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REVIEW

Cellular polarity in aging: role of redox regulation and nutrition

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Abstract Cellular polarity concerns the spatial asymmetric organization of cellular components and structures. Such organization is important not only for biological behavior at the individual cell level, but also for the 3D organization of tissues and organs in living organisms. Processes like cell migration and motility, asymmetric inheritance, and spatial organization of daughter cells in tissues are all dependent of cell polarity. Many of these processes are compromised during aging and cellular senescence. For example, permeability epithelium barriers are leakier during aging; elderly people have impaired vascular function and increased frequency of cancer, and asymmetrical inheritance is compromised in senescent cells, including stem cells. Here, we review the cellular regulation of polarity, as well as the signaling mechanisms and respective redox regulation of the pathways involved in defining cellular polarity. Emphasis will be put on the role of cytoskeleton and the AMP-activated protein kinase pathway. We also discuss how nutrients can affect polarity-dependent processes, both by direct exposure of the gastrointestinal epithelium to nutrients and by indirect effects elicited by the metabolism of nutrients, such as activation of antioxidant response and phase-II detoxification enzymes through the transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2). In summary, cellular polarity emerges as a key process whose redox deregulation is hypothesized to have a central role in aging and cellular senescence.

Keywords AMPK · Hydrogen peroxide · Cytoskeleton · Reactive oxygen species · Tight junctions · Asymmetrical inheritance · Nrf2

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Introduction

In 1956, Denham Harman formulated the free radical theory of aging, suggesting that free radicals produced during aerobic respiration cause cumulative oxidative damage, resulting in aging and death (Harman 1956). This theory gained support due to several observations including the cellular increase in reactive oxygen species (ROS) levels and the accumulation of oxidation products with aging (for a review Beckman and Ames 1998). The prevailing view was that ROS were damaging species that would oxidize biological targets, such as lipids, DNA, and proteins, causing a series of cellular malfunctions that would result in pathologies and aging. In this context, oxidative stress was defined as the "a disturbance in the pro-oxidant–antioxidant balance in favor of the former, leading to potential damage" (Sies 1986).

At the end of the eighties, several near simultaneous observations confirmed the previous speculative hypothesis that ROS could also have an important regulatory role in the cell, opening the field of redox regulation. Concerning hydrogen peroxide (H_2O_2), the most common and stable

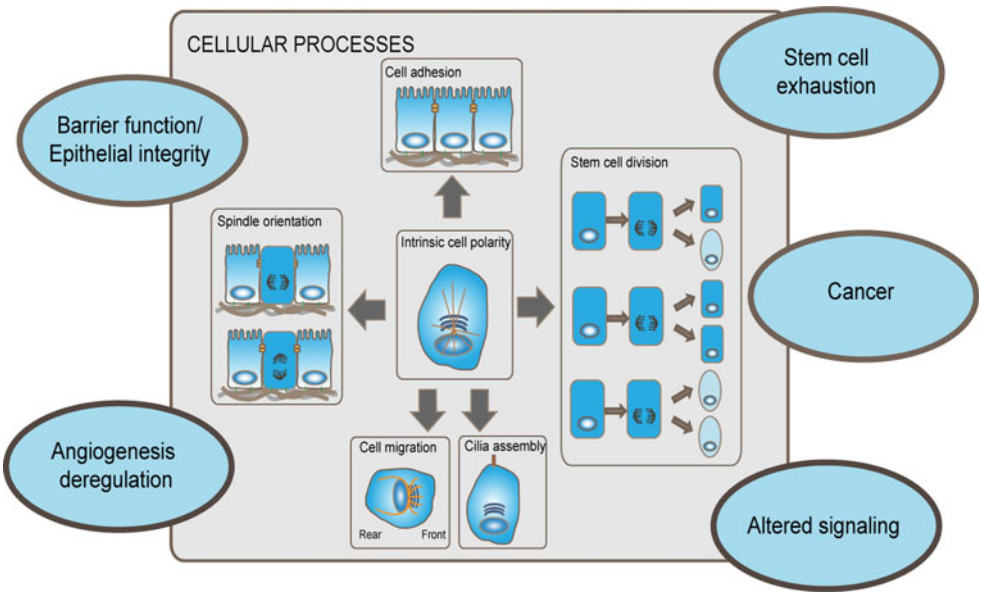

Fig. 1 Polarity at cellular and multicellular level. Intrinsic polarity is at basis of front and back polarity in migrating cells, focalized cilia assembly, asymmetrical inheritance as in stem cell division, and correct positioning of daughter cells in tissues by coupling with the position and orientation of the mitotic spindle. All these processes are altered during aging, resulting in stem cell exhaustion, cancer, deregulation of angiogenesis, and cell signaling and compromised barrier function

ROS, several transcription factors—such as activator protein-1 (AP-1), hypoxia-inducible factor 1 (HIF-1), nuclear factor (erythroid-derived 2)-like 2 (Nrf2), and OxyR or nuclear factor kappa B (NF-κB)—and signaling molecules—such as protein kinases and phosphatases—were identified as being under its regulation. This redox regulation by H_2O_2 involves direct or indirect oxidation and reduction of reactive protein thiols (Flohé 2010). The direct reactions of H_2O_2 with phosphatases and kinases containing reactive SH groups are apparently not relevant in vivo, since the rate constants are too low (Winterbourn and Hampton 2008). However, H_2O_2 derivatives, like peroxymonophosphate (LaButti et al. 2007) and peroxymonocarbonate (Trindade et al. 2006; Zhou et al. 2011), show higher reactivity toward SH groups. Also, alternatively, sensor molecules such as peroxiredoxins may act as intermediates in the activation of the H_2O_2-dependent signaling cascade (D'Autréaux and Toledano 2007; Woo et al. 2010; Flohé 2010). According to this role of ROS in redox regulation, oxidative stress was redefined as a disruption of redox signaling and control (Jones 2006). In this framework, it was proposed that the contribution of oxidative damage to senescence-related functional losses is relatively minor compared to that emanating from the derangement of the redox state (Sohal and Orr 2012). Here, we apply this hypothesis to analyze whether redox deregulation of cell polarity could be a cause of aging and associated pathologies, also taking into consideration that oxidative damage accumulation has to be dealt with in senescent cells.

Cell polarity can be viewed as an asymmetric distribution and spatial arrangement of biomolecules, cellular components (e.g., membrane domains and organelles such as the Golgi apparatus, mitochondria, cilia, and others), and structures (e.g., cytoskeletal filaments and centrosome) where their specific positioning in the cell, in close relationship with their functions, generates a structural/functional asymmetry that can be conserved and transmitted to new cells during cell division (Bornens 2008; Budovsky et al. 2011).

This organizational/functional asymmetry is required for a variety of cell functions in both unicellular and multicellular organisms (see Fig. 1) such as correct cell division, differentiation (where it is critical to determine the fate of daughter cells upon division as in the case of the asymmetric division of stem cells), protrusion, motility, and cell migration (Bornens 2008; Etienne-Manneville 2008; Tavares et al. 2012; Yamashita and Fuller 2008). There is also growing evidence that disruption of cell polarity is a hallmark of cancer (for review, see Lee and Vasioukhin 2008). Moreover, in mammalian cells, polarity can be challenged by environmental cues and cells are able to remodel their intrinsic polarity (Fig. 1), e.g., by repositioning the centrosome, the major microtubule-organizing center (MTOC) of animal cells, Golgi apparatus, and assembling primary cilia (Bornens 2008; Dupin and Etienne-Manneville 2011; Luxton and Gundersen 2011). Alterations of the polarized architecture of epithelia tissue may also underlie cancer development and progression by affecting cell–cell adhesion and cell matrix adhesion, which are key players in the establishment of tissue polarity (McCaffrey and Macara 2011).

