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Cell Cycle News & Views

Sperm(idine) answers the next generation

Comment on: Bauer MA, et al. Cell Cycle 2013; 12:346-52;
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Our life starts with the fusion of oocytes with spermatozoa, eventually leading to the development of an embryo. Though the cellular events involved in fertilization are well-described, their molecular underpinnings remain poorly understood.[1] Substances controlling the elementary fusion processes are of utmost socioeconomic and medical relevance. Spermidine is a naturally occurring polyamine vital for life,[2] regulating multiple cellular processes, including gene expression, autophagy and aging.[2,3] In this issue, Bauer et al.[4] establishes a fundamental role for spermidine in this fusion process in budding yeast *S. cerevisiae*.[4] Subsequently, the authors also extended the role of the polyamine spermidine for oocyte fertilization in the nematode *Caenorhabditis elegans* as well, implying that the fundamental mechanisms underlying fertilization are highly conserved across phlyla.

Fertilization efficiency has been reported to decline gradually with advanced paternal age,[5] in line with the age-related decrease in polyamines level.[6] However, it remained unclear whether the very fusion process of oocytes and spermatozoa is in fact directly modulated by polyamines. To test this, Bauer et al.[4] studied sexual reproduction in the baker's yeast *S. cerevisiae*, during which two haploid cells of opposed mating type ("MATa" and "MATα") combine to generate one diploid cell. To specifically determine the role of the spermidine *SPE2*, an enzyme essential for spermidine production was deleted in one of the mating strains ("MATa"Δspe2). This disruption resulted in drastic reduction of overall mating efficiency. Interestingly, supplementation of exogenous spermidine in cultures of "MATα" wild type and "MATa"Δspe2 cells restored the mating efficiency, indicating that spermidine is necessary for efficient mating in *S. cerevisiae*.

In response to mating pheromone, sexually reproducing yeast cells of opposite mating type differentiate into a specialized pear-shaped functional form, known as "shmoo." Consistent with the idea, while the phermone-treated viable "MATa"Δspe2 cells hardly developed shmoos, the administration of spermidine rescued the deficient shmoo formation in *SPE2* disruptants to wild-type control levels. Thus, polyamines appear largely indispensable for shmoo formation, explaining the reduced mating efficacy in *SPE2* disruptants.

The authors then extended their finding to a metazoan animal, *C. elegans*. Here, impairing spermidine biosynthesis by deleting of spermidine synthase (*Spds-1*), resulted in a significant reduction of the total number of fertilized

