Effects of Environmental Stress Factors on the Actin Cytoskeleton of Fungi and Plants: Ionizing Radiation and ROS

Octavian O. H. Stephan[1]

[1]Department of Biology, Friedrich-Alexander University of Erlangen-Nuremberg

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Abstract

Actin is an abundant and multifaceted protein in eukaryotic cells that has been detected in the cytoplasm as well as in the nucleus. In cooperation with numerous interacting accessory-proteins, monomeric actin (G-actin) polymerizes into microfilaments (F-actin) which constitute ubiquitous subcellular higher order structures. Considering the extensive spatial dimensions and multifunctionality of actin super-arrays, the present study analyses the issue if and to what extent environmental stress factors, specifically ionizing radiation (IR) and reactive oxygen species (ROS), affect the cellular actin-entity. In that context, this review particularly surveys IR-response of fungi and plants. It examines in detail which actin-related cellular constituents and molecular pathways are influenced by ionizing radiation and related ROS. This comprehensive survey concludes that the general integrity of the total cellular actin cytoskeleton is a requirement for IR-tolerance. Actin´s functions in genome organization and nuclear events like chromatin remodelling, DNA-repair, and transcription play a key role. Beyond that, it is highly significant that the macromolecular cytoplasmic and cortical actin-frameworks are affected by IR as well. In response to IR, actin-filament bundling proteins (fimbrins) are required to stabilize cables or patches. In addition, the actin-associated factors mediating cellular polarity are essential for IR-survivability. Moreover, it is concluded that a cellular homeostasis system comprising ROS, ROS-scavengers, NADPH-oxidases, and the actin cytoskeleton plays an essential role here. Consequently, besides the actin-fraction which controls crucial genome-integrity, also the portion which facilitates orderly cellular transport and polarized growth has to be maintained in order to survive IR.

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Octavian O. H. Stephan

Department of Biology, Friedrich-Alexander University of Erlangen-Nuremberg, Erlangen, Bavaria 91058, Germany.

Corresponding author's e-mail address: octavian.stephan@fau.de

ABSTRACT

Actin is an abundant and multifaceted protein in eukaryotic cells that has been detected in the cytoplasm as well as in the nucleus. In cooperation with numerous interacting accessory-proteins, monomeric actin (G-actin) polymerizes into microfilaments (F-actin) which constitute ubiquitous subcellular higher order structures. Considering the extensive spatial dimensions and multifunctionality of actin super-arrays, the present study analyses the issue if and to what extent environmental stress factors, specifically ionizing radiation (IR) and reactive oxygen species (ROS), affect the cellular actin-entity. In that context, this review particularly surveys IR-response of fungi and plants. It examines in detail which actin-related cellular constituents and molecular pathways are influenced by ionizing radiation and related ROS. This comprehensive survey concludes that the general integrity of the total cellular actin cytoskeleton is a requirement for IR-tolerance. Actin´s functions in genome organization and nuclear events like chromatin remodelling, DNA-repair, and transcription play a key role. Beyond that, it is highly significant that the macromolecular cytoplasmic and cortical actin-frameworks are affected by IR as well. In response to IR, actin-filament bundling proteins (fimbrins) are required to stabilize cables or patches. In addition, the actin-associated factors mediating cellular polarity are essential for IR-survivability. Moreover, it is concluded that a cellular homeostasis system comprising ROS, ROS-scavengers, NADPH-oxidases, and the actin cytoskeleton plays an essential role here. Consequently, besides the actin-fraction which controls crucial genome-integrity, also the portion which facilitates orderly cellular transport and polarized growth has to be maintained in order to survive IR.

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1. INTRODUCTION

Different naturally occurring as well as anthropogenic stress factors cause changes in the biosphere and impose selection pressure to diverse living systems on earth. For human society, especially the adverse consequences of altered environmental determinants on cellular growth and development of organisms have severe implications for numerous areas of interest. The hereby compromised superordinate topics range from accelerated aging, carcinogenesis, and cell death in animals and humans, over decrease of agricultural yields, up to shifts in the biotic composition of entire ecosystems [**1**].

Numerous abiotic stress factors, such as extreme drought, heat, or salinity affect molecular constituents of cells and impair functionality of essential cellular processes. Among the multiplicity of harmful external influences on cells are ionizing radiation (IR) (e.g. γ-rays, X-rays, α-particles) and related oxidative stress. In our environment, the stressor IR originates from natural sources (geological activities and cosmic radiation) as well as from human activities (e.g. radioactive waste, nuclear accidents, and nuclear weapons). On the other hand, humanity uses IR also in various beneficial life science applications, as for example in medical cancer treatment, and in plant breeding.

The consequences of exposure to ionizing radiation are damage of molecular constituents of cells through direct energy transfer, and in addition, also through indirect effects which are mediated by chemical reactions of IR-induced free radicals, such as reactive oxygen species (ROS) [**2-8**]. Since Wilhelm Conrad Röntgen first discovered electromagnetic X-rays in 1895, the effects of IR particularly on the genome have been extensively studied by numerous cell biological, biochemical, and genetic analyses [**9-13**], whereby the nuclear DNA-macromolecule has been postulated as the major target of radiation damage [**2, 14**]. However, apart from that, the question which other molecular constituents, metabolic pathways, or signal transduction mechanisms of cells are affected by ionizing radiation, specifically in plants and fungi, has received too little attention to this day and still requires precise clarification. Most importantly, this scientific issue is particularly interesting, because some plant cells (pollen) and fungal hyphae are extremely tolerant of acute high dose ionizing radiation. Generally their lethal doses are far beyond those of animal or human cells [**15**]. Deeper analysis of the molecular basis for these differences between IR-susceptibility of plant gametophytes, plant sporophytes, fungi, and animals can provide new concepts and approaches for improvement of human cancer treatment, for radiation protection, and eventually for dealing with contaminations of the biosphere. Above all, related research contributes to understanding of fundamental processes which govern cell growth and division.

Especially the actin cytoskeleton presumably represents a substantial target for ionizing radiation, when considering the large microfilament structures within eukaryotic cells and its wide range functionality in numerous physical and biochemical processes.

The term '*skeleton*' represents a widely used paraphrase in biology. Macroscopic skeletal structures range from the steady bone endoskeleton which gives shape to vertebrate organisms, up to the protecting exoskeleton that coats the body of arthropods and molluscs. However, in contrast, this study

will focus on the microscopic '*cytoskeleton*' which represents lively changing, and yet, steady aggregates of polymer molecules within eukaryotic cells. Thereby, this comprehensive review article specifically examines implications of ionizing radiation on the abundant subcellular framework of actin filaments. For this purpose, especially data about diverse cells of fungi and plants were comparatively surveyed to gain insights into relations between ionizing radiation, ROS, and the actin-entity. Here, primarily IR-effects from the high-dose range were evaluated.
In this context, such IR- and ROS-related research specifically sheds light on the crucial connections between actin dynamics, membrane transport, and polarized cell growth. In addition, the results particularly point to a role for actin in configuration of nuclear chromatin and transcriptional regulation. What´s more, in that respect, this survey especially provides in-depth analysis of protein-networks which conduct actin-dynamics and allocate the cytoskeleton of fungal and plant taxa to eclectic cellular tasks.
For a review of IR-effects on the cytoskeleton of animal tissues, primarily regarding the mechanisms of cellular adhesion and migration, see [**16**].

In general, actin molecules are ATP-binding globular protein monomers (G-actin) which polymerize into microfilaments (F-actin) that associate with a large set of different actin-binding proteins to generate abundant macromolecular structures within cells of diverse eukaryotic organisms [**17-19**]. Actin has been conserved extraordinarily high during evolution, whereby actin homologs have been verified throughout the phylogenetic tree in eukaryotic cells of fungi, plants, and animals, but also in archaea and bacteria [**20**]. Cells of the baker´s yeast *Saccharomyces cerevisiae*, for example, express only one essential actin gene (*ACT1*), whereas plants have numerous homologs. *Arabidopsis thaliana* cells possess more than a dozen actin variants, which are differentially expressed with respect to the specific plant organs, tissues, and cell types.
The establishment and maintenance of the eukaryotic actin cytoskeleton depends on a dynamic equilibrium of assembly and disassembly, which is controlled by associated proteins of different function [**21-23**]. Actin-dynamics is mediated with the assistance of nucleating, branching, cross-linking, and bundling proteins (ARP2/3-complex, fimbrin, formin), in combination with proteins that facilitate severing (ADF/cofilin, villin) and capping (CP, AIP1) of microfilaments [**24, 19, 25**]. The range of actin-binding proteins (ABPs) is diverse, and comprises numerous evolutionary conserved protein families with specific functions [**26**]. These are: AIPs (actin-interacting proteins) [**27, 28**], ARPs (actin-related proteins) [**29, 30**], ADFs (actin-depolymerizing factors) [**31, 32**], cofilin [**27, 31**], profilin [**33, 34**], fimbrins [**35, 36**], formins [**37, 38**], villins [**39**], myosins [**40**], and tropomyosins [**26**]. Actin and these associated proteins act concertedly, in order to generate microfilaments and organize them spatially in higher order patterns which constitute patches, linear bundles, intricate networks, as well as ring- or fringe-like arrays [**17, 22, 41**]. Those large subcellular microfilament aggregates fulfil roles in different physiological processes, such as cell division, differentiation, growth, morphology, and motility [**42, 43**].

The distinct shapes of superordinate cytoskeletal structures, such as, for example, '*actin-fringes*' in polarized growing cells, are specifically organized and individually composed of microfilaments according to the respective requirements of cell type and its functionality in different environments [**41, 25**]. Here, particularly, individual cellular growth rates very likely play a role [**41**]. Elements of the actin cytoskeleton are widely known to act as mechanical framework and tracks for guidance of intracellular transport throughout the cytoplasm and at the cellular cortex, thereby facilitating polar cell growth [**44, 18**]. Yet, how the precise molecular composition of each individual cytoskeleton super-structure is specifically controlled, is still unknown today.

In the context of this article it is important to note that the actin-entity is not restricted to cytoplasm or cellular cortex, but increasing publications during the last twenty-five years have corroborated that actin and especially actin-related proteins (ARPs) hold additional roles within the cell´s nucleus. Here specifically in organization and remodelling of chromatin-structure, as well as in transcription, and nuclear transport processes [**45-48**]. However, studies on intranuclear F-actin have mainly been done on yeast and animal systems. Plants are under-researched concerning this matter.

In view of ongoing environmental-changes and increasing pollution problems on earth, particularly the response of the plant and fungal cytoskeleton to external stimuli should receive more attention. Biotic stimuli that trigger actin dynamics during plant immune response gained some attention, however abiotic stimuli, such as radiation are under-researched [**49, 50**].

In general, the key factor for IR-induced impairment of cellular viability is the damage of the macromolecular DNA entity through various detrimental molecular alterations, such as single-strand breaks (SSBs), double-strand breaks (DSBs), base modifications, as well as protein-DNA cross-linking [**11, 7**]. Normally, these molecular defects like broken DNA are mended by the cell´s enzymatic repair systems which mediate 'homologous recombination' (HR) or 'non-homologous end joining' (NHEJ) [**51**]. However, the ability of cells to survive specific IR-doses depends on both, the level of deterioration of the chromatin, and the impairment of protein-complexes which facilitate DNA-repair. In that respect, the present article illuminates contributions of the nuclear actin cytoskeleton to DNA-repair. Moreover, this survey shows that actin-dynamics and maintenance of higher order cytoskeleton structures in the cytoplasm and cortex are affected by ionizing radiation as well. Hence, it is concluded that actin and its accessory proteins represent a key target of IR-related damage.

Abnormally high amounts of intracellular reactive oxygen species, which are generated by IR, might additionally play a role here. Reactive oxygen and nitrogen species (ROS & RNS) are being produced by radiolysis of intra- and extracellular water [**3, 4, 52**]. Reactive oxygen species represent a highly reactive group of molecules which comprise hydrogen peroxide (H_2O_2), superoxide anion radical ($^{\cdot}O_2^-$), singlet oxygen ($^1O_2$), and the hydroxyl radical ($^{\cdot}OH$) [**3, 7**]. Based on their enormous chemical reactivity and ubiquity, the IR-induced ROS can damage diverse categories of biological molecules, such as DNA, RNA, proteins, and lipids.

