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Cell Cycle News & Views

Complexity of IFNγ signaling gets another twist

Comment on: Burova EB, et al. Cell Cycle 2011; 10:2197–205

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Initially, IFNγ was viewed solely as an antiviral molecule with biological effects elicited through straightforward activation of the JAK–STAT pathway. Nowadays, it has become explicitly clear that IFN-γ coordinates an amazing variety of diverse cellular programs,[1] and that signaling cascades triggered by IFNγ are by no means limited to JAK-STAT.[2,3] It is now clear that in addition to direct antiviral effects, IFNγ actively participates in immunoregulation, antigen presentation, respiratory burst, leucocyte-endothelial interactions, tryptophan metabolism and apoptosis.[1] Even though IFNγ (type II interferon) was not among the first human proteins to be used successfully in cancer therapy (this honor belongs to type I interferons), the antitumor effect of IFNγ is well-established. IFNγ enhances cellular susceptibility to apoptosis in tumor cells of different origin, and the antiproliferative and proapoptotic actions of IFNγ are associated with STAT1-dependent expression of interferon regulatory factor 1 (IRF1), a member of a family of related proteins that operate as

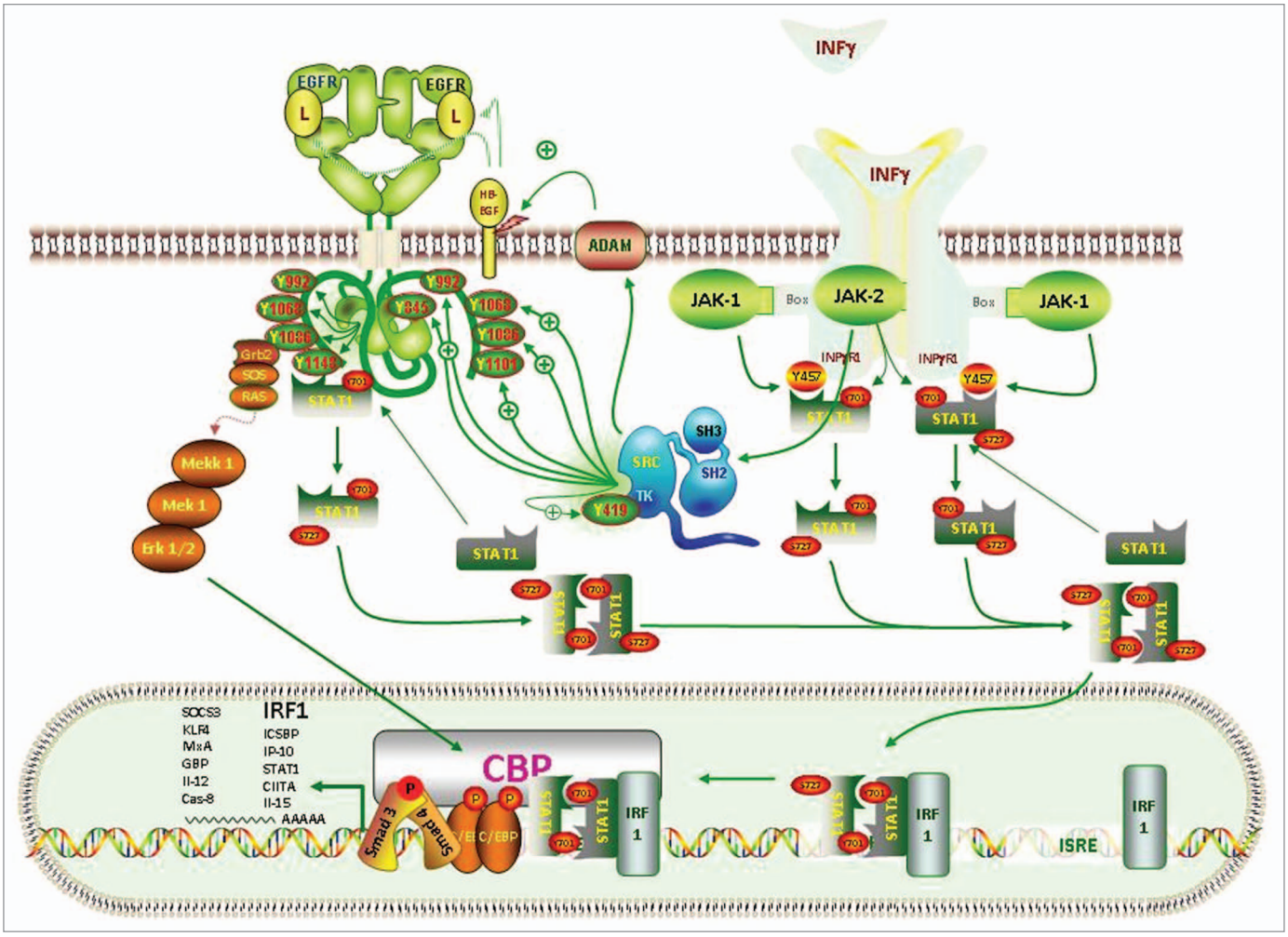

Figure 1. IFNγ signaling through STAT1 and EGFR in A431 cells. Stimulation of the canonical JAK-STAT signaling pathway by IFNγ is shown along with the proposed contribution of EGFR, transactivated via JAK2-mediated activation of a member of the Src family of kinases. STAT1 is phosphorylated on Y701 either by JAKs or, after recruitment to phosphorylated Y1148 in the C terminus of EGFR, by EGFR. STAT1 interacts directly with the CREB-binding protein (CBP)/p300 family of transcriptional coactivators, and this interaction is facilitated by phosphorylation of STAT1 S727. Activation of ERK 1/2 signaling in IFNγ-treated A431 cells occurs exclusively through transactivation of EGFR. Since ERK is capable of interaction functionally with CBP to mediate upregulation of CBP transcriptional activity, it can be proposed that ERK activation contributes to recruitment of CBP/p300 to mediate IFNγ-induced IRF1 expression.

transcriptional activators or repressors.

The study by Burova et al. published recently in *Cell Cycle*[4] presents exciting new data which demonstrate that IFNγ recruits EGFR kinase activity and ERK 1/2 signaling to inhibit proliferation of the human epidermoid carcinoma cell line A431. This group has previously shown that in A431 cells, IFNγ is able to transactivate EGFR, likely through JAK2-dependent activation of the Src family of kinases.[5] New data add that EGFR signaling and ERK activity are required for IFNγ-induced A431 cell death.