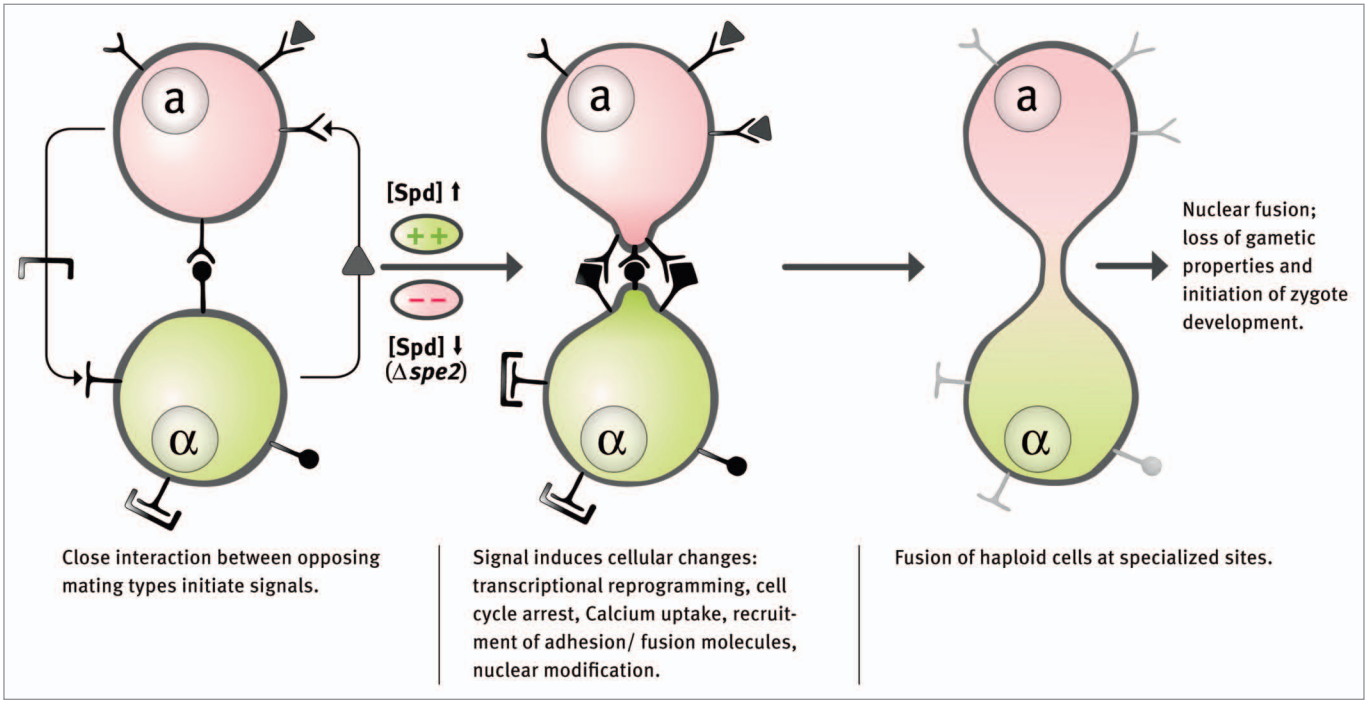

Figure 1. Model representing the major events of fertilization in yeast. Several responses are induced in response to reciprocal pheromone stimulation in oppositely mating strains, which might be regulated by spermidine (Spd).

eggs laid, indicating spermidine to be important for effective fertilization in worms.

How about the mechanistic underpinnings of the spermidine effects on fertilization? Spermidine was recently shown to induce autophagy in a range of model organisms, including mice, worms, flies and yeast.[3] However, deletion of *ATG7*, which is indispensable for autophagy-mediated clearance in *S cerevisiae*, did not influence its mating efficiency. Similarly, RNAi-driven knockdown of beclin-1, an autophagic regulator in *C. elegans*, did not influence egg fertilization rates. Thus, in both cases, spermidine-mediated effects on fertilization seem autophagy-independent. Alternative pathways involving different molecular target(s) should then be responsible for these spermidine-mediated effects. In yeast, when opposite mating type are mixed together, reciprocal pheromone stimulation via a GTP-binding protein-mediated pathway triggers a plethora of responses: increased expression of agglutinins, cell division arrest, increased Ca^{2+} uptake, competence for nuclear fusion, transcriptional reprogramming and formation of a projection that becomes the site of cell fusion.[7,8] A key question for the future is how and which response(s) of fertilization processes are regulated by spermidine?

Recently, structural similarities between proteins involved in sperm-egg recognition in eukaryotes and such involved in fusion of haploid yeasts was proposed.[8] This study here demonstrated the power of the "simple" yeast model in the genetic dissection of fundamental eukaryotic fertilization mechanisms.[4] An important preset for male fertility in higher eukaryotes is the physiological priming of spermatozoa, collectively referred to as capacitation; during capacitation, several signaling cascades are initiated, thereby rendering spermatozoa competent for acrosome reaction, a process driving the sperm to penetrate the oocyte.[1] Ca^{2+} is reported to play a critical role for both capacitation as well as acrosome reaction.[1] As Ca^{2+} channels are known to be regulated by spermidine, an interesting (though speculative) possibility is that Ca^{2+} influx could be modulated by spermidine. In addition, cross-talk between cAMP and phosphatidylinositol pathways is highly critical for the fertilization process[1] and, again, spermidine is reported to modulate these pathways.

