

■ FROM THE DIRECTOR'S OFFICE

Retreating to Advance—CCR Tenure Track Investigators Taking Charge of Their Future

Do you know your SIP from your CRTA? How about your NCI Tenure Review Panel from your CTC? And what is this site visit thingyabobber all about anyway?!

Being a new tenure track investigator is an exciting time, having the opportunity to build and run the laboratory group you always wanted and the ability to pursue the science you think is *most* important. But new tenure track investigators face many challenges in trying to navigate the CCR, NCI, and NIH to get the answers they need to their questions. With shrinking budgets and fewer new recruits, it becomes difficult to find colleagues who have been “in the trenches” recently and can show you the ropes, particularly when the trenches keep moving and the ropes keep changing! To address issues pertinent to tenure track investigators and to facilitate communication within the tenure track community and between tenure track investigators and the CCR, the Tenure Track Investigators’ Committee (TTIC) has been established.

Goals of the Tenure Track Investigators’ Committee

The TTIC was established to accomplish several goals outlined in a TTIC charter. First, the committee seeks “to raise the visibility of tenure track investigators at CCR.” Second, the committee seeks to establish an infrastructure to facilitate communication within the tenure track community and between tenure track investigators and the Office of the Director as well as the scientific community as a whole. The recently developed Tenure Track Investigator Web site (<http://ccrintra.cancer.gov/TTI>) and the CCR Tenure Track Investigator email listserv have been excellent resources for investigators to find the answers to their questions and to increase communication for the tenure track community. The third goal of the TTIC is “to raise awareness about issues specific to junior principal investigators and pertinent to obtaining tenure at NCI/NIH.” As part of this effort, members of the TTIC have raised awareness of the issue of mentoring over the last year, and this has led to a shift within the CCR toward a more institution-wide recognition of the importance of second mentors and advisory committees for tenure track investigators. To continue raising visibility, promoting communication, and addressing issues for the tenure track investigator community, the TTIC has organized an annual retreat in the spring to bring together tenure track investigators and other members of the CCR, NCI, and NIH communities. In addition to providing information directly to tenure track investigators, these retreats will provide useful information to lab and branch chiefs and program

directors, who play a role in mentoring these investigators.

The 2006 Tenure Track Investigator Retreat: Educating Investigators on the Tenure Process

The first Tenure Track Investigator Retreat was held June 16, 2006, in Rockville, Maryland. The retreat had an instructional focus and excellent representation by the senior leadership. Many diverse topics relevant to the success of tenure track investigators were presented, and the PowerPoint slides for most of the presentations are available at the Tenure Track Investigator Web site (<http://ccrintra.cancer.gov/TTI/retreat.asp>). The morning session focused on the process of getting tenure, with Michael Gottesman, MD, Doug Lowy, MD, Robert Wiltout, PhD, and Lee Helman, MD, providing an overview of different aspects of the tenure process. Ira Pastan, MD, and Arlyn Garcia-Perez, PhD, presented the perspective of the NCI Tenure Review Panel and the Central Tenure Committee, respectively, while Frank Balis, MD, and Thomas Waldmann, MD, described the aspects of the tenure process specific to clinical investigators. The morning session concluded with a presentation on mentoring initiatives by Beverly Mock, PhD, and a presentation on the site visit process by Florence Farber, PhD. This session gave an excellent overview of the tenure process and provided tips for tenure track investigators to consider from the very beginning on their road to tenure.

The afternoon session of the retreat was entitled “Making the Most of Your Money” and focused on how to take advantage of NIH resources to maximize your budget, a topic of great interest in this age of shrinking budgets. Rick McGee, PhD, began the session by describing the Graduate Partnership Program at NIH and the various mechanisms for bringing students to work in the laboratory. Next, David Goldstein, PhD, presented information on the Office of Science and Technology Partnerships and the Research Technology Program. Finally, Karen Maurey, Director of NCI’s Technology Transfer Branch (TTB), discussed the issues of technology transfer at NCI, including patents, licenses, and cooperative research and development agreements (CRADAs). Overall, this session was very informative on the resources currently available to investigators, and even the most seasoned of tenure track investigators learned something new!

The 2007 Tenure Track Investigator Retreat: Highlighting the Science of Tenure Track Investigators

Communication is a two-way street. The first tenure track investigator retreat successfully informed tenure track investigators about different aspects of the CCR, NCI, and NIH. Now, the TTIC thinks the tenure track community should inform colleagues about the work it is doing! For this reason, the second retreat has been scheduled for May 11, 2007, in Bethesda to highlight the science of current tenure track investigators. One of the goals of this retreat will be to increase interactions between CCR tenure track investigators and DCEG, as well as senior leaders from other NIH institutions. A take-home message from the last retreat was that to get tenure at NIH, tenure track investigators should become full-fledged members of the broader trans-NIH community through collaboration and interaction. The TTIC will

invite leaders in various fields from other institutions to present keynote speeches, followed by presentations by CCR tenure track investigators and panel discussions. A preliminary agenda will be available soon. The TTIC hopes this retreat will build ties between tenure track investigators and other institutions that will lead to future collaborations and possibly better visibility with members of the Central Tenure Committee. The TTIC encourages all tenure track investigators to participate and to consider how they might benefit from inter-institution collaboration. The TTIC also encourages senior investigators to make suggestions and help build bridges between individual tenure track investigators and colleagues throughout NIH. Any questions or comments on the TTIC or the upcoming retreat can be addressed to the TTIC chair, Esta Sterneck, PhD (sterneck@ncifcrf.gov), or any of the other TTIC members listed at <http://ccrintra.cancer.gov/TTI/contact.asp>.