How do EGFR signaling and the ERK cascade contribute to the long-term IFNγ effect in A431 cells? Burova and colleagues in the laboratory of N. Nikolsky demonstrate that, whereas EGFR tyrosine kinase inhibitor protects A431 cells from IFNγ-mediated apoptosis by initiating G_1 arrest, the blockade of ERK 1/2 acts through suppression of caspase-3 activation.[4] On the other hand, since IRF1's role in IFNγ-mediated apoptosis of cancer cells can't be overestimated, it seems important that in A431 cells, ligand-activated EGFR was shown to induce expression of a module of genes known to be inducible by IFNγ, including IRF1.[6] Consequently, transactivation of EGFR by IFNγ could contribute to IRF1 production. The ability of IFNγ to induce STAT1 phosphorylation was partially dependent on EGFR kinase activity,[5] raising the question of whether inhibition of both EGFR and ERK1/2, which caused protection of A431 from IFNγ-mediated cell death, would also decrease IFNγ-induced IRF1 production in these cells. The complex picture of IFNγ-induced, STAT1-dependent, IRF1-mediated proapoptotic signaling is getting an additional twist with new data arguing that, besides canonical activation of STAT1 transcription factors by JAKs, we now must take into consideration the involvement of EGFR and ERKs in promoting apoptotic and antiproliferative actions of IFNγ (**Fig.1**).

A431 cells are among the most suitable cell lines to study EGF receptor transactivation, since they are characterized by huge overexpression of EGF receptors (2–3 x 10^6 receptors/cell), and the high density of receptors is expected to favor efficiency of transactivation. It is of note that methods used by authors to demonstrate involvement of the Src family of kinases in IFNγ-induced EGFR activation (treatment with CGP77675, inhibitor of Src kinases)[5] do not allow them to distinguish between individual members of the Src family. It seems that in this particular case, the member of the Src family responsible for IFNγ-induced EGFR transactivation could be Fyn. Fyn was shown to mediate IFNγ-induced signaling pathways,[7] and after IFNγ treatment, Fyn is recruited to the signaling complex of IFNγ receptor and, particularly, to IFNγ-dependent JAK-2 kinase.[8] Whether EGFR transactivation in IFNγ-treated A431 cells specifically involves Fyn but not Src or Yes (as is the case with EGFR transactivation triggeed by M2 muscarinic receptors in human neuroblastoma cells)[9] should be tested using either dominant-negative mutants of the Src family of kinases or Fyn kinase-specific siRNA. Independently of these speculations, the study by Burova et al.[4] provides new research opportunities to further unravel the complexity of IFNγ signaling and to understand how cooperation of IFNγ with EGFR promotes IFNγ-induced apoptosis in cancer cells.

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What's going on at stalled forks?

Comment on: Trego KS, et al. Cell Cycle 2011; 10:1998–2007

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XPG is an endonuclease that functions in nucleotide and base excision repair. WRN is a RecQ-like family helicase with 3′–5′ exonuclease activity that functions in DNA replication and repair. In a recent report, Trego et al. showed that XPG and WRN may physically and functionally interact at sites of DNA replication, and that XPG contains strand annealing activity that is independent of its endonuclease activity.[1] This work connects two DNA repair proteins in a novel complex in mid-to-late S-phase cells. The authors present a model in which helicase and strand annealing activities of XPG and WRN are required at stalled DNA replication forks to prevent formation of potentially lethal chromatid breaks. These results add a new member to the cast of characters that assemble at DNA replication forks that are stalled at natural and induced replication barriers.

By using a stringent extraction procedure, Trego et al. were able to show that XPG was concentrated at discrete nuclear foci in S-phase cells. Nuclear fractionation also showed that a fraction of XPG was associated with chromatin and the nuclear matrix, and this was enhanced by synchronization of cells to mid-S phase. UV irradiation (30 J/m^2) to induce severe fork stalling further increased XPG binding to chromatin and the nuclear matrix. WRN was found to interact with XPG by coimmunoprecipitation and colocalized with XPG in un-irradiated and irradiated mid-S phase cells. Previous work had shown that WRN accumulates at DNA replication forks that are stalled by pool depletion (HU treatment) or inhibition of topoisomerase I (camptothecin), and WRN colocalized with RPA and Rad51 at these sites.[2,3] Thus, the presence of colocalized WRN and XPG during mid to late-S phase implies that during the normal course of DNA replication, forks may become stalled at natural barriers. Many natural barriers to DNA replication have been described, and cells appear to have evolved a variety of tactics for successful replication through these barriers.[4] As WRN-defective cells display chromosomal instability,[5] it is possible that WRN functions to stabilize replication forks that are stalled at natural replication barriers.

The physical association of XPG and WRN in human S-phase cells prompted a search for a functional interaction. XPG was found by Trego et al. to stimulate WRN helicase activity. Remarkably, when the strand annealing activity of WRN was assessed, it was found that XPG independently could stimulate strand annealing. The two proteins appear to stimulate strand annealing cooperatively. The strand annealing property of XPG was found to reside

in R- and C-terminal domains that interacted with WRN, and the endonuclease domain was not required for WRN interaction or strand annealing. These results revealed a new function of XPG, independent of its endonuclease activity, that helps to explain how truncation mutations of XPG give rise to the combined XPG/CS phenotype with severe progeroid symptoms.[6] In addition to the role of the endonuclease domain in nucleotide excision repair, the R- and C-terminal domains may work with WRN to preserve or restore DNA replication forks that become stalled at natural or induced barriers.

The concept of DNA replication fork stabilization is of considerable interest in the cancer research field, as this appears to be the essential function of the ATR- and Chk1-dependent intra-S checkpoint[7] and an early response in oncogene-transformed cells.[8] An expanding cast of players at stalled replication forks includes WRN, Metnase, a hominid lineage-specific chimera of a SET family methyl transferase and the Mariner transposase[9] as well as the replication fork protection complex of Timeless, Tipin and Claspin.[7] The Trego et al. study expands the list to include XPG. One can anticipate the day when structural biologists will solve the structure of the multisubunit DNA replication complex as it encounters natural and induced barriers.