Taking into account the fundamental importance of intrinsic cell polarity in the maintenance of tissue 3D organization, signaling pathways, and correct cell division, it is conceivable that the molecular mechanisms required

for the establishment and maintenance of this general cellular feature may be at an intersection with the regulating molecular mechanisms and signaling pathways underlying aging and age-related diseases. In this article, we review the mechanisms by which ROS regulate polarity, how this regulation can be impaired during aging and whether nutritional interventions can be helpful in reversing the age-dependent alterations in polarity.

Aging and polarity

Several important physiological processes that are dependent on cellular polarity are altered during aging. Next, we briefly describe age-associated changes found in angiogenesis, barrier function, cancer, and stem cell asymmetric division.

Angiogenesis

Endothelial dysfunction is one of the major causes of morbidity and mortality in the elderly. The regulation of blood vessel formation and maintenance is a crucial step for the development of many diseases, particularly those associated with aging such as ischemic injury, atherosclerosis, age-related nephropathy, and cancer.

New blood vessel formation in the adult occurs mainly through sprouting of new capillaries from preexisting vessels in a process known as angiogenesis (Herbert and Stainier 2011). More recently, the formation of blood vessels from endothelial progenitor cells has also been described in cases of angiogenic stress such as cancer and ischemic injury in the adult (Real et al. 2008). Endothelial cells have a quiescent phenotype until they are activated by angiogenic cues such as vascular endothelial growth factor (VEGF). Only a small number of the endothelial cells (tip cells) present in blood vessels will be selected to form new sprouts, acquiring a motile and invasive phenotype; they lose cell–cell contacts and polarize in the direction of the source of the pro-angiogenic factor. These events are crucial for the formation of a functional vessel network. Sprouting proceeds in a directional way until the newly formed vessel connects and undergoes anastomosis (Eilken and Adams 2010).

Elderly people have increased vessel stiffness and thickness, impairing vascular function, and show reduced vessel density and reduced angiogenesis after ischemia. These alterations are strongly associated with cardiovascular disease, hypertension, and kidney disease (Lähteenvuo and Rosenzweig 2012). This indicates that the molecular mechanisms underlying the angiogenic process depend both on the injury site and physiological context.

Barrier function

Permeability barriers have a key role in the maintenance of organism entity and function. In multicellular organisms, endothelial and epithelial barriers have important physiological roles keeping fluids apart and maintaining solute gradients. They are also important as a defense barrier against microorganisms. These barriers are often leakier in many diseases, and strong evidence has been found showing that several barriers become leakier with aging, namely the gastrointestinal barrier (Hollander and Tarnawski 1985; Katz et al. 1987), the lung (Tankersley et al. 2003), the blood–brain barrier (Blau et al. 2012; Lee et al. 2012), and the epididymis (Levy and Robaire 1999). More specifically, the compromise in the barriers occurs at the level of tight junctions, which seal adjacent cells just beneath their apical surface and decrease the paracellular flux across the barrier. The main components of tight junctions connecting cells are several claudins and occludins, which are connected to the cytoskeleton by the zonula occludens proteins. Decreases in the number of structural components of the barrier are often observed in aging organisms (Lee et al. 2012; Levy and Robaire 1999; Mooradian et al. 2003; Reznick et al. 2007), but changes in signaling pathways, like protein kinase C (PKC), rapidly accelerated fibrosarcoma kinase/mitogen-activated protein kinase kinase/extracellular-signal-regulated kinase (RAF/MEK/ERK), and glycogen synthase kinase 3 (GSK-3) and AMP-activated protein kinase (AMPK) that regulate those structural components or the assembly of the tight junctions may also be responsible for the age-related changes (Mullin et al. 2005; Zhang et al. 2006, 2011). Whether the decreased barrier function is a cause or an effect of aging, it remains to be determined.

Cancer

Many critical proto-oncogenes, e.g., apical cell polarity-protein aPKC, and tumor suppressor genes, e.g., *Drosophila* polarity proteins lethal giant larvae (Lgl), scribble (Scrib), and discs large (Dlg) proteins, have been identified as polarity factors. Conversely, polarity factors have been implicated in tumor formation and progression, as in the case of the human polarity liver kinase LKB1 (PAR-4 ortholog) whose mutations cause the Peutz–Jeghers syndrome, which is characterized by gastrointestinal polyps and predisposition to several types of cancer, including pancreatic, ovarian, and breast cancer (Katajisto et al. 2007). LKB1 mutations have also been found in sporadic carcinomas of the lung, pancreas, ovary, and endometrium as well as in cervical cancer (Hezel and Bardeesy 2008; Partanen et al. 2009; Wingo et al. 2009). More recently, it has been shown that LKB1 has a tumor suppressor function in the mammary gland, which is coupled to the maintenance of epithelial integrity (Partanen et al. 2012).

Alterations in the polarization of epithelia tissue may underlie cancer development and progression (McCaffrey and Macara 2011). The event of epithelial–mesenchymal transition that is associated with many carcinomas leads to the loss of apical–basolateral polarity and is characterized by the loss of several markers of epithelial differentiation such as E-cadherin in adherens junctions, transmembrane proteins, including claudins, occludins, and junctional adhesion molecules (Thiery and Sleeman 2006). The loss of cell–cell junctions accompanied by the loss of primary cilia and deregulated proliferation due to the loss of contact inhibition may underlie the appearance of invasiveness phenotypes of cancer cells (Christiansen and Rajasekaran 2006) and metastasis. These alterations may also be reflected in the deregulation of asymmetric divisions of stem cells that may be in the origin of abnormal self-renewal and the appearance of cancer stem cells.

Stem cells

Somatic stem cells have a key role in tissue homeostasis, and a decline in either stem cell number or proliferation may lead to tissue degeneration associated with disease or aging (Liu and Rando 2011; Yamashita et al. 2010). There are clear evidences that in tissues with a high cellular turnover, stem cells undergo a time-dependent functional decline (reviewed in Rando 2006; Sharpless and Depinho 2007).

The hallmark of stem cells is their ability of self-renewal while also being capable of generating differentiated daughter cells (Lin 2008). Stem cell division can be symmetric or asymmetric (Florian and Geiger 2010; Yamashita et al. 2010), and the self-renewing ability of stem cells is tightly related to their ability to undergo self-renewing asymmetric divisions. A typical asymmetric division will give rise both to a daughter stem cell and to a more differentiated daughter cell. This unique asymmetry allows a stem cell to self-replicate while producing a differentiated progeny (Lin 2008). A possible mechanism used by mammalian stem cells in order to protect themselves from cellular senescence could be a selective segregation of damaged cellular biomolecules to their differentiating rather than to their self-renewing progeny (Liu and Rando 2011). Such a protective mechanism has been identified in yeast cells and *E. coli* (Aguilaniu et al. 2003; Erjavec et al. 2007, 2008; Lindner et al. 2008; Liu et al. 2010, 2011; Tessarz et al. 2009) and will be described in detail below.

