Chapter 2
Complex Systems Biology of Networks: The Riddle and the Challenge

Miguel A. Aon

Abstract There is no direct relationship between metabolite, mRNA, protein, and gene; the expression of a gene is not necessarily correlated with the abundance of the corresponding protein product, and the activity of a protein may depend on posttranslational modifications, e.g., phosphorylation, redox-modulation/modification, and acetylation. It is believed that the diverse nature and outcomes of networks composed of genes, transcripts, proteins, and metabolites remain an obstacle for tracing the flux from genes to proteins in order to be able to capture or explain developmental programs or the underlying mechanisms of a disease. A different approach is needed to address this problem, and accordingly an alternative view based on the dynamic integration of three different kinds of networks, mass–energy, information, and signaling, is proposed and developed in this chapter. From this perspective, the spatio-temporal expression of mass–energy transformation and information-carrying networks is modulated by signaling networks associated with fundamental cellular processes such as cell division, differentiation, and autophagy. The dynamic network of reaction fluxes (i.e., the fluxome) represents the ultimate integrative outcome of the whole process. This approach—which accounts for the basic biological fact that cells and organisms make themselves—can only be realized by networks connected by overall cyclic topologies. Thereby, the output of mass–energy/information networks, composed of proteins, transcriptional factors, metabolites, is at the same time input for signaling networks which output activates or represses those same networks that produced them.

> (. . .) If the genes are "essentially the same," what then is it that makes one organism a fly and another a mouse, a chimp, or a human? The answer, it seems, is to be found in the structure of gene networks—in the way in which genes are connected to other genes by the complex regulatory mechanisms that, in their interactions, determine when and where a particular gene will be expressed. But unlike the sequence of the genome, this regulatory

M.A. Aon (✉)
Division of Cardiology, Johns Hopkins University, Baltimore, MD 21205, USA
e-mail: maon1@jhmi.edu

M.A. Aon et al. (eds.), *Systems Biology of Metabolic and Signaling Networks*,
Springer Series in Biophysics 16, DOI 10.1007/978-3-642-38505-6_2,

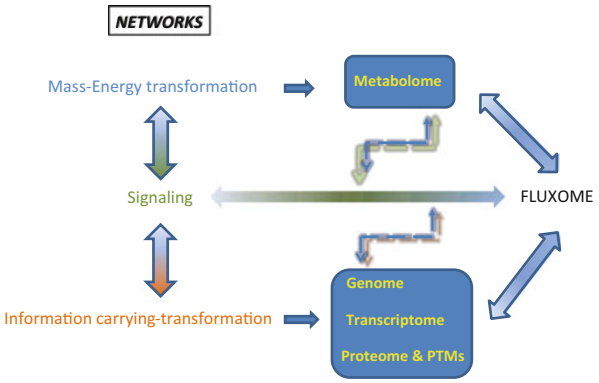

Fig. 2.1 The fluxome and the overall integration of mass–energy/information and signaling networks. Signaling networks connect and modulate the mass–energy–information networks. The fluxome represents the complete ensemble of fluxes in a cell, and as such it provides a true dynamic picture of the phenotype because it captures, in response to the environment, the metabolome (mass–energy) in its functional interactions with the information (genome, transcriptome, proteome, and posttranslational modifications, PTMs) and signaling networks (Cortassa et al. 2012). As a result of this integration between several cellular processes, the fluxome represents a unique phenotypic signature of cells (Cascante and Marin 2008).
The double sense of the arrows denote reciprocal interactions and an overall cyclic topology and connectivity that results in circular causality. Thus, an output from a network (metabolome, e.g., ROS or AMP:ATP ratio) is the input of the next network (signaling, e.g., AMPK network), which after processing will feedback on the same network that produced the initial triggers (e.g., ROS, AMP), thus modulating their levels.

> circuitry is not fixed: it is dynamic rather than static, a structure that is itself changing over the course of the developmental cycle. It is just this dynamic system that I am calling the developmental program." (Fox Keller 2000)
>
> If regulatory state (transcription factors, signaling pathways, etc.) is accepted to control metabolic state, is it not also unconditionally certain that metabolic state will reciprocally control the regulatorystate itself? Understanding this reciprocity, and digging to the bottom of it, is where the future lies (McKnight 2010)

Cell function can be visualized as the outcome resulting from the unfolding in space and time of three different kind of interacting networks: mass–energy, information, and signaling (Fig. 2.1). *Mass–energy transformation networks* comprise metabolic and transport processes, e.g., metabolic pathways and electrochemical gradients, that give rise to the metabolome. *Information-carrying networks* include the genome, transcriptome, and proteome, which account for the whole set of genes, transcripts, proteins, and their posttranslational modifications, respectively. *Signaling networks*, distinct in composition, dynamics, and topology, modulate by activating or repressing the function in space and time of the mass–energy/information networks to which they relate, e.g., metabolome, genome. The overall outcome of this process is the phenotype represented by the fluxome, which accounts for the whole set of fluxes sustained by a diverse range of processes

associated with vital cellular functions such as division, differentiation, autophagy, apoptosis/necrosis, or the response to key environmental signals such as starvation or hypoxia. As such, the fluxome provides a true dynamic picture of the phenotype thereby constituting a unique phenotypic signature of cells (Cascante and Marin 2008) while integrating a myriad of cellular processes. In the mouse, for example, there are only ~600 metabolites (i.e., low-molecular-weight intermediates) (Griffin 2006), when as there are ~10,000 proteins, and ~22,000 protein-encoding genes (Cortassa et al. 2012). Thus, an unique advantage of fluxomics over genomics and proteomics is that the former is based on information from metabolites, which are far fewer than genes or proteins (Gherardini and Helmer-Citterich 2013; Raamsdonk et al. 2001).

The riddle is schematized in Fig. 2.1 and can be summarized as follows. Transcriptional factors, proteins, and metabolites are, at the same time, the products of mass–energy/information networks and their modulators by participating in the signaling networks that activate or repress the same networks that produced them. The presence of these control loops, in which network components are both cause and effect, together with their self-organizing properties sustained by a continuous exchange of energy and matter with the environment, is where the riddle of the unique complexity of the living state lies.