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SIRT1, p53 and mitotic chromosomes

Comment on: Fatoba ST, et al. Cell Cycle 2011; 10:2317–22

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Mammalian SIRT1 is regarded as an epigenetic regulator of major importance in mammalian development, differentiation and maintenance of homeostasis.[1] SIRT1 is also linked with aging and with human diseases, including Alzheimer disease, Parkinson disease, diabetes and cancer.[1,2] Biochemically, SIRT1 functions as an NAD-dependent deacetylase. Through its capacity to deacetylate target histone proteins, SIRT1 participates in the regulation of chromatin structure and of DNA accessibility for processing and repair. SIRT1 contributes to the silencing of major DNA satellite repeats[3] and also to the formation of facultative heterochromatin.[4] In addition, SIRT1 participates in transcriptional control networks via deacetylation of transcription factors and co-factors. One target of SIRT1 is the tumor suppressor p53, and by downregulating p53 following DNA damage, SIRT1 favors cell survival over apoptosis.[1] Elegant studies using ChIP and genomic promoter tiling arrays have revealed that SIRT1 constitutively represses DNA repeats and diverse functional genes in mouse embryonic stem cells.[3] Following DNA damage, SIRT1 relocalizes from its constitutive loci to sites of DNA damage where it promotes DNA repair and hence genomic stability.[3] Thus both SIRT1 and p53 are chromatin/DNA responders that help maintain genomic stability and are coordinated so that SIRT1 favors repair and survival, while p53 elicits programmed removal of overly damaged cells via apoptosis.

At mitosis, there is profound reorganization of chromosomal architecture as cells prepare to exit G_2 of the cell cycle and enter the prophase of mitosis. Initial steps involve SIRT2-dependent chromosomal condensation via histone H4 deacetylation.[5] In a previous issue of *Cell Cycle,* Fatoba and Okorokov reported that SIRT1 also contributes to mitotic chromosomal condensation, and that this involves histone H3 deacetylation.[6] Using human HCT116 cells, originally derived from a colorectal carcinoma, the authors showed that SIRT1 appears to colocalize with condensed chromosomes during transit from prophase to prometaphase and remains associated with mitotic chromatin into telophase. When cells were arrested in G_2/M of the cell cycle by treatment with nocodazole, the level of SIRT1 protein increased,[6] indicating that SIRT1 protein levels may naturally rise in early mitosis. Depletion of SIRT1 by RNAi correlated with aberrant mitotic figures and an increase in anaphase chromatin bridges known to be linked with chromosomal breakage and aneuploidy. Based upon these and additional biochemical data, the authors present a model in which SIRT1 is globally recruited to chromosomes subsequent to SIRT2 and enables condensin 1 and histone H1 loading onto mitotic chromatin. In this way, it is proposed that SIRT1 in coordination with SIRT2 contributes towards global chromosomal condensation in a highly ordered sequence as mammalian cells enter and transit mitosis and cytokinesis.[6]

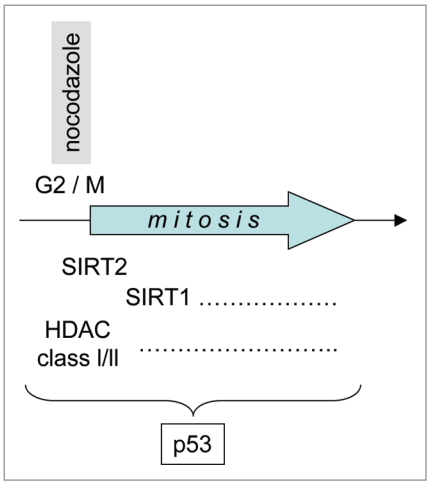

Figure 1. Overlapping roles and regulatory interplay between SIRT1, SIRT2, class I/II HDACs and p53 during chromosomal condensation and mitotic cell division in mammalian cells (see text).

Using the same HCT116 cell line, others have previously demonstrated that SIRT1 depletion induces apoptosis, whereas non-cancer cells survived, thus identifying SIRT1 as a putative cancer-specific survival factor.[7] Massive apoptosis is observed within 48 hours, and it will be of interest to determine if the apoptotic effect is triggered by abnormal chromosomal processing due to SIRT1 deficiency during mitosis.

Other histone deacetylases have been found to play a major role in chromosomal modification and mitotic progression. For example, class I/II HDACs (sensitive to the pan-HDAC class I/II inhibitor trichostatin A, TSA) appear necessary for the continual cycling

and deacetylation of HCT116 histone H3 under normal conditions of cell growth.[8,9] The effect is site-specific for lysine K9, since other acetylated residues on histone H3 are affected to a much lesser extent or not at all.[8,9] Phosphorylation of serine 10 (S10), which requires Aurora B and is important for maintaining normal ploidy, is also coupled with HDAC I/II activity at lysine K9.[8,9] Interestingly, acetylation of K9 (AcK9) and phosphorylation of S10 (S10P) are linked in a p53-dependent manner.[8,9] Moreover p53 is also required (1) to enable recovery of G_2/M arrested cells (after release from nocodazole arrest) and (2) to coordinate events necessary for re-entry and transit through mitosis into the cell cycle.[8,9] Given the fundamental importance of p53 in maintaining genomic integrity and suppressing cancer, it would be interesting to determine the regulatory interplay between p53, SIRT1 and other chromatin-modifying enzymes during chromosomal condensation and mitotic cell division in mammalian cells (**Fig. 1**).

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News on hypomethylating agents: Chasing the FOX

Comment on: Thépot S, et al. Cell Cycle 2011; 10:2323–30

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The hypomethylating agents azacitidine and decitabine found widespread application in the treatment of high-risk myelodysplastic syndrom (MDS) and certain subtypes of acute myeloid leukemia (AML).[1-3] In an international randomized phase III study, azacitidine demonstrated its ability to prolong survival and its superiority as compared to conventional care regimens.[1,2]

Although an abundance of preclinical studies aimed to define the mode of action of hypomethylating drugs, the exact mechanisms of response and resistance remain unclear.[3] Despite many advances, crucial questions remain to be answered. Most importantly, the clinician's daily practice (and the patient's daily life) is complicated by the lack of predictive markers that enable one to foretell the likelihood of response to hypomethylating agents.

The study by Thépot et al.[4] published in a previous issue of *Cell Cycle* links the antileukemic effects of the hypomethylating agents azacitidine and decitabine to their ability to revise aberrant FOXO3A signaling. In detail, the study provides evidence that hypomethylating agents dephosphorylate FOXO3A, enabling FOXO3A's passage from the cytoplasm to the nucleus and the subsequent resumption of its role as a transcriptional regulator (**Fig. 1**).

