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Review

Hallmarks of Health

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SUMMARY

Health is usually defined as the absence of pathology. Here, we endeavor to define health as a compendium of organizational and dynamic features that maintain physiology. The biological causes or hallmarks of health include features of spatial compartmentalization (integrity of barriers and containment of local perturbations), maintenance of homeostasis over time (recycling and turnover, integration of circuitries, and rhythmic oscillations), and an array of adequate responses to stress (homeostatic resilience, hormetic regulation, and repair and regeneration). Disruption of any of these interlocked features is broadly pathogenic, causing an acute or progressive derailment of the system coupled to the loss of numerous stigmata of health.

INTRODUCTION

Pathology and pathophysiology usually focus on the identification of etiological agents and the elucidation of pathogenic mechanisms that accompany the transition from health to disease, whereas biotechnology and pharmacology seek remedies to delay or reverse this transition. Hence, in biomedical research, health is commonly defined in a negative fashion as the absence of disease (Conti, 2018). Given the overwhelming multiplicity of disease-inducing conditions and pathways, this negative definition of health as the nonexistence of any kind of pathology is impractical (Ayres, 2020). An alternative (and descriptive) definition of health might contemplate signs of physical and mental fitness coupled to normal function of all organs measurable by specific medical exams. Here, we will endeavor to define health in positive terms, while enumerating its underlying biological causes or "hallmarks" in a didactic fashion.

In the past, *Cell* published landmark papers describing the hallmarks of cancer and aging, summarizing the properties of malignant cells (Hanahan and Weinberg, 2000) and their interactions with their non-malignant environment (Hanahan and Weinberg, 2011), as well as the molecular and cellular pathways that explain the time-dependent deterioration of living organisms (López-Otín et al., 2013). When attempting to identify and categorize the molecular and cellular hallmarks of health, we came to the conclusion that they are not simply opposed to those of cancer (as a paradigmatic partially age-independent disease) or aging (as an inexorable time-dependent process) but that they must be conceived in a fundamentally different fashion. In our view, the hallmarks of health reside in the overall "organization" of organisms and hence are not confined to a particular class of molecules (such as DNA, RNA, proteins, and metabolites), organelles (such as nuclei, mitochondria, and lysosomes), cell types (such as parenchymatous, auxiliary/stromal, and inflammatory/immune cells), supracellular units constituting the minimal functional units of organs (such as villi and crypts in the intestine, hepatic lobules, pancreatic acini and islets, thyroid follicles, nephrons in the kidney, …), entire organs within their anatomical boundaries, organ systems (such as cardiovascular or nervous systems and gastrointestinal, respiratory, or genitourinary tracts), systemic circuitries (such as endocrine, neurological, or immune connections), or the meta-organism (that integrates the host and the microbiota). Being "organizational," the hallmarks of health reflect a series of dynamic features that maintain the precarious equilibrium preceding disease and infirmity across the aforementioned microscopic and macroscopic strata.

Similar to the preceding hallmark articles on cancer and aging (Hanahan and Weinberg, 2000, 2011; López-Otín et al., 2013), we suggest that the "hallmarks of health" are not mere indicators of vigor but rather are "causatively" involved in its homeostatic maintenance. Thus, each hallmark of health should ideally fulfill the following requisites: (1) it should be associated with the healthy state; (2) its experimental or real-life perturbation should be vastly pathogenic; and (3) its experimental or medical maintenance or restoration should have a broad pro-health activity. This set of ideal criteria—especially the third—is met to varying degrees by the eight proposed hallmarks (Figure 1). For this reason, not all of the hallmarks are fully sustained yet by interventions that succeed in improving health. This caveat is assuaged by

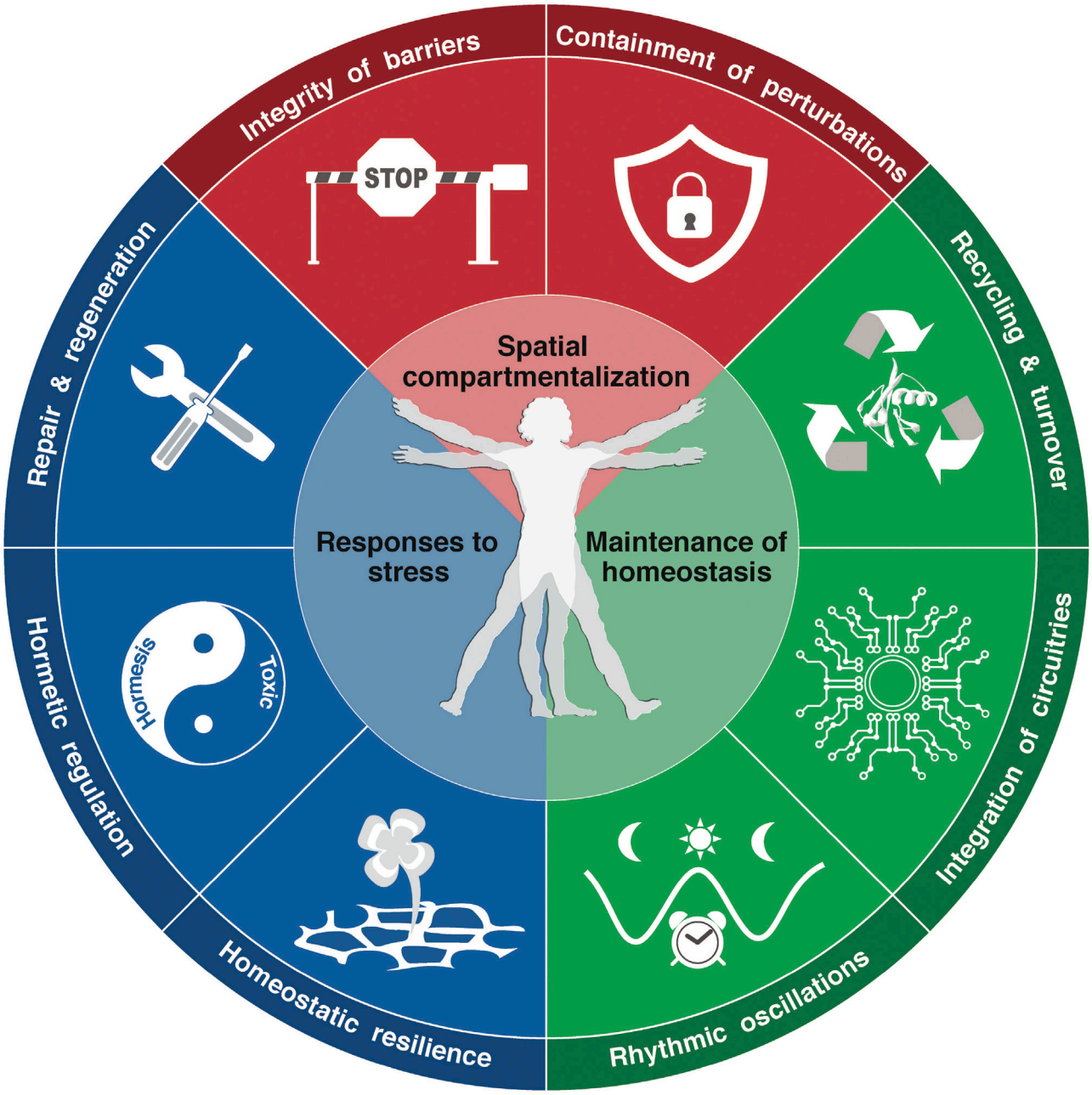

Figure 1. The Hallmarks of Health

The scheme compiles the eight hallmarks of health proposed in this review: integrity of barriers, containment of local perturbations, recycling and turnover, integration of circuitries, rhythmic oscillations, homeostatic resilience, hormetic regulation, and repair and regeneration. These hallmarks are grouped into three categories: spatial compartmentalization, maintenance of homeostasis over time, and adequate responses to stress.

the far-reaching interconnectedness among the stigmata of health, denoting that experimental reinvigoration of one particular hallmark may impinge on others.

Hallmark 1: Integrity of Barriers

All living beings must shield from their environment by erecting selective barriers that allow for maintaining their identity (as a frontier between the internal and the external world) and the reduction of entropy (which requires compartmentalization) (Marín et al., 2009). None of the subcellular, cellular and supracellular compartments would exist or function without organism-intrinsic barriers that assure their delimitation, allow for vital electrophysiological and chemical gradients to establish, yet facilitate their permeation for the exchange of gases and osmolytes, the replenishment of metabolic circuitries, the communication/coordination among compartments, as well as for

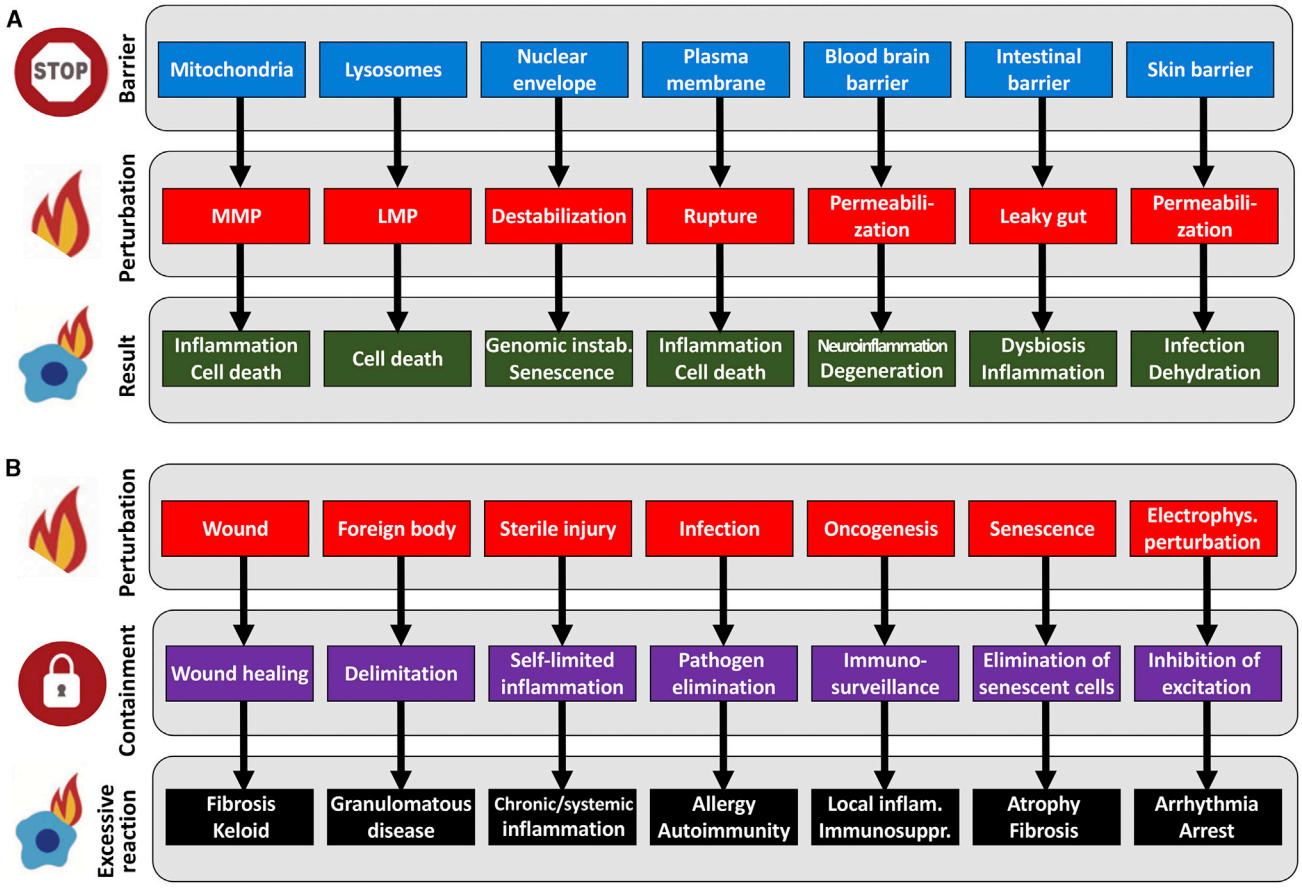

Figure 2. Effect of the Disruption of the Integrity of Barriers and of Local Perturbations on Health

(A) Consequences of the alteration of barriers at the cellular and organismal levels. Note that lesions to macroscopic barriers (epidermis, blood vesicles, meninges, peritoneum, pleura, pericardium, etc.) are not listed here.

(B) Mechanisms of containment of local perturbations and consequences of their excessive activation. LMP, lysosomal membrane permeabilization; MMP, mitochondrial membrane permeabilization.

detoxification. Here, we illustrate the importance of the integrity of selective barriers for the maintenance of health by evoking a limited number of representative examples at the levels of organelles (mitochondria and nuclei), cell membranes, internal barriers (blood-brain) and barriers with the outside world (intestinal, respiratory and cutaneous) (Figure 2A).

Mitochondrial Membrane Integrity

The inner mitochondrial membrane must remain close to impermeable to maintain the electrochemical gradient for oxidative phosphorylation, but at the same time must facilitate the transport of ions and metabolites. This is achieved by a specific cholesterol-free, cardiolipin-containing lipid bilayer with specific channels, transporters, and antiporters, as well as by transient mitochondrial permeability transition that occurs in a flash-like fashion accompanied by the generation of reactive oxygen species (ROS) and facilitates the transport of molecules <1,500 Da (Kuznetsov et al., 2017). The outer mitochondrial membrane must retain potentially dangerous molecules such as cytochrome *c*, which activates the apoptosome (a cytosolic caspase activation complex), and apoptosis-inducing factor, which ignites caspase-independent cell death pathways when it translocates to the cytosol and the nucleus (Bock and Tait, 2020).

Mitochondrial membrane permeabilization (MMP), which can differentially affect the outer (MOMP) and inner (MIMP) membranes, constitutes the central coordinating event of the intrinsic pathway of apoptosis and many instances of necrotic cell death (e.g., in neuronal excitotoxicity and ischemia reperfusion damage) (Kroemer et al., 2007). Apoptosis has been linked to preferential MOMP mediated by pro-apoptotic proteins of the BCL2 family such as BAX (Kalkavan and Green, 2018), while necrosis has been linked to MIMP initiated by the opening of the permeability transition pore (PTP) that likely involves several components of the ATP synthasome and the regulatory protein cyclophilin D/PPIF (Karch et al., 2019). MOMP and MIMP are stressful and often lethal events that can be triggered by multiple stimuli ranging from deficient ion, bioenergetic or redox imbalance, exposure to toxins, and activation of damage-sensing pathways in organelles other than mitochondria (Galluzzi et al., 2012). Irreversible PTP opening with subsequent loss of the electrochemical gradient stimulates mitophagy (the autophagic removal of depolarized mitochondria) and compromises

bioenergetic and redox metabolism (Youle, 2019). Full-blown MOMP results in the release of cell death-igniting proteins, while partial MOMP may cause sublethal caspase activation resulting into DNA damage and genomic instability (Ichim et al., 2015). The combination of MIMP and MOMP facilitates the protrusion or release of mitochondrial DNA (usually confined to the matrix), resulting in the activation of the cytosolic cyclic GMP-AMP synthase (cGAS):stimulator of interferon genes (STING) pathway, setting off a pro-inflammatory response and potentially causing cellular senescence (Riley et al., 2018). Knockout of the MOMP inducer *BAX* confers protection in mouse models of stroke, heart attack, neurodegeneration, and other diseases of unwanted cellular demise (Walensky, 2019). Knockout of the MIMP facilitator *PPIF* reduces the severity of ischemia reperfusion damage in multiple organs (Briston et al., 2019) and attenuates oxalate-induced acute kidney injury, high glucose-induced cognitive decline, hepatosteatosis, osteoporosis, myopathy, and acute pancreatitis, spurring the development of inhibitors targeting cyclophilin D/PPIF and other PTP components (Panel et al., 2019).

Altogether, it appears that the avoidance of excessive MMP is cardinal for maintaining cellular and organismal health. A similar, but less well-characterized role may be attributed to the avoidance of lysosomal membrane permeabilization (LMP) (Papadopoulos et al., 2020), which constitutes a disease-initiating event in lysosomal storage diseases but may also contribute to neurodegeneration induced by α-synuclein aggregates (Jiang et al., 2017) or brain trauma (Sarkar et al., 2020).