2.1 Signaling Networks: Connecting and Modulating the Mass–Energy-Information Networks

Information (e.g., gene, mRNA, and protein circuits) and signaling (e.g., AMPK, MAPK) networks can be clearly distinguished, by the following differences (Kiel et al. 2010):

- Signaling systems operate rapidly (ms to min) whereas transcriptional responses are slow, ranging from minutes (prokaryotes) to hours (eukaryotes)
- Subcellular localization plays an important role in signaling
- Protein structure and folding are involved in signaling (Mitrea and Kriwacki 2013); these processes are less predictable than DNA conformational changes present in information networks
- Genetic circuits tend to be noisy because they involve fewer molecules compared with signaling pathways, which usually involve larger number of molecular steps and thus tend to be less stochastic
- Amplification cascades occur in signaling thus spontaneous activation is avoided through negative feedback regulation or duplicated triggering signal.

Time-dependent regulation is of utmost importance for cellular responses, resulting from sudden, transient changes in environmental conditions. The earliest cellular response to an external cue usually consists in the activation of upstream

signaling networks, which in turn regulate transcription factors. The modulation of gene expression therefore represents a later event (Gherardini and Helmer-Citterich 2013). The rapid relaxation provided by molecular mechanisms involved in signaling networks is crucial for fast adaptation (Aon and Cortassa 1997; Aon et al. 2004; Lloyd et al. 1982). Signaling networks exhibit their own intrinsic dynamics (Bhalla 2003; Bhalla and Iyengar 1999; Eungdamrong and Iyengar 2004) (see Chap. 4). Several different kinds of dynamic behaviors have been described, among them ultrasensitivity, bistability, hysteresis, and oscillations (Dwivedi and Kemp 2012; Kholodenko et al. 2012). Ultrasensitive behavior arises in protein modification cycles, whereas bistability stems from positive feedback loops, e.g., MAPK cascades, present in signaling cascades that may result in all- or none responses. Positive feedback loops alone or in combination with negative feedbacks can trigger oscillations. Emergent properties such as negative-feedback amplification could be demonstrated in the Raf-MEK-ERK signaling network with negative feedbacks. The "negative-feedback amplifier" confers resistance to perturbations of the amplifier resulting in resistance to inhibitors (e.g., that account for drug resistance) (Kholodenko et al. 2012).

The output of mass–energy networks is the metabolome as constituted by the ensemble of intracellular metabolites, e.g., cAMP, AMP, phosphoinositides, Reactive oxygen species (ROS), or nitrogen (RNS) species (Fig. 2.2). The outcome of information networks is represented by mRNAs, proteins or small peptides, and growth and transcriptional factors. Metabolites such as ROS, cAMP, and ADP exhibit a dual role; on the one hand, they are essential constituents from mass–energy networks that produce them, but on the other hand, they may act as intracellular sensors/messengers with allosteric effects (positive or negative) that react on enzymatic activities present in signaling networks. These dual roles of metabolites compose crucial cellular mechanisms in response to increasing environmental challenges (e.g., oxygen or substrate restriction) or cues (e.g., light, temperature). For example, the alterations of AMP:ATP ratio in response to starvation activates the AMPK signaling pathway, and at the same time AMP functions as an allosteric activator of the AMP kinase within the signaling network thus contributing to modulation of its dynamics. This results in the activation of catabolic and repression of anabolic processes thereby modulating the spatio-temporal unfolding of the mass–energy networks by, e.g., favoring organelle autophagy over biogenesis.

The spatio-temporal dynamics of the fluxome (Fig. 2.2) changes in response to signaling networks, through which cells can modulate, suppress, or activate gene expression (transcription, translation), whole metabolic pathways (e.g., respiration and gluconeogenesis during carbon catabolite repression), or specific enzymatic reactions within them.

Signaling networks are characterized by specific: (1) components and mechanisms; (2) metabolic pathway targeted; (3) conditions for signaling activation; and (4) physiological response (Cortassa et al. 2012). Each one of these characteristics can be identified in the AMP-activated protein kinase (AMPK) signaling pathway.

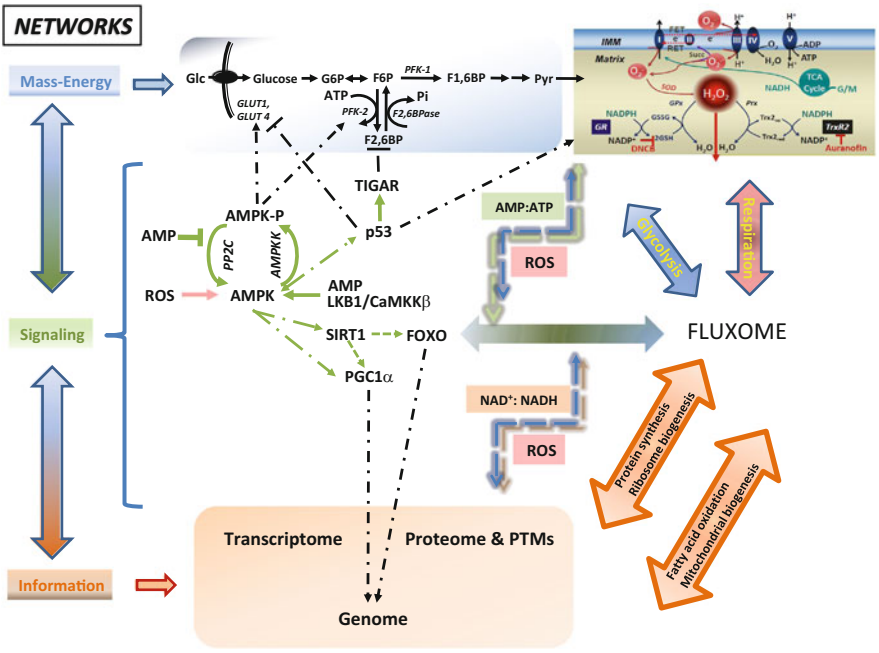

Fig. 2.2 The AMP-activated protein kinase (AMPK) signaling network and interactions with mass–energy and information networks. This figure depicts the main components of the AMPK signaling network and its interactions with the metabolome, genome-transcriptome-proteome, and other signaling paths.

AMPK: this network includes the kinase (AMPKK) and the phosphatase (PP2C), representing an ultrasensitive cellular energy sensor, as it is allosterically modulated by AMP (Hardie and Hawley 2001; Hardie et al. 1999). Environmental stressors such as starvation or hypoxia produce changes in the metabolome (e.g., rising AMP and falling ATP). An increased AMP binds to sites located on the γ subunit of AMPK, whereas high concentrations of ATP are inhibitory. Apart from being an allosteric activator, AMP also inhibits dephosphorylation of AMPK. AMPK is activated 1,000-fold by the combined effect of activation by its upstream kinases, neuronally enriched calcium/calmodulin-dependent protein kinase β (CaMKKβ) or LKB1, together with its allosteric stimulator AMP (Suter et al. 2006).