Forkhead O transcription factors (FOXO) are critically involved in the regulation of cell cycle arrest, apoptosis, differentiation and DNA damage repair.[5] Inactivation of FOXO promotes tumorigenesis, as demonstrated for breast cancer, prostate cancer, glioblastoma, rhabdomyosarcoma and leukemia.[5] A large series of studies shows that activation of different oncogenic pathways (such as PI-3-kinase/AKT- or RAS/MAPK-mediated signaling) suppresses FOXO transcriptional activity through phosphorylation at different sites, leading to its nuclear exclusion and subsequent degradation.[5] This identification of FOXOs as bona fide tumor suppressors makes them attractive therapeutic targets. Although the antagonization of oncoproteins is still the predominant anticancer strategy, the treatment paradigm currently shifts towards the (concomitant) restoration of tumor suppressor mechanisms.

In particular, the previous demonstration that increased phosphorylation of FOXO3A is linked to a poor prognosis in AML[6] emphasizes the relevance of the findings described by Thépot and colleagues. Although their study only assesses a small number of patient samples for a functional link between hypomethylating agent-induced apoptosis and concomitant nuclear FOXO3A translocation, the positive correlation of these two parameters

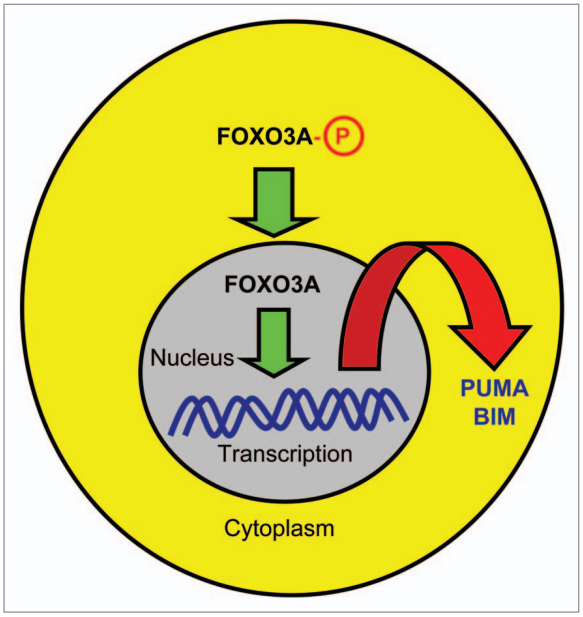

Figure 1. Hypomethylating agents dephosphorylate FOXO3A, followed by its shift to the nucleus and upregulation of its transcriptional targets.

upon azacitidine exposure (and its absence upon decitabine incubation) is intriguing.[4]

Besides the more academic aspects, notably the necessity to evaluate whether hypomethylating agents are also able to curtail the deleterious role of FOXO3A in leukemia initiating cells,[7] the study by Thépot et al. might present a first step towards a better definition of predictive biomarkers. Nonetheless, future studies will have to confirm the clinical relevance of the described observations, in particular, whether the pre-treatment status of aberrant FOXO3A signaling in malignant cells (and the potential "correction" under treatment) is predictive of an in vivo response to these agents.

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XPG and WRN: An unexpected partnership

Comment on: Trego KS, et al. Cell Cycle 2011; 10:1998–2007

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Rare inherited disorders frequently yield powerful insights into normal biological processes. Xeroderma pigmentosum (XP) is an inherited disease manifested by deficits in DNA repair; patients with XP have a 1000-fold increase in skin cancers in areas exposed to UV irradiation.[1] All genes mutated in XP function in nucleotide excision repair (NER) to excise DNA lesions, including UV-induced DNA adducts. One of the genes mutated in XP, XPG, is a structure-specific endonuclease that cleaves DNA at single strand-double strand junctions 3′ to the DNA lesion.[2] Mutations in the nuclease active site render cells defective for NER and result in XP. Mutations in XPG are also associated with a more severe disorder, Xeroderma pigmentosum/Cockayne syndrome (XP-G/CS), believed to result from impaired activated transcription and characterized by developmental and neurological abnormalities.[3] Notably, phenotypes of both XP and XP-G/CS are apparent at birth. In contrast, at the other end of the developmental spectrum is Werner syndrome (WS), a disorder of premature aging manifested in adolescence and associated with an elevated risk of specific types of cancer.[4] The gene mutated in WS, *WRN*, encodes a 3′→5′ DNA helicase and a 3′→5′ DNA exonuclease.[5,6] Nearly all *WRN* mutations result in loss of both helicase and exonuclease activity. Though WS shares no obvious phenotypic abnormalities with XP and XP-G/CS, Trego et al.[7] now report multiple findings that surprisingly and functionally link XPG and WRN.

In addition to its essential role as an endonuclease in NER, XPG has been implicated in RNA transcription through its tight physical interaction with the transcription activator protein, TFIIH.[3] XPG alleles with C-terminal mutations or truncations are proficient for DNA excision in vitro yet are unable to bind TFIIH and, thus, are unable to anchor the TFIIH accessory Cdk-activating kinase subunit, resulting in impaired activated RNA polymerase II-mediated transcription.[3] In vitro evidence also suggests a role for XPG in the repair of oxidative DNA lesions by a NER-independent pathway.[8] Trego et al.[7] recnetly reported yet another activity of XPG that could implicate it in other DNA transactions. They showed that XPG has intrinsic single-strand annealing activity; importantly, this activity is independent of its nuclease activity but requires the N- and C-terminal domains of XPG. Intriguingly, ATP-independent single-strand DNA annealing activity has also been reported for WRN.

WRN, like XPG, is a structure-specific enzyme, catalyzing the unwinding and degradation of DNA containing alternate secondary structures that arise during DNA replication, recombination, repair and RNA transcription.[4] The prevailing notion is that WRN functions at the interphase of DNA replication and recombination by helping to prevent replication fork stalling and to resolve forks once stalled. Studies have shown that WRN colocalizes and coimmunoprecipitates with many proteins in vivo, and, likewise, that purified recombinant WRN binds to many other proteins in vitro.[4] However, functional interactions of WRN have been limited to only a few proteins. Trego et al.[7] demonstrated a direct physical interaction between WRN and XPG; domain-mapping studies identified the C-terminal 180 amino acids of XPG and two small C-terminal regions, aa 1070-1142 and aa 1382-1432, of WRN as the interaction motifs. They also reported that XPG stimulates the helicase activity of WRN, and that XPG and WRN work cooperatively to anneal two DNA single strands. Together, these studies provide evidence for an association between these two proteins.