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■ CARCINOGENESIS

Effect of Estrogen on Arsenic-induced Urogenital Carcinogenesis

Waalkes MP, Liu J, Ward JM, Powell DA, and Diwan BA. Urogenital carcinogenesis in female CD1 mice induced by *in utero* arsenic exposure is exacerbated by postnatal diethylstilbestrol treatment. *Cancer Res* 66: 1337–45, 2006.

Environmental inorganic arsenic exposure from contaminated drinking water is a serious problem throughout the world. In humans, arsenic, a carcinogen, targets various tissues and is associated with urogenital system tumors, including urinary bladder cancers. Although arsenic is clearly carcinogenic in humans, in adult animals it has proven difficult to induce tumors with inorganic arsenic alone. Gestation, however, is a period of high sensitivity to chemical carcinogenesis in animals and probably humans. This is because of factors like rapid global proliferative growth during the fetal life stage.

We performed a series of transplacental carcinogenesis studies in which mice were exposed in the womb to arsenic via the maternal system (Waalkes MP et al. *Toxicol Appl Pharmacol* 186: 7–17, 2003; Waalkes MP et al. *Carcinogenesis* 25: 133–41, 2004; Waalkes MP et al. *J Natl Cancer Inst* 96: 466–74, 2004). This prior work showed that arsenic exposure *in utero* induced tumors and pretumorous lesions in several tissues of the offspring when they became adults. The targets of transplacental arsenic in mice included the ovaries, liver, adrenal glands, uterus, and oviducts, which are also potential targets of carcinogenic estrogens, such as synthetic estrogen diethylstilbestrol, in humans and rodents. Because arsenic showed an estrogen-like tumor spectrum in mice, we hypothesized that aberrant estrogen signaling plays a role in transplacental arsenic carcinogenesis. Estrogen receptor- α (ER- α), a key factor in estrogen signaling, helps control estrogen-induced cellular proliferative responses. ER- α overexpression increases sensitivity to estrogen carcinogenesis in mice. In our prior work, a marked overexpression of both ER- α and estrogen-related genes important in carcinogenesis was observed in adult mice bearing transplacental arsenic-induced tumors. Furthermore, in an arsenic-exposed human population with increased arsenic-associated cancers, ER- α was clearly overexpressed (Waalkes MP et al. *J Natl Cancer Inst* 96: 466–74, 2004). Thus, ER- α overexpression was associated with arsenic carcinogenicity.

In the present study, we directly tested the hypothesis that aberrant stimulation of estrogen response pathways plays a role in transplacental arsenic carcinogenesis. Specifically, the effects of postnatal diethylstilbestrol exposure on the carcinogenicity of *in utero* arsenic

exposure were explored. Pregnant mice received drinking water containing arsenic, and female offspring received diethylstilbestrol for several days after birth. In adulthood, arsenic alone induced adrenal adenomas and some urogenital tumors, including, mostly, benign tumors of the ovaries and uterus. Diethylstilbestrol alone induced some tumors (primarily cervical), but when given after *in utero* arsenic, it synergistically increased urogenital tumor incidence, multiplicity, and progression. For instance, compared with the incidence of urogenital malignancies in the control (0%), arsenic alone (9%) and diethylstilbestrol alone (21%) groups, arsenic plus diethylstilbestrol induced a 48% incidence of malignant urogenital tumors. Of the urogenital tumors induced by arsenic plus diethylstilbestrol, 80% were malignant, and 55% were in multiple sites, while 60% precipitated early death. Arsenic plus diethylstilbestrol increased ovarian, uterine, and vaginal tumors and urinary bladder proliferative lesions (tumors plus preneoplasias; **Figure 1**), including several transitional cell carcinomas, the urinary bladder tumor type seen in humans exposed to arsenic. Uterine and bladder carcinomas induced by arsenic plus diethylstilbestrol greatly overexpressed ER- α and *pS2*, an estrogen-regulated gene. In neonatal uteri, prenatal arsenic increased ER- α expression and enhanced estrogen-related gene expression induced by postnatal diethylstilbestrol. Thus, arsenic acts with estrogens to enhance production of female mouse urogenital cancers.

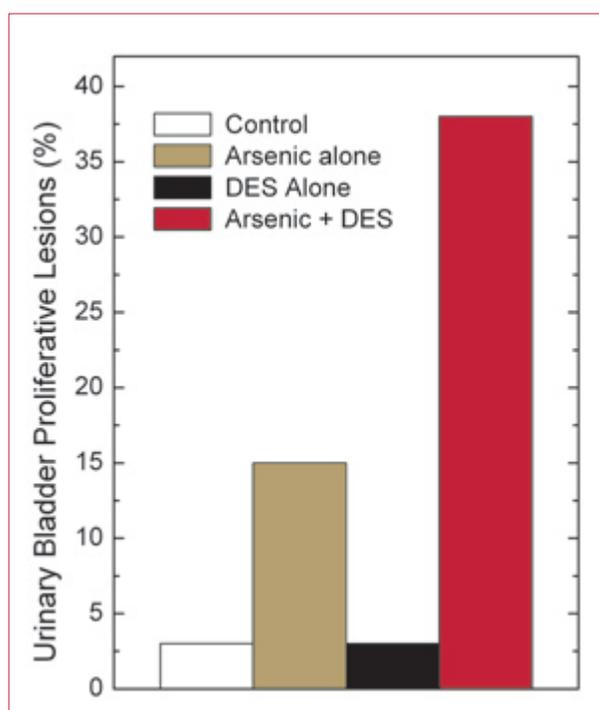


Figure 1. Urinary bladder proliferative lesions (tumors plus preneoplasias) in female mice after *in utero* arsenic exposure combined with postnatal diethylstilbestrol (DES). The urinary bladder proliferative lesions included three transitional cell carcinomas, the tumor type seen in humans exposed to arsenic.