Nuclear Envelope Integrity

As opposed to other intracellular membranes or the plasma membrane, the nuclear envelope contains pores that enable selective movement of molecules across the membrane. The pores allow free diffusion of metabolites and proteins up to 30–60 kDa, while actively importing or exporting larger proteins, facilitating the export of RNA transcribed from DNA, and retaining DNA in the nucleus (Beck and Hurt, 2017). The disruption of nuclear pore complexes involved in nucleocytoplasmic transport has been associated with aging and a broad spectrum of diseases, in particular neurodegenerative conditions (Sakuma and D'Angelo, 2017). Another peculiarity of the nuclear envelope is that it is periodically dismantled during each mitosis when chromosomes compact (Carlton et al., 2020). Leakage of genomic DNA may occur as a result of aberrant mitoses or from herniation of the envelope during the interphase. If nuclear DNA comes into contact with the cytoplasm, it can be "confounded" with DNA from invading pathogens and hence perceived by cytosolic pattern recognition receptors to set off the cGAS/STING pathway and to activate pro-inflammatory and pro-senescence pathways (Lan et al., 2019). Accordingly, mutations affecting nuclear pores or nuclear lamina components yield an age-accelerating phenotype that derives from increased genomic and epigenomic instability, reduced proliferative and regenerative capacities, and increased inflammation (Gordon et al., 2014).

Plasma Membrane Integrity

The barrier function of the plasma membrane is essential for maintaining cellular viability, as well as for avoiding the spilling of intracellular material into the extracellular space, which has potent pro-inflammatory consequences. Rupture of the plasma membrane occurs passively when ion homeostasis fails (e.g., in the context of a bioenergetic catastrophe or when cells are exposed to toxins that inhibit ion pumps) or may result from the activation of pore-forming proteins. As an example, the pore-forming protein gasdermin E is proteolytically activated by caspase-3 during end-stage apoptosis to mediate post-apoptotic necrosis, while gasdermin D is activated by inflammatory caspases in the context of inflammatory cell death (pyroptosis). As yet another example, mixed lineage kinase domain like pseudokinase (MLKL) can be activated by receptor-interacting protein 3 (RIP3) to permeabilize the plasma membrane in necroptosis. Although these pathways (apoptosis, pyroptosis, and necroptosis) may be important for the clearance of infected or malignant cells, they also play a major role in unwarranted cell loss (Tang et al., 2019). Notably, interruption of the pyroptotic cascade protects from non-alcoholic steatohepatitis (Xu et al., 2018a), cisplatin-induced acute kidney injury (Miao et al., 2019), and disseminated intravascular coagulation induced by bacterial lipopolysaccharide (Yang et al., 2019). Similarly, the inhibition of necroptosis has a wide-ranging health-improving effect in models of stroke, acute kidney injury, and cardiac ischemia/reperfusion, suggesting its usefulness for the suppression of inflammatory and degenerative diseases (Martens et al., 2020).

Blood-Brain Barrier Integrity

The blood-brain barrier (BBB) is maintained by multiple cell types within so called neurovascular units, including brain microvascular endothelial cells (BMVECs), pericytes, astrocytes, glia, neurons, and extracellular matrix (ECM). BMVECs form complex tight junctions that impose transcytosis as the only mechanism for allowing the transport of molecules from the bloodstream through the capillary wall into the CNS or vice versa. Moreover, BMVECs express multiple broad-spectrum efflux pumps that actively prevent many lipophilic small molecules from passively diffusing through the BBB and extrude metabolic waste products and amyloid-β from the brain's interstitial fluid into the blood. BBB dysfunction is associated with numerous neurological diseases (Zhao et al., 2015). Dysfunctions of the BBB can result from aberrant endothelial-pericyte and/or astrocyte-pericyte signaling, causing the local accumulation of blood-derived neurotoxic proteins or iron and the reduced clearance of neurodegeneration-associated proteins in a complex self-amplificatory system influenced by genetic risk factors (e.g., the E4 allele of *APOE* gene for Alzheimer's disease), environmental and lifestyle factors, and arterial hypertension (Montagne et al., 2020; Zhao et al., 2015).

Intestinal Barrier Integrity

The intestinal barrier is composed by mucus, the epithelial layer, and the epithelial-mesenchymal barrier. Mucus is produced by goblet cells and constitutes a reservoir of antimicrobial peptides and immunoglobulin A (IgA), because it represents the first structure that must be overcome by mucosal pathogens to establish an infection (Johansson and Hansson, 2016). The gut epithelium constitutes another barrier composed by multiple distinct specialized cell types originating from stem cells located in the crypts: enterocytes (for transepithelial transport of nutrients), goblet cells (for mucus production), Paneth cells (that produce antimicrobial peptides), M cells (that sample antigens in the lumen),

chemosensory Tuft cells, and enteroendocrine cells. All these cells are linked by tight junctions that form a selective and semipermeable barrier between the apical and basolateral compartments, allowing for the paracellular transport of solutes to occur (Kurashima and Kiyono, 2017). Yet another frontier, the epithelial-mesenchymal barrier (not only found in the gut but also at any other epithelium) plays a cardinal role in communicating alterations of the epithelia to intestinal immune cells that either cluster in the gut-associate lymphoid tissues or disseminate throughout the intestinal *lamina propria* and the overlying epithelium, producing essential factors for anti-pathogen defense and epithelium repair (Nowarski et al., 2017).

Collectively, imbalances or deviations in the gut microbiota ("dysbiosis") can compromise intestinal barrier function ("leaky gut") and vice versa, and both phenomena are tightly linked to multiple pathologies including inflammatory bowel disease (Fasano, 2020), celiac disease (Odenwald and Turner, 2017), type 1 diabetes (in which leaky gut may trigger the immune-mediated destruction of pancreatic β-cells) (Sorini et al., 2019), type 2 diabetes (in which hyperglycemia compromises tight junctions in gut epithelia) (Thaiss et al., 2018), and Kawasaki disease (in which the pro-inflammatory cytokine interleukin [IL]-1β causes leaky gut, which in turn amplifies cardiovascular inflammation) (Noval Rivas et al., 2019). Dietary composition has a major impact on the gut microbiota, directly impacting whole-body physiology, gut homeostasis, and general health. A dietary fiber-deprived gut microbiota erodes the colonic mucus barrier, thus enhancing susceptibility to bacterial colitis, but also compromising general immune function (Xavier et al., 2020). Leaky gut syndrome allows bacteria and their products to reach the liver through the portal circulation causing local damage that contributes to the highly prevalent non-alcoholic fatty liver disease (NAFLD) or systemic inflammation and infection (Tilg et al., 2020). Although there is a large body of evidence suggesting that leaky gut contributes to human disease, and interruptions of the circuitries leading to this condition prevent or attenuate pathogenesis, no such disease can be cured by simply normalizing intestinal barrier function. However, repair of this barrier may be indispensable for other therapeutic measures to be efficient (Camilleri, 2019).

Barrier Function in the Respiratory Tract

Respiratory mucosae composed of ciliated cells, mucous-producing cells, and undifferentiated basal cells separate the airway lumen and the parenchyma from the nasal passage to alveoli, where the ~1-μm thick alveolar-capillary barrier is composed by alveolar epithelium plus endothelial cells to permit the exchange of O_2 and CO_2 between air and blood, while assuring the right height and composition of the airway surface liquid. Acute respiratory distress syndrome is primarily characterized by increased exudation and impaired clearance of alveolar and interstitial fluids. Defects in the mucociliary apparatus, secreted antimicrobial substances, and the intercellular junctions, as well as shifts in the local microbiota, are involved in a wide spectrum of pathologies ranging from hereditary cystic fibrosis and ciliary dyskinesis, to acute pneumonitis and cigarette smoke-induced chronic obstructive pulmonary disease (Bhattacharya and Matthay, 2013).

Skin Integrity

The skin is the largest organ of the body, covers its entire external surface, and serves many functional roles that are essential for maintaining health. The skin constitutes a multilayered anatomical barrier protecting against microbial pathogens, physical or chemical damage, and excessive water loss, while assuring thermal regulation and selectively absorbing specific ultraviolet wavelengths for vitamin D synthesis. This highly adaptive organ also plays a critical role in sensory perception and immunologic surveillance and hosts a multitude of bacterial species that provide health benefits by boosting multiple aspects of its barrier function. However, microbial skin residents can also cause damage and promote a variety of skin pathologies. Deficiencies in different regulatory and structural components of the intricate molecular network—including microRNAs (miRNAs), filaggrin, collagens, and proteolytic enzymes—that contributes to the establishment and maintenance of the skin architecture cause severe pathologies. In addition to these dermatological diseases that have their origin in the skin, aging and most systemic disorders lead to characteristic alterations of cutaneous morphology and function as well.

In sum, integrity of barriers is a common hallmark of health (Figure 2A). There are multiple examples of the broadly active pro-health effects of maintaining barrier function, while the permeabilization of these internal or external barriers is intrinsically pathogenic (Table 1).

Hallmark 2: Containment of Local Perturbations

The human organism is constantly subjected to indolent or manifest local perturbations that may stem from intrinsic "accidents" occurring during incomplete and asymmetric cellular division, as a result of failed DNA repair, loss of the (epi)genetic cellular identity, and accumulation of dysfunctional organelles or proteins, among others. Moreover, external agents including invading pathogens, mechanic, chemical, or physical trauma frequently cause local perturbations and compromise barriers. In all these cases, for the maintenance of a healthy state, it is essential to confine the perturbation, avoiding its spread to a systemic level that might cause permanent loss of functional units and surpass the capacity of the organism to repair the damage. This situation would result in disease and eventually death from systemic inflammation, uncontrolled infection or malignant disease (Figure 2B).

Barrier Healing

Within cells, ruptured nuclear envelopes may self-heal (Lan et al., 2019). It appears plausible that both BANF1 mutations and lamin A mutations, causing Néstor-Guillermo and Hutchinson-Gilford progeria syndromes, respectively, compromise this process (Halfmann et al., 2019), explaining why these two syndromes mostly affect mechanically stressed organs. Limited lysosomal damage and focal plasma membrane permeabilization are repaired by a process involving endosomal sorting complexes required for transport (ESCRT) (Papadopoulos et al., 2020). Thus, the ESCRT-III complex may prevent excessive cell death by necroptosis (that involves permeabilization of the plasma membrane) in the context of renal transplantation (Gong et al., 2017). At the tissue scale, single epithelial cell loss in the intestinal or respiratory tract activates immediate closure of the gap by

Table 1. Hallmarks of Health across Strata of Organismal Organization: Examples of Pathological Deviations

Hallmark	Stratum	Phenomenon	Pathological Deviations (Examples)	Consequences (Examples)
Integrity of barriers	organelles	mitochondrial integrity	mitochondrial membrane permeabilization	cell stress and death, genomic instability, inflammation, senescence
		lysosomal integrity	lysosomal membrane permeabilization	cell death, neurodegeneration
		nuclear envelope integrity	leakage of nuclear DNA into cytoplasm	genomic instability, inflammation, aging
	cells	plasma membrane integrity	rupture resulting from defective bioenergetics or ionic homeostasis, activation of pore-forming proteins	cell death, leakage of cellular content, and inflammation in acute and chronic diseases (liver, heart, brain, kidney, etc.)
	supracellular units	blood-brain barrier maintained by neurovascular units	failure to exclude blood born neurotoxic factors and to clear locally produced neurodegeneration-associated proteins	acute and chronic neurological diseases including encephalitis, multiple sclerosis, Alzheimer's disease, etc.
	organs	skin barrier	enhanced permeability, desquamation	infection, inflammation, dehydration
	organ systems	barrier function in the respiratory tract	reduced gas exchange, defects in the mucociliary apparatus, secreted antimicrobial substances, and the intercellular junctions	acute respiratory distress syndrome, pneumonitis, cystic fibrosis, chronic obstructive pulmonary disease, etc.
	systemic circuitries	endothelial barrier	enhanced leakage of plasma proteins and extravasation of leukocytes	edema, inflammation, disseminated intracellular coagulation, arteriosclerosis, etc.
	meta-organism	intestinal barrier	leaky gut with dysbiosis	inflammatory bowel disease, celiac disease, diabetes, Kawasaki disease, non-alcoholic fatty liver disease, etc.
Containment of local perturbation	molecules	prion-like neurotoxic proteins	failure to contain protein aggregates	neurodegenerative disease
	organelles	repair of permeabilized lysosomes	leakage of lysosomal hydrolases into the cytosol	cell loss
	cells	repair of limited plasma membrane damage	failure to maintain cell viability on mechanic damage or activation of pore-forming proteins	cell loss
		neutrophil extracellular traps	failure to control invading pathogens	systemic inflammation, if excessive
	supracellular units	extrusion of dysfunctional cells from epithelia	failure to extrude dysfunctional cells	breach of epithelial integrity, inflammation, persistence of pre-malignant cells
		foreign body granulomas	excessive granuloma formation	granulomatous inflammation
		removal of senescent cells	failure to clear senescent cells by macrophages, NK, and T cells	tissue aging, inflammation
	organs	compensatory proliferation of basal layer cells in the skin	failure to replace apoptotic or exfoliated cells	loss of skin barrier function

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Table 1. *Continued*

Hallmark	Stratum	Phenomenon	Pathological Deviations (Examples)	Consequences (Examples)
		wound healing	failure to repair the breach or excessive wound healing	chronic ulcers or fibrosis and keloids
	organ systems	containment of electrophysiological perturbations	unrestrained spread of local electrophysiological perturbations	epilepsy, cardiac arrhythmias
	systemic circuitries	self-limited inflammation	failure to spatially limit and to resolve inflammation	chronic inflammation, inflammaging, autoinflammatory syndromes
	meta-organism	innate and acquired immune responses including anticancer immunosurveillance	failure to eliminate infectious agents and (pre-)malignant cells	acutely lethal or chronic infectious disease cancer
Recycling and turnover	molecules	ubiquitin-proteasome system, secretion by cells, targeted autophagy	failure to eliminate misfolded and aggregated proteins	proteinopathies such as Alzheimer's, Parkinson's, or Huntington's disease, amyotrophic lateral sclerosis, and frontotemporal dementia
	organelles	general or organelle-specific autophagy	accumulation of dysfunctional organelles	accelerated aging, organ dysfunction, inflammation, cancer
	cells	cell death, removal, and replacement	deficient cell death and efferocytosis and exhausted stem cell pools	accumulation of dysfunctional, mutated, and senescent cells, autoimmunity
Integration of circuitries	molecules	pleiotropic functions of proteins	mutations in protein-encoding genes	monogenetic diseases such as cystic fibrosis
		coding versus non-coding RNAs	epigenetic instability	loss of cellular identity
	organelles	inter-organellar contact sites	failed coordination in organelle biogenesis and stress response	cell death, myopathy, neuropathy, metabolic syndrome
	cells	inside-outside communication	failed communication of cellular stress to immune cells and distant organs	failed elimination of stressed cells
		outside-inside communication	deficient integration of paracrine and (neuro) endocrine signals	insufficient coordination among cellular functions
	supracellular units	cooperation between parenchymatous and supportive cells	deficient trophic and mechanic support	compromised units (intestinal villi and crypts, hepatic lobules, pancreatic acini, thyroid follicles, nephrons, etc.)
	organs and organ systems	participation of all organs in local and systemic neuro-endocrine circuitries	failed (neuro)endocrine and metabolic coordination among organs	deregulated appetite, neurovegetative circuitries, and whole-body metabolism
	meta-organism	systemic effects of the gut microbiota and its products	shifts in the intestinal microbiota compromising metabolism, triggering inflammation and affecting cancer immunosurveillance	obesity, cardiometabolic disorders, cancer, psychiatric diseases
Rhythmic oscillations	molecules	circadian oscillations of gene expression, pulsatile secretion of hormones	failure of maintenance of organismal homeostasis	dysregulated stress responses, endocrinopathies, hypertension

