virtual BBs

and rule-based modeling

modeling in synthetic biology

Computational modeling is integral to synthetic biology, eg the early toggle switch and repressilator (Nature 403 2000) used a model.

Modeling (as in any engineering discipline) is good if quick, agile, cheap and helps zeroing in on picking parts that will work ok

-so:

1. standardisation: one needs well characterised parts =: BBs and their "data sheets"
-what's a data sheet? when are two bricks the same? in which context?

2. executable & composable designs: one needs in numero BBs- to be assembled into virtual systems =: models

digression: use bionumbers!
(bionumbers.hms.harvard.edu)

B10NUMB3R5

THE DATABASE OF USEFUL BIOLOGICAL NUMBERS

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Didn't find what you looked for? Let us know and we will try to help! (include email for an answer)

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Search tips: Try not limiting organism. Try abbreviations, full names etc., e.g. 'Oxygen' or 'O2'.
Disclaimer: Numbers in biology depend highly on conditions.
Use values as order of magnitude estimates or refer to experimental details in cited literature.

Search

Most Popular BioNumbers | Most Recent BioNumbers | Random BioNumbers

Find Terms: cell cycle

e.g., ribosome, p53, glucose, CO2

Organism: Bacteria Escherichia coli (836)

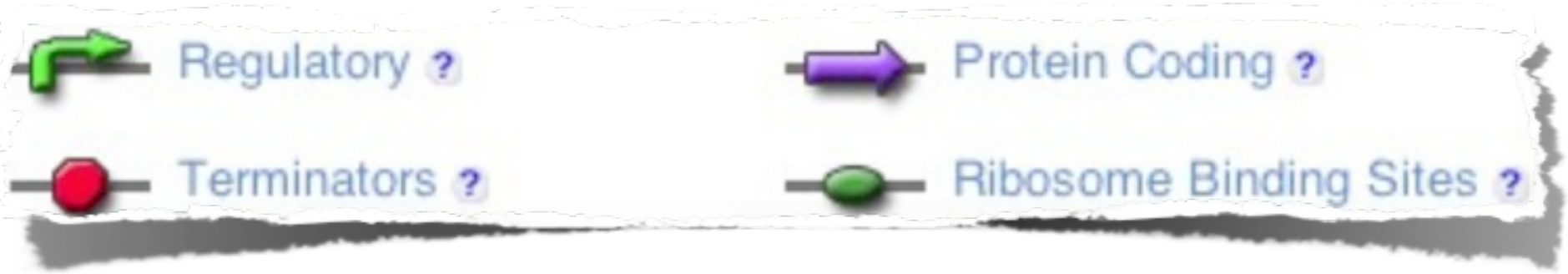
search | reset

Found 450 results

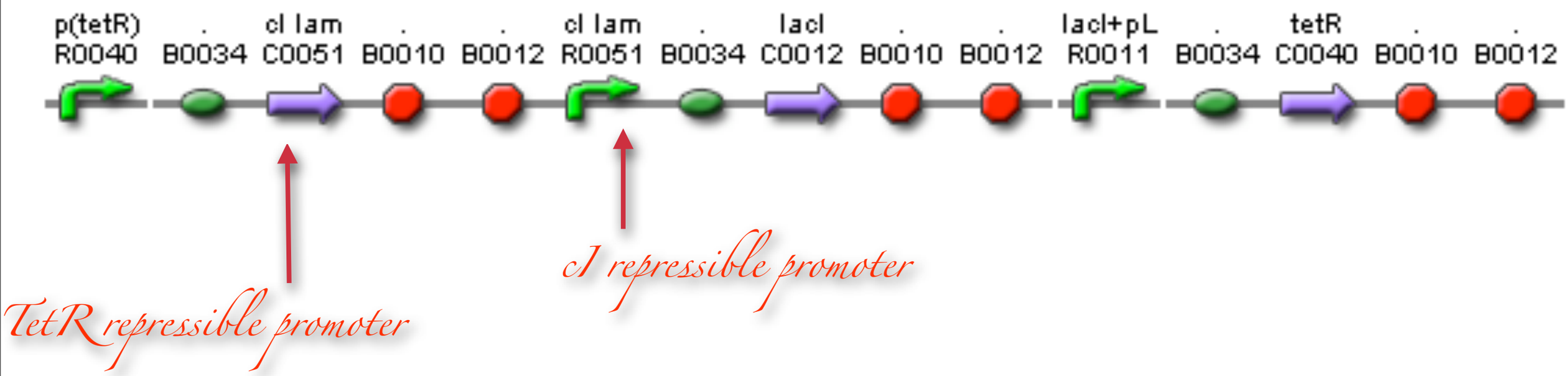
Click a row for more details.

ID	Property	Organism	Value	Units	Range	Details
103514	Minimal generation time	Bacteria Escherichia coli	20	min		more
102047	Translation bursts of beta-galactosidase per cell cycle	Bacteria Escherichia coli	0.16	unitless		more
102046	Translation bursts of tsr-venus fusion protein per cell cycle	Bacteria Escherichia coli	1.2	unitless		more
101790	"Rule of thumb" for the cell cycle (generation time)	Bacteria Escherichia coli	3000	sec		more

Bio-Bricks



eg repressilator, a TetR repressible promoter drives the expression of the cI coding sequence etc.



partsregistry.org

page discussion view source history Log in / create account

BBa R0051 Main Page Part Design Physical DNA Hard Information Experience Tools

Part:BBa_R0051

Designed by Vinay S Mahajan, Brian Chow, Peter Carr, Voichita Marinescu and Alexander D. Wissner-Gross Group: Registry

Regulatory
cI lam

DNA Available
1 Registry Star
Get This Part

promoter (lambda cI regulated)

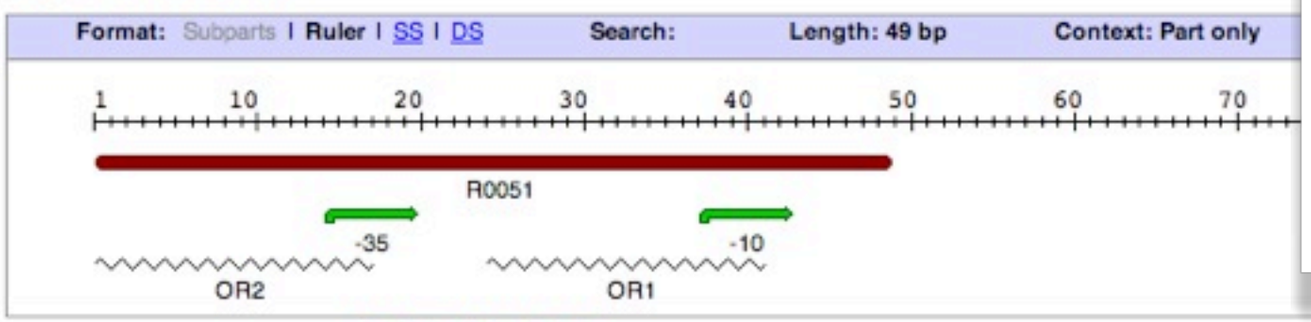
The cI regulated promoter is based on the pR promoter from bacteriophage lambda. The promoter has two DNA binding sites for lambda cI repressor BBa_C0051. cI binding results in repression of transcription. The specific sequence used here is based on the cI repressible promoter used in the Elowitz repressilator (and references therein).

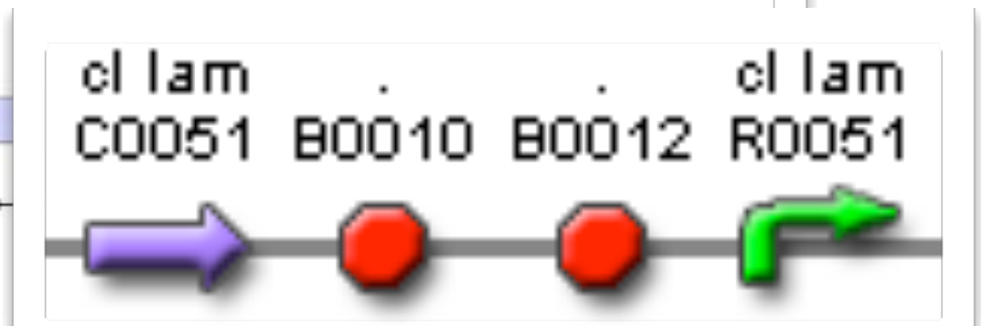
Usage and Biology

Strong promoter. [jb, 5/24/04]

Sequence and Features

Format: Subparts | Ruler | SS | DS Search: Length: 49 bp Context: Part only





Assembly Compatibility: 10 21 23 25

[edit]

Parameters

control	lambda cI
direction	Forward
negative_regulators	1
o_h	
o_l	
positive_regulators	

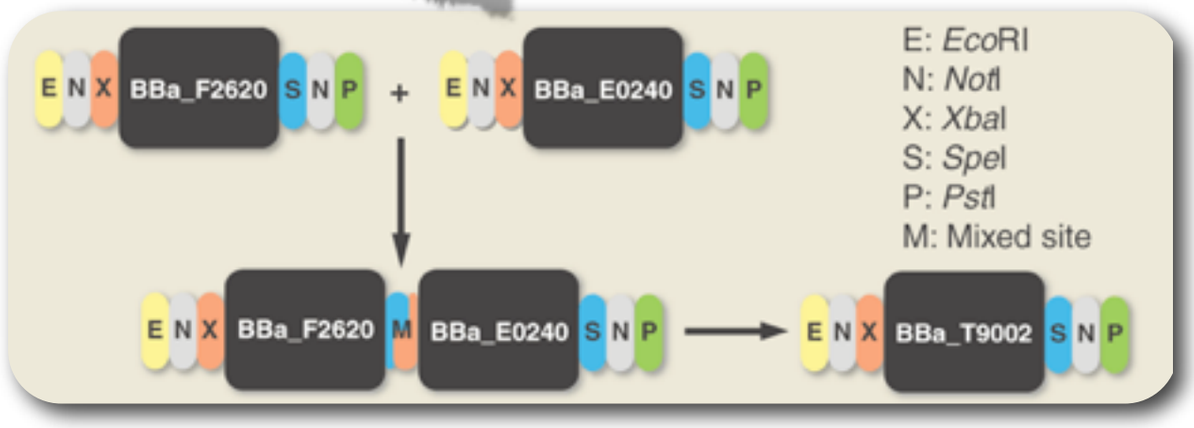
Reviews

1 Registry Star
Experience: Works

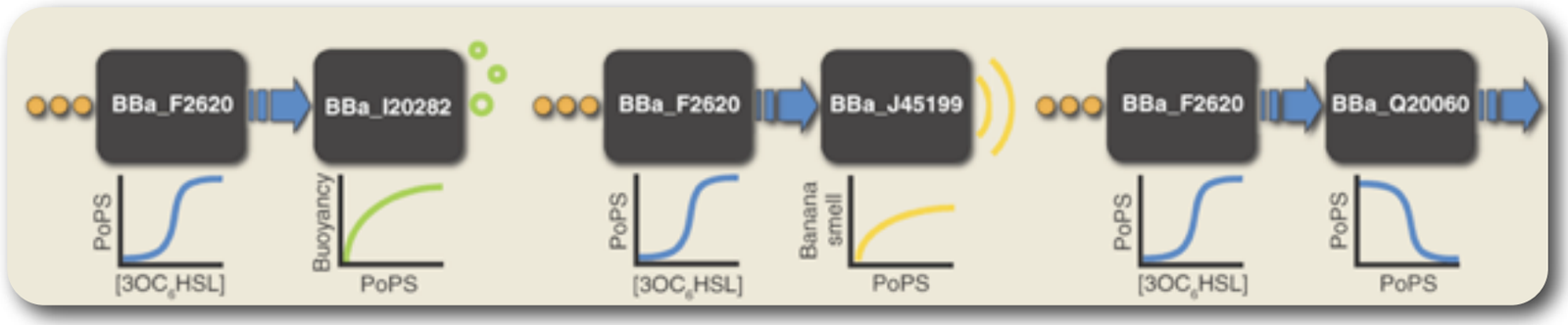
Categories

//chassis/prokaryote/ecoli
//direction/forward
//promoter
//regulation/negative
//rnap/prokaryote/ecoli/sigma70