These tantalizing findings raise many interesting questions. Is the functional interaction of XPG unique to WRN? If so, does XPG play a role in resolving stalled replication forks by virtue of its cooperative single-strand annealing activity with WRN? Alternatively, based on the following lines of evidence, is WRN involved in any of the known XPG transactions? WS cells are sensitive to bulky DNA adduct-generating agents, such as cisplatin, which is repaired through an XPG-dependent pathway[9] WRN is implicated in the repair of oxidative DNA damage through the base excision repair pathway,[10] and WS cells show reduced Pol II-dependent transcription.[11] Notably, the C-terminal region of XPG that interacts with WRN also interacts with TFIIH to activate transcription. Do XPG and WRN function together in an as-yet-unidentified process? Are XPG and WRN part of a large, master DNA repair complex that recognizes and recruits proteins to DNA lesions and/or alternate DNA secondary structures in vivo? The identification of XPG as another interacting partner of WRN adds a complicated yet interesting new twist on the role of these proteins in vivo.

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The "suppressive side" of yeast mitotic cyclins

Comment on: Signon L. Cell Cycle 2011; 10:1655–68

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Cyclin-dependent kinases (Cdks) are the main drivers of the eukaryotic cell division cycle. The activities and substrate specificities of Cdks are regulated by their association with the regulatory subunits, the cyclins.[1] In budding yeast *Saccharomyces cerevisiae,* Cdc28 (Cdk1) is the sole cyclin-dependent kinase that drives cell cycle progression by partnering different cyclins. Like vertebrate cells, M-phase onset in yeast is critically dependent on the activity of the Cdk1/cyclin B complex. Yeast cells express four mitotic cyclins (Clb1, Clb2, Clb3 and Clb4)[2] with considerable functional overlap, though the Cdk1/Clb2 complex is the major contributor to the total Cdk1/cyclin B activity during mitosis.[3] While assigning the unique functions to different Cdk1/cyclin complexes has been difficult, their "collective" roles in specific aspects of mitosis, such as the biogenesis of a mitotic spindle, have become increasingly clear. The requirement of the Cdk1/cyclin B complex for breaking of the inter SPB (spindle pole body) bridge to allow assembly of a short bipolar spindle,[4] the maintenance of the spindle midzone integrity[5] and for spindle elongation during anaphase[6] have been documented. These reports ascribe Cdk1/cyclin B complexes a "positive role" in spindle biogenesis and dynamics.

The findings described in the paper by Signon[7] give this "positive tale" a new twist. The study analyses chromosome segregation in *rad51*Δ *clb2*Δ diploid cells "adapting" to a single double-strand break (DSB). These cells, unable to undergo gene conversion in the absence of Rad51, repair the DSB by break-induced repair (BIR) and progress through mitosis. A significant proportion of these cells lose chromosomes during anaphase, with increased frequency of monopolar and syntelic segregations. Detailed analysis using both fixed samples and live cell imaging revealed two major defects in *rad51*Δ *clb2*Δ cells: (1) The heightened oscillatory behavior of chromosomes and (2) formation of new spindles and the inability to undergo anaphase. Importantly, a larger proportion of *clb2*Δ cells also displayed these defects, albeit to a milder extent, suggesting that these defects are not specifically associated with cells recovering from or adapting to DNA damage. Instead, these abnormalities develop due to a lack of Clb2 during normal growth and are exacerbated in DNA damaged cells. Keeping in view the functional redundancy among the Clbs, this may also mean that contributions by Clb1, Clb3 and Clb4 are diminished in cells exposed to genotoxic stress, causing a heightened requirement for Clb2 in the execution of mitotic affairs.

Oscillatory movement of chromosomes during metaphase is caused, in part, by growth and shrinkage of the kinetochore microtubules once chromosomes are laterally attached to the spindle.[8] However, growth of kinetochore microtubules diminishes (and so do chromosome-oscillations) following the establishment of bi-orientation. Hence, an increase in chromosome oscillation in the absence of Clb2 would be consistent with the notion that Clb2 plays a role in suppressing the growth of kinetochore microtubules following bi-orientation. The second type of defect in *clb2*Δ cells, i.e., the formation of a new spindle (or strands of microtubules resembling a bipolar spindle that are able to capture the kinetochores), is surprising. The author proposes that the delay in anaphase progression in the absence of Clb2 is not due to the lack of spindle extension but is caused by the formation of new bipolar spindle, resulting in abnormal spindle structures (multi-stranded or triangle-shaped spindles) that prevent progression through anaphase. Although the abnormal spindle shapes generated by the reformation of bipolar spindle are not multipolar spindles (resulting from overduplication of centrosomes under certain circumstances in vertebrate cells); such deformed spindles can lead to chromosome segregation defect and aneuploidy. Hence, this study suggests that Cdk1/Clb kinase suppresses new spindle formation during early anaphase, thus preventing the emergence of abnormal spindle structures. An interesting extension of this notion is that Cdk1/Clb kinase mediates centrosome separation and the biogenesis of a bipolar spindle in late-S phase but suppresses the formation of a new bipolar spindle in early anaphase to prevent missegregation of chromosomes. This dichotomous behavior has a parallel in S-phase kinase in that Cdk1/Clb5,6 complex participates in the firing of replication origins but subsequently prevents re-firing later in S phase.[9] It will indeed be instructive to uncover the mechanism by which the mitotic kinase suppresses the formation of a new bipolar spindle during the transition to anaphase.

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Novel findings on actin regulation in asymmetric division of mouse oocytes

Comment on: Sun SC, et al. Cell Cycle 2011; 10:1853–60

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A unique feature of oocyte maturation is the formation of the meiotic spindle followed by two asymmetric cell divisions to achieve haploidy in the unfertilized oocyte. The meiotic spindle is formed at the center of the oocyte and migrates to the oocyte's cortex driven by actin-mediated mechanisms, thereby clearly indicating oocyte polarization. Microfilaments become reorganized to form an actin-enriched cap in the region overlaying the meiotic spindle, while cortical granules (CGs) become redistributed to form a CG-free domain (CGFD) that is devoid of microvilli. When cortical reorganization is complete, the oocyte undergoes the first asymmetric cell division, resulting in the extrusion of the small polar body; the large oocyte retains the maternal components that are critical for embryo development. Understanding the cellular and molecular mechanisms driving this unique maturation event has been a challenge; so far, only very few molecules have been identified to be involved in this process (see references 1–3).