Apart from IR-treatment, ROS are usually generated through high metabolism rates in cellular compartments such as chloroplasts and mitochondria. ROS are important stressors for molecular constituents of cells, however, on the other hand, a growing set of studies demonstrate that ROS play versatile roles in cell physiology [**53**]. ROS also act as key signalling molecules that regulate processes like plant cell growth, development, sexual reproduction, plant defence, and responses to different environmental stimuli [**54-56**]. Thus, cellular ROS-homeostasis represents an essential requirement that needs to be tightly controlled for correct cellular functionality. This is achieved by a balance between ROS-production through NADPH oxidases (NOX), and ROS-scavenging through detoxifying enzymes, such as superoxide dismutase (*SOD*), glutathione peroxidase (*GPX*), thioredoxin (*TRX*), glutaredoxin (GRX), and catalase (*CAT*) [**57**].

For the first time, this comprehensive survey integrates biochemical, genetic, and cell biological data about effects of ionizing radiation on the cellular actin-entity specifically of fungi and plants. To this end, the available literature was screened to identify IR-sensitive yeast deletion mutants which are specifically related to the actin cytoskeleton. Moreover, functionalities of these genes were reviewed in detail and conceptionally evaluated. In addition, the effects of IR on gene expression of fungi and plants were investigated, with a special focus on elements of the actin array, heat shock proteins, and antioxidants. Finally, mutual connections between actin-dynamics and IR-induced ROS were thoroughly examined and discussed, hence resulting in a simplified model.

2. ACTIN-RELATED IR-SENSITIVE YEAST DELETION MUTANTS

Despite uncertainties regarding the holistic determination of the total biomass on earth, current estimations designate plants as the largest global taxon, while fungi are placed on the third rank, sorted after bacteria [**58**]. Ontogeny and reproduction of fungi is diversified, consequently they represent a heterogeneous polymorphic eukaryotic kingdom. Fungal taxa comprise unicellular microorganisms, such as yeasts, but also multicellular entities. In the latter case, polarized growing hyphae of filamentous fungi generate large mycelial structures and sporocarps (fruitbodies). Members of the multifaceted taxon fungi thrive in diverse habitats, yet their tolerance of ionizing radiation is in general very high, which is similar to the extreme IR-resistance of pollen performance [**15**]. Concerning collective plant microgametophytes the mean value of 50% inhibition doses (ID_{50}) for gamma-radiation amounts to significantly more than 1000 Gy. Whereas the mean ID_{50} of plant sporophytes is substantially below 100 Gy [**15**]. Also the IR-tolerance of fungal hyphae is considerably higher than that of plant sporophytes. When comparing diverse species of fungi, the ID_{50} ranges approximately between 500 and 5000 Gy [**15**].

Similar to other members of the kingdom fungi, cells of the unicellular budding yeast *Saccharomyces cerevisiae* exhibit significantly higher IR-tolerance (X- and γ-irradiation) than cells of plant sporophytes, or particularly animal tissues (< 50 Gy). The 50% lethal doses (LD_{50}) are ~300 Gy for haploid (By4741) and ~600 Gy for diploid (By4743) wild-types of *S. cerevisiae* [**59, 60**].

Genetic mutations which cause X-ray sensitivity of *S. cerevisiae* were initially discovered in the early 1950´s [**61**]. Those genetic loci have been termed *rad* mutations [**62**], and since that time, numerous studies categorized their functions in epistatic groups which are primarily involved in cell cycle arrest, recombination, and DNA repair [**63, 64**]. Later, various screening studies identified additional genes which affect the IR-tolerance of yeast [**65, 59, 60, 66**].

For this study, all IR-sensitive yeast deletion mutants have been surveyed in order to identify and investigate pathways which are specifically related to the actin cytoskeleton. Hereinafter, those IR-sensitive yeast deletion mutants which were found to be particularly related to the cellular actin-entity have been discussed and, in conclusion, categorized in two groups: i) Actin-related genes that are involved in nuclear genome-related events like chromatin remodelling. ii) Genes that are associated with regulation of cytoplasmic/cortical actin cytoskeleton structure, cell polarity and growth.

Their functional interaction capacity is comprehensively analysed in Figure1.

2.1 The Relation between Actin and Chromatin Remodelling: *ARP8* and *TAF14* (*ANC1*)

In order to characterize genes that are involved in cellular response to ionizing radiation, genome-wide screens were performed by Game *et al*. [**59**] and Bennett *et al*. [**65**], thereby utilizing a comprehensive set of homozygous diploid *S. cerevisiae* deletion mutants. Regarding genes which are related to the actin cytoskeleton, it is particularly significant that homozygous *arp8Δ* deletion mutant strains demonstrate considerably reduced survival rates compared to the congenic wild-type when subjected to γ-ray sensitivity assays [**59**]. Multidose X-ray cell survival curves show ~1% survival of *arp8Δ* cells after irradiation with 800 Gy, as compared to still more than 30% surviving cells of the wild-type [**59**].

Actin-related proteins (ARPs) represent actin-like proteins that share sequence and structural similarity with actin [**67, 68**]. ARPs are a heterogeneous class of unconventional actins with diverse functions and subcellular localization [**29**]. Like actins, ARPs are conserved from yeast over plants to animals [**29, 30**]. Members of the actin/ARP-superfamily of proteins possess a common structural motif termed the ‘actin-fold’, which has the potential to bind ATP/ADP, thereby causing conformational changes [**69**]. Yeast Arp1, Arp2, and Arp3 are cytoplasmic proteins [**67, 70**] whereas Arp4-9 are localized in the nucleus [**67, 45**]. Actin-related protein 8 (Arp8) [**67**] physically binds to Act1 (**Fig.1**) and has also been related to inhibition of actin polymerization and stabilization of monomeric actin in the nucleus [**71**].

Particularly in yeast and animal cells, nuclear actin has been a matter of intense research and discussion since its discovery more than 45 years ago, whereas in plants this issue has been mostly disregarded [**72**]. Numerous works, which are to some extent reviewed in this study, demonstrate that specifically monomeric actin functions as a component of nuclear protein complexes and thereby is involved in structural organization of chromatin, DNA damage response, gene expression and mRNA transport.

Figure 1

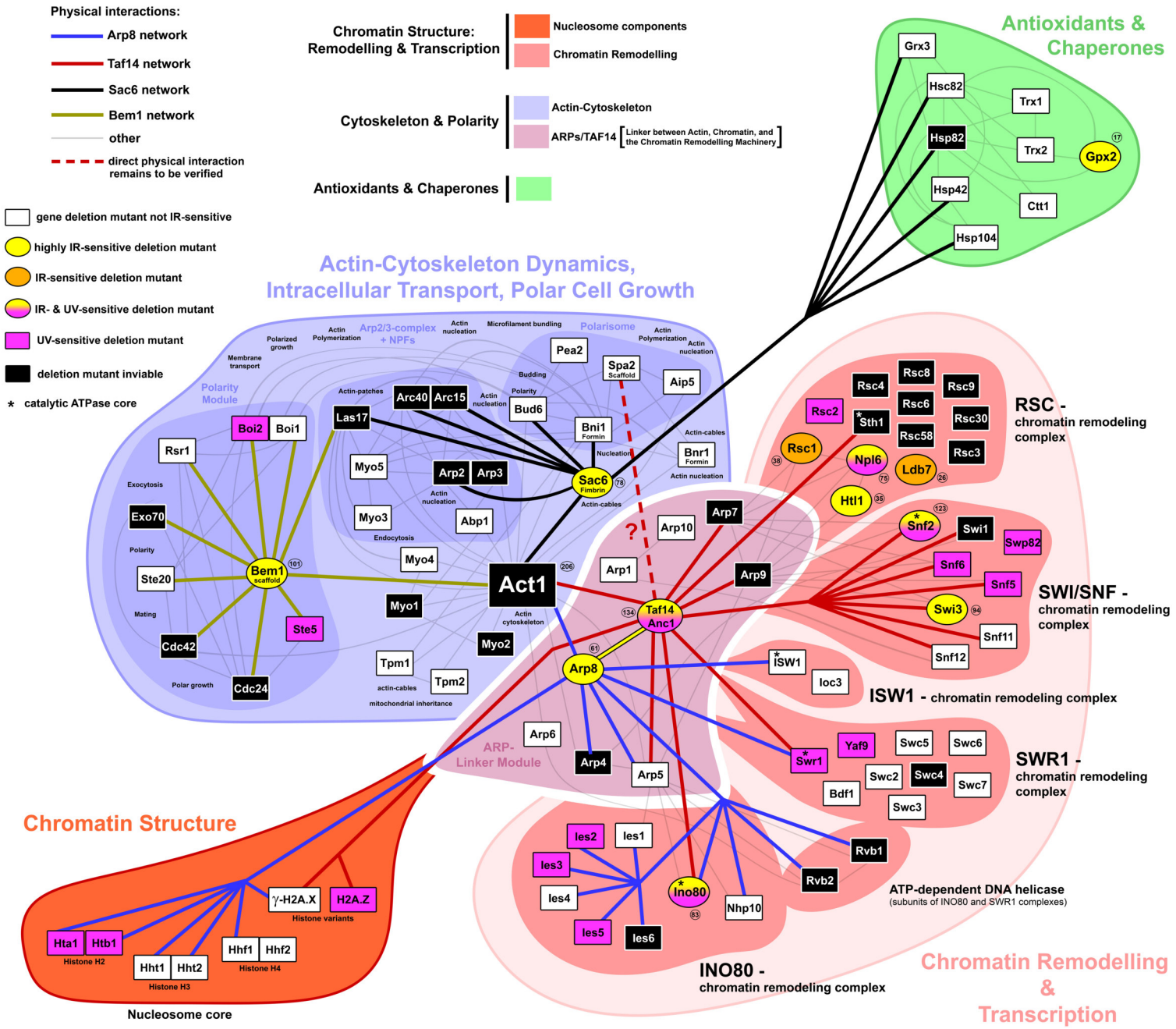


Figure1. Schematic Overview of the Actin-related Protein Interaction Network Highlighting the Deletion Mutants which Exhibit IR-Sensitivity.

This schematic illustration depicts the relations and specifically the physical protein-interaction network of IR-effectors which are related to the actin cytoskeleton. Proteins, whose gene deletions effect cellular sensitivity to ionizing radiation are marked in yellow. Note the three modules: I) actin cytoskeleton, intracellular transport and polar cell growth (blue), II) chromatin remodelling complexes and transcription (light red), III) chromatin structure / nucleosome components (dark red), IV) antioxidants and chaperones (green). The modules are interconnected via the ARP/TAF14 adaptor module (violet). The central 'Arp8-Taf14' unit connects, with assistance of Arp4, Arp5, Arp7, and Arp9, the chromatin remodelling machinery to the chromatin and the nuclear actin-entity. Moreover, Sac6 establishes multiple connections to members of the module IV comprising heat shock proteins, chaperones, and antioxidants. Coloured lines indicate the direct interactions between IR-effectors and physical binding partners. Blue: Arp8 interaction network; Red: Taf14 network; Black: Sac6 network; Beige: Bem1 network). Grey lines indicate other protein-protein interactions. Those are comprehensively shown for the actin module and within the antioxidants module. Note: for conciseness and better visual clarity the interactions between subunits of chromatin remodelling complexes are not indicated. The number of total physical interactions for individual proteins are indicated in circles next to the respective protein.

The physical interaction network in this schematic diagram was developed by analysis of literature cited in this study, and additionally, by utilization of Saccharomyces genome database (https://www.yeastgenome.org) and Yeast BioGRID version 4.4 (https://thebiogrid.org/32710).

It should be noted, that some studies also report polymerized filamentous actin in the nucleus of diverse organisms, however, its structural properties are still under-researched, and outside of the animal kingdom this issue has received little attention to date [**73-78**]. For reviews see [**79-81**]. Specific nuclear import and export receptors for active transport of actin-profilin complexes have been identified in animal cells (importin-9 and exportin-6) [**82, 83**]. Furthermore, some ABPs like profilin, cofilin, and gelsolin, which effect F-actin assembly, have been suggested to shuttle between nucleus and cytoplasma [**80**]. Nuclear F-actin has been associated with spatial organization of transcriptional regulation, replication, and DNA-repair [**80, 77, 84**].