Targets of the AMPK signaling network are components of the metabolome in glycolysis (PFK-2, F2,6BP, GLUT), oxidative phosphorylation (ROS), and other pathways; the latter are not indicated but may comprise fatty acid (FA) oxidation and anabolism, e.g., triacylglycerol synthesis, glycogen, FAs, protein, and cholesterol. In the feedback from AMPK signaling to the metabolome, the dashed lines indicate activation through phosphorylation by AMPK-P of PFK-2 and glucose transport by increasing the levels of glucose transporters (GLUT1 and GLUT4). The increase in PFK-2 activity augments the level of the allosteric regulator F2,6BP that in turn activates PFK-1; the activity of the latter is also enhanced by the decrease in ATP. Thus, activation of glycolysis under ischemic conditions results in alteration of the fluxome as a result of the concerted action of AMPK signaling and the metabolome.

SIRT1: depicted is the interaction of the AMPK network with SIRT1 and their impact on the acetylation status of PGC-1α and other transcriptional regulators such as the FOXO family of transcription factors. Activation of AMPK in muscle by means of pharmacological intervention (metformin) or physiology (fasting or exercise) triggers an increase in the NAD^+/NADH ratio which activates SIRT1. AMPK induces the phosphorylation of PGC-1α and primes it for subsequent deacetylation by SIRT1(Canto et al. 2009). Deacetylation of PGC-1α is a key mechanism by which AMPK triggers PGC-1α activity in cultured myotubes and in skeletal muscle.

2.2 Reciprocal Interactions Between Signaling and Mass–Energy/Information Networks: Two Case Studies

2.2.1 The AMPK Signaling Network

This evolutionarily conserved signaling network functions as a cellular switch that activates catabolic pathways and turns off anabolic processes thereby restoring cellular energy levels (Poels et al. 2009). In physiological situations, AMPK senses energy deficiency (in the form of an increased AMP/ATP ratio), but it is also activated by metabolic stress such as glucose or oxygen deprivation triggering transient behavior regulation (Fig. 2.2). It has recently been shown that mitochondria-generated ROS induces autophagy mediated by the AMPK pathway under starvation conditions (Li et al. 2013). The decline in the responsiveness of AMPK signaling toward cellular stress with aging impairs metabolic regulation, increases oxidative stress, and reduces autophagic clearance (Salminen and Kaarniranta 2012).

The AMPK signaling network is paradigmatic, because the molecular components and mechanisms involved (i.e., kinetic properties of AMPK toward main effectors), demonstrating physiological impact as well as conditions in which the signaling operates, are all well understood and thus clearly identifiable (Fig. 2.2). As a specific example of the AMPK signaling network function in the context of ischemia in the heart: (1) components: AMPK allosterically modulated by AMP and phosphorylation (Hardie and Hawley 2001); (2) targets (changes in the metabolome): 6-phosphofructo-2-kinase (PFK-2) activity, fructose 2,6-bisphosphate (F2,6BP)

Fig. 2.2 (continued) SIRT1 appears to be a mediator of AMPK action on PGC-1α transcriptional activity. The acute actions of AMPK on lipid oxidation (fluxome) alter the balance between cellular NAD^+:NADH (metabolome), which acts as a messenger to activate SIRT1 (signaling), and the latter closes the circle by acting on the genome (information) which then again modifies the fluxome (mitochondrial respiration, lipid oxidation).

*Tumor suppressor protein P53:*a transcription factor that acts in response to cellular stress signals (e.g., DNA damage, hypoxia, oxidative, and nitrosative stress) and is redox sensitive because of the presence of conserved Cys residues that contain redox-sensitive thiol groups (see Fig. 2.3) (Vurusaner et al. 2012). P53 is also able to inhibit the nutrient-sensitive kinase target of rapamycin complex 1, mTORC1, by activation of AMPK, which is subsequently followed by induction of autophagy (Li et al. 2013; Melnik 2012; Poels et al. 2009). The interaction between p53 and TOR plays an important role in normal cell growth and proliferation (Jones et al. 2005), and it is likely that the AMPK-dependent induction of autophagy by p53 contributes to its role in tumor suppression (Poels et al. 2009).

Abbreviations: *AMPK* AMP-activated protein kinase, *CaMKKβ* calcium/calmodulin-dependent protein kinase kinase β, *p53* tumor suppression protein, *TOR* target of rapamycin, *PGC-1α* peroxisome proliferator-activated receptor-γ coactivator 1α, *PTMs* posttranslational modifications

concentration, and glucose transporters (GLUT1 and GLUT4) levels and translocation; (3) conditions for signaling activation: any stress that interferes with ATP synthesis and readily affects the AMP:ATP ratio, e.g., interruption of blood supply (ischemia); (4) physiological response (changes in the fluxome): activation of glycolysis that increases ATP availability (Marsin et al. 2000) [see also Chap. 11 in this book, Chap. 10 in Cortassa et al. (2012) and Fig. 2.2 for further explanation].

2.2.2 ROS-Signaling Networks

Redox signaling can be exemplified by the regulation of protein activity and the transduction of signals to downstream proteins through oxidative modification of reactive cysteine (Cys) residues by ROS (Finkel 2000; Paulsen and Carroll 2010). Cellular functions can be signaled by ROS in essentially two ways (Fig. 2.3): (1) through direct oxidation of specific Cys or (2) indirectly through changes in the activity of kinases or phosphatases that in turn modulate protein phosphorylation. The switch-like nature of the sulfenic acid (SOH) and disulfides that are formed after the initial reaction of a Cys thiolate with H_2O_2 and by reaction of SOH with neighboring Cys or reduced glutathione (GSH), explains their potential to function as reversible modifications that regulate protein function, analogous to phosphorylation (Haddad 2004). For example, myofilament activation and contractile function may be altered during oxidative stress by direct oxidative modifications of specific sites on contractile proteins or by ROS-induced changes in the activity of kinases or phosphatases that regulate sarcomeric protein phosphorylation (Santos et al. 2011; Sumandea and Steinberg 2011).