The paper by Sun SC et al. now provides clear evidence for actin nucleation that is critical and directly involved in the oocyte's asymmetric division. The authors showed that the Arp2/3 complex,[4] JMY[5] and newly released WAVE2[6] regulate spindle migration and cytokinesis. The Arp2/3 complex is an actin nucleator that binds to the side of an existing actin filament and initiates new filament assembly. WAVE2 and JMY are nucleation-promoting factors (NPFs), which could activate the Arp2/3 complex. In this elegant research, using RNAi, antibody injection and inhibitor treatment approaches, the authors showed that after disruption of these molecules, spindles became arrested at the central location in the oocyte. Subsequently, some spindles failed to initiate cytokinesis, which was associated with failure of polar body emission. Although some oocytes were able to initiate cytokinesis, these oocytes underwent symmetric division, perhaps as a result of the central spindle location. The actin cap and cortical granule-free domain (CGFD) also became disrupted, indicating failure of oocyte polarization. These results provide evidence that actin nucleators regulate oocyte polarization and affect asymmetric division (**Fig. 1**).

The observation that disruption of WAVE2 causes aberrant spindle formation is interesting considering that recent work has shown that WAVE2 is regulated by MAPK.[7] These two findings may link actin nucleation to spindle formation, as MAPK is a well-known regulator of spindle formation.

In summary, the findings on WAVE2, JMY and the Arp2/3 complex extend our knowledge on the oocyte's asymmetric division and implicate actin nucleators in this process. Whether other actin nucleators, such as N-WASP, are involved in the oocyte's asymmetric division remains to be investigated.

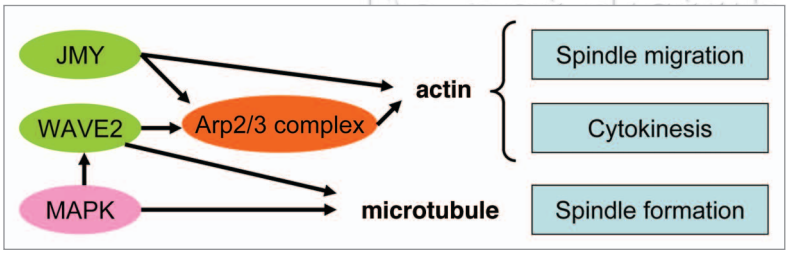

Figure 1. Diagram of relationship between actin nucleators and oocyte polarization.

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Braking with 30 fingers: ZNF521 holds on Ebf1-dependent B-cell development

Comment on: Mega T, et al. Cell Cycle 2011; 10:2129–39

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Regulation of the hematopoietic stem cell (HSC) compartment and maintenance of an appropriate pool of HSCs during hematopoiesis are critical processes, both in the physiological and pathological contexts. The development of B lymphocytes from HSCs is a well-characterized and tightly regulated process that requires transcriptional reprogramming, turning on one set of B cell-specific genes while silencing the expression of others. This is achieved by the concerted action of a network of transcription factors and epigenetic effectors.[1] Early B-cell factor 1 (Ebf1), a zinc knuckle (an atypical zinc finger motif)-containing transcription factor, is required for B-cell development, as demonstrated by the complete lack of mature B cells in Ebf1-deficient mice.[2] During B-cell differentiation, Ebf1 expression and/or activity needs to be modulated in order to maintain the progenitor pool and to establish a correct balance between B- and T lymphopoiesis.[2] Although several inhibitors of Ebf1 have already been identified, such as Bmi1,[3] zinc finger protein 521 (ZNF521 in humans, Zfp521 in mice, also known as Evi3 or EHZF) has emerged as a key player.

ZNF521 is a large 30 zinc finger-containing protein first cloned in an experimental B-cell lymphoma mouse model and as an enriched mRNA transcript in CD34-positive human

hematopoietic progenitor cells.[4,5] Recent work has shown that, in addition to hematopoietic cells, ZNF521 is expressed in embryonic stem cells, osteoblasts, chondrocytes and in subsets of neurons.[6-8] In all these cell types, it appears to control cell differentiation as well as the function of mature cells by modulating the activity of specific transcription factors.

Previous studies in non-hematopoietic cells have shown that ZNF521 suppresses Ebf1 activity via its C-terminal zinc fingers. It also harbors a putative nucleosome remodeling and histone deacetylation (NuRD) complex-binding domain that could be involved in transcriptional repression. However, what has been lacking is direct evidence that ZNF521 could modulate B-cell development by suppressing Ebf1 activity and its molecular mechanism of action.

In their study, Mega et al. addressed the question of whether ZNF521 could regulate Ebf1 activity in a B-cell context and thus modulate their differentiation.[9] They first showed that ZNF521 binds to Ebf1 via its C-terminal domain, and that this interaction is required for efficient suppression of Ebf1 activity. Deletion of the N-terminal NuRD domain had some, but modest, effects on ZNF521's capacity to suppress Ebf1 activity in reporter assays or Ebf1 target gene expression in lymphoblastoid cell lines. This suggests that, at least in overexpression systems and in the context of B-cell development, the interaction of ZNF521 with NuRD is not essential for suppression of Ebf1 activity. However, NuRD interaction is required for suppression of GATA-1 activity by ZNF521 during erythropoiesis,[10] and its role in other contexts of ZNF521 biology remains to be tested.

More importantly, Mega et al. showed for the first time that downregulation of ZNF521 in human or mouse hematopoietic progenitor cells resulted in increased B-cell differentiation in vitro. Although not directly shown in this paper, this can reasonably be attributed to increased Ebf1 transcriptional activity in absence of its suppressor, ZNF521. Future work will be necessary to confirm these findings in an in vivo setting and to determine whether ZNF521 works only through Ebf1 in B cells or whether other molecular mechanisms are in play. Also of importance is the fact that ZNF521 has been implicated in the development of B lymphoid neoplasias in animal studies. The data in humans are still sparse, and it remains to be tested whether ZNF521-Ebf1 interplay is involved in human B-cell malignancies. Further, as ZNF521 and Ebf1 are co-expressed in several other cell types, including neuronal cells and osteoblasts, the interplay of these factors is likely to regulate progenitor pools and cell differentiation and function in several other cellular lineages. Determining the molecular mechanisms involved in these various systems will be of significant interest.

