10.2.2 Robustness by Backup Elements

Many loss-of-function mutations in cells have little effect on cell viability. Only about 300 of the 4000 genes in *E. coli*, for instance, have been classified as *essential* [23]. Genes can be dispensable because of different reasons. On the one hand, many gene products are used only under special circumstances: A metabolic pathway, for instance, may be required during growth on minimal medium, but not in a medium in which the pathway product is already provided. On the other hand, some proteins (e.g., isoenzymes) and metabolic pathways can replace each other and serve as backups when one of them is knocked out. All these genes, while being dispensable under ideal standard conditions, may be needed under harder conditions or in environments that provide special opportunities.

10.2.2.1 Backup Genes and Gene Loss

Backup components can make biological as well as technical systems more robust: If a component exists twice (e.g., the two alleles of a gene in diploid cells), the system can still function when one of the components fails. Gene duplications can happen randomly and be conserved if they turn out to be beneficial. In the yeast *Saccharomyces cerevisiae*, about 60% of all genes have duplicates because of a whole-genome duplication that occurred in ascomycetes [25]. Once genes become duplicated – or once a gene copy disappears – their expression levels will change and may have to be readjusted. Quantitatively, robust protein levels can be ensured, for instance, by gene silencing (e.g., of one copy of the X chromosomes in females) or by feedback control of expression levels. Moreover, if protein levels still vary, downstream robustness mechanisms can ensure that the physiological output, for example, the performance of a signaling pathway, remains unaffected.

While gene duplications can provide advantages, they may also come at a cost, especially in cells that need to keep their genomes small. A selection pressure on small genomes, for instance, has been suggested for hummingbirds [26]: Since genome size, nucleus size, and red blood cell size are positively correlated in vertebrates and since smaller red blood cells facilitate gas exchange, a high metabolic energy demand would favor smaller genomes. In fact, hummingbirds, whose energy demand during flight is particularly high, show the smallest known genomes among birds. In general, cells that reduce their genome size during evolution may develop "anti-backup" strategies: Obligate intracellular parasites have lost many genes that are dispensable during life in host cells – not only because of the material and energy, but also because of the relatively constant environment that host cells provide. Similarly, virus strains can lose genes and profit from gene products produced by wild-type viruses residing in the same cell [27]. By reducing their genome, these "selfish" virus mutants can replicate faster. However, they also depend on other viruses and thus become vulnerable. A similar dependence arises in cells that have lost the ability to produce certain compounds: These substances, for example, certain amino acids, must be taken up from food and thus become essential nutrients for the organism.

10.2.2.2 Backup Pathways

Backup strategies exist not only between genes but also between entire pathways. Whether a metabolic pathway is used depends upon external conditions, that is, on whether it *can be* run and whether it *needs to be* run. In the absence of oxygen, a yeast cell cannot use respiration, but it may still rely on glycolysis for energy generation. Likewise, when a pathway is blocked by a gene loss, it may be bypassed by redirecting the metabolic fluxes through alternative pathways. On the contrary, pathways may become dispensable in certain environments: For instance, if the product of an anabolic pathway can also be imported, the pathway need not be expressed [24]. In the yeast *S. cerevisiae*, such higher level robustness mechanisms turn out to be more important than single-gene backups [28].

10.2.3 Feedback Control

A basic task in control engineering is to keep output quantities of a technical system constant despite perturbations. In living systems, such a behavior is called homeostasis, and it is one of the preconditions for life.

10.2.3.1 Feedback Regulation Changes the System Dynamics

A simple and powerful way to buffer an output quantity against perturbations is *negative feedback* (see Section 8.2.3). Consider a linear model:

$$\frac{\mathrm{d}\mathbf{x}}{\mathrm{d}t} = \mathbf{A}\mathbf{x} + \mathbf{B}\mathbf{u}, \tag{10.18}$$

with the Jacobian matrix $\mathbf{A}$ and an input vector $\mathbf{u}(t)$. Such models can be obtained from biochemical models by linearization (see Section 6.3.4). Fluctuations of $\mathbf{u}$ will affect the output $\mathbf{x}$, and the system's robustness against such fluctuations depends on the eigenvalues of $\mathbf{A}$. In technical systems, the eigenvalue spectrum can be shaped by coupling the system to a feedback controller. The controller measures the current state $\mathbf{x}$, computes a linear function