The present data provide compelling evidence that arsenic can initiate or induce urogenital tract cancers, potentially including tumors of the urinary bladder, and that this response is exacerbated by estrogen. In this regard, prolonged arsenical exposure can produce urinary

bladder tumors in adult rats, but unlike the case with most other bladder carcinogens, females appear more sensitive than males (Wei M et al. *Carcinogenesis* 23: 1387–97, 2002; Shen J et al. *Toxicol Appl Pharmacol* 210: 171–180, 2006), which would be in keeping with a role for estrogens in this response. Furthermore, our assessment of early molecular events in transplacental arsenic carcinogenesis indicates that arsenic precipitates and can further facilitate aberrant estrogen signaling in urogenital target tissues of arsenic carcinogenesis, potentially leading to the reprogramming of critical signaling pathways. Estrogen levels during pregnancy are much higher than in other periods of adult life, which could provide an endogenous stimulus for *in utero* arsenic carcinogenesis. Because fetal arsenic exposure initiates cancer in so many sites within the female mouse urogenital system, we now hypothesize that arsenic *in utero* attacks a critical pool of progenitor cells in the urogenital system and induces aberrant genetic reprogramming as part of its carcinogenic mechanism, in a fashion similar to early life exposure to diethylstilbestrol (Cook JD et al. *Proc Natl Acad Sci U S A* 102: 8644–49, 2005).

These findings have important public health implications. For instance, pharmacological or environmental estrogen exposure could possibly enhance arsenic-initiated cancers, whereas prenatal arsenic exposure may predispose people to develop estrogen-related carcinogenesis. In addition, the fetal life stage is clearly a period of high sensitivity to arsenic carcinogenesis in mice and a comparable sensitivity in humans would be cause for great alarm. A transplacental component of human arsenic carcinogenesis may be difficult to prove because populations exposed to arsenic during gestation only do not appear to exist. However, in areas where chronic exposure to elevated environmental arsenic is common, all life stages are involved and significant *in utero* exposure inevitably occurs. Because of this, protection of pregnant women from excessive arsenic exposure may be a valid intervention strategy in preventing human cancer induced by environmental arsenic.

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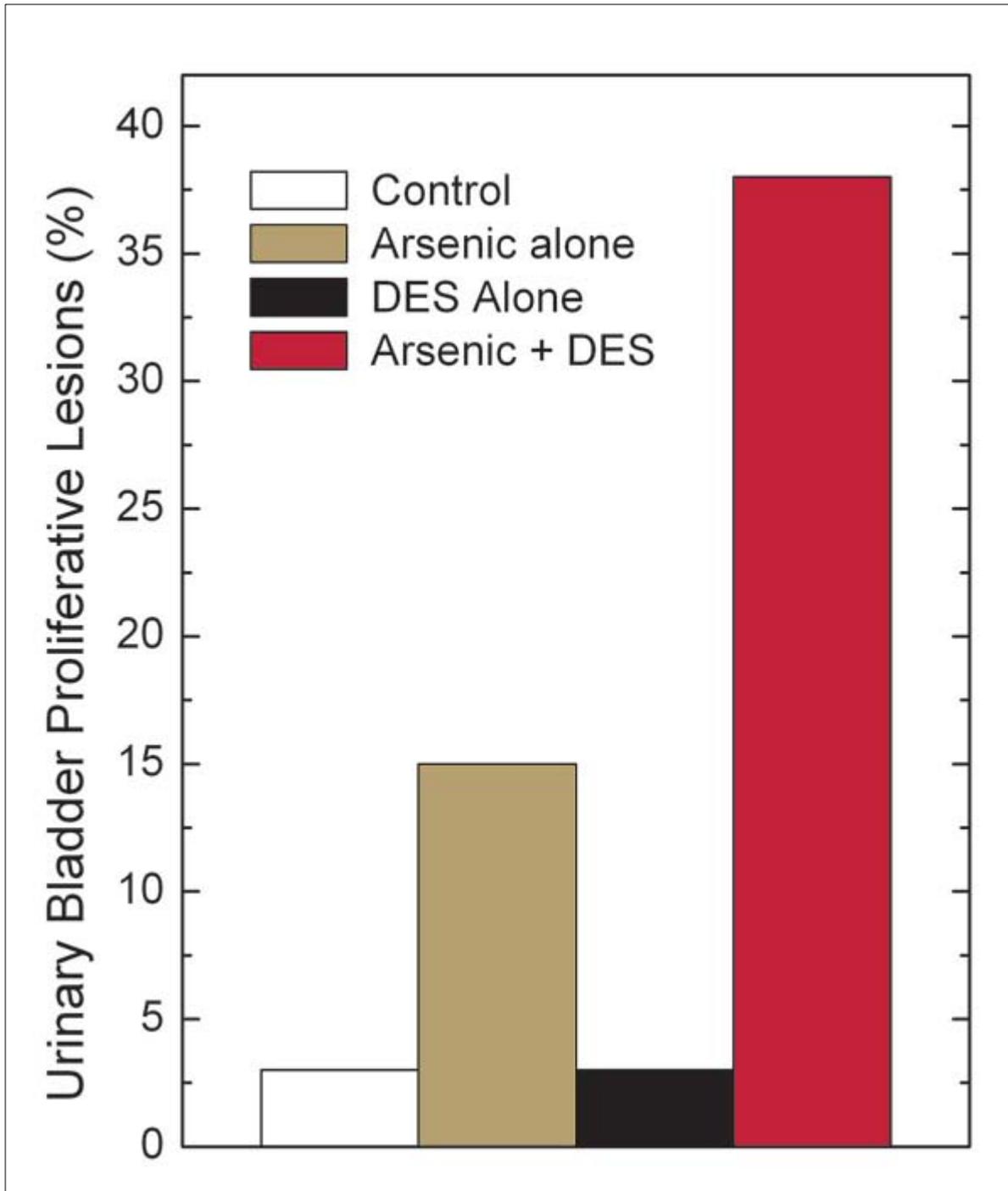


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■ CELL BIOLOGY

Rap Signaling Regulates Stem Cell Anchoring in *Drosophila* Testis

Wang H, Singh SR, Zheng Z, Oh SW, Chen X, Edwards K, and Hou SX. Rap-GEF signaling controls stem cell anchoring to their niche through regulating DE-cadherin-mediated cell adhesion in the *Drosophila* testis. *Dev Cell* 10: 117–26, 2006.