Our understanding of the biology of ZNF521 is increasing rapidly. It serves as a brake for cell differentiation in many cell lineages (erythrocytes, B cells, osteoblasts) by functioning as a repressor for several transcription factors.[7,9,10] However, in embryonic stem cells, it promotes neural differentiation, and instead of acting as a repressor, ZNF521 appears to function as a transcriptional coactivator together with p300,[6] adding to the functional diversity of ZNF521. Based on these data, it is clear that ZNF521 is emerging as a major regulator of cell differentiation in multiple cellular contexts, and we will surely see its fingers mingle into many more stories.

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An Aurora A-Lats-Aurora B axis ensures proper chromosome segregation

Comment on: Yabuta N, et al. Cell Cycle 2011; 10:2724–36

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Aneuploidy, aberrant numbers of chromosomes, is a common characteristic of cancer cells and is often associated with poor patient prognosis. Aneuploid cells can arise from diploid cells due to mitotic abnormalities such as incorrect attachment of the mitotic spindle and chromosome missegregation, centrosome amplification or perturbation, problems in the spindle checkpoint mechanism, defective chromosome cohesion and others.[1] Another proposed model suggests that the generation of aneuploidy occurs via polyploid intermediates that are inherently genomically unstable.[2] Although a spectrum of mechanisms can generate aneuploid cells, the crucial common denominator is regulation of chromosome segregation during mitosis. The work of Yabuta et al. in a previous issue of *Cell Cycle*[3] proposes a novel axis that regulates mitosis progression and chromosome segregation and involves four known players: Aurora A/B and Lats1/2 (large tumor suppressor 1 and 2).

Aurora kinase A and B have well-established roles in several aspects of mitotic regulation; inhibition of either of them causes altered ploidy.[4] Interestingly, their binding partners, kinase substrates and cellular localization in dividing cells are distinct, leaving an open question regarding their respective roles in the regulation of proper chromosome segregation during mitosis. Now, Yabuta et al. have proposed that the Lats1/2 kinases "connect" the two Aurora kinases to form an Aurora A-Lats1/2-Aurora B (ALB) axis that ensures accurate chromosome segregation.

Lats1 and Lats2 are mostly known for their participation in the Hippo signaling cascade, which controls cell proliferation and apoptosis.[5,6] Lats2 also responds to mitotic and oncogenic stress in conjunction with the p53 tumor suppressor pathway.[7,8] As reported by Toji et al.,[9] Lats2 is phosphorylated by Aurora A on Ser83, and this modification regulates Lats2 centrosomal localization during interphase. Yabuta et al. now show that yet another phosphorylation of Lats2 by Aurora A, this time on Ser380, leads to a distinct mitotic localization of phosphorylated Lats2, which is shared with Aurora B. This phosphorylation and the consequent nuclear localization of Lats2 are

required to ensure proper chromosome partitioning and cytokinesis. The final mechanistic link of this axis is the phosphorylation of Aurora B by Lats2-associated Lats1, which enables the accurate activation of Aurora B. This new function of Lats2 in the regulation of mitotic chromosome segregation joins its previously documented activity in establishing a tetraploidy checkpoint together with p53 after microtubule damage,[7] which also serves to prevent aneuploidy.

As already mentioned, the Lats proteins are also active in the Hippo signaling pathway, where they phosphorylate and inhibit the YAP and TAZ proteins and thus control cell proliferation, apoptosis and organ size determination.[5,6] The new emerging role of Lats kinases in regulating proper chromosome segregation, mitosis and cytokinesis together with known mitotic regulators, such as Aurora kinases, may suggest that different post-translational modifications and protein-protein interactions act as switches that engage Lats proteins in different cellular signaling cascades. In fact, Lats proteins are known to respond to a variety of signals, including cell density, DNA damage, microtubule damage and oncogenic stress. Intra- and intercellular cues that activate different signaling cascades may converge onto Lats proteins, and this may orchestrate different cellular responses depending on the initial cue and the specific protein(s) mediating Lats activation. It may very much be that modifications by different upstream components, such as the phosphorylation of Lats2 by Aurora A, channel Lats signaling toward the desired biological outcome. Epigenetic silencing of *lats* genes is implicated in human cancers;[10,11] this is expected to compromise the different signaling cascades in which Lats proteins participate, eventually leading to genome instability and cancer.

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Defining bad stroma in human breast tumors

Comment on: Witkiewicz AK, et al. Cell Cycle 2011; 10:1794–809

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Profound changes in gene expression occur not only in tumor epithelial cells, but also in the tumor stroma during breast cancer development. Ma et al.[1] used laser capture to separate stroma and epithelium in normal breast lobules, ductal carcinoma in situ and invasive breast cancer. In in situ carcinoma, 2338 genes were upregulated and 1234 downregulated compared with normal lobules. There were relatively few additional gene expression changes in the stroma microdissected from invasive carcinomas compared with in situ carcinoma (76 upregulated and 229 downregulated genes).[1]

We are used to defining patient prognosis and response to therapy on the basis of the microscopic appearance of the epithelium of the tumor cells (e.g., grade), the expression of epithelial cell antigens (e.g., ER, HER2) and gene expression profiles. In most gene expression studies, stroma and epithelium are not separated. However, several groups have assessed the expression of putative stromal genes in relation to prognosis.[2,3] For example, Chang et al.[2] reported that the expression of genes altered by serum treatment of fibroblasts (equivalent to a wound) was related to a poor prognosis when the same genes were expressed in primary human tumors. However, Finak et al.[4] were the first to use laser capture to define the relationship of stroma alone to prognosis. Increased expression of stromal genes related to angiogenesis, the immune response and hypoxia were associated with a poor prognosis. This landmark study indicated that the type of stroma was related to outcome and was probably more predictive than older stromal markers of outcome, such as lymphocyte infiltration or vascularity.