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Table 1. *Continued*

Hallmark	Stratum	Phenomenon	Pathological Deviations (Examples)	Consequences (Examples)
	organelles	rhythmicity of mitochondria biogenesis and function	alterations in mitochondria number, morphology, and activity, defective response to hypoxia and oxidative stress	metabolic, cardiovascular and neurodegenerative diseases caused by disturbances in mitochondrial rhythmicity
	cells	stem cell regulation	accumulation of DNA damage, failure of stem cell renewal, and differentiation	disorders associated with stem cell deficiency, aging
	supracellular units	time-dependent tissue homeostasis and repair	loss of tissue homeostasis, deficiency of tissue repair	lack of tissue renewal, carcinogenesis
	organs	function of the suprachiasmatic nucleus master clock	mutations in master clock genes	hereditary sleep disorders (familial advanced sleep phase disorder, familial delayed sleep phase disorder)
	organ systems	coordinated peripheral clock rhythmicity	failure of organ coordination in response to external/internal cues	loss of organ homeostasis
	systemic circuitries	endocrine signaling, responses to light-dark cycles, feeding, and exercise	systemic deficiencies in responses to external and internal stressors	metabolic alterations, obesity
	meta-organism	temporal control of immune responses and microbiota composition	hyperactivation or deficiency of immune responses, loss of temporal balance in immune and inflammatory reactions, dysbiosis	multiple diseases (cancer, depression, diabetes, accelerated aging, etc.)
Homeostatic resilience	molecules	genetic factors associated with resiliency	variants associated with resilience deficiency	pathological responses to stress and adverse events
	organelles	organellar resilience to stressful factors	loss of organellar integrity and functional failure in stress responses	metabolic disorders
	cells	cellular resilience to acute/chronic stress	dysregulated cell death, decreased cell plasticity	excessive cell loss
	supracellular units	tissue resilience to stressful conditions	loss of tissue resilience and plasticity	tissue damage, aging
	organs	brain-mediated regulation of responses to stress	abnormal psycho-biological responses to stress	major depressive disorder, anxiogenesis
	organ systems	organ systems-mediated control of acute/chronic stress	loss of adaptive responses to stress in organ systems	gastrointestinal and cardiovascular pathologies
	systemic circuitries	resilience mediated by HPA axis and sympathetic nervous system	deficiency of systemic homeostatic resilience under stress conditions	neural damage, hypertension, cardiovascular diseases
	meta-organism	microbiota resilience and immune response under stress conditions	dysbiosis, deficient immune response to stress factors	immunosuppression, metabolic diseases, mental-health disorders, accelerated aging
Hormetic regulation	molecules	hormetins, low levels of ROS, and other oxidants	failure to elicit preconditioning protective responses to toxins and other stressors	deficiency of beneficial hormetic responses neurotoxicity cardiotoxicity

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Table 1. *Continued*

Hallmark	Stratum	Phenomenon	Pathological Deviations (Examples)	Consequences (Examples)
	organelles	mitohormesis	failure of mitochondria-mediated compensatory adaptations to future stressors	aging and age-related pathologies, loss of cardiac preconditioning effects against myocardial damage
	cells	enhancement of stem cell function by hormesis	loss of appropriate tissue renewal	diseases associated with stem cell deficiencies, aging
	supracellular units	tissue-specific induction of hormesis	tissue-specific failure in adaptive responses to stress	tissue-specific disorders
	organs	hormetic prevention of brain damage	neurotoxicity, neural damage, cell death	neurological disorders
	organ systems	hormetic regulation of organ systems	loss of hormetic responses operating at organ systems	organ-system deficiencies mainly affecting to neural, cardiovascular, respiratory, or gastrointestinal systems
	systemic circuitries	hormesis induced by metabolic, neural, endocrine, and immune pathways	failure of appropriate preconditioning responses at systemic levels	cardiometabolic, neurodegenerative, hormonal, and immunological disorders
	meta-organism	hormetic regulation of microbiota and immune responses	loss of global hormetic responses beneficial for the organism	aging and age-linked diseases
Repair and regeneration	molecules	DDR components, proteostasis factors, reprogramming factors	genomic/epigenomic damage, loss of proteostasis, defective repair	accumulation of mutated and dysfunctional cells, cancer, neurodegeneration
	organelles	UPR, mitoUPR, ELDR	organelle dysfunction, metabolic alterations	metabolic diseases
	cells	stem cells, pluripotent cells	age-related diseases, macular degeneration, myocardial infarction, Parkinson's disease	promising applications of reprogrammed and differentiated or transdifferentiated cells for treatment of numerous diseases
	supracellular units	tissue repair and regeneration	tissue dysfunction defective wound healing	neurodegenerative, skin, cardiovascular, and retinal diseases
	organs	organ regeneration	functional loss or deficiency of specific organs	organ-specific diseases
	organ systems	organ systems regeneration	defective function of organ systems	multiple diseases affecting to the different organ systems
	systemic circuitries	systemic mechanisms of repair and regeneration	failure of systemic mechanisms involved in repair and regeneration	neurodegenerative, cardiovascular, and ocular diseases
	meta-organism	global functional maintenance of the whole organism	dysbiosis, endocrine, neural, cardiometabolic, and immune alterations	aging and age-linked pathologies

DDR, DNA damage response; ELDR, endo-lysosomal damage response; HPA, hypothalamo-pituitary-adrenals; ROS, reactive oxygen species; UPR, unfolded protein response.

adjacent epithelial cells (Gagliardi and Primo, 2019), whereas removal of keratinocytes from the upper layer of the skin triggers rapid compensatory proliferation of cells in the basal level coupled with exudation of a microbicidal fluid. At the supracellular level, damage by local trauma such as cuts, frostbite, or burns gives rise to a rapid wound healing response designed to fill the breach, activating a stepwise series of responses including local inflammation with rapid recruitment of neutrophils and macrophages, capillary angiogenesis, and compensatory proliferation of fibroblasts and epithelial cells. Reduced wound healing capacity, as it occurs in the elderly, increases the susceptibility to chronic and systemic complications (Willyard, 2018). Excessive

wound healing can lead to fibrosis and keloids through a process that involves local overproduction of transforming growth factor-β.

Delimitation of Foreign Bodies

Foreign bodies including invading pathogens that trespass the skin or mucosal barriers give rise to multiple reactions that isolate them from the surrounding tissues and limit their advancement, especially if they cannot be eliminated by phagocytosis. One of the most rapid mechanisms involves extracellular traps, in which neutrophils and other immune cells create extracellular nets by extruding DNA and antimicrobial proteins, a phenomenon that is useful for the local control of invading pathogens, yet may be pathogenic if occurring at the systemic level (Daniel et al., 2019; Silvestre-Roig et al., 2019). Local vasoconstriction and thrombus formation is not only useful for stopping hemorrhage, but may also help to prevent the dissemination of invading pathogens and the diffusion of toxins (Berling and Isbister, 2015). Encapsulation is a slower process, in which the extraneous object is surrounded by fibroblasts and collagen to isolate it from healthy tissues through creation of a foreign body reaction. This occurs in the context of splinters and invading parasites, but also during tumor suppression (Qin et al., 2002). If the cause of granuloma formation is not locally circumscribed, systemic granulomatous inflammation may occur in a range of infectious diseases, sarcoidosis, Crohn's disease, and rheumatoid arthritis, illustrating a maladaptive inflammatory response (Brooks et al., 2019).

Self-Limited Inflammation

In a physiological context, inflammation is spatially and temporally limited by multiple mechanisms (Furman et al., 2019). Spatial limitation is assured by the local action of inflammatory mediators, thus avoiding systemic reactions secondary to cytokine storms that typically cause fever and affect systemic circuits with subsequent reallocation of resources in the context of the sickness behavior (Wang et al., 2019a). For example, when components and regulators of inflammasomes required for the proteolytic maturation of IL-1β are mutated and become abnormally sensitive to activation (or insensitive to inactivation), systemic inflammation causes repeated episodes of fever in response to challenges that usually cause localized, non-systemic inflammation (Kesavardhana et al., 2020). Temporal limitation or resolution of inflammation is facilitated by the removal of its primary cause (e.g., removal of the pathogen or healing of the wound), as well as multiple negative feedback loops that act locally (resulting from the decay of inflammatory cells and factors or from the production of anti-inflammatory mediators) or systemically (e.g., glucocorticoids) (Basil and Levy, 2016). The resolution of inflammation is required for the avoidance of tissue damage and fibrosis that ultimately lead to permanent organ dysfunction, as exemplified by keloids for the skin, emphysema, and fibrosis for the lung, cirrhosis for the liver, glomerulosclerosis for the kidney, or gliosis for the brain (Weiskirchen et al., 2019). Chronic, systemic inflammation can result from the failure to remove the pathogenic agent, be it infectious (e.g., in malaria or tuberculosis) or non-infectious (e.g., urate crystals in gout). This type of limitless inflammation is highly prevalent and contributes to aging ("inflammaging"). Anti-inflammatory agents including aspirin and inhibitors of pro-inflammatory cytokines are used for the treatment of chronic inflammatory diseases and may have relatively broad health-improving effects, as exemplified by the fact that IL-1β inhibition improves arteriosclerosis and prevents heart failure but also reduces the incidence of lung cancers in clinical trials (Everett et al., 2019; Ridker et al., 2017).

Innate and Acquired Immune Responses

The most primitive innate immune responses occur at the cellular level, allowing cells to reduce the translation of mRNAs coding for viral proteins by activating the "integrated stress response" (ISR), consisting in the phosphorylation of eukaryotic initiation factor α (eIF2α) by a set of stress-responsive kinases and then detect, isolate, and destroy intracellular pathogens by their autophagic machinery (Costa-Mattioli and Walter, 2020). Type-1 interferons (IFN) that are secreted by infected cells act in a paracrine fashion on neighboring cells by eliciting the induction of IFN-response genes, as well as by stimulating ISR (Schoggins, 2019). Innate immune effectors are rapidly activated by microbe-associated molecular patterns (MAMPs), which are produced by different viruses, bacteria, fungi, and parasites. At sites of tissue damage, such innate effectors are also triggered by danger-associated molecular patterns (DAMPs), which usually are endogenous metabolites and highly abundant proteins that are secluded within the intracellular space, yet become exposed on the cell surface or extruded into the extracellular space. Intriguingly, the exposure of the DAMP calreticulin (that triggers phagocytosis of stressed cells by macrophages and dendritic cells) and the secretion of the DAMP ATP (that acts on purinergic receptors to attract and activate mobile immune effectors) rely on ISR and autophagy, respectively (Galluzzi et al., 2017). DAMPs and MAMPs act on an overlapping set of pathogen recognition receptors (PRRs) mostly expressed by myeloid cells to act as adjuvants (Gong et al., 2020), resulting into the formation of tertiary lymphoid organs in proximity of the insult or facilitating the transport of antigenic material toward secondary lymphoid organs including lymph nodes. Such lymphoid organs provide the appropriate context for the ignition of immune responses by T and B lymphocytes (Kabashima et al., 2019). Under ideal circumstances, these responses are so rapid that the pathogen becomes neutralized before it has spread through the body, which can happen when there is immunological memory of the successful defense against antigenically related microbes (Le Bert et al., 2020). Failure to mount a fast and efficient immune response, either because of pathogenicity of the infectious agent or because of genetic or acquired immunodeficiency, results into systemic and potentially life-threatening infection (Casanova and Abel, 2018). Moreover, failure to contain the inflammatory-immune response at the local level results in systemic autoinflammatory or autoimmune diseases (Savic et al., 2020).

Anticancer Immunosurveillance

The oncogenic transformation of cells caused by accumulation of genetic and epigenetic alterations only results in cancer if immunosurveillance fails. According to the three "E" hypothesis, nascent cancer cells are usually eliminated by immune effectors, establish an equilibrium state between proliferation and immune clearance in smoldering lesions, and finally escape from immunosurveillance to locally infiltrate tissues and disseminate as metastases to distant locations (Dunn et al., 2004). Many of the

mechanisms that allow for the containment of infection by microbial pathogens may also apply to antitumor immune responses that depend on IFN, DAMPs conferring adjuvant signals, as well as the expression of tumor-associated antigens that are different from normal self but ideally cross-reactive with microbial antigens (Fluckiger et al., 2020; Galluzzi et al., 2017). These immunological mechanisms of containment are successful if they elicit a response by cytotoxic T lymphocytes, often in the context of intratumoral tertiary lymphoid structures (Sautès-Fridman et al., 2019). In contrast, containment responses that resemble wound healing and fibrotic encapsulation are maladaptive because they favor cancer cell proliferation and prevent T lymphocytes from accessing tumor nodes, respectively (Jerby-Arnon et al., 2018). Cancer cells undergo a genetic or epigenetic selection within the hostile tumor microenvironment to actively suppress the anticancer immune response or, conversely, remove adjuvant signals (DAMPs) and "hide" tumor-associated antigens (Burr et al., 2019). In part for this reason, even after a phase of initial success, anticancer immunotherapies usually fail when they are administered at an advanced stage. In contrast, the preventive stimulation of immunosurveillance may reduce the incidence of cancer (Buqué et al., 2020).

Cellular Senescence and Its Clearance

Genotoxic agents, inflammatory factors, and metabolic signals can induce cellular senescence, consisting in a close-to-irreversible arrest of the cell cycle and the acquisition of the senescence-associated secretory phenotype (SASP). This phenotype may mobilize immune effectors and trigger inflammation, thus causing spreading of cellular senescence. Although senescent cells formed after local damage may have positive effects in the sense that they stimulate wound healing and contribute to tumor suppression, their accumulation in tissues and at the systemic level drives organ dysfunction and aging, respectively (Xu et al., 2018b). Indeed, with age, the cell-intrinsic damage affecting proliferating cells drives cells into senescence, coinciding with reduced clearance of senescent cells by macrophages (He and Sharpless, 2017).

Containment of Other Perturbations

Numerous neurotoxic proteins behave like prions (proteinaceous infectious particle) and transmit their misfolded three-dimensional structure to force nearby protein molecules into a similar shape. Failure to contain such proteins in defined areas hence propagates the disease (Iadanza et al., 2018). Epilepsy and cardiac arrhythmias exemplify another type of perturbation containment diseases, in which the spatially or temporarily unrestrained spread of local electrophysiological perturbations is pathogenic, requiring therapeutic measures that consist in the removal of the focus or the inhibition of excitatory circuits. Of note, genetic manipulations leading to a reduction of excitability in the neuronal system can increase lifespan in nematodes and mice (Zullo et al., 2019), pointing to, as yet, poorly understood general implications of these findings.

In sum, there are multiple mechanisms that allow containment of physical or chemical damage and inflammation; elimination of pathogens, nascent cancers and senescent cells; or the containment of other perturbations. Failure to isolate such lesions, to spatially confine them, and to resolve them over time, results into systemic disease (Table 1). Paradoxically, the failure to limit containment reactions is also pathogenic, meaning that excessive wound healing or foreign body reactions, as well as exaggerated or persistent inflammatory and immune responses that trespass the local context, are incompatible with human health (Figure 2B). Measures to improve wound healing, to limit inflammation, to enhance immune responses against infectious agents, to improve immunosurveillance, and to prevent the spreading of senescence have a broad positive impact on health.

Hallmark 3: Recycling and Turnover

Even in a context of close-to-perfect spatial compartmentalization due to the maintenance of barrier functions and appropriately tuned containment mechanisms, each of the subcellular, cellular, and supracellular units composing the organism undergoes modifications that result from endogenous damage (like the oxidative modifications of proteins, lipids and nucleic acids, or the spontaneous denaturation and degradation of macromolecules that lose their native conformation) or from exogenous stress (resulting in an acceleration of damage). To avoid degeneration, most cellular components and most cell types must therefore be constantly recycled, meaning that they must undergo active destruction followed by their replacement without errors (Figure 3).