Stem cells can either self-renew or differentiate into short-lived cell types. Cancer cells also possess the potential for self-renewal; tumors may originate from a few transformed cancer stem cells (Reya T et al. *Nature* 414: 105–11, 2001).

Understanding the molecular mechanisms that control stem cell self-renewal versus differentiation is crucial to the use of stem cells in regenerative medicine and the development of effective anticancer therapies. Accumulated evidence suggests that stem cells are controlled by particular microenvironments known as niches (Spradling A et al. *Nature* 414: 98–104, 2001; Fuchs E et al. *Cell* 116: 769–78, 2004). A niche is a subset of neighboring stromal cells and extracellular substrates. The stromal cells often secrete growth factors to regulate stem cell behavior.

The *Drosophila* testis provides an excellent *in vivo* system to study stem cells and niches at the cellular and molecular levels (Fuller MT. *Semin Cell Dev Biol* 9: 433–44, 1998; Yamashita YM et al. *J Cell Sci* 118: 665–72, 2005). At the tip of the *Drosophila* testis (the apex) is a germinal proliferation center, which contains the germline and somatic stem cells that maintain spermatogenesis. Each adult male fly testis has five to nine germline stem cells (GSCs), each encysted by two somatic stem cells (SSCs, also called cyst progenitor cells). Both GSCs and SSCs attach to a group of 12 nondividing somatic cells called the hub (Hardy RW et al. *J Ultrastruct Res* 69: 180–90, 1979; Gonczy P and DiNardo S. *Development* 122: 2437–47, 1996). The hub defines the stem cell niche by expressing the growth factor Unpaired (Upd), which activates the JAK/STAT pathway in GSCs to regulate the stem cell self-renewal process (Kiger AA et al. *Science* 294: 2542–5, 2001; Tulina N and Matunis E. *Science* 294: 2546–9, 2001). Meanwhile, a member of the transforming growth factor- β (TGF- β) family, glass bottom boat (Gbb), is also expressed in the hub and plays a part in regulating GSC self-renewal by activating its corresponding signal transduction pathway in the GSCs (Kawase E et al. *Development* 131: 1365–75, 2004). Because Upd and Gbb are expressed in the hub, they have very limited ability to diffuse; therefore, the GSCs must first be anchored to the hub to receive the signals and maintain their stem cell identity.

The cell adhesion molecules E-cadherin and β -catenin (named Armadillo [Arm] in *Drosophila*) are concentrated at the hub-GSC interface and may anchor the stem cells to the niche (Yamashita YM et al. *Science* 301: 1547–50, 2003). However, how the adherens junctions are specifically formed at the hub-GSC interface is not clear. We recently identified in a genetic screen a *Drosophila* small GTPase Rap guanine nucleotide exchange factor (Gef26) as a major regulator of the anchoring of stem cells to their niche. The Gef26 protein has a PDZ domain, a Ras-binding domain, a cAMP/cGMP-binding domain, and a Rap-binding domain. Mutations of Gef26 cause loss of both GSCs and SSCs in the fly testis. We demonstrated that the Rap-Gef (Gef26)/Rap signaling controls stem cell anchoring to the niche through regulation of E-cadherin-mediated cell adhesion. The *Gef26* mutation specifically impairs adherens junctions at the hub-stem cell interface, which results in the stem cells “drifting away” from the niche and losing stem cell identity (Figure 1). Thus, the Rap signaling/E-cadherin pathway may represent one mechanism that regulates polarized niche formation and stem cell anchoring (Wang H et al. *Dev Cell* 10: 117–26, 2006).

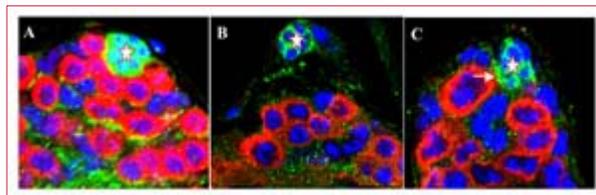


Figure 1. Germline stem cells (GSCs) “drift away” from the niche in *Gef26* mutant testes because of impaired adherens junctions. The Wild-type (A), *Gef26*⁴ (B), and *Gef26*⁶ (C) testes of adult flies were immunostained with anti-E-cadherin (green), anti-Vasa (red), and DAPI (blue). In (A), all GSCs are anchored to the hub (star) through E-cadherin-positive adherens junctions. In (B), all Vasa-positive germ cells drifted away from the hub (star). In (C), only one GSC is still attached to the hub (star) through E-cadherin-positive adherens junctions (arrow).