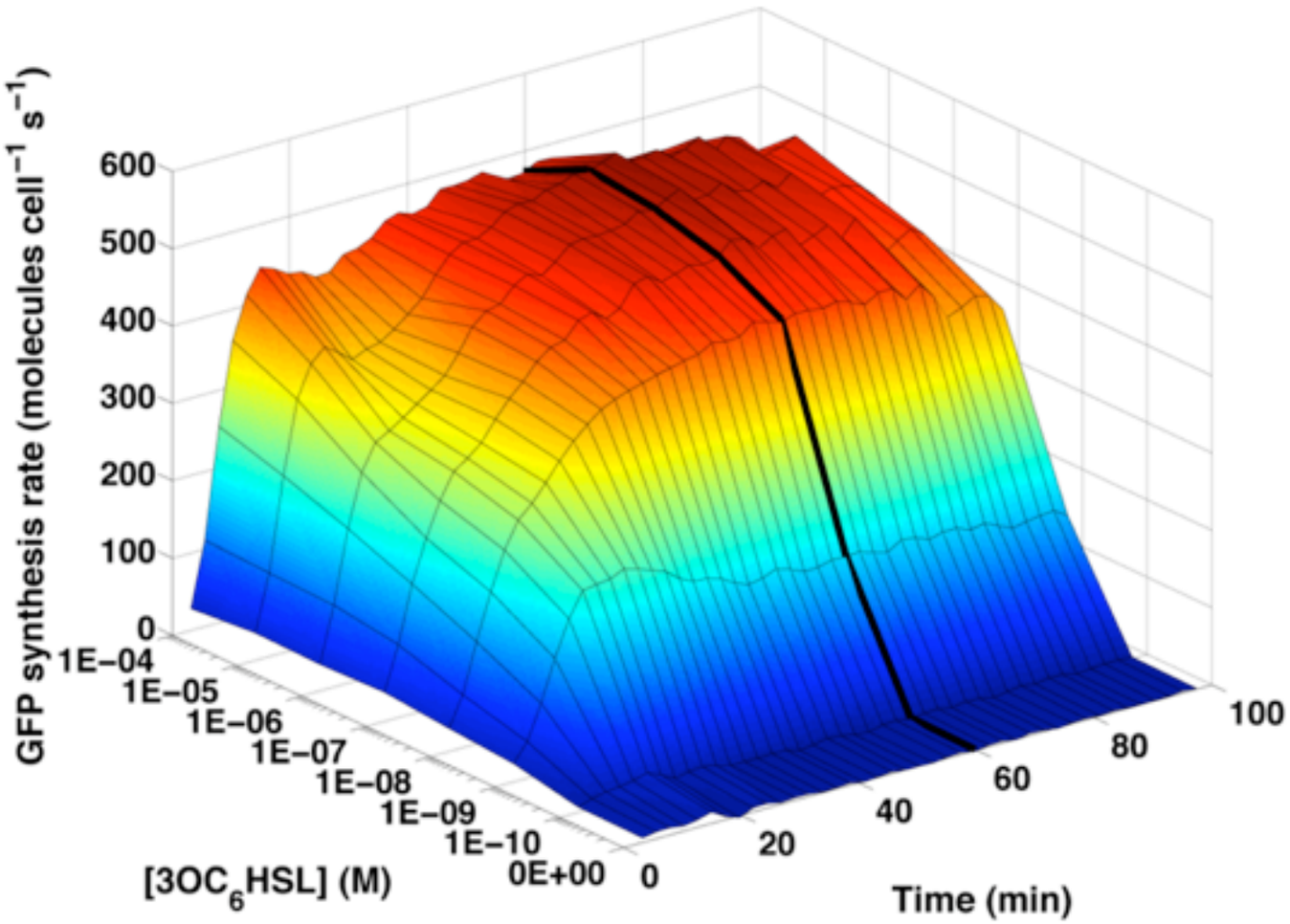
physical composition



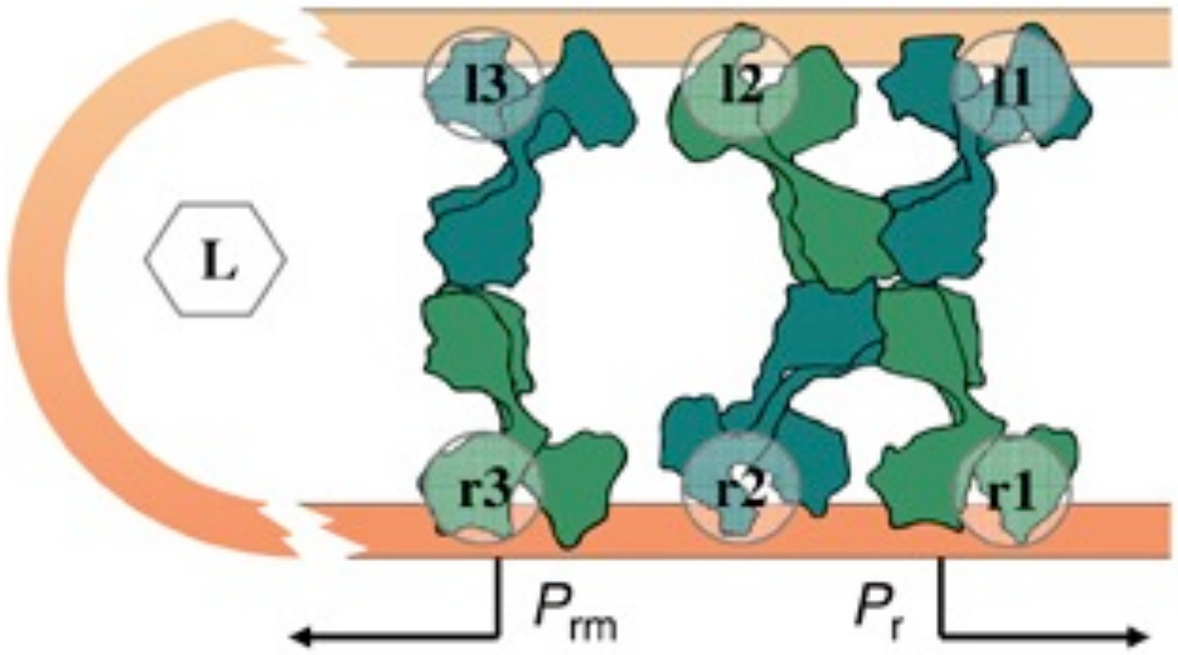
flux composition : pops, ribs, ...



BBa_F2620 Transfer Function

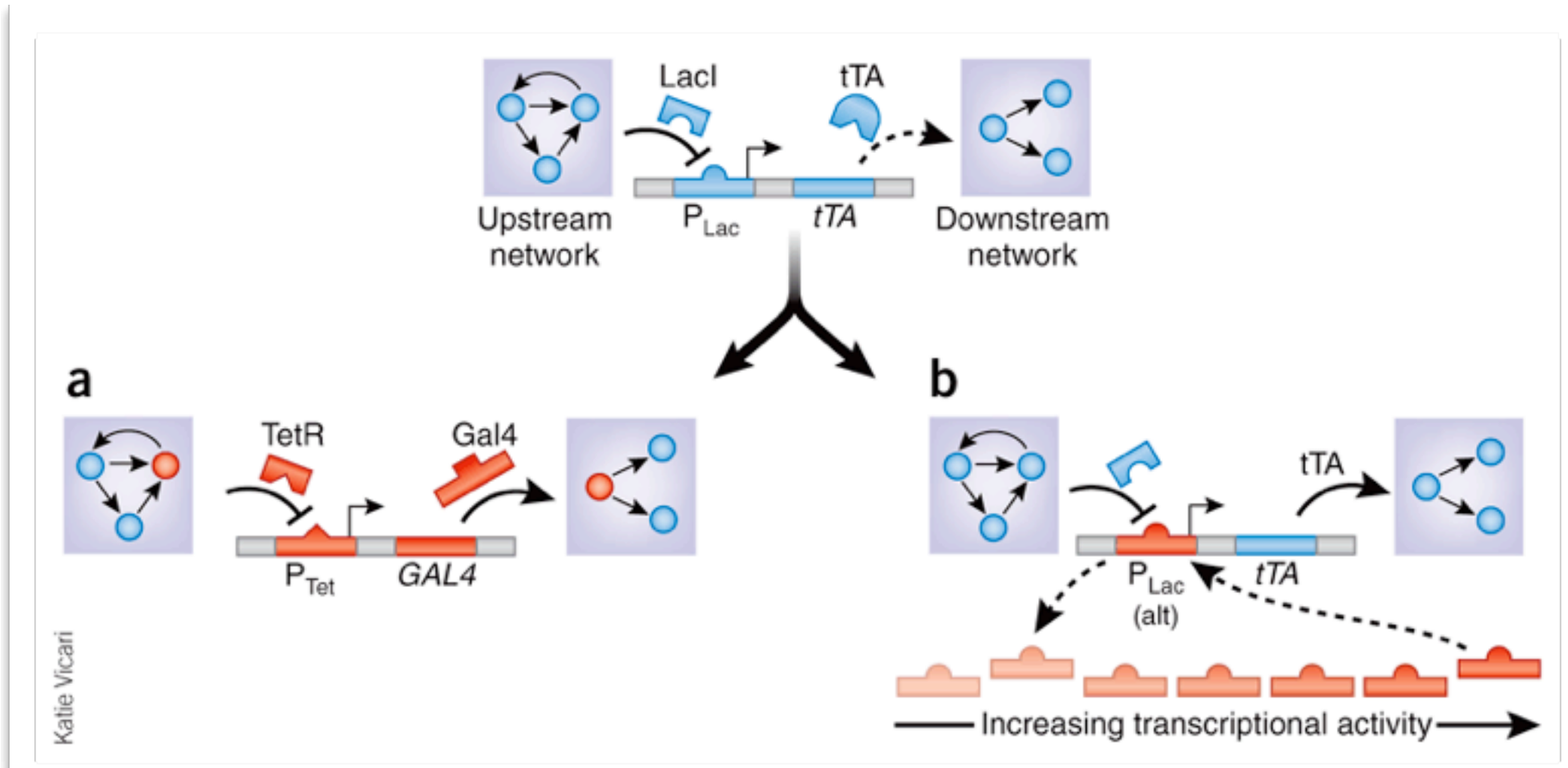


cI repressor–DNA complex formation



a data sheet can be more complex!!

Strength and orthogonality - or how it is useful to have many versions of (eg) a promoter



- The upstream network must be reconfigured to produce TetR instead of LacI
- the downstream network must receive a new input Gal4p (Fig. 1a) because TetR and tTA interfere

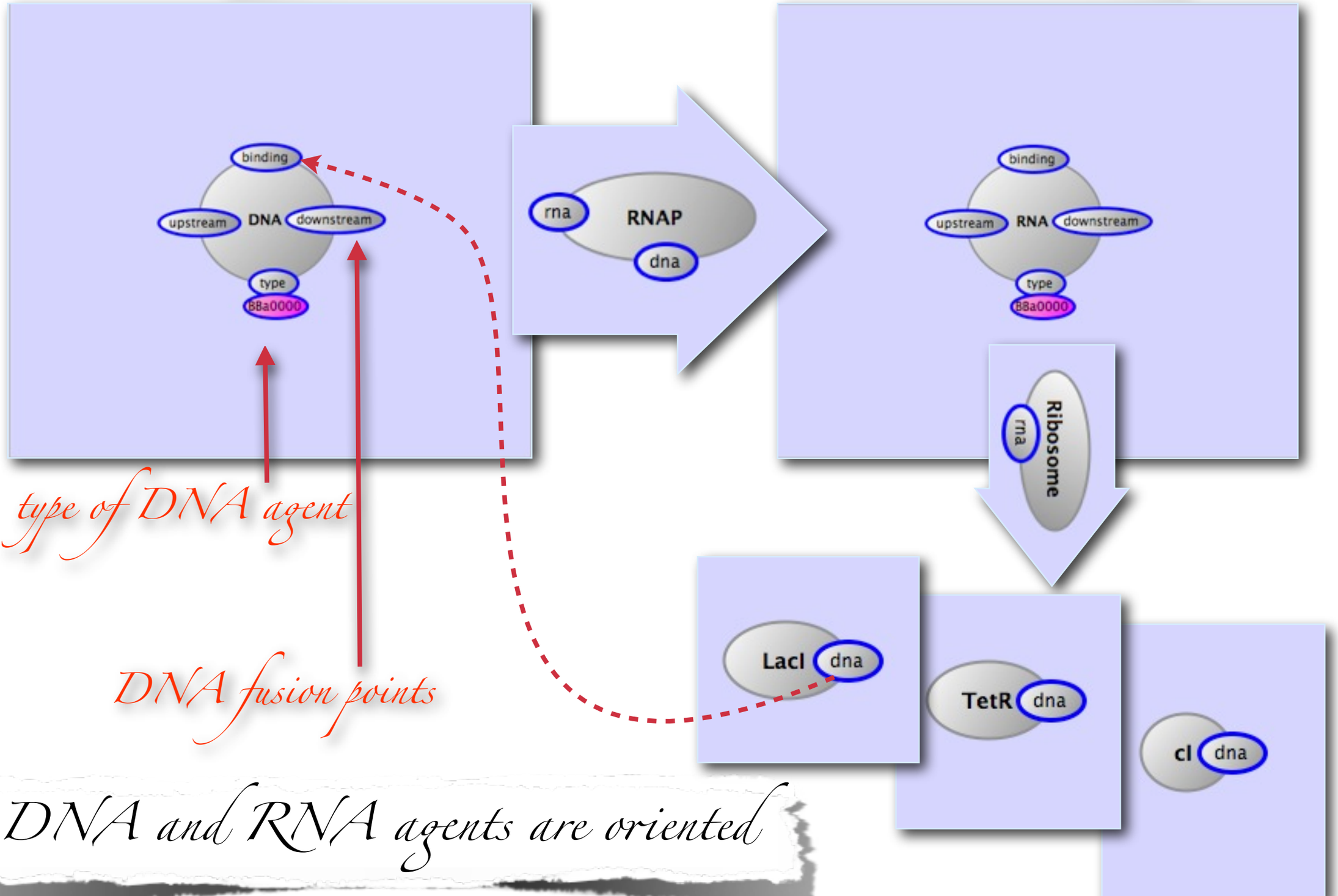
reactions vs. rules

we propose that a data sheet is a set of rules for each BB part

- [modularity] at the BB level of granularity, we can write rules for each BB independently
- [contention, jams, queues] competition for resources (RNAp, Ribosomes) is captured naturally
- as is stochastic behaviour

+ rules are really easy to write!
especially for "solid state evolution"

agents (for the repssor)



type of DNA agent

DNA fusion points

+ DNA and RNA agents are oriented

DNA "axioms"