Another relevant example is given by the tumor suppressor protein p53, a transcriptional factor that in response to environmental challenge (e.g., hypoxia, oxidative stress) can sense cellular redox status. When p53 is oxidized by ROS its DNA binding capacity is decreased (Sun et al. 2003). Thus, under stressful conditions, ROS from the metabolome oxidizes p53: the latter when oxidized decreases its DNA binding capacity (Sun et al. 2003) thus inhibiting gene expression (genome) (Fig. 2.3). In turn, p53 can influence the metabolome through decreasing F2,6BP and glucose transporter levels that affect the fluxome by diminishing glycolysis and stimulating mitochondrial respiration (Fig. 2.2) (Lago et al. 2011). p53 is also able to interact with the AMPK signaling network inducing its activation after inhibition of the nutrient-sensitive kinase mTORC1: these effects are followed by induction of autophagy (Fig. 2.2).

2.2.3 Sensing H_2O_2 through Cysteine Oxidation

Cells can "sense" changes in redox balance through the specific reactions of H_2O_2 (D'Autreaux and Toledano 2007; Paulsen and Carroll 2010; Pourova et al. 2010; Schroder and Eaton 2009). In proteins, the thiol side chain of the amino acid Cys is

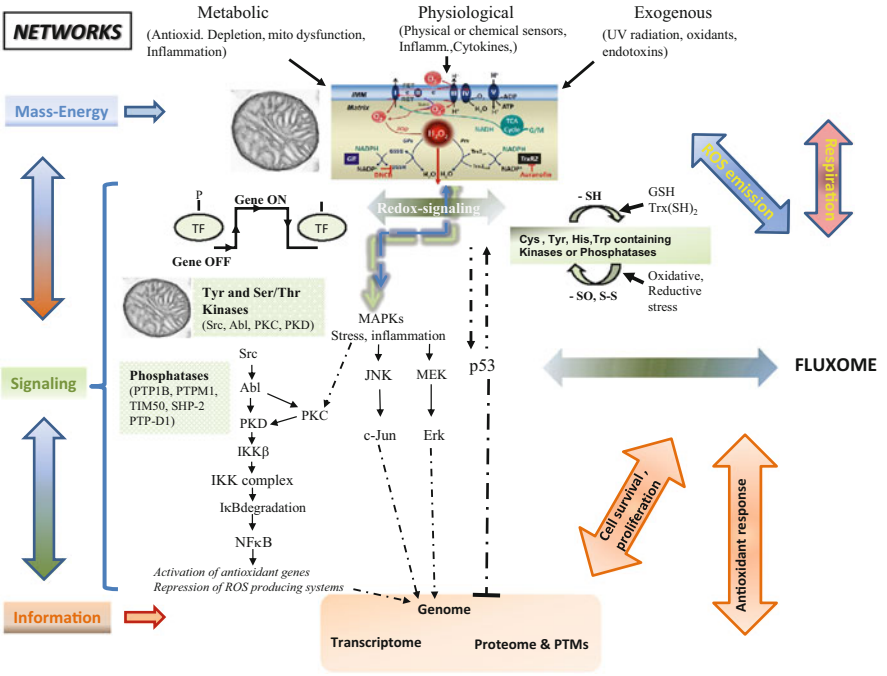

Fig. 2.3 ROS-activated signaling networks and interactions with mass–energy and information networks. Reactive oxygen species (ROS) can signal cellular functions through direct oxidation of specific Cys or indirectly through changes in the activity of kinases or phosphatases that in turn modulate protein phosphorylation. The redox-sensitive family of components from signaling networks use Cys motifs as redox-sensitive sulphydryl (-SH) switches (Paulsen and Carroll 2010). ROS-activated signaling networks comprise two distinct protein families—the Mitogen Activated Protein Kinase (MAPK) and the redox-sensitive kinases (Waris and Ahsan 2006).

MAPKs: serine (Ser)/threonine (Thr) kinases that transduce signals from the cell membrane to the nucleus in response to a wide range of stimuli, and modulate gene expression through phosphorylation of a wide array of transcription factors. MAPKs consist of three family members: the extracellular signal-regulated kinase (ERK); the c-Jun NH2-terminal kinase (JNK); and the p38 MAPK (Wada and Penninger 2004). MAPKs regulate processes important in carcinogenesis including proliferation, differentiation, and apoptosis (Waris and Ahsan 2006).

NFκB: the modification of gene expression by ROS has direct effects on cell proliferation and apoptosis through the activation of transcription factors including MAPK and NFκB pathways. The NFkB signaling network is significantly altered by dysregulated ROS that activates NFkB signaling through elimination of the IkB inhibitor. An increase in ROS levels induces the activation of the IkB kinase (IKK), which in turn phosphorylates IkB, leading to its proteasome-dependent degradation (Maryanovich and Gross 2012),while releasing NFκB for nuclear translocation and gene transcription (Chiu and Dawes 2012). NF-κB modulates the expression of several genes involved in cell transformation, proliferation, and angiogenesis, including bcl-2, bcl-x*L*, SOD (Chiu and Dawes 2012; Waris and Ahsan 2006). The NFκB link to carcinogenesis derives from of its roles in inflammation, differentiation, and cell growth (Rahman et al. 2006). Carcinogens and tumor promoters (e.g., UV radiation, phorbol esters, asbestos, alcohol) activate NF-κB.

Src and Abl: tyrosine kinases that constitute the signaling pathway leading to H_2O_2-mediated tyrosine phosphorylation of PKD that enhances NFkB activation (Storz and Toker 2003).

Protein tyrosine phosphatases (PTPs): regulated by H_2O_2 through induction of intramolecular disulfide bond formation that inactivates PTPs (Paulsen and Carroll 2010). The activity of the PTP

particularly sensitive to oxidation. Thiolate anions (RS^-) are intrinsically better nucleophiles and show enhanced reactivity with H_2O_2, compared to the thiol form (Winterbourn and Metodiewa 1999). Thus, the p*K*a value of the thiol group can modulate Cys reactivity. Other determinants of Cys reactivity toward H_2O_2 include access of the oxidant to its target and the presence of specific binding sites, e.g., low pKa catalytic Cys from peroxiredoxins or protein tyrosine phosphatases that react with $H_2O_2$with different second-order rate constants likely due to the unique conformation of their active site (Paulsen and Carroll 2010).

Thus, the initial reaction of a Cys thiolate with H_2O_2 yields a SOH, which once formed can lead to formation of additional posttranslational modifications (PTMs). The stability of a SOH is influenced, in part, by the presence of nearby Cys residues and by the accessibility of the modification site to GSH (Paulsen and Carroll 2010). The reaction of SOH with either a neighboring cysteine or GSH will generate a disulfide bond that, in the case of GSH, corresponds to *S*-glutathiolation (Mieyal et al. 2008). Both disulfide products can be reduced back to the thiol by the action of either the GSH/glutathione reductase or the thioredoxin/thioredoxin reductase systems (Aon et al. 2012a; Berndt et al. 2007; Ghezzi and Di Simplicio 2009; Stanley et al. 2011).