$\mathbf{z} = \mathbf{F}\mathbf{x}$, and adds it to the system input. The resulting *closed-loop system* (see Figure 15.14) follows the dynamics

$$d\mathbf{x}/dt = \mathbf{A}\mathbf{x} + \mathbf{B}(\mathbf{u} + \mathbf{z}) = (\mathbf{A} + \mathbf{B}\mathbf{F})\mathbf{x} + \mathbf{B}\mathbf{u}, \tag{10.19}$$

with a new Jacobian matrix $\mathbf{A} + \mathbf{B}\mathbf{F}$. With an appropriately chosen feedback matrix $\mathbf{F}$, all eigenvalues of the Jacobian may become negative and the coupled system will be stabilized against perturbations (see Section 15.5). The stabilizing effect of negative feedback has been shown, for instance, for proteins that repress their own transcription [29]. In theory, such a self-repression could also have the opposite effect: By pushing some complex eigenvalues to the right side of the complex plane, feedback control can destabilize otherwise stable systems, allowing them to show sustained oscillations (see Section 8.2). This typically happens in cases of negative feedback with long time delays. In reality, the possibilities to control systems through feedback mechanisms are limited. First, a single feedback arrow (e.g., an allosteric regulation in a metabolic network) will not specifically change a single eigenvalue, but all eigenvalues at the same time. Second, real feedback controllers cannot freely sense and manipulate the state vector $\mathbf{x}$, but sense some of the outputs and affect some of the inputs only. Two resulting issues, called observability and controllability, are discussed in Section 15.5.

In kinetic metabolic models, the Jacobian $\mathbf{A}$ is obtained by multiplying the stoichiometric matrix with the elasticity matrix. For the pathway in Figure 10.6, with a fixed influx rate $v_0 > 0$, the Jacobian $\mathbf{A}$ reads

$$\mathbf{A} = \mathbf{N}\tilde{\varepsilon} = \begin{pmatrix} 1 & -1 & 0 \\ 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} 0 & 0 \\ \tilde{\varepsilon}_{11} & \tilde{\varepsilon}_{12} \\ 0 & \tilde{\varepsilon}_{22} \end{pmatrix} = \begin{pmatrix} -\tilde{\varepsilon}_{11} & -\tilde{\varepsilon}_{12} \\ \tilde{\varepsilon}_{11} & \tilde{\varepsilon}_{12} - \tilde{\varepsilon}_{22} \end{pmatrix}. \tag{10.20}$$

Its diagonal elements describe a direct self-regulation of individual compounds and are usually negative. In a reaction, an increased substrate level will lead to a higher rate (positive reaction elasticity), and therefore to a higher consumption of the substrate itself (stoichiometric coefficient −1). Likewise, a higher product level leads to a smaller rate, and thus decreases its own production. In both cases, the net effect is negative, so fluctuations of individual metabolites tend to be washed out. Despite this stabilizing effect (described by the diagonal elements of the Jacobian), the overall dynamics (described by the Jacobian's eigenvalues) may still be unstable. The dynamics is further shaped by allosteric regulation, which adds elements to the elasticity matrix or changes the existing ones, which affects the Jacobian and the dynamic behavior.

10.2.3.2 Allosteric and Transcriptional Feedback

Organisms employ feedback regulation on various levels of organization. In metabolism, the main mechanisms are transcriptional, posttranslational, and allosteric regulation of enzyme activities. While allosteric regulation via metabolites acts on the metabolic time scale of seconds (determined by metabolite diffusion), transcriptional regulation is much slower: Its characteristic time is set by the protein's effective average lifetime. In growing bacteria with stable proteins, it is given by the cell cycle period,