- BBs (DNA agents) are glued (for good) along the downstream/upstream sites

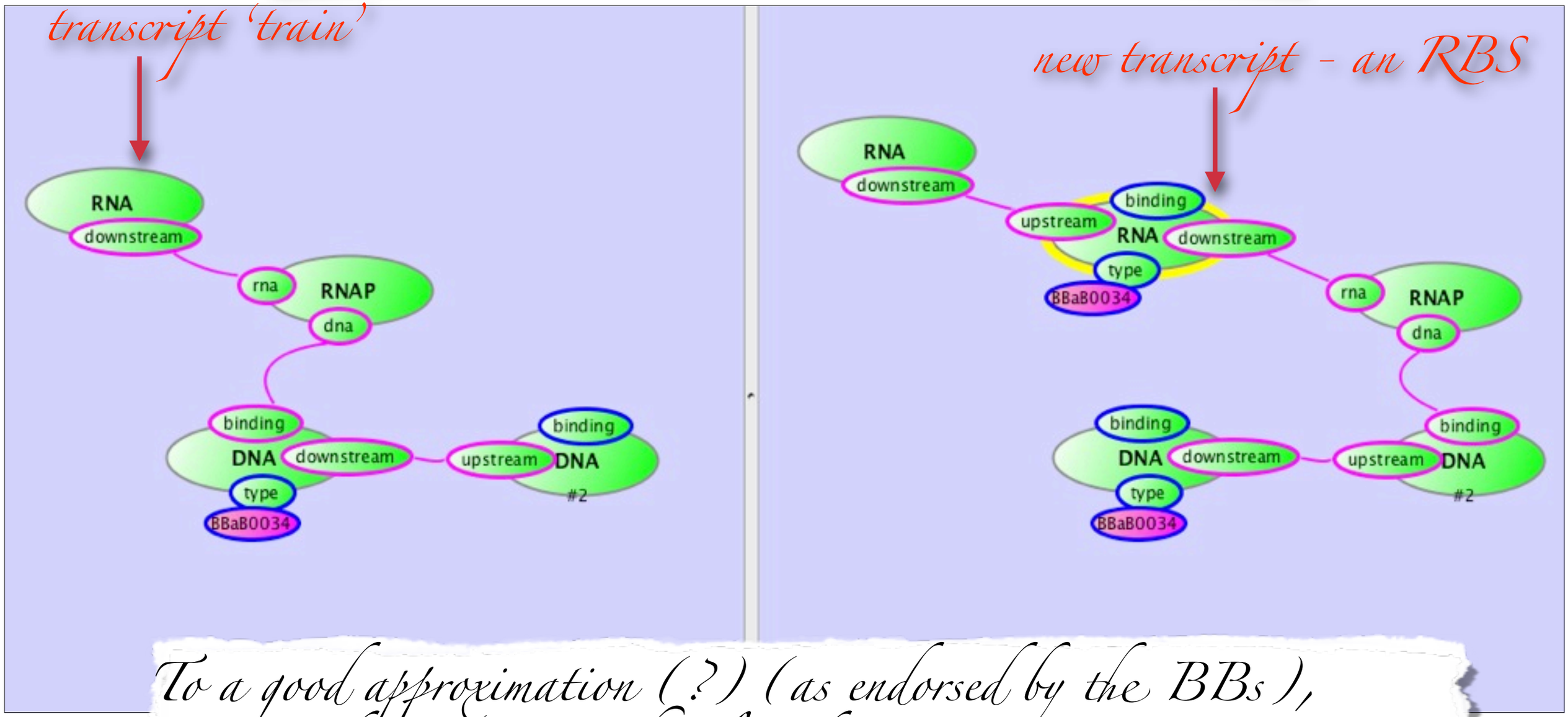
- Each BB is able to receive an RNAP agent from upstream, or directly if it has no upstream neighbour (eg RNAP must be accepted by the binding site of the upstream-most DNA agent)

- Each BB has one or more rules for its transcription.

- a transcriptional step adds one RNA agent to the RNA train on the RNAP and passes the RNAP along to the next BBs part

- transcription initiation - after RNAp binds to promoter, it moves down and creates a fictitious RNA agent, the "seed" <- this way we can tell when RNAp is in processive mode/elongation (cf the video)

RBS BBa_B0034 transcription



To a good approximation (?) (as endorsed by the BBs), RNAp does not care what lies downstream

DNA "axioms" (2)

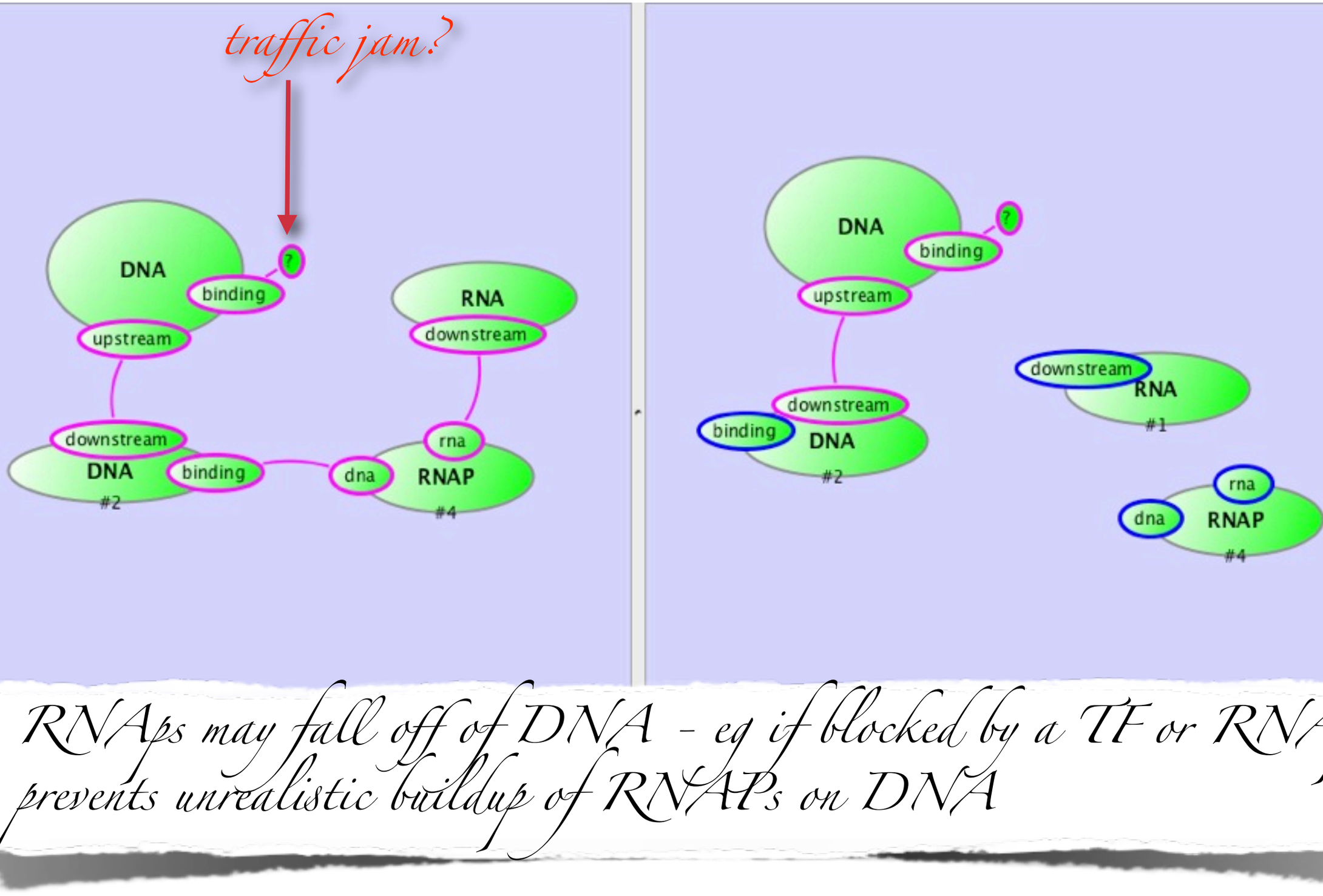
- a promoter is not made of parts, but of sub-parts (they could be sites in fact)

- rules for the binding of RNAP for promoter sub-parts will depend on activator or repressor occupancy of the part

- BB parts may have rules for the dissociation of RNAP from the part. Eg terminators would have a very high rate of RNAP dissociation compared to their rate of transcription (the ratio of which is related to the termination efficiency).

- release of the RNAP agent from DNA causes the release of its RNA train as well

RNAP falloff



+ RNAps may fall off of DNA - eg if blocked by a TF or RNAp
+ prevents unrealistic buildup of RNAPs on DNA

RNA axioms

- The RNA representation of BBs parts must be composed of one or more RNA agents connected in a row, with the downstream site of one RNA agent bound to the upstream site of the next.

- For simplicity no polycistronic messages: any RNA equivalent of a BB part will consist of a single RNA agent, and any RNA site that can be translated must include both a start codon and an stop codon (neither represented explicitly).

- Any RNA agent that can be translated must have one or more rules to take a Ribosome off of the upstream RNA agent, and produce the appropriate protein agent and release the ribosome.

- RNA agents can be degraded only if they have a free binding site. We do not consider endonucleases and exonucleases independently. Instead, each RNA agent has equal likelihood to be degraded. A further extension could include a more detailed model of RNA degradation.

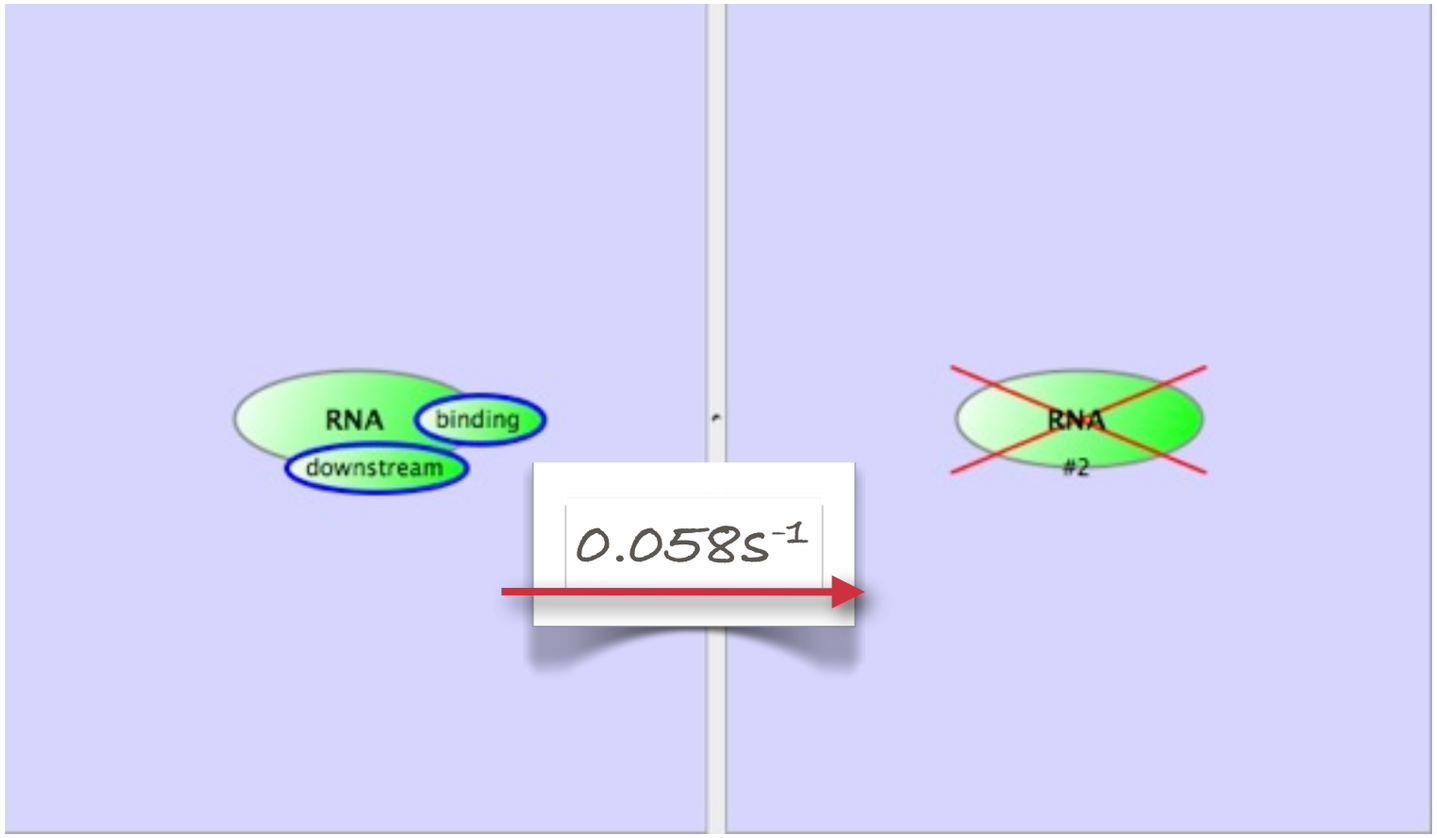
RNA axioms (2)

- RNA agents may have rules that describe the binding of a Ribosome agent to the binding site of the RNA agent. Typically, RNA agents that can bind Ribosomes will be RNA equivalents of Ribosome Binding Sites (RBS's).

- RNA agents that cannot be translated do not take Ribosome agents, and thus have no rules involving the Ribosome.

- Unlike for the RNAP during transcription, the Ribosome is not passed on to the next RNA agent, but is instead taken by a downstream agent.