Particularly actin monomers in the nucleus are very well documented to specifically act in configuration and assembly of ATP-dependent chromatin remodelling complexes, and furthermore, G-actin binds to RNA Polymerase II and promotes formation of the pre-initiation complex. In this connection it is significant that Arp8 plays a role in transcription, DNA-repair, DNA-replication, and cell cycle progression, which is based on its contribution to the functionality of genome-structure regulators (**Fig.1**). Arp8 represents a key component of the evolutionary conserved INO80 (inositol requiring 80) ATP-dependent chromatin-remodelling complex (overall molecular mass ~1.3 MDa), which determines mobilization and positioning of the nucleosomes. Here, Arp8 interacts with the catalytic core Ino80 and numerous other subunits, such as Ies1-6, Rvb1, Rvb2, Nhp10, and Taf14 (Anc1) (**Fig.1**) [**85, 86**]. The key topological submodule (Arp8-module), which organizes with the Ino80-IES2 core, consists of Arp4-Arp8-Act1-Taf14-Ies4 [**87, 85, 88, 86**]. In this context, it is important that Bennett *et al*. specifically identified homozygous *taf14Δ* (*anc1Δ*) yeast cells as highly radio-sensitive (γ-rays) (**Fig.1**) [**65**]. The gene product of *TAF14* (**T**ATA binding protein (TBP) - **A**ssociated **F**actor), also known as *ANC1* (**A**ctin **N**on-**C**omplementing), has been described as regulator of actin organization and functionality of the cytoskeleton, as well as of RNA polymerase II transcription [**89, 90**]. Here, Taf14 acts as subunit of the general transcription factor complexes TFIID and TFIIF in stabilization of the transcription pre-initiation complex (PIC), particularly as negative regulator of stress-responsive genes [**91-93**].

Arp8, Arp4, Arp5, and actin are essential stoichiometric components of the overall 15 subunits comprising INO80-complex, whereby Arp8 acts as conformational switch for orderly complex-assembly, and represents the connector module linking Ino80 and actin (Act1) (**Fig.1**) [**94, 86**]. Moreover, Arp8 mediates recruitment of the epigenetic structure regulator INO80 to the chromatin through interaction with histones and extranucleosomal DNA (**Fig.1**) [**95-98, 86**]. The yeast Arp8 dimer specifically interacts with the nucleosome core histones H3/H4, and H2A/H2B, thereby inducing conformational changes in the protein-complex (**Fig.1**) [**94, 96, 86**]. Hence, the observation that *ino80Δ*, *arp8Δ*, and *taf14Δ* cells demonstrate sensitivity to ionizing radiation is consistent with a significant role in DNA-repair processes (**Fig.1**) [**99, 65, 59, 100, 101**]. In this connection, Kashiwaba *et al*. demonstrated that Arp8 is required for recruitment of INO80-complex to laser-induced DNA-damage sites (DSBs) which are marked by the phosphorylated histone H2A variant (γH2A.X foci) (**Fig.1**) [**102**].

The analysis of physical interactions of Arp8 and Taf14 shows that both proteins represent highly interconnected network hubs that link the actin-entity with a functional module of different genome-structure regulators (**Fig.1; Fig.2**). Arp8 and Taf14 are direct interaction partners (**Fig.1;** *yellow line*). Like Arp8, Taf14 also interacts with DNA [**103**] and the 'YEATS' domain of Taf14 mediates binding to acetylated H3 of the nucleosome core [**104, 105**]. Arp8 and Taf14 associate with multiple subunits of various ATP-dependent chromatin remodelling complexes (**Fig.1**), suggesting that both, Arp8 and Taf14 play central roles in wide organization of chromatin remodellers (**Fig.2**). Aside from its central 'switch-like' function in the INO80-complex, Arp8 also associates with the SWR1(SWI2/SNF2 related 1)- and the ISW1(Imitation Switch 1)-nucleosome remodelling complex via direct interaction with their ATPase catalytic core units Isw1 and Swr1 [**99**] (**Fig.1**).
Taf14 is associated with four chromatin remodellers in total, whereby it directly interacts with their catalytic core units: Ino80, Swr1, Snf2, and Sth1 (**Fig.1**). In particular, Taf14 is widely interconnected among numerous subunits of the SWI/SNF-chromatin remodelling complex (**Fig.1**). Apart from Taf14´s direct interaction with Sth1, its binding partners Arp7 and Arp9 additionally connect to multiple subunits of the RSC-complex (Rsc1, Htl1, Npl6) (**Fig.1**) [**106, 91**]. Most interestingly, independent genome-wide screens correspondingly reported *rsc1Δ*, *htl1Δ*, and *npl6Δ* cells (RSC-complex subunits), as well as *snf2Δ* and *swi3Δ* cells (SWI/SNF-complex subunits) as radio-sensitive [**65, 59**] (**Fig.1**).

It should be added that, besides the mentioned members of chromatin remodelling complexes, several subunits of histone acetyltransferase complexes (HATs), histone deacetylase complexes (HDACs), methylases, demethylases, and the SAGA-complex have been identified as gene deletions that induce IR-sensitivity (**Fig.3**). These IR-sensitive mutants highlight the key role of related post-translational modifications, particularly of nucleosome components. And that is not surprising when considering the essentiality of genome-integrity. However, data about direct connections between these chromatin structure regulators and the actin cytoskeleton are limited. In this context it should be noted that Taf14 associates with the histone H3–acetyltransferase complex NuA3 [**91**]. Furthermore, a fraction of total cellular Arp4 recruits the NuA4 HAT complex to γH2A.X, thus mediating progressive chromatin restructuring [**107**].

Regarding plants, it should be added that orthologs of yeast *TAF14* have been found in diverse species (At*YAF9*), and the few existing data correspondingly show a role in chromatin remodelling (SWR1-complex). In particular, interactions with TAFs, transcription factors, and NuA4-acetyltransferase complex which are involved in flowering timing [**108, 109**].
The closest homolog of yeast *ARP8* in Arabidopsis is *AtARP9* [**30**], and limited data suggest a role in drought and salt stress response [**110**].

Hence, all things considered, the yeast 'actin-Arp-Taf'-aggregate represents a versatile chromatin-associated adaptor-module which recruits different structure regulators in a spatial, timely and function-related manner. Specifically regarding the cellular response to ionizing radiation, this

Figure 2

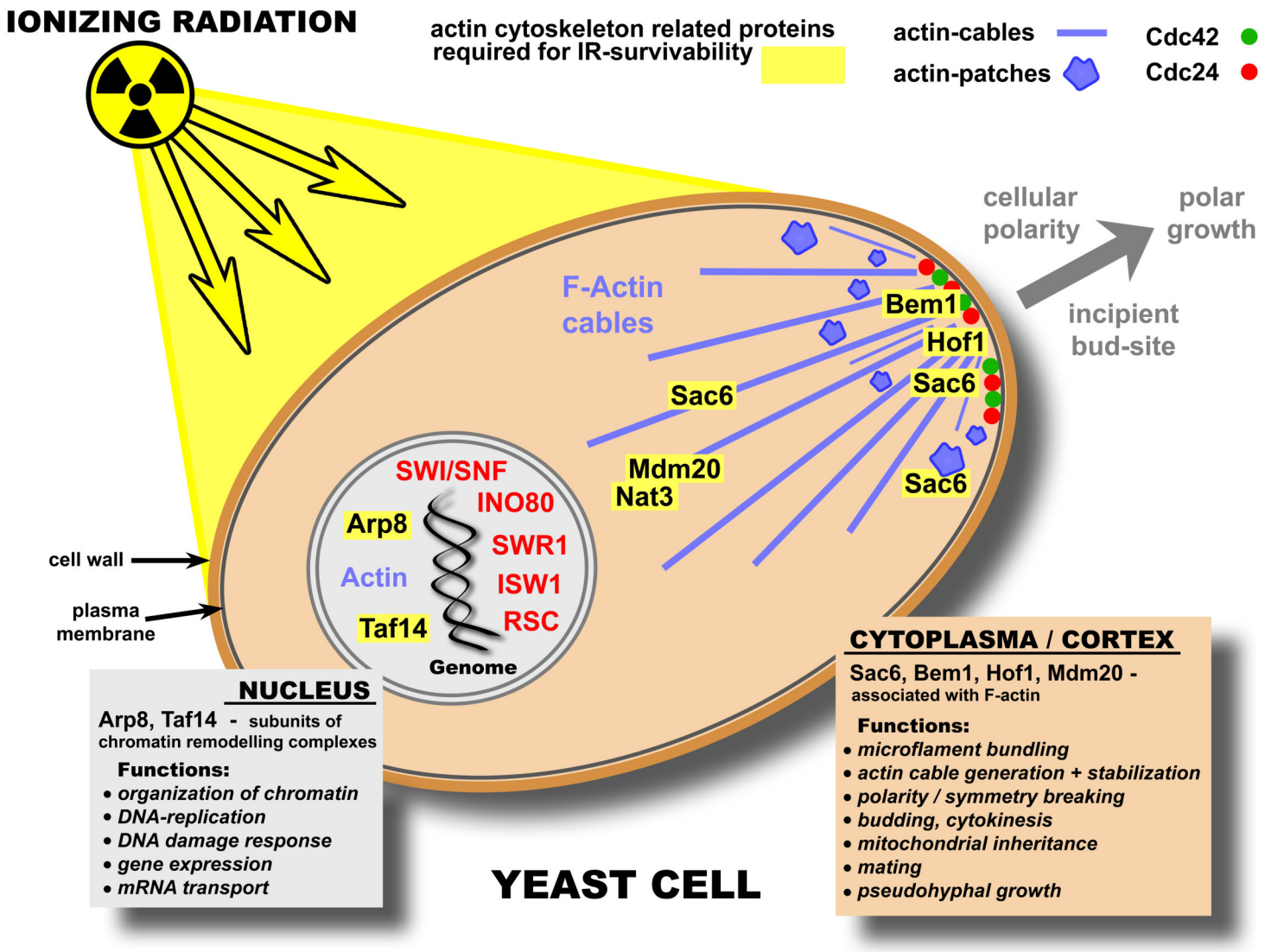


Figure2. Graphical Illustration Showing the Subcellular Localization of Yeast IR-Effectors Which Are Related to the Actin Cytoskeleton.

This succinct and simplified graphic portrayal shows the subcellular localization of reviewed proteins which are involved in cellular response to ionizing radiation. Particular significant is the functional partition of overall seven proteins into three operational modules that are spatially separated: 1) Nuclear-events, which are performed by chromatin-remodelling complexes that depend on subunits Arp8 and Taf14. Their functional repertory comprises organization of chromatin, DNA-repair, and transcription. 2) Cytoplasmic events, that are related to pathways involving *SAC6* (fimbrin), and *MDM20* (actin cable stabilizer) that is linked to *NAT3* (acetyltransferase). 3) Cortical events, which are mediated by *SAC6* (fimbrin), *BEM1* (polarity scaffold), *HOF1* (cytokinesis scaffold). Note the multifaceted localization of fimbrin Sac6, which acts as F-actin cross-linker and stabilizer at cytoplasmic actin cables, cortical actin patches, and at the apex. For conciseness and clarity, Sac6 has been exemplary indicated only three times. Respectively at one actin cable, at one actin patch, and at the apical cortex. This simplified representation symbolizes the ubiquitous involvement of numerous Sac6 molecules in the entirety of cables and patches in a real yeast cell.

central connector role of Arp8 and Taf14 is of significance, because both influence chromatin dynamics, DNA-repair, and transcription (**Fig.1**).

Yet, apart from a role in nuclear chromatin regulation, it is intriguing that *arp8Δ* and *taf14Δ* (*anc1Δ*) strains specifically exhibit abnormal actin cytoskeleton, altered cell morphologies, impaired growth, abnormal budding, and defective shmoo formation. Especially *anc1Δ* and *anc1-1* cells demonstrate disorganized cortical actin, which resembles mutations in *ACT1* or *SAC6* (fimbrin) [**35, 89, 111**]. In that respect Welch *et al.* further state that expression levels of actin and Sac6 are not changed in *taf14Δ* (*anc1Δ*) cells [**111**].

Most genes are regulated by TFIID-complex (>85%) [**112-114**]. However, the functionality of individual TFIID-components (TAFs) is different at diverse promoters [**115, 116**]. In this context it should be mentioned that the deletion of *TAF14* affects the expression of only 843 genes (~13% of all genes) [**104, 92**]. A closer look at data of Shanle *et al*. shows that expression of actin (*ACT1*) and its accessory proteins (*SAC6*, *BNI1*, *BNR1*, *ARP2/3*-complex, other *ARP*s, and *MYO1-5*) remains principally unaffected in *taf14Δ* cells compared to wild-type [**104**].