SOH can undergo further reaction with H_2O_2 to generate the SO_2H (sulfinic) and SO_3H (sulfonic) oxoforms, though the rates of these reactions are slower than those observed for a thiolate (Hugo et al. 2009). Both the SO_2H and SO_3H modifications are considered irreversible, and the latter is deemed a hallmark of diseases such as cancer, diabetes, cardiovascular, and neurodegenerative disorders that are associated with oxidative stress (Aggarwal and Makielski 2013; Andersen 2004; Jeong et al. 2012; Kembro et al. 2013; Klaunig and Kamendulis 2004; Leloup et al. 2011; Lowell and Shulman 2005). In a subset of eukaryotic peroxiredoxins, the SO_2H modification can be reversed by sulfiredoxin (Biteau et al. 2003). To prevent over oxidation of critical Cys residues, SOH may be converted to a disulfide or be *S*-glutathiolated or form sulfenamide and hypervalent sulfur species (Paulsen and Carroll 2010).

Fig. 2.3 (continued) family (e.g., PTP1B, PTPα) of phosphatases can be SOH-regulated, which is facilitated by the low p*K*a of catalytic Cys that can oxidize to SOH with concomitant inactivation. Protein kinases also undergo redox control.
Tumor suppressor p53: its gene is mutated in 30–50 % of human cancers, representing a checkpoint protein that elicits cell cycle arrest, DNA repair, and apoptosis in response to stressors (Sun et al. 2003). To perform its tumor suppressor activity p53 binds, as a tetramer, to DNA elements within promoters of its target genes and enhances transcription. P53 is sensitive to redox signaling: oxidation of Cys residues (some of the ten present in p53), and formation of disulfide bonds inhibits p53 tetramerization and DNA binding activity (Lago et al. 2011; Sun et al. 2003). P53 stimulates mitochondrial respiration and decreases glycolysis by affecting F2,6BP and the plasma membrane glucose transporters (Lago et al. 2011).
Abbreviations: *NFκB* nuclear factor κB, *MAPK* mitogen-activated protein kinase, *PKD* protein kinase D, *PKC* protein kinase C, *Src, Abl* tyrosine kinases, *IKK* IkB kinase, *JNK* c-Jun N-terminal kinase, *MAP kinase* mitogen-activated protein kinase, *β-MHC* b-myosin heavy chain

We should also keep in mind that, under oxidative stress, Cys thiols that are not redox sensors can also become oxidized. Thus, it is important to differentiate true Cys redox sensors that participate in redox signaling from other Cys that become oxidized but without biological consequences (Chiu and Dawes 2012).

2.3 Complex Systems Biology of Networks

The intricate networks of reactions and processes within living systems (Figs. 2.1, 2.2, and 2.3) exhibit complex dynamic behavior (Lloyd and Lloyd 1993, 1995; Lloyd and Stupfel 1991) (see Chaps. 12, 8 and 5). This complexity arises in part from the existence of multiple topological, structural, as well as functional interactions among components of these networks organized as molecular (e.g., enzymes), supra-molecular (e.g., cytoskeleton, respiratory, or enzymatic supercomplex), and organellar assemblies (e.g., in mitochondria) (see Chaps. 7, 8 and 11). Consequently, a full description of a biological system involves the *structure*, the *pattern of organization*, and the *function* (Capra 1996; Kitano 2002). *Structure* refers to the catalog of individual components (e.g., proteins, genes, enzymes, transcriptional factors); *pattern of organization* indicates how the components are wired (linked) and organized (e.g., topological relationships, morphology, feed-forward, and feed-back), and *function* implies how the ensemble works, i.e., unfolding in space and time of functional interrelationships, mass–energy-information fluxes, response to stimuli, growth, division (Figs. 2.2 and 2.3).

The collective dynamic function of networks is characterized by novel properties that cannot be anticipated from the behavior of network components in isolation. These novel properties are called *emergent*. As a fundamental trait of complexity, emergence is a manifestation of the interdependent function of processes within cells, organs, organisms (see Chap. 10). Ultimately, what we seek is to understand how function is coordinated in a cell that exhibits spatially distributed and compartmentalized subsystems, and the dynamics which unfolds simultaneously, although in sequentially consecutive temporal scales. Consequently, in the following, we attempt to dissect key organizational and functional traits of living cellular systems.

- *Function occurs in spatially distributed, compartmentalized, systems in which process dynamics unfolds in different successive but overlapping temporal scales*

  Processes of different nature occur in distinct compartments connected by transport mechanisms, temporally unfolding on different timescales from few milliseconds (electric), hundreds of milliseconds (mechanical) to few seconds (energetic) (see Chaps. 11 and 5).

- *Network organization*

  Mass–energy/information/signaling networks exhibit an overall loop topology. They comprise reaction and transport processes, and some nodes in these networks represent hubs since they exhibit multiple inputs and outputs while most nodes only possess a few of them. This feature confers these networks the trait of "scale-free." The topologically circular connectivity present in these networks bestows them with self-making and -maintaining properties that combined with continuous energy and matter exchanges allow them to self-organize in space and time (Aon and Cortassa 2009; Aon et al. 2012b). Although constructed with a high degree of redundancy that confers these networks resilience to attack (Barabasi 2009), under stress they may reach critical conditions that make them collapse, especially if hubs fail (see Chap. 5).
- *Top—down and bottom—up interrelationships (heterarchies)*

  Cells, tissues, and organs can be viewed as networks within networks. One of the most distinctive features of these networks is that all components interact one way or another, constituting a heterarchy (Aon and Cortassa 2012; Aon et al. 2012b; Lloyd and Rossi 2008; Yates 1993). In a heterarchy, but unlike in a strict hierarchy, interactions between network components and relevant functional interrelationships (including regulatory ones) flow between levels in both directions, top-down and bottom-up. This has important consequences for control and regulation of integrated metabolic and transport networks where every reaction, metabolite, ion, and process, may contribute, although to differing extents, to the overall control and regulation of the network (Aon and Cortassa 2012; Cortassa et al. 2012).
- *Control is distributed, and operates through "diffuse loops"*

  Systemic analysis of extensive networks as given by Metabolic Control Analysis shows that every process (edge, e.g., enzymatic reaction, channel) controls and is controlled by every other process in the network. However, the strength of control exerted by different processes may vary significantly, a trait that relates the fact that control is distributed (see Chaps. 3, 9 and 13). In the case of nodes (e.g., metabolites, second messengers, cofactors), every node can regulate other processes and in turn can be controlled (e.g., its concentration) by a process. The character of "distributed control" relates the fact that different processes (edges) exert control, and can be "diffuse" as well as direct. A diffuse control was first described in an integrated computational model of the cardiomyocyte and corresponds to the control exerted by seemingly indirectly related processes through shared ubiquitous cofactors such as ATP, ADP, and Ca^{2+} (Cortassa et al. 2009a, b).