RNA degradation



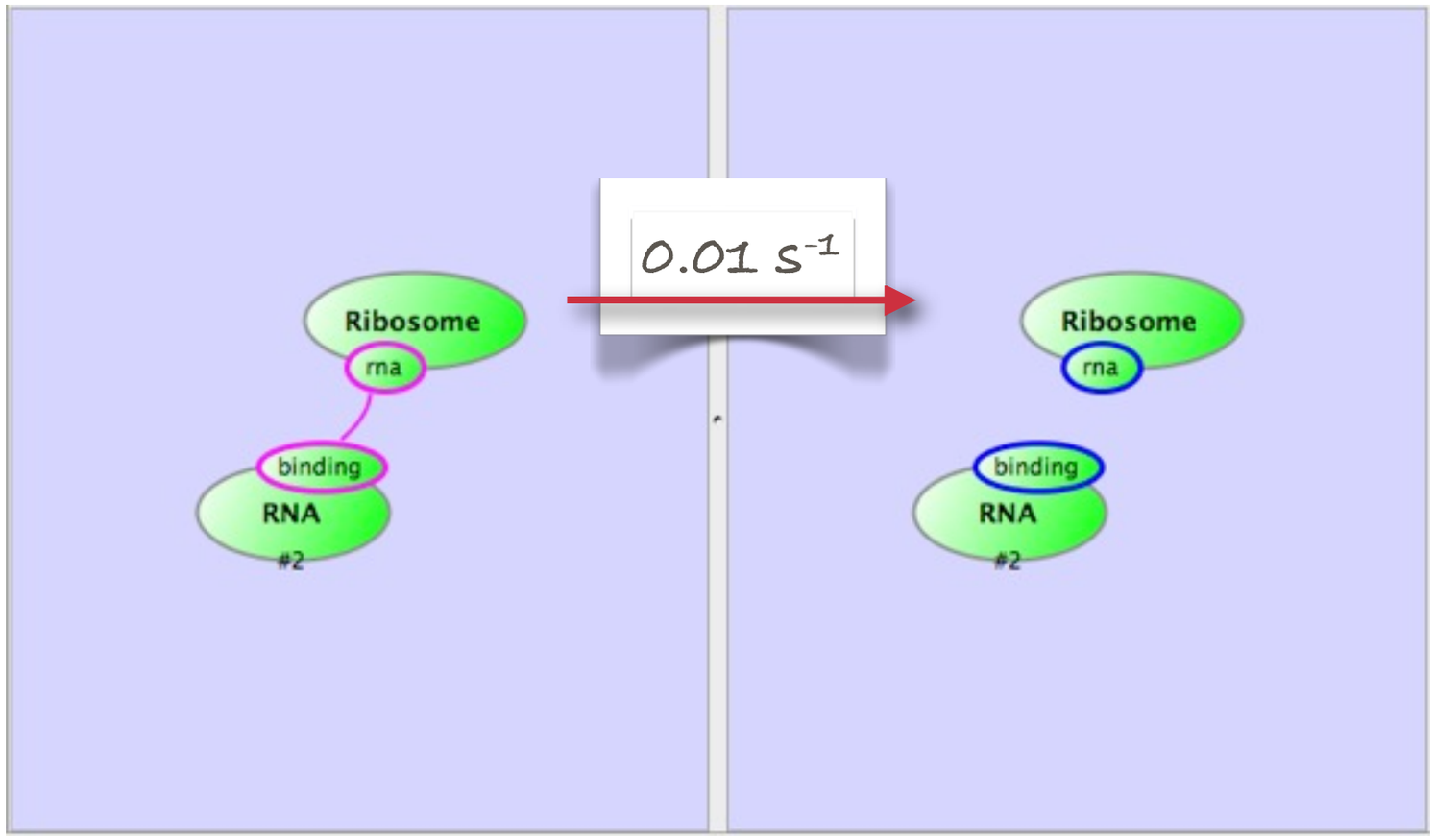
+ when Ribo is bound to an mRNA it prevents degradation

+ could model deg otherwise; rate might depend on transcript

Ribosome fall-off

when Ribo is bound to an mRNA message with no start codon/coding sequence, it should fall off

BB parts migth have an RBS upstream of something other than a cds, thus translation cannot proceed to clear the ribosome off of the RBS



on-line modeling tool

www.cellucidate.com

Foreword

Copy book | Link to book

Rule-Based Modeling of BioBrick Parts

Owner: Ty Thomson

CREATED
April 3rd

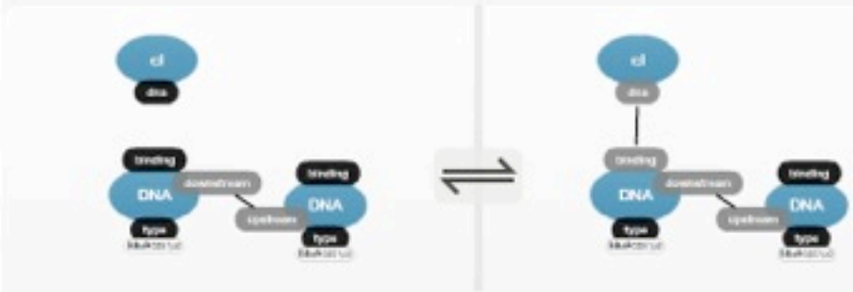
BOOKSHELF
Synthetic Biology

In this cBook, we introduce a framework for creating modular and reusable models of individual BioBrick parts, such that these parts models can be trivially combined to produce full models of systems composed of BioBrick parts. This framework uses the power of rule-based modeling to acheive this level of modularity.

cI binding to R0051p2 (no cI)

cI binds the cI operator sequence when no other cI protein is bound. The rate constants were taken from Elowitz et al. and the units converted assuming a cell volume of 1 fL.

Diagram & Kappa



Forward rate: **6** uM^-1*s^-1

Backward rate: **2.24** s^-1

Kappa string:

```
DNA(binding,type~BBaR0051p3,upstream!2),
DNA(downstream!2,binding,type~BBaR0051p2),
cI(dna) <->
DNA(binding,type~BBaR0051p3,upstream!3),
DNA(downstream!3,binding!1,type~BBaR0051 ...
```

Annotations (0)

Contact Map

Influence Map

Related Agents

- DNA
- cI

Related Simulations

- cI simulation
- Repressilator

Need help? For answers to common questions, tips, ideas and more check out the Cellucidate Discussions.

Comments

Add a comment

Page History

Ty Thomson created page on May 9.

Initial Conditions (5)

700 RNAP(dna,rna)

18000 Ribosome(rna)

1
DNA(upstream,downstream!1,binding,type~BBaR0040p1),
DNA(upstream!1,downstream!2,binding,type~BBaR0040p2),
DNA(upstream!2,downstream!3,binding,type~BBaR0040p3),
DNA(upstream!3,downstream!4,binding,type~BBaR0040p4),
DNA(upstream!4,downstream!5,binding,type~BBaB0034),
DNA(upstream!5,downstream!6,binding,type~BBaC0051),
DNA(upstream!6,downstream,binding,type~BBaB0011)

1
DNA(upstream,downstream!1,binding,type~BBaR0051p1),
DNA(upstream!1,downstream!2,binding,type~BBaR0051p2),
DNA(upstream!2,downstream!3,binding,type~BBaR0051p3),
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DNA(upstream!6,downstream,binding,type~BBaB0011)

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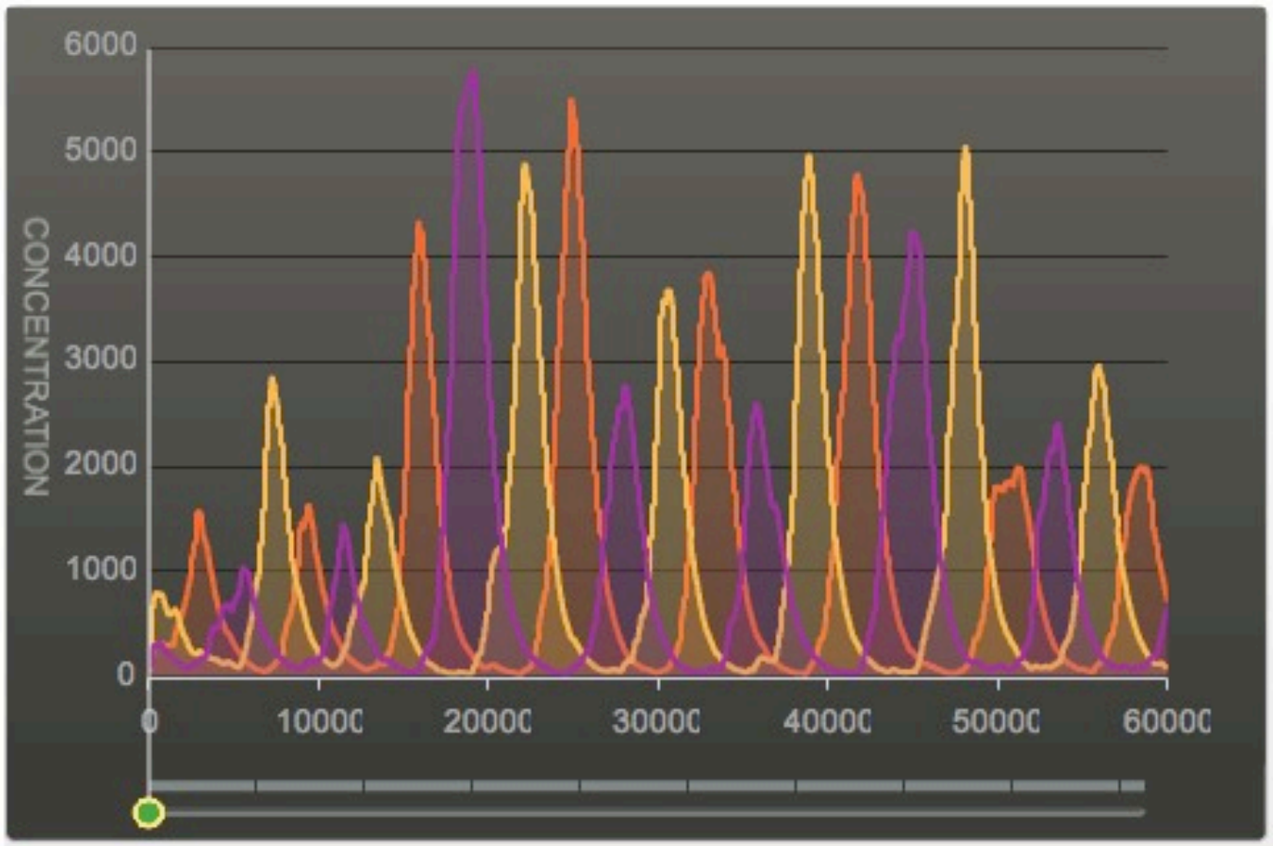
DNA(upstream!2,downstream!3,binding,type~BBaR0010p3),
DNA(upstream!3,downstream!4,binding,type~BBaR0010p4),
DNA(upstream!4,downstream!5,binding,type~BBaB0034),
DNA(upstream!5,downstream!6,binding,type~BBaC0040),
DNA(upstream!6,downstream,binding,type~BBaB0011)

simulation (rates from Elowitz et al.)

Reaction Volume: 1e-15 Liters

Simulation results

Simulation Results



Solution observables:

- TetR
- cI
- LacI

Time: 59.97

60000 total time elapsed

3935247 events

1004 data points returned

so what?

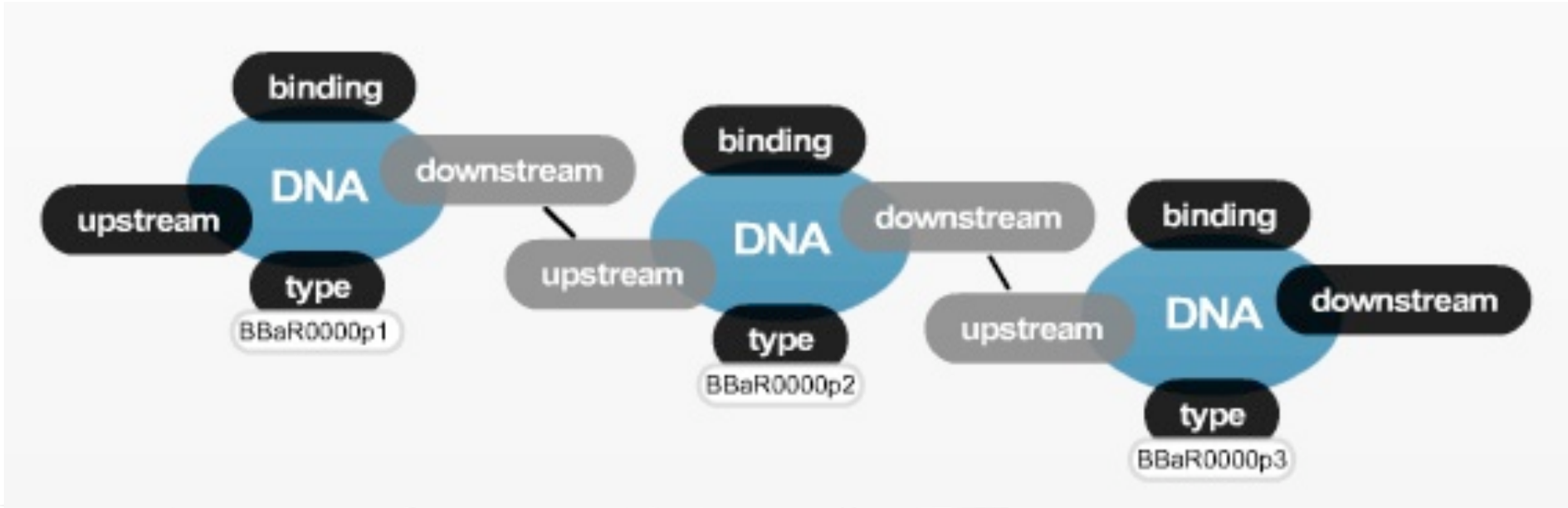
this shows how versatile rules are in describing the part's behaviours