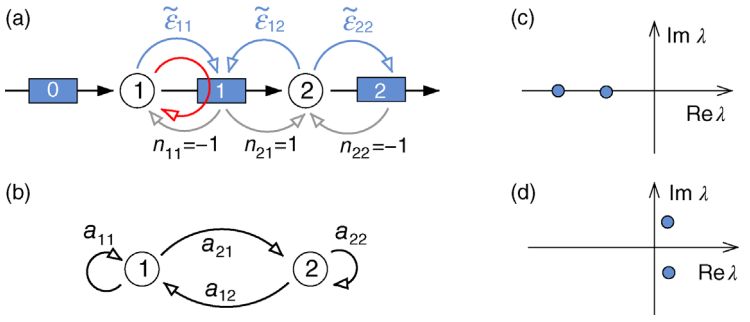

Figure 10.6 Interpretation of the Jacobian matrix. (a) A chain of metabolic reactions (boxes 0, 1, and 2) with a fixed influx v_0 and metabolites (circles). Around a steady state, concentration fluctuations affect the reaction rates via the elasticity coefficients (blue arrows). In the system equation, reaction rates act back on the concentrations via stoichiometric coefficients (gray arrows). (b) Jacobian matrix $\mathbf{A} = \mathbf{N}\tilde{\varepsilon}$. Individual matrix elements arise from paths of length 2 in scheme (a): The element $a_{11} = -\tilde{\varepsilon}_{11}$, for instance, is the product of $\tilde{\varepsilon}_{11}$ and n_{11} on the loop between metabolite 1 and reaction 2 (red arrow). If there are several paths with the same start and end points, the resulting values are added. (c) Eigenvalues of the Jacobian matrix, shown as points in the complex plane. If all real parts are negative, the steady state is stable. (d) Changes in the elasticity matrix (e.g., an addition of allosteric regulation) can change the eigenvalues. The pair of Jacobian eigenvalues shown indicate an unstable steady state, giving rise to spontaneous oscillations.