Rap1 was first identified as a gene that can reverse the transformed phenotype of fibroblasts by one of the mutated Ras genes, *K-ras* (Kitayama H et al. *Cell* 56: 77–84, 1989). *Rap1* belongs to the Ras family of small GTP-binding proteins. Its apparent tumor suppressor properties were initially proposed to antagonize the activity of Ras by competing for a common target (or regulatory protein). However, recent studies have suggested that *Rap1* may actually regulate adherens junctions. In a recent study, a *Rap1* GTPase activator, *Dock4*, was identified as a tumor suppressor (Yajnik V et al. *Cell* 112: 673–84, 2003). *Dock4* specifically activates *Rap1* and regulates the formation of adherens junctions. Our recent results show that the Rap-Gef (Gef26)/Rap signaling controls the anchoring of stem cells to their niche through regulation of E-cadherin-mediated cell adhesion in the *Drosophila* testis. The mammalian homolog of Gef26 may regulate cancer stem cell anchoring and function as a tumor suppressor. We are in the process of knocking out the homologous *Gef26* gene in mice (*RapGef2*) and searching for an association between *RAPGEF2* and human diseases. Thus, the powerful genetic manipulations available in *Drosophila* in combination with the mouse knockout studies may make this an ideal system to study cancer stem cells and cancers.

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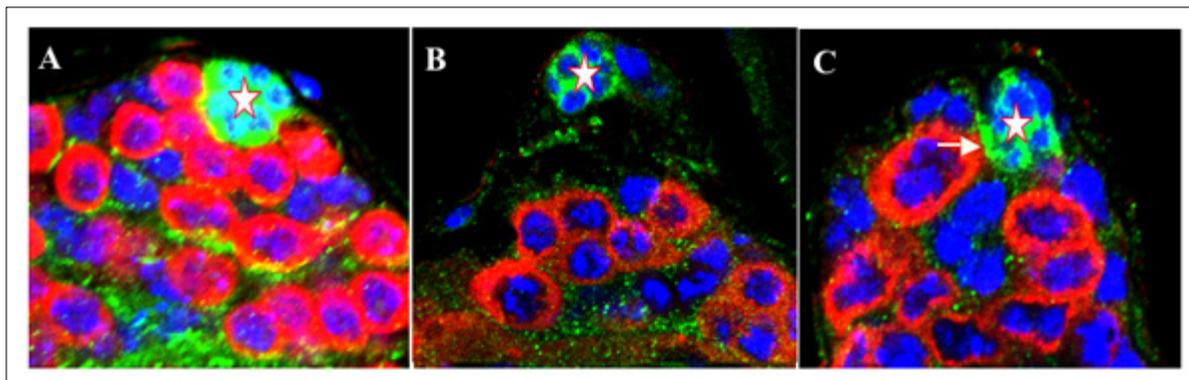


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■ MOLECULAR BIOLOGY/PHARMACOLOGY

Novel Interfacial Inhibitors of Topoisomerase I

Marchand C, Antony S, Kohn KW, Cushman M, Ioanoviciu A, Staker BL, Burgin AB, Stewart L, and Pommier Y. A novel norindenoisoquinoline structure reveals a common interfacial inhibitor paradigm for ternary trapping of the topoisomerase I-DNA covalent complex. *Mol Cancer Ther* 5: 287–95, 2006.

Human DNA topoisomerase I (Top1) is a ubiquitous and essential enzyme because it relaxes DNA supercoiling during replication and transcription. Top1 generates DNA single-strand breaks and allows rotation of the cleaved strand around the double helix axis. During relaxation, the 3' end of the cleaved DNA strand is covalently linked to a Tyr residue on the protein. After relaxation, Top1 religates the cleaved strand and regenerates intact duplex DNA. Under normal conditions, the covalent Top1-cleaved DNA intermediates, referred to as “cleavage complexes,” are transient and remain at a very low level because the religation (“closing”) step is much faster than the cleavage (“nicking”) step (Figure 1, part A).

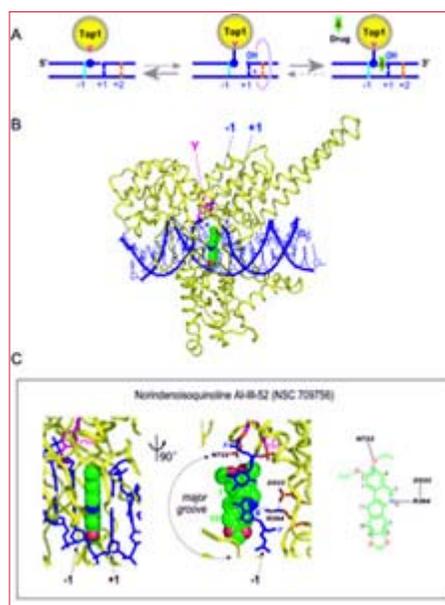


Figure 1. (A) The human DNA topoisomerase I (Top1)–mediated cleavage and religation of DNA. (B) Structure of the norindenoisoquinoline AI-III-52 in a Top1 cleavage complex. The 3' end of the cleaved strand is covalently linked to the catalytic Tyr residue 723 (Y). (C) An expanded view of AI-III-52 bound inside the Top1 cleavage complex in the same orientation as in part B (left panel) or with a 90° rotation (center panel). The –1 base pair (capped sticks) covers and stacks against the

entire drug (space filling).

Top1-specific inhibitors, such as camptothecins, can trap Top1 cleavage complexes. These potent anticancer drugs bind at the Top1-DNA interface in a ternary complex and prevent DNA religation. Their anticancer activity is therefore not directly driven by the inhibition of Top1 catalytic activity *per se*, but by the generation of lethal DNA lesions. Many camptothecin derivatives have been synthesized for clinical development, and two have been approved by the U.S. Food and Drug Administration: topotecan (Hycamtin) for ovarian and lung cancer and irinotecan (CPT11, Campto) for colon carcinomas. To circumvent the clinical limitations of camptothecins, other Top1 inhibitors have been developed. Among them, the indolocarbazole edotecarin is the most advanced in clinical development.