Laser capture and stromal gene expression profiles are not for the routine diagnostic pathology laboratory. A simple biomarker of stromal "activation" was discovered by Michael Lisanti and his colleagues.[5] They demonstrated that a protein which is found in cell caveolae, caveolin-1 (Cav-1), is downregulated in the stroma of tumors with a poor prognosis, irrespective of standard epithelial markers. This important observation has been confirmed and validated by others.[6,7] To determine the mechanism of Cav-1 downregulation, the investigators performed co-culture experiments of human immortalized fibroblasts and breast tumor cell lines (MCF-7, MDA-MB-231).[8-10] These studies demonstrated that hydrogen peroxide (H_2O_2) released from tumor cells causes downregulation of Cav-1 and is associated with a number of other changes in fibroblasts, including increased HIF1α, NFκB activation and autophagy of mitochondria (mitophagy).[8,9] The fibroblasts activated by adjacent tumor cells in co-culture were metabolically glycolytic and produced lactate, which is exported by the upregulated lactate transporters MCT4 in the fibroblast and MCT1 in the tumor cell. This occurs in order to, it is hypothesized, "feed" the TCA cycle of adjacent tumor cells, which are relatively non-glycolytic.[10] The relatively high stromal glycolysis and epithelial cell respiration has been termed the "reverse Warburg effect" and the tumor-induced autophagy within fibroblasts, the "autophagic stromal model of cancer metabolism;"[8-10] these are shown schematically in **Figure 1**.

In the study outlined in a previous issue of *Cell Cycle*, the Lisanti group reported differences in stromal gene expression profiles between in Cav-1 (–) and Cav-1 (+) human primary tumors. They used laser capture in order to determine whether the processes they reported in the fibroblasts of the co-culture experiments are also detectable in primary tumors.[11]

Compared with Cav-1 (+) stroma, Cav-1 (–) stroma showed 238 gene transcripts upregulated and 232 gene transcripts downregulated (> 1.5 fold). Gene set enrichment analysis indicated that the upregulated genes in Cav-1 (–) stroma were associated with "stemness", inflammation, DNA damage, oxidative stress, hypoxia, autophagy and mitochondrial

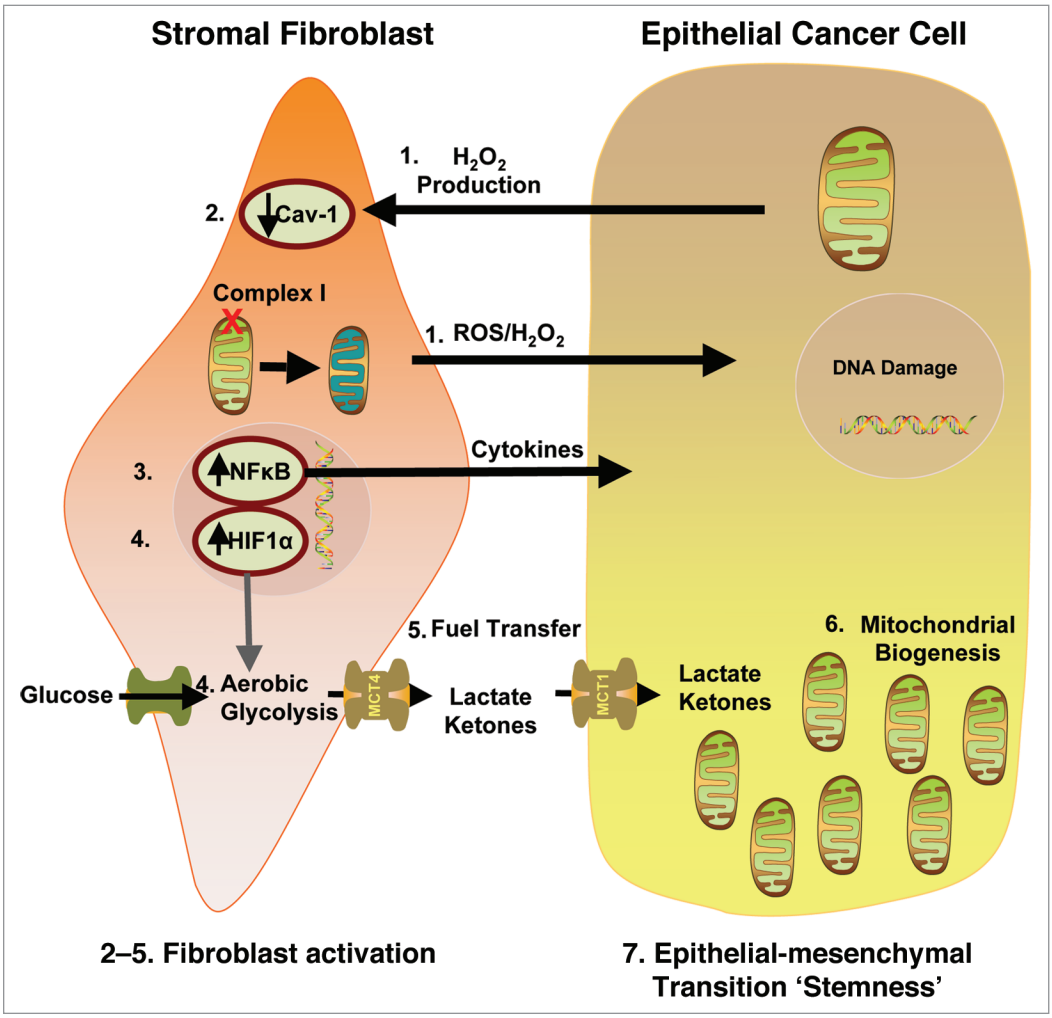

Figure 1. Defining bad stroma in human breast tumors. The putative metabolic interactions shown to occur in co-culture experiments between human fibroblasts and the MCF-7 human tumor cell line. Gene expression changes in separated primary tumor and stoma described in this paper support this model.

dysfunction, similar to findings in the co-culture studies. These results directly support the use of the co-culture system as a model to investigate metabolic interactions between tumor stroma and the epithelium.

Collectively, the studies outlined above suggest the hypothesis that large changes occur in the stroma of DCIS and invasive cancer compared with normal breast stroma. The currently reported data indicate that some human mammary tumors co-opt the stroma [Cav-1 (-)] to allow them to metastasize, whereas others [Cav-1 (+)] do not.

Most importantly, we now have a marker of bad stroma [Cav-1 (-)] and an indication of the metabolic mechanisms of how tumor epithelial cells influence stromal cells to support the metastatic process.

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