Moreover, it should be stressed, *anc1Δ*, *anc1-1*, and *anc1-2* cells demonstrate divergent phenotypes, and Welch *et al*. suggest that those differences might be due to partial loss of function of an actually multifaceted acting Taf14 [**111**]. Altogether, this allows to hypothesize about differential functionality of Taf14, in both, the nucleus as well as potentially in cytoplasm and cortex. This idea is supported by the observation of delocalized Spa2 in *taf14Δ* cells [**111**]. Normaly, in wild-type cells strictly polarized Spa2 acts as key subunit of the 'polarisome-complex' which regulates cytoskeleton dynamics at the imminent bud-site and bud-tip during polar cell growth [**117, 118**] (see next chapter) (**Fig.1; Fig.2**). Similar to deletion mutants of polarisome-subunits (*spa2Δ, bni1Δ, pea2Δ*, bud6*Δ*), yeast *taf14Δ* mutants exhibit dysfunctional shmoo formation and mating [**119, 111, 120-122**].

Consequently, further analysis of additional roles for Taf14 in the cytoplasm seems worthwhile, despite the fact that it has been characterized as sole nuclear protein [**111**] (**Fig.2**). It is conceivable that earlier studies detected primarily the abundant nuclear fraction, and failed to demonstrate a minor cytoplasmic subset.

2.2 Organization of the Cytoplasmic/Cortical Actin Filament Array and Cellular Polarity: *SAC6*, *BEM1*, *HOF1*, and *MDM20*

Regarding functional connections to the actin cytoskeleton, four additional deletion mutants could be identified among all known IR-sensitive yeast. Those are *sac6Δ*, *bem1Δ*, *hof1Δ*, and *mdm20Δ* [**65, 60**] (**Fig.1; Fig.2**). These genes play roles in cytoplasmic cytoskeleton dynamics and establishment of polarity during budding, pseudohyphal growth, mating, and cytokinesis.

2.2.1 The Fimbrin *SAC6*

The *SAC6* gene product (**S**uppressor of **AC**tin) is a highly conserved actin-binding fimbrin (Abp67) which mediates microfilament bundling by side-interactions (**Fig.1**) [**123-126**]. In budding yeast, the

fimbrin Sac6 cross-links actin filaments, thereby mediating actin cable assembly and stabilization [**127, 128**]. On the other hand, Sac6 has no influence on structuring of actin patches, but solely stabilizes their microfilament networks against depolymerization [**128, 129**]. This is facilitated in a cell cycle dependent manner in response to Sac6 phosphorylation by Cdk1 [**130**]. Sac6 is localized at actin cables, cortical actin patches, and at the cytokinetic-ring structure (**Fig.2**) [**123**], thereby playing a role in the endocytic pathway [**131**].

The interaction capacity of Sac6 is mainly restricted to proteins which are involved in the actin assembly-promoting machinery (**Fig.1**). Sac6 interacts with three subunits of the Arp2/3 complex, namely Arp2, Arc15 and Arc40 (**Fig.1**), and with the nucleation promoting factor Las17 as well, which jointly act in actin-nucleation and filament-branching, hence controlling especially composition and stability of actin patches [**132, 129, 133, 134**]. Moreover, Sac6 establishes physical contacts to one of the two yeast formins, Bni1, but not to Bnr1 (**Fig.1**). Both formins belong to an evolutionary conserved family of proteins containing formin homology domains (FH) [**135-138**]. They function as nucleation factors, which facilitate polymerization of actin-monomers and promote actin-cable formation [**139, 136, 140**]. However, they are differently localized: Bni1 at the budding site and at the tip of the daughter cell, whereas Bnr1 is exclusively found at the bud-neck. Hence, these formins differentially change the higher order structure of F-actin [**141**], and thus assemble polarized actin cables that guide directed transport for polar cell growth [**135**].

Sac6 also physically interacts with Bud6 (**Bud** site selection), which is involved in establishment of polarity, apical bud growth, shmoo formation, and pseudohyphal growth in yeast and filamentous fungi (**Fig.1**) [**142**]. Bud6 is a nucleation factor and interacts with both formins [**135**] thereby mediating the formation of bud tip-directed actin cables to facilitate transport of post-Golgi vesicles for polarized secretion. Bud6, the formin Bni1, Pea2, and Aip5 are assembled on the scaffold Spa2 (**Fig.1**) [**121, 143-146**]. This assembly constitutes the above mentioned 'polarisome-complex' which triggers cortical actin structures at the imminent bud site to induce polarized growth cones for budding, mating, and pseudohyphal growth [**143**]. Moreover, interaction of Bud6 and Bni1with Boi1 and Boi2 is required for correct localization of the cortical actin structure and directed vesicular transport (**Fig.1**) [**142**].

Regarding the radio-sensitivity of *SAC6* and *TAF14* deletion mutants, it is particularly significant that both are involved in functionality of the polarisome. Yet, a direct physical interaction between Taf14 and any subunit of the polarisome has not been detected to date (**Fig.1**).

The Arabidopsis homolog of Sac6 is Fimbrin5, which is an important organizer of actin filaments in tip-growing plant cells. AtFIM5 generates microfilament bundles and is essential for pollen germination as well as polarized growth of pollen tubes and root hairs [**36, 147-149**]. AtFIM5, just as AtFIM1, is involved in spatial and temporal assembly of higher-order actin structures like the actin-fringe, and stabilizes apical actin filaments for tip-directed vesicle trafficking [**36, 150, 41**].

Altogether, yeast and plant fimbrins are primarily required for orderly actin-dynamics and directed membrane trafficking during polarized cell growth. In general, Sac6/fimbrin specifically

stabilizes the polymer structure of actin cables, patches, and higher order networks. Therefore the reduced microfilament cohesion in *sac6Δ* cells might lead to generally increased vulnerability of the actin cytoskeleton. Consequently, this most likely causes the high IR-sensitivity of the yeast deletion mutant cells.

Moreover, in reaction to diverse cellular stress situations, Sac6 establishes physical contacts to several heat shock proteins with chaperone-function (Hsc82, Hsp82, Hsp42, Hsp104), as well as to the Grx3 oxidoreductase (**Fig.1**) [**151, 130, 152**]. In this connection it is relevant that *sac6Δ* cells demonstrate significant sensitivity to elevated temperatures [**153**].

Regarding a role in oxidative stress response, *SAC6* has been associated with ROS-related signal transduction in filamentous fungi. Here, particularly through inhibition of transport of transcription factor Cap1 from cytosol to nucleus [**154**]. Retaining of Cap1 in a complex with Sac6 and actin within the cytoplasm is suggested to repress transcription of genes involved in oxidative stress response [**154**]. Deletion of *SAC6* in *Candida albicans* leads to reduced polarized growth and defects in hyphal development and pathogenicity [**154**]. Similarly, *sac6Δ* mutants of *Ashbya gossypii* demonstrate abnormal localization of the hyphal actin cytoskeleton and defects in endocytosis [**155**].

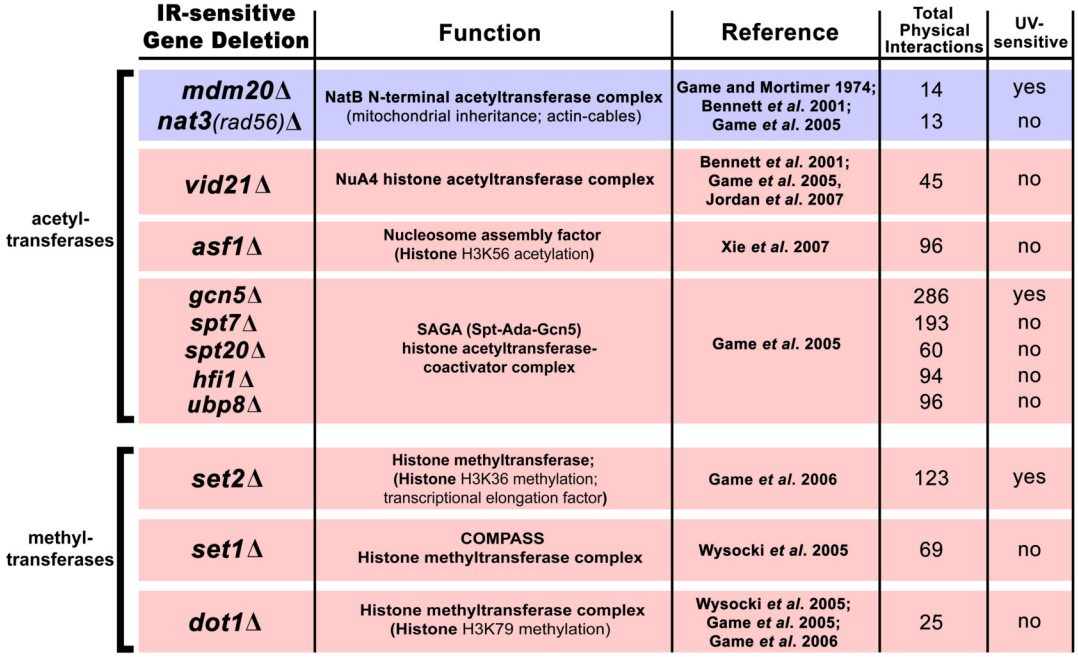
It´s worth noting that eukaryotic translation elongation factor 1 (eEF1) of *Saccharomyces cerevisiae* and *Aspergillus fumigatus* has been demonstrated to bind actin and mediate bundling of microfilaments [**156, 157**]. Furthermore, eEF1 is a downstream effector of Rho1 and interacts with formin Bni1 [**156, 158**]. Most interestingly, in *A. fumigatus ΔelfA* cells (eEF1Bγ deletion mutants) *SAC6* expression is upregulated particularly under oxidative conditions [**159**].

In summary, the current available data specifically demonstrate Sac6 as microfilament bundling and actin cable stabilizing fimbrin that is essential for maintenance of the cytoplasmic cytoskeleton and cortical patches in the bud-tip (**Fig.2**). It is concluded that, by this functionality, Sac6/fimbrin is required for viability upon high-dose irradiation.

In addition, Sac6 acts in oxidative stress response. Numerous interactions with members of the Hsp90-family and antioxidant enzymes suggest a central role for Sac6 in securing integrity of the actin array under unfavourable conditions. Potentially by recruiting those enzymes to the actin cytoskeleton. This particular function of Sac6 is very likely a requisite for tolerance of high IR-doses and the resulting ROS.

According to today´s knowledge, the fimbrin Sac6 is solely localized out of the nucleus and especially involved in functionality of large F-actin structures in cytoplasm and cortex (**Fig.2**), which corresponds to localization of fimbrins in other fungi [**125, 160, 161**]. Therefore, the gathered data allow to deduce that specifically those fimbrin-dependent microfilament arrays in that subcellular area are susceptible to IR, or rather required for IR-survivability. What´s more, it shows that also such IR-effects impair viability which are unrelated to the genome but are specifically imposed on the cytoplasmic/cortical microfilament subpopulation.

Figure 3



	IR-sensitive Gene Deletion	Function	Reference	Total Physical Interactions	UV-sensitive
acetyl-transferases	***mdm20*Δ**	**NatB N-terminal acetyltransferase complex** (mitochondrial inheritance; actin-cables)	**Game and Mortimer 1974; Bennett *et al.* 2001; Game *et al.* 2005**	14	yes
	***nat3*(*rad56*)Δ**			13	no
	***vid21*Δ**	**NuA4 histone acetyltransferase complex**	**Bennett *et al.* 2001; Game *et al.* 2005, Jordan *et al.* 2007**	45	no
	***asf1*Δ**	**Nucleosome assembly factor (Histone** H3K56 acetylation**)**	**Xie *et al.* 2007**	96	no
	***gcn5*Δ**	**SAGA (Spt-Ada-Gcn5) histone acetyltransferase-coactivator complex**	**Game *et al.* 2005**	286	yes
	***spt7*Δ**			193	no
	***spt20*Δ**			60	no
	***hfi1*Δ**			94	no
	***ubp8*Δ**			96	no
methyl-transferases	***set2*Δ**	**Histone methyltransferase; (Histone** H3K36 methylation; transcriptional elongation factor**)**	**Game *et al.* 2006**	123	yes
	***set1*Δ**	**COMPASS Histone methyltransferase complex**	**Wysocki *et al.* 2005**	69	no
	***dot1*Δ**	**Histone methyltransferase complex (Histone** H3K79 methylation**)**	**Wysocki *et al.* 2005; Game *et al.* 2005; Game *et al.* 2006**	25	no

Figure3. Compilation of IR-Sensitive Yeast Deletion Mutants Which Act in Diverse Acetyltransferase or Methyltransferase Protein Complexes.