Control of polarity

Cellular mechanisms

Cells are able to remodel their intrinsic polarity, for example by repositioning the centrosome, Golgi apparatus, and assembling primary cilia (Bornens 2008; Dupin et al. 2009; Luxton and Gundersen 2011). In most of the cells, the microtubule and actin networks play a critical role in the definition of the cell polarity. In the case of microtubules, in animal interphase cells, they constitute an aster that radiates from the centrosome, located at the cell center in close association with the nucleus, to the periphery (de Anda et al. 2005). During cell migration, this symmetric arrangement is broken, which may be accomplished by (1) repositioning the centrosome, (2) by adding an asymmetric microtubule nucleation site, (3) by reorganizing the microtubules network, or (4) by altering microtubules dynamics (Vinogradova et al. 2009).

The relative position of the centrosome to the nucleus is generally a good indicator of the orientation of cell polarity and has been shown to be essential, for several cellular functions and for the early development of *Caenorhabditis elegans* (Malone et al. 2003). Moreover, it is under regulation and changes during cell-state transitions such as the establishment of immune synapses, wound healing, cell migration, and cell growth and differentiation (de Anda et al. 2005; Yvon et al. 2002). The positioning of the centrosome and cytoplasmic organization is highly dependent on geometrical constraints imposed by both the substratum/cellular matrix and cell–cell interactions (Dupin et al. 2009; Pouthas et al. 2008), which should be relevant for cells in tissues. In addition, forces exerted by microtubules at the cell cortex and forces exercised on them by actomyosin and dynein are also crucial to position the centrosome (Burakov et al. 2003).

Remarkably, a structural and functional asymmetry is already observable between the two centrioles in the centrosome (Yamashita and Fuller 2008), which creates an intrinsic polarity of this structure. This is a consequence of the duplication and maturation processes of the centrosomes during cell cycle (for review, see Brito et al. 2012). In G1, the centrosome contains an older mature centriole (mother centriole) and an immature centriole (daughter centriole). The mother centriole has appendages on its distal and subdistal ends and is more robust in microtubule anchoring (Bornens 2002). Also, the distal appendages allow membrane anchorage during its transformation in basal body during primary cilium assembly.

The stem cells asymmetric cell divisions seem to depend on this structural and functional centrosomal asymmetry. In *Drosophila melanogaster*, male stem cells and neuroblasts present asymmetric divisions that control which of the two sibling cells will possess the stem cell identity or has the fate to differentiate. These asymmetric divisions seem to depend on the regulated orientation of the mitotic spindle (Cheng et al. 2008; Yamashita et al. 2007). Interestingly, the centrosome with the oldest, "grandmother," centriole, which localizes cortically close to the stem cell hub, is kept

by the germline stem cell, whereas the other is inherited by the cell committed to differentiation (Cheng et al. 2008; Rebollo et al. 2007; Wang et al. 2009). It has been proposed that the fate of the two daughter cells can be related to the fact that the one inheriting the older centriole is the first to assemble a primary cilium (Anderson and Stearns 2009), rendering each daughter cell differentially prompted to be challenged by environmental signals.

On the other hand, the Golgi apparatus was recently shown as having the ability to nucleate microtubules (Efimov et al. 2007; Rivero et al. 2009), playing a role in the generation of asymmetry in microtubule networks. Centrosome repositioning is also involved in the relocation of the pericentrosomal Golgi. Perturbing Golgi apparatus positioning, by disconnecting it from the centrosome, has a more dramatic effect on directional cell migration than disrupting the Golgi apparatus itself (Hurtado et al. 2011). This intrinsic polarity axis is also probably interlinked to the establishment of a close relationship between the cytoskeleton and the compartmentalization of membrane lipid microdomains such as lipid rafts. For example, Gómez-Moutón et al. (2001) have observed that acquisition of a motile phenotype in T lymphocytes results in the asymmetric redistribution of ganglioside GM3- and GM1-enriched raft domains to the leading edge and to the rear, respectively, that requires an intact actin cytoskeleton, but is unaffected by microtubule depolymerization. These authors also showed that membrane cholesterol depletion inhibits acquisition of the polarized cell phenotype and prevents both cell–cell interaction and cell chemotaxis.

In vertebrates, almost every cell type can assemble a primary cilium, usually in G0 or G1. To assemble the cilium, the centrosome migrates to the cell surface where the mother centriole docks to the plasma membrane becoming a basal body, which assembles the ciliary axoneme (Satir et al. 2010). Cilia are important sensory/signaling organelles playing crucial roles in both the physiology and development of vertebrates. Therefore, the assembly of primary cilia creates a specific cellular polarized territory devoted to concentrate and transmitting signals from environment to the cell body (see Fig. 1). Indeed, it is now well established that cilia have major roles in signaling pathways such as Hedgehog (Hh), Wingless (Wnt), planar cell polarity, and platelet-derived growth factor receptor-α signaling (PDGFRA) (Gonçalves et al. 2010). Defective ciliary signaling may have direct implications in development and cancer, as deregulation of ciliary Hh has been associated with several types of human cancers. Disruptions of the primary cilium are also linked with ciliopathies, a plethora of human diseases, like Alstrom, Bardet-Biedl, Joubert and Meckel-Gruber syndromes, retinal degeneration, obesity, polycystic kidney, and neural tube defects (Goetz and Anderson 2010; Hildebrandt et al. 2011; Nigg and Raff 2009).

Signaling mechanisms, redox regulation, and oxidative damage

AMPK as a key sensor

The molecular mechanisms involved in the establishment of cell polarity and their implications in cell function and organisms' physiology are far from being completely understood mostly because many of the proteins involved in cell polarity are usually involved in a myriad of different signal pathways. For example, the four protein kinases of the microtubule-affinity regulating kinase family (MARK/Par-1) have a variety of crucial cellular roles ranging from the establishment of cell polarity, cell cycle control, and intracellular transport to cell migration (for review, see Matenia and Mandelkow 2009). Many of these functions are related to the dynamics and polarity of the cytoskeletal microtubule and actin networks. In fact, MARK2/Par-1b phosphorylates the microtubule-associated protein *tau,* which causes the release of the protein from the microtubules and their consequent destabilization. Hyperphosphorylation of *tau* is an early mark of Alzheimer's disease (Chin et al. 2000). Although other kinases like cyclin-dependent kinase 5 (CDK5), GSK-3, and ERK2 phosphorylate *tau* (Duan et al. 2012), MARK2/Par-1b has a key role in neuronal polarity because its depletion by using RNAi induces multiple axons in hippocampal neurons, whereas its overexpression inhibits axon and dendritic formation. Moreover, MARK2/Par-1b is also involved in neuronal migration through its ability to regulate cellular polarity and microtubule dynamics (Sapir et al. 2008). Besides these data, studies in knockout mice have implicated MARK2/Par-1b in the regulation of fertility (Bessone et al. 1999), immune cell function (Hurov et al. 2001), spatial learning and memory (Segu et al. 2008), the positioning of nuclei in pancreatic beta-cells (Fu et al. 2009; Granot et al. 2009), as well as adiposity, insulin hypersensitivity, and glucose metabolism (Hurov et al. 2007). More recently, studies in knockout mice showed that loss of MARK3/Par-1a leads to reduced adiposity (animals present decreased white and brown adipose tissue mass), resistance to hepatic steatosis, and defective gluconeogenesis not acquiring weight under a high-fat diet (Hurov et al. 2007).