With fertility rates steadily decreasing in western societies, a better understanding of the mechanistic details that underlie the pro-fertilization effects is needed. It might not be too far-fetched to see a role of polyamines for fertilization medicine one day.

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Prognostic value of LIPC in non-small cell lung carcinoma

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Lung carcinoma is the primary cause of death by cancer. In the last decades, together with improvements in anesthetic and surgical techniques, several new drugs for chemotherapy or biotherapy have been made available. Thus, while metastatic patients are treated by chemotherapy and/or biologically targeted therapies, in initial stages of disease (I–II), surgery remains the cornerstone of treatment. Locally advanced disease is generally treated in the setting of multimodal combinations, including chemotherapy, radiotherapy and surgery, whose respective indications have been refined in the last years. However, these developments provided only limited increase in survival, and a large room for improvement persists.[1]

If clinical stage of non-small cell lung cancer (NSCLC) is the major determinant of treatment strategy, pathologic stage is currently considered the most important determinant of prognosis in resected patients and is the most important parameter determining the choice of adjuvant treatments. However, within the same stage of disease, large prognostic variability obviously exists and sensitivity to adjuvant treatments is heterogeneous. Furthermore, at present there is no consensus regarding the usefulness of post-operative follow-up. Repeated chest X-ray, CT scan, fiberoptic bronchoscopy or PET scans have been proposed, with enormous variations in medical resource utilization and costs. The identification of patients with poor prognosis within a determined stage would be useful for individual tailoring follow-up procedures.[2]

The identification of prognostic factors is of major importance to develop adequate management strategies. For instance, subgroups of patients who benefit more from peri-operative chemotherapy still need to be identified more precisely, to define those for whom the benefit/risk ratio of neo-adjuvant or adjuvant treatments is the most favorable. This issue is of particular importance in early stages, especially stage IB, in which no strict guidelines are available and which represent the majority of stage I–II resected lung cancer. Several prognostic markers have been proposed in the last years, including characteristics of tumoral immune microenvironment, growth factors and their receptors, markers of systemic inflammation, peptides/proteins/enzymes produced by tumor cells with impact on cell cycle, metabolism and sensitivity to chemotherapy, suggesting the paramount importance of this topic.[3-5]

The study by Galluzzi et al.[6] reports evidence that intratumoral levels of hepatic lipase LIPC, as assessed by immunohistochemistry,

positively correlate with disease outcome in two independent series of non-metastatic NSCLC patients treated by surgery, possibly in a multimodality setting. Furthermore, the authors found that in one of two series, patients with tumors expressing low levels of LIPC had better survival when receiving adjuvant cis-platinum-based chemotherapy, whereas patients with tumors expressing high levels of LIPC had survival unaffected by post-operative chemotherapy. Although validation by specifically designed prospective clinical trials is mandatory to draw definitive conclusion on possible clinical applications, LIPC seems an extremely interesting marker in resected NSCLC from both a prognostic and a predictive point of view.

The same team previously identified LIPC as a cisplatinum response modifier by a genome-wide siRNA-based screen in human NSCLC A549 cells.[7] Together with LIPC, 84 other functional cisplatinum response modifiers were identified, including several proteins known to regulate platinum-induced cell death (e.g., the pro-apoptotic cytoplasmic adaptor APAF-1 and the anti-apoptotic Bcl-2 family member BCL-XL) as well as factors with no obvious links with platinum-elicited signaling pathways, including another enzyme (pyrixodal kynase) and the hepatic lipase LIPC. Thus the mechanisms responsible for the impact of LIPC on the effect of platinum-based chemotherapy remain to be elucidated.

Although discovered as a platinum-response modifier with possible impact on survival in patients receiving platinum-based chemotherapy, LIPC was shown to have a strong prognostic impact also in patients who did not undergo chemotherapy. Thus, the mechanisms explaining the impact on prognosis of LIPC are even more obscure and probably intriguing. Response to therapy and survival in cancer patients probably depends not only on the ability of treatments in removing or killing proliferating tumor cells, but on a complex interaction between disease, treatments and respective impacts on host reaction, whose determinants are not completely elucidated.[8] Identification of mechanisms responsible for LIPC impact on response to chemotherapy as well as on survival in chemotherapy naïve patients could provide interesting insights in the general knowledge of mechanisms of survival in cancer patients.

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Sensing hyperploidy and immune surveillance: A pas-de-deux

Comment on: Boilève A, et al. Cell Cycle 2013; 12:473–9; PMID:23324343; http://dx.doi.org/10.4161/cc.23369

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The immune surveillance hypothesis[1] posits that tumor cells are subject to control by the immune system based on the recognition by T cells of tumor-associated antigens presented in the context of major histocompatibility complex (MHC) molecules. Operationally, this requires phagocytosis of tumor cells by host antigen presenting cells, such as macrophages and dendritic cells, which leads to the generation of tumor antigen-specific T cells, thus promoting the elimination of tumor cells. Mechanisms by which tumor cells evoke attention from immune cells are not fully understood.

In a previous issue, Boilève et al.[2] suggest that hyperploid colon cancer cells are subject to "hardwired" immune surveillance that results in immunological control of hyperploid neoplastic cells. They use a system of transformed colon cell organoids, in which the tumor suppressor gene *Tp53* is silenced to facilitate hyperploidization. Interestingly, while wild-type colon cancer cells are not readily inducible to tetraploidy, *Tp53* silencing renders them responsive to pharmacologically induced hyperploidy as well as spontaneous hyperploidization, consistent with previous data demonstrating that *Tp53* control of the G_1 checkpoint is a barrier to pharmacologically induced hyperploidization.[3] Hyperploid *Tp53*$^{-/-}$ colonocytes initially grow in syngeneic immunocompetent hosts but fail to progress. Histologically, these tumors are fibrotic and display chaotic cellular architecture. Conversely, hyperploid *Tp53*$^{-/-}$ colon cancer cells grow progressively in immunodeficient hosts and form organized structures. Additionally, the nuclei of graft cells in immunodeficient mice are larger than those of cells growing in immunocompetent mice, implying increased ploidy. Taken together, these results suggest that the immune system senses hyperploid colonocytes and, in turn, controls their growth.

Investigating how tetraploid tumor cells are sensed by the immune system, the authors show that *Tp53*$^{-/-}$ tetraploid colonocytes heterogeneously upregulate cell surface expression of calreticulin, which is trafficked to the cell membrane during apoptosis serving as an "eat-me" signal for macrophages and dendritic cells.[4] They additionally show that hyperploid *Tp53*$^{-/-}$ colonocytes in immunodeficient mice express the phosphorylated form of eukaryotic initiation factor (eIF) 2α, suggesting that these cells undergo ER stress response.[5] Recently the same group showed that an intact ER stress response is necessary for calreticulin upregulation in hyperploid cancer cells.[6] Thus, the increased immunogenicity of hyperploid neoplastic cells is driven by an ER stress response-mediated upregulation of calreticulin, leading to increased uptake of hyperploid cells by phagocytes, and initiation of a specific cellular immune response. The nature of the immune response against hyperploid transformed cells should be the subject of future study.

Why does increased uptake of hyperploid cancer cells lead to their selective elimination? While increased protein content in a hyperploid cancer cell would ostensibly lead to

greater quantitative presentation of antigens to T cells, this alone would not necessarily lead to selective elimination of hyperploid cancer cells. Rather, one would expect the elimination of all tumor cells presenting the same antigen, including diploid cancer cells. An intriguing possibility is that hyperploidization of cancer cells changes their antigenic repertoire, driving the expansion of T cells specific for hyperploidy-associated antigen(s). Alternatively, hyperploid cancer cells may be simply "better" targets than neighboring diploid cancer cells for cytotoxic T cells due to increased cell-surface display of antigen, even though target recognition and killing by cytotoxic T cells was shown to require as little as a single MHC-antigen complex.[7]

The role of the ER stress response in the tumor microenvironment remains an open question. Boilève et al.,[2] as well as previous work from the same group, suggests that the ER stress response enforces expression of calreticulin on hyperploid cells, thus promoting tumor immune surveillance. On the other hand, others have shown that the ER stress response is a cell-intrinsic survival mechanism for cancer cells.[8] Further supporting a tumorigenic role for the ER stress response, recent work has uncovered a novel cell-extrinsic role for the tumor ER stress response in polarizing myeloid cells to a pro-inflammatory/suppressive phenotype that impairs $CD8^+$ T cell priming and facilitates tumor growth in vivo.[9] Reconciling these seemingly contrasting effects, we suggest that the tumor ER stress response may fulfill both functions, perhaps promoting cellular immunity against hyperploid cells while simultaneously undermining the immune response against cancer cells. The fact that clinical tumors samples exhibit heterogeneous ploidy suggests that this might indeed be the case.

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A new BET on the control of HIV latency

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Although intensive regimens of antiretroviral therapy (HAART) reduce viral loads to undetectable levels in the circulation, HIV quickly resumes active replication when treatment is interrupted due to the emergence of the virus from latent reservoirs.[1,2] Although it is difficult to exclude the possibility that slowly replicating viruses persist in sanctuary sites that are poorly accessed by the antiviral drugs, the consensus in the field is that the virus emerges from a small population of resting memory CD4 T cells (~1 in 10^6 cells) harboring silenced HIV proviruses.

Eliminating this latent reservoir is particularly challenging since it is established early during infection, is extremely stable (with an estimated half-life of 44 mo), and can be replenished during episodes of viremia or by homeostatic replacement of latently infected cells. Since latently infected cells express minimal levels of viral proteins they are invisible to the immune system and unaffected by antiretroviral drugs. Recent curative strategies have therefore focused on developing pharmaceutical agents that can induce HIV expression in latently infected cells and then purging these cells by antiviral immune responses, viral cytopathic effects or even cell-targeted killing strategies (the rhetorically named "shock and kill" strategy).[1,2]

In the last three months there has been a flurry of provocative papers, including the report by Boehm et al.[3] published in a previous issue of *Cell Cycle*, demonstrating that JQ1 and other clinically useful bromodomain (BET family) inhibitors can efficiently reverse HIV latency in established cell lines and in certain primary cell models for HIV latency.[4-7] Thus the BET proteins have been validated as potential new targets for HIV induction strategies. But, how do these compounds work?

All BET proteins are highly conserved transcriptional regulators capable of binding to acetyl-lysine residues found on histones and many transcription factors through tandem bromodomains. Perhaps the best-studied BET family member is BRD4, which binds the positive transcription elongation factor b (P-TEFb) the essential cofactor for the HIV Tat gene (**Fig. 1**). It was therefore postulated that BET family inhibitors induce HIV expression by inhibiting BRD4 interactions with P-TEFb and thereby favoring enhanced Tat binding.[4,6,8] Consistent with this model, and the inhibitor studies, knockdown of BRD4 by shRNA also results in potent HIV induction.

Boehm et al.[3] have now discovered that in addition to BRD4, a second BET protein, BRD2, also regulates HIV latency. Knockdown of BRD2 by shRNA activates HIV transcription to an even higher extent than knockdown of BRD4 and to levels comparable to JQ1 treatment of cells. In contrast to BRD4, BRD2 associates directly with transcription complexes and proteins required for chromatin remodeling. Thus it seems likely that BRD2 can enhance HIV transcription in response to JQ1 and other BET inhibitors. But how can it act as a repressor in the absence of BET inhibitors?

One clue comes from the observation that BRD2 interacts directly with the E2F1 transcription factor. Earlier studies showed that E2F1 can bind together with NFκB p50 to the HIV enhancer and block HIV transcription mediated by the NFκB p50/p65 heterodimer.[9] It seems reasonable to postulate that BRD2 is recruited to the HIV LTR by E2F1/p50 heterodimers, and then recruits repressor complexes

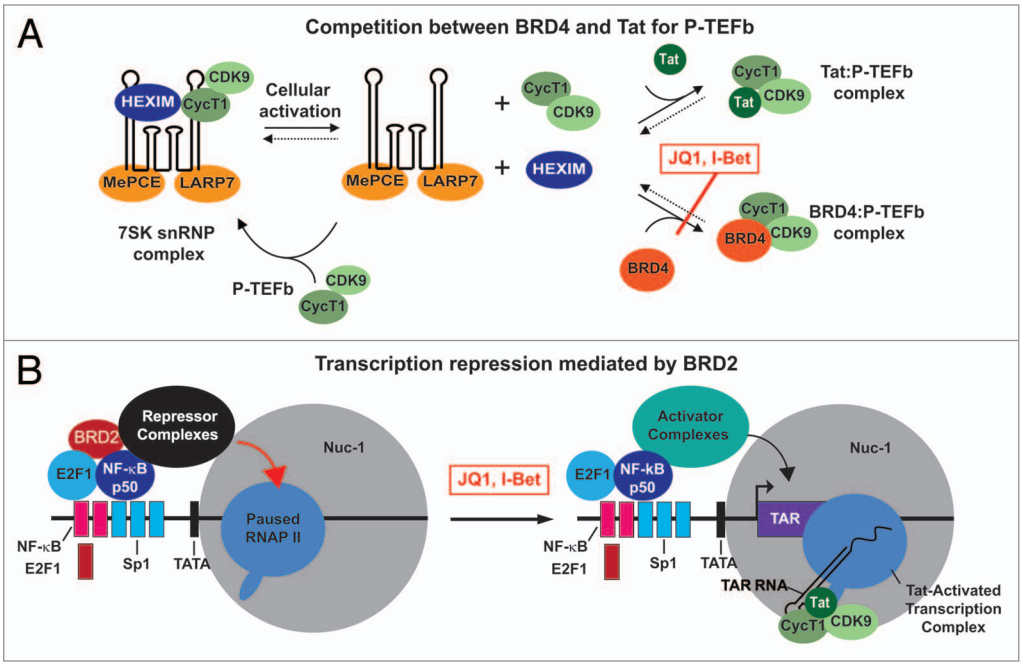

Figure 1. Models for HIV induction by BET inhibitors. (**A**) Inhibition of BRD4 blocks its association with P-TEFb and permits enhanced association with the HIV transactivator protein Tat. The Tat:P-TEFb complex is recruited to the HIV promoter and induces transcription. (**B**) Inhibition of BRD2 blocks its association with E2F1:NFκB p50 heterodimers and co-repressor complexes. In the absence of BRD2, the repressor complexes are replaced by activator complexes, and HIV transcription is induced.

carrying acetylated lysine residues (**Fig. 1**). Thus when BRD2 is inhibited, these interactions are blocked, and repressor complexes are exchanged for activators.

Although further work will be needed to confirm the molecular details of the two mechanisms outlined in **Figure 1**, it is important to note at this stage that the high potency of JQ1 and related BET inhibitors in mediating HIV induction could be due to the targeting of multiple bromodomain proteins that regulate HIV transcription. Indeed, preliminary data emerging from several laboratories suggests that several other bromodomain proteins in addition to BRD4 and BRD2 can also contribute to the maintenance of HIV latency. It's a safe bet that studies of these important regulatory mechanisms will reshape our understanding of HIV latency in the years to come.

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