+ also proposes an answer to the data sheet question, ie a part data sheet is the set of rules (at some granularity) associated to the part (which we may not know of course)

+ rules also reproduce the parts registry social aspects -one can publish and discuss virtual parts on line

example: a repressible promoter

Definition of a promoter



An example of a **repressible** promoter with a single binding site for the repressor. The part thus consists of three DNA agents:

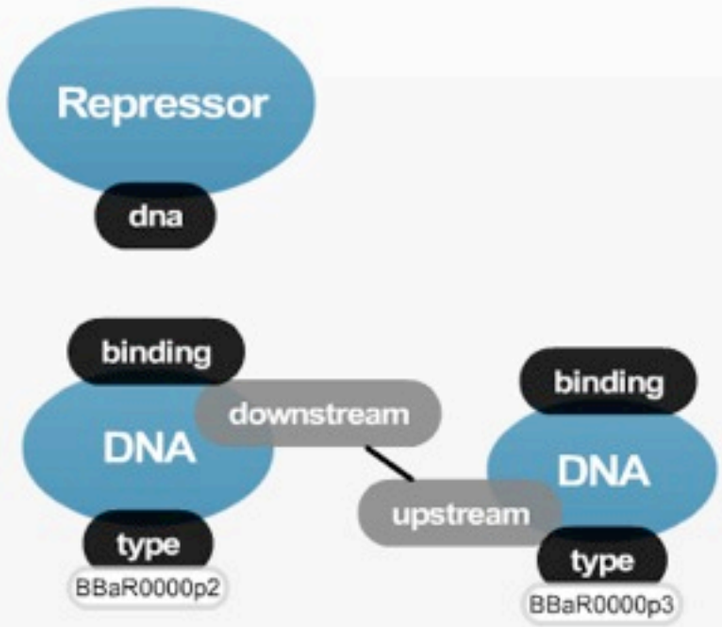
- the first (BBaR0000p1) agent is the mandatory agent that receives RNAP agents passed on by upstream parts

- the middle agent (BBaR0000p2) is where a repressor agent (defined later) can bind,

- and the last (BBaR0000p3) agent is where the promoter recruits a new RNAP to bind.

Repressor binding (no RNAP)

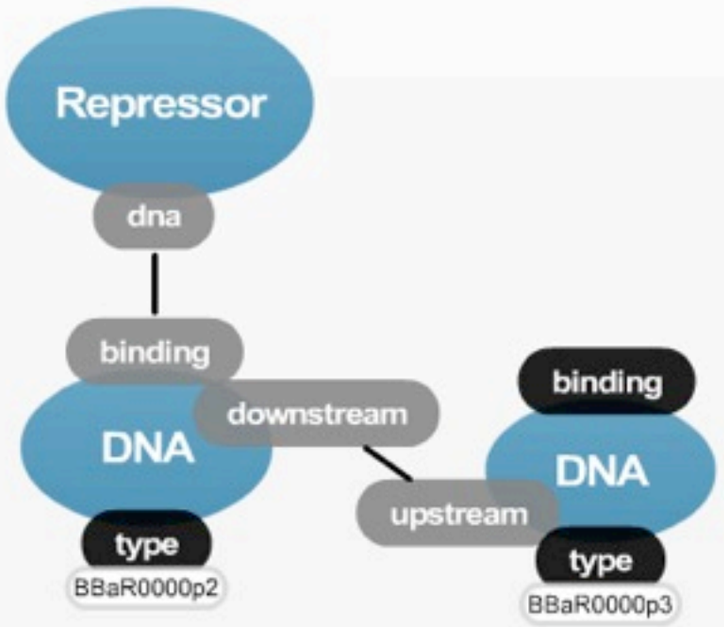
The repressor can bind to the middle binding site (BBaR0000p2) of the promoter with a high affinity when the RNAP is not bound to the third binding site (BBaR0000p3). By showing the "binding" site of BBaR0000p3, but having nothing bound to it, we are saying that the rule only applies when nothing is bound to this site. Note that the rule is reversible. Also, the status of the DNA upstream and downstream of these agents do no impact the rule.



$10\mu M^{-1}s^{-1}$

$\rightleftharpoons$

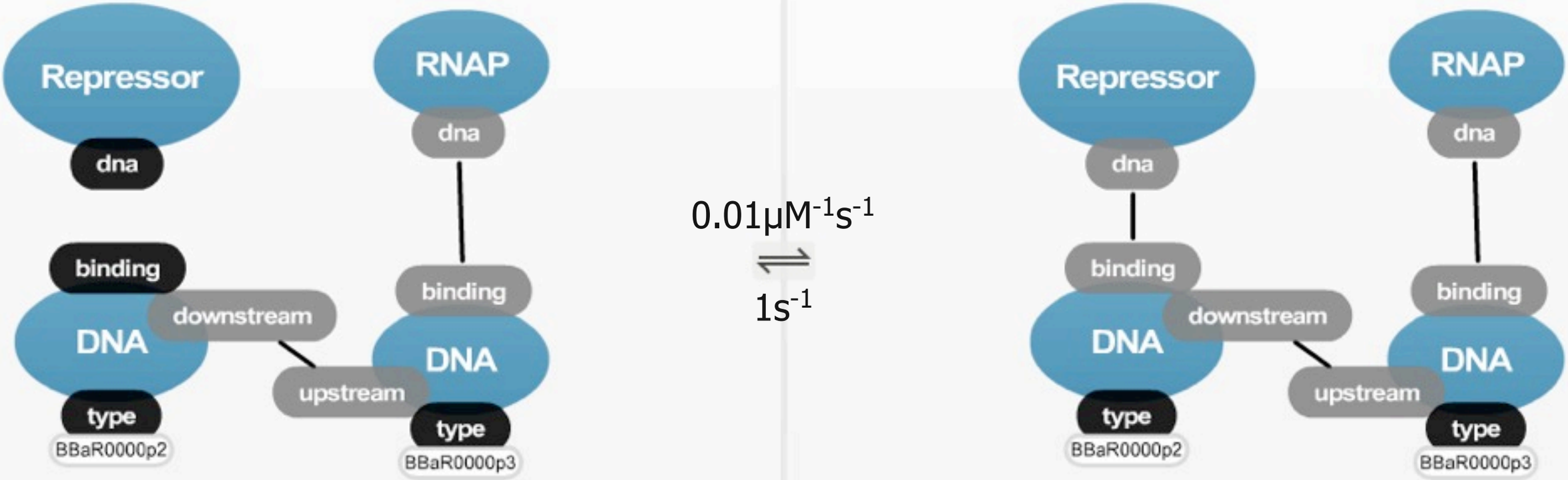
$1s^{-1}$



NB: p1, p2, p3 are non compositional subparts (could be sites in fact)

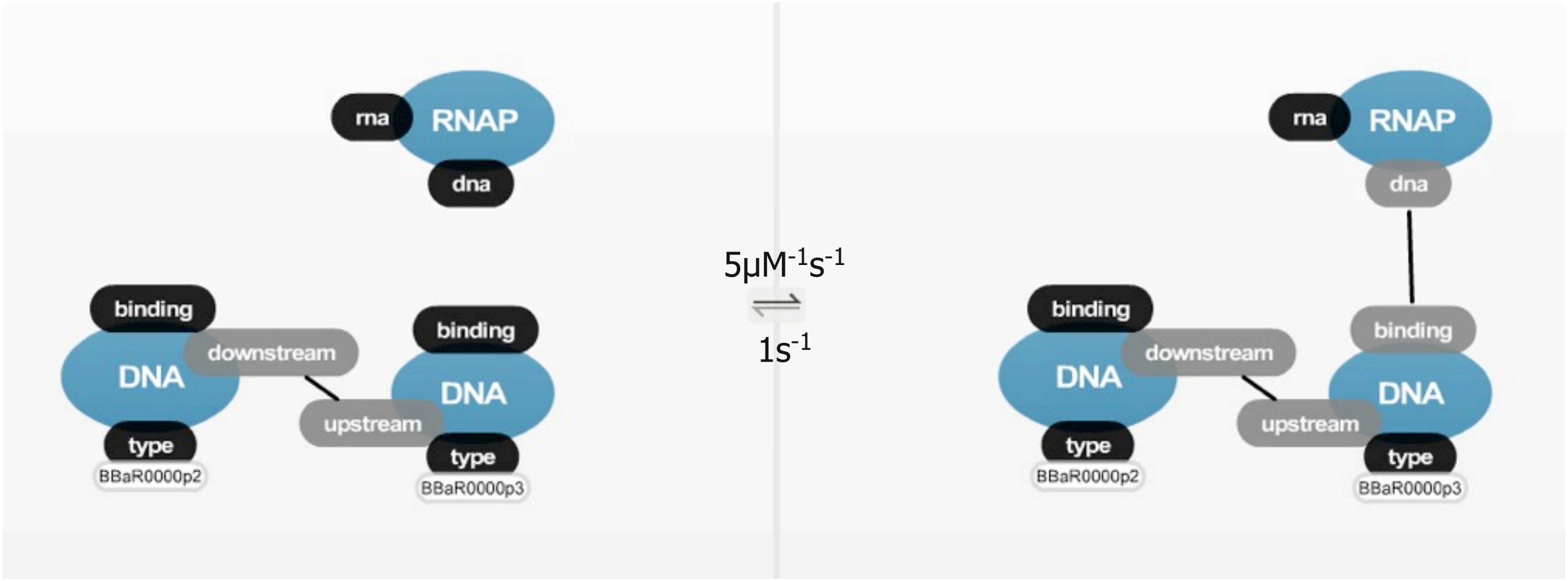
Repressor binding

The repressor can bind to the middle binding site (BBaR0000p2) of the promoter with a low affinity when the RNAP is bound to the third binding site (BBaR0000p3). The association rate is 1000 times smaller than the association rate with no RNAP present. Note that the rule is reversible (both forward and reverse arrows are black). Also, the status of the DNA upstream and downstream of these agents do no impact the rule.



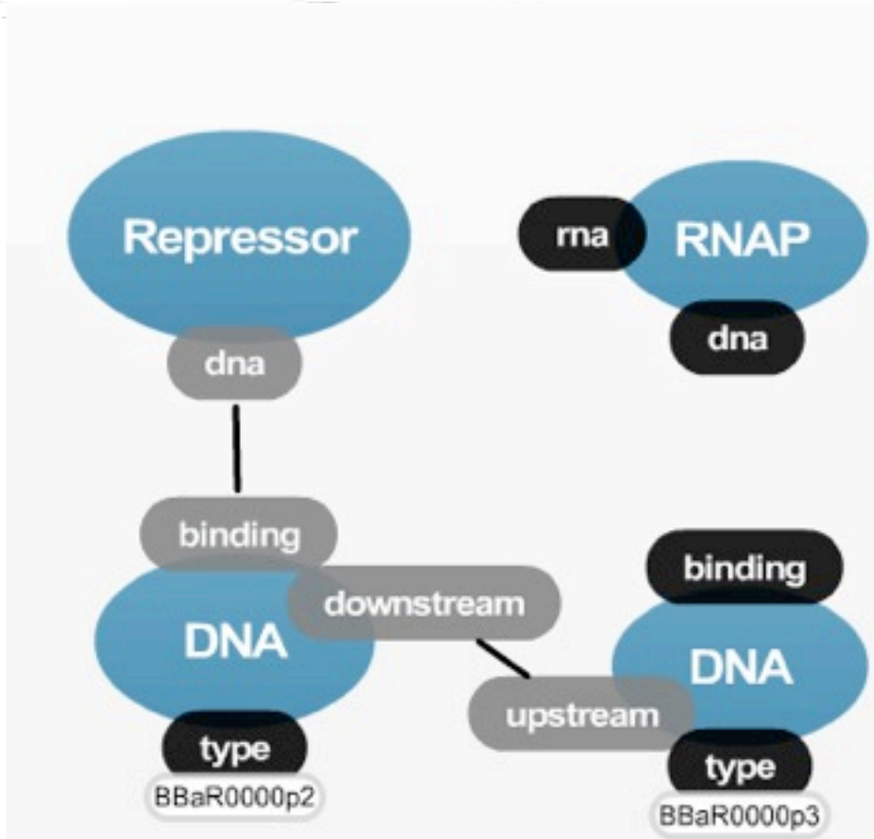
RNAP binding (no repressor)

The RNAP can bind to the third site (BBaR0000p3) of the promoter with a high affinity when the repressor is not bound to the middle binding site (BBaR0000p2). By showing the "binding" site of BBaR0000p2, but having nothing bound to it, we are saying that the rule only applies when nothing is bound to this site. Note that the rule is reversible (both forward and reverse arrows are black). Also, the status of the DNA upstream and downstream of these agents do no impact the rule.