Another key factor in the establishment of cell polarity relayed through MARK/PAR-1 in different cell types, including neurons and epithelial cells in invertebrates and vertebrates, is the tumor suppressor kinase LKB1 (for review, see (Mirouse and Billaud 2011). LKB1 activates the evolutionary conserved AMPK family (Lizcano et al. 2004) that is also activated by increases in cellular ADP:ATP and AMP:ATP ratios and is involved in the maintenance of energy balance by favoring catabolic

versus ATP-consuming anabolic pathways and a suppressor of cell proliferation (Hardie 2011).

AMPK is also required for tight junction assembly since hyperactivation of AMPK can improve the stability of tight junctions under conditions of calcium depletion (Zhang et al. 2006; Zheng and Cantley 2007). This suggests that the establishment of epithelia tight junctions may be able to respond to energy stresses. Tight junction assembly can also be stimulated by lymphocytes. This assembly requires AMPK activation, which is dependent on the pro-inflammatory cytokine TNF-α but independent of changes in cellular ATP levels (Tang et al. 2010). On the other hand, activation of the AMPK pathway suppresses axon initiation differentiation and neuronal polarization by preventing PI3K targeting to the axonal tip (Amato et al. 2011). It was also shown that phosphorylation of the microtubule plus end protein CLIP-170 by AMPK is required for microtubule dynamics and the regulation of directional cell migration (Nakano et al. 2010). Therefore, AMPK has highly conserved roles across metazoan species not only in the control of metabolism, but also in the regulation of tissue/cellular structures. Supporting this idea is the fact that in *Drosophila,* the LKB1-AMPK polarity pathway leads to the activation of Myosin II with impact on actin cytoskeleton (Lee et al. 2007). Moreover, it seems plausible that polarization involving the actin cytoskeleton will require a cross talk with the extracellular matrix because it has been shown that in epithelial cells, dystroglycan controls the localization of Myosin II in the basal membrane (Kachinsky et al. 1999; Mirouse and Billaud 2011).

Active AMPK localizes at the centrosome, spindle midzone, and midbody during cell division (Vazquez-Martin et al. 2009), which led to the suggestion that AMPK could regulate cell division by integrating information coming from the physiological/metabolic state of the cell. Altogether, these data sustain that the LKB1-MAPK pathway acts as a "tumor suppressor" due to the fact that it links cell polarity to proper cell division, which in turn responds to signals from extracellular matrix, cell–cell contacts, and the energetic status of the cell. Additionally, AMPK is p53-dependent up-regulated in response to DNA damage, which results in the inhibition of the mammalian target of rapamycin complex 1 (mTORC1) activity. The inhibition of mTORC1 has several consequences, namely inhibition of lipid biosynthetic pathways that participate to the malignant process in a wide range of cancers (Mirouse and Billaud 2011), and inhibition of mitochondria proliferation and function (Zoncu et al. 2011). mTORC2, a rapamycin-insensitive TOR complex, is also able to directly regulate spatial cell growth through actin cytoskeleton remodeling by acting upstream Rho-GTPases (Jacinto et al. 2004).

AMP:ATP ratios and AMPK activity are increased in replicative senescence (Wang et al. 2003) and in the aged brain (Liu et al. 2012) but not in the aged muscle (Qiang et al. 2007; Reznick et al. 2007). It is clear that independent of the levels of AMPK activity, the responsiveness of the activation of AMPK in aging is decreased (Salminen and Kaarniranta 2012). AMPK is activated by ROS in a LKB1-dependent and LKB1-independent manner: (1) adriamycin, a redox-cycling drug that produces superoxide radical, induces senescence by activating LKB1 (Sung et al. 2011); (2) H_2O_2 (<0.1 mM) activates the LKB1-AMPK pathway through the kinase ataxiatelangiectasia mutated (Alexander et al. 2010); and (3) ROS produced during hypoxia activate AMPK by the activation of calcium release-activated calcium channels in a LKB1-independent manner (Mungai et al. 2011). Thus, the impairment of the redox regulation of AMPK activation may impair the central role of AMPK in integrating metabolic and cellular information to regulate cellular polarity (see Fig. 2).

Redox regulation of endothelial cell polarity

In endothelial cells, small GTPases activated by VEGF, such as Ras homolog gene family member A (RhoA), Ras-related C3 botulinum toxin substrate 1 (Rac1), and cell division control protein 42 homolog (Cdc42), are signaling intermediates involved in the changes in cell polarity and in the cytoskeleton reorganization that are essential for cell migration and lumen formation (Koh et al. 2008; Soga et al. 2001). During endothelial cell migration, Cdc42 activation promotes the formation of actin-reach protrusions called filopodia, followed by Rac1-dependent deposition of polymerized actin at the cell periphery. This creates a forward movement of the cell that is concomitant with the Rho-induced cytoskeleton contraction at the cell rear by the phosphorylation of the myosin light chain. Downstream of Cdc42, there is a sequential activation of p38, MAPK-activated protein kinase 2 (MAPKAP K2), and heat-shock protein 27 (HSP27) that leads to the formation of stress fibers (Lamalice et al. 2007). HSP27 is an actin-capping protein, and its phosphorylation has been proposed to release actin, allowing filament elongation.

The angiogenic process, particularly in hypoxic situations, is strongly regulated by AMPK activation on endothelial cells, inducing its migration and differentiation into vessel-like structures (Nagata et al. 2003; Stahmann et al. 2010). More recently, in vivo experiments have revealed the importance of LKB1 in AMPK activation during the revascularization process after tissue ischemia (Ohashi et al. 2010).

These molecular processes regulating endothelial cell polarization and directional migration are fine-tuned by ROS production, either directly or indirectly (Fig. 2). NADPH oxidases (NOX) are the major source of superoxide anion radical ($O_2^{\cdot-}$) in the vasculature and are

