');
ylabel('phosphorylated protein ');
legend('rate of phosphorylation of DNA complex');
```



The mathematical model was set up to describe the experimental results. The model has a simple structure, temporary complexes between ATP and sensor kinase or between sensor kinase and response regulator were neglected to reduce the number of uncertain parameters. The parameters were estimated based on a number of experiments.
The analysis led to a system with O.D.Es expressing the dynamics of the binding of the response regulator which is a mirror of the dynamics of the autophosphorylation and phosphoryl transfer.

Assumptions:

- In this model only one stimulus is under investigation and it represented as a fixed parameter.
- Phosphorylation of response regulator by alternative phosphor donor is ignored.
- The de-phosphorylation of response regulator is solely dependent on sensor kinase and rate of de-phosphorylation decay is taken into consideration.
- Non- specific binding of the response regulator to other DNA binding site is neglected in the model.

- Sensor kinase acts as an enzyme that de-phosphorylated phosphorylated response regulator.

- The model was done by considering ATP as an input because of the complexity in modelling extracellular response of E-coil (considering CIA 1) and it was thought of, that it best to model the response of downstream gene by modelling it from intacellular point of view(considering ATP) and this is backed by reference and some assumptions.

- The mathematical model was set up to describe the experimental results. The model has a simple structure, temporary complexes between ATP and sensor kinase or between sensor kinase and response regulator were neglected to reduce the number of uncertain parameters. The parameters were estimated based on a number of experiments.

Comments from simulations point of view.

```
% the k1 because of it affinity with ATP in the equation has a great
effect on the
% response of the output. The k_1 affect the output because of it
rate at
% which it return to the original position has great impact on the
output.
%k3 and k2 only lower the speed the which steady state of the
reactions.

kB has great impact on the output because it
%expresses the forward rate of reaction of the DNA binding, the K_b
has some
% impact on the output response.

ADP been a product of ATP (which is
%the assumed input) has little impact on the general output or
mechanism. so
%the k1,kb,k3, K_b and ATP have great influence on the glowing of the
GFP.
```

The response of the autophosphorylation and phosphorylation of the sensor kinase the response regulator and the expression of the gene respectively show that the system is stable and under any condition it should respond well and the model and simulation validate the in-vitro experiment results.

Sensitivity/Dynamics of the system

Considering the sensitivity of the system modelled, the parameters with highest sensitivity were `k1, kb, k3, k_b.`

BarA-Bacteria Adaptive Response Gene A
UvrY Response Reulatory Protein for BarA

Appendix and References:

Parameter values for the in vitro data set

In vitro parameters

```
k1=0.0029  1/hµM;
k_1=0.00088  1/hµM;
k2=108  1/hµM;
k_2=1080  1/hµM;
k3=90  1/hµM;
kb=5400  1/hµMM;
k_b=360 1/h;
ATP=100 µM;
ADP=8  µM;
```

DNA_o =100 µM
$BarA_o$=1µM
$UvrY_o$= 4 µM

The goal of parameter estimation is to find a set of parameters that can describe the experimental results. Here, was based on experiment done in vitro

A.1 (just an illustration)
Reconstruction of the BarA/Uvry signal transduction cascade in vitro

We construct the complete signal transduction cascade of BarA/Uvry system in vitro. Purified BarA in proteoliposomes and purified Uvry in a ration of 1 to 4μM were mixed with 100μM DNA comprising of the DNA binding site of Uvry in buffer (50mM Tris/HCl pH,7.5, 10% glycerol (v/v), 0.5M NaCl, 2mM dithiotreitol). The double-stranded DNA fragment comprising the Uvry-binding sites were obtained by annealing of two complementary oligonucleotides. The upper strand sequence (from 5' to 3') has the following sequence:
5'-CATTTTTATTACTTTTTTTACACCCCGCCCG-3'. The reaction was started by additionof 100μM [γ-32P]ATP (0.476 Ci/mmol), 8μM ADP (ratio of 1 to 12.5, refecting the ratio in living cells), and 110μM MgCl. At the time indicated, sample were taken and the reactions were stopped by addition of an equal volumes of 2* concentration sodium dodecyl sulphate (SDS) sample buffer. In each case, samples were immediately subjected to SDs polyacrylamide gel electrophoresis. Gels were dried, the amount of radio-labeled proteins was detected by exposure of the gel to a phosphor screen, and the images were analyzed with a phosphorlmager SI system(molecular Dynamics) using [γ-32P] ATP as a standard. In parallel, the phosphorylation degree of Uvry~p was determined in a gel-free detection system. This method consist of direct spotting of the phosphorylated sample on nitrocellululose after removal of BarA~p (ultracentrifugation) and [γ-32p]ATP (gel filtration).

References:

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