RNAP binding

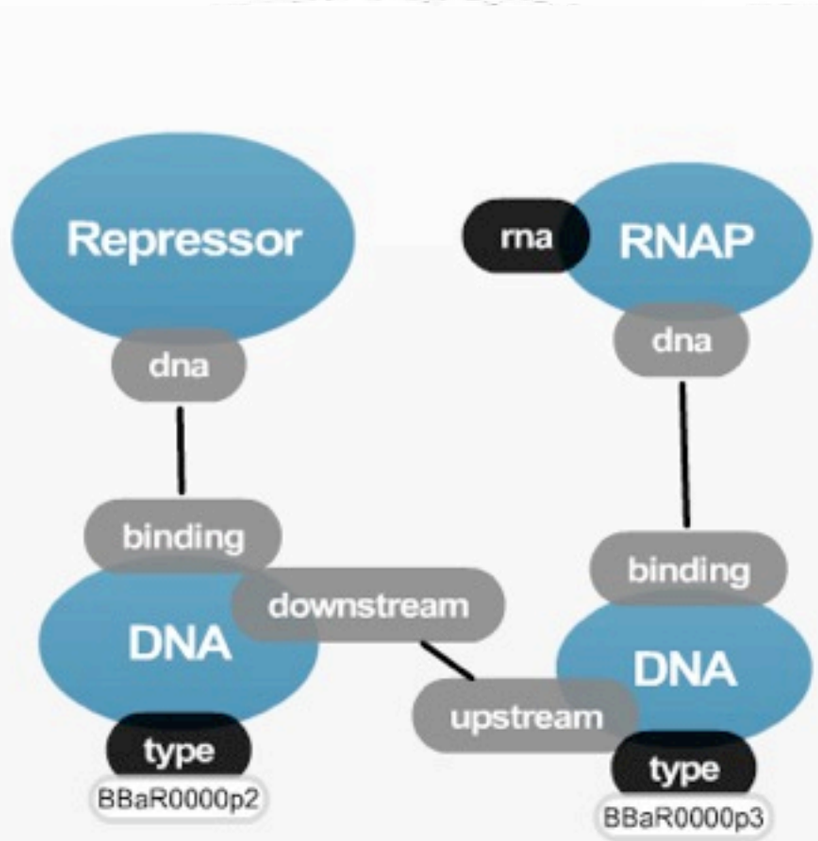
The RNAP can bind to the middle third site (BBaR0000p3) of the promoter with a low affinity when the repressor is bound to the middle binding site (BBaR0000p2). The association rate is 10^4 times smaller than the association rate with no repressor.



$0.0005\mu M^{-1}s^{-1}$

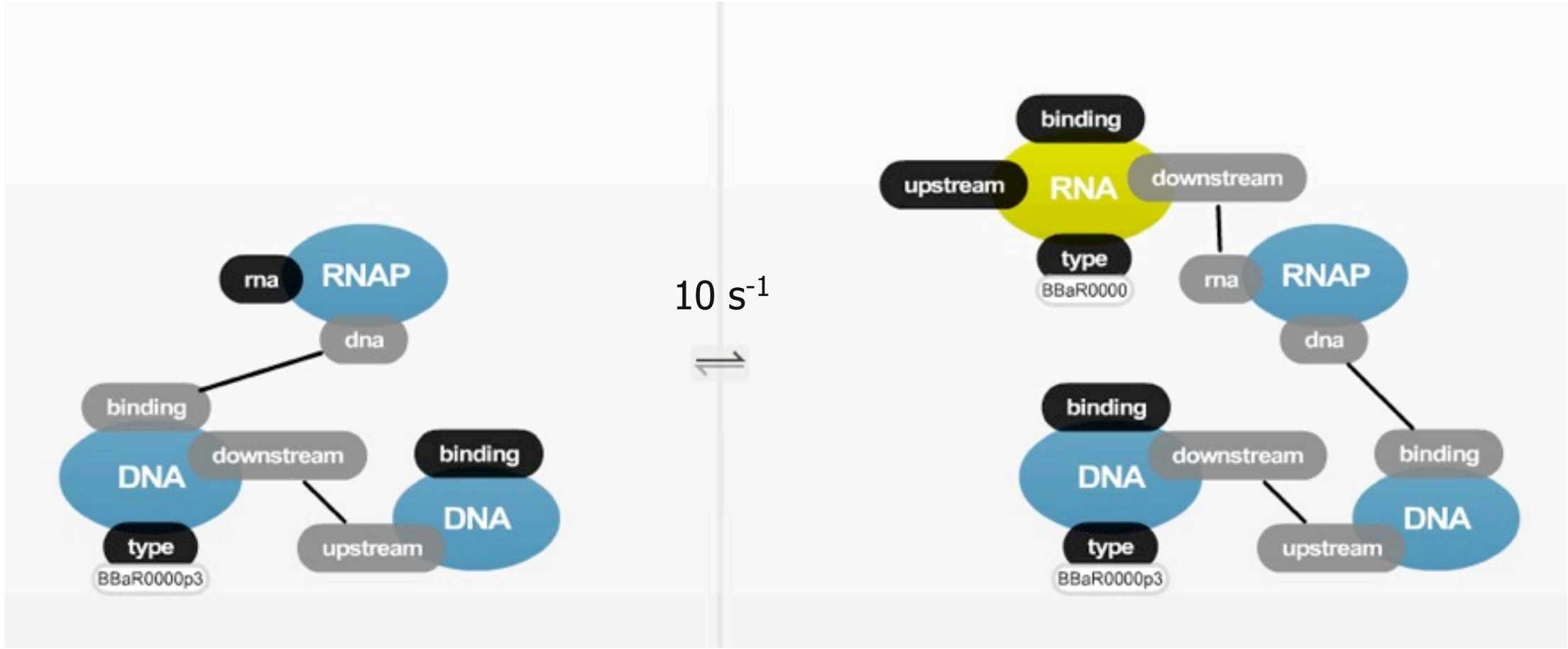
$\rightleftharpoons$

$1s^{-1}$



Transcription initiation

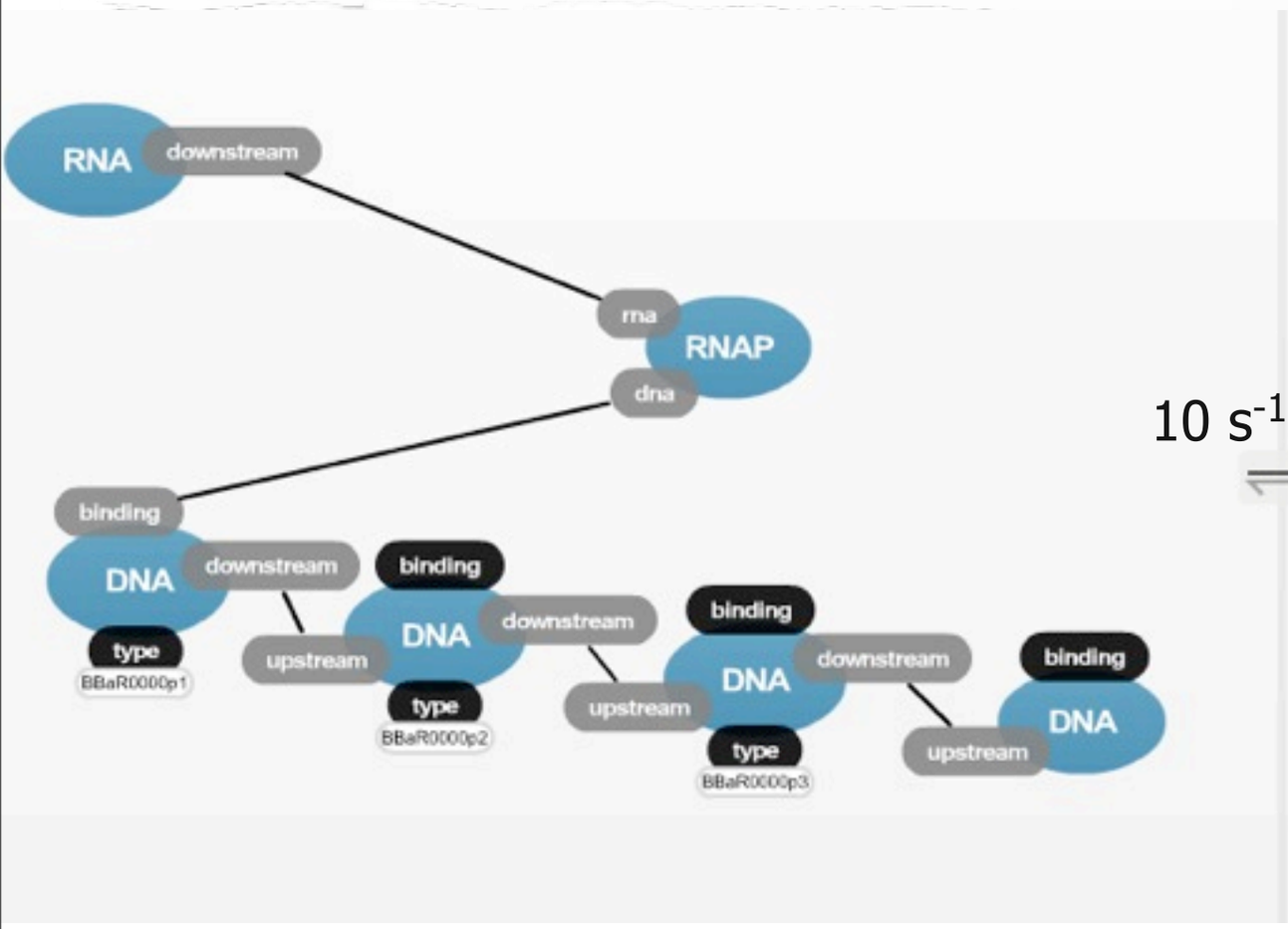
When an RNAP is bound to the third site (BBaR0000p3) of the promoter, and the binding site of the DNA agent of the downstream part is unoccupied, the RNAP can initiate transcription. Transcription initiation involves the RNAP moving to the free binding site of the next part, and an RNA copy of the promoter being made and attached to the RNAP. Obviously, the newly made RNA agent has nothing attached to its upstream site (since it's the 5' end of the mRNA) and nothing attached to its binding site.



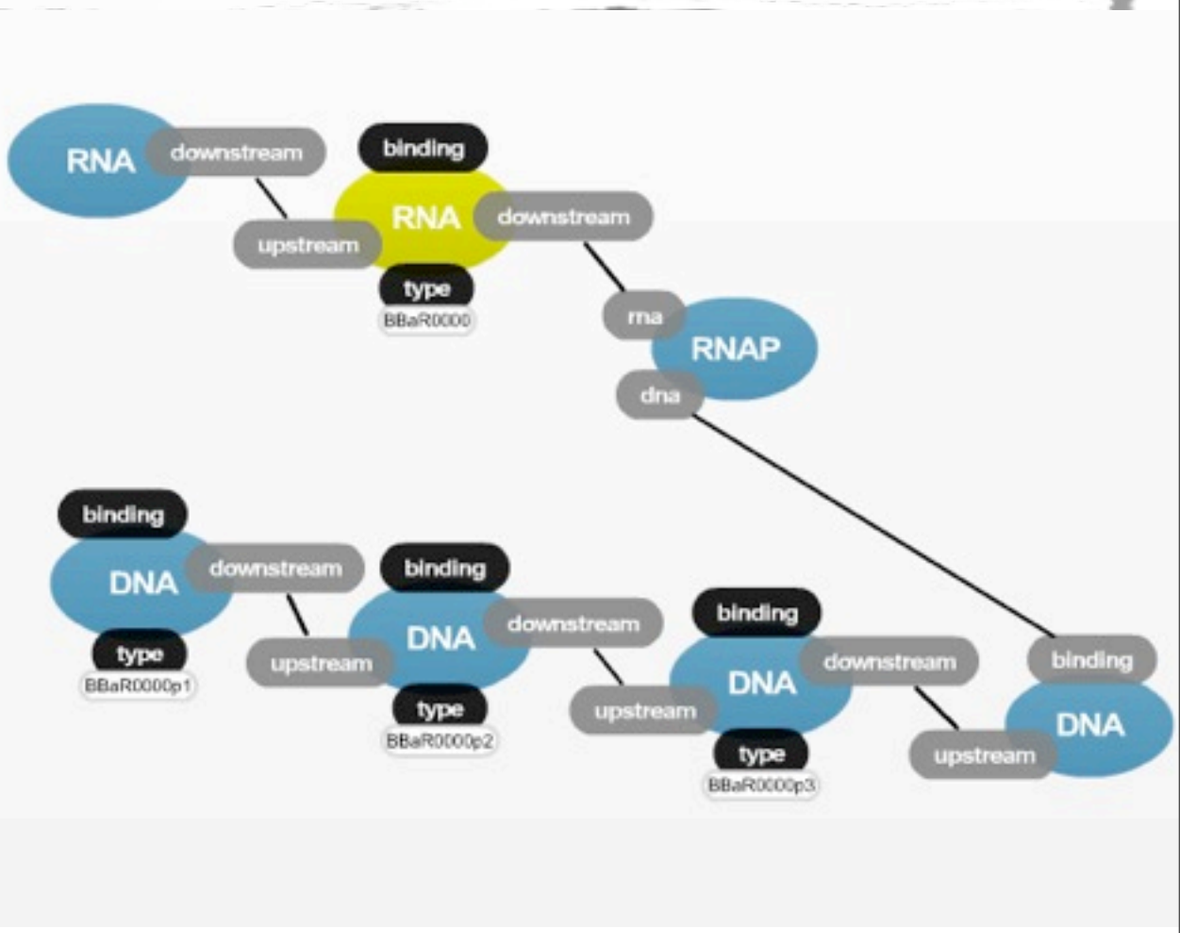
Promoter transcription (readthrough)

When an RNAP is bound to the third site (BBaR0000p3) of the promoter, and the binding site of the DNA agent of the downstream part is unoccupied, the RNAP can initiate transcription.

We can model cyclic DNA, failed terminations, etc.