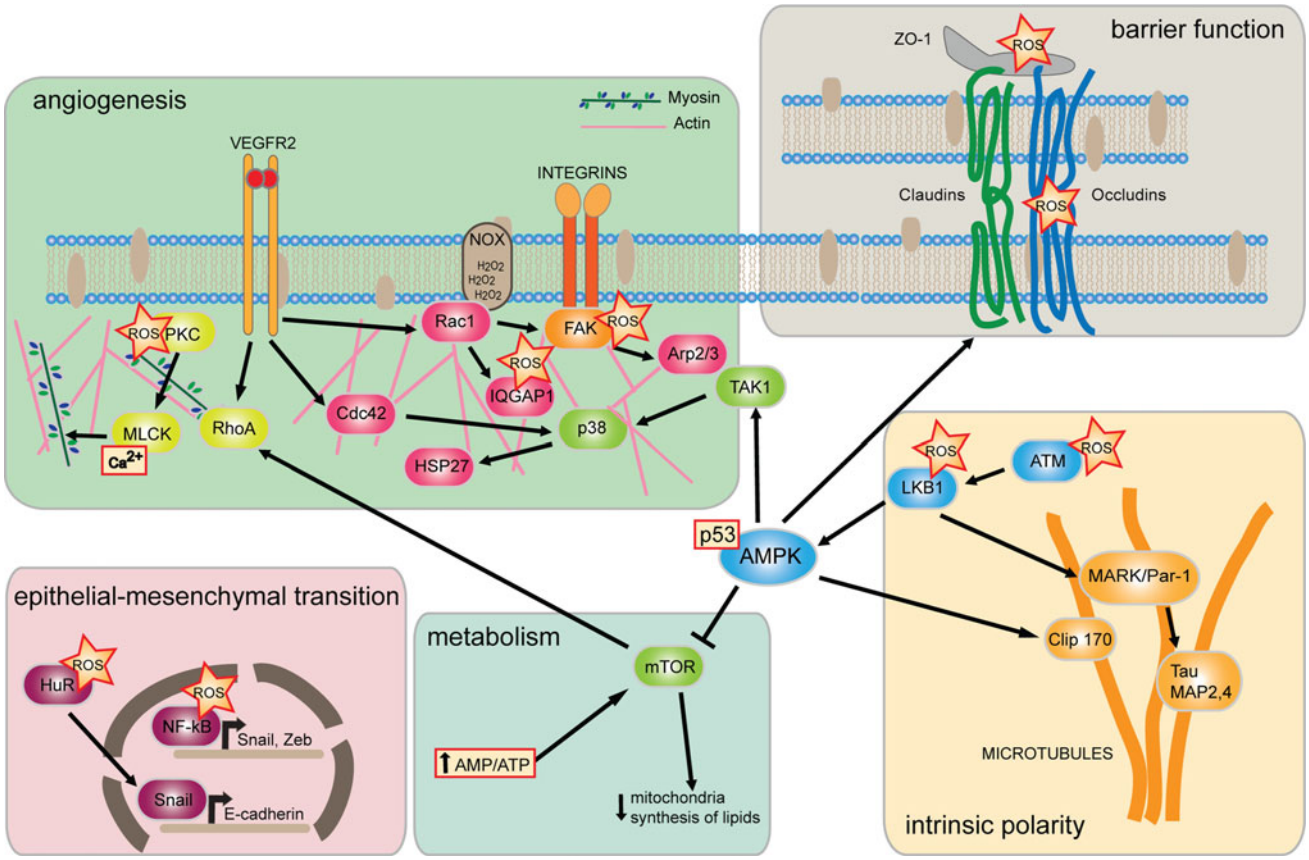

Fig. 2 Molecular pathways of cellular polarity that are regulated by ROS, in different physiological processes altered during aging. AMPK has a central role in regulating several molecular pathways that are master players in the establishment of cellular polarity. ROS are responsible for adjusting cellular polarity to the oxidative status, through redox regulation of angiogenesis, tight junctions, transcription, and cytoskeleton

activated by VEGF signaling (Ushio-Fukai 2007). $O_2^{\cdot-}$ is rapidly dismutated to H_2O_2, which plays a key role in physiological and pathophysiological angiogenesis, regulating endothelial cell proliferation, migration, and vessel morphogenesis. In vitro studies have shown that actin polymerization is induced by H_2O_2 in endothelial cells, promoting cell polarization and migration (Omann et al. 1994). Actin reorganization induced by H_2O_2 is dependent on HSP27 phosphorylation (Huot et al. 1997) and Calcium/calmodulin-dependent protein kinase II (CaM Kinase II) activation (Nguyen et al. 2004). Rac1 is also a key regulator of ROS-dependent cell polarization. Activation of this small GTPase leads to loss of cell–cell adhesions (van Wetering et al. 2002) and cytoskeleton reorganization (Moldovan et al. 1999) with simultaneous production of H_2O_2. More recently, it was shown that Rac1 promotes NOX2 binding to actin and IQGAP1, a scaffold protein, at the leading edge of the migrating endothelial cells promoting ROS-dependent endothelial cell directional migration (Ikeda et al. 2005; Yamaoka-Tojo et al. 2004). Low concentrations of H_2O_2 are able to induce the phosphorylation of focal adhesion kinase (FAK) at Tyr-925 and Tyr-861 in human microvascular endothelial cells (Roy et al. 2006; Vepa et al. 1999). Downstream Cdc42, FAK supports actin reorganization through the activation of actin-related proteins, Arp2/3 (Serrels et al. 2007).

ROS are also implicated in cellular contraction, particularly H_2O_2, which induces myosin light-chain phosphorylation via myosin light-chain kinase (MLCK) and PKC. However, high concentrations of H_2O_2 inhibit PKC, promoting opposite effects (Zhao and Davis 1998).

Redox regulation of the barrier function

Reactive oxygen species and, in particular, H_2O_2, cause an increase in the permeability of the tight junction, in several cellular systems. Several mechanisms are involved in this regulation (Fig. 2), but occludins are a main target for this redox regulation (Blasig et al. 2011). Complex posttranslational modifications of occludins triggered by H_2O_2 deregulate the interaction of occludins with ZO proteins and the actin cytoskeleton, disrupting the tight junctions and increasing paracellular permeability. In addition, H_2O_2 can also trigger changes in the cellular levels of occludins.

For example, in Caco-2 cell monolayers, 20 μM H_2O_2 enhances translocation of protein phosphatase 2 (PP2A) by a Src kinase-dependent mechanism, leading to dephosphorylation of occludin threonine residues; the same concentration leads to tyrosine phosphorylation in occludins (Basuroy et al. 2006; Sheth et al. 2009). In the same cells, exposure to higher H_2O_2 concentrations (500 μM) causes a decrease in the expression of ZO-1 and occludins (Wang et al. 2012) and a re-localization of claudin-4 out of the tight junction that is dependent on the activation of p38 MAP kinase (Oshima et al. 2007). In HUVEC monolayers, treatment with 1 mM H_2O_2 led to a decreased paracellular permeability, occludin disorganization on the endothelial surface with loss of ZO-1 association and increased phosphorylation of occludin serine residues mediated by ERK1/ERK2 signal pathways (Kevil et al. 2000). The involvement of this signaling pathway was also observed in Madin Darby canine kidney (MDCK II) cells, where H_2O_2 concentrations in the mM range caused a temporary increase in tight junction permeability that correlated with a decrease in the expression of occludins, in a process that involved activation of both ERK1/ERK2 and p38 (Gonzalez et al. 2009). On the other hand, generation of H_2O_2 is coupled with the self-restorative capacity of the endothelial barrier function after micrometer-scale disruptions induced by transmigrating leukocytes (Martinelli et al. 2013).

The role of occludins in tight junctions depends not only on its phosphorylation status, but also on its dimerization through a disulfide bond, a process that is dependent on the cellular redox status (Walter et al. 2009).