Cell Death, Removal, and Replacement

Keratinocytes located at the surface of the skin undergo desquamation as they are replaced through proliferating cells in the basal level that move upward while going through terminal differentiation and keratinization. Cells at mucosal surfaces can experience live-cell delamination or apoptosis-mediated extrusion in which neighboring cells use the actin-myosin cytoskeleton to generate a contractile ring that closes as the apoptotic cell is expulsed in the lumen (Gagliardi and Primo, 2019). As they die, cells contained within internal organs must be silently cleared by phagocytosis, a process known as efferocytosis (Morioka et al., 2019). For this, dying cells must emit soluble "find-me" signals that attract phagocytes, membrane-bound "eat-me" signals that facilitate their recognition and engulfment by phagocytes, as well as anti-inflammatory signals that avoid an unwarranted overreaction (Medina et al., 2020). Defective clearance leads to accumulation of dead cells, spillage of their content into the tissue, inflammation, and autoimmune reactions (Morioka et al., 2019). Neoplastic cells tend to downregulate "eat-me" signals and to upregulate "do not eat-me" signals (such as CD47) on their surface to escape from phagoptosis (programmed death by phagocytosis). For this reason, antibodies that neutralize "do not eat-me" signals might become clinically useful as anticancer agents (Feng et al., 2019). In addition, CD47 is upregulated during atherogenesis, and its blockade can prevent arteriosclerosis in mice (Kojima et al., 2016), illustrating yet another example of the pro-health effects of efferocytosis.

The challenge of cellular turnover consists in matching cell loss, disposal, and proliferation without any disequilibrium (Figure 3). For the replacement of dead cells, the stem cell pool must maintain its size, its genomic integrity, and its epigenetic identity, three features that tend to be lost with old age. Cell competition between different cell clones is also important for

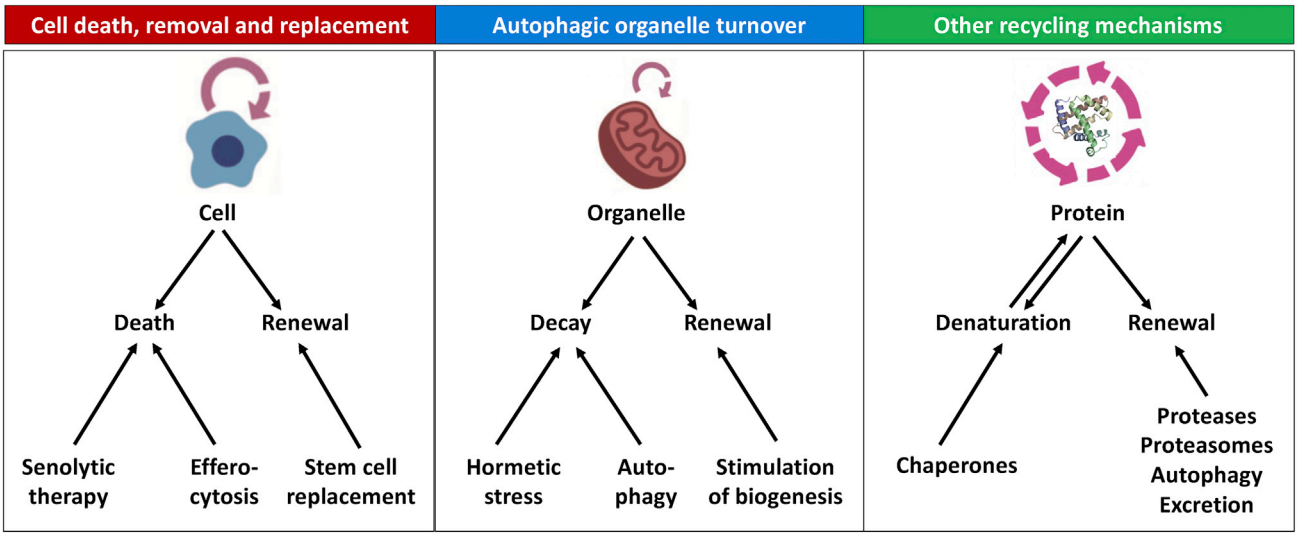

Figure 3. Recycling and Turnover Mechanisms in Tissues and Cells

The appropriate recycling and turnover of different components of the organism is essential for the maintenance of a healthy status. The turnover of entire cells involves a coordinated triad of regulated cell death/efferocytosis/replacement that can be stimulated for therapeutic purposes. Moreover, the turnover of the cytoplasm, mostly by autophagy, as well as other recycling mechanisms involving chaperones and proteolytic systems are necessary for maintaining and promoting health. Mitoch., mitochondrial; prot., protein; resp., response.

the maintenance of tissue homeostasis and in certain diseases such as cancer (Baker, 2020). The expansion of (epi)genetically altered cells, as in clonal hematopoiesis, accompanies aging and predisposes to inflammatory and malignant diseases (Cheung et al., 2018; Yokoyama et al., 2019). The rate of turnover is very different among distinct cell types and tissues: rapid for neutrophil granulocytes and enterocytes, very slow for neurons in the CNS, and even slower for cardiomyocytes after the neonatal phase. This may explain the specificities of the short-term toxicity of chemotherapy or full-body radiation, as well as the fact that the CNS and the heart are among the organs that manifest the most prevalent slowly degenerative phenotypes after chemotherapy and during aging (Baar et al., 2017).

There are two general therapeutic strategies to improve cellular turnover. In the first case, differentiated, somatic cells are reprogrammed to a pluripotent state, for instance by transient and cyclic expression of the Yamanaka transcription factors (Oct4, Sox2, Klf4, and c-Myc). This reduces the manifestation of age-associated phenotypes and improves the resistance of mice to toxin-induced type 1 diabetes or muscle damage, presumably by favoring the replacement of damaged or dead cells (Ocampo et al., 2016). In the second case, apoptotic cell death is preferentially induced in senescent cells, which accumulate in aging tissues and need to be replaced by non-senescent, functional cells that arise from compensatory proliferation (He and Sharpless, 2017). Such a "senolytic" therapy can be achieved by expression of "suicide genes" under the control of inducible promoters (such as that of $p16^{Ink4}$), causing a reduction of the signs of aging in mice (Baker et al., 2011). Moreover, "senolytic drugs" that overcome the intrinsic apoptosis resistance of senescent cells (such as the BCL2 antagonist navitoclax) counteracts aging in mice, but also prevents diabetes induced by high-fat diet (Aguayo-Mazzucato et al., 2019). Other senolytic agents (such as dasatinib plus quercetin) have broad health-improving effects in mouse models of arteriosclerosis, cardiac damage, neurodegeneration, hepatosteatosis, and type 2 diabetes, as well as in human idiopathic pulmonary fibrosis (Khosla et al., 2020).

Autophagy

In proliferating cells, each division cycle leads to a dilution of the cytoplasm by a factor of two, facilitating its renewal. Thus, especially in non-dividing or slowly proliferating cells, the cytoplasm must undergo turnover by alternative mechanisms, including macroautophagy (usually called "autophagy"), a mechanism by which large protein aggregates and entire organelles can be sequestered in double-membraned vesicles, the autophagosomes, that later fuse with lysosomes for the digestion of luminal content by hydrolases that operate at low pH (Figure 3). Autophagy can occur in generalized mode, especially when it is induced in response to starvation following the activation of energy sensors or a reduction in trophic hormones, but may also occur in a selective fashion to eliminate cargo that has been marked for destruction, for instance on ubiquitinylation and/or binding of specific autophagy receptors (Levine and Kroemer, 2019).

Autophagy operates at low baseline levels, and its disruption by knockout of specific autophagy genes (*ATG*s) results in the accumulation of inclusion bodies (composed by misfolded protein aggregates) and degenerating organelles, in particular, mitochondria that tend to reduce the efficiency of oxidative phosphorylation and overproduce ROS (Levine and Kroemer, 2019). Hence, genetic inhibition of autophagy drives the dysfunction and death of most cell types in which *ATG* genes are ablated. After the inducible knockout of *Atg7* in mice, all examined organs undergo degenerative changes, and animals die from generalized neurodegeneration within 2–3 months (Karsli-Uzunbas et al., 2014). Restoration of baseline autophagy after its transient inhibition reverts part of this premature aging phenotype, yet reveals a

major increase in cancer incidence, supporting the notion that autophagy is tumor suppressive (Cassidy et al., 2020). There are multiple human genetic disorders that mostly spare the core machinery of autophagy and rather concern regulators and autophagy receptors, causing partial defects in the pathway that lead to cancer, organ-specific diseases (most often neurodevelopmental and neurodegenerative disorders), or multi-organ syndromes (that frequently share an inflammatory component) (Levine and Kroemer, 2019). Moreover, obesity—with its underlying excess in nutrients and trophic hormones—may accelerate aging and the precocious manifestation of age-related diseases, at least in part due to the inhibition of autophagy (López-Otín et al., 2016).

Autophagy may be conceived as the most important cytoplasmic recycling mechanism, explaining why the direct stimulation of autophagy by genetic manipulation, caloric restriction, fasting cycles, ketogenic diet, inhibition of insulin/IGF1 signaling, or pharmacological manipulation of nutrient sensors (for instance, with rapalogs or with spermidine) extends the healthspan and lifespan of model organisms (Madeo et al., 2019). Beyond its general antiaging activity in mice (Eisenberg et al., 2016; Harrison et al., 2009), pharmacological autophagy enhancement has a broad effect on the time-dependent manifestation of major diseases including hereditary mitochondrial disorders, metabolic syndrome, arteriosclerosis, hepatosteatosis, hypertension-induced cardiac decompensation, and numerous neurodegenerative diseases. Indeed, autophagy protects cells from premature death, reduces inflammation, and improves anticancer immunosurveillance. Mechanistically, cytoprotection is achieved by the autophagic sequestration of damaged mitochondria, the removal of potentially toxic aggregates of misfolded proteins, and the destruction of pro-necroptotic proteins including RIP3 (Xie et al., 2020). Inflammation is reduced because autophagy prevents the release of DNA from leaky mitochondria, sequesters micronuclei, reduces the abundance of components of the inflammasome, and counteracts the cGAS/STING pathway (Hopfner and Hornung, 2020). Immunosurveillance is enhanced due to a favorable impact on immunogenic cancer cell death (Pietrocola et al., 2016), as well as improved T cell renewal, preventing the exhaustion of tumor-infiltrating T lymphocytes (Vodnala et al., 2019).

Mitophagy, which is mitochondrion-specific autophagy, stands out among the specific autophagy pathways because genetic defects in mitophagy are involved in neurodegenerative conditions like Parkinson's disease. Moreover, activation of mitophagy by nicotinamide riboside dinucleotide (NAD^+) precursors has broad health-improving effects in rodent models of vascular aging and dilated cardiomyopathy (Das et al., 2018; Katsyuba et al., 2018) and reduces the age-associated elevation of inflammatory cytokines in patients (Elhassan et al., 2019).

Other Recycling Mechanisms Affecting Proteins

Intracellular proteins that misfold or lose their function due to posttranslational modifications can be destroyed by additional mechanisms that depend on their structure and subcellular localization (Boland et al., 2018). Several aging-associated neurodegenerative disorders including Alzheimer's, Parkinson's, or Huntington's disease, amyotrophic lateral sclerosis, and frontotemporal dementia are "proteinopathies" characterized by the accumulation of aberrantly processed and misfolded proteins (such as amyloid-β, α-synuclein, mutant huntingtin, tau, and TDP-43). The turnover of these proteins involves multiple mechanisms. Within neurons and glial cells, elimination of neurotoxic proteins is predominantly executed by the ubiquitin-proteasome system (UPS) (see also hallmark 8) or by autophagy, but such proteins can also be liberated by exosomes into the extracellular space through a process involving the autophagy machinery. The lymphatic system and the BBB extrude neurotoxic proteins from the interstitial and cerebrospinal fluids, where they may also be degraded by proteases or phagocytosed by microglia and astrocytes (Figure 3). Deterioration of all these mechanisms has been incriminated in the pathogenesis of aging-associated neurodegenerative disorders, whereas their restoration is being explored as a possible treatment strategy (Boland et al., 2018).

In synthesis, the balanced turnover of different components of the organism is required for the maintenance of a healthy status (Figure 3; Table 1). Artificial stimulation of recycling acts to dilate biological time, to reduce entropy, and hence to delay aging and age-associated diseases.

Hallmark 4: Integration of Circuitries

Organisms are built in a way that facilitates the integration of circuitries within and between strata of organization, conferring them the capacity to maintain the stability of the whole system over time (Figure 4).

Intracellular Circuitries

Each molecule, molecular complex, or organelle can engage in several functional circuitries. For example, metabolites convey information beyond their implication in anabolic or catabolic reactions. Intracellular metabolites act as second messengers, as exemplified for AMP, which activates the energy sensor AMP-dependent kinase (Steinberg and Carling, 2019), or acetyl coenzyme A, which influences the acetylation of metabolic enzymes, autophagy-related proteins and histones (Pietrocola et al., 2015). Similarly, in sharp contrast to prior assumptions (like the one gene-one protein-one function hypothesis), each protein has multiple functionalities. Thus, enzymes are usually modulated by other factors than their substrates and their products; ion channels like cystic fibrosis transmembrane receptor (CFTR) may have scaffold functions affecting proteostasis (Strub and McCray, 2020); and pattern recognition receptors capture information from both pathogens and host-intrinsic danger signals (Fitzgerald and Kagan, 2020), just to give a few examples. Intracellular proteins undergo multiple post-translational modifications that, like in a combinatorial code, affect their subcellular localization, stability, activity, and physical interactions, thus connecting them to multiple regulatory systems (Conradi and Shiu, 2018). The transcription of genes is influenced by a complex interplay of epigenetic modifiers and transcription factors, each of which is influenced by posttranslational modifications and often by allosteric modulators. Coding and non-coding RNAs influence each other with respect to their stability, thus creating a network that may suppress random fluctuations and increase the robustness of biological processes (Ebert and Sharp, 2012). Central organelles are interconnected by defined micro-anatomical structures, as exemplified by the mitochondria-associated membranes (MAMs) that connect the endoplasmic reticulum (ER) with the outer mitochondrial membrane

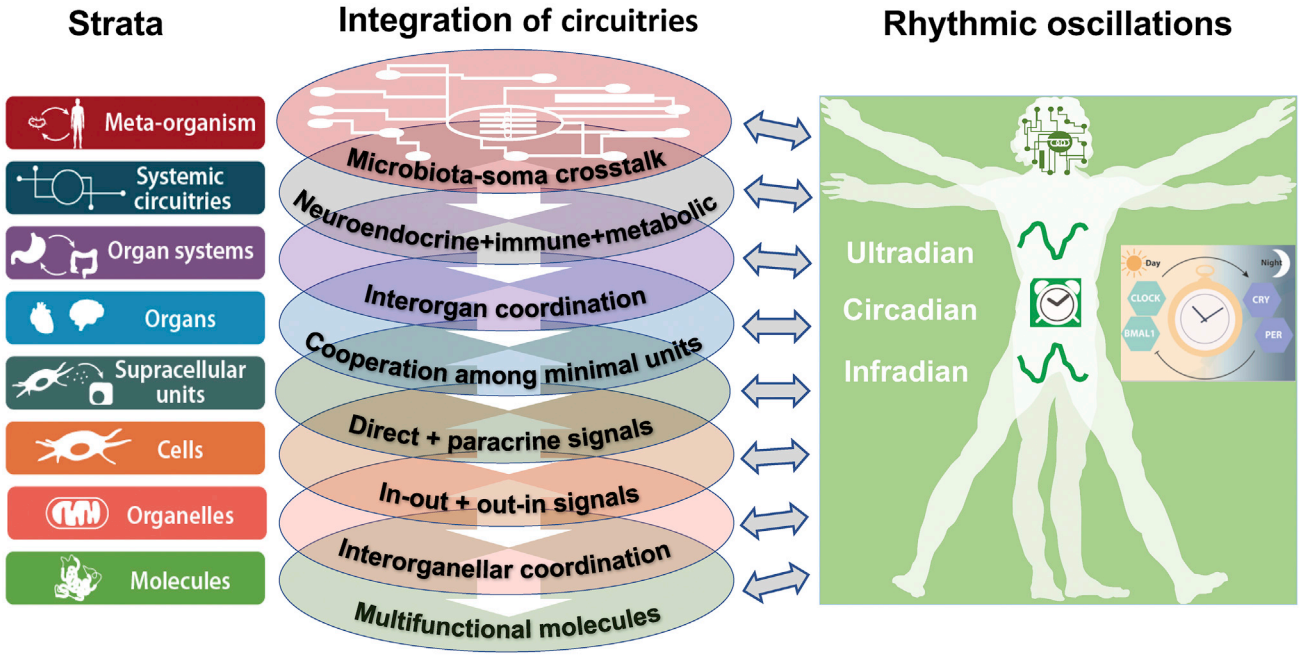

Figure 4. Integration of Circuitries and Oscillations
The maintenance of a healthy organism involves the successful crosstalk among different circuitries (from cells to tissues organs and organ systems), their synchronization with rhythmic oscillations (circadian, infradian, or ultradian) determined by central and peripheral clocks, as well as their homeostatic integration from subcellular compartments (molecules and organelles) to the meta-organismal level (the dialog between the microbiota and the human organism).

for coordinating lipid metabolism or for transmitting Ca^{2+} signals that stimulate respiration or open the PTP (Perrone et al., 2020). All these features allow cells to cope with fluctuating and often stressful internal or external conditions.