Indenoisoquinolines are a novel family of Top1 inhibitors with several advantages over camptothecins. First, they do not require metabolic activation and have a prolonged half-life. Second, they induce Top1 cleavage complexes at different sites and therefore offer a different biological profile. Third, Top1 cleavage complexes induced by indenoisoquinolines are markedly more stable and persistent than those trapped by camptothecins.

We recently analyzed the co-crystal structures of Top1-mediated DNA cleavage complexes with five potent and highly specific Top1 inhibitors: AI-III-52 norindenoisoquinoline, MJ-238 indenoisoquinoline, SA315F indolocarbazole, topotecan, and camptothecin. The norindenoisoquinoline AI-III-52 is bound deeply inside the protein and intercalated between the base pairs flanking the cleavage site (positions -1 and +1) (Figure 1, part B). The 3' end of the cleaved strand is covalently linked to the catalytic Tyr residue 723 (Y). The -1 base pair covers and stacks against the entire drug (Figure 1, part C). Extensive stacking is also observed for the +1 base pair, which is completely covered by the drug (and therefore not shown in Figure 1, part C). The drug is stabilized by two critical hydrogen bonds with residues Asn 722 and Arg 364 on Top1 (Figure 1, part C, center and right panels).

We found that all five inhibitors exhibit a common mechanism of action. They all bind at the Top1-DNA interface by intercalating between the base pairs flanking the DNA cleavage site and by forming a network of hydrogen bonds with Top1, as observed for the norindenoisoquinoline AI-III-52 (Figure 1, part B). Our results add critical information to the understanding of the biological effect of Top1 inhibitor substitutions and offer new insights for the rational design of novel Top1 inhibitors.

The camptothecins (camptothecin, topotecan, and irinotecan) and non-camptothecins (indenoisoquinolines and indolocarbazole) represent a paradigm for interfacial inhibition because they bind at the interface of two macromolecules (Top1 and its DNA substrate) as these macromolecules undergo a catalytic conformational change (cleavage complex) (Pommier Y and Cherfilis J. *Trends Pharmacol Sci* 26: 138–45, 2005). Interfacial inhibition is reversible and uncompetitive. Interfacial inhibitors trap a catalytic intermediate of the macromolecular complex in a specific conformation. The interfacial inhibitors identified so far are natural products that stabilize a wide range of macromolecular complexes: brefeldin

A (for the Arf-GTP exchange factor), colchicine, vinblastine, Taxol, epothilones (for alpha- and beta-tubulin), rapamycin (for the FKBP-TOR complex), antibiotics within the ribosome, and alpha-amanitin (within the elongating RNA pol II complex) (Pommier Y and Marchand C. *Curr Med Chem Anticancer Agents* 5: 421–9, 2005). The mode of interfacial inhibition for these camptothecins and non-camptothecins has implications for drug discovery because interfacial inhibitors stabilize rather than inhibit the formation of macromolecular complexes. Hence, screening through the use of methods to measure the stabilization of macromolecular complexes has the potential to lead to the discovery of highly selective interfacial inhibitors.

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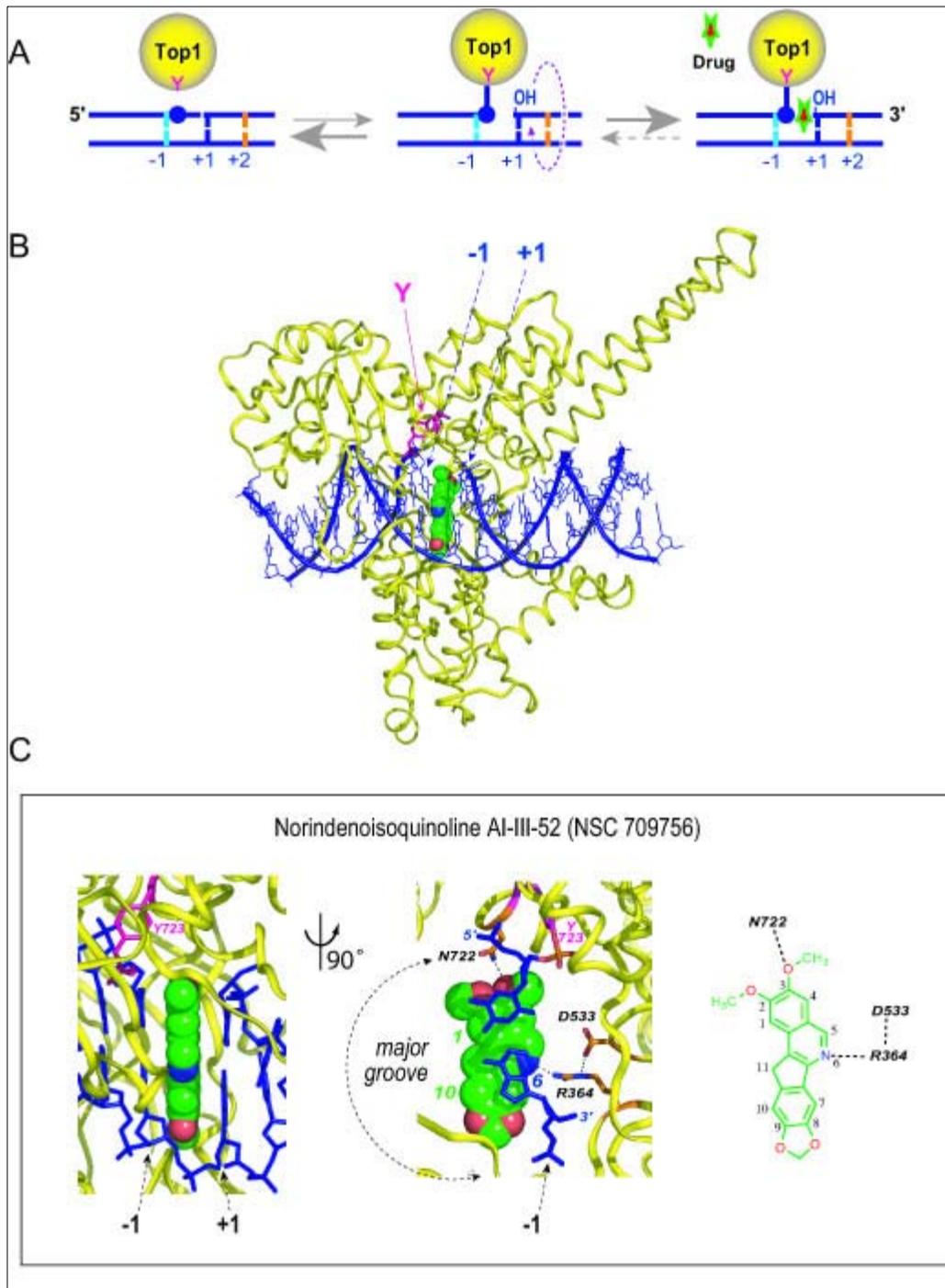