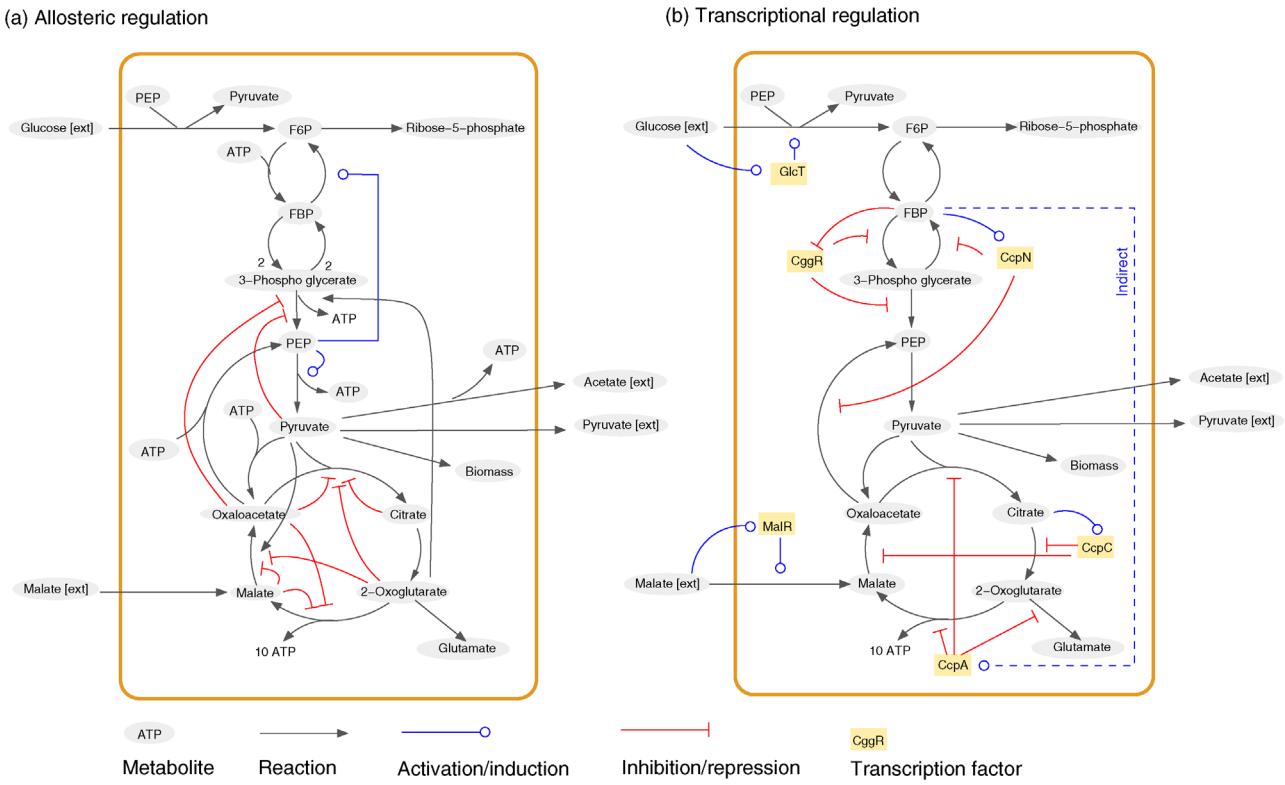

Figure 10.7 Regulation of central metabolism in *B. subtilis*. (a) Metabolic reactions and allosteric regulation in a simplified scheme of glycolysis and citric acid cycle. Only some metabolites are shown. Allosteric regulation by small molecules allows enzyme activities to adapt rapidly to changing concentrations (blue arrows: activation; red arrows: inhibition). (b) The same network, with transcriptional regulation (blue: effective activation; red: effective repression). Transcription factors (yellow boxes), regulated by small molecules, establish a number of feedback loops. The glucose and malate transporters are induced by their own substrates (feed-forward activation by supply). Fructose 1,6-bisphosphate (FBP) acts as a central regulator: It induces gluconeogenesis at low levels and glycolysis when its level is high. (Data from Ref. [31].)

that is, typically more than half an hour. Its delayed and smooth response makes transcriptional regulation less effective. On the other hand, transcriptional regulation is more cost-efficient: When enzymes are not needed, they will not be produced and do not occupy space. Allosteric and posttranslational inhibition, in contrast, decrease an enzyme's activity, but not its concentration – so the effort for producing and maintaining the protein remains (see Section 11.1). Figure 10.7 shows both types of regulation in the central carbon metabolism of the soil bacterium *Bacillus subtilis*. The metabolite fructose 1,6-bisphosphate (FBP) plays a central role in controlling the glycolytic flux: Whenever its level is high, it induces the production of glycolytic enzymes by inhibiting the transcriptional repressor CggR. At low levels, in contrast, it induces enzymes that revert the glycolytic flux and enable gluconeogenesis. This regulation can be seen at work in Figure 9.20. The same metabolite, FBP, has also been found to be a sensor of the glycolytic flux in *E. coli* [30].

10.2.3.3 Integral Feedback

Integral feedback, used to stabilize system outputs, is a standard method in control engineering. The task is to steer a system output toward a defined target value, making the system return to this value under any change of system inputs. It is achieved by continuously sensing the deviation between target value and output, integrating it over time, and feeding the resulting value back into the system as a control.

As an example, consider a linear system whose output variable $y(t) = ku(t)$ depends directly on the input variable $u(t)$. Our goal is to stabilize the output at a value y_0 despite variation in u. To achieve this, a controller continuously measures the difference $\Delta y(t) = y(t) - y_0$, computes its negative time integral $z(t)$, and adds it to the input u (see Figure 10.8). The resulting differential equations read as follows:

$$\begin{aligned} z(t) &= -\int_{t_0}^{t} y(t) - y_0 \mathrm{d}t, \\ y(t) &= ku(t) + k'z(t). \end{aligned} \tag{10.21}$$

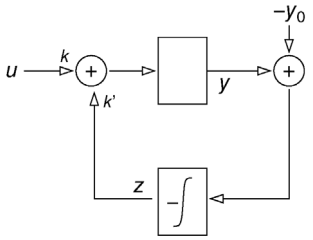

Figure 10.8 Integral feedback controller shown as a wiring diagram.

Now imagine that k and u assume arbitrary static values. In this case, the output y will be static as well and from the stationarity condition $dy/dt = 0$, we directly obtain $y = y_0$, no matter which values for u, k, and even k' we assume. After any change of u, as soon as u becomes constant again, the feedback will steer the system back to its target value y_0. Importantly, integral feedback does not depend on parameters, but only on the structure of the equation system. Several biochemical pathways, including the yeast osmostress system [31] and the bacterial chemotaxis system [32], which we shall discuss below, implement integral feedback by their structure: This allows them to show perfect adaptation and to maintain this property even when their biochemical parameters are changing. Whether metabolic systems can also be controlled by integral feedback is not clear. For a linear synthesis pathway in which enzymes are transcriptionally controlled by the pathway product, an integral feedback mechanism would require enzymes to be degraded at a constant rate (molecules per time) independent of their own current levels [33] – a condition that cannot be satisfied by cells.