Redox regulation of epithelial to mesenchymal transition

H_2O_2 affects cell polarity by inducing the epithelial to mesenchymal transition through the regulation of the transcription factor NF-κB (for review, see Oliveira-Marques et al. 2009). In MCF-7 cells, 50 μM H_2O_2 stimulated cell migration by inducing translocation of Hu antigen R (HuR) to the cytosol, leading to the stabilization the mRNA of Snail, a known inducer of epithelial to mesenchymal transition (Dong et al. 2007). In fact, NF-κB directly activates the transcription of other zinc finger transcription factors normally involved in the control of development, such as Snail and Zeb (Chua et al. 2007; Julien et al. 2007) that in turn down-regulate the E-cadherin gene (Fig. 2). On the other hand, the reduced ability of thymus to produce naive T cells during aging is one of the key determinants of reduced immune surveillance in elderly (Linton and Dorshkind 2004). Recent data showed that caloric restriction specifically inhibits the age-related adipogenic programming of thymic stroma and preserves thymopoiesis. In fact, caloric restriction increased cellular density in the thymic cortex and medulla, preventing the age-related increased levels of FoxC2 and fibroblast-specific protein-1 (FSP-1), which are epithelial–mesenchymal transition regulators, and the reduction in lipid-laden thymic fibroblasts (Yang et al. 2009).

Oxidative damage and asymmetric inheritance during cell division

(a) Yeast replicative aging

Evidence linking polarity and aging has been obtained in unicellular organisms (for reviews, see Lindner and Demarez 2009; Macara and Mili 2008; Nystrom 2011), such as yeast and *E. coli,* where several studies suggest that polarity is used to ensure that deleterious material is differentially inherited by aging cells (Aguilaniu et al. 2003; Erjavec et al. 2007, 2008; Lindner et al. 2008; Liu et al. 2010, 2011; Tessarz et al. 2009).

Two types of asymmetry occur during cell divisions of *S. cerevisiae* cells (Macara and Mili 2008). Morphologically, the formation of daughter cells does not occur randomly over the surface of the mother cell. The new bud occurs either adjacent to the previous bud site (axial) or, in the case of diploids, at the opposite end to the previous bud site (bipolar) (Macara and Mili 2008). Functionally, asymmetry occurs since the mother can switch the mating type during cell division. Furthermore, new material produced in the mother cell—RNAs, proteins, and vesicles—is differentially inherited by the bud. This material is delivered to the bud along actin cables that extend from the mother to the bud (see Fig. 3a). At the bud neck, a cortical ring, composed of GTP-binding proteins called septins, forms (Gladfelter et al. 2001). This ring acts as a diffusion barrier to the entry into the bud of older material, which is retained in the mother cell. Therefore, the mother cell undergoes age-related deterioration and, with repeated divisions, eventually loses the capacity to divide. However, the aging mother cell is still capable of generating daughter cells with a renewed reproductive capacity (Steinkraus et al. 2008). The life span of a yeast cell is generally around 20–30 divisions.

Important contributors to the replicative aging of yeast cells are extra-chromosomal rDNA circles (Shcheprova et al. 2008; Sinclair and Guarente 1997) and protein carbonyls and other forms of oxidatively damaged proteins. Several studies have showed that protein carbonyls and aggregated proteins accumulate during replicative aging in yeast and that their levels are much higher in mother cells than in daughter cells (Fig. 3a) (Aguilaniu et al. 2003; Erjavec et al. 2007, 2008; Lindner et al. 2008; Liu et al. 2010, 2011; Tessarz et al. 2009). The most likely mechanism causing the asymmetry of protein aggregates distribution during yeast cells aging is that these aggregates do

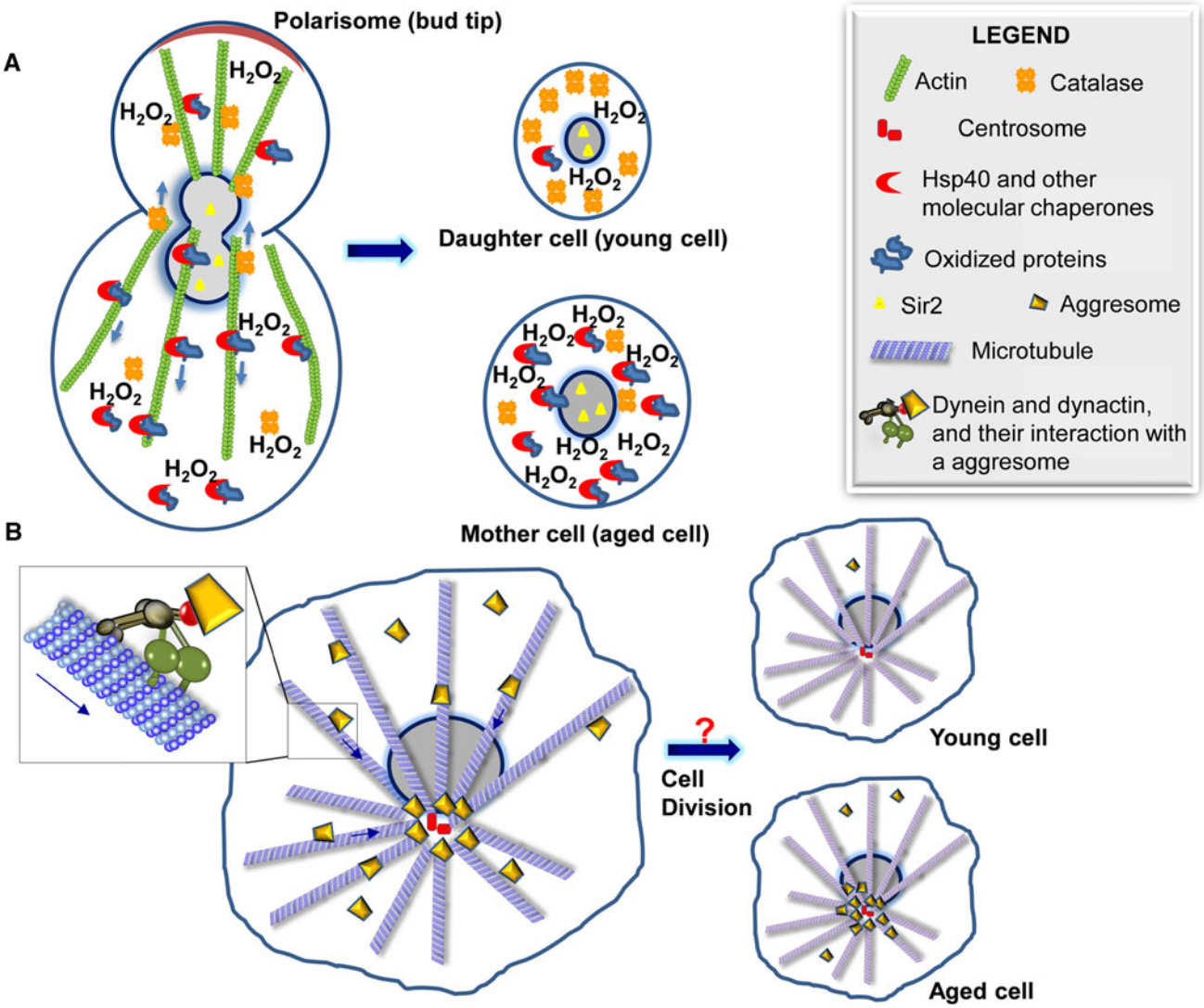

Fig. 3 Schematic representation of asymmetric inheritance of damaged/aggregated proteins and protective molecules during yeast and mammalian cell division. In yeast, **a** misfolded and oxidatively damaged proteins form aggregates that preferentially accumulate in mother cells (long lived) not entering in the bud. At the same time, there is an increased catalase concentration in the bud contributing to the reduced levels of H_2O_2 found in the daughter cell. This asymmetrical distribution is dependent on the actin cytoskeleton, molecular chaperones (e.g., Hsp40), and the NAD^+-dependent histone deacetylase Sir2p. Similarly, in mammalian cells, **b** aggresomes (damaged proteins) are asymmetrically distributed to one of the daughter cells and concentrate close to the centrosome. This transport relies on dynein/dynactin complexes and on the microtubule cytoskeleton. In both types of cells, the asymmetrical distribution of damaged materials and protective molecules seems to be an important event for cell aging. A similar mechanism may possibly be used by adult mammalian stem cells in order to protect their self-renewing progeny of damaged molecules. For more details, please see the text