  In networks involving various compartments, not all the control of the flux, e.g., in an organelle, resides within the organelle itself. In the heart, the control of mitochondrial respiration is exerted by cytoplasmic and sarcolemmal membrane-linked processes, e.g., the myofibrillar and Na/K ATPases, in addition to processes residing in the mitochondrion (Aon and Cortassa 2012). This is especially true under working conditions, when the interaction between cytoplasmic and mitochondrial processes is quantitatively more important.

- *Scaling—Fractal dynamics*

  Scaling tackles the question of functional coordination in a living cell that exhibits spatially distributed and compartmentalized subsystems with time constants in sequentially consecutive and overlapping temporal scales. Scaling involves both spatial and temporal levels of organization and reveals the interdependence between processes happening at different spatio-temporal coordinates (Aon and Cortassa 2009; Aon et al. 2012b; Lloyd et al. 2012). Genome-wide expression (transcriptome, ~5300 transcripts) during the time frame period provided by the ~40 min ultradian clock revealed the existence of two blocks of redox superclusters manifested in two phases of ~600 and ~4,700 maximally expressed genes during active respiration (oxidative) and low respiration (reductive), respectively (Klevecz et al. 2004; Lloyd and Murray 2005) (see also Chap. 12). Within the 40 min time frame of the clock, there is a 10–15 min period conducive to DNA synthesis and replication, a time window that opens during the reductive phase of the clock cycle: this suggests an evolutionary strategy to avoid oxidative damage.

  A bottom up modeling strategy provides an insight into how scaling arises, and what it reveals. For the sake of example, during a heartbeat, macroscopic and measurable properties of the cardiac cell such as action potentials, cell shortening-relaxation, and concomitant Ca^{2+} transients emerge from the integrated dynamic behavior of excitation–contraction and mitochondrial energetics (Aon and Cortassa 2012; Aon et al. 2012b). Underlying key electromechanical macroscopic functional properties, fast ionic currents operating in the few milliseconds range are revealed. These, in turn, are fueled by relatively slower (few seconds) mitochondrial energetic processes involving rapid transport processes in different subcellular compartments: sarcolemma, mitochondria, sarcoplasmic reticulum (see Chaps 5 and 10). The processes involved in the phenomenon of a heartbeat are simultaneous, and their apparent sequential nature results from the differential relaxation properties exhibited by the processes involved. Thus, the scale-free dynamic behavior exhibited by mitochondrial network energetic-redox function is based on the simultaneous operation of processes of different nature (electrical, mechanical, metabolic) in distinct compartments. Faster to slower temporal relaxation reflects the time it takes a process to return to the state previous to the stimulation that elicited the response, e.g., the initial potential depolarization triggered by the opening of Na channels in the sarcolemma.

  The inverse power law behavior of the power spectrum and the invariant relative dispersion across temporal scales obtained from the analysis of experimentally obtained time series in yeast and cardiac mitochondria support the existence of scale-free dynamics. The multi-oscillatory behavior of yeast and heart cells corresponds to statistical fractal dynamics, a behavior consistent with scale-free dynamics spanning a wide range of frequencies of at least three orders of magnitude (Aon et al. 2007, 2008; Lloyd et al. 2012). Scale-free temporal organization for organelle, cell, and organism implies timekeeping occurring across temporal scales in living systems (Aon et al. 2008; Sasidharan et al. 2012) (see also Chap. 12).

2.4 Concluding Remarks

The fundamental complexity and uniqueness of living systems resides in their capacity for self-making and -repairing (Luisi 2006; Varela et al. 1974). This distinctive trait is possible to be accomplished through closed loop topologies of nonlinearly interrelated processes operating in thermodynamically open systems thereby subjected to continuous energy dissipation and exchange of matter, e.g., substrates, and gases.

Another consequence of the self-making ability of living systems is that some network components (nodes, e.g., metabolites like AMP or TFs such as NFκB) can be both cause and effect at the same time (or output and input) for the same network, i.e., mass–energy and information, respectively, in these examples. A plethora of computational and experimental network-based methods are being developed and applied to different biological systems including complex diseases (Cho et al. 2012; Kholodenko et al. 2012; Neph et al. 2012; Przytycka and Cho 2012). It is worth remarking that the data and meaningful information that these approaches can provide are just the starting point for testable hypotheses.

The dynamic diversity arising from the interactions between spatially distributed mass–energy/information/signaling networks organized in circularly connected topologies has potentially explosive combinatorial possibilities. The modulation exerted by signaling networks on the spatio-temporal unfolding of mass–energy/information networks, together with the countless available dynamic paths emerging from these interactions, generates both the uniqueness and diversity of living creatures. Interestingly, recent findings have highlighted the marked cell type specificity of human transcriptional regulatory networks, with only ~5 % of overlap across 41 tested cell types, thereby underscoring the high regulatory diversity within humans (Neph et al. 2012).

Acknowledgments This work was performed with the financial support of R01-HL091923 from NIH.

References

Aggarwal NT, Makielski JC (2013) Redox control of cardiac excitability. Antioxid Redox Signal 18:432–68

Andersen JK (2004) Oxidative stress in neurodegeneration: cause or consequence? Nat Med 10 (Suppl):S18–25

Aon MA, Cortassa S (1997) Dynamic biological organization. Fundamentals as applied to cellular systems. Chapman & Hall, London

Aon MA, Cortassa S (2009) Chaotic dynamics, noise and fractal space in biochemistry. In: Meyers R (ed) Encyclopedia of complexity and systems science. Springer, New York

Aon MA, Cortassa S (2012) Mitochondrial network energetics in the heart. Wiley Interdiscip Rev Syst Biol Med 4:599–613

Aon MA, Cortassa S, Lloyd D (2012a) Chaos in biochemistry and physiology. In: Meyers R (ed) Encyclopedia of molecular cell biology and molecular medicine: systems biology. Weinheim, Wiley-VCH

Aon MA, Cortassa S, O'Rourke B (2007) On the network properties of mitochondria. In: Saks V (ed) Molecular system bioenergetics: energy for life. Wiley-VCH, Weinheim, pp 111–35