This list comprises IR-sensitive yeast deletion mutants which are involved in regulation of the nuclear chromatin-structure (red) or of cytoplasmic proteins (blue). The listed genes either represent subunits of acetyltransferase complexes or methyltransferase complexes. Number of total physical protein-protein interactions is respectively indicated. Besides, the UV-sensitivity for each gene deletion mutant is given.

2.2.2 The polarity scaffold *BEM1*

Bennett *et al* show that diploid *bem1Δ* cells are highly sensitive to ionizing γ–radiation (**Fig.1**) [**65**]. Bem1 interacts with actin and functions as central organizing scaffold protein that plays a specific role in establishment of cell polarity during budding, mating and pseudohyphal growth of yeast [**162**] (**Fig.1; Fig.2**). At the incipient bud site the plasma membrane bound GTPase Cdc42 in its GTP-bound ('on'-state) recruits scaffold Bem1 which compiles the p21-activated kinase Ste20, the pheromone-responsive MAPK Ste5, and the GEF (GDP/GTP exchange factor) Cdc24, thereby inducing a positive feedback loop that generates a polar localized GTP-Cdc42 cluster [**163**] (**Fig.1; Fig.2**). GTP-Cdc42 further recruits the actin nucleating formin Bni1 [**164**], and the thereupon formed actin cables in turn direct localization of Bem1. Those polarity site directed actin cables mediate vesicular transport for secretion via myosin5. Bem1 also interacts with the exocyst subunit Exo70 (**Fig.1**). The landmarks (Axl2, Bud8, Bud9) associated Ras-GTPase Rsr1 interacts with Bem1 and Cdc24-GEF, thereby directing localized Cdc42 activation, and hence specific positioning of the polar growth site. For reviews about yeast polarity establishment see [**163, 165, 166**].

In summary, Bem1 fulfils a key function as factor for selection of the bud site and bud emergence, as well as for control of pseudohyphal growth, and mating. Bem1 achieves this regulatory role in conjunction with growth site directed actin microfilaments (**Fig.2**). Bem1 connects actin organization with Cdc42-dependent polarity establishment, vesicular transport, and exocytosis. The central scaffold function of Bem1 evinces a role as molecular network hub that organizes signal transduction for symmetry breaking and creation of cellular polarity. Thus, the observed IR-sensitivity of *bem1Δ* mutants meets the expectations of hubs representing crucial network connections. Deletion of *BEM1* impairs integrity of this symmetry breaking pathway, what therefore enhances its vulnerability to effects of ionizing radiation.

Generally, IR-sensitive deletion mutants comprise to a large extent genes that are involved in control of genome organization and transcription. Whereas *BEM1* and *SAC6* represent IR-susceptibility triggering factors, which specifically connect actin to the establishment of cell polarity and growth. The IR-sensitivity of *bem1Δ* cells shows that this actin-related pathway, which distinctively acts in the cortical area, is particularly required for IR-survivability. Consequently, this cytoskeleton-associated pathway, which acts in cell growth and morphology, represents another vital target of ionizing radiation apart from the nuclear genome.

2.2.3 Organisation of Actin Cables, Vesicle Trafficking, and Cytokinesis: *HOF1*

Diploid *hof1Δ* strains are moderately sensitive to γ–radiation [**65**]. Budding yeast *hof1Δ* mutants demonstrate punctuate actin clusters, multiple nuclei and a large elongated bud [**167**]. Hof1 represents an F-BAR domain containing protein that is part of the cytokinetic machinery during asymmetric cell division [**167-169**]. Dependent on the cell cycle phase, Hof1 localizes to the mother–bud neck (**Fig.2**), where it is involved in assembly of the actomyosin ring and regulation of its constriction [**167**]. Current data demonstrate Hof1 as a linkage in the coordination of septum synthesis

and actomyosin ring contraction [**170**]. Hof1 binds and bundles actin filaments hence generating actin cables and organizing them aligned between mother cell and bud, thereby physically connecting them with septins of the septin ring [**171**]. Furthermore, Hof1 interacts with type II myosin Myo1, and inhibits formin-mediated actin nucleation through binding to Bnr1 [**167, 172**].
BAR domains have been described as differentially bend 'arc'-shaped frameworks that associate with lipid bilayers and mediate membrane curvature [**173, 174**]. Present data suggest that Hof1´s F-BAR domain acts as tether between the plasma membrane and the cytokinetic apparatus [**170**]. Yet a function in F-actin assembly, via interaction with formin Bnr1, has also been shown [**172**]. In fungi and animals F-BAR (**B**in-**A**mphiphysin-**R**vs) domain proteins constitute a superfamily which can be categorized into sub-families according to their additional functional domains, such as RhoGEF-, RhoGAP-, SH3-, and Cdc42-binding domain, as well as tyrosine kinase-domain [**175**]. To date, in plants no F-BAR domain containing protein could be identified.

Altogether, issuing from its stationary position at the mother-bud neck, Hof1 plays a role as actin cytoskeleton structure regulator, which represents a central linking conductor of polarized membrane traffic and different events during cytokinesis [**172, 171**]. In short, Hof1 acts as scaffolding network hub in bud growth and cytokinesis. In the light of this, the IR-sensitivity of *hof1Δ* cells demonstrates another example for the susceptibility of the cytoplasmic and cortical actin cytoskeleton to ionizing radiation. Hence, integrity of the extranuclear actin-entity is required for cellular IR-survivability.

2.2.4 The Actin-related NatB N-terminal Acteyltransferase Complex: *MDM20* and *NAT3*

Finally, in this connection two additional yeast deletion mutants, which demonstrate high sensitivity to X-rays and γ–rays, should be mentioned: *mdm20Δ* and *nat3Δ* [**65, 60**]. Mdm20 (**m**itochondrial **d**istribution and **m**orphology) as auxiliary subunit constitutes, together with the catalytic core Nat3 (**N**-terminal **a**cteyl**t**ransferase), the NatB N-terminal acteyltransferase complex [**176-178**].
In general, N-terminal acetylation represents a multifaceted signal that has been related to regulation of protein-protein interactions, subcellular localization as well as degradation of proteins. Orthologs of yeast Mdm20 have been identified in diverse taxa of fungi and animals. The Nat-complexes of plants are still under-researched in comparison to those of fungi and humans. For a review see [**179**]. The closest Arabidopsis ortholog is the NatB auxiliary unit NAA25 (AT5G58450) which has been associated with plant growth [**180**]. The Arabidopsis NatB catalytic unit (NAA20) has been implicated in diverse developmental processes, in plant defence, in response to toxins, salt and osmotic stress, as well as oxidative stress [**180**].

Yeast Mdm20 plays a role in distribution of mitochondria into growing buds, specifically by influencing integrity and dynamics of the actin cytoskeleton [**181, 182**] (**Fig.2**). *MDM20* deletion mutants exhibit no detectable actin cables, whereas cortical patches are largely unaffected, and moreover, alleles of actin (*ACT1*) and tropomyosin (*TPM1*) were identified as suppressor mutations

[**181, 182**]. In detail, the available data suggest that Mdm2 controls acetylation of tropomyosin Tpm1 as well as of actin Act1 through the NatB N-terminal acetyltransferase, and thereby regulates actin-tropomyosin interactions which mediate generation and stability of microfilaments and cables [**181, 182, 177, 178**] (**Fig.3**). Yet notably, the mutant phenotypes of *mdm20Δ* and *tpm1Δ* cells exhibit differences, despite of synthetic lethality of double mutants [**181**]. Those observations imply that both gene products influence distinct portions of the actin cytoskeleton. Moreover, Mdm20 potentially effects additional targets apart from tropomyosin. Interestingly, a role for Mdm20 in regulation of the actin cytoskeleton has been corroborated in human cell lines, where Mdm20 acts via the Rictor/mTORC2 pathway on cell growth and motility [**183**].

In conclusion, current data depict yeast Mdm20 as cytoplasmic regulator involved in maintenance of cytoplasmic actin cables, which particularly mediate mitochondrial inheritance. This again highlights the multifaceted functionality of the cellular actin cytoskeleton. The reviewed studies support a view which depicts the global actin-entity as differentiated set-up with spatially distinct parts that are specifically regulated by individual effectors (**Fig.2**). In this context, the IR-sensitivity of *mdm20Δ* cells implies that functionality of the related cytoplasmic actin array represents a requirement for IR-survivability. Hence, this particular pathway constitutes a substantial target of ionizing radiation.

3. IR-EFFECTS ON TRANSCRIPTION OF CYTOSKELETON- AND STRESS-RELATED GENES

3.1 Fungi

Genomewide microarray transcriptional profiling of budding yeast after IR-treatment during the recovery period was performed in a variety of studies. Wild-types that were exposed to high-dose IR exhibit significant changes of their transcription levels (up-/down-regulation > twofold) which depends on the received irradiation doses [**184-187**]. Global transcriptional patterns and the total number of influenced genes in wild-type cells differ between haploid and diploid cells, as well as depending on the mating type (1523 genes in MATα; 1485 genes in MATa; 1274 genes in diploid yeast cells) [**186**]. A key subfraction of all modulated genes, which was correspondingly identified in haploid as well as diploid wild-type yeast strains, comprises particularly genes involved in DNA damage response and replication (RNR2/4, RAD51/52/54, POL4) [**184-188**]. Up-regulation of *RAD51* and *RAD54* transcription was also found in the radiation-resistant fungi *Cryptococcus neoformans* [**189**] and the black yeast *Exophiala dermatitidis* [**190**].

It should be highlighted that in *S. cerevisiae* and other fungi the mRNA levels of actin (*ACT1*) and related genes, such as *ARP*s, formins, *SAC6*/fimbrin, and *BEM1* are not significantly altered by high dose ionizing radiation (> 200 Gy) [**186, 189**]. This allows to conclude that upregulation of their transcription is not required after irradiation. Presumably because most mRNAs and proteins which are related to the actin array are relatively robust against IR-effects.

However on the other hand, Benton *et al*. show differently altered transcript levels of some genes which are involved in mating and pseudohyphal growth (*HO*, *ASH1*, *CDC24*, *STE11*, *STE5*, *BEM3*, *TEC1*) [**187**].

Most notably, numerous IR-induced genes are related to protection from cellular damages through heat or oxidation. In this context it is interesting that previous heat-shock of cells induced following increase of their IR-tolerance [**191, 192**].

DeSanctis *et al*. report the IR-dependent transcriptional modulation of ~200 yeast genes after irradiation with sublethal X-ray doses (80 Gy), whereby induction of several heat-shock protein genes (*HSP10*, *HSP12*, *HSP26*, *HSP30*, and *SSA4*) was particular significant [**184**]. Mercier *et al*. corroborate transcriptional activation of heat-shock genes after exposure to 200 Gy γ–rays [**186**].

In addition, upregulation of genes involved in response to oxidative stress was observed, as for example catalase (*CTT1*), superoxide dismutase (*SOD*), glutathione-dependent oxidoreductase (*GRX1*), glutathione-S-transferase (*GTT1*), glutathione peroxidase (*GPX1/HYR1*) [**184-187**]. A role for antioxidant enzymes particularly in IR-response is corroborated by the observation that deletion of catalase (*ctt1Δ*) or superoxide dismutase (*sod1Δ*) increased IR-sensitivity of *S. cerevisiae* [**193, 194**].

Similarly in *Schizzosaccharomyes pombe*, among 200 changed transcription levels in total, the induction of antioxidant (*GRX1*, *TRX2*, *GST2*) and heat-shock (*HSP16*) genes was observed in response to 500 Gy γ–rays [**195**]. Also in the IR-resistant melanized fungus *Cryptococcus neoformans* heat-shock protein genes (*HSP31*, *HSP78*) were induced at 3 kGy [**189**], and antioxidant enzyme transcript levels of *SOD1*, *TRX1*, *GPX5* were up-regulated at 100 Gy and 300 Gy γ–irradiation [**196**].