Inside-Outside Communication

Intracellular stress can be communicated to the extracellular space to favor systemic adaptive responses (Galluzzi et al., 2018). In the extreme scenario of cell death, cells change the properties of their surface and release a range of distinct DAMPs. The precise nature of these cell surface alterations and DAMPs depends on the activation of premortem stress pathways and the cell death modality, thus generating a combinatorial code that determines the fate of the corpse, its engulfment by one or another phagocyte type, and the functional consequence (inflammation or its suppression, immunity, or tolerance) (Morioka et al., 2019). Sublethal stress is communicated as well. Thus, DNA damage usually elicits the production of IFN, but also facilitates the recognition of cells by T and natural killer (NK) cells due to the upregulation of MHC class I molecules and NK-activating ligands, respectively. Similarly, mitochondrial stress and ER stress are relayed to the external world. For example, mitochondrial stress in muscle leads to the secretion of growth differentiation factor 15 (GDF15) and fibroblast growth factor 21 (FGF21) into the systemic circulation, which then adjust eating behavior and lipid metabolism in adipocytes (Galluzzi et al., 2018). Starvation-induced autophagy causes the release of acyl coenzyme A binding protein (ACBP) into the extracellular space, thus activating feedback mechanisms that inhibit autophagy and stimulate appetite to increase nutrient uptake (Bravo-San Pedro et al., 2019).

Outside-Inside Communication

Specialized cells functioning in our sensory organs (like photoreceptor cells in the retina or hairy cells in the inner ear) receive specific inputs (like light or sound). However, even non-specialized cells must constantly integrate information from the external world. Such information may be physical, as exemplified by temperature, shear stress, arterial vessel tension and deformation, or chemical, such as changes in pH, partial pressure of oxygen and carbon dioxide, osmolarity, and extracellular metabolites that act on nutrient transporters and specific receptors. Moreover, cells receive cell-to-cell contact-dependent inputs by direct connections including gap junctions and microchannels, as well as neuroendocrine inputs in the form of a myriad of amines, peptides, proteins, eicosanoids, and steroids (Lee et al., 2019). The short half-life of many of these mediators, as well as the existence of binding proteins limiting their bioavailability, allows the creation of local gradients, assuring local and paracrine rather than systemic, endocrine effects. Moreover, many cells are connected to synaptic terminations of the vegetative nervous system, assuring that they receive instructions in a spatially defined fashion.

Functional Units in Organs

Organs are composed by parenchymatous (specific) and connective or supportive (non-specific) cell types. Macrophages and fibroblasts, two cell types that are found in most human tissues, engage in direct cell-to-cell contacts and exchange growth factors to create a stable and robust circuitry that includes feedback mechanisms increasing resilience in the context of environmental perturbation (Zhou et al., 2018). It appears plausible that such contact-dependent and cytokine-based circuitries also

facilitate the functional organization of the smallest functional units of organs, which are characterized by a stereotyped geometry juxtaposing non-parenchymatous and parenchymatous cells, the latter usually arising from a common stem cell population. Each of these functional units behaves like a micro-ecosystem that continuously adapts to changing external cues.

Organs, Tracts, and Systemic Circuitries

Hormones, cytokines, growth factors, alarmins, and immunoglobulins connect distinct organs throughout the body. Most cell types are able to secrete multiple cytokines and neuroendocrine factors in the same way as they are equipped with dozens or hundreds of distinct receptors for such extracellular mediators, meaning that the traditional separation of endocrine versus non-endocrine organs and cell types has lost its contours. Moreover, most cell types express neurotransmitter receptors, meaning that they can respond to inputs from the vegetative nervous system, and participate in multiple neuroendocrine circuitries and stress responses, in the same way as the stomach, the small and large bowel, the liver, or skeletal muscle assume endocrine functions to regulate appetite, behavior, and whole-body metabolism. The facts that the colon can generate corticosteroids (Bouguen et al., 2015) or that myeloid cells produce catecholamines (Staedtke et al., 2018) illustrate the existence of largely unexplored circuitries through which multiple organs contribute to local and systemic stress hormone responses.

The Meta-Organism

Multicellular organisms are meta-organisms comprised of the host and the bacteria, archaea, fungi, phages, viruses, and parasites that inhabit them. The gut microbiota influences the digestion and absorption of nutrients, local synthesis of vitamins, gut motility, clearance of pathogens, elimination of xenobiotics, inflammation, and colon carcinogenesis (Walter et al., 2020). In addition, it exerts long-distance effects by interfering with neuroendocrine circuitries (Valles-Colomer et al., 2019), by determining the tonus of the inflammatory and immune systems (Arpaia et al., 2013), or by shaping the immune repertoire (Fluckiger et al., 2020) to prevent overt inflammation, autoimmunity, allergy, and oncogenesis. Major diseases including obesity, cardiometabolic disorders, cancer, and psychiatric conditions have been linked to shifts in the composition of the gut microbiota (Gilbert et al., 2018). Conversely, the healthspan and lifespan of mice that were genetically manipulated to develop accelerated aging can be extended with fecal microbiota transplantation (FMT) from healthy young mice (Bárcena et al., 2019), underscoring that the microbiota can be both a source of disease and a source of health.

In sum, a myriad of communication systems integrates the functionalities of distinct building blocks from subcellular structures to organ systems and the body-microbiota crosstalk (Figure 4). Integration is facilitated by the fact that most of these elements communicate at several levels, simultaneously playing several roles. Thus, the mental representation of simplified linear pathways (element 1 → element 2 → element 3, and so forth) should be replaced by multidimensional networks in which each element is integrated in numerous interwoven circuitries (Topol, 2019). The multifunctionality of each subcellular, cellular, and supracellular building block of the organism culminates in the successful integration of rhythmic oscillation, homeostatic circuitries, hormetic stress responses, and repair pathways (see the forthcoming hallmarks 5–8), increasing the robustness of the system. Health relies on the permanently successful integration of multiple circuitries. The flip side of this vision is that there is no "localized" disease, implying, for example, that psychiatric states are usually connected to somatic perturbations (and vice versa), and any kind of major pathology will alter the microbiota (and vice versa). Indeed, there is ample evidence that common mental diseases such as refractory depression and therapy-resistant schizophrenia are linked to metabolic syndrome and thus associated with a higher risk of mortality (Godin et al., 2019). Moreover, mental or metabolic diseases, as well as cancer, are associated with shifts in the intestinal microbiota (Gentile and Weir, 2018) (Table 1).

The integration of circuitries can be lost as a result of multiple perturbations including the rarefaction of essential elements (e.g., due to genetic deficiencies, the loss of specific sensory, neural or endocrine cell types, or the development of dysbiosis), a deficiency in communication systems (e.g., due to denervation or neuroendocrine deregulation), or the saturation of signaling systems (e.g., due to an excess of metabolites such as glucose in diabetes or a cytokine storm paralyzing the normally localized coordination of inflammatory responses) that are incompatible with organismal health. Beyond a point-of-no-return that determines the irreversible loss of health, the restoration of circuitries is likely impossible, as this occurs in advanced age-linked sarcopenia, cancer-associated cachexia, septic shock, or vital organ failure. Thus, reestablishing integrated circuitries is a difficult task requiring timely and rather complex interventions as exemplified by enzyme and hormone replacement strategies, systemic neutralization of excessive cytokines, organ and stem cell transplantation, or FMT.

Hallmark 5: Rhythmic Oscillations

The precise order, temporal control, as well as the timing itself of molecular and cellular events (e.g., in embryonic development or in regeneration) is essential for life. In addition, ultradian, circadian, and infradian oscillations provide rhythmicity to physiological functions and contribute to the maintenance of organismal homeostasis. Ultradian rhythms (with a periodicity shorter than 24 h) are exemplified by the function of vital organs (e.g., brain electrical activity, heart rate, respiration, and peristalsis), the ~90 min pulsatile secretion of cortisol and ACTH as part of stress responses (Russell and Lightman, 2019), the ~5 h oscillatory pattern of activation of the tumor suppressor TP53 after DNA damage (Stewart-Ornstein and Lahav, 2017), or the cell cycle with its stereotyped succession of phases and checkpoints. Infradian rhythms (with a periodicity well above 1 day) are illustrated by the menstrual cycle or the seasonal variation in biological parameters. However, the best-studied rhythmic oscillation is the evolutionarily conserved circadian clock (Cederroth et al., 2019) (Figure 4).

Mechanics of the Circadian Clock

The central component of the circadian synchronization system is a master clock comprising ~20,000 neurons in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN receives information on environmental light-darkness cues from photoreceptive retinal cells and then confers circadian rhythmicity to

peripheral clocks present in virtually every cell of our body, via autonomic innervation and through the regulation of systemic cues such as endocrine signaling, body temperature, and food intake (Chaix et al., 2016). Recent works with tissue-specific mutant mice have revised this hierarchical model of circadian clocks, replacing it by a network of peripheral clocks (Ray et al., 2020; Welz et al., 2019). The molecular mechanisms that drive these circadian oscillations rely on complex transcriptional-translational feedback loops whose interplay induces the rhythmic expression of clock-controlled genes and causes subsequent oscillations in the cellular proteome (Trott and Menet, 2018). The principal circadian feedback loop consists of a series of core clock elements such as the transcription factors BMAL1 and CLOCK, which transactivate genes coding for the cryptochromes (CRY1 and CRY2), and the transcriptional repressors PER1 to PER3, which in turn inhibit the expression of BMAL1 and CLOCK (Greco and Sassone-Corsi, 2019). Mutations in core clock genes disrupt circadian rhythms and cause hereditary sleep disorders (Kurien et al., 2019). More than half of human genes exhibit circadian oscillations in their expression patterns in at least one body tissue or organ (Ruben et al., 2018). Circadian transcriptional alterations affect major homeostatic mechanisms converging on stem cell regulation, mitochondrial function, immune responses, and microbiota control.

Stem Cell Regulation

Through an effect of stem cells functions, circadian clocks influence a variety of processes such as hematopoietic cell migration, bone remodeling, adipogenesis, hair cycle, myogenesis, and neurogenesis (Sato et al., 2017; Solanas et al., 2017). Circadian clocks in stem cells may contribute to reduce DNA damage caused by UV light during daytime by giving preference to advancement through the S phase of the cell cycle during nighttime, when the probability of genomic damage is reduced. The identification of circadian oscillations in stem cells may provide novel mechanistic insights into their biological roles, but can also contribute to optimizing stem cell therapeutics. Accordingly, hematopoietic stem cell transplantations can be rendered more efficient by appropriate timing of both the extraction of these cells from donors and their subsequent infusion into patients (Weger et al., 2017).

Mitochondrial Function

Mitochondria are at the core of multiple metabolic pathways that exhibit a close bidirectional relationship with the circadian clock (Chaix et al., 2016). The diurnal rhythmicity of mitochondria biogenesis mainly results from the reciprocal interaction between PGC1α—the master regulator of this process and the core clock component BMAL1. PGC1α controls BMAL expression, and *Pgc1α* mutant mice exhibit alterations in circadian-dependent oscillations of locomotor activity, body temperature, and metabolic rate (Liu et al., 2007). Likewise, genetic disruption of *Bmal1* in mice reduces PGC1α levels, abolishes the diurnal changes in mitochondrial architecture, and causes alterations in number and morphology of these organelles. Other proteins involved in mitochondrial fission (FIS1) and fusion (DRP1) are also under circadian control (Schmitt et al., 2018). The activity of fatty acid oxidation enzymes and electron transfer flavoproteins, which are required for β-oxidation and oxidative phosphorylation, respectively, also follow a circadian fluctuation (Peek et al., 2017). Hypoxia-inducible factor 1α (HIF1α) expression is diurnal, and its levels are increased on BMAL depletion in response to hypoxia. Several enzymes involved in redox homeostasis, such as mitochondrial superoxide dismutase 2 (SOD2) and some peroxyredoxins, exhibit circadian activity in mouse liver. Reciprocally, the redox state influences the rhythmic function of the master clock, and factors such as HIF1α bind to clock gene promoters and facilitate adaptation to hypoxia. The NAD^+-dependent SIRT1 deacetylates PER2, diminishes its activity, and alters the circadian rhythmicity of the core clock machinery. Circadian oscillation of nicotinamide phosphoribosyltransferase (NAMPT)—the rate-limiting enzyme in NAD^+ biosynthesis—affects NAD^+-dependent metabolic reactions in mitochondria and creates a feedback mechanism to regulate the activity of SIRT1 and thereby the transcription of master clock genes (Nakahata et al., 2009). Therefore, the molecular clock orchestrates mitochondrial oxidative rhythms linked with the fasting-feeding cycle to maximize energy production during the resting period. SIRT1 also contributes to the circadian clock regulation of mitophagy, a process that occurs predominantly during the active phase in the light-dark cycle (Ramsey et al., 2009).

Immune Response

The circadian clock confers rhythmicity to immunity under normal conditions and in response to inflammatory challenges (Man et al., 2016). These circadian oscillations drive the appropriate trafficking of immune cells, influence the susceptibility to microbial infections, determine the temporal expression of pattern recognition receptors and the components of their signaling pathways, and establish the timing of synthesis and secretion of chemokines, cytokines, complement proteins, coagulation factors, granzymes, and perforins. This clock-mediated regulation of immunological functions may be part of a strategy to anticipate environmental changes and provide an optimized protection for each time of the day. The rhythmicity of immune responses may also contribute to the temporal separation of mutually incompatible programs—tolerance versus immunity—or to avoid cooperative interactions that may cause pathological hyperactivation of immune reactions (Downton et al., 2020). Mutant mice deficient in core-clock proteins lose the temporal balance between immune and inflammatory reactions and develop severe diseases (Scheiermann et al., 2018).