Aon MA, O'Rourke B, Cortassa S (2004) The fractal architecture of cytoplasmic organization: scaling, kinetics and emergence in metabolic networks. Mol Cell Biochem 256–257:169–84

Aon MA, Roussel MR, Cortassa S, O'Rourke B, Murray DB, Beckmann M, Lloyd D (2008) The scale-free dynamics of eukaryotic cells. PLoS One 3:e3624

Aon MA, Stanley BA, Sivakumaran V, Kembro JM, O'Rourke B, Paolocci N, Cortassa S (2012b) Glutathione/thioredoxin systems modulate mitochondrial H_2O_2 emission: an experimental-computational study. J Gen Physiol 139:479–91

Barabasi AL (2009) Scale-free networks: a decade and beyond. Science 325:412–3

Berndt C, Lillig CH, Holmgren A (2007) Thiol-based mechanisms of the thioredoxin and glutaredoxin systems: implications for diseases in the cardiovascular system. Am J Physiol Heart Circ Physiol 292:H1227–36

Bhalla US (2003) Understanding complex signaling networks through models and metaphors. Prog Biophys Mol Biol 81:45–65

Bhalla US, Iyengar R (1999) Emergent properties of networks of biological signaling pathways. Science 283:381–7

Biteau B, Labarre J, Toledano MB (2003) ATP-dependent reduction of cysteine-sulphinic acid by S. cerevisiae sulphiredoxin. Nature 425:980–4

Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J (2009) AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 458:1056–60

Capra F (1996) The web of life. Anchor books Doubleday, New York

Cascante M, Marin S (2008) Metabolomics and fluxomics approaches. Essays Biochem 45:67–81

Chiu J, Dawes IW (2012) Redox control of cell proliferation. Trends Cell Biol 22:592–601

Cho DY, Kim YA, Przytycka TM (2012) Chapter 5: Network biology approach to complex diseases. PLoS Comput Biol 8:e1002820

Cortassa S, Aon MA, Iglesias AA, Aon JC, Lloyd D (2012) An introduction to Metabolic and Cellular Engineering, 2nd edn. World Scientific, Singapore

Cortassa S, O'Rourke B, Winslow RL, Aon MA (2009a) Control and regulation of mitochondrial energetics in an integrated model of cardiomyocyte function. Biophys J 96:2466–78

Cortassa S, O'Rourke B, Winslow RL, Aon MA (2009b) Control and regulation of integrated mitochondrial function in metabolic and transport networks. Int J Mol Sci 10:1500–13

D'Autreaux B, Toledano MB (2007) ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Nat Rev Mol Cell Biol 8:813–24

Dwivedi G, Kemp ML (2012) Systemic redox regulation of cellular information processing. Antioxid Redox Signal 16:374–80

Eungdamrong NJ, Iyengar R (2004) Computational approaches for modeling regulatory cellular networks. Trends Cell Biol 14:661–9

Finkel T (2000) Redox-dependent signal transduction. FEBS Lett 476:52–4

Fox KE (2000) The century of the gene. Harvard University Press, Cambridge, MA

Gherardini PF, Helmer-Citterich M (2013) Experimental and computational methods for the analysis and modeling of signaling networks. N Biotechnol 30:327–32

Ghezzi P, Di Simplicio P (2009) Protein glutathiolation. In: Jacob C, Winyard PG (eds) Redox signaling and regulation in biology and medicine. Weinheim, WILEY-VCH Verlag Gmbh & Co. KGaA, pp 123–41

Griffin JL (2006) The Cinderella story of metabolic profiling: does metabolomics get to go to the functional genomics ball? Philos Trans R Soc Lond B Biol Sci 361:147–61

Haddad JJ (2004) Oxygen sensing and oxidant/redox-related pathways. Biochem Biophys Res Commun 316:969–77

Hardie DG, Hawley SA (2001) AMP-activated protein kinase: the energy charge hypothesis revisited. Bioessays 23:1112–9

Hardie DG, Salt IP, Hawley SA, Davies SP (1999) AMP-activated protein kinase: an ultrasensitive system for monitoring cellular energy charge. Biochem J 338(Pt 3):717–22

Hugo M, Turell L, Manta B, Botti H, Monteiro G, Netto LE, Alvarez B, Radi R, Trujillo M (2009) Thiol and sulfenic acid oxidation of AhpE, the one-cysteine peroxiredoxin from Mycobacterium tuberculosis: kinetics, acidity constants, and conformational dynamics. Biochemistry 48:9416–26

Jeong EM, Liu M, Sturdy M, Gao G, Varghese ST, Sovari AA, Dudley SC Jr (2012) Metabolic stress, reactive oxygen species, and arrhythmia. J Mol Cell Cardiol 52:454–63

Jones RG, Plas DR, Kubek S, Buzzai M, Mu J, Xu Y, Birnbaum MJ, Thompson CB (2005) AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Mol Cell 18:283–93

Kembro JM, Cortassa S, Aon MA (2013) Mitochondrial ROS and arrhythmias. In: Laher I (ed) Systems biology of free radicals and anti-oxidants. Springer, Heidelberg

Kholodenko B, Yaffe MB, Kolch W (2012) Computational approaches for analyzing information flow in biological networks. Sci Signal 5:re1

Kiel C, Yus E, Serrano L (2010) Engineering signal transduction pathways. Cell 140:33–47

Kitano H (2002) Systems biology: a brief overview. Science 295:1662–4

Klaunig JE, Kamendulis LM (2004) The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol 44:239–67

Klevecz RR, Bolen J, Forrest G, Murray DB (2004) A genomewide oscillation in transcription gates DNA replication and cell cycle. Proc Natl Acad Sci USA 101:1200–5

Lago CU, Sung HJ, Ma W, Wang PY, Hwang PM (2011) p53, aerobic metabolism, and cancer. Antioxid Redox Signal 15:1739–48

Leloup C, Casteilla L, Carriere A, Galinier A, Benani A, Carneiro L, Penicaud L (2011) Balancing mitochondrial redox signaling: a key point in metabolic regulation. Antioxid Redox Signal 14:519–30

Li L, Chen Y, Gibson SB (2013) Starvation-induced autophagy is regulated by mitochondrial reactive oxygen species leading to AMPK activation. Cell Signal 25:50–65

Lloyd AL, Lloyd D (1993) Hypothesis: the central oscillator of the circadian clock is a controlled chaotic attractor. Biosystems 29:77–85