Interestingly, Schultzhaus *et al*. state that melanin, which is considered as radical scavenger, per se does not protect against IR and related ROS. However, melanization and prior metabolic state of *Cryptococcus neoformans* substantially influenced the general composition of its transcriptome, which thus increased cellular IR-tolerance [**197**]. This conclusion is in line with the argument that the specialized and rationalized transcriptome of plant microgametophytes (pollen) represents a foundation for their high IR-tolerance compared to plant sporophytes [**15**]. Particularly the transcription of heat-shock and antioxidant enzyme genes is generally up-regulated during germination and tube growth of untreated/non-irradiated pollen [**198, 15**], and this hence might serve as a base for control of the molecular consequences of ionizing radiation.

Yet, it should be said that up-regulation of transcription by irradiation does not necessarily cause increased protein levels, but it might complement protein loss due to protein damage and degradation which are induced by IR. This is supported by the results of Schultzhaus *et al*. [**199**].

3.2 Plants

While profiling of genome-wide transcripts has been analysed numerously in human cell lines exposed to IR, plants on the other hand have been under-researched in this connection.

The model plant *Arabidopsis thaliana* is a highly robust ‘weed’ that copes with various detrimental environmental factors. Accordingly, thale cress has been described as exceptionally tolerant to high

doses of ionizing radiation, much more than most other plants. Kim *et al*. analysed gene expression profiles of Arabidopsis rosette leaves after exposure to 200 Gy γ–radiation and observed induction of 2165 genes and repression of 1735 genes [**200**]. Notably, among this large number of modulated genes, the actin-associated Arabidopsis formins *AFH1* and *SCAB3* are induced. Interestingly, AtSCAB3 is a myosin-related protein that bundles and stabilizes actin filaments [**201**]. Moreover, a variety of genes which are related to the antioxidant system of plant cells were particularly affected (*SOD*, *CAT*, *APX*, POD, glutarredoxine family proteins, FeII-oxidoreductase) [**200, 202, 203**]. Increased mRNA levels of these ROS-scavenger genes were also observed in *Nicotiana tabacum* plants after irradiation with 50 Gy γ–rays [**204**] and in *Solanum lycopersicum* [**205**] as well as in *Vigna unguiculata* [**206**] between 100 Gy - 300 Gy. In addition, transcription of Arabidopsis NADPH oxidases (AtRBOH) and NAD(H) kinase (AtNADK-1), which are involved in control of ROS levels, is up-regulated after γ–irradiation [**207, 208**].

Regarding low-dose ionizing radiation (< 1 Gy), it should be mentioned that IR-doses which correspond to conditions in the radioactive contaminated soil of the Chernobyl or Fukushima zone induce transcriptional upregulation of antioxidant enzyme genes in leaves of poaceae like *Oriza sativa* [**209-211**]. Moreover, Zaka *et al.* conclude that chronic low-dose radiation at the Semipalatinsk nuclear test site lead to selection of genotypes demonstrating high antioxidant enzyme activities [**212**]. Interestingly, proteomic analyses of the second generation flax seeds that were cultivated in the contaminated soil at Chernobyl demonstrate increased expression and potential post-translation modification of several NADP-dependent enzymes of carbohydrate and lipid metabolism [**213**].

All in all, in fungi and plant tissues the transcription of antioxidant genes is particularly modulated by IR, whereas, mRNA levels of most actin cytoskeleton related genes remain largely unchanged even at high doses. Yet, the transcriptional up-regulation of microfilament-bundlers *AFH1* (formin) and *SCAB3* in Arabidopsis suggests a tendency for stabilization of the cytoskeleton.

4. THE RELATION BETWEEN ACTIN AND IR-INDUCED ROS

High doses of ionizing radiation generate extensive amounts of intracellular and extracellular reactive oxygen species that are potentially toxic to cells. However, usually under natural conditions (without elevated IR-levels), ROS are already physiologically generated in the cytosol, as well as in metabolic highly active organelles like chloroplasts, mitochondria, and peroxisomes, or are delivered into the apoplast [**56**]. ROS do not only act as toxic agents, but function as signalling molecules and support cellular processes as for example cell growth, cell division, differentiation, as well as responses to biotic and abiotic stimuli [**54-56**]. Particularly, a tip-focused accumulation of ROS has been observed in polarized growing cell types, such as pollen tubes [**214-216**], root hairs [**217-219**], fungal hyphae [**220-222**], and fucoid algae [**223**]. Plasma membrane localized NADPH oxidases (NOX) cause an intracellular ROS-gradient and apoplastic accumulation in the cell wall [**217, 224, 218, 214, 215, 225**]. This apical accumulation of ROS in the cytoplasm as well as in the cell wall of

above-mentioned polarized cell types represents an essential requirement for the orderly tip growth process. However, apart from cell growth, it is crucial for numerous additional physiological processes that a cellular ROS-homeostasis system controls and maintains the steady-state level of ROS-production and ROS-scavenging. In essence, this multifunctional '*NOX / ROS / ROS-scavenger*' system (**Fig.4**) appears to be conserved between plants, fungi, and animals.

It is of special significance that available data point to a mutual interrelation between reactive oxygen species and the actin cytoskeleton (**Fig.4**). Several lines of evidence establish that production of ROS by NADPH oxidases is required for actin cytoskeleton stability in yeast [**226**], in plants during immune response and self-incompatibility (SI) response [**227-229, 49**], and in animals regarding cellular migration, development of the nervous system, various diseases, as well as defence against pathogens (reviewed by [**230, 231**]. Yet, in biomedical research the interconnection particularly of actin, ROS, and NOX still remains poorly understood, and even less is known in fungi and plants.

Regarding yeasts, for example, the NADPH oxidase deletion mutant *yno1Δ* exhibits reduced integrity of the actin cytoskeleton, which is hence hypersensitive to the inhibitors of actin-polymerization Latrunculin-B and Wiskostatin [**226**].
On the other hand, some studies suggest that the actin cytoskeleton is involved in regulation of the cellular ROS content as well. Consequently, this would mean that there is an interdependency between actin and ROS (**Fig.4**). The view of mutual connections between actin dynamics and cellular ROS levels is supported by studies which suggest that stabilization of F-actin induces ROS production and vice versa (**Fig.4**) [**232-234**]. In this context, overexpression of yeast *SCP1*, which acts as actin filament cross-linker in the cell cortex, has been described to cause increased amounts of intracellular ROS [**232**]. Whereas *SCP1* deletion promotes cell survival under high oxidative stress conditions [**232**]. Deletion of genes that enhance actin dynamics (*END3*, *SLA1*), on the other hand, leads to actin-aggregation and elevated ROS-levels [**235**]. Cellular ROS levels were also increased through treatment with the F-actin stabilizing drug Jasplakinolide [**232**].

Moreover, Farah *et al.* suggest that the actin cytoskeleton executes protective effects at high levels of oxidative stress, thereby generating numerous distributed actin patches which they term '*oxidation-induced actin bodies'* (OABs), while actin cables fully disintegrate [**234**]. These characteristics of the actin cytoskeleton resemble those of '*miconazole*'-treated wild-type yeasts or untreated *hof1Δ* mutants [**233**]. The fungicide '*miconazole*' induces ROS-generation [**236**]. Importantly, IR-sensitive yeast *hof1Δ* mutants are also miconazole-hypersensitive [**233**]. Taken together, this shows that impairment of viability by IR, particularly regarding the above-mentioned actin-related *HOF1*-pathway (*chapter 2.2.3*), might be imposed via IR-induced ROS.
Interestingly, OABs are massively associated with fimbrin Sac6 and are resistant to Latrunculin-B [**234**]. When additionally considering the increased IR-sensitivity of *sac6Δ* cells (*chapter 2.2.1*), these observations altogether allow to hypothesize that Sac6 plays a key role as F-actin stabilizer and recruiter of antioxidant enzymes (GRX) as well as HSPs, specifically under high ROS conditions which are induced by IR (**Fig.1**).

In the context of the yeast OABs, the SI-stimulated formation of '*actin punctate foci*' in incompatible pollen tubes should be mentioned [**228**]. Wilkins *et al*. show that activation of self-incompatibility response increases ROS levels in pollen tubes, which in turn correlates with appearance of '*actin punctate foci*' [**228**]. Here, live-cell imaging demonstrated ROS throughout the pollen tube cytoplasm, yet ROS appeared massed at hot spots, and thus most probably originated from intracellular organelles [**228, 237**]. Application of ROS-scavengers reduced occurrence of these actin foci and partially restored normal filamentous actin cytoskeleton [**228**]. Hence, in addition to yeast cells, these observations corroborate the actin-stabilizing effect of ROS for plant cells as well (**Fig.4**). Similar to yeast OABs, the pollen tube F-actin foci are likewise resistant to depolymerization by Latrunculin-B and colocalize with fimbrin and a subset of ABPs (ADF/cofilin, CAP) [**238**]. Furthermore, treatment of leaf epidermal cells with exogenous H_2O_2 increased F-actin abundance and stability [**229, 239**]. Cao *et al*. suggest that ROS enhance F-actin assembly probably by inhibiting the actin capping protein (CP) [**239**]. In turn, disruption of F-actin by Latrunculin-B leads to compensatory cellular counteraction, thus enhancing ROS production [**229**].

Thus, generally, it can be stated that actin-dynamics is sensitive to the cellular redox status (**Fig.4**). However, most probably there is a difference between physiological low-level ROS signalling, and cellular response to extensive ROS levels which are imposed by IR. Abundant IR-induced ROS can more severely affect organization of cytoskeleton arrays (**Fig.4**). Redox modifications of specific residues in actin´s amino acid sequence can lead to conformational changes of protein structure. This, as a consequence, can cause impairment of actin´s interaction capacities. Hence, actin polymerization and association with ABPs are affected by the redox status within cells. This is particularly caused by oxidation of cysteine and methionine residues that are normally important for actin´s functionality [**240, 234, 241**]. Most importantly, current data, which are largely collected from animal studies, indicate that actin apparently acts as sensor and buffer against high ROS concentrations through formation of covalent disulfide bonds between sulfhydryl groups of cysteines [**240, 234**]. This scenario implies intra- and inter-molecular linkages of microfilaments, which foster the generation of large F-actin aggregates, like yeast OABs or plant '*actin punctate foci*'. In conclusion, this means that the cellular actin-entity most probably plays a role as universal scavenger of radicals in redox homeostasis, apart from its specific functions in chromatin organization, DNA-repair, transcription, membrane transport, growth, and morphogenesis.

In this connection, the IR-induced ROS have an exceptional role due to their high intrinsic and extrinsic levels. Yet, the exact mechanism by which IR-induced ROS cause spatial reorganization of the actin array still remains to be determined. Which molecular actin-related events, ABPs, and signalling pathways are specifically involved? Is the postulated buffer function a general feature of the collective actin-entity, or is merely a particular subset involved?

Finally, it can be concluded, brief and concisely, that ROS-levels and actin-dynamics are interconnected (**Fig.4**). Regarding a potential involvement of regulatory networks, it should be highlighted that small RHO-GTPases (Rac-like/Rho-like GTPases in plants termed 'RAC/ROPs') play

a role in both, regulation of actin-dynamics as well as in production of ROS by NADPH-oxidases [**242, 214, 243**]. Moreover, concerning filamentous fungi (*A. nidulans*; *E. festucae*), it should be mentioned that Bem1, Cdc24, and Cdc42 have been shown to act in polarized localization of NADPH-oxidases, which are required for apical hyphal growth [**220-222**].
Yet, starting from current available data, still further scientific work is needed to reveal a precise picture of the interrelation between actin, small GTPases, NADPH-oxidases, and ROS.

Apart from the influence of endogeneous ROS on normal cell functionality, the implications of stress-related ROS on plant and fungal cells remain under-researched. In particular, regarding the relation between actin and ROS that are induced by IR. In short, high-dose IR generates extensive amounts of intra- and extracellular ROS, besides other reactive organic peroxides. In addition to the damages that are caused by IR through direct energy transfer on biomolecules, these abundant ROS attack a wide-variety of cellular constituents. Most significantly, high ROS quantities interfere with the fine-tuned homeostasis system comprising actin / GTPases / NOX / ROS / ROS-scavengers. The eclectic action spectrum of the actin-entity implies that perturbations of actin-dynamics through abnormal ROS levels potentially affect a multiplicity of cellular pathways, which hence, dramatically impair cellular viability.