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■ MOLECULAR BIOLOGY

Nature of the Nucleosomal Barrier to RNA Polymerase II

Kireeva ML, Hancock B, Cremona GH, Walter W, Studitsky VM, and Kashlev M. Nature of the nucleosomal barrier to RNA polymerase II. *Mol Cell* 18: 97–108, 2005.

Eukaryotes use the ordered packaging of template DNA into nucleosomes for regulation of gene expression. Examples of genes regulated at the level of transcription elongation in a nucleosome-dependent manner are *c-Fos* and *c-Myc* in mammals, *Hsp* genes in *Drosophila*, and *Met16* in yeast. In all these cases, the nucleosome positioned within the transcribed regions close to the promoter is required to establish an elongation checkpoint. When gene repression takes place, the elongation complex is stalled there. In response to an activation signal, RNA polymerase is rapidly released to start the elongation process. Establishing the sequence of events leading to chromatin-dependent changes in gene expression is an intriguing fundamental scientific problem and has important biomedical significance. Targeted regulation of the expression of oncogenes and tumor suppressors reveals new opportunities for the development of anticancer therapies.

Nucleosomes have long been considered powerful barriers to transcription elongation. This view is based on *in vitro* observations of a nucleosome-dependent inhibition of elongation by human RNA polymerase II (Izban MG and Luse DS. *Genes Dev* 5: 683–96, 1991). The observations suggest that *in vivo*, the nucleosomes must be modified so that the DNA-histone contacts are weakened enough to allow passage of the RNA polymerase (reviewed by Sims RJ 3rd et al. *Genes Dev* 18: 2437–68, 2004). However, despite multiple attempts, none of the nucleosome remodeling or modification events tested (including H2A/H2B dimer dissociation, or histone acetylation) brings the transcription rate on the chromatin template close to the physiological level. Our work challenges the hypothesized obligatory nature of chromatin remodeling and modification for efficient transcription elongation. We established that, in principle, the nucleosome does not present a specific barrier to RNA polymerase II. The observed transcription block is attributable to stabilization of intrinsic, sequence-specific pauses and arrests. Our findings emphasize the primary role of the elongation complex properties over the chromatin modification state for overcoming the nucleosome barrier by RNA polymerase II.

The first key observation we made was that transcription arrests induced by nucleosomes occur in the same sites as the sequence-dependent pauses on the nucleosome-free DNA. Notably, two nucleosomes, differently positioned on the same DNA sequence, caused the

same pattern of nucleosome-induced arrests. Furthermore, polymerase stalling at most of the positions within the nucleosomes leads to transcription arrest. Thus, interruption of the processive movement of RNA polymerase II on the nucleosomal template is necessary for the development of the nucleosome-specific transcription arrest and is the first step in this process (Figure 1).

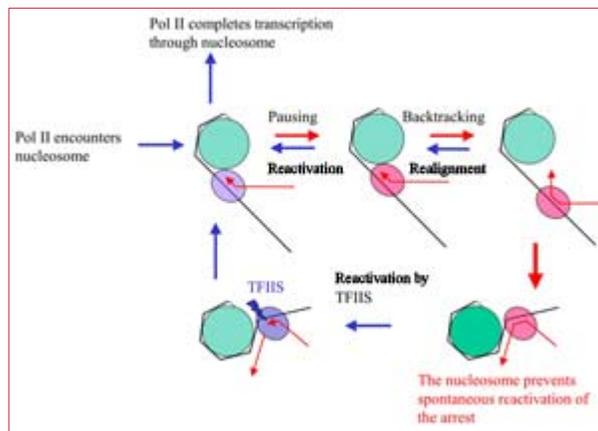


Figure 1. Nucleosome-induced transcription arrest. The nucleosome is viewed from top. Only one superhelical turn of the DNA around a histone octamer (green circle) is shown. The RNA in the elongation complex is shown as a red arrow. RNA polymerase in the active elongation complex is shown in blue; in the paused or arrested elongation complex, it is shown in pink. Pol II, RNA polymerase II; TFIIS, transcription factor S-II.

Next, we examined the mechanism of arrest. On free DNA, arrest occurs by backtracking (Komissarova N and Kashlev M. *Proc Natl Acad Sci U S A* 94: 1755–60, 1997). Not surprisingly, blocking RNA polymerase II backtracking prevented nucleosome-induced arrest both in the stalled complexes and during continued transcription. Thus, we showed that nucleosome-induced arrest also occurs by the backtracking mechanism (Figure 1). Moreover, backtracking of RNA polymerase II leads to stabilization of the histone-DNA contacts in the nucleosome. From this we conclude that during processive RNA synthesis, the polymerase successfully disrupts DNA-histone contacts. However, when the elongation complex stalls and backtracks, the disrupted DNA-histone contacts are established again, preventing spontaneous reactivation of the elongation complex (Figure 1).