Lloyd AL, Lloyd D (1995) Chaos—Its significance and detection in biology. Biol Rhythm Res 26:233–52

Lloyd D, Cortassa S, O'Rourke B, Aon MA (2012) What yeast and cardiomyocytes share: ultradian oscillatory redox mechanisms of cellular coherence and survival. Integr Biol (Camb) 4:65–74

Lloyd D, Murray DB (2005) Ultradian metronome: timekeeper for orchestration of cellular coherence. Trends Biochem Sci 30:373–7

Lloyd D, Poole RK, Edwards SW (1982) The cell division cycle: temporal organisation and control of growth and reproduction. Academic, London, p 523

Lloyd D, Rossi EL (2008) Epilogue: a new vision of life. In: Lloyd D, Rossi EL (eds) Ultradian rhythms from molecules to mind. Springer Science+Business Media B.V., New York, pp 431–39

Lloyd D, Stupfel M (1991) The occurrence and functions of ultradian rhythms. Biol Rev Camb Philos Soc 66:275–99

Lowell BB, Shulman GI (2005) Mitochondrial dysfunction and type 2 diabetes. Science 307:384–7

Luisi PL (2006) The emergence of life. From chemical origins to synthetic biology. Cambridge University Press, Cambridge, UK

Marsin AS, Bertrand L, Rider MH, Deprez J, Beauloye C, Vincent MF, Van den Berghe G, Carling D, Hue L (2000) Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia. Curr Biol 10:1247–55

Maryanovich M, Gross A (2012) A ROS rheostat for cell fate regulation. Trends Cell Biol 23:129–34
McKnight SL (2010) On getting there from here. Science 330:1338–9
Melnik BC (2012) Leucine signaling in the pathogenesis of type 2 diabetes and obesity. World J Diabetes 3:38–53
Mieyal JJ, Gallogly MM, Qanungo S, Sabens EA, Shelton MD (2008) Molecular mechanisms and clinical implications of reversible protein S-glutathionylation. Antioxid Redox Signal 10:1941–88
Mitrea DM, Kriwacki RW (2013) Regulated unfolding of proteins in signaling. FEBS Lett 587:1081–8
Neph S, Stergachis AB, Reynolds A, Sandstrom R, Borenstein E, Stamatoyannopoulos JA (2012) Circuitry and dynamics of human transcription factor regulatory networks. Cell 150:1274–86
Paulsen CE, Carroll KS (2010) Orchestrating redox signaling networks through regulatory cysteine switches. ACS Chem Biol 5:47–62
Poels J, Spasic MR, Callaerts P, Norga KK (2009) Expanding roles for AMP-activated protein kinase in neuronal survival and autophagy. Bioessays 31:944–52
Pourova J, Kottova M, Voprsalova M, Pour M (2010) Reactive oxygen and nitrogen species in normal physiological processes. Acta Physiol (Oxf) 198:15–35
Przytycka TM, Cho DY (2012) Interactome. In: Meyers RA (ed) Systems biology: advances in molecular biology and medicine. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 85–115
Raamsdonk LM, Teusink B, Broadhurst D, Zhang N, Hayes A, Walsh MC, Berden JA, Brindle KM, Kell DB, Rowland JJ, Westerhoff HV, van Dam K, Oliver SG (2001) A functional genomics strategy that uses metabolome data to reveal the phenotype of silent mutations. Nat Biotechnol 19:45–50
Rahman I, Yang SR, Biswas SK (2006) Current concepts of redox signaling in the lungs. Antioxid Redox Signal 8:681–9
Salminen A, Kaarniranta K (2012) AMP-activated protein kinase (AMPK) controls the aging process via an integrated signaling network. Ageing Res Rev 11:230–41
Santos CX, Anilkumar N, Zhang M, Brewer AC, Shah AM (2011) Redox signaling in cardiac myocytes. Free Radic Biol Med 50:777–93
Sasidharan K, Tomita M, Aon M, Lloyd D, Murray DB (2012) Time-structure of the yeast metabolism in vivo. Adv Exp Med Biol 736:359–79
Schroder E, Eaton P (2009) Hydrogen peroxide and cysteine protein signaling pathways. In: Jacob C, Winyard PG (eds) Redox signaling and regulation in biology and medicine. Wiley-VCH Verlag Gmbh & Co. KGaA, Weinheim, pp 181–195
Stanley BA, Sivakumaran V, Shi S, McDonald I, Lloyd D, Watson WH, Aon MA, Paolocci N (2011) Thioredoxin reductase-2 is essential for keeping low levels of H(2)O(2) emission from isolated heart mitochondria. J Biol Chem 286:33669–77
Storz P, Toker A (2003) Protein kinase D mediates a stress-induced NF-kappaB activation and survival pathway. EMBO J 22:109–20
Sumandea MP, Steinberg SF (2011) Redox signaling and cardiac sarcomeres. J Biol Chem 286:9921–7
Sun XZ, Vinci C, Makmura L, Han S, Tran D, Nguyen J, Hamann M, Grazziani S, Sheppard S, Gutova M, Zhou F, Thomas J, Momand J (2003) Formation of disulfide bond in p53 correlates with inhibition of DNA binding and tetramerization. Antioxid Redox Signal 5:655–65
Suter M, Riek U, Tuerk R, Schlattner U, Wallimann T, Neumann D (2006) Dissecting the role of 5′-AMP for allosteric stimulation, activation, and deactivation of AMP-activated protein kinase. J Biol Chem 281:32207–16
Varela F, Maturana H, Uribe R (1974) Autopoiesis: the organization of living systems, its characterization and a model. Biosystems 5:187–96
Vurusaner B, Poli G, Basaga H (2012) Tumor suppressor genes and ROS: complex networks of interactions. Free Radic Biol Med 52:7–18

Wada T, Penninger JM (2004) Mitogen-activated protein kinases in apoptosis regulation. Oncogene 23:2838–49

Waris G, Ahsan H (2006) Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinog 5:14

Winterbourn CC, Metodiewa D (1999) Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide. Free Radic Biol Med 27:322–8

Yates FE (1993) Self-organizing systems. In: Boyd CAR, Noble D (eds) The logic of life. The challenge of integrative physiology. Oxford University Press, New York, pp 189–218

http://www.springer.com/978-3-642-38504-9

Systems Biology of Metabolic and Signaling Networks
Energy, Mass and Information Transfer
Aon, M.A.; Saks, V.; Schlattner, U. (Eds.)
2014, X, 375 p. 90 illus., 67 illus. in color., Hardcover
ISBN: 978-3-642-38504-9