not enter the daughter cells. These aggregates are recognized by the protein remodeling factor Hsp104p (Erjavec et al. 2007). Liu et al. (2010, 2011) proposed that Hsp40p-containing protein aggregates associate with actin cables and that aggregates tethered on cables do not enter the buds (Fig. 3a). In addition, they found that some protein aggregates showed a retrogade flow along the actin cables away from the daughter cell bud tip and back into the mother after a transient heat stress (Liu et al. 2010). This retrograde segregation requires the expression of *SIR2*, a gene coding for histone NAD^+-dependent deacetylase, essential for normal longevity in several organisms, and which is a key regulator of the polarisome, a protein complex located at the distal end of the bud. Disruption of *SIR2* results in an impaired segregation of the oxidized proteins and reduces the life span of the daughter cells. This impairment can be amended by the overexpression of Hsp104p, which leads to anchoring of the damaged proteins by the mother cell (Erjavec et al. 2007). Sir2p is also involved in the reduction of reactive oxygen species levels

in the daughter cell after completion of cytokinesis (Erjavec and Nystrom 2007). For example, the reduction in the levels of H_2O_2 in the daughter cell is a consequence of a twofold higher activity of cytosolic catalase (Cct1p) in the daughter cell due to a Sir2p- and actin cytoskeleton-dependent asymmetric segregation of this enzyme (see Fig. 3a).

Recent studies have shown that this partitioning of damaged and potentially toxic protein species, known as spatial quality control (SQC) (Nystrom 2011), also occurs in unicellular systems where cytokinesis is accomplished by binary fission, including *E. coli* and *Schizosaccharomyces pombe*, and that the damage-enriched sibling lineages show signs of aging and decreased fitness (Erjavec et al. 2008; Lindner et al. 2008).

(b) Mammalian cell aging and stem cells

Like it happens with yeast cells, mammalian cells during aging also accumulate misfolded and oxidatively damaged proteins. This occurs because in some situations, such as when the ubiquitin–proteasome system capacity is exceeded, these non-degraded proteins may form microaggregates (Koga et al. 2011; Kopito 2000; Rujano et al. 2006). Those microaggregates are microtubule-dependent transported by dynein/dynactin and accumulate specifically at the centrosome in inclusion bodies, the aggresomes (Fig. 3b). The formation of aggresomes requires an intact microtubule cytoskeleton but also a dramatic reorganization of the intermediate filament cytoskeleton (Johnston et al. 1998) and when in the aggresomes proteins are not degraded by proteasomes (Holmberg et al. 2004).

The accumulation of aggresomes has been proposed as being one of the important factors of cell aging (Bucciantini et al. 2002; Terman 2001) and also a reason for the dysfunction and death of long-lived postmitotic mammalian cells such as neurons or cardiomyocytes (Rodriguez et al. 2010). However, it may also possible that cells that exist in the tissues during the entire life span, such as self-renewable tissue stem cells or germ-line cells, may use storage of aberrant proteins in aggresomes to preserve fitness and functionality. Since aggresomes are accumulated at the centrosomes, which is an organelle crucial for proper spindle formation, it could be hypothesized that the formation of aggresomes could interfere with proper cell division and hence impair cell renewal in proliferating tissues (Johnston et al. 1998). However, Rujano et al. (2006) showed in *D. melanogaster* embryonic neuroblasts that the accumulation of misfolded proteins in aggresomes does not impair cell division leading instead to an asymmetric inheritance of the aggregated proteins. Alternatively, the structural intrinsic polarity of centrosomes may be required for the establishment of this asymmetry, a hypothesis that deserves investigation. Thus, aggregated proteins are asymmetrically distributed to one of the daughter cells, leaving the other daughter free of accumulated protein damage (Fig. 3b). This asymmetric inheritance occurs with a stringent polarity in progenitor cells and prevents the transmission of damaged proteins to the longest lived daughter cell. A similar mechanism may possibly be used by adult mammalian stem cells (Liu and Rando 2011).

How nutrients affect polarity in aging

Polarity-dependent processes that are potentially regulated by ROS are altered during aging. Taking in account that ROS are increased during aging (Beckman and Ames 1998), could some of the deleterious alterations in polarity-dependent processes that occur during aging be avoided by nutritional interventions with redox-active molecules? Answering to this question is a formidable task, and the ultimate evidence has to come from clinical trials. The lack of confirmation of the beneficial effects of antioxidants as cancer prevention agents observed in clinical trials illustrates the difficulties in this topic (Goodman et al. 2011). Methodological considerations that are important to consider in the design of the clinical trials include the duration of trials, choice of end points, and intervention dose.

Trial duration is critical when accessing effects that occur over a long period of time, such as aging-related processes. For example, it takes one to three decades of molecular changes to produce a detectable neoplastic lesion (Aggarwal et al. 2009). It is very expensive and difficult to design a clinical trial that has the duration needed to detect such effects. A useful alternative to clinical end points that take decades to be detectable is the use of biomarkers of aging-related polarity processes. Changes in these biomarkers could be detected in shorter periods. For example, trials of antioxidant supplements that used biomarkers of cell proliferation as the end points of interest were successful (Bussey et al. 1982; Cahill et al. 1993; Paganelli et al. 1992), showing decreased proliferation, while antioxidants failed to reduce the risk of clinically detectable neoplasias.

Another critical aspect is the selection of the intervention dose. The occurrence of biphasic effects or hormesis, in which a potential beneficial effect turns into a harmful effect by changing the dose, is common for many chemical species, including reactive oxygen species and phytochemicals (Calabrese et al. 2012). In this context, it is also worth pointing out the concept of para-hormesis, which describes the process by which non-toxic compounds maintain an adaptive and defense system, i.e., a hormetic-like response that does not necessarily require an activating or initiating stress (Forman et al. 2013). Thus, combined

with doubts concerning the bioavailability of many nutrients, the choice of an intervention dose from the data acquired in cell systems is close to impossible. In other words, several intervention doses should be tested. Again, the design of relative short-term clinical trials where biomarkers are the end point may be a viable alternative to choose the intervention dose.

The identification of biomarkers for changes in polarity-dependent processes should be an important research focus. Inflammatory mediators are a likely candidate as end points for short-term trials with nutrients because, in general, barrier failure of epithelia increases inflammation. However, the interpretation of changed levels of inflammatory mediators is challenging because inflammation could also have other origins, such as cancerization or other pathologies.