One example is the dose-dependent inhibition of bivalent chromosome mobility in the pachytene stage during meiosis of yeast cells through 40 Gy X-rays, which can be recovered by radical-scavenging antioxidants [**244**]. Illner *et al*. show that both, IR-induced ROS as well as treatment with H_2O_2 similarly disrupt integrity of actin cables, thereby compromising meiotic chromosome movement. Whereas on the other hand, H_2O_2 treatment only marginally caused double-strand breaks per cell in comparison to IR [**244**]. This demonstrates that oxidative-stress specifically affects functions of the actin cytoskeleton.

Notably, current data establish that the actin cytoskeleton is involved in apoptosis and programmed cell death in all three eukaryotic kingdoms, namely animals, fungi, and plants (for a review see [**245**]). Data, which indicate actin-dynamics, particularly F-actin-stabilization, plays a role in triggering of programmed cell death [**232**], support the here proposed key function for the actin-entity in IR-response.

Moreover, it thus seems reasonable to hypothesize that the abundance and exceptional high turnover rate of the actin cytoskeleton in typically fast growing pollen tubes might be a basis for the extreme IR-tolerance of plant male gametophytes [**15**]. In other words, the extraordinary lively actin-dynamics of pollen tubes, in comparison to other cell types, can facilitate buffering of excessive IR-induced ROS.

Figure 4

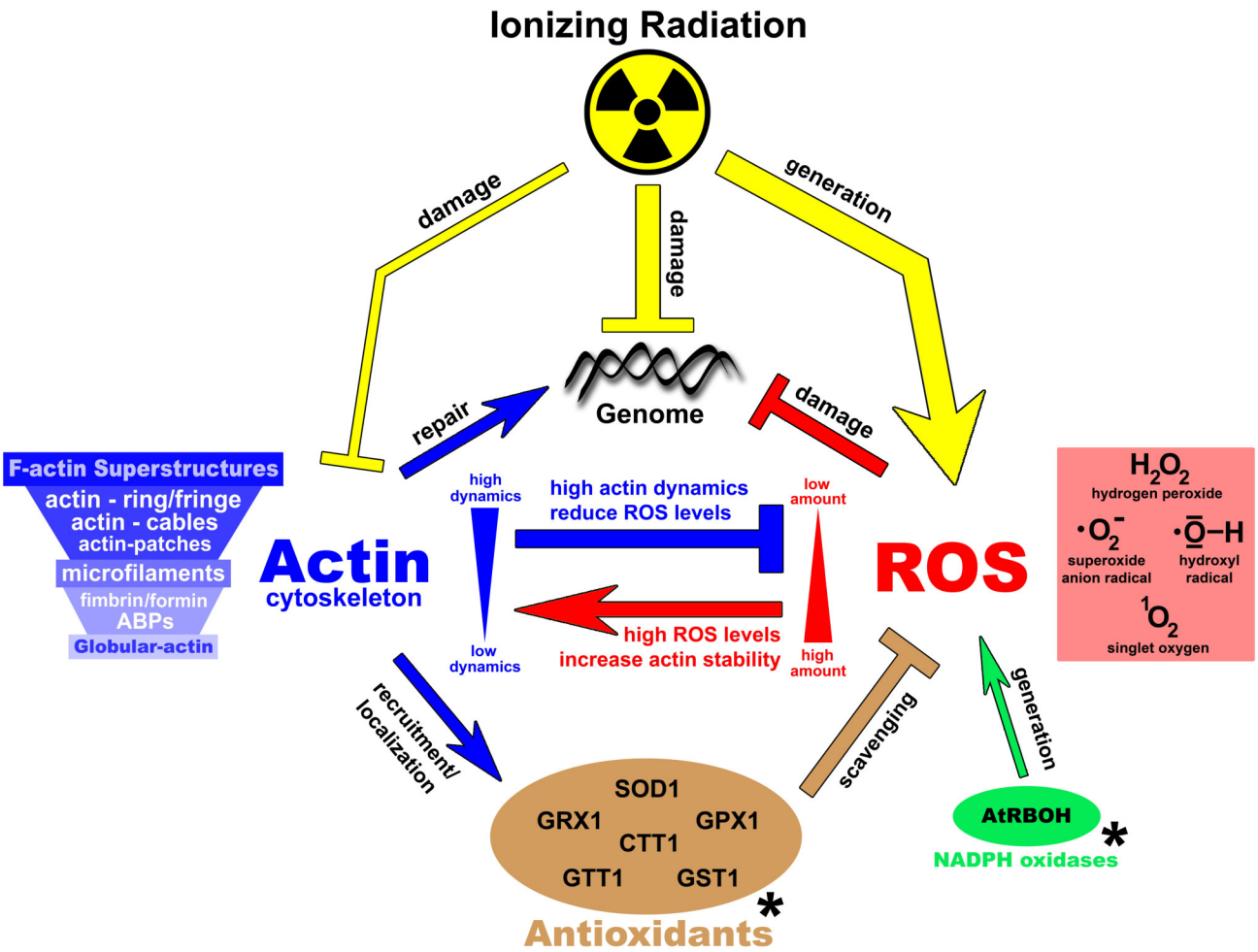


Figure4. Diagram Depicting the Interrelation between Ionizing Radiation, the Actin Cytoskeleton, Reactive Oxygen Species, and Antioxidants.

This schematic overview outlines implications of ionizing radiation on the genome, the cellular actin cytoskeleton, ROS, and antioxidant enzymes. Moreover, the mutual effects and interrelations between the actin-entity and ROS are highlighted. Note: 1) High ROS levels induce F-actin stabilization. 2) High actin-dynamics reduce ROS levels. And vice versa. Arrows indicate positive effects (e.g. induction, stabilization), whereas the T-shaped indications represent inhibitory effects (damage). Colours correspond to the respective source of effect. Yellow: Ionizing radiation (IR). Blue: Actin cytoskeleton. Red: Reactive oxygen species (ROS). Brown: Antioxidant enzymes. Green: NADPH oxidases (NOX). Asterisks: Gene expression induced by IR.

5. CONCLUSION AND PERSPECTIVES

This survey highlights the multifaceted character of the microfilament cytoskeleton and demonstrates that actin and its accessory proteins represent a key entity in the eukaryotic cell. All in all, the whole cellular actin-framework consists of distinct actin arrays in nucleus, cytoplasma, and cortex which fulfil diversified functions. The collective actin-entity is universally involved in a wide-range of cellular pathways, however, the spatially discrete submodules of this abundant subcellular assembly are assigned to specific physiological processes. These comprise the organization of chromatin and other nuclear events (e.g. DNA-repair and transcription), as well as cytoplasmic transport and cortical actions like endo- and exocytosis, which are required for polarization and growth. Based on this ubiquitous functionality, the present review particularly concludes that the globally acting total actin-polymer entity has a key role in IR-tolerance. This is highly plausible considering its extended subcellular dimension and its multifaceted actions in multiple compartments of the cell. *ARP*8, *TAF14*, *SAC6*, *BEM1*, *HOF1*, and *MDM20* are respectively related to specific actin-aggregates which constitute spatially and functionally distinct subsets of the cellular cytoskeleton (**Fig.1** and **Fig.2**). Consequently, the collective IR-sensitivity of their deletion mutants allows to conclude that actin and its accessory proteins represent one multifaceted key entity whose integrity is required for survival of high-dose IR. In overall terms, the cellular actin framework is a substantial target of IR-effects.

Regarding the herein suggested key role of the actin cytoskeleton in radiation-susceptibility, it should be mentioned that potential '*non-genetic causes of death*' have been thematized since Mortimer [**246**], Sarachek *et al*. [**247**], and Magni [**248**]. The widely accepted '*basic principle of radiation biology*' postulates that the genome is the main target for IR-effects, and thus its functional integrity undeniably represents the major factor for survivability of dividing cells under high-dose ionizing radiation [**2**]. However, the degree of chromosomal deterioration and viability depends not only on direct DNA-damages through IR (e.g. SSBs, DSBs or DNA-histone cross-linking), but also on the capabilities of the cellular repair machinery. The present study especially highlights the significance of actin arrays in this connection. Particularly, the IR-sensitivity of *arp8Δ* and *taf14Δ* strains shows that IR-effects on the actin-entity specifically impair functionality of the DNA repair system (e.g. functionality of chromatin-remodelling complexes).

It is of great significance that actin and accessory proteins are involved in maintenance of genome integrity and transcription. However, given that the cellular DNA repair machinery is fully operational and enabled to maintain the genome functional at specific IR doses, then more sensitive factors, which regulate other cellular processes, can become essential. This study depicts the cytoplasmic and cortical microfilament array as such a vital constituent for cellular survivability of IR. The observed requirement of *SAC6* (fimbrin), *BEM1* (polarity scaffold), *HOF1* (cytokinesis scaffold), and *MDM20* (actin cable stabilizer) for cellular IR-tolerance allows the deduction that particularly extra-nuclear events have great importance for viability as well. It should be highlighted that Sac6, Hof1, and Mdm20, altogether are specifically involved in actin cable integrity and stabilization of the

cytoplasmic/cortical cytoskeleton. The requirement for stabilizers of actin filament arrays, when cells are exposed to ionizing radiation, corresponds to a model in which the large abundant cytoplasmic cytoskeleton superstructures represent major targets of IR.

To put it concisely, this article highlights that other cellular key processes, apart from maintenance of genome integrity, are also significant targets of IR-destruction, and thus required for viability. For this, cellular pathways that are associated with Sac6, Bem1, Hof1, and Mdm20 represent examples which are specifically involved in polarity establishment and anisotropic cell growth.

It should be said that data about plant homologs of the above reviewed yeast genes are very limited. Despite some few hints for involvement in stress response, little is known about the exact role for plant orthologs of yeast *ARP8* (*AtARP9*), *TAF14* (*AtYAF9*), or *MDM20* (*AtNAA25*). Much more experimental work is required here. Moreover, hitherto no relevant homologs of *HOF1* or *BEM1* could be identified in Arabidopsis or other plants.

It is a matter of ongoing discussion if direct IR-damages or indirect ROS-damages are the major source for general impairment of cellular constituents. Either way, both, IR as well as the IR-induced ROS affect the individual constituents of cells differentially. Diverse classes of molecules (DNA, RNA, proteins, lipids) are specifically damaged by ionizing radiation [**7**]. Even molecules of the same class can be differently impaired. For example, based on the individual amino acid sequence and 3-dimensional structure of diverse proteins it is evident that IR differentially affects their specific functionality. Consequently, diverse protein-complexes or macromolecular protein-assemblies like the cytoskeleton have the potential to respond in different ways towards IR.

In this context, it should be mentioned that IR-related protein damage, which imposes lethal effects on cells, has been made a subject of discussion by Dale [**249, 250**]. Later also by Daly *et al*., through examinations on the radiation resistant bacterium *Deinococcus radiodurans* [**251**]. In this particular case, Daly *et al.* state proteome damage through the IR-induced ROS represents the major factor for cell death. They further attribute this to the resulting inhibition of enzymes, which are specifically required for replication and repair of DNA [**252**].

By being the central storage for genetic information, it is self-evident for cellular systems that integrity of DNA is the central requirement for viability. Hence, in this connection, functionality of the enzymatic DNA-repair machinery becomes essentially important after genome damage through irradiation. Therefore, put in simplified terms, both, genome and proteome are respectively crucial for proper functionality of the cell, and what´s more, they are interdependent.

Finally it should be mentioned that the vast majority of cells, and especially, proliferative cells which perform continuous mitotic division, are highly susceptible to IR, because they are specifically dependent on integrity and propagation of the genome. IR-induced damage of the chromatin is the main factor which impairs cellular viability after irradiation. The IR-sensitivity of *arp8Δ* and *taf14Δ* cells substantiates the crucial function of the nucleus-specific subset of actin and its accessory proteins for genome maintenance after DNA damage through ionizing radiation (**Fig.2**). However, apart from

that, this article shows that other, actin-related pathways (*SAC6*, *BEM1*, *HOF1*, *MDM20*), which are required for cytoplasmic and cortical events (**Fig.2**), are significant for IR-survivability as well.

It should be mentioned that some cellular processes which are partially independent of a functional genome, such as particularly pollen germination and early tube growth, are less sensitive to ionizing radiation **[15]**. High-dose irradiated pollen, which thus harbor annihilated genomes, still perform germination and polarized growth. Evidently, in pollen that have received high-dose IR, the DNA represents the most susceptible molecule, comparably more than RNA, proteins, or lipids [**15**]. Since effects of IR on various cellular constituents are differential, ionizing radiation imposes distinct implications on different cell types. However, not only on cells of diverse taxa, but also on cell types of one species, which is based on their individual subcellular composition (e.g. cells of gametophyte and sporophyte).

In this connection, reactive oxygen species most probably play an essential role. Particularly the abundant IR-induced ROS affect the actin cytoskeleton through generation of intra- and inter-molecular covalent disulfide linkages between sulfhydryl groups of cysteines in actin and actin-associated proteins. As a consequence, normal dynamics of microfilaments, actin cables, and actin patches is impaired. Generation of F-actin aggregates is fostered.

This allows to develop a scenario, in which diverse cell types respond differently to IR-induced ROS, and that respectively depends on the specific abundance and dynamics of their actin-entity. In this connection, it is highly significant that actin-dynamics of fast tip-growing pollen tubes is much higher than that of sporophytic cell types. Pollen tubes exhibit a high percentage of cellular G-actin in comparison to other cell types [**17**]. Thus, despite their extensive filamentous actin arrays in shank and subapex, F-actin merely represents less than one-tenth of the total cellular actin content [**253, 254**]. This means that numerous G-actin monomers are available for continuous polymerization and buffering of IR-induced ROS. Hence an abundant cellular actin-entity represents a potent scavenger of extensive harmful ROS.

Relations between actin and mitochondria play a role in initiation of apoptosis. Deregulation of the tightly controlled dynamics between G- and F-actin triggers commitment to programmed cell death. Reduction of F-actin levels in pollen tubes through depolymerization via Latrunculin-B treatment induces programmed cell death, whereas F-actin stabilization by Jasplakinolide prevents this [**255**]. However, in contrast, stabilization of the actin cytoskeleton by drugs, actin mutations, or actin-bundling proteins causes high intracellular ROS levels and cell death of yeast [**232**]. Gourlay *et al*. attribute this to ROS production by mitochondria, which is evoked by effects of stabilized F-actin on membrane channels. Thus there are deviations between plants, yeast, and animals concerning the events that initiate cell death [**256, 232, 255**]. The above-mentioned differences between diverse cell types regarding total actin content and G/F-actin ratio can be considered the cause for this.

Finally it should be said that the connection between actin, mitochondria, and apoptosis might be relevant for the IR-sensitivity of *mdm20Δ* mutants.

Hence, this leads to an important question. How does ionizing radiation affect the actin cytoskeleton at large? By direct energy deposition through IR, or indirect damage via IR-induced abundant ROS? Most likely both, yet heterogeneously and to varying extents. Specifically the exceptional high ROS levels dramatically impair actin-dynamics in various ways. In contrast to the reactive oxygen species which are normally produced in diverse plant organelles at differential levels (e.g. the tip-directed ROS-gradient in pollen tubes), ionizing radiation homogeneously induces extensive amounts of ROS throughout the cell. This gives IR-related ROS an exceptional position. Moreover, it is of particular importance to consider that the proportional composition of the collective intracellular ROS-entity is different between the biogeneously generated and the IR-induced oxidants. This means that, depending on its causative source (stressor), the ratio between the diverse reactive oxygen species varies.

Among the actin-associated proteins which affect IR-tolerance, members of the ARP-linker module have a special place. It is striking that both, Arp8 and Taf14 represent highly interconnected network hubs which link the actin-entity with a functional module of multiple different genome-structure regulators (**Fig.1**). Therefore, one might simply suspect that the number of physical protein interactions might, in general, play a role regarding IR-sensitivity of deletion mutants. However, by comparing the number of interactions this could not be corroborated by this study. The number of total protein-interactions (e.g. Taf14 = 134; Arp8 = 61) regarding IR-sensitive deletion mutants ranges between 13 and 286 (**Fig.1, Fig.3**). Their mean value shows no statistically significant difference from the entirety of all proteins (**Fig.1, Fig.3**). Thus the respective total number of links most probably plays a minor role. This study rather suggests that relative connectivity and topology within specific protein-interaction networks is far more important for susceptibility to IR. Here, those proteins have a particular significance which play a role as interface that links and coordinates essential modules of diverse functionality.

This is particularly given for Arp8 and Taf14 which represent highly connected elements in a conformational switch module. This module organizes various chromatin remodellers and spatially links them to actin and distinct regions of the chromatin, particularly in response to DNA-damage (**Fig.1**).

Bem1 represents a central network hub thereby acting as scaffold that is crucial for tethering of various proteins which are required for polarity establishment and growth (**Fig.1**).

Sac6 is the sole fimbrin in yeast and interacts with key actin-associated proteins (ARP2/3-complex) and the polarisome. Furthermore, Sac6 links actin filaments and stabilizes the actin array. Regarding IR-related damages it is moreover significant that Sac6 variously connects to members of the HSP/antioxidants-module.

Altogether, the reviewed actin-associated proteins in figure 1 (Act1, Arp8, Taf14, Sac6, Bem1,) constitute a hierarchic-decentralized interaction network structure. Whereby actin itself has a key role here.

Remarkably, among the 25 analysed IR-sensitive yeast deletion mutants only 7 are also UV-sensitive (**Fig.1, Fig.3**). Specifically regarding actin-related genes, sensitivity to UV and IR has solely been demonstrated for *taf14Δ* and *mdm20Δ* cells. This suggests distinct functionality for Arp8 and Taf14, despite their cooperation in the same functional module. However, correspondingly Arp8 is primarily linked with INO80- and ISW1-complex, whereas Taf14 mainly associates with SWI/SNF- and RSC-complex with extensive assistance by Arp7 and Arp9 (**Fig.1**).
Generally speaking, the mentioned divergences between cellular sensitivity to IR and UV demonstrate the different characteristics and effects of the various types of electromagnetic radiations.

In summary, this study highlights the eclectic character of the actin-entity and its multifaceted spheres of action comprising distinct functionality in nucleus, cytoplasma, and cortex. This fact provides the basis for actin´s role in IR-response. Moreover, it is of special significance that several IR-sensitive pathways have roles apart from genome maintenance.
This survey shows that various facets of the actin cytoskeleton are affected by ionizing radiation: i) The cytoplasmic/cortical actin-polymer framework which facilitates cellular transport, polarization, growth, and cytokinesis. ii) The nuclear actin-entity which is involved in organization of protein complexes that regulate chromatin-dynamics, DNA-repair, replication, and transcription. Whether and how potential actin filaments in the nucleus are affected still requires more experimentation and precise clarification, particularly in fungi and plants. Presumptive properties and function of filamentous actin-structures in the nucleus represent a fascinating and currently hot debated topic particularly concerning animal cells [**257**]. Interestingly, studies on mammalian cell lines suggest that assembly of actin filaments in the nucleus is induced through DNA-damage with involvement of formins and Spire nucleators [**77**].

In a wider view, the presented results do not only contribute to general research about functionality of the cellular actin-entity, but can furthermore help to identify potential drug targets for improvement of radiation therapy or human radiation protection. However, it should be stressed that much more experimental work is needed to exactly elucidate the interrelationship between actin, ABPs, and ROS. Specifically regarding their multifacted role in chromatin regulation and transcription in the nucleus, as well as membrane transport, cellular polarization and growth in the cytoplasm and cortex.

All in all, the present study takes a holistic view, thereby postulating that numerous physiological modules, as for example genome or cytoskeleton, jointly contribute to IR-survivability of eukaryotic cells. These modules are interconnected and mutually influence each other. They have different molecular composition, functionality, essentiality, and thus, exhibit diversified individual IR-susceptibility. Considering this, it is important to emphasize that IR-tolerance generally represents a highly complex trait which is differentially related to cellular components and hence to diverse cell types.

6. ACKNOWLEDGEMENTS

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7. DECLARATION OF INTEREST

None

8. FIGURES:

Figure1. Schematic Overview of the Actin-related Protein Interaction Network Highlighting the Deletion Mutants which Exhibit IR-Sensitivity.

This schematic illustration depicts the relations and specifically the physical protein-interaction network of IR-effectors which are related to the actin cytoskeleton. Proteins, whose gene deletions effect cellular sensitivity to ionizing radiation are marked in yellow. Note the three modules: I) actin cytoskeleton, intracellular transport and polar cell growth (blue), II) chromatin remodelling complexes and transcription (light red), III) chromatin structure / nucleosome components (dark red), IV) antioxidants and chaperones (green). The modules are interconnected via the ARP/TAF14 adaptor module (violet). The central 'Arp8-Taf14' unit connects, with assistance of Arp4, Arp5, Arp7, and Arp9, the chromatin remodelling machinery to the chromatin and the nuclear actin-entity. Moreover, Sac6 establishes multiple connections to members of the module IV comprising heat shock proteins, chaperones, and antioxidants.

Coloured lines indicate the direct interactions between IR-effectors and physical binding partners. Blue: Arp8 interaction network; Red: Taf14 network; Black: Sac6 network; Beige: Bem1 network). Grey lines indicate other protein-protein interactions. Those are comprehensively shown for the actin module and within the antioxidants module. Note: for conciseness and better visual clarity the interactions between subunits of chromatin remodelling complexes are not indicated. The number of total physical interactions for individual proteins are indicated in circles next to the respective protein. The physical interaction network in this schematic diagram was developed by analysis of literature cited in this study, and additionally, by utilization of Saccharomyces genome database (https://www.yeastgenome.org) and Yeast BioGRID version 4.4 (https://thebiogrid.org/32710).

Figure2. Graphical Illustration Showing the Subcellular Localization of Yeast IR-Effectors Which Are Related to the Actin Cytoskeleton.

This succinct and simplified graphic portrayal shows the subcellular localization of reviewed proteins which are involved in cellular response to ionizing radiation. Particular significant is the functional partition of overall seven proteins into three operational modules that are spatially separated: 1) Nuclear-events, which are performed by chromatin-remodelling complexes that depend on subunits Arp8 and Taf14. Their functional repertory comprises organization of chromatin, DNA-repair, and transcription. 2) Cytoplasmic events, that are related to pathways involving *SAC6* (fimbrin), and *MDM20* (actin cable stabilizer) that is linked to *NAT3* (acetyltransferase). 3) Cortical events, which are

mediated by *SAC6* (fimbrin), *BEM1* (polarity scaffold), *HOF1* (cytokinesis scaffold). Note the multifaceted localization of fimbrin Sac6, which acts as F-actin cross-linker and stabilizer at cytoplasmic actin cables, cortical actin patches, and at the apex. For conciseness and clarity, Sac6 has been exemplary indicated only three times. Respectively at one actin cable, at one actin patch, and at the apical cortex. This simplified representation symbolizes the ubiquitous involvement of numerous Sac6 molecules in the entirety of cables and patches in a real yeast cell.

Figure3. Compilation of IR-Sensitive Yeast Deletion Mutants Which Act in Diverse Acetyltransferase or Methyltransferase Protein Complexes.

This list comprises IR-sensitive yeast deletion mutants which are involved in regulation of the nuclear chromatin-structure (red) or of cytoplasmic proteins (blue). The listed genes either represent subunits of acetyltransferase complexes or methyltransferase complexes. Number of total physical protein-protein interactions is respectively indicated. Besides, the UV-sensitivity for each gene deletion mutant is given.

Figure4. Diagram Depicting the Interrelation between Ionizing Radiation, the Actin Cytoskeleton, Reactive Oxygen Species, and Antioxidants.

This schematic overview outlines implications of ionizing radiation on the genome, the cellular actin cytoskeleton, ROS, and antioxidant enzymes. Moreover, the mutual effects and interrelations between the actin-entity and ROS are highlighted. Note: 1) High ROS levels induce F-actin stabilization. 2) High actin-dynamics reduce ROS levels. And vice versa.

Arrows indicate positive effects (e.g. induction, stabilization), whereas the T-shaped indications represent inhibitory effects (damage). Colours correspond to the respective source of effect. Yellow: Ionizing radiation (IR). Blue: Actin cytoskeleton. Red: Reactive oxygen species (ROS). Brown: Antioxidant enzymes. Green: NADPH oxidases (NOX). Asterisks: Gene expression induced by IR.

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