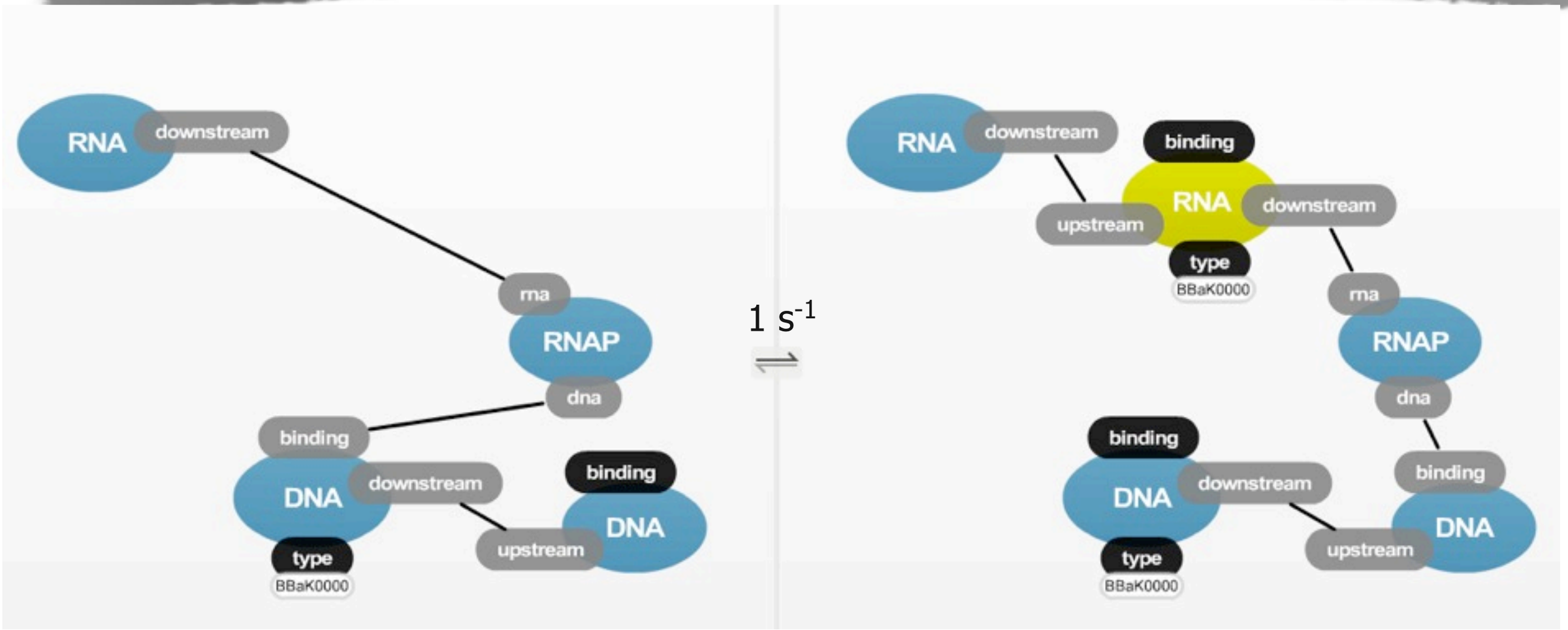


$10\ s^{-1}$ ⇌



Terminator transcription (readthrough)

At a lower rate than termination, a bound RNAP will transcribe the terminator instead of terminating transcription. An RNA copy of the terminator is added to the 5' end of the transcript that is attached to the RNAP.



example 2: an RBS

Definition of an RBS

We define an example BB ribosome binding site (RBS), BBa_B0000. The RBS part consists of a single DNA agent. We need a few rules:

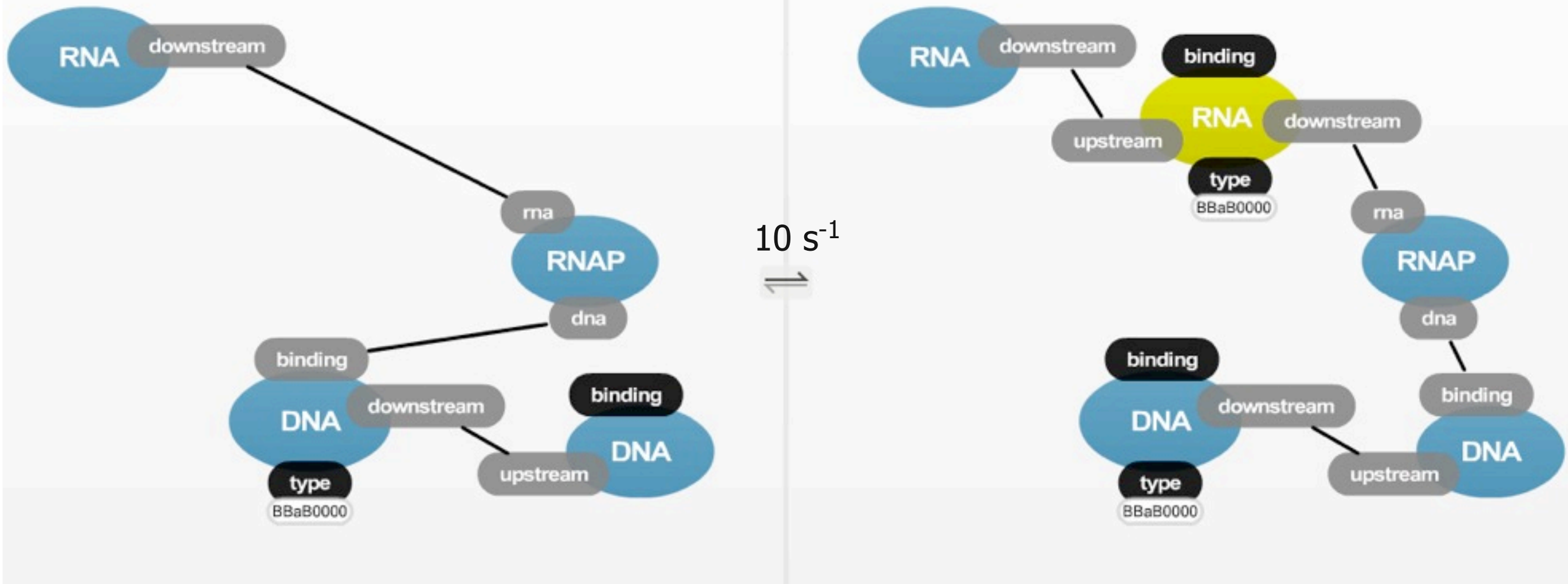
- **RBS transcription**

- **Ribosome binding**

Note that translation initiation depends on the presence of an appropriate coding sequence (with a start codon) immediately downstream of the RBS RNA agent on an mRNA molecule. Thus, the translation initiation rules are coding sequence dependent and included in the rules for each coding sequence.

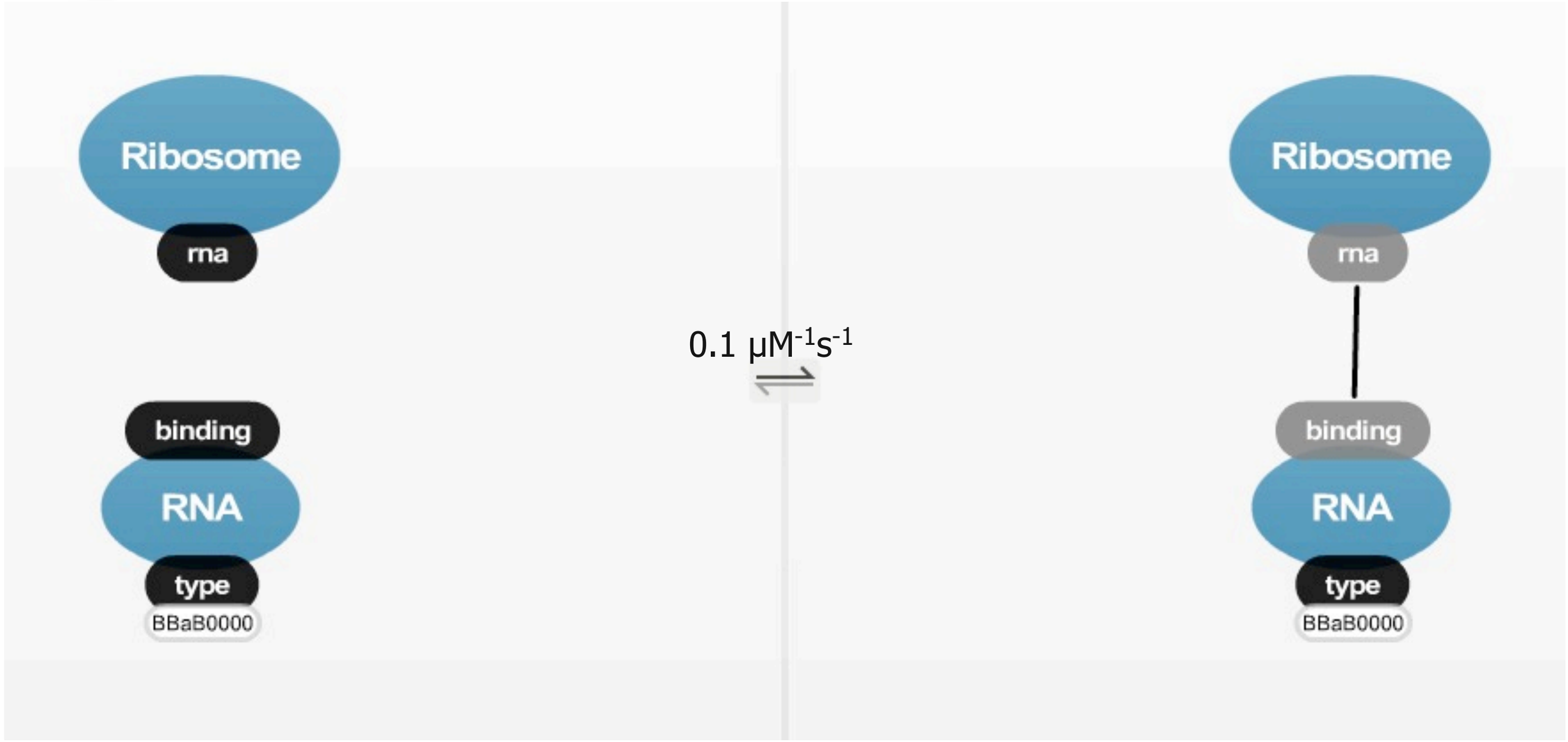
RBS transcription

RBS transcription requires that the "binding" site of the downstream part be free to take the RNAP. An RNA copy of the RBS is added to the 5' end of the mRNA molecule that is attached to the RNAP. The identity of the downstream part, and the presence or identity of an upstream part do not affect this transcription rule.



Ribosome RBS

Ribosomes can bind the "binding" site of the RBS RNA agent.



example 3: coding sequence

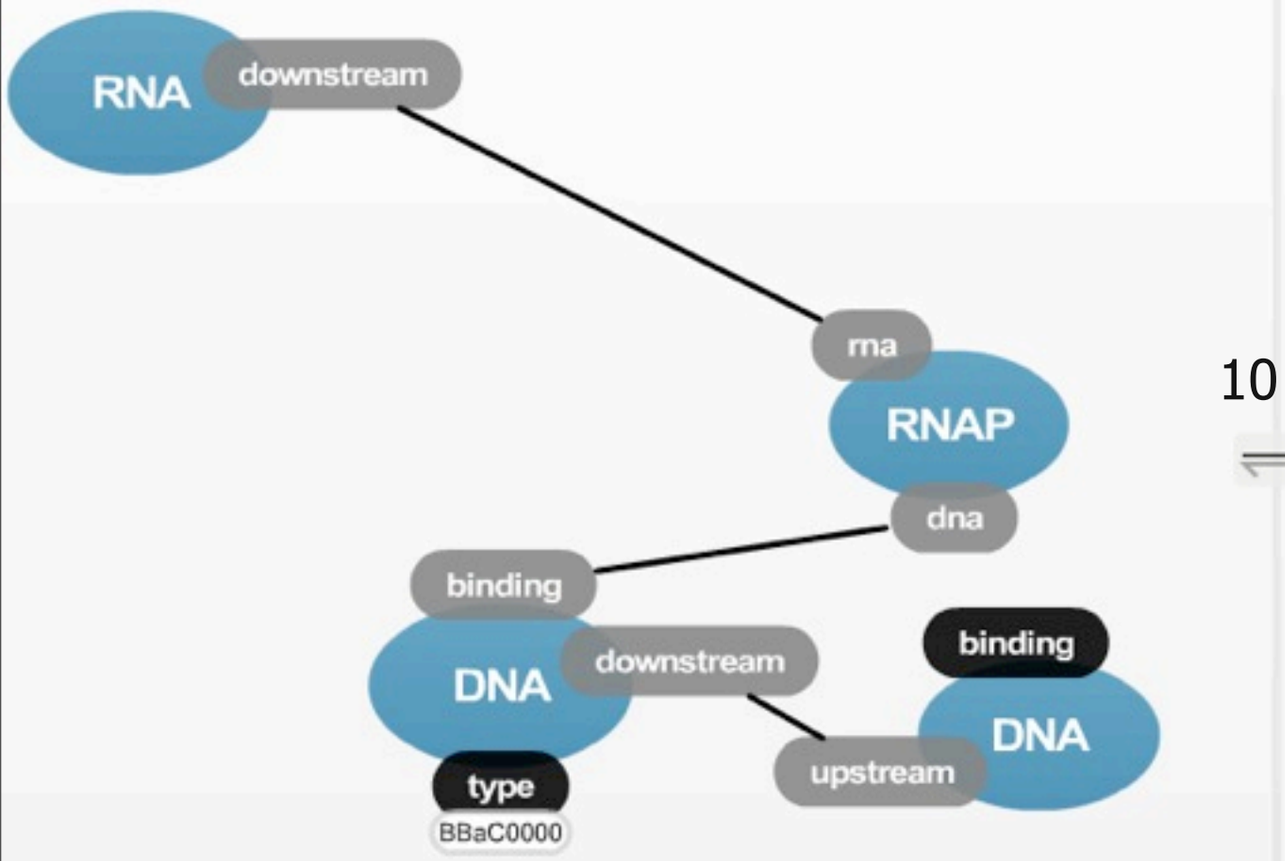
Definition of a repressor coding

The coding sequence for a repressor, BBa_C0000, consists of a single DNA agent with a few rules:

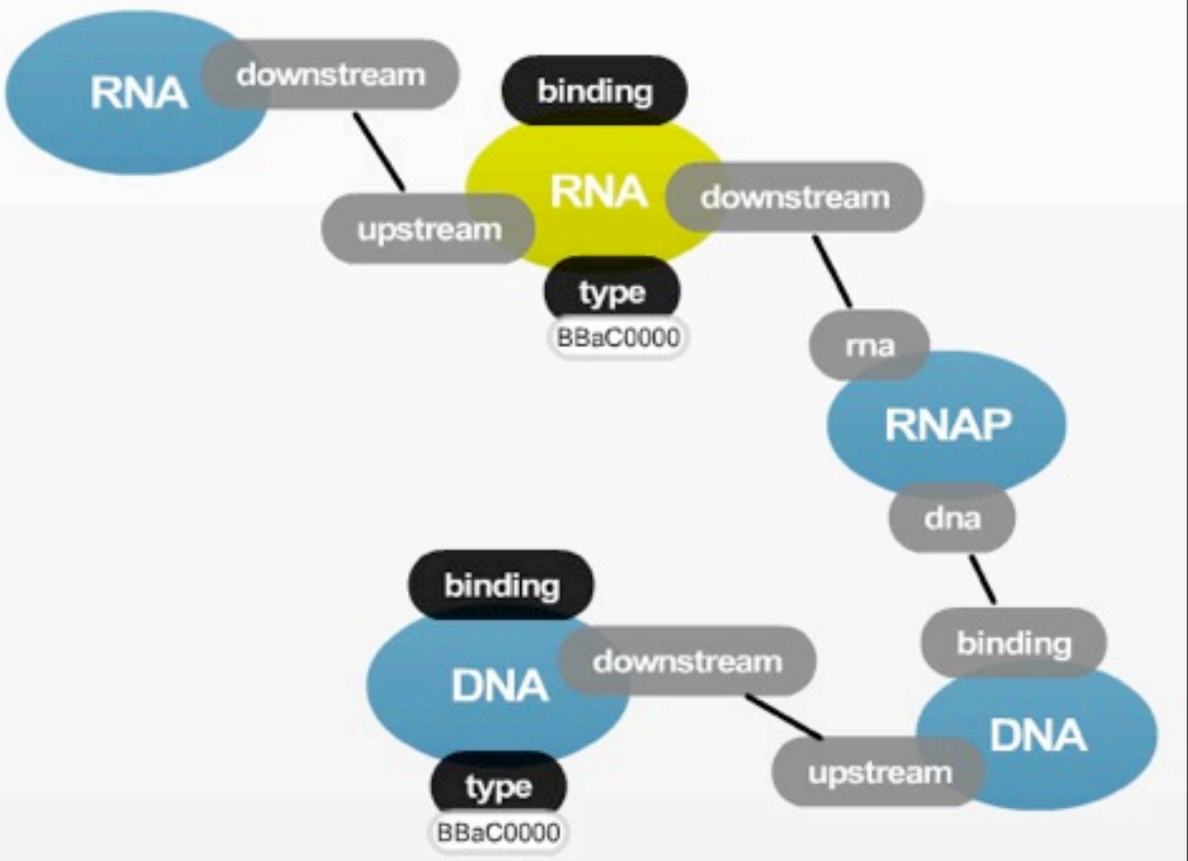
- Coding sequence **transcription**
- **Translation initiation**
- **Coding sequence translation**
- **Protein degradation**

Coding sequence transcription

Transcription requires that the "binding" site of the downstream part be free to take the RNAP. An RNA copy of the coding sequence is added to the 5' end of the mRNA molecule that is attached to the RNAP. The identity of the downstream part, and the presence or identity of an upstream part do not affect this transcription rule.

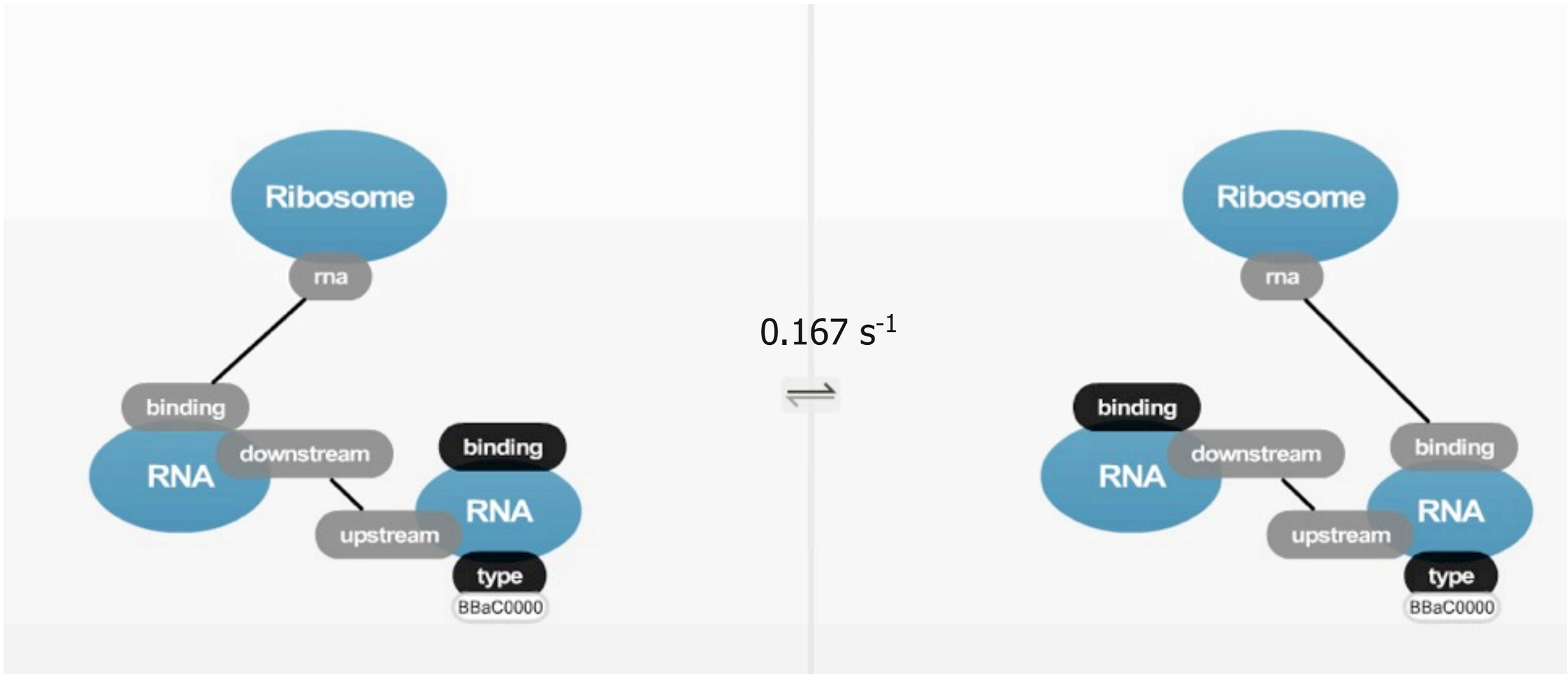


$10\ s^{-1}$



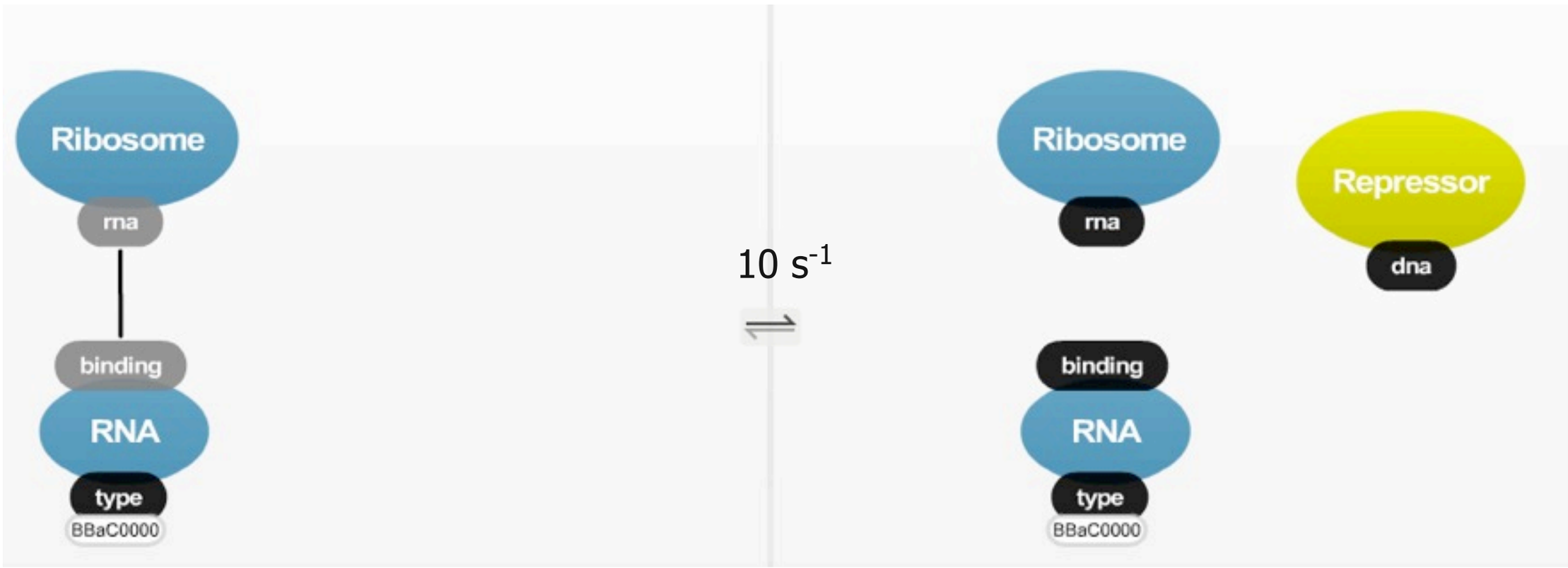
Translation initiation

During translation initiation, the coding sequence accepts a Ribosome agent from the upstream RNA agent. The rule does not depend on the identity of the upstream agent (although presumably it is a ribosome binding site) nor the presence or identity of a downstream RNA agent.



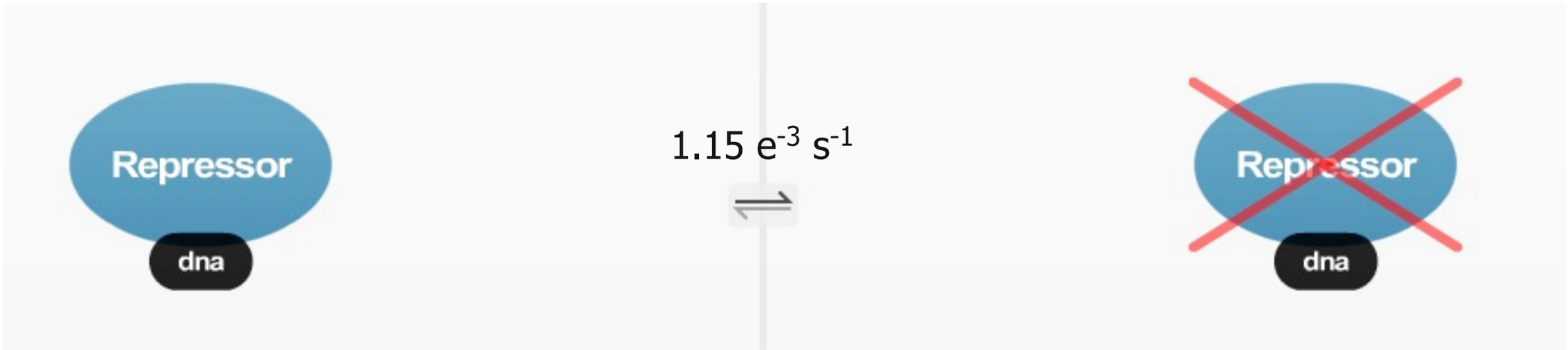
Coding sequence

The translation of the coding sequence occurs when a Ribosome is bound to the coding sequence, and is independent of the presence or identity of upstream or downstream RNA agents. The Ribosome is released, and a single copy of the encoded protein, here **Repressor**, is produced (shown in green).



Repressor degradation

The Repressor protein is degraded at a constant rate when not bound to anything via the "dna" binding site.



example 4: terminator

Definition of a terminator

We define an example BB terminator, BBa_K0000. The terminator part consists of a single DNA agent with rules:

- **Termination**

- **Terminator transcription** (failed termination)

Sometimes, an RNAP that is bound to the terminator DNA will not fall off and will instead read through and transcribe the terminator (failed termination).

Termination

An RNAP agent that is bound to the terminator dissociates from the DNA relatively rapidly. When it dissociates, the RNAP releases the mRNA molecule. Termination does not depend on the presence or identity of upstream or downstream DNA agents, nor the identity of the mRNA molecule.