There is plenty of evidence that several bioactive compounds present in the diet may reverse epithelial barrier disruption induced by reactive oxygen species that are observed during aging. The result obtained in cell lines may be particularly relevant for the gastrointestinal epithelium where there is a direct contact between nutrients and the epithelium. The hydrogen peroxide-induced disruption of tight junctions and barrier function in Caco-2 cell monolayers is ameliorated by curcumin, which up-regulates heme oxygenase-1 (Wang et al. 2012), and by probiotics that modulate PKC- and MAPK-dependent mechanisms (Seth et al. 2008). In ECV304 monolayers, quercetin is able to reverse H_2O_2-induced disruption of tight junctions and hyperpermeability by inhibiting the H_2O_2-dependent increase of phosphorylated p38 MAPK (Chuenkitiyanon et al. 2010). Cancer-preventive activities in the digestive tract have also been identified for the tea flavonoid epicatechin gallate and the non-flavonoid polyphenol ellagic acid. In this regard, it is interesting to note that both compounds have a negligible transcellular absorption in epithelial Caco-2 cells but their cellular uptake is very high (Vaidyanathan and Walle 2003; Whitley et al. 2003). This further reinforces the notion that flavonoids may exert most of their bioactivity in epithelial gastrointestinal cells (Walle 2004).

At the animal level, probiotics prevent intestinal barrier dysfunction in acute pancreatitis in rats via induction of glutathione biosynthesis in the ileal mucosa, which is expected to help maintain the intestinal barrier and reduce the potential for sepsis (Lutgendorff et al. 2009). In this model, probiotic bacteria help also to prevent disruption of intestinal epithelium tight junction proteins by maintaining normal levels of claudin-1 and claudin-2 incorporated into tight junctions, and prevent the mucosal damage that has been associated with the oxidative stress induced by acute pancreatitis. In addition, systemic glutathione levels are increased. Dietary methionine restriction, which has been associated with increased life span, improves colon tight junction barrier function and alters claudin expression and occludin posttranslational modifications in rats (Ramalingam et al. 2010). Effects of nutrients are not restricted to the gastrointestinal epithelium. For example, oral treatment with *Lactobacillus plantarum* of rats with obstructive jaundice assisted in the return of active hepatic barrier function. In this model, Lactobacillus prevents the oxidation of plasma glutathione and the down-regulation of claudin-1 and claudin-4 caused by the oxidative stress associated with obstructive jaundice (Zhang et al. 2010).

A key notion is that phytochemicals, once in the liver, undergo rapid detoxification mechanisms that can further activate or deactivate their bioactivity (Justino et al. 2004; Santos et al. 2008). At the molecular level, phytochemicals up-regulate antioxidant response- and phase-II detoxification enzymes through the activation of the transcription factor Nrf2 (Forman et al. 2013). For a comprehensive list of phytochemicals, and respective structures, that activates Nrf2, see (Birringer 2011). Phytochemicals activate Nrf2, either by an S-alkylation of reactive cysteine residues present in the Nrf2 partner Keap1, or by the generation of H_2O_2 that subsequently oxidizes Keap1 reactive thiols (Birringer 2011). However, H_2O_2 induces Nrf2 in a narrow range of conditions (Covas et al. 2013) and some authors considered that the S-alkylation of Keap1 is the operating reaction in vivo (Forman et al. 2013). Irrespectively of the mechanism of activation, phytochemicals elicit an antioxidant response that may protect against oxidative damage associated with aging. In this context, the concept of vitagenes, a group of genes involved in preserving cellular homeostasis during stressful conditions and that promotes longevity, has been introduced (Calabrese et al. 2012). At the animal level, the flavonoid phloretin was shown to increase glutathione content and heme oxygenase-1 expression in rat liver through the activation Nrf2 (Yang et al. 2011). In addition, diet rich in olive oil phenolics decreases oxidative stress in the heart of senescence-accelerated mouse-prone 8 by inducing Nrf2-dependent gene expression (Bayram et al. 2012). Nrf2 activation by phytochemicals is not restricted to liver or the heart, being observed also in intestine, liver, kidney, and spleen (Balstad et al. 2011). So, in principle, the activation of Nrf2 could elicit an antioxidant response that ameliorates the aging-associated polarity dysfunctions described in section III and that are under redox-control. In support of this, Nrf-2 activation by isothiocyanates is a key for the protection against the blood–CSF barrier disruption caused by H_2O_2 (Xiang et al. 2012). Furthermore, in *Drosophila,* the life span extension dependent of *vang-1*, a core member of the Wnt/planar cell polarity signaling pathway, partially requires skn-1, a Nrf1/2/3 protein ortholog (Honnen et al. 2012).

Both at the cellular and animal levels, there are evidences that phytochemicals can regulate the LKB1/AMPK signaling axis that is central in the control of polarity. For example, baicalin, one of the major flavonoids in traditional Chinese herb medicine, protects against the development of hepatic steatosis by stimulating AMPK activity through an enhancing of its phosphorylation in liver and skeleton muscle (Guo et al. 2009; Ma et al. 2012). Administration of resveratrol to spontaneously hypertensive rats, a well-established genetic model of hypertension and subsequent cardiac hypertrophy, also increases AMPK activity by increasing its phosphorylation resulting in a reduced left ventricular hypertrophy. Resveratrol elicits its protective effect by inhibiting the formation of adducts of 4-hydroxy-2-nonenal, a product of lipid peroxidation, with LKB1, resulting in LKB1 inhibition (Dolinsky et al. 2009). The beneficial effect of quercetin on endothelial cell function is also mediated via the AMPK pathway (Shen et al. 2012). Oral administration of epigallocatechin gallate (200 mg/kg body weight) to mice also increases AMPK activity, being the gallocatechin moiety or the galloyl residue responsible for AMPK activation (Murase et al. 2009). It was also shown that epigallocatechin gallate inhibits VEGF transcription in tumor cells, preventing tumor growth by targeting neoangiogenesis (Sartippour et al. 2002).

Conclusions

ROS have a fundamental role in cell signaling, working as key regulators of normal cell function, and the unbalanced production of these species and/or its general overproduction may have as outcome cell dysfunction and, ultimately, cell death. Unbalanced production of ROS occurring during aging may be responsible for alterations in cell polarity, because many pathways that control cell polarity are redox regulated. Moreover, polarity deregulation may have an impact in proper cell division and deregulate the pathways leading to cilia assembly, which will expose cells to deficient signaling from the environment. Permeability barrier compromise, decreasing endothelial cell migration, and angiogenesis dysfunction may ensue. These events will render cells susceptible to cancer development and other age-associated pathologies.

The hypothesis that redox deregulation of cellular polarity is a central process during aging that integrates both intracellular and extracellular signals is speculative but can be used as a guide for future nutritional interventions. Besides being dependent on the general energetic status of the cell, cellular polarity is also modulated by several components of the diet that elicit redox regulation of the molecular pathways involved. However, additional in vivo studies are needed to corroborate the importance of ROS in the regulation of cell polarity as well as the central role of polarity for age-associated cellular and tissue changes. It is also important to identify biomarkers associated with polarity deregulation. Such biomarkers are needed as end points of short-term pilot clinical trials to address whether nutritional interventions could ameliorate age-associated dysfunctions. If successful, these could help to define both the composition and the dose of the nutritional intervention to be applied in large-scale expensive long-term clinical trials that have as end points clinical alterations that take a long time to manifest. Such a focused and structured approach holds the promise of elucidating the relationship between nutrition and the aging process.

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