10.2.4 Perfect Robustness by Structure

Integral feedback is a way to realize *perfect robustness*: the fact that a system output y becomes completely independent of some perturbation parameter x. Even in cases where dependencies would be expected (e.g., between the expression level x of a signaling pathway and the pathway's output signal y), perfect robustness can be ensured by special network structures. To see how perfect robustness is realized in biochemical signaling pathways, let us now study models of the bacterial two-component signaling system [34] and the bacterial chemotaxis pathway [36–37].

10.2.4.1 The Two-component System

The two-component system, consisting of a sensor protein and a regulator protein, is a common type of signaling pathway in bacteria. In the EnvZ/OmpR system in *E. coli*, the membrane-bound sensor EnvZ senses osmolarity and activates the diffusible transcription factor OmpR, which triggers the osmotic stress response. In experiments [38], the system output (in this case, the expression level of target genes of OmpR) was found to be robust against overexpression of the two signaling proteins: It changed by roughly 20% even if the EnvZ and OmpR levels were increased by factors of 10. This robustness clearly improves information transmission: The less an output responds to protein variation, the more precisely it will reflect changes in the pathway's input signal.

As shown in Figure 10.9, signal transduction in the EnvZ/OmpR system consists of three steps: (i) the sensor EnvZ (called X) is phosphorylated under consumption of ATP; (ii) its phosphate group is transferred to the regulator OmpR (called Y); and (iii) the phosphorylated regulator (called Y_P) is dephosphorylated again. A surprising feature of this mechanism, which calls for an explanation, is that the last step requires the presence of ATP, but it does *not* rely on ATP as an energy source. This requirement for ATP has been experimentally shown for the EnvZ/OmpR system, for the envelope stress system CpxA/CpxR in *E. coli*, and for the oxygen limitation system PrrB/PrrA in *Rhodobacter sphaeroides* (see Ref. [34]).

Both properties, the remarkable robustness and the unusual ATP-dependence of the last step, have been explained by a kinetic model [34]. In the model, it is not ATP itself but the first intermediate complex X·ATP that catalyzes the dephosphorylation. As shown in Figure 10.9c, the reactions are broken down into pairs of elementary reactions (1, 1′), (2, 2′), and (3, 3′). Each reaction consists of two mass-action steps: a reversible binding and an irreversible dissociation. The rates read as follows:

$$\begin{aligned}
\text{Autophosphorylation}: \quad & \nu_1 = k_1[\mathrm{X}][\mathrm{ATP}] - k_{-1}[\mathrm{X}\cdot\mathrm{ATP}], \\
& \nu_{1'} = k_{1'}(u)[\mathrm{X}\cdot\mathrm{ATP}]; \\
\text{Phosphotransfer}: \quad & \nu_2 = k_2[\mathrm{X_P}][\mathrm{Y}] - k_{-2}[\mathrm{X_P}\cdot\mathrm{Y}], \\
& \nu_{2'} = k_{2'}[\mathrm{X_P}\cdot\mathrm{Y}]; \\
\text{Dephosphorylation}: \quad & \nu_3 = k_3[\mathrm{Y_P}][\mathrm{X}\cdot\mathrm{ATP}] - k_{-3}[\mathrm{X}\cdot\mathrm{Y_P}\cdot\mathrm{ATP}], \\
& \nu_{3'} = k_{3'}[\mathrm{X}\cdot\mathrm{Y_P}\cdot\mathrm{ATP}].
\end{aligned} \tag{10.22}$$

The signal is transduced as follows. The external osmolarity signal u sets the rate constant $k_{1'}(u)$, which regulates the autophosphorylation rate: Each value of $k_{1'}$ leads to a certain steady-state level of Y_P, which acts as the system output (blue arrows in Figure 10.9c).

The model allows for a steady-state flux that transfers a phosphate group from ATP to the proteins and finally converts it into inorganic phosphate (see Figure 10.9c). An external stress signal will change the autophosphorylation rate of X, which shifts the steady state and changes the output concentration $[Y_P]$. The steady-state output