Microbiota Control

The composition of bacterial communities in the intestine exhibits diurnal variation and is entrained by host circadian rhythms. Reciprocally, gut microbiota influences the biological function of the intestinal oscillator. Disruption of this bidirectional communication between bacteria and host results in dysbiosis and causes ulcerative colitis and metabolic disorders. The brain and gut microbiome are connected by several communication systems, including the vagus nerve, hormones, immunological factors, neurotransmitters, and microbial metabolites such as bile acids and short-chain fatty acids (Cryan et al., 2019). Alterations in this communication axis—associated with gene polymorphisms, environmental insults, dietary changes, gastrointestinal disturbances, or aging—may contribute to the

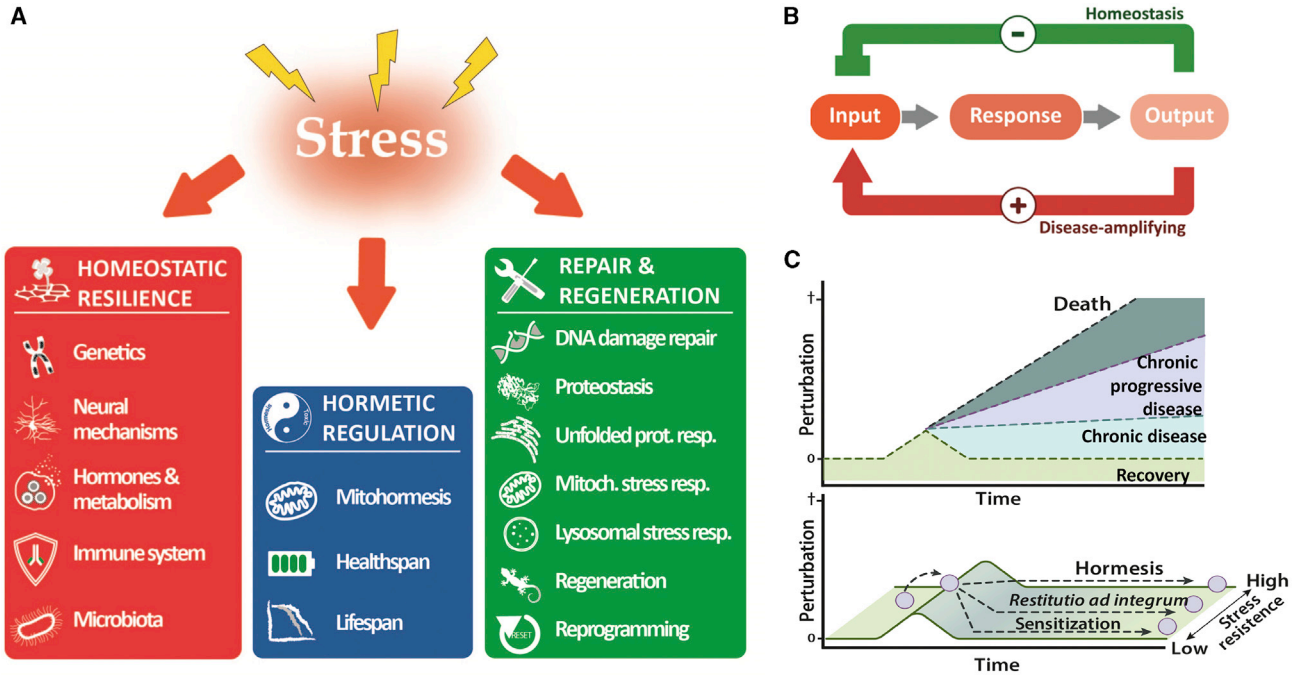

Figure 5. Responses to Stress

(A) Health is continuously threatened by multiple sources of stress. To achieve biological stability, organisms use different strategies such as homeostatic resilience, hormesis, repair, and, whenever possible, regeneration of damaged tissues and organs.

(B) Homeostatic versus disease amplifying effects. Negative feedback loops allow correcting the effects of perturbations (input), whereas positive feedback loops contribute to disease amplification.

(C) Hormetic regulation. In response to a sublethal stress, the status quo ante can be restored. This response ideally leads to an increase in stress resistance (hormesis) that confers "memory" to the system.

development of neurological and psychiatric pathologies such as Parkinson's disease, Alzheimer's disease, anxiety, major depressive disorder, and autism spectrum disorders (Walker et al., 2020).

Alterations in circadian rhythms caused by shift work, irregular sleep-wake patterns, poor sleep quality, frequent travel across time zones, social jetlag, and changes in the timing of food intake are associated with an increased risk of a variety of human pathologies ranging from cancer and depression to diabetes and dysbiosis (Table 1) (Roenneberg and Merrow, 2016). Myocardial infarctions occur more frequently in the morning and have worse clinical outcome because ischemia tolerance is reduced early during the day. Diseases such as cancer, inflammatory processes, psychiatric disorders, diabetes, asthma, or allergies typically present daily oscillations in symptoms and responses to drugs. Unsalutary dietary habits and eating schedules disturb the alignment of feeding-fasting cycles to the circadian cycle and cause metabolic perturbations. Nutritional interventions, such as time-restricted feeding, intermittent fasting, and ketogenic diets, regulate expression of oscillating genes mainly via the mTOR pathway and improve metabolic health in part by restoring the temporal orchestration between master and peripheral pacemakers (Ramanathan et al., 2018). In this context, chronopharmacological methods for directly drugging circadian clocks or chrononutritional and chronotherapeutic strategies might be useful to anticipate or ameliorate dysfunctions in the circadian cycle.

Hallmark 6: Homeostatic Resilience

Homeostatic circuitries maintain myriads of biological parameters (like blood pH, serum osmolarity, arterial oxygen and carbon dioxide, glycaemia, blood pressure, body temperature, body weight, or the concentrations of hormones) at close-to-constant levels unless the setpoint of the regulator is altered, resulting in chronic disease. Endocrine feedback loops that become deregulated due to the lack or overproduction of specific hormones illustrate the cardinal importance of homeostatic regulation. The term "homeodynamics" describes the fact that equilibrium does not rely on the static maintenance of a unique state but rather must evolve through adaptive interactions among the components of the system (Lloyd et al., 2001). The "homeodynamic space" delimits the buffering capacity (resilience) of biological systems and hence determines the ability to survive and maintain health by damage control, adequate stress responses, reduction of biological noise, and constant remodeling (Eling et al., 2019; Rattan, 2014). Homeostatic resilience involves genetic, neural, metabolic, immunological, and microbiome-based mechanisms (Figures 5A and 5B).

Neural Mechanisms

Resilience is largely mediated by adaptive changes in the function of multiple brain circuits that regulate the psychobiological responses to stress ("fight and fly" versus "rest and digest"). These changes involve the participation of a myriad of neurotransmitters, neuropeptides, hormones, receptors, and their associated signaling pathways, which together orchestrate

homeostatic responses to acute or chronic stressors. The sympathetic adrenal-medullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axes constitute two major arms of neuroendocrine responses to stress, which eventually generate the paradigmatic "stress hormones" catecholamines (epinephrine and norepinephrine) and glucocorticoids (GCs), respectively. Following an instantaneous (but transient) response via the SAM axis, a more delayed activation of the HPA axis initiates the cascade-like biosynthesis of corticotropin-releasing hormone (CRH) by hypothalamic neurons, adrenocorticotropic hormone (ACTH) by the hypophysis, and finally GCs by the adrenal gland. If the responsiveness of SAM is limited, the homeostatic resilience process is promoted, but if the system is hyperactivated, cardiovascular disorders and mental health problems are triggered. Acute actions of GCs are protective and elicit adaptive responses, whereas chronic exposure to high GCs levels causes a number of pathological conditions such as neural damage, hypertension, cardiovascular diseases, immunosuppression, and dysbiosis (Rothman and Mattson, 2013).

Acting in close coordination with GCs, neurotrophins such as BDNF are also part of neural circuits of stress resilience. Chronic stressors decrease *BDNF* expression in the hippocampus and cause depression-like effects that can be reversed by antidepressant drugs and physical exercise. Serotonin is involved in the circuits that mediate mood and emotion and may result in anxiogenic or anxiolytic effects. Acute stressors increase the brain turnover of this neurotransmitter and cause depression. Dopamine signaling bidirectionally modulates reward and aversion, contributes to fear extinction, and plays a key role in stress susceptibility and resilience. The neuropeptide NPY has anxiolytic-like effects under stressful conditions and counteracts anxiogenic effects of CRH in different brain regions (Cathomas et al., 2019).

The neurobiological mechanisms promoting resilience involve both physical and molecular adaptations of all these neural circuits. GC release induced by stress decreases hippocampal neurogenesis. Prolonged stress causes atrophy of brain structures, loss of glial cells, and extensive shrinkage of the apical dendritic tree, hampering adaptive plasticity and compromising resilience. Of note, administration of serotonin reuptake inhibitors increases hippocampus volume (Maller et al., 2018), whereas lithium treatment increases the volume of gray matter (Anand et al., 2020). Mechanistically, transcription factors, epigenetic modulators, and chaperones are fundamental mediators of the adaptive responses in brain circuits.

Genetic Factors

Genome-wide association studies (GWAS) analyses and meta-analyses of biological and emotional factors have demonstrated a moderate influence of genetic factors on the heritability of resilience. Pro-resilience variants have been identified in genes encoding neurotrophic factors or modulators of the norepinephrine stress response. Conversely, resilience deficiency due to the presence of variants in *COMT* (catechol-O-methyltransferase), *BDNF* (brain-derived neurotrophic factor), *SLC6A4* (serotonin transporter), and *NPY* (neuropeptide Y), enhances the risk of mental disorders (Zhou et al., 2008). Likewise, studies of the stress-related gene *FKBP5*, which modulates glucocorticoid receptor responses, have identified specific genetic-environment interactions in the context of childhood trauma (Qi et al., 2020). Notably, the same genetic polymorphisms that confer an increased risk of pathological responses to adverse events may also provide substantial benefits in favorable environments, perhaps illustrating antagonistic pleiotropy (Reiss et al., 2013).

Hormones and Metabolism

Homeostatic resilience is not only controlled by the brain, but also involves endocrine and metabolic circuitries. On acute stress, like injury, catecholamines transiently increase thermogenesis through activation of mitochondrial uncoupling proteins in the brown adipose tissue (BAT) (Townsend and Tseng, 2014). By contrast, chronic stress induces a BAT-independent adaptive mechanism or may lead to energetic deficiencies (Turkson et al., 2019). In this scenario, GCs promote the mobilization of macromolecules by activating gluconeogenesis and glycogenolysis, proteolysis and lipolysis, as they facilitate a metabolic switch from anabolic to catabolic reactions, thereby providing energy sources and building blocks (glucose, amino acids, and fatty acids) for stress responses. In addition, GCs increase blood pressure, suppress immune responses, and influence mitochondrial physiology (Machiela et al., 2020).

The metabolic actions of GCs are modulated by other hormones, such as leptin and ghrelin (Tomiyama, 2019). Leptin is mainly produced by adipocytes, inhibits appetite, and informs the brain on the status of energy reserves. Ghrelin is produced by gastrointestinal cells and acts on the hypothalamus to stimulate appetite, but also mediates neuroprotective effects (Yanagi et al., 2018). Other hypothalamic hormones with important functions on metabolic control are oxytocin, which protects cardiomyocytes, and arginine vasopressin, an antidiuretic peptide. Somatostatin contributes to resilience by reducing CRH release during chronic stress conditions. Sex hormones have a strong impact on homeostatic resilience and explain the sexual dimorphism in the responsiveness to chronic stressors (Hodes and Epperson, 2019). Notably, patients with stress-induced neuropsychiatric diseases exhibit metabolic phenotypes, which substantially overlap with metabolic syndrome (Raue et al., 2019).

Immune System

Both innate and adaptive immune systems represent key components of the homeostatic resilience response (Cathomas et al., 2019). Chronic exposure to stress promotes extensive immune changes, and numerous studies have associated stress vulnerability and development of affective disorders with immunological alterations. Importantly, treatment with anti-inflammatory drugs may elicit anti-depressive effects, whereas patients under antidepressant therapies exhibit a reduction in the levels of prototypical pro-inflammatory cytokines such as IL-1β and IL-6. The sympathetic and parasympathetic systems induce and inhibit, respectively, the production of inflammatory cytokines. After subjecting mice to pain-induced stress, vulnerable animals exhibit higher IL-6 levels than resilient mice. The genetic knockout or antibody-mediated neutralization of IL-6 promotes the resilience phenotype (Hodes et al., 2014). High levels of stress-elicited proinflammatory cytokines stimulate the HPA axis (and hence

the production of GCs) but interfere with the function of glucocorticoid receptors (GRs). The resulting glucocorticoid resistance interferes with the HPA/GR-mediated downregulation of cytokine synthesis, thereby interrupting a homeostatic feedback loop and creating a vicious cycle (Quax et al., 2013). Interestingly, proinflammatory cytokines also induce GC resistance in patients with major depressive disorder, suggesting that inflammation and GC signaling act on the same processes to cause cumulative damage. Epigenetic mechanisms also contribute to modulate immune responses to stress. Several miRNAs, such as miR-25-3p (that belongs to the miR-106b-25 cluster), are induced in monocytes of mice exposed to social defeat stress. Selective elimination of this cluster in peripheral leukocytes promotes behavioral resilience to this type of stress (Pfau et al., 2019).

As for the adaptive immune system, several studies have evaluated B and T lymphocyte numbers and functions in normal and pathological stress responses (Miller and Raison, 2016). Patients with major depressive disorder (fLMDD) exhibit T cell lymphopenia, pointing to neuroprotective or proresilient effects of these cells. Immunization of rats with myelin basic protein before application of chronic low-intensity stress induces the generation of autoreactive T cells and reduces depression-associated behaviors. The recruitment of T cells to the CNS positively correlates with stress resilience. Moreover, lymphocytes from chronically stressed reportedly mice reduce levels of proinflammatory cytokines and confer behavioral resilience and antidepressant effects to naive mice (Brachman et al., 2015).

Gut Microbiota

The maintenance of a stable gut microbiota is important for the stability of the host immune system and the cognitive/emotional balance through the production of biologically active metabolites, giving rise to the "microbiota-gut-brain axis" (Teichman et al., 2020). The gut microbiota is highly variable among individuals. However, once bacterial diversity and functional redundancy are well established during childhood, it exhibits strong resilience, meaning that its composition and activity remain substantially stable. The resilience of the healthy microbiota protects from a variety of dysbiosis-related pathologies, such as inflammatory bowel disease, metabolic syndrome, cardiovascular dysfunctions, depression, asthma, rheumatoid arthritis, colon cancer, and autism spectrum disorders.

Oral intake of diverse prebiotics, probiotics, and postbiotics may increase the resilience of gut bacterial communities, although for most of the currently available products there is no clear evidence yet to support beneficial effects on human health. FMT has been successful for the treatment of recurrent infections with *Clostridium difficile*, prompting its evaluation in other pathogenic conditions associated with intestinal dysbiosis (Allegretti et al., 2019). FMT may affect multiple phenotypes ranging from behavior to aging. For example, transfer of microbiota derived from MDD patients to germ-free mice confers them depression-like behavior (Cheung et al., 2019). Similar findings have been reported in the context of metabolic syndrome (Zhang et al., 2019) and immunotherapy responsiveness of cancer patients (Routy et al., 2018). Further research will be necessary to identify the mechanisms by which some bacterial species and their metabolites exert such effects.

In summary, local, organ-wide, and whole-body communication systems are built in a way that they can respond to perturbations by rapid adaptation and counter-regulation, yielding homeostatic resilience thanks to the activation of mostly negative feedback loops. Failure of such resilience mechanism due to excessive stress or enfeeblement of the reserve capacity finally lead to aging and disease (Table 1). Interventions aimed at enhancing homeostatic resilience represent promising strategies for the promotion of health.

Hallmark 7: Hormetic Regulation

Hormesis relies on biological processes in which low doses of toxins elicit a protective response that prevent the organism from experiencing harm on exposure to a higher dose of the same toxins (Gems and Partridge, 2008). The term "hormesis" is now widely used to describe the situation where low doses of stressors induce an adaptive response in cells and organisms to maintain homeostasis while increasing biological plasticity (Figures 5A and 5C) through the action of factors called hormetins (Calabrese, 2018).

Mitohormesis

This term defines the situation in which a mild and transient mitochondrial stress induces beneficial responses in a cell, tissue, or organism (Tapia, 2006), for instance in the context of exercise, caloric restriction, intermittent fasting, and dietary phytochemicals, which elicit the production of ROS by the respiratory chain (Ristow and Zarse, 2010). Low levels of mitochondrial ROS (mtROS) decrease the susceptibility to anoxia/reoxygenation damage in rat ventricular myocytes and induce strong protective actions in models of ischemia/reperfusion (Granger and Kvietys, 2015). mtROS might also contribute to explaining the so-called "cardiac preconditioning" effect, whereby brief periods of ischemia (e.g., with exercise) before prolonged coronary artery occlusion are cardioprotective, because they reduce the subsequent myocardial lesion size or the risk of ventricular fibrillation (Thijssen et al., 2018). Cardiac preconditioning effects also involve the activation of pro-survival kinases, an improved capacity of mitochondria to retain calcium, and the induction of myocardial heat shock proteins. Low levels of mtROS activate hormetic responses in stressed neurons by promoting the expression of protective genes including BCL2 and SOD2 (Sivandzade et al., 2019). This adaptive response protects neurons against more severe oxidative stress and diminishes the risk of oxidative and ischemic injuries. The broad relevance of ROS for triggering beneficial mitohormetic responses may explain the failure of clinical trials that aimed at revealing the health-promoting effects of antioxidants (Ristow, 2014). Conversely, several compounds widely used in clinical routine—metformin for diabetes and statins for lowering cholesterol—moderately increase mtROS levels (Piskovatska et al., 2020).