Once the polymerase is trapped in the inactive state by the DNA-histone contacts reestablished downstream of the backtracked elongation complex, the nucleosome indeed becomes a block to further elongation. The transcription resumes only in the presence of the anti-arrest factor transcription factor S-II (TFIIS). TFIIS promotes endonucleolytic cleavage of the RNA, which creates a new 3' end properly positioned in the active center. The reactivated polymerase acquires another chance to overcome the nucleosome. We observed that in the presence of TFIIS, the polymerase was more likely to transcribe through the nucleosome. However, even with TFIIS, the elongation remained slow, presumably because of the multiple transcript cleavage and re-synthesis cycles occurring in the presence of TFIIS. It is likely that the nucleosome-induced arrest rarely develops in the cell, because it is blocked at any of the three preceding stages (pausing, backtracking, and reestablishment of

the DNA-histone contacts downstream of the backtracked elongation complex, such that the nucleosome prevents spontaneous reactivation of the arrest).

In summary, we have uncovered the following sequence of events leading to nucleosome-induced transcription arrest:

- RNA polymerase II efficiently disrupts the DNA-histone contacts until it encounters an intrinsic pause site and stops there.
- The elongation complex pauses and then backtracks.
- The histone-DNA contacts, formed downstream of the polymerase, stabilize the inactive backtracked conformation of the elongation complex.

Notably, only the last step is nucleosome specific and may be dependent on chromatin remodeling and modification. The first two steps, as well as TFIIS-dependent reactivation, are nucleosome independent. Thus, transcription factors that change the efficiency of sequence-specific pausing and backtracking should be powerful regulators of transcription in the context of chromatin, along with chromatin remodeling and modification factors.

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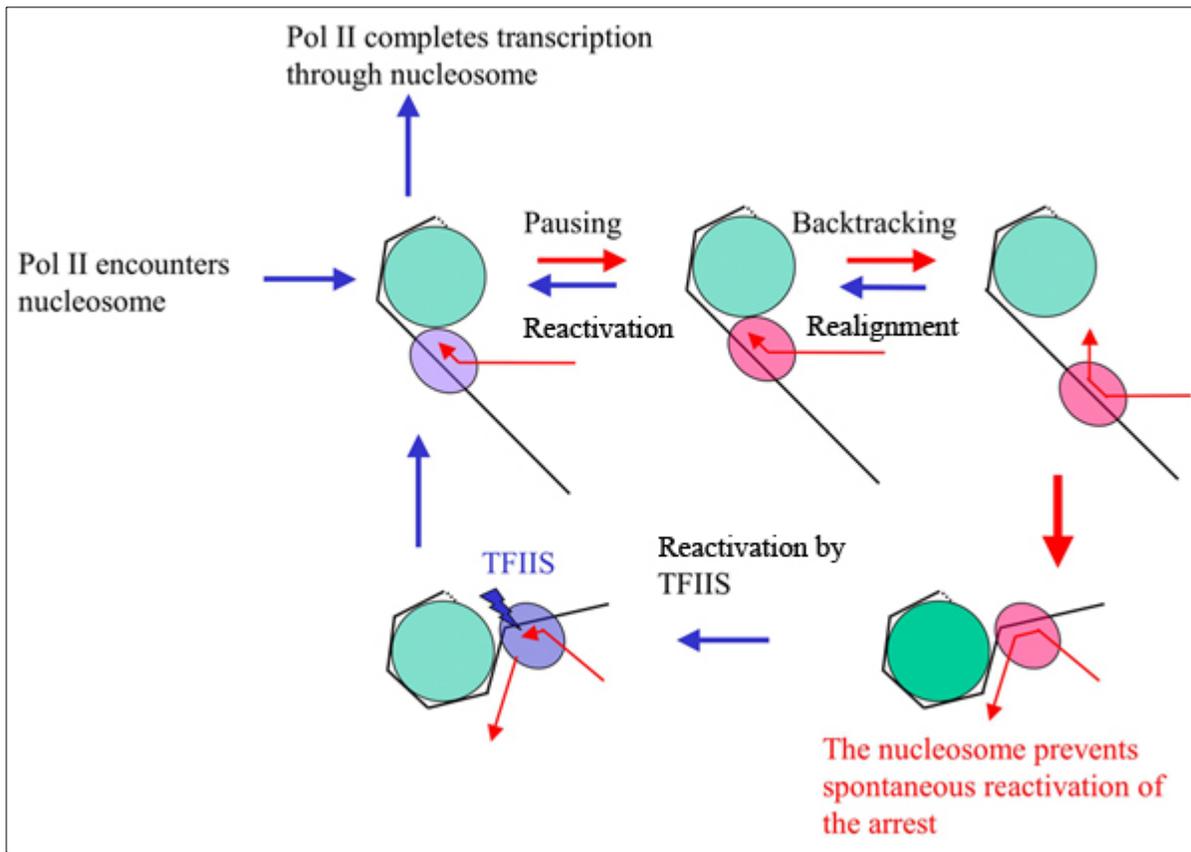


Figure 1. Nucleosome-induced transcription arrest. The nucleosome is viewed from top. Only one superhelical turn of the DNA around a histone octamer (green circle) is shown. The RNA in the elongation complex is shown as a red arrow. RNA polymerase in the active elongation complex is shown in blue; in the paused or arrested elongation complex, it is shown in pink. Pol II, RNA polymerase II; TFIIIS, transcription factor S-II.

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