Downstream of ROS, several transcription factors can be activated to trigger efficient cytoprotective mechanisms, long-term metabolic alterations and enhanced stress resistance (Merry and Ristow, 2016). Transient knockdown of mitochondrial SOD in mice induces a mitohormetic response depending on the activation of the NRF2 antioxidant and PPARγ/PGC-1a mitochondrial signaling pathways (Cox et al., 2018). Similarly, the endogenous metabolite *N*-acetyl-L-tyrosine (NAT) triggers mitohormetic

responses that do not only involve mitochondrial ROS production but also activate FoxO, which in turn transactivates antioxidant genes and KEAP1 to elicit cytoprotective responses. NAT also represses tumor growth, likely through KEAP1 activation (Matsumura et al., 2020). The future elucidation of mitohormesis-stimulatory pathways may lead to the development of strategies to extend healthspan and lifespan (Table 1).

Healthspan

Healthspan, the length of time during which an individual is in reasonably good health, may be influenced by hormesis, for instance in the context of adequate diet and physical activity. These hormesis-based interventions also provide moderate but significant protection against cerebrovascular accidents, myocardial infarctions and neural degeneration. Such beneficial actions may rely on direct short-range cytoprotection through the induction of ROS, heat shock proteins, sirtuins, and thioredoxins (Calabrese et al., 2011). Alternatively, they may involve long-range intercellular communication systems via metabolic pathways, neural circuits, endocrine signals, and immune responses, such as the polarization of proinflammatory M1 toward the M2 phenotype, which facilitates protective, reparative, and anti-inflammatory responses (Calabrese et al., 2018).

Remarkably, stem cells exhibit hormetic responses to low doses of ionizing radiation, hypoxia, and chemical compounds (Gopi and Rattan, 2019), and this might help to improve their therapeutic potential in the repair of cardiovascular or neurological damage. The hormesis concept may also offer a new framework to preclinically evaluate and clinically develop novel drugs against neurodegenerative diseases or to improve biological performance and human health (Leri et al., 2020). Low doses of chemical carcinogens may protect against genotoxic and cytotoxic damage caused by later exposure to higher doses (Nohmi, 2018), and similarly, antineoplastic drugs may elicit hormetic-like dose responses in cultured cancer cells (Cho et al., 2018). Thus, the hormetic theory may guide dose finding studies and help optimizing the timing of drug administration to maximize beneficial effects.

Lifespan

Pioneering experiments in insect models revealed that low chronic exposure to ionizing radiation increased longevity (Shibamoto and Nakamura, 2018). Similarly, low-dose irradiated mice exhibited an increase in lifespan by ~20% coupled to features characteristic of healthy aging, such as weight maintenance, muscular strength, and fur quantity and quality (Shibamoto and Nakamura, 2018). Human fibroblasts subjected to low-dose ionizing radiation exhibited a hormetic response in terms of genomic stability and increased replicative lifespan. Moreover, there are isolated reports indicating that humans exposed to low-dose radiation have a reduced cancer incidence and increased longevity (Sutou, 2018), but large-scale epidemiological evidence in favor of this contention is still elusive.

These findings based on radiation-induced hormesis were extended to a series of longevity-increasing compounds, exemplified by synthetic chemical hormetins and dietary phytochemicals that are abundant in vegetables, fruits, spices, and seeds (Rattan, 2012). This particular form of hormesis has been called xenohormesis to emphasize the mutualistic relationship between plant and animal species (Howitz and Sinclair, 2008). Xenohormetins include a variety of phytochemicals such as flavonoids, organosulfur compounds, diferuloylmethanes, and stilbene derivatives. At high levels, these phytochemicals exhibit direct free radical-scavenging properties, but at the low concentrations present in the diet they have pro-oxidant and electrophilic properties that induce adaptive cellular stress response mechanisms via the KEAP1-NRF2 signaling pathway. Moreover, many xenohormetins induce autophagy, which maintains homeostasis and promotes healthy lifespan by removing damaged organelles and macromolecular structures (Menendez et al., 2013). Hormetic response patterns have also been detected after treatment with caloric restriction mimetics that show geroprotective actions likely through the induction of autophagy (Madeo et al., 2019). Low-intensity dietary interventions improve proteostasis and increase lifespan through ER hormesis. This process involves the activity of the IRE-1-XBP-1 branch of the unfolded protein response (UPR) of the ER (UPR-ER) and results in increased ER-associated degradation of misfolded proteins (Matai et al., 2019).

Hormetic responses are frequently diminished in aged model organisms. For example, preconditioning with various stressors reduces ischemia-induced heart damage in young adult mice and rats, but this protection is lost in aged rodents (Calabrese, 2018). An age-related decline in UPR-ER has also been detected (Ferguson and Bridge, 2016). Moreover, the hormetic response to persistent organic pollutants in patients with diabetes was blunted in the elderly (Lee, 2011). These findings are consistent with the idea that hormetic preconditioning pathways decay over time, thus eroding the capacity of the aging organism to adapt to endogenous and exogenous stressors.

In summary, hormetic responses to low doses of a broad spectrum of chemical, physical, pharmacological, and nutritional stressors can protect from subsequent threats. Hormetic responses are evolutionarily conserved and have been widely studied in animal models, but their application to human pathophysiology still presents serious limitations (Thayer et al., 2005). Unfortunately, hormetic effects resulting from low exposure to any of these stressors are moderate and may be difficult to assess, unless quantifiable endpoints are clearly defined and analyzed in clinical trials.

Hallmark 8: Repair and Regeneration

Organismal health is constantly threatened by multiple sources of intrinsic and extrinsic damage. This damage must be repaired and, whenever possible, lost functional elements must be regenerated to achieve full recovery (*reconstitutio ad integrum*). In contrast to turnover (see hallmark 3), which occurs without specific stimulation, repair and regeneration are stimulated responses that occur in a specific fashion in response to the precise type of damage inflicted to the system. Accordingly, cells have developed intricate signaling networks that systematically sense, and react to, specific types of damage in all strata of the body (Figure 5A) (Table 1).

DNA Damage and Repair

The integrity and stability of DNA is constantly challenged by exogenous chemical, physical, and biological cues, as well as by endogenous threats, such as DNA replication errors or spontaneous hydrolytic and oxidative reactions. This genotoxic stress

causes a variety of alterations in DNA including point mutations, insertions and deletions, translocations, chromosomal aneuploidies, telomere attrition, adduct formation, DNA-protein crosslinks, and gene disruptions resulting from retrovirus or transposon integration. These genomic lesions may be at the origin of numerous chronic diseases, as well as physiological and pathological aging. Cells possess a dense network of constitutive and inducible DNA repair systems—collectively conforming the DNA damage response (DDR)—to deal with the diversity of damages inflicted to nuclear and mitochondrial genomes (Colombo et al., 2020). Mutations in DDR genes cause a number of inherited diseases that are usually linked to accelerated aging and cancer, supporting the relevance of DNA repair for healthspan (Keijzers et al., 2017). The DDR machinery is also directly implicated in determining the fate of cells that have irreparable genomic damage. DDR effectors, such as TP53, drive cellular senescence or apoptosis and contribute to homeostasis preservation. Immune effectors participate in the elimination of DNA-damaged cells by inducing the expression of high levels of MHC class I molecules on the cell membrane, thus facilitating their destruction by cytotoxic T cells (Galluzzi et al., 2018). DNA damage also increases the expression of ligands for NK cells, which favors the removal of damaged cells. Cytosolic DNA accumulated during the DDR engages the cGAS/STING pathway and stimulates a local response that may facilitate tissue homeostasis. However, deregulated and excessive release of IFN causes uncontrolled inflammation and tissue damage, resulting in autoimmune disorders or pathological maladaptation to stressful situations such as myocardial infarctions (Burdette et al., 2011). In summary, cells respond to DNA damage by activating a series of integrated mechanisms that maintain microenvironmental and systemic homeostasis.

Protein Damage and Proteostasis

Proteins accumulate multiple types of damage including formation of advanced glycation end products, deamidation of Asn and Gln residues, aberrant disulfide crosslinks, amino- or carboxy-terminal truncation, internal cleavage, carbonylation, and formation of inappropriate aggregates. Protein aggregation typically occurs in neurodegenerative disorders such as Alzheimer's, Parkinson's, or Huntington's disease, as well other age-associated diseases (Kaushik and Cuervo, 2015). Therefore, cells use a series of sophisticated and energy consuming strategies to maintain protein homeostasis ("proteostasis") and to ensure that protein synthesis, folding, modification, trafficking, localization, concentration, and turnover are optimal. Proteostasis involves a network of factors that either assure the refolding and stabilization of misfolded proteins or target them for degradation (Morimoto, 2020). Protein (re)folding and stability is mediated by a large collection of cytosolic and organelle-specific molecular chaperones, including heat shock proteins (Hipp et al., 2019). Pharmacological induction of the heat shock response and overexpression of molecular chaperones exhibit protective activity in several disease models. For instance, delivery of a fragment of the chaperonin TRiC enhances proteostasis and improves cellular phenotypes in Huntington disease (Sontag et al., 2013). The two principal proteolytic systems implicated in protein quality control are the ubiquitin-proteasome system and the autophagy-lysosomal system (Pohl and Dikic, 2019). Deficiencies in either system collapse proteostasis and accelerate age-associated diseases, while genetic or pharmacological interventions that enhance proteostasis improve age-related phenotypes in animal models. Notably, inhibition of deubiquitinases, upregulation of proteasome subunits, and administration of autophagy inducers promote healthspan and lifespan in mice (Madeo et al., 2019).

The ER Stress Response

After ribosomal synthesis, most secreted or membrane proteins enter the ER, where they fold and assemble. To assess fidelity in protein folding and to avoid the accumulation of unfolded proteins in the ER lumen, cells have developed an adaptive response involving the activation of a series of signaling pathways collectively called the ER stress response or unfolded protein response (UPR) (Walter and Ron, 2011). There are three mechanistic modules of the UPR that together maintain homeostasis in the ER or induce apoptosis to remove cells that have been unable to recover proteostasis. These modules are based on the function of three stress sensors (PERK, ATF6, and IRE1) that evaluate the proteome in the ER lumen and induce bZIP transcription regulators, which in turn activate transcription of UPR target genes. The final outcome of this transcriptional activity is a decrease in the flux of proteins entering the ER and an increase of the protein folding capacity of the organelle. The molecular components of the UPR are transcriptionally regulated by the UPR itself, underscoring that this proteostatic response follows the same strategy of feedback loops used for most mechanisms involved in the functional maintenance of the whole organism under stress conditions. The ER stress response has a substantial impact on health, and deficiencies in different components of the system are at the heart of numerous diseases. Deficiency or saturation of the ER stress response does not only compromise proteostasis, but also triggers excessive cell death and inflammatory responses that—if unresolved—contribute to chronic maladaptation, metabolic disorders and accelerated aging (Hetz et al., 2020).

Mitochondrial Stress Responses

The mitoUPR is associated with a metabolic switch to glycolysis that favors mitochondrial repair (Costa-Mattioli and Walter, 2020). Similar to the UPR-ER, the mitoUPR involves a series of stress sensors, transducers, and transcription regulators, such as ATF4, ATF5, CHOP, and C/EBPβ4, and is relayed across the plasma membrane to modulate local and systemic adaptations to mitochondrial stress. For example, myocytes undergoing mitochondrial stress induce a transcriptional response that finally results in the release of mitokines such as GDF15 and FGF21 into the systemic circulation. On binding to GDNF family receptor α-like (GFRAL) on the surface of neurons of the brainstem, GDF15 controls appetite and feeding behavior, whereas FGF21 binds to its receptor FGFR1 and modulates lipid metabolism in the adipose tissue. This adaptive stress response pathway finally results in beneficial systemic effects via endocrine signaling (Klaus and Ost, 2020).

Lysosomal Damage Response

Lysosomal membrane permeabilization (LMP) and full rupture of late endosomes represent severe cellular stress conditions that play important roles in the context of degenerative diseases, microbial infection, and tumor progression. LMP is

either lethal for the cell or elicits a defense and repair mechanism known as the endo-lysosomal damage response (ELDR) (Papadopoulos et al., 2020). HSP70 chaperone binds to lipids of the damaged lysosomal membrane and recruits the ESCRT machinery to repair the perforated organelle. Lysophagy can be initiated by the influx of cytosolic galectins into the damaged organelles, followed by a process of massive ubiquitination of lysosomal proteins regulated by the ubiquitin-conjugating enzyme UBE2QL and final sequestration of the lysosome in autophagosomes (Koerver et al., 2019). In parallel, local damage stimulates lysosomal biogenesis through a signaling cascade that involves dissociation of the mTORC1 complex from lysosomes, the dephosphorylation of TFEB in the cytoplasm, its translocation into the nucleus, and the transactivation of TFEB-inducible genes.

Tissue-Level Regeneration

Regeneration consists in the full restoration of elements that have been injured or lost. Stem and progenitor cells present in rodents and humans possess the ability to repair damaged tissues and to favor adaptive and compensatory responses (Wu and Izpisua Belmonte, 2016). Such stem cells are even present in the adult brain, an organ long-time believed to be irreparable. Neural stem cells can self-renew and generate terminally differentiated neurons and glial cells. However, tissue-specific stem cells cannot regenerate entire organs (that are built of multiple cell types with a broad structural and functional diversity), perhaps with the exception of the liver that can regenerate fully functional hepatic lobules. Although mammals have lost most of their regenerative potential during phylogeny, they still exhibit surprising capacities in this regard during development and early in life. For example, neonatal mouse heart exhibits a substantial regenerative capacity, and regeneration of the digital phalanx has been described both in neonatal mice and young children (Miller et al., 2019). Furthermore, there is solid evidence that the ability of stem cells to repair or rejuvenate adult tissues declines with age (López-Otín et al., 2013). Thus, humans possess a latent capacity of regeneration that has been progressively silenced by evolution, development, and aging.

Cell Identity Reprogramming

Stem cells constitute the principal biological tool proposed by regenerative medicine to repair diseased or aged tissues and organs, as an alternative to the transplantation of cells and organs. Takahashi and Yamanaka (2006) demonstrated in 2006 that the introduction of four transcription factors (Oct3/4, Sox2, Klf4, and c-Myc) into somatic cells was sufficient to reprogram them to become induced pluripotent stem cells (iPSCs). Since then, reprogramming strategies have been employed *in vivo* in mouse models for the rejuvenation or reinvigoration of specific tissues (Abad et al., 2013; Kurita et al., 2018; Ocampo et al., 2016), or in combination with gene editing by CRISPR-Cas9, to correct inborn gene defects (Giacalone et al., 2018). The first successful utilization of autologous reprogrammed cells in humans concerns the treatment of age-related macular degeneration (Mandai et al., 2017). The differentiation of clinical-grade iPSCs into progenitor or somatic cells may lead to new cellular therapies for a wide range of diseases including diabetes, myocardial infarctions, Parkinson's disease, and spinal cord injury. This list is rapidly growing due to technological advances in this field, including the possibility of using cellular transdifferentiation methods to avoid traversing a pluripotent state, which would minimize the tumorigenic potential of these cells (Karagiannis et al., 2019). iPSCs are also being employed for producing xeno-organs and human organs in animals, which may eventually be used as transplants, pending the solution of technical and ethical issues (Wu et al., 2017).

Integration of Hallmarks

The current stigmata of health include features of spatial compartmentalization (hallmarks H1 and H2), maintenance of homeostasis over time (H3, H4, and H5) and an array of adequate responses to stress (H6, H7, and H8). Disruption of any of these features is highly pathogenic, causing an acute or progressive derailment of the system, meaning that manifest disease is usually connected to the loss of more than one of the hallmarks of health. Obviously, these hallmarks do not exist in isolation since they are highly interconnected at multiple levels. Thus, each of the organizational strata (molecules, organelles, cells, supracellular units, organs, organ systems, systemic circuitries, and meta-organism) of the human body crosstalk to any of the hallmarks of health (Figure 6A), as we will exemplify here.

Macromolecular Integration

Macromolecules such as proteins are simultaneously affected by many, if not all, of the eight hallmarks, as illustrated in pathological processes. For example, proteins like CFTR participate in the control of respiratory and intestinal barrier function (H1), as well as in the local limitation of chloride flux imbalances (H2). CFTR present in the plasma membrane must undergo constant recycling, and loss-of-function variants in the *CFTR* gene encoding this receptor cause cystic fibrosis and affect general proteostasis including autophagy (H3). CFTR is involved in multiple regulatory circuitries (H4), and its mutation can even disrupt circadian rhythm (H5). *CFTR* mutations associated with cystic fibrosis trigger a pathogenic—rather than homeostatic—feedforward mechanism (H6), causing tissue damage that self-perpetuates (H7) and cannot be fully repaired (H8), finally resulting in permanent respiratory and intestinal dysfunction that even can drive colorectal oncogenesis (Scott et al., 2020) (Figure 6B). A similar logic can be applied to proteins accumulating in neurodegenerative diseases that disrupt the blood-brain barrier (H1), propagate their misfolded state in a prion-like fashion (H2), subvert and avoid proteostasis (H3), disrupt neuroglial and synaptic communication (H4), abolish circadian rhythms (H5), engage in vicious feedforward (rather than homeostatic feedback) mechanisms (H6), fail to elicit hormesis (H7), and damage neurons and neuronal circuitries that cannot be repaired (H8).

Organellar Integration

The elementary building blocks of cells, the organelles, participate in each of the hallmarks. For example, mitochondria must maintain the integrity of their internal and external membranes to fulfill their function (H1) and to avoid unwarranted cell death as well as the aberrant activation of inflammatory pathways. The permeabilization of a few mitochondria can be spatially contained (H2) by fission/fusion cycles affecting the mitochondrial network coupled to mitophagy, as well as by the interruption of feedforward mechanisms that otherwise would cause cell death. Without continuous renewal by mitophagy/autophagy,

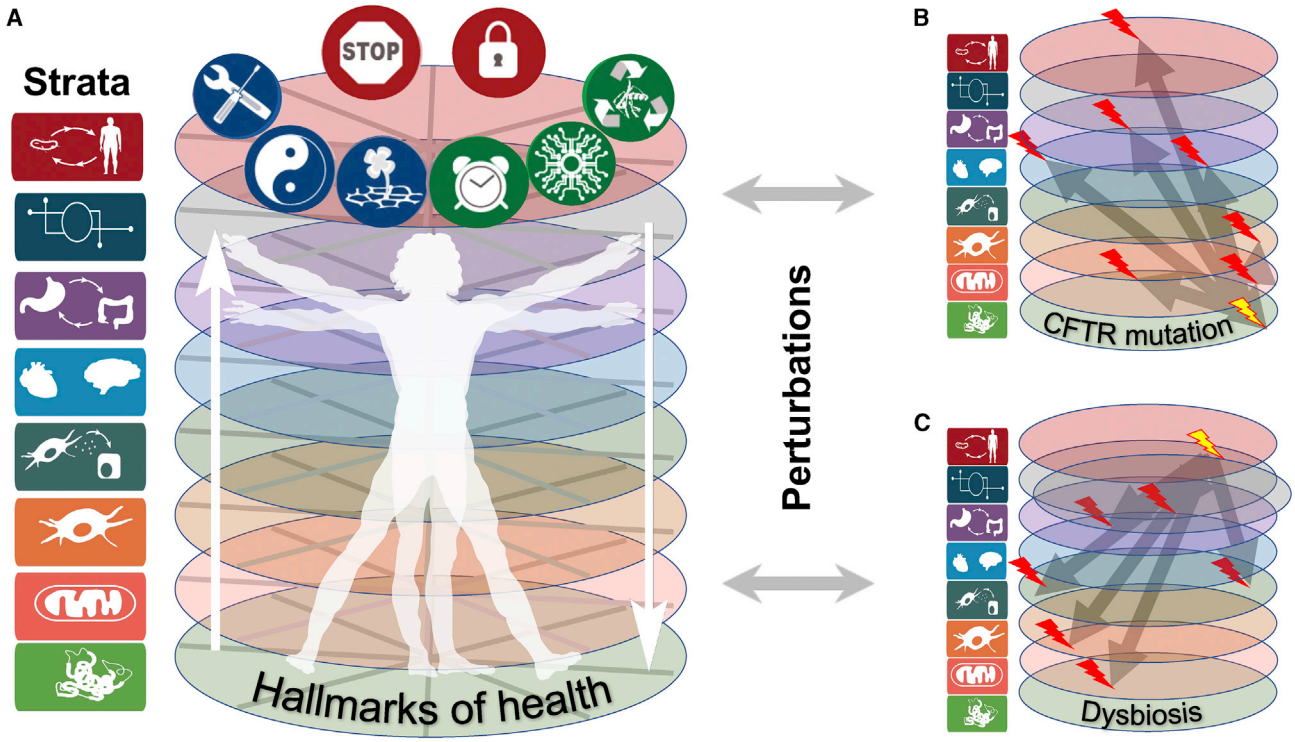

Figure 6. Spatiotemporary Trajectories of Health Perturbations

(A) The eight hallmarks of health integrate the multifunctionality of each hierarchical stratum and orchestrate the complex interactions of distinct subcellular, cellular, and supracellular compartments, supporting the multidimensional basis of health.

(B) Examples of trajectories emanating from one single molecular perturbation. CFTR mutations primarily affect turnover of the protein, but then spread through several strata of the organization affecting several hallmarks including, in the upper layer, the microbiota of the gut, and the respiratory tract.

(C) Examples of trajectories starting from the overgrowth of one single toxin-producing bacterium in the gut, hence saturating the organismal system down to the organellar and cellular levels, overcoming homeostatic and hormetic regulation, and durably damaging cells and organs. CFTR, cystic fibrosis transmembrane receptor.

mitochondria lose their function (H3). Mitochondria participate in many metabolic, signaling, and stress pathways assuring their integration into subcellular circuitries (H4). Embedded in multiple physiological networks that contribute to metabolic homeostasis (H5), they contribute to, and are influenced by, circadian rhythms (H5). Mitochondria recall, and mediate adaptation to, a diverse array of stress signals in the context of mitohormesis (H7). Finally, mitochondria may repair their DNA or undergo a mechanism of turnover favoring the selective replacement of damaged organelles (H8). Similar broad implications in all the hallmarks of health can be delineated for most if not all organelles including autophagosomes, lysosomes, the ER, or the nucleus. This implies that alterations in organelle-specific enzymes/pathways or structures (e.g., mitochondrial DNA, nuclear lamina, and pores) can affect all aspects of normal physiology, yielding a broad spectrum of pathological perturbations.

Cellular Integration

Normal epithelial stem cells contribute to homeostasis by maintaining plasma membrane integrity to avoid death (H1), repair perturbations in their membranes or replace missing cells at endangered barriers (H2), renew permanently (H3), participate in larger functional units (H4), respond to rhythmic oscillations (H5), and engage in homeostatic/hormetic resilience, for instance by recalling local perturbation in the skin to mediate accelerated wound healing responses on a second insult (H6–H8) (Naik et al., 2017). Serving as counterexamples, senescent and malignant cells perturb health at multiple layers. Senescent cells tend to lose the integrity of their nuclear envelope (H1), transmit the senescent phenotype to other cells (H2), fail to renew due to permanent cell cycle block (H3), lose their normal function and integration in the tissue (H4), become refractory to circadian oscillations (H5), engage in pro-inflammatory feed-forward loops (H6), trespass the threshold for hormetic regulation (H7), and cannot actively participate in tissue regeneration (H8), although they may emit signals to favor wound healing. Cancers arising from malignant cells disrupt the integrity of epithelial barriers (H1), overcome local containment by immunosurveillance (H2), undergo excessive proliferation beyond the limits of mere renewal (H3), perturb the integration of circuitries at the levels of the circulatory and neurovegetative systems (H4), escape from or subvert circadian rhythms (H5), and selfishly resist therapeutic challenges by homeostatic, hormetic, and regenerative pathways (H6–H8) at the expense of organism-wide health-preserving circuitries. Thus, cell-autonomous alterations may endanger superior levels of organization to compromise organismal health.

Integration of Supracellular Units

Tissue-sessile macrophages which, together with fibroblasts, participate in the stroma of all organs, sense perturbations at external and internal barriers (H1), engulf and eliminate dead cells (H2), signal for their replacement (H3), execute and regulate inflammatory and immune responses (H4), follow rhythmic oscillations (H5), recall a prior exposure to TLR4 ligands to reduce a subsequent response (H6), but increase such a TLR4-elicited arousal after prior exposure to viral TLR agonists (H7) (Wang et al., 2019b), and play a major role in tissue repair (H8). Intestinal crypts exemplify minimal units of an organ that maintains the integrity of the local barrier (H1), contains local perturbations (H2), constantly recycles (H3), communicates with the microbiota and the immune system while producing metabolism-relevant hormones (H4), constitutes a peripheral clock (H5), adapts to changing nutritional and microbial challenges (H6 and H7), and is endowed with regenerative capacities (H8). In contrast, intestinal dysbiosis is broadly pathogenic because it can erode the barrier function of the ileum and the colon (H1), with systemic effects on metabolism that trespass the local environment (H2), affect the recycling and turnover of intestinal epithelial stem cells (H3), saturate neuroendocrine circuitries by bacterial metabolites and toxins (H4), perturb peristalsis while uncoupling the gastrointestinal tract from circadian rhythms (H5), durably disrupt the microbial ecosystem (H6), and activate systemic inflammatory responses while dampening the immune tonus (H7), with long-distance effects on tissue repair and aging (H8) (Figure 6C). Similarly, obesity with metabolic syndrome subverts health at multiple levels because it affects the integrity of the intestinal barrier (H1), compromises cancer immunosurveillance (H2), blocks autophagy (H3), perturbs metabolic and hormonal circuitries by excessive levels of glucose and insulin (H4), desynchronizes circadian rhythms (H5), abolishes appetite control (H6), subverts hormetic longevity pathways (H7), and compromises wound healing (H8). These examples illustrate some of the connections among supracellular regulation and the hallmarks of health.

Integration among Distinct Strata

In the aforementioned examples, perturbations affecting distinct strata of organismal building blocks (from molecules to the meta-organism) have been enumerated. However, the hallmarks of health may also provide a theoretical framework to explain vertical connections (“leaps”) between distinct levels of (dis)organization (Figures 6B and 6C). Thus, a monogenic disease affecting the structure/function of a single protein may compromise the hallmarks of health across all layers of organization, well beyond the molecular stratum, as we already discussed for cystic fibrosis. Thus, *CFTR* mutation ultimately even disrupts subtle equilibria of the meta-organism, affecting not only the respiratory but also the intestinal microbiota. Similarly, Wilson’s disease (caused by a mutation in *ATP7B* encoding a copper-extruding enzyme), does not only increase hepatocyte vulnerability to copper toxicity but also induces a characteristic spectrum of psychiatric and behavioral abnormalities (Członkowska et al., 2018). An early sign of Parkinson’s disease, theoretically a molecular and cellular disease of dopaminergic neurons in the striatum, is constipation (Hustad and Aasly, 2020), while Huntington’s disease, another neurodegenerative condition, is tied to prodromal alterations in whole-body metabolism causing an increase in energy expenditure and subsequent weight loss (Pratley et al., 2000). Neurodegenerative and psychiatric diseases are also tightly correlated to alterations in the microbiota, illustrating yet another consequence of the intimate connections among all strata of organization. The current understanding of these interconnections is in its infancy, calling for efforts to understand “leaps” in disease manifestations across all layers of the organism.

Loss of Health and Spreading of Disease

The principal characteristics of health enumerated here are usually not lost one by one. Rather, the collapse of the organizational features that normally maintains a salutary state occurs in a domino-like cascade. For this reason, a major “event” like stroke, myocardial infarction, or cancer is usually followed by the accelerated advent of another “event,” as compared to age-matched healthy controls (Narayan et al., 2020). There are several non-exclusive hypotheses to explain spreading of health deterioration beyond nosological entities and anatomical boundaries. First, the manifestation of a major, life-threatening disease may reflect a general, often age-linked derailment of health, indicating an individual’s descending trajectory. For example, cancer and atherosclerosis share common risk factors, as well as disease mechanisms including chronic inflammation and poor clearance of aberrant cells. Second, the catastrophic event marking the clinical manifestation of a disease may itself trigger a further deterioration of health as suggested for an excessive, pro-inflammatory activation of the sympathetic system post-myocardial infarction, thereby accelerating atherosclerosis (Dutta et al., 2012). Thus, the disorganization of health-preserving circuitries implies common patterns in disease pathogenesis and interconnections between diseases, explaining some of their common features, as well as the epidemiological connections between distinct diseases that tend to progress from mono- to oligo- or polypathological states.

In sum, health can be viewed as the holistic property of a multidimensional framework of distinct vertical/hierarchical strata that are organized in horizontal hallmarks (Figure 6). Health deterioration follows a spatiotemporary trajectory across strata and hallmarks, leading to pathological perturbations that usually spread to the system when the capacity of any of strata/hallmarks to recover their function has been lost. This has major implications for medical interventions that only can be fully efficient if they succeed in restoring or maintaining all the hallmarks of health.

Final Speculation

There are numerous challenges ahead with respect to the final definition and interconnectedness of the proposed biological hallmarks of health, as well as their further integration with the organization of health care systems and socioeconomic aspects (Berwick and Cassel, 2020; Snyder-Mackler et al., 2020). Prospective and integrative personalized omics profiling—increasingly at the single-cell level—are discovering clinically actionable conditions, as well as novel molecular pathways associated with oncologic, cardiovascular, and metabolic pathophysiology (Schüssler-Fiorenza Rose et al., 2019). Thus, large-scale epigenomic, metabolomic, and metagenomic studies are providing valuable information about epigenetic marks, specific metabolites, and microbiota components associated with human health

(Bell et al., 2019; The Integrative, 2019; Koh and Bäckhed, 2020). Longitudinal and deep multi-omics profiling of healthy individuals revealed different types of aging patterns (ageotypes), depending on specific molecular pathways that changed over time in each individual (Ahadi et al., 2020). Comprehensive proteomic analyses of 16,894 individuals identified plasma protein patterns for 11 different health indicators: liver fat, kidney filtration, percentage body fat, visceral fat mass, lean body mass, cardiopulmonary fitness, physical activity, alcohol consumption, cigarette smoking, diabetes risk, and primary cardiovascular event risk. This approach opens the possibility of using the blood proteome for "liquid health checks" (Williams et al., 2019). Moreover, the combination of multidimensional information provided by connected objects and ever more sophisticated sensors, data-driven science, as well as by artificial and human intelligence (Topol, 2019), will transform our vision of health into a high-definition model similar to that recently proposed for high-definition medicine (Torkamani et al., 2017).

Potentially, a future "medicine of health" might detect perilous trajectories to intercept them by targeted interventions well before the traditional "medicine of disease" comes into action. In this context, the conceptualization of the hallmarks of health might establish a framework for future mechanistic studies, for programming algorithms that integrate biomedical parameters, and for designing interventions on human healthspan and lifespan.

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The authors declare that they do not have any manifest conflict of interest. C.L.-O. has ownership interest including stock and patents in DREAMgenics. G.K. has been holding research contracts with Bayer Healthcare, Daiichi Sankyo, Glaxo Smyth Kline, Genentech, Eleor, Institut Mérieux, Kaleido, Lytix Pharma, PharmaMar, Sotio, and Vasculox/Tioma. G.K. is on the Board of Directors of the Bristol Myers Squibb Foundation France. G.K. is a scientific co-founder of everImmune, Samsara Therapeutics, and Therafast Bio. G.K. is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis, and metabolic disorders. G.K.'s wife, Laurence Zitvogel, has been holding research contracts with Glaxo Smyth Kline, Incyte, Lytix, Kaleido, Innovate Pharma, Daiichi Sankyo, Merus, Transgene, Tusk, and Roche, is on the Board of Directors of Transgene, a co-founder of everImmune, and holds patents covering the treatment of cancer and the therapeutic manipulation of the microbiota. G.K.'s brother, Romano Kroemer, is an employee